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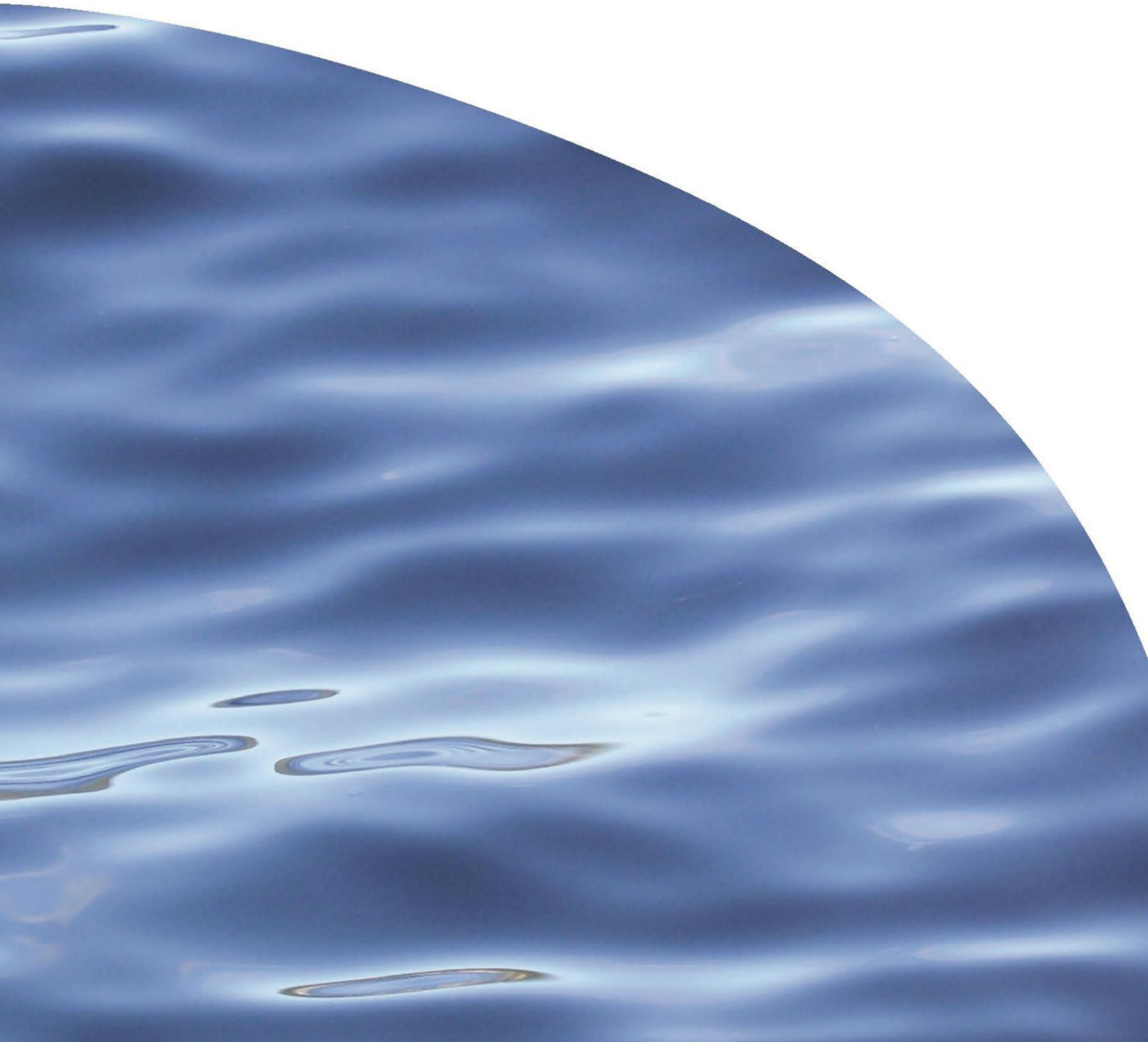
INVESTIGATING ENVIRONMENTAL DRIVERS OF *PHORMIDIUM* BLOOMS



INVESTIGATING ENVIRONMENTAL DRIVERS OF *PHORMIDIUM* BLOOMS

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EXECUTIVE SUMMARY

The aims of this project were to collate *Phormidium* data from all known datasets across the country, identify correlative relationships between *Phormidium* and environmental drivers, and to recommend experimental studies to provide further evidence of any identified relationships. The key outcomes from six areas of work were:

1. **Identifying factors driving temporal and spatial variation in *Phormidium* cover in three regions (analysis of weekly resolution datasets).** We explored the temporal variation in *Phormidium* data collected at weekly intervals in three regions (Manawatu, Nelson and Canterbury) in relation to potential driver variables using a generalised additive mixed model (GAMM). Model output showed that *Phormidium* blooms are influenced by a complex interplay of seasonality, river flow and water chemistry. In general, streams with dissolved inorganic nitrogen (DIN) of greater than 0.05 mg L^{-1}, dissolved reactive phosphorus (DRP) of less than 0.01 mg L^{-1} and flows between ca. 0.1 and 1.0 times median flow were more likely to experience *Phormidium* proliferations. However, there were exceptions which suggested that different hierarchies of importance in physicochemical variables occur at some sites.

2. **Identifying factors driving spatial variation in *Phormidium* cover (Manawatu monthly dataset).** We explored the spatial variation in periphyton data from 61 river sites that covered a wide range of nutrient and river flow conditions in the Manawatu-Wanganui region using boosted regression tree (BRT) models. Three periphyton response variables were included in the models: *Phormidium,* filamentous algae and chlorophyll-*a*. For each response variable, two separate BRT models were built, one with a catchment-scale descriptor of land use (heavy pastoral land cover) and other descriptors of environmental variability, and a second model with measures of proximate stressors (e.g. nutrient concentration, turbidity) and environmental variables. All three periphyton indicators responded similarly to heavy pastoral land cover. In the first model, when pastoral land cover exceeded 20% there was a near linear increase in *Phormidium* cover until pastoral land cover was greater than 80%. In the second model, replacement of the pastoral land cover variable by measures of nutrients and fine sediment improved BRT model fit for all three periphyton response variables, providing insights into possible factors influencing *Phormidium* blooms. Model output suggested that *Phormidium* cover increased with rising DIN to a threshold of ca. 0.6 mg L^{-1}, after which further changes had little effect, and that streams with more frequent flushes (≥ 3 times the long-term median flow) are more likely to experience *Phormidium* proliferations.

3. **Developing a model to predict river susceptibility to *Phormidium* nationally.** We developed a generalised additive model (GAM) using *Phormidium* and environmental data from 492 sites across the North and South Island and predicted maximum *Phormidium* cover in all New Zealand streams. The model explained 67% of the

deviance in *Phormidium* cover and had satisfactory performance with a coefficient of determination value (R^2) of 0.51, a Nash-Sutcliffe efficiency value of 0.5 and a relative root mean square error of 0.7. The percent bias performance statistic indicated that the model moderately overestimated maximum percentage *Phormidium* cover by 7.1%. In general the model predictions conformed to our expectations and showed that proliferations generally occur in lowland streams in regions where water quality is relatively good, and do not occur in slower flowing, nutrient-rich habitats. The model predictions provide a description of regional to national scale patterns in streams which might experience *Phormidium* proliferations. The predictions should be interpreted with caution at a site scale, as other processes that can influence *Phormidium* proliferations along with seasonal and inter-annual variability are not accounted for.

4. **Independent model validation of the national river susceptibility model.** We tested the national river susceptibility model by collecting *Phormidium* cover data from 32 stream sites during the 2016/17 summer. Despite *Phormidium* being predicted to occur at all of these sites it was only observed at 11 sites. At 10 of the 11 sites, cover was lower than predicted and there was only one site where the cover exceeded the levels predicted. The independent model validation provided a number of interesting insights into the range of river types where *Phormidium* occurs and proliferates, and ways in which the river susceptibility model could be refined (e.g. exclusion of large rivers). The site visits also indicated that the river susceptibility model overpredicts *Phormidium* cover.

5. **National observed data analysis.** We developed a GAM using the national *Phormidium* dataset assembled during the development of the river susceptibility model to explore whether the relationships identified in previous sections of this study were observed at a national scale. The number of observations and environmental variables measured varied considerably between regions. To maximise the size of our dataset we focused on the four most commonly-measured environmental variables: DIN, DRP, conductivity and water temperature. The model had low (16%) explanatory power, which may be due to sample error (i.e. variability in the ways data are collected and analysed among regions) and the exclusion of variables such as flow. Despite the low explanatory power of the model, the modelled relationships between *Phormidium* cover and water quality variables were consistent with those previously identified in our analyses. The two environmental variables that showed the strongest relationships with *Phormidium* cover were DIN and DRP.

6. **Recommendations for experiments to test for causal relationships.** The Ministry for the Environment Benthic Cyanobacteria Working Group met in March 2017 to discuss a range of possible experimental projects to better quantify the relationship between *Phormidium* and environmental drivers. The following three projects were identified as high priority by the Working Group:

- *Phormidium* inoculation experiments (described in detail in this report) conducted in a range of streams across the country varying in land use and environmental gradients.
- Further research on the role of fine sediment. This could involve manipulative experiments either in stream and/or using *ex situ* channels.
- Obtaining further weekly resolution data including corresponding site descriptors, such as water depth and substrate composition.

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1. INTRODUCTION

Phormidium is a genus of benthic mat-forming cyanobacteria that blooms (defined as >20% cover of a stream bed) in streams around New Zealand. It commonly produces toxins (anatoxins) which pose a human health risk through ingestion and skin contact. There has been an apparent increase in *Phormidium* blooms in streams across New Zealand in the past decade and ingestion of mats and/or stream water has resulted in numerous animal fatalities (Wood et al. 2015a; McAllister et al. 2016).

In 2014, the Ministry for the Environment commissioned a literature review ('the Review') to summarise existing knowledge on benthic cyanobacteria (including *Phormidium*) and associated toxins:

> Wood SA, Hawes I, McBride G, Truman P, Dietrich D 2015. Advice to inform the development of a benthic cyanobacteria attribute. Prepared for Ministry for the Environment. Cawthron Report No. 2752. 91 p.

Following this, a Benthic Cyanobacteria Expert Panel ('the Panel') was convened to discuss the review. The Panel concluded that there were significant knowledge gaps regarding the environmental drivers of *Phormidium* blooms and that these needed to be addressed to allow for the development of a *Phormidium* attribute suitable for application within the National Objectives Framework. The Panel identified that a number of research groups have collected data. However, the datasets have been analysed independently and, as a result, there is a limited understanding at a national level of what environmental factors promote *Phormidium* blooms and where and when blooms are likely to occur.

The Cawthron Institute (Cawthron) and University of Canterbury were tasked by the Ministry for the Environment to collate and analyse data from all known datasets across the country, and to design potential experimental studies to confirm any correlative relationships identified during the data analyses. The six research objectives were;

1. To combine high resolution (weekly) datasets and identify and quantify relationships between water chemistry, flow and *Phormidium* percent cover within and across regions.
2. To use one or more monthly resolution dataset(s) to characterise the relationship between *Phormidium* proliferations and potential driver variables and identify potential thresholds in the relationships if present.
3. To collate information about stream sites which experience *Phormidium* blooms from Regional Council datasets into a 'National Dataset', including land use and catchment characteristics from the River Environment Classification (REC), Freshwater Ecosystems of New Zealand (FENZ), and Landcover Database v3.0

(LCDB3). This dataset will then be used to predict the susceptibility of rivers to *Phormidium* blooms across New Zealand (risk susceptibility model).

4. To select approximately 30 new stream sites identified as having a high likelihood of experiencing *Phormidium* blooms in the risk susceptibility model and undertake a survey of *Phormidium* during periods when blooms would be expected (i.e. independent model validation the risk susceptibility model).
5. To use the 'National Dataset' to identify drivers of *Phormidium* proliferations and potential thresholds at a national scale.
6. To provide recommendations of manipulative experiments that could be undertaken to test for causal relationships based on the correlative patterns identified during this study.

2. IDENTIFYING FACTORS DRIVING TEMPORAL AND SPATIAL VARIATION IN PHORMIDIUM COVER IN THREE REGIONS (ANALYSIS OF WEEKLY RESOLUTION DATASETS)

2.1. Introduction

The abundance of benthic *Phormidium* in streams has been observed on a weekly basis by several regional councils and researchers. The weekly data allow for an in-depth analysis of the effects of physicochemical variables on *Phormidium* percent cover and accrual rates. To date, these datasets have been analysed separately. Our research objective was to combine and analyse these datasets to obtain a cross-regional perspective of how the measured environmental variables influence *Phormidium* abundance. The dataset is also used to explore accrual rates and carrying capacity (see Section 6.1).

2.2. Methods

2.2.1. Data sets

We collated three weekly datasets from a total of 21 stream sites (Table 1):

1. Canterbury region. This dataset contains weekly measurements from a single site in each of eight rivers in the Canterbury region. These rivers were sampled from November 2014 to June 2015. Locations and detailed sampling methods are described in McAllister et al. (in review).
2. Maitai River in Nelson. This dataset contains weekly measurements at three sites in the lower Maitai River over three summer periods (2012/13, 2013/14 and 2015/16). Locations, detailed sampling methods and analysis of the 2012/13 and 2013/14 datasets are described in Wood and Bridge (2014) and Wood et al. (2015b). The 2015/16 data had not been collated or analysed previously.
3. Manawatu region. One or two sites at each of seven rivers were sampled weekly from January 2012 to June 2013. Locations, detailed sampling methods and analyses are described in Wood and Young (2012), Wood et al. (2014) and Wood et al. (2016).

Sampling methods were generally consistent between studies. To assess *Phormidium* cover all studies followed the transect method outlined in Ministry for the Environment and Ministry of Health (2009). Five transects (total of 25 views) were used in the Canterbury study, whereas four transects (total of 20 views) were used in the Nelson and Manawatu studies.

The following environmental variables were measured consistently among studies: spot measures of water temperature, conductivity, dissolved inorganic nitrogen (DIN), and dissolved reactive phosphorus (DRP), and continuous measurement of river flow at nearby flow gauges (Wood et al. 2015b; McAllister et al. 2016; Wood et al. 2016; Table 1).

Table 1. Physical and hydrological characteristics and maximum *Phormidium* cover of the 21 study sites. Long-term median flow is from the gauging site closest to each sampling site. Catchment data were retrieved from the Land cover Database v3.0. d/s = downstream, u/s = upstream, STP = sewage treatment plant.

River - Site	Site No.	Region	Catchment area (km^2)	Heavy pastoral land cover (%)	Native vegetation cover (%)	Other land cover (%)	Median flow (m^3 s^{-1})	Max. *Phormidium* cover (%)
Ashley River	1	Canterbury	11,500	25	70	5	10.2	42.8
Opihi River	2	Canterbury	17,400	61	35	4	8.7	72.8
Orari River	3	Canterbury	5,400	5	93	2	6.2	28.0
Paraora River	4	Canterbury	4,300	68	29	3	1.4	20.9
Selwyn River	5	Canterbury	2,400	46	40	14	2	24.6
Te ana a wai River	6	Canterbury	5,000	69	29	2	1.8	31.4
Temuka River	7	Canterbury	5,600	62	30	8	3.4	43.4
Waipara River	8	Canterbury	4,100	34	62	4	0.9	12.4
Makakahi River	9	Manawatu	163	79	18	3	3.18	46.5
Manawatu River d/s STP	10	Manawatu	4,022	73	21	6	73.4	46.8
Manawatu River u/s STP	11	Manawatu	3,915	74	20	6	73.4	2.1
Mangatainoka River	12	Manawatu	11	0	98	2	2.13	1.0
Mangatainoka River	13	Manawatu	413	76	20	4	8.90	56.0
Oroua River d/s STP	14	Manawatu	585	75	16	9	7.10	45.3
Oroua River u/s STP	15	Manawatu	582	76	16	8	7.10	10.8
Oruakeretaki River	16	Manawatu	54	67	30	3	1.42	14.3
Tiraumea River	17	Manawatu	744	83	12	5	7.21	15.3
Tokomaru River	18	Manawatu	56	1	77	22	1.25	74.3
Maitai River – Avon Terrace	19	Nelson	91	2	58	40	0.87	54.0
Maitai River – Campground	21	Nelson	91	2	58	40	0.58	41.8
Maitai River – Dennes Hole	20	Nelson	91	2	58	40	0.95	61.3

Table 2. Descriptive summary of each predictor variable included in the *Phormidium* cover generalised additive mixed models. * calculated from the maximum daily mean flow between sampling periods divided by the long-term median flow

Variable	Units	Mean	Median	SD	Minimum	Maximum	Transformation
Accrual DIN	mg L^{-1}	0.395	0.237	0.467	0.002	2.409	log (x)
Accrual DRP	mg L^{-1}	0.0095	0.0056	0.0135	0.0005	0.1300	log (x + 0.00025)
Conductivity	µS cm^{-2}	149	140	76.41	29	481	log (x)
Maximum median flow*	ratio	2.78	0.92	5.60	0.001	87.77	log (x + 0.0005)
Temperature	°C	14.5	14.8	4.17	4.0	26.2	untransformed

2.2.2. Data analysis

We explored non-linear trends in percent *Phormidium* cover (n = 1,102) in relation to time (week) of the year and environmental variables using generalised additive mixed models (GAMMs, Hastie & Tibshirani 1990). We used log-normal errors because cover data were strictly positive, highly right-skewed, over-dispersed and with a large proportion of zeros.

Based on initial data exploration six non-collinear predictor variables were considered in the analyses: week of the year, accrual DIN, accrual DRP, water temperature, conductivity and maximum median flow (calculated from the maximum daily mean flow between sampling periods divided by the long-term median flow; Table 2). Accrual DIN and DRP were used rather than spot measurements as Biggs and Close (1989) suggest that simple point-by-point correlations between nutrient concentrations and periphyton biomass do not provide a true indication of the water column nutrients that periphyton has been exposed to over its accrual period. Accrual DIN and DRP were calculated as the cumulative mean of the DIN (the sum of NH_4-N, NO_2-N and NO_3-N) and DRP concentrations over the accrual period were calculated for each time-point (i.e., the mean concentrations since the previous sampling point where the *Phormidium* cover was zero). Where biomass was present at the beginning of sampling, accrual nutrients were calculated from the start of the sampling period. Log-transformed accrual DIN and DRP were included as fixed effects covariates in the model. Because many of the accrual DRP values were very low (0.0005), half of observed minimum (0.00025) was added for transformation. The influence of flow was considered as a fixed effect in the model by including log-transformed 'maximum mean flow' + 0.0005 (i.e. half of minimum). Water temperature and log transformed conductivity were included as continuous fixed effect covariates. Water temperature and log-transformed conductivity were included as continuous covariates. Week of the year was included as continuous covariate (1 to 52) to account for seasonal trends, using a cyclic cubic spline to allow the continuity between January and December. Site was included as a random effect to account for spatial variability among sites within the three regions.

Collinearity among predictor variables was checked using the variance inflation factor (VIF < 5, Zuur et al. 2010). To reduce the effect of temporal autocorrelation in the weekly data, models were fitted using auto-regressive moving average correlation structure of order 1.

The non-linear interaction between accrual DIN and DRP on *Phormidium* cover was further investigated using a tensor product smoother in a GAM model, and visualised using a contour plot of the predicted values.

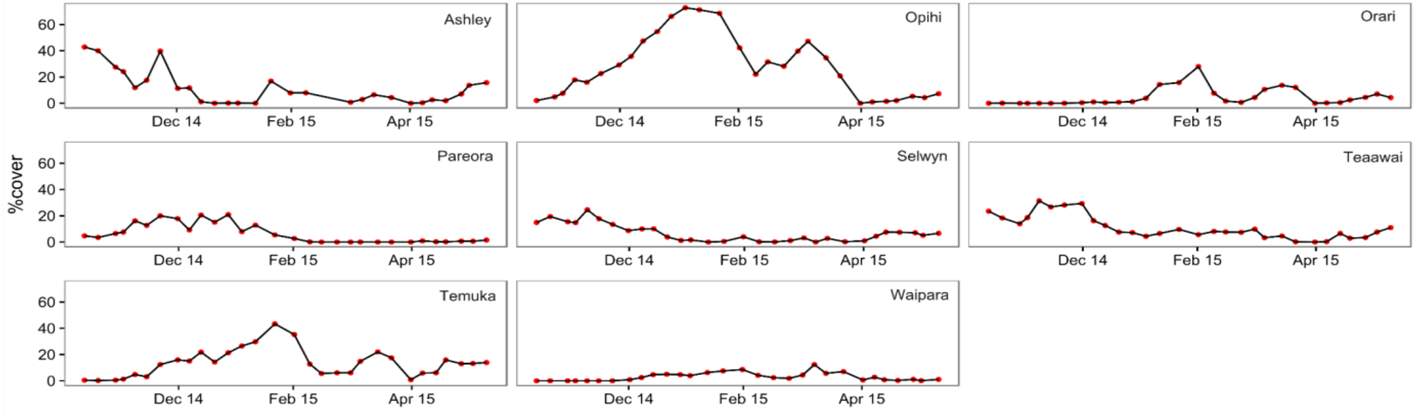
Competing models were evaluated using a stepwise procedure based on the Generalised Akaike Information Criteria (GAIC) approach, and inspecting the deviance residuals. Selected models were summarised using partial effects plots, which show the effect of each predictor variable conditional to others in the model. The partial effects of each predictor are displayed as cubic splines showing either negative or positive effects relative to the overall mean of the response variable centred on zero. Partial plots also show standard errors around the fitted spline and partial residuals for each observation. All statistical analyses were performed with the software R (R Core Team 2014) using the 'gamlss' package (Rigby & Stasinopoulos 2005).

2.3. Results

2.3.1. Temporal trends in Phormidium cover

The period with the highest observed *Phormidium* cover was generally from November through to the end of May. However, months with high cover varied among regions and sites (Figure 1). For example, among the Canterbury sites, *Phormidium* cover was highest in the Ashley River in November and December (the river dried up later in the summer), but in January and February in the Opihi River (Figure 1). The Manawatu dataset spans two summer periods enabling inter-annual comparisons. At some sites bloom intensity varied markedly between 2012 and 2013, e.g. the Manawatu and Oroua rivers downstream of the sewerage treatment plant (STP), (Figure 1). In Nelson's Maitai River blooms were most pronounced in spring or early summer, and with the exception of the Avon Terrace site, blooms disappeared in December and did not reform over the summer sampling period (Figure 1).

A. Canterbury region



B. Manawatu region

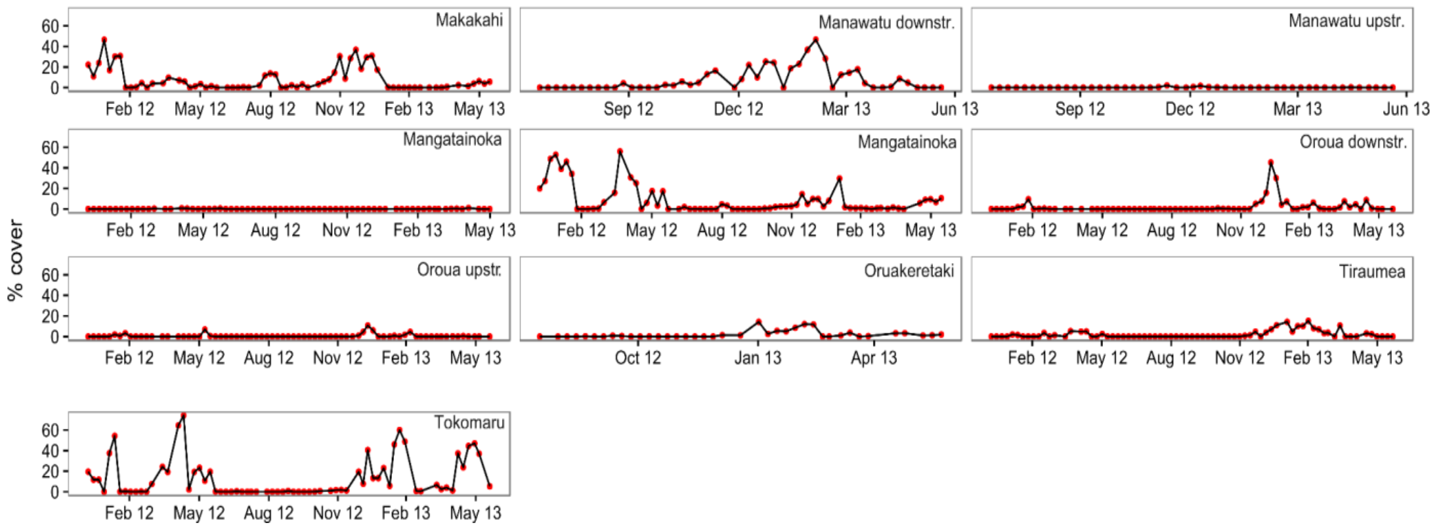


Figure 1. Percentage *Phormidium* cover assessed weekly at each stream site in three regions: (A) Canterbury, (B) Manawatu, and (C) Nelson (next page).

C. Nelson region – Maitai River

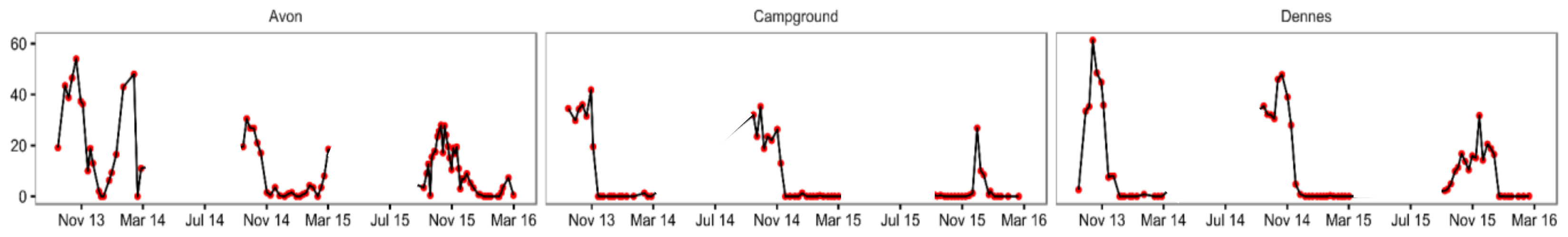


Figure 1, continued. Percentage *Phormidium* cover assessed weekly at each stream site in three regions: A) Canterbury, B) Manawatu (previous page), and C) Nelson.

2.3.2. Environmental variables

In general, accrual DIN and DRP was lowest at the Nelson sites and highest at the Manawatu sites (Figure 2). Variation in median accrual DIN and DRP concentrations within sites was often less than variation between sites in each region. The range in temperature observed across the regions was similar and the slightly higher medians in the Canterbury and Nelson datasets are likely because sampling only occurred over the summer in these studies. Within the Canterbury and Manawatu regions there were notable differences in conductivity between sites. For example, conductivity at sites 4 (Paraora River) and 8 (Waipara River) in Canterbury and 17 (Tiraumea River) in Manawatu were markedly higher than the other study sites (Figure 2).

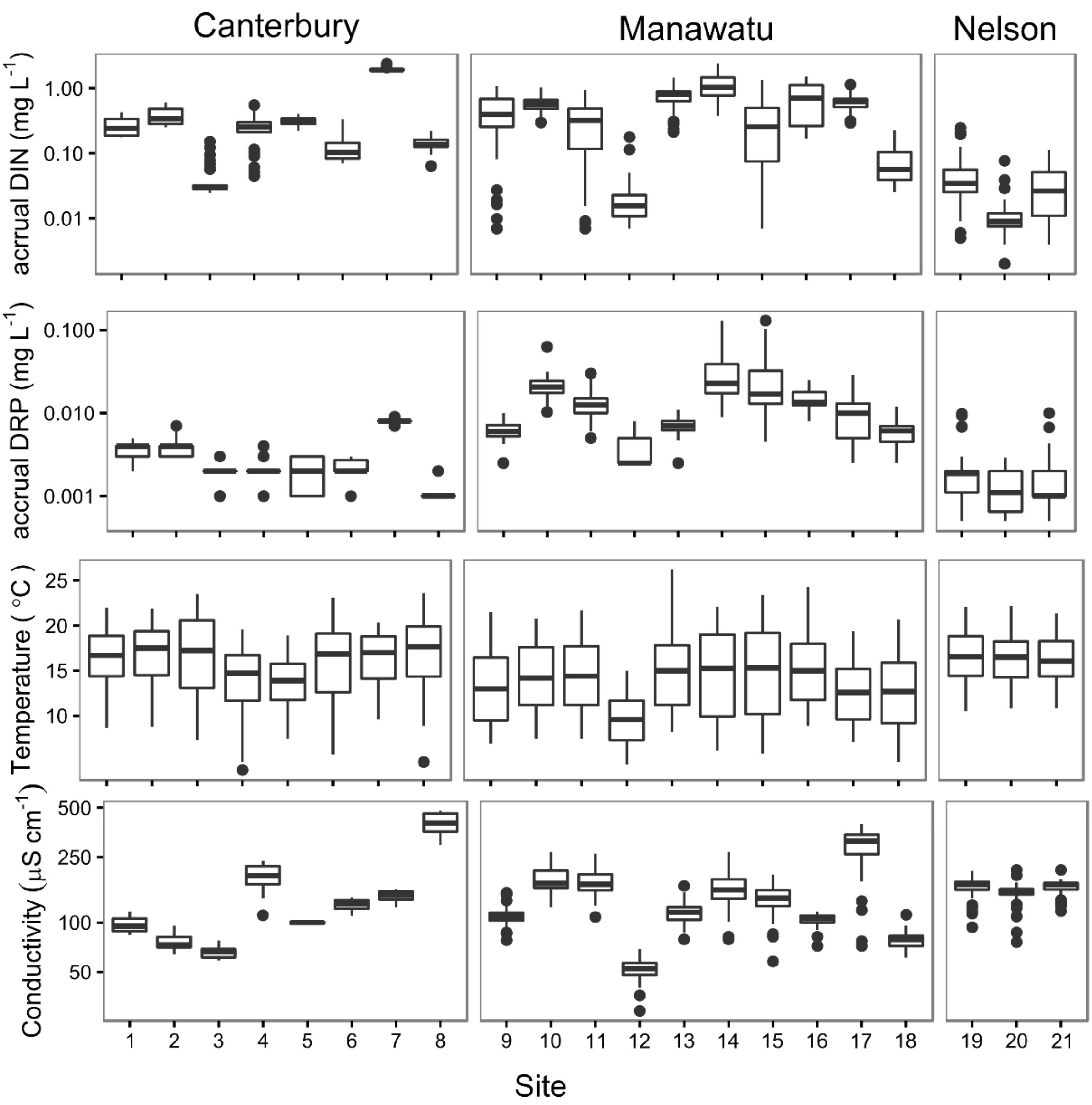


Figure 2. Physicochemical variables assessed at each site: (a) accrued dissolved inorganic nitrogen (DIN), (b) accrued dissolved reactive phosphorus (DRP), (c) temperature and (d) conductivity. Y-axes (except temperature) in log scale. The solid black line in each box is the median; the box is confined by the 1st and 3rd quartiles; and whiskers extend to the lowest or highest data point still within 1.5 times the inter-quartile range and black dots are outliers as they extend beyond this range.

2.3.3. Driver-response model

The most parsimonious GAMM explained 52% of the total deviance in *Phormidium* cover and included six predictor variables: week of the year, accrual DIN, accrual DRP, maximum flow, conductivity and site (Table 3, Figure 3, $P < 0.001$). Water temperature was eliminated during the stepwise model selection process based on the GAIC. Week of the year had a weak and marginal effect ($P = 0.12$, Figure 3a), with a positive effect on *Phormidium* cover predicted for the first nine weeks of the year and for the last 11 weeks, that is from November through February (Figure 3a).

Accrual DIN had a positive effect on *Phormidium* cover up to a concentration of0.05 mg L^{-1} after which further increases had no effect (Figure 3b). Increasing accrual DRP, on the other hand, was associated with reducing cover, although this effect was weaker from about 0.025 mg L^{-1} (Figure 3c). There was a significant effect of maximum medium flow, with a weak positive effect predicted for flows up to 0.1 times the long-term medium (although there are limited data at flows less than this), and a strong negative effect for maximum median flow when greater than 1 (Figure 3d). There was a relatively weak, but statistically significant positive effect of conductivity, with *Phormidium* cover increasing monotonically with conductivity, particularly above 200 µS cm^{-1} (Figure 3e). 'Site' had a strong, statistically-significant effect, indicating that the large variability between sites that could not be explained by other explanatory variables (Figure 3f).

Table 3. Results of the generalised additive mixed model for *Phormidium* cover with log-normal error terms. Bold values are statistically significant (P < 0.05). DIN = Dissolved inorganic nitrogen, DRP = Dissolved reactive phosphorus.

	Estimate	**Std. Error**	**t value**	***P*-value**
Intercept	-10.70	0.84	-12.67	**< 2e-16**
Week of year	- 0.01	0.00	-1.55	0.121
Accrual DIN	0.57	0.05	10.73	**< 2e-16**
Accrual DRP	-1.01	0.08	-12.02	**< 2e-16**
Maximum median flow	-0.74	0.05	-13.65	**< 2e-16**
Conductivity	1.17	0.15	7.64	**4.81e-14**

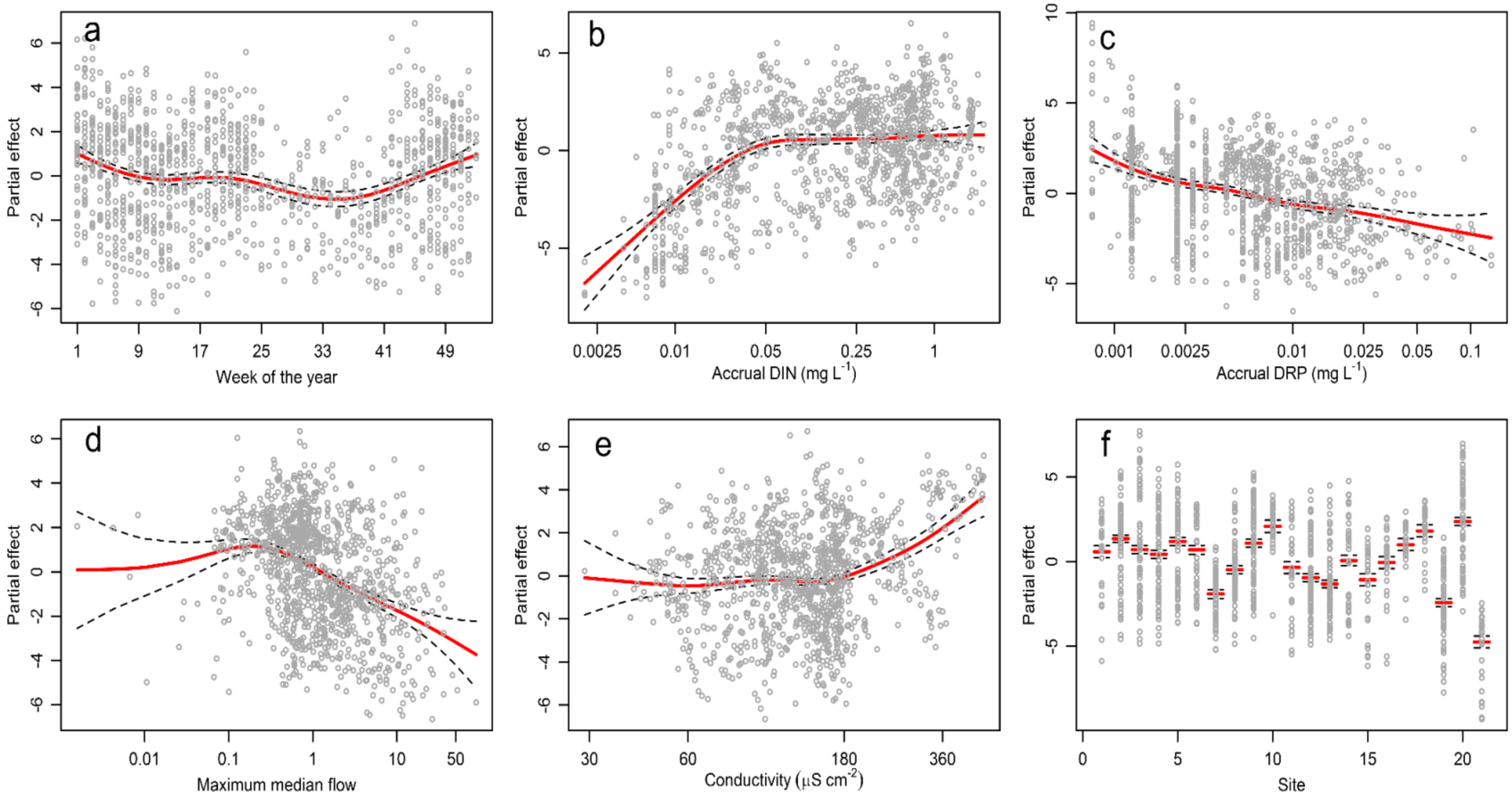


Figure 3. The percentage cover of *Phormidium* (partial plots) in relation to: (a) week of the year, (b) accrual dissolved inorganic nitrogen (DIN), (c) accrual dissolved reactive phosphorus (DRP), (d) maximum median flow (= daily mean flow divided by long-term median flow), (e) conductivity, and (f) site. Red lines represent cubic splines (± standard error, dashed black lines) fitted using a log-normal generalised additive model. See methods for description of x-axis partial effect scale. Note differences in the scales on the y-axes.

2.3.4. Potential DRP and DIN thresholds for bloom formation

A comparison of the accrual DRP and DIN concentrations from the three regions showed they loosely fall into three clusters (Figure 34a).

1. In the Maitai River (Nelson), where DRP and DIN tended to be low and low-moderate respectively, observations of high *Phormidium* cover occurred when accrual DRP was low (below 0.05 mg L^{-1}) and a range of accrual DIN from 0.01 to 0. 25 mg L^{-1}.
2. In Canterbury, DIN and DRP concentrations overlapped those of Nelson, and high cover was again observed at low accrual DRP (below 0.01 mg L^{-1}). There was a greater range in accrual DIN concentrations at which blooms occurred (ranging from 0.02 to 2.39 mg L^{-1}; Figure 4a).
3. Accrual DIN and DRP concentrations showed the greatest range among sites in the Manawatu region. With the exception of two sites (both downstream of sewerage treatment plants), cover greater than 20% only occurred when accrual DRP concentrations were less than 0.01 mg L^{-1} (Figure 4a). As in other regions, blooms occurred with DIN concentrations from 0.02 to >1 mg L^{-1}.

There was a significant interactive effect of accrual DIN and DRP on the cover of *Phormidium* ($P < 0.001$). The interactive nutrient effect model, using data from all sites, predicted greater than 20% cover when accrual DIN was ca. 0.3 mg L^{-1} , and DRP ca. 0.003 mg L^{-1} (Figure 4b).

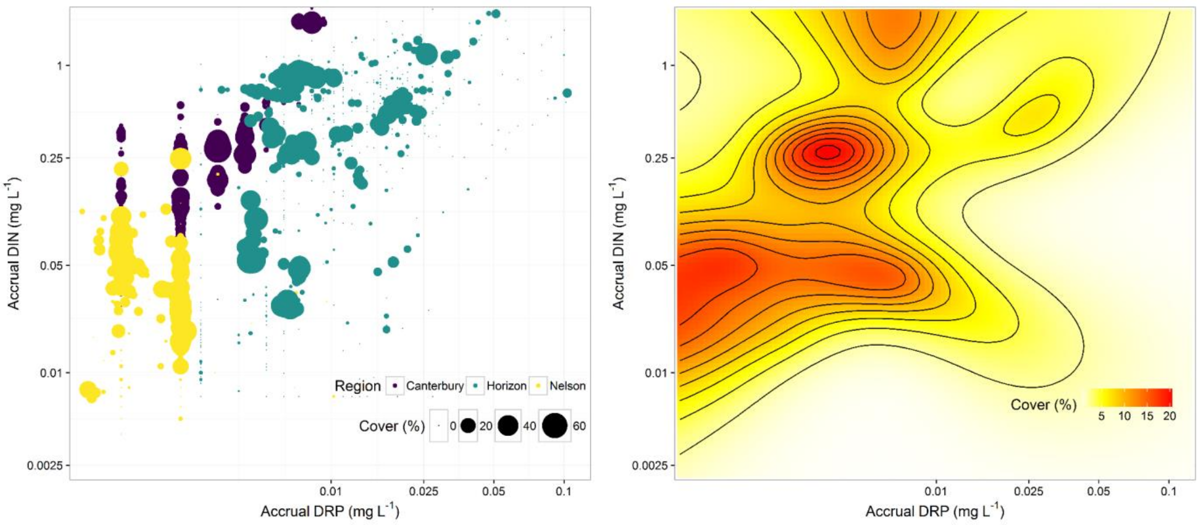


Figure 4. Relationship between accrual dissolved reactive phosphorus (DRP) and accrual dissolved inorganic nitrogen (DIN) on the percentage cover of *Phormidium*: (a) observed cover data for each region [Nelson is yellow, Manawatu is green, Canterbury is purple], and (b) contour plot of *Phormidium* cover predicted by generalised additive modelling. Note the y- and x-axis are in log-scale.

2.4. Discussion

Streams in the three study regions which experience *Phormidium* blooms (> 20% cover) generally have relatively good water quality. Our analyses showed that increasing DIN concentrations up to ca. 0.05-0.1 mg L^{-1} positively affected *Phormidium* cover, after which increasing DIN had no further positive effect. Blooms could occur, however, at DIN concentrations as low as 0.02 mg L^{-1}. *Phormidium* cover, on the other hand, decreased when DRP concentrations exceeded ca. 0.01 mg L^{-1}, and no blooms were seen at DRP concentrations above 0.025 mg L^{-1}.

The ability of *Phormidium* to form blooms under relatively low nutrient concentrations is at first counterintuitive, based on previous studies which usually associate high algal biomass with nutrient enrichment. *Phormidium*, however, has a growth form quite different to many other types of periphyton, forming thick and cohesive mats. This growth structure is likely critical to understanding the prevalence of *Phormidium* mats in rivers with relatively low DRP concentrations. Additionally, *Phormidium* mats go through different successional stages—colonisation, growth and expansion, and dispersal (Wood et al. 2015a). Environmental variables that are important to thin, early-stage *Phormidium* films may not be essential to thick well-established mats. Sand-Jensen (1983) noted that the relationships between the water column nutrients and algal mats are most important and evident during early growth stages. As mats mature and internal processes such as nutrient recycling begin, water column nutrients are less important.

A process that may give *Phormidium* a competitive advantage over other algae during the initial colonisation phase is luxury phosphorus uptake. Luxury uptake is the ability to store phosphorus as polyphosphate which can be used for subsequent growth when phosphorus concentrations are low (Rier et al. 2016). It occurs during periods when phosphorus is available at a higher concentration than is needed for immediate growth. During experimental studies, where small fragments of *Phormidium* mats were placed in low nutrient environments, we observed very rapid growth over 2-3 days, before mat expansion ceased (Wood, unpub. data). Based on the assumption that the fragments contained excess P at the beginning of the experiment, this suggests that *Phormidium* may have the ability to store polyphosphates, however further research is required to confirm this.

Wood et al. (2015c) used microelectrodes to show that a boundary layer (a layer directly above the mats where a velocity gradient exists) of ca. 0.1 mm to 0.2 mm thick exists between the surface of well-developed *Phormidium* mats and river water, which may restrict fluxes of nutrients in and out of the mat. While the boundary layer can limit nutrient exchange, it facilitates the formation of geochemical conditions (e.g. pH and dissolved oxygen) within mats that are very different to the surrounding water column (Wood et al. 2015c). Wood et al. (2015c) suggest these conditions enable release of DRP bound to sediments entrapped in the mat. The authors showed much higher (320-fold) DRP of the water contained within the mats compared to that of the overlying water column. They suggest this may partially explain how *Phormidium* can form high biomass in rivers with very low DRP. Aristi et al. (2016) provide evidence of alkaline phosphatase activity within *Phormidium* mats. This involves the conversion of organic phosphorus into forms that can be used for growth. Based on these observations, it is likely that once *Phormidium* mats are established, water column DRP concentrations are of little relevance to further biomass accrual. This could help explain why no significant responses to increased DRP were observed in this study, despite such relationships having been reported for other periphyton in New Zealand rivers (Biggs & Close 1989). The negative response to increased DRP might be related to competition, with other forms of periphyton that are more effective at taking up nutrients from the water column (i.e. filamentous green), dominating under these conditions.

Molecular analysis of *Phormidium* cultures isolated from New Zealand rivers indicates that they cannot fix nitrogen (Heath 2015). This, in concert with culture-based studies (Heath et al. 2016), suggests that increased DIN concentrations should enhance *Phormidium* proliferations. However, Brasell et al. (2015) demonstrated how diverse bacterial communities exist among *Phormidium* mats including taxa capable of nitrogen fixation. Thus, although *Phormidium* itself cannot fix nitrogen, other organisms within the mat may provide sufficient quantities for growth once mats are established. Therefore, as described above for DRP, DIN in the water column may be critical during the initial colonisation phase, but less important once cohesive, multi-functional mats have established.

Previous studies have suggested water column DIN concentrations greater than 0.10-0.20 mg L^{-1} promote *Phormidium* proliferations (Wood & Young 2012; Heath et al. 2015; McAllister et al. 2016). In general this threshold holds true but in the present study however, there were sites in each region that did not confirm to this threshold. The interaction between DIN and DRP predicted that the highest *Phormidium* cover would occur when DIN concentrations were 0.3 mg L^{-1}. However, blooms have been reported at DIN concentrations much lower than this. Analysis of the Maitai River (Nelson) data showed that a reduction in DIN in early summer did not have an effect on *Phormidium* cover. However, despite prolonged stable flows following a detachment event, *Phormidium* mats did not re-establish at sites where blooms previously persisted. Wood et al. (2015b) suggest that monitoring did not start early enough in the season 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. They also speculate that the inability of the mats to re-establish in summer is due to low water column DIN. In the Manawatu dataset Site 18 (Tokomaru River) has very low DIN concentrations yet experiences prolonged proliferations. A possible reason for this observation is that a high intensity flow (ca. > 200 times the long-term medium, Wood et al. 2016) is predicted to be required to reduce *Phormidium* cover at this site. The high substrate stability at this site provides long accrual periods, and may also result in larger starting inoculums following flushing events. These 'relic' patches may already contain nitrogen-fixing organisms enabling rapid mat expansion.

Conductivity was identified as having a positive relationship with *Phormidium* cover, however caution is required when interpreting this result as it is primarily driven by two sites (8 and 17) with high conductance and cover values. These sites also had high concentrations of some metals such as iron (data not shown), but the paucity of such data precluded their inclusion in the current analysis. Conductivity is often correlated to periphyton biomass, and highly related to catchment geology and water source in New Zealand rivers (Biggs 1990, 1995). It is also integrally linked with river flow, with concentrations generally increasing during periods of low flow because of lack of dilution from lower ionic content water, a trend also evident in our dataset (data not shown). Many of the major ions contributing to conductivity e.g. calcium, chloride, sodium, potassium, and magnesium, influence important metabolic processes or enhance/supress growth in cyanobacteria (Seale et al. 1987; Parker et al. 1997; Carneiro et al. 2011). We suggest that individual ions/compounds that contribute to conductivity may play a role in regulating *Phormidium* accrual and further research is required.

Our analysis highlighted the importance of time of year, with higher *Phormidium* cover predicted in summer and early autumn. This aligns with periods of stable flow and elevated temperature at most sites (data not shown). A recent analysis of a 20 years' dataset of benthic algae from two pristine Norwegian streams also forecasted greater *Phormidium* cover with increasing temperatures (Schneider 2015) and previous studies have highlighted the importance of stable flows in providing long accrual periods (Heath et al. 2015).

River flow in the region of 0.1 to 1x the long-term median flow was neutral to *Phormidium* cover, but at flows above 1x median there was a tendency for cover to decline (refer Figure 3). Heath et al. (2015) developed a habitat suitability criteria model (based on velocity, depth and substrate type) for *Phormidium* and showed a similar relationship, with highest coverage observed at moderate velocities (1 m s^{-1}). Gas bubble formation (due to photosynthesis) is often visible within *Phormidium* mats and is more likely under low flow regimes, since diffusion of oxygen will be slowed by the thicker boundary layer (Hawes et al. 2014). *Phormidium* growth in slower flow may therefore be coupled with more frequent autogenic detachment reducing biomass accrual in low flows (Boulêtreau et al. 2006). Thus, flows that are sufficient to enhance nutrient and gas flux, yet insufficient for shear stress which results in biomass loss, are postulated to be optimal for *Phormidium* accrual.

In summary, the analysis of the combined weekly datasets demonstrates that *Phormidium* blooms are influenced by a complex interplay of seasonality (likely strongly linked with temperature and frequency of flushing flows), stream flow and water chemistry. As suggested in previous studies, in general streams with accrual DIN greater than 0.05 mg L^{-1}, accrual DRP less than 0.01 mg L^{-1} and median flow between ca. 0.1 times and 1.0 times median were more likely to experience proliferations. However, there are exceptions to this, suggesting that different hierarchies of importance in physicochemical variables occur between sites (discussed in further detail in Section 6). Water conductivity was identified as having a positive effect on *Phormidium* cover. The relationship appears complex and further investigations into specific ions are recommended.

3. IDENTIFYING FACTORS DRIVING SPATIAL VARIATION IN *PHORMIDIUM COVER* (MANAWATU MONTHLY DATASET)

3.1. Introduction

Horizons Regional Council are the only council who have collected monthly observations of periphyton data suitable for the analysis proposed in this section, where the aim is to explore the spatial variation in *Phormidium* cover in relation to land cover, proximate stressors and environmental variables.

Pasture is the predominant land use in the Manawatu-Wanganui region, and stressors associated with this are thought to be linked to periphyton proliferations (Wagenhoff et al. 2017). The Horizons Regional Council's periphyton monitoring program was established to identify the main drivers of these proliferations. In our analysis we used a sub-set (61 sites) of data from the monitoring programme. In addition to investigating the response of *Phormidium* to a range of variables, we also included filamentous algae and chlorophyll-*a* in our analysis. Filamentous algae, while not toxic, can reach high biomass, change river habitat and functioning, and reduce amenity values. Knowledge of whether the responses of *Phormidium* and filamentous algae to land use and proximate stressors are similar could guide management and mitigation strategies. For example, reducing a specific nutrient to control filamentous algae may result in *Phormidium* blooms, or vice versa. Chlorophyll-*a* is a commonly-used proxy for total periphyton biomass, however, the measurement does not discriminate among different types of algae. We included it in this analysis to assess whether environmental variables that influenced chlorophyll-*a* concentrations were similar to those for *Phormidium* and/or filamentous algae, and therefore determine whether it is a good proxy to assess how noxious periphyton types respond to land use and proximate stressors.

3.2. Methods

3.2.1. Study sites

Data from 61 study sites that covered a wide range of nutrient and river flow conditions in the Manawatu-Wanganui region were selected (Figure 5). Land cover in each catchment was derived from a satellite-imagery-based layer from the New Zealand Land Cover Database 3 (http://www.lcdb.scinfo.org.nz/home). Pastoral land cover varied from 0-93% (Table 4) and was considered the main land cover stressor variable for data analysis. Exotic forest accounted for less than 3% at two-thirds of the sites, with only four sites having values between 10-50%. Urban land cover was generally low with a maximum cover of 4% across all sites. The sites varied in catchment size (11–4,240 km^2; stream orders 2–7) and surficial geologies (hard-sedimentary, soft-sedimentary, volcanic and alluvial categories; Snelder & Biggs 2002). The climate at the sites was classified as cold with a mean annual temperature of < 12°C and ranged from dry to extremely wet (mean annual effective precipitation < 500 mm to ≥ 1500 mm; Snelder & Biggs 2002).

Table 4. Description and summary statistics of site descriptors including land cover, proximate stressors (nutrients and fine sediment), and environmental variables. (*) Median of monthly measurements taken during the period of *Phormidium* and periphyton assessments, (**) Freshwater Ecosystems of New Zealand (FENZ) database (Leathwick et al. 2011).

Descriptor variables	Predictor	Description	Min	Mean	Median	Max
Land cover stressors	*PastoralHeavy*	Percentage of catchment covered by intensive pastoral land-use categories	0	48	58	93
Proximate stressors	*DIN**	Dissolved inorganic nitrogen (mg L^{-1})	0.010	0.371	0.300	1.240
	*DRP**	Dissolved reactive phosphorus (mg L^{-1})	0.004	0.016	0.009	0.176
	*TSS**	Total suspended solids (mg L^{-1})	1.0	3.7	3.0	16.0
	*Turbidity**	Turbidity (NTU)	0.4	2.2	1.6	6.0
Environmental variables	*MALF*	Mean annual low flow (m^3 s^{-1})	0.02	3.1	1.2	15.9
	FRE3	Mean number of floods per year greater than three times the long-term median flow (ecosystem disturbance index)	5.4	11.6	11.2	17.4
	*USPhosphorus***	Catchment average of phosphorus concentration of underlying rocks, 1 = very low to 5 = very high	1.0	1.7	1.6	3.0
	*SegSumT***	Summer air temperature (°C)	14.3	16.4	16.8	17.4
	RipShading	Visual assessment of the canopy cover (%)	3	32	26	94

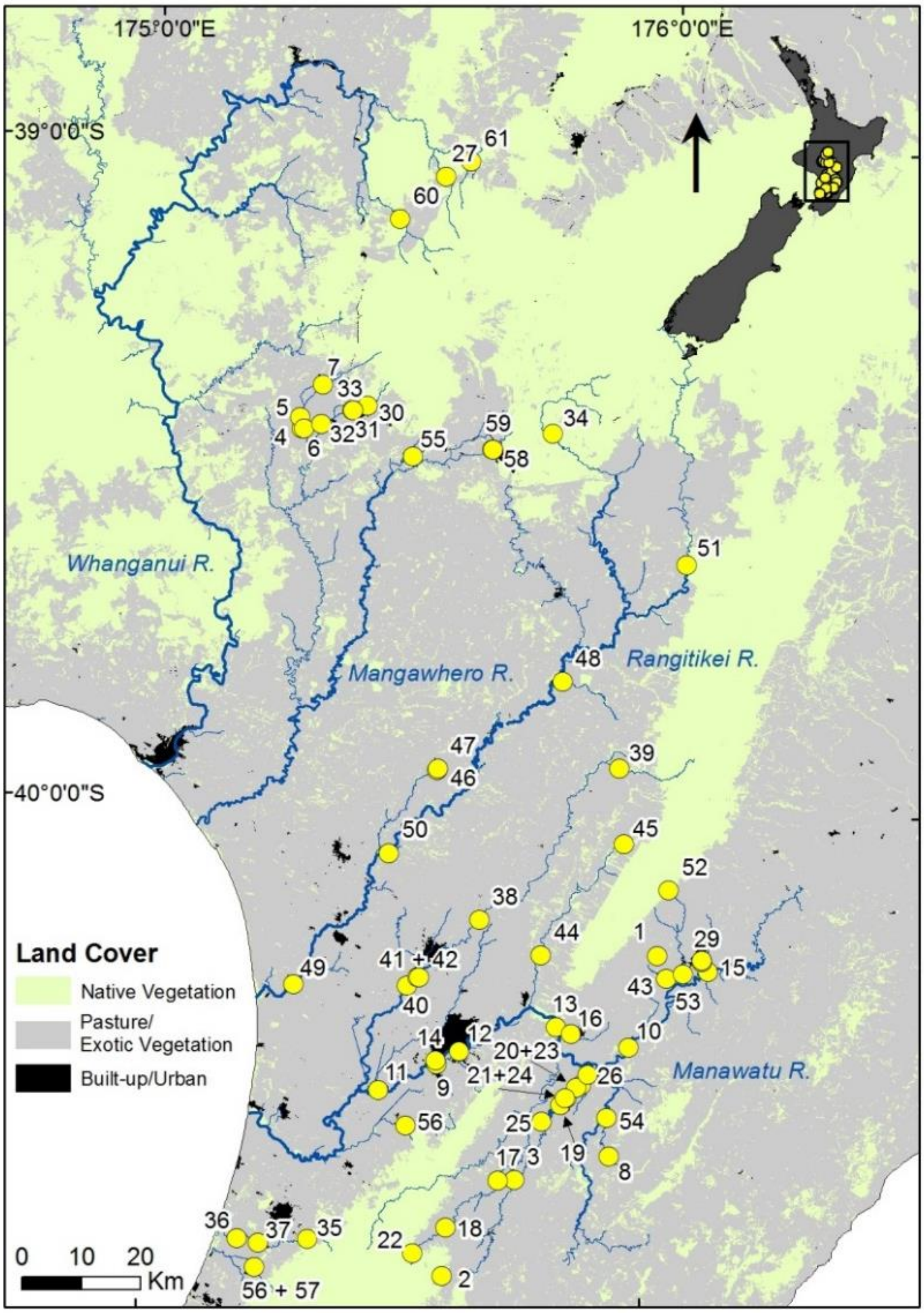


Figure 5. Locations of study sites in the Manawatu region.

3.2.2. Phormidium and periphyton data

Each site was visited monthly for up to three consecutive hydrological (October 1 to September 30) years from July 2011 to June 2014. At each site, a visual estimate of *Phormidium*-dominated mats and coarse filamentous algae (usually green or brown, can also be composed of multiple filamentous diatom or green algal species) cover was conducted using an underwater viewer with a clear acrylic bottom marked with a grid. Estimates were repeated at five locations along each of four transects spaced 10 m apart. An average percent cover value was calculated for each site.

Periphyton biomass was determined at each site by measuring the chlorophyll-*a* concentration of adhered biofilms. The periphyton sample was collected by scraping a standardised area from ten rocks, located along one or two transects, and combining the scrapings. Chlorophyll-*a* was spectrophotometrically determined in the laboratory after ethanol extraction according to Biggs and Kilroy (2000).

Phormidium and periphyton are temporally highly variable but management is generally concerned with the episodes of high cover of nuisance periphyton types (Snelder et al. 2014), thus we calculated the annual maxima of values recorded during each hydrological year. Subsequently, two years (4 sites) or three years (50 sites) of annual maxima were averaged to provide the mean annual maximum for each periphyton variable (Table 5).

Table 5. Description of the periphyton response variables along with summary statistics. All three variables are mean annual maxima estimated from monthly measurements taken during 2-3 hydrological years.

Periphyton response variables	Description	Min	Mean	Median	Max
Phormidium Cover	Visual assessment of the percentage of the riverbed covered by *Phormidium*	0	10	4	47
Filament Cover	Visual assessment of the percentage of the riverbed covered by coarse filamentous algae	0	7	3	37
Chl-a	Chlorophyll-*a* (mg m^{-2})	4	94	88	322

3.2.3. Stressors and environmental predictors

Water samples were taken monthly for determination of DIN and DRP concentrations and total suspended solids (TSS). These were analysed in the laboratory using standard methods (APHA 2005). Turbidity was recorded on each sampling occasion in the field. In order to characterise the typical nutrient and suspended fine sediment conditions for each site, medians were calculated for DIN, DRP, TSS and turbidity over the periphyton sampling period (i.e. up to 3 years; Table 5).

Environmental variables known to influence *Phormidium* and periphyton growth and accrual were also collected. Long-term flow data were available from flow gauging stations at or near the sampling sites for 53 sites, resulting in 8 missing values. From the flow data, variables describing flow magnitude were calculated: mean annual low flow (MALF) and the mean number of floods per year greater than three times the long-term median flow (FRE3), a commonly-used ecosystem disturbance index in New Zealand (Booker 2013) (Table 4). Riparian shading was derived from visual estimates of the percentage of the canopy covered by trees by standing mid-stream at ten locations along the reach or standing on the bank of non-wadeable sites (Table 4). Variables describing catchment geology and temperature were retrieved from the FENZ database (Leathwick et al*.* 2011). These were catchment average of phosphorus concentration of underlying rocks (USPhosphorus) and segment summer air temperature (SegSumT; Table 4).

3.2.4. Data analysis

We used boosted regression tree (BRT) models to explore the spatial variation in periphyton variables in response to land cover, proximate stressors and environmental variables. The merits of the multi-predictor BRT model approach, which is a machine-learning technique, include the ability to automatically handle interactions between predictors, to rank the predictors according to their relative contributions and describe their potentially complex response curves that can be visualised in partial dependence plots. These plots show the marginal effects of a predictor on a response, i.e. the effects that are solely attributed to this predictor when all other predictors are held at their mean values. BRT models were built in the statistical program R using the ‘gbm’ package (Ridgeway 2013) and modified functions based on Elith and Leathwick (2014), in particular the function ‘gbm.step’ which uses cross-validation (CV) to estimate the optimal number of trees. Cross-validation is a technique that is used to develop and evaluate a model when large amounts of data are unavailable by testing the model on withheld data while still using all data to fit the final model (Elith et al. 2008).

The periphyton response variables *PhormidiumCover* and *FilamentCover* were ln(x+1) transformed and *Chl.a* was square-root transformed to meet the model assumptions (Gaussian distribution). All three response variables were standardised by dividing by 1 standard deviation to compare the magnitude of the effects of the predictors among the periphyton variables. For each response variable two separate BRT models were developed, one containing the land cover variable '*PastoralHeavy'* and all environmental variables, and a second model containing the proximate stressors and environmental variables (Table 4). The inclusion of potentially irrelevant predictors was not of concern as these are unlikely to be selected during tree fitting and hence cause minimal effect on prediction (Elith et al*.* 2008). Spearman rank correlation coefficients for all pairs of predictors were also calculated (Figure 6). Model parameterisation was done according to suggestions by Elith et al. (2008). Tree complexity was specified to three allowing for up to three-way interactions although, due to the small sample size, this study is unlikely to provide reliable information on the nature of potential interactive effects between predictors.

									RipShd
								SegSumT	**-0.61**
							USP	-0.29	0.37
						FRE3	-0.11	-0.27	0.15
					MALF	-0.29	0.23	**0.51**	**-0.60**
				Turb	**0.54**	**-0.50**	0.16	**0.62**	-0.40
			TSS	**0.81**	0.42	**-0.55**	0.26	**0.57**	-0.23
		DRP	0.42	0.38	0.09	-0.39	**0.41**	-0.09	0.14
	DIN	0.06	0.18	0.28	-0.01	-0.01	0.12	0.38	-0.10
PastoralHeavy	**0.77**	0.13	0.42	**0.62**	0.27	-0.21	0.14	**0.57**	-0.32

Figure 6. Spearman rank correlation coefficients between all predictors. Bold values are statistically significant at α = 0.001. See Table 4 for description of predictors.

3.3. Results

Phormidium was detected at all but three sites over the study period and reached a maximum cover of 80% at two sites on the Mangatainoka River (State Highway 2 and downstream of DB brewery; Figure 7). Filamentous algae were present at all but five study sites and the highest cover recorded was 72% (Makotuku River upstream of the sewerage treatment plant in Raetihi) during the study period (Figure 8).

The land cover BRT model for *Chl.a* had the best goodness of fit (Total Deviance Explained (TDE) = 45%), followed by *PhormidiumCover* (25%) and *FilamentCover* (22%; Table 6). The land cover variable, *PastoralHeavy,* was ranked first, second and third in order of importance among the six predictors included in these three models respectively (Table 6), also reflected in the overall magnitude of the positive effect (Figure 9). The shapes of these stressor-response relationships were similar regardless of order of importance, with an initial increase in response at about 20% heavy pastoral land cover and a cessation in response at about 70% *PastoralHeavy* (Figure 9).

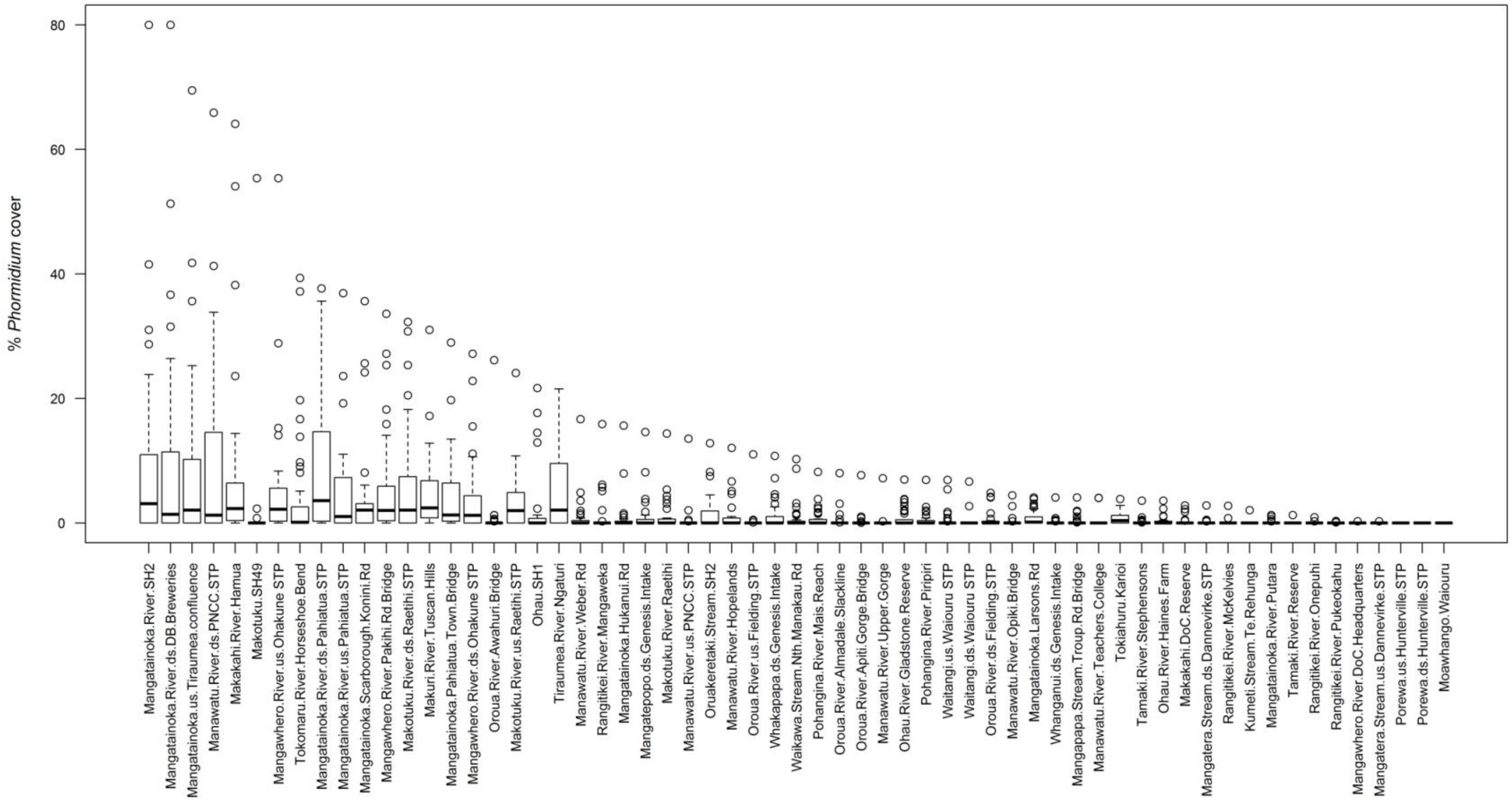


Figure 7. Percent *Phormidium* cover at each of the study sites in the period July 2011 to June 2014. The solid black line in each box is the median, the box is confined by the 1st and 3rd quartiles, and whiskers extend to the lowest or highest data point still within 1.5 times the inter-quartile range of the lower or higher quartile, respectively. Circles represent data points that extend beyond this range.

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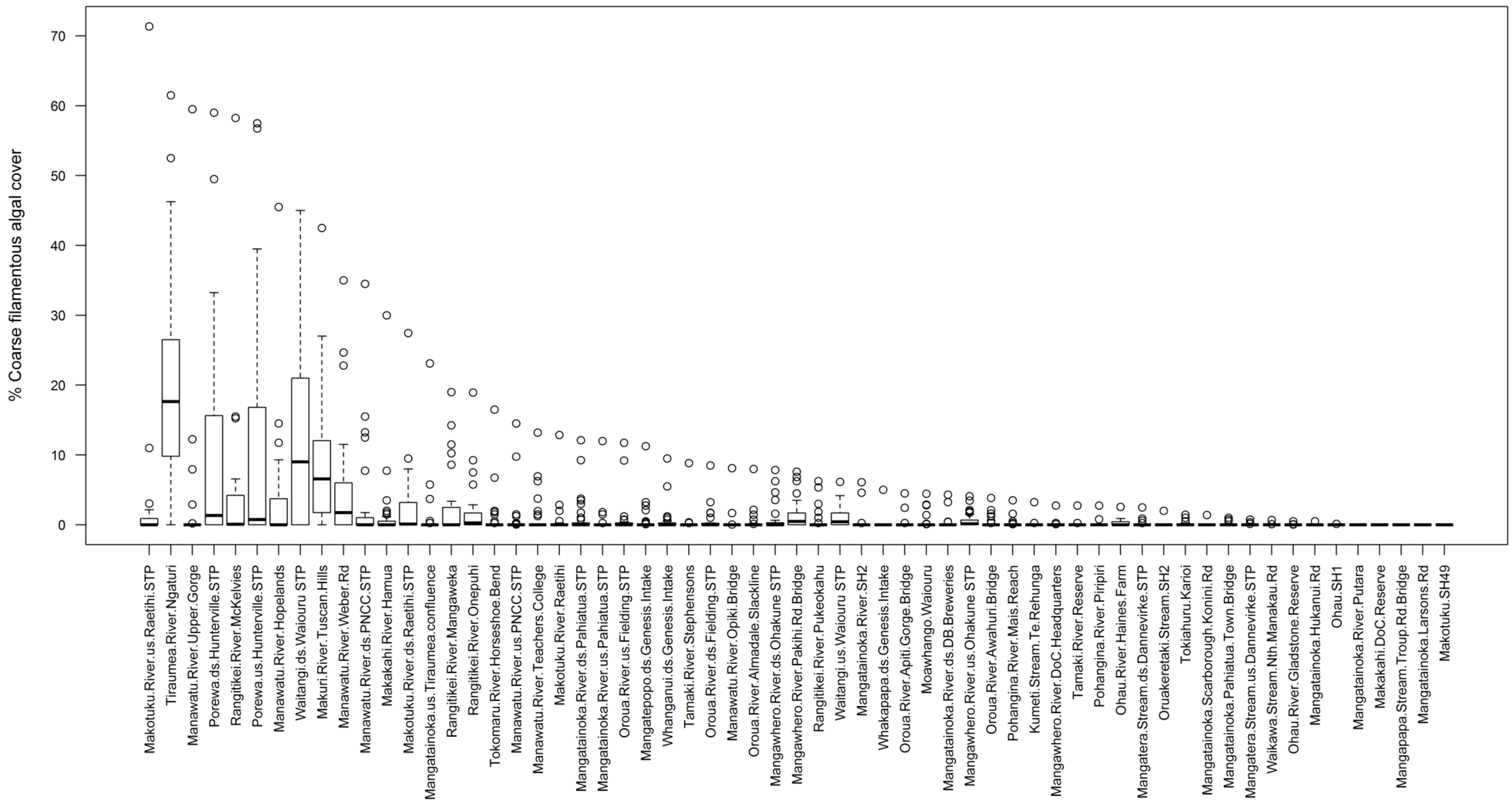

Figure 8. Percent coarse filamentous algal cover at each of the study sites in the period July 2011 to June 2014.

Table 6. Boosted regression tree (BRT) model results for the percentage cover of *Phormidium* mats (*PhormidiumCover*) and coarse filamentous algae (*FilamentCover*) and for chlorophyll-*a* (*Chl.a*). For each periphyton response variable a land cover model and a proximate stressor model were developed. Results include the percentage total deviance explained (% TDE), the mean cross-validation (CV) correlation coefficient and its standard error (SE), and the relative contributions (%) of the predictors in each model. The contributions of the two highest-ranked predictors in each model are presented in **bold**, and the overall shape of the relationship, positive (+) or negative (-), is given for predictors unless the effect was very small based on partial dependence plots. The shapes of the fitted functions for the *PastoralHeavy* gradient (Figure 9) and for all other predictors of the proximate stressor models (Figure 10) are presented in partial dependence plots

		Land cover models			Proximate stressor models		
		PhormidiumCover	*FilamentCover*	*Chl.a*	*PhormidiumCover*	*FilamentCover*	*Chl.a*
	BRT model fit (% TDE)	25	22	45	38	28	47
	CV correlation coefficient (SE)	0.55 (0.09)	0.52 (0.14)	0.66 (0.06)	0.56 (0.07)	0.51 (0.12)	0.66 (0.09)
	Predictors	Relative contributions of the predictors (%)					
Land cover	*PastoralHeavy*	**18.4 (+)**	15.0 (+)	**40.8 (+)**			
Proximate stressors	*DIN*				**29.8 (+)**	1.8	**28.5 (+)**
	DRP				0.7	1.1	5.8 (+)
	TSS				1.7	13.4	5.6
	Turbidity				4.2	**49.2 (+)**	15.7 (-)
Environmental variables	*MALF*	13.0	**47.1 (+)**	3.0	9.5	12.5 (+)	3.0
	FRE3	**59.8 (+)**	**27.4 (-)**	10.7 (+)	**47.4 (+)**	**14.2 (-)**	6.1 (+)
	USPhosphorus	2.6	8.0 (+)	**32.0 (+)**	1.8	6.6 (+)	**24.6 (+)**
	SegSumT	2.4	1.2	5.5	1.4	0.7	3.5
	RiparianShading	3.8	1.4	8.0 (-)	3.6	0.6	7.2 (-)

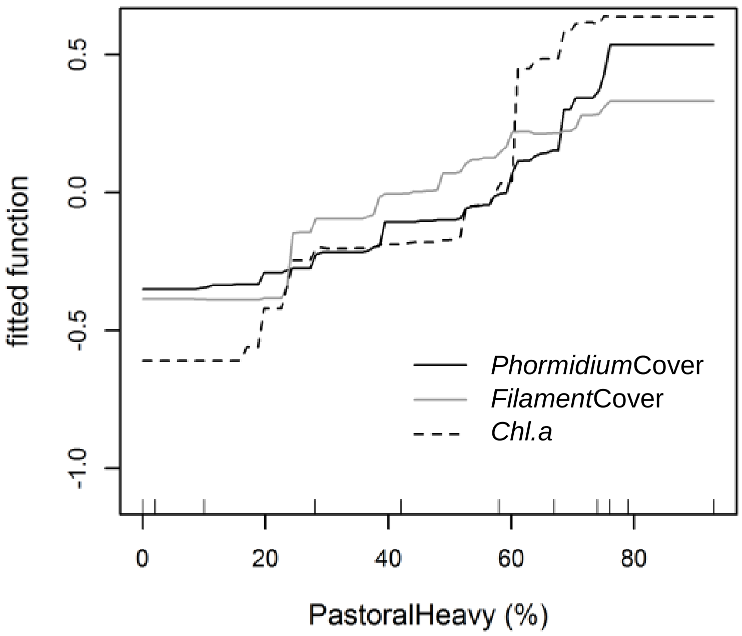

Figure 9. Partial dependence plots from the land cover Boosted Regression Tree models. The fitted functions depict the marginal effects of *PastoralHeavy* on the percentage cover of *Phormidium* mats (*PhormidiumCover*) and coarse filamentous algae (*FilamentCover*) and on chlorophyll-*a* (Chl.a) when all other predictors are held at their mean values. *PastoralHeavy* ranked second, third and first for these three periphyton responses, respectively. The fitted functions for the environmental variables are not presented (see Table 6 for rankings). A rug plot on the x-axis shows the data distribution (in steps of 10%). The y-axis values represent deviation from the mean predicted response value (zero) in units of standard deviation.

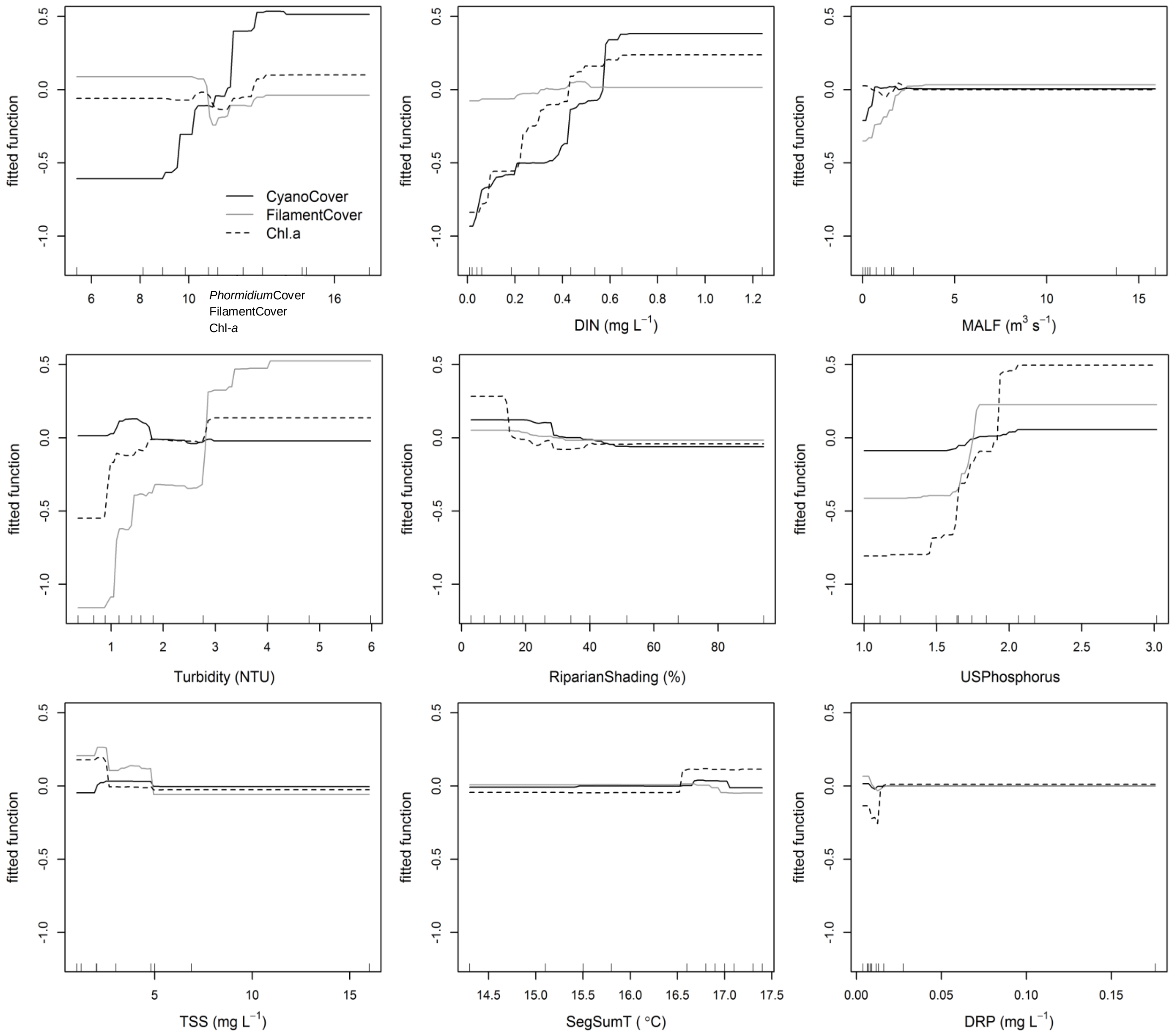

Figure 10. Partial dependence plots of the proximate stressor Boosted Regression Tree (BRT) models. The fitted functions depict the partial effects of each predictor on *Phormidium* (*PhormidiumCover*) and coarse filamentous algae (*FilamentCover*) cover, and chlorophyll-*a* (*Chl.a*) when all other predictors are held at their mean values. The predictors are ordered by the relative contributions of the predictors for *PhormidiumCover* (see Table 6). A rug plot on the x-axis shows the data distribution (in steps of 10%). The y-axis values represent deviation from the mean predicted response value (zero) in units of standard deviation; ranges were kept equal across plots for all rankings)

Replacement of the land cover variable, *PastoralHeavy,* by the proximate stressor variables describing nutrients and fine sediment improved BRT model fit for all three periphyton response variables. The proximate stressor model for *Chl.a* still had the best fit (TDE = 47%), followed by the model for *PhormidiumCover* (38%) and *FilamentCover* (28%; Table 6). For *PhormidiumCover*, the second rank and strong positive effect of *PastoralHeavy* was replaced by a strong positive effect of *DIN* but all other stressor variables had weak effects (Table 6, Figure 10). Conversely, for *FilamentCover* the strong positive effect of *PastoralHeavy*, ranked third, was replaced by a strong positive effect of *Turbidity* ranked first, but all other stressor variables also had weak effects (Table 6, Figure 10). Finally, for *Chl.a* the first rank and positive effect of *PastoralHeavy* was replaced by a strong positive effect of *DIN* and a positive but smaller effect by *Turbidity*, ranked third (Table 6, Figure 10). When comparing the land cover and proximate stressor models, there were no or only minor changes to the ranking of the environmental predictors, with the exception of *MALF*, which was the best predictor in the land cover model for *FilamentCover* but lost importance and only ranked fourth in the proximate stressor model (Table 6).

For interpretation of the drivers of the three periphyton variables, the proximate stressor models were used. For *PhormidiumCover*, FRE3 was the most important predictor having a strong and larger positive effect than *DIN*, all other predictors had weak or negligible effects (Figure 10). For *FilamentCover*, *Turbidity* was the most important predictor having a strong positive effect, but *USPhosphorus* also had positive effects while the positive effects of *MALF* and negative effects of *FRE3* were much smaller (Table 6, Figure 10). Finally, for *Chl.a*, *DIN* was the most important predictor and had a strong positive effect followed by also relatively strong positive effects of *USPhosphorus*, ranked second, and positive effects of *Turbidity*, ranked third (Table 6, Figure 10). *RiparianShading* had weak negative effects and *FRE3* weak positive effects on *Chl.a* (Figure 10). Overall, *TSS*, *SegSumT* and *DRP* were unimportant predictors for all three periphyton variables (Figure 10).

Potential thresholds for important stressor variables were visually determined from Figure 10. Across the *DIN* gradient, both *PhormidiumCover* and *Chl.a* increased from very small values while a cessation threshold at about 0.6 mg DIN L^{-1} could be identified. Across the *Turbidity* gradient, both *FilamentCover* and *Chl.a* had an initiation threshold at about 1 NTU while cessation thresholds were located at about 3 NTU and 4 NTU, respectively.

3.4. Discussion

A similar response from all three periphyton indicators (*PhormidiumCover*, *FilamentCover* and *Chl.a*) to increasing pastoral land cover was observed. When pastoral land cover exceeded 20% there was a near linear increase until pastoral land cover was greater than 80%. This result indicates that among the rivers included in this study, there is an association between increasing *Phormidium* proliferations and heavy pastoral land cover. This pattern was also identified in Section 4 of this report (National susceptibility model) with pastoral land cover (or highly correlated variables) identified as significant explanatory variables. However, *Phormidium* proliferations also occur in rivers whose catchment contains very limited pastoral land cover, for example the Hutt (Wellington; Heath et al. 2011) and Maitai rivers (Aristi et al. 2016). Aristi et al. (2016) showed that *Phormidium* cover in the Maitai River increased with the proportion of exotic forestry in the catchment (the Maitai catchment has very little pastoral land use). The authors suggest that the observation that *Phormidium* proliferations can occur in relatively low nutrient-yielding forested catchments supports the view that high nutrient export is not a prerequisite for proliferations. They note that their study supports the notion that fine sediment plays a key role in promoting *Phormidium* proliferations. Collectively these results indicate that multiple land uses are associated with *Phormidium* proliferations. Rather than the land use *per se*, it is likely that it is the stressors associated with specific land use that in combination promote *Phormidium* mats e.g. increased runoff of fine sediment.

Replacement of the land cover variable, *PastoralHeavy,* by the proximate stressor variable describing nutrients and fine sediment improved BRT model fit for all three periphyton response variables. The proximate stressor models also enabled further insights into which stressors related to pastoral land use are predicted to be involved in promoting *Phormidium* proliferations.

Phormidium cover was predicted to increase in response to increasing DIN concentrations, up to ca. 0.6 mg L^{-1}. This output is consistent with findings from other sections of our research, e.g. Section 2 and 5, although the analysis of the weekly dataset indicated that the threshold above which increasing DIN did not enhance cover was markedly lower (ca. 0.05 mg L^{-1}). The relationship between *Phormidium* and elevated DIN is discussed in detail in Section 2. In contrast, increasing DIN concentrations had no effect on filamentous algae cover. This may partly reflect the growth form of these taxa which tends to be filamentous, forming long tails out in to the water and thereby having more immediate access to water column DIN, compared to the low profile cohesive mat structure of *Phormidium*. Finally, in this study chlorophyll-*a* concentrations (a proxy for total algae biomass) were the lowest when DIN was low, indicating that efforts to reduce periphyton biomass by reducing DIN are likely to be effective. However managers should be aware that shifts in the dominant species are likely to occur as nutrient concentrations and ratios are manipulated.

An interesting finding of the BRT modelling was the positive relationship between turbidity, a measure of suspended fine sediment, and filamentous algal cover. *Phormidium* cover on the other hand was unaffected by turbidity, suggesting that one of the key stressors caused by increasing pastoral land cover is an increase in suspended fines which favours filamentous green algae over *Phormidium*. A possible explanation for this finding is that filamentous species tend to form long tails, which as they increase in biomass float on the river/stream surface. Thus an increase in turbidity may have less impact on these filamentous forms, compared to the mat-forming *Phormidium*. Although it is unlikely that the *Phormidium* is light-limited under the range of turbidity observed in this study, high concentrations of fine sediment might prevent or reduce colonisation of *Phormidium* filaments. In this study, we did not have a reliable estimate of deposited sediment and hence were not able to tease apart the effects of suspended and deposited sediment. However, increased suspended fine sediment, a proxy of the sediment loading, is not always linked to increased deposition rates. Increased sediment deposition rates and percent deposited sediment cover have been shown by several studies to be associated with increased *Phormidium* cover, likely because *Phormidium* can utilise phosphorus attached to fine sediment (e.g. Wood et al. 2015b, 2015c). Future studies, via experimental or survey approaches, could explore how suspended and deposited fines differentially affect the prevalence of these Phormidium or filamentous periphyton.

The highest explanatory factor in the *Phormidium* model was FRE3, the frequency of floods that are at least three times the long-term median flow. The model predicts that rivers with a higher FRE3 are more likely to experience *Phormidium* proliferations. Initially this seems counterintuitive, as previous research has suggested that prolonged stable periods allow longer accrual periods, enabling more extensive proliferations (Heath et al. 2016). There are a number of potential explanations for this observation. The first relates to the use of FRE3 as an indicator of a flushing flow. Recent studies have shown that *Phormidium* mats often tolerate higher flows than other periphyton classes (Hart et al. 2013), and that there are marked difference in the magnitude of a flushing flow required to reduce *Phormidium* cover among sites (Wood et al. 2016). For example, Wood et al. (2016) predicted in the Mangatainoka River (State Highway 2, Site 13 in Section 2) a flushing flow event 5.36 times the median flow is needed to reduce *Phormidium* cover below 20% (using a 90th percentile quantile regression approach). In contrast, a flow of 16.5 times the median flow was predicted to be required to reduce *Phormidium* cover below 20% at Tokomaru River at the Horseshoe Bend site (Site 18 Section 1, 85th percentile). Based on these data it is probable that rivers with a high frequency of flushing flows of magnitude of three times the long-term median flow may favour *Phormidium* proliferations, provided these do not exceed a flow that cause detachment of mats (which will be site specific). Further in-depth analysis of site-specific flows and removal processes are required to confirm this suggestion.

More frequent flushes, provided they don't remove the *Phormidium* mats (see paragraph above), will also be inherently linked to greater inputs of fine sediment, and in some catchments, nitrate runoff, which have both been linked to increased *Phormidium* proliferations (See Section 2.4). A final reason for the observation of increased *Phormidium* proliferation in rivers with higher FRE3 could be linked to their ability to rapidly colonise bare substrate. Brasell et al. (2015) showed that *Phormidium* could quickly (within ca. 2 weeks) obtain high cover and out-compete diatoms and green algae. This process is likely to be further enhanced when relic populations exist due to incomplete removal during flushing events (Thiesen 2015).

Chlorophyll-*a* follows the pattern of the most dominant periphyton type in the sample, thus providing no information on how different algal types respond to management actions. For example, our BRT model results suggest that management actions that decrease the export of DRP into streams may result in a reduction of filamentous algae, but may promote *Phormidium*. If the response to this management action was assessed by only measuring chlorophyll-*a*, this changes in dominant taxa would not be observed.

In summary, the results from our exploration of spatial variation in periphyton in response to land cover and proximate stressor variables indicate:

- There is a relationship between *Phormidium* cover and increased pastoral land cover in the Manawatu region. This correlation was also observed for filamentous algae.
- Streams with more frequent flushes (≥ 3 times the long-term median flow) are more likely to experience *Phormidium* proliferations. Possible reasons for this require further research but include that *Phormidium* mats are able to withstand greater shear stress and are therefore more resilient to flushes (up to a certain magnitude which is site specific); frequent flushes that do not cause abrasion of mats deliver increased sediment and nitrate which may promote growth; and that *Phormidium* may be able to colonise bare substrate more rapidly than other algae.
- *Phormidium* cover increased with rising DIN to a threshold of ca. 0.6 mg L^{-1}, after which further changes in DIN had little effect. This result is congruent with previous research and analysis performed in other sections of this study, although the specific thresholds which are required to initiate increased *Phormidium* cover, and above which have no effect, vary among studies.

4. DEVELOPING A MODEL TO PREDICT RIVER SUSCEPTIBILITY TO *PHORMIDIUM* NATIONALLY

4.1. Introduction

The aim of this section was to produce a model to predict which rivers across New Zealand are susceptible to *Phormidium* proliferations. The Ministry for the Environment and regional councils are interested in using such predictive models to identify regions where additional monitoring or data collection might be required. The model may also identify which types of rivers are more likely to experience *Phormidium* proliferations and catchment scale drivers. The model was developed based on data from 493 sites across the North and South Island. It was then was used to predict maximum *Phormidium* cover in all New Zealand river segments.

This section of the report also covers objective 4 (see Introduction) of independent model validation by visiting approximately 30 unmonitored sites that were identified in the risk susceptibility model as having a high likelihood of experiencing *Phormidium* blooms. Each site was visited once during the summer of 2016/2017 and assessment of percent *Phormidium* cover undertaken, in concert with general observation about the site. Approximately half of these sites were in regions where we did not have any training data (e.g. West Coast, Gisborne) to help assess whether the model was able to extrapolate successfully in these regions.

4.2. Methods

4.2.1. Phormidium data

We collated *Phormidium* cover datasets from 11 regional councils including information from a total of 493 stream sites (Table 7). No *Phormidium* monitoring is undertaken by Auckland Council, Waikato Regional Council, Marlborough District Council or the West Coast Regional Council. The sample data were collected as part of State of the Environment monitoring, recreational bathing sampling, and specific periphyton or *Phormidium* monitoring programmes, and therefore the frequency of sampling and the types of environmental data collected in parallel varied greatly. Most regional councils used the transect method outlined in Ministry for the Environment and Ministry of Health (2009) to collect sample data. Some regional councils also used a bankside visual estimate of *Phormidium* cover. When both sampling methods were used, we selected the higher *Phormidium* cover value for analysis.

We calculated maximum *Phormidium* cover for sites with at least five independent observations, resulting in a total 492 sites. Sites were distributed unevenly among regional councils. The lowest number of sites per region was five (Otago Regional Council; ORC), compared to 161 from Environment Canterbury (ECAN, Table 7).

Similarly, the sampling effort, i.e. total and mean number of observation (n) per site, differed considerably between regional councils. The lowest sampling effort was recorded in ORC with a mean of 30 observations per site. The maximum mean number of observations was recorded by the Nelson City Council (NCC) with 86.3 per site, whereas the highest total number of observations came from ECAN (total n = 8,342). The maximum *Phormidium* cover measured was 90% at a site in the Canterbury region (Table 7).

Observed maximum *Phormidium* cover was mapped to assess the spatial representativeness of monitoring sites. Density plots were used to assess the environmental representativeness of sites. Representativeness refers to the degree to which the distribution of environmental predictor variables at monitoring sites matches the national distribution of the same environmental variables for all stream segments. Poor representativeness can reduce the reliability of the model predictions because certain sets of environmental conditions are not represented.

Table 7. Summary statistics for maximum *Phormidium* cover and number of sites and observations recorded for each site (n) for the eleven regional councils. BoPRC = Bay of Plenty Regional Council, ECAN = Environment Canterbury, GWRC = Greater Wellington Regional Council, HBRC = Hawkes Bay Regional Council, NCC = Nelson City Council, NRC = Northland Regional Council, ORC = Otago Regional Council, ES = Environment Southland, TDC = Tasman District Council, TRC = Taranaki Regional Council.

	***Phormidium* max. cover**				**Sites**		
Council	**mean**	**SD**	**min.**	**max.**	**Sites**	**mean n**	**total n**
BoPRC	32	19	0	61	12	16.6	199
ECAN	18	23	0	90	161	51.8	8342
GWRC	18	24	0	80	66	65.6	4330
HBRC	19	20	0	81	45	22.1	995
Horizons	20	22	0	80	61	42.2	2574
NCC	8	7	0	21	26	86.3	2244
NRC	15	18	0	70	36	29.1	1047
ORC	30	19	4	58	5	6.0	30
ES	28	20	13	72	7	11.4	80
TDC	17	21	0	80	66	21.0	1385
TRC	25	17	8	48	8	35.3	282
Total					493		21,508

4.2.2. River and land use predictor data

Catchment and segment-scale environmental descriptors were extracted from the Freshwater Ecosystems of New Zealand database (FENZ; Leathwick et al 2011). A total of 19 predictor variables were considered in the analyses, based on our knowledge of the potential drivers of *Phormidium* blooms (Table 8). Land cover variables were was derived from the most recent satellite imagery available in the Land Cover Database Version 3 (LCDB3, Landcare Research 2013).

Table 8. Predictor variables included in the generalised additive model. Data were obtained from the Freshwater Ecosystems of New Zealand database (Leathwick et al. 2011) and the New Zealand Landcover Database version 3 (http://www.lcdb.scinfo.org.nz/home).

Predictor	Abbreviation	Description	Units
Climate and flow	SEGJANAIRT	Average summer (January) air temperature	°C
	SEGRIPSHAD	Riparian shading proportion	%
	USDAYSRAIN	Days/year with rainfall greater than 25 mm in the upstream catchment to indicate the likely frequency of elevated flows	Days/year
	SEGFLOWSTA	Annual low flow/annual mean flow (ratio)	
	USAVGSLOPE	Average slope in the upstream catchment	degrees
	SEGLOWFLOW	Mean annual 7-day low flow	m^3/sec
	SEGSLOPE	Segment slope	degrees
Land cover	T2ExoticForest	Proportion of catchment occupied by exotic forest	%
	T1NativeVeg	Proportion of catchment occupied by native vegetation	%
	T1Urban	Proportion of catchment occupied by built-up area, urban parkland, surface mine, dump and transport infrastructure	%
	T2PastoralHeavy	Proportion of catchment occupied by heavy pasture	%
	T1BareGround	Proportion of the catchment with bare ground	%
	CATCHAREA	Catchment area	m^2
Geography and topography	USPHOSPHOR	Phosphorus content of surface rocks	Ordinal
	LOCSED	Weighted average of proportional cover of bed sediment using categories of: 1–mud; 2–sand; 3–fine gravel; 4–coarse gravel; 5–cobble; 6–boulder; 7–bedrock	Ordinal
	LOCHAB	Weighted average of proportional cover of local habitat using categories of: 1–still; 2–backwater; 3–pool; 4–run; 5–riffle; 6–rapid; 7–cascade	Ordinal
Geology	USCALCIUM	Calcium content in surface rocks	Ordinal
	USHARDNESS	Average hardness (induration) of surface rocks	Ordinal
	LOGNCONC	Log10 transformed values of nitrogen concentration (ppb) as estimated from CLUES, a leaching model combined with a regionally-based regression model, implemented within a catchment framework (Woods et al. 2006)	ppb

4.2.3. Generalised additive model

We initially used two fundamentally different modelling approaches, namely BRT and GAM. Boosted regression tree analysis has proven to be a suitable method for ecological data exploration and prediction (Leathwick et al. 2006; De'ath 2007; Elith et al. 2008) as discussed in Section 3. GAMs is a flexible modelling technique where relationships between the individual predictors and the dependent variable follow smooth patterns that can be linear or nonlinear. It allows fitting of models with a range of error distributions, including binomial which is particularly suited for percentage cover data. In both methods the response curves can be visualised in partial dependence plots that show the marginal effects of a predictor on a response, i.e. the effects that are solely attributed to this predictor when all other predictors are held at their mean values. The BRT approach significantly under predicted *Phormidium* cover and here we present only the results from the GAMs approach.

Generalised additive modelling was used to model maximum percent *Phormidium* cover in relation to environmental and land use variables for each sampled river segment. Initial data exploration was conducted following the protocol suggested by Zuur et al. (2010). Collinearity among predictor variables was tested using the variance inflation factor (VIF). There was considerable correlation among predictor variables, and 'T2PastoralHeavy' had a VIF > 5 (indicating high correlation). This was removed from all subsequent analysis and reassessment of collinearity resulted in VIF values of < 4 for all variables. Maximum *Phormidium* cover data (a continuous variable bounded by 0 and 100) was modelled using a binomial error with a logit link in relation to a range of environmental predictor variables (Table 8). Monitoring site data was weighted based on the number of observations (n) used to calculate maximum *Phormidium* cover. This gave more importance to data points calculated from a larger number of observations.

Candidate models were selected via a generalised likelihood ratio test, the AIC and the residual deviance values, and were validated by inspecting the deviance residuals. A spatial correlation structure was not included in the model, as no evidence of correlation was detected after plotting residuals by their geographical coordinates.

The final selected model was presented as partial effects plots, which show the effect of each predictor variable conditional to others in the model. The partial effects of each predictor were displayed as cubic splines showing either negative or positive effects relative to the overall mean of the response variable centred on zero. Partial plots also show standard errors around the fitted spline and partial residuals for each observation. GAMs were conducted in in the 'mgcv' package (Wood 2006) in R (R Core Team 2014).

4.2.4. National predictions and model performances

The final GAM model was used to predict *Phormidium* cover for all stream segments across the country, excluding still and backwater habitats (i.e. FENZ ReachHab 1 and 2), small streams (i.e. stream order < 3) and segments located in glacial mountains (source of flow G - M, data from the REC; Snelder & Biggs 2002). These factors were excluded as *Phormidium* blooms rarely occur in these habitat types.

Model performance was checked by plotting observed versus predicted values and calculating the following summary statistics:

- regression R^2, the coefficient of determination derived from a regression of the observations against the predictions
- Nash-Sutcliffe efficiency (NSE), indicates how closely the observations coincide with predictions (Nash & Sutcliffe 1970). NSE values range from $-\infty$ to 1. An NSE of 1 corresponds to a perfect match between predictions and the observations. An NSE of 0 indicates the model is only as accurate as the mean of the observed data. Values less than 0 indicate the model predictions are less accurate than using the mean of the observed data.
- percent bias (PBIAS) measures the average tendency of the predicted values to be larger or smaller than the observed values. Optimal bias is zero, positive values indicate underestimation bias and negative values indicate overestimation bias (Piñeiro et al. 2008).
- relative root mean square error (RSR), the ratio of the root mean square error to the standard deviation of the observations as a measure of the characteristic model uncertainty (Moriasi et al. 2007).

A rule of thumb is that model predictions are satisfactory if NSE > 0.50, RSR < 0.70, and if PBIAS < ±25% and are good if 0.65 < NSE > 0.75, 0.5 < RSR < 0.60, and if 25% < PBIAS < ±40% (Moriasi et al. 2007).

4.2.5. Independent model validation

River segments were grouped by river name, and the segment with the highest predicted *Phormidium* cover in each river selected. Rivers with existing monitoring sites were excluded. The 30 segments with the highest predicted *Phormidium* cover in each council region are shown in Figure 11. Of these segments, 32 were selected and visited to sample *Phormidium* in the summer of 2016/2017 (Figure 11). These sites were selected to cover a range of river types and sizes, and where possible, to cover a variety of geographic locations. In five cases the incorrect conversion of coordinates resulted in sites being visited that were predicted to have low *Phormidium* cover. At each visited site, *Phormidium* cover was visually estimated from the edge of the stream. Photographs were taken at each site and general descriptions of the site and surrounding land recorded.

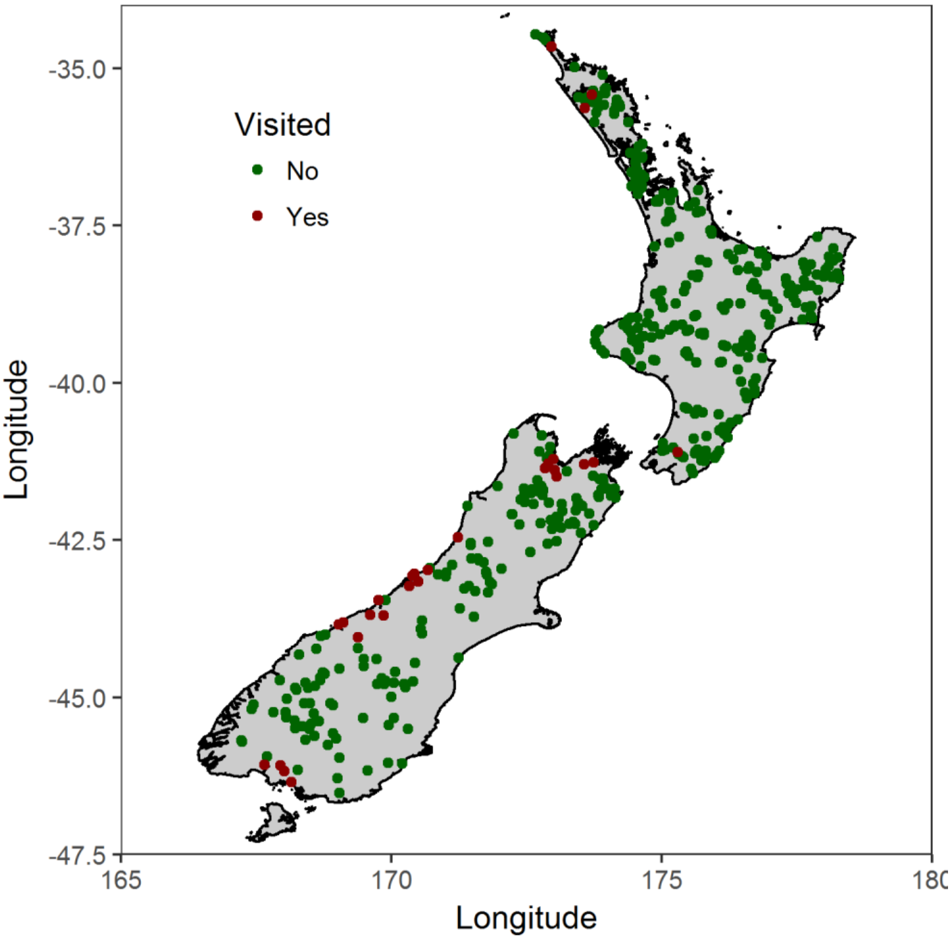

Figure 11. The 30 sites (green dot) predicted to have the highest *Phormidium* cover within each regional council boundary (only one site per river). Red dots show the 33 sites visited during the independent model validation section of this study.

4.3. Results

4.3.1. Phormidium cover and predictor variables

The mean maximum *Phormidium* cover at 493 sites was 18% (Figure 12, ± 21% SD, range 0-90%), with sites experiencing high and low *Phormidium* cover being relatively evenly distributed across the country (Figure 12). Some less represented areas included the central region of the North Island and the West Coast of the South Island (Figure 12).

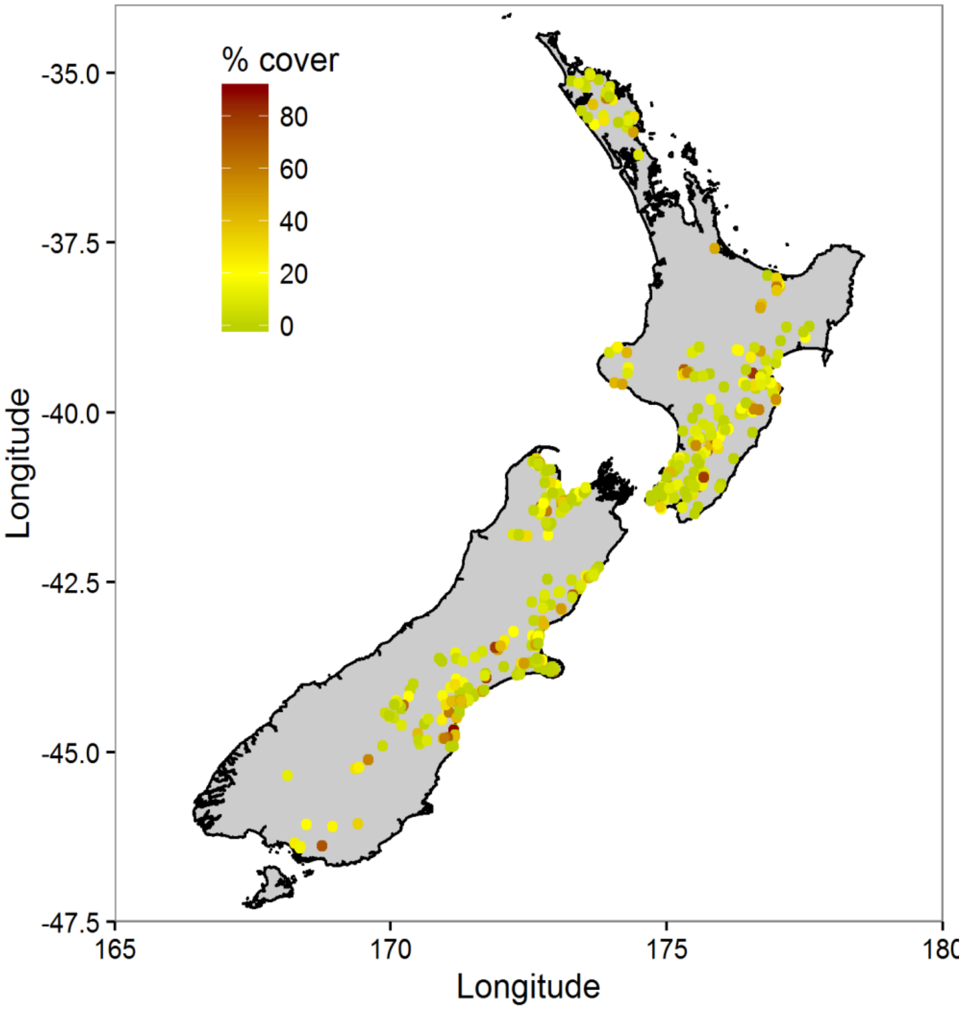

Figure 12. Maximum observed *Phormidium* cover observed at each of the 493 sites used as training data in model development.

The degree of correlation between predictor variables considered in the models is presented in a correlogram (Figure 13). Colour intensity and symbol size are proportional to the corresponding correlation coefficient. The correlogram indicates that nutrient related variables (USPHOSPHOR and LOGNCONC) and pastoral heavy cover in the catchment are highly and positively inter-correlated, whereas they are negative correlated with native vegetation cover (T1NativeVeg), days of rain (USDAYSRAIN), ratio of annual low flow/annual mean flow (SEGFLOWSTA), catchment mean slope (USAVGSLOPE), segment bed sediment (LOCSED) and segment habitat (LOCHAB) (top mid-left of Figure 13), which were positive correlated. Other predictor variables were generally weakly correlated (mid- and bottom-right Figure 13).

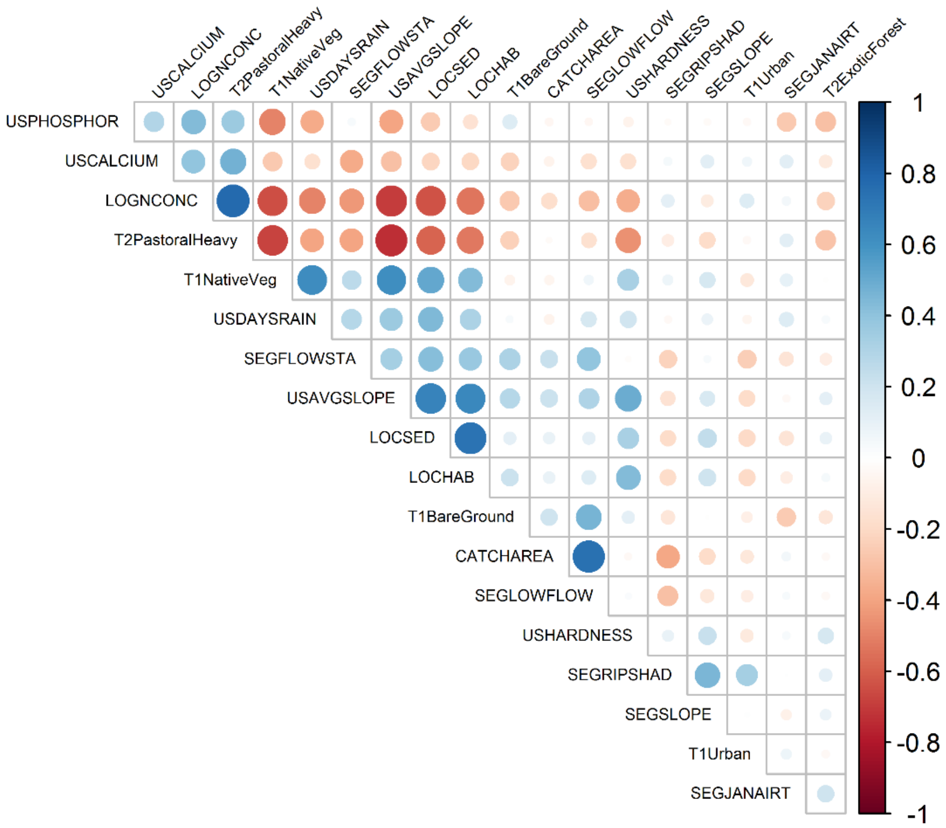

Figure 13. Correlations of predictor variables considered in national river susceptibility model. Positive correlations are displayed in blue and negative correlations in red. Colour intensity and the size of the circle are proportional to the correlation coefficients. The scale bar shows the correlation coefficients represented as a colour spectrum. For variable abbreviations see Table 8.

The distribution of *Phormidium* monitoring sites across the environmental gradients defined by the 19 predictor variables were generally consistent with the distribution of all segments in the river network (excluding sites as described in Section 4.2.4) (Figure 14). Ranges were comparable, but for some predictor variables there was a minor over- and under-representation of monitoring sites compared to the national river network. For example, *Phormidium* monitoring sites were slightly under-represented in environments characterised by high segment annual low flow/annual mean flow ratio (SEGPLOWSTA, Figure 14) and were over-represented by sites medium to high heavy pastoral land use (T2PastoralHeavy, Figure 14). There was also under-representation of sites with low catchment average summer air temperature (SEGJANAIRT, Figure 14).

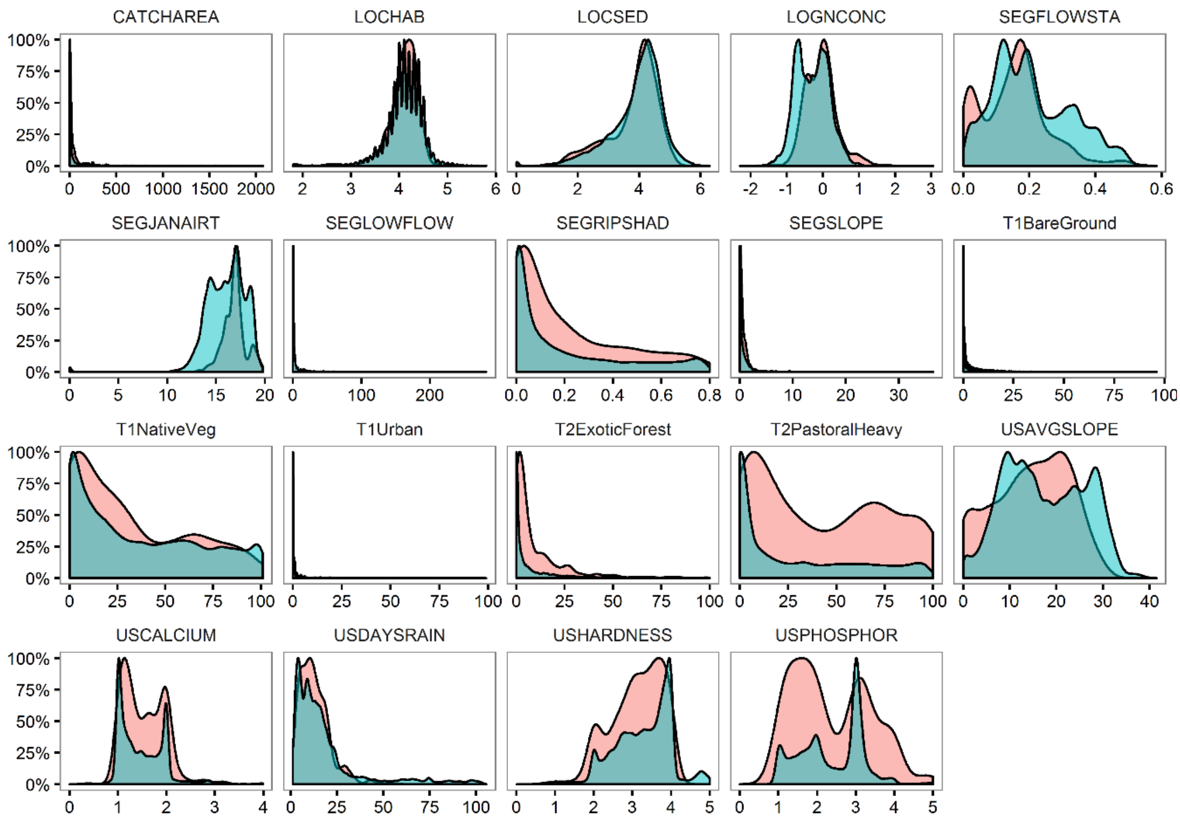

Figure 14. The distributions of predictor variable values at *Phormidium* monitoring sites with all river segments nationally (excluding still and backwater habitats, stream order < 3, and segments located in glacial mountains). The national pool of river segments is represented by blue density profiles and the monitoring sites are represented by pink density profiles. Similarities in the distributions shown in the two histograms in each panel provide an indication of the degree to which environmental variation across the monitoring sites represents environmental variation across all river segments in New Zealand. Complete representativeness would be indicated by exact matches between the density profiles. See Table 8 for predictor variables definitions.

4.3.2. Generalised additive model

The final GAM model explained 67% of deviance in maximum *Phormidium* cover data and included all predictor variables, except T1Urban that was not significant and T2PastoralHeavy that was eliminated during by the VIF selection. Partial effect plots (Figure 15) demonstrate USCALCIUM had the largest amplitude with a positive effect on *Phormidium*. LOCHAB also had large amplitude, with values < 4 (i.e. pool, backwater and still water) having a strong negative effect on *Phormidium* cover. However, this negative effect was highly variable due to the small number of sites having LOCHAB < 4. Other important variables included LOGNCONC, SEGFLOW, CATCHAREA, SEGSLOPE and T1BareGround (Figure 15).

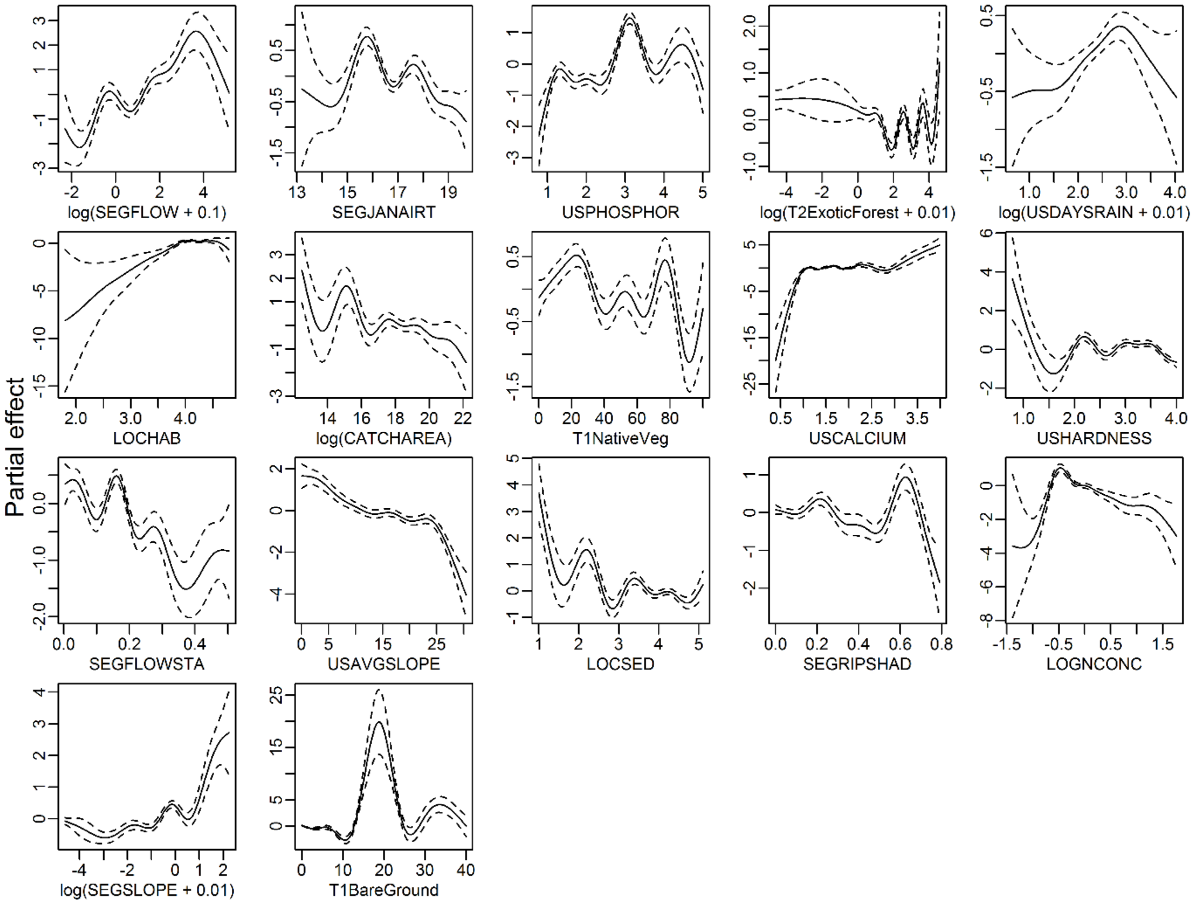

Figure 15. The effects of each predictor variable included in the generalised additive model (GAM) predicting national river susceptibility to *Phormidium* proliferation. Solid lines represent cubic splines (± standard error, dashed black lines). Note different y-axis scales. See Table 8 for variable names, description and units.

4.3.3. Model performance

The GAM predictions had a satisfactory performance, as indicated by the determination coefficient value (R^2) of 0.51 for the regression between the observed and predicted values (Figure 16 and Table 9).

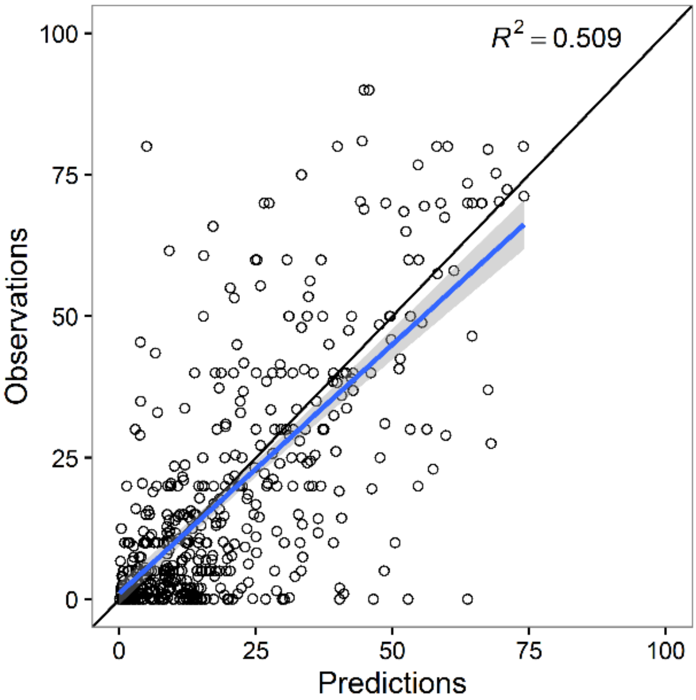

Figure 16. Relationship between observed and predicted maximum percent *Phormidium* cover by generalised additive model. Note that the observed values are plotted on the Y-axis and predicted values on the X-axis, following Piñeiro et al. (2008). The blue line is the best fit linear regression of the observed and predicted values with pointwise standard error bands (shading). The solid black line is one-to-one relationship.

The final model had a Nash-Sutcliffe efficiency value of 0.5 (Table 9), which is also indicative of a satisfactory performance (Moriasi et al. 2007). Similarly, the relative root mean square error indicated a satisfactory model performance (RSR = 0.7, Table 9). The PBIAS performance statistic indicated that the model moderately over-estimate maximum percentage *Phormidium* cover by 7.1%. This moderate over-prediction is evident in Figure 16, where the best fit linear regression is slightly below the 1:1 line.

Table 9. Model performance statistics for the generalised additive model. Interpretation of the values is based on the rule of thumb proposed by Moriasi et al. (2007). R2 = coefficient of determination, NSE = Nash-Sutcliffe efficiency, RSR = relative root mean square error and PBIAS = percent bias.

Performance statistic	Value	Interpretation
R^2	0.51	Satisfactory
NSE	0.5	Satisfactory
RSR	0.7	Satisfactory
PBIAS (%)	7.1	Moderate overestimation

4.3.4. National predictions

Maximum *Phormidium* percentage cover was predicted for 68,631 river segments across the country (Figure 17), excluding still and backwater habitats, stream order < 3, and segments with glacial mountains source of flow. In general the predictions confirm to our expectations with high *Phormidium* cover in lowland areas with cobble-bed rivers, and in regions with lower rainfall (i.e. east coast of the South Island).

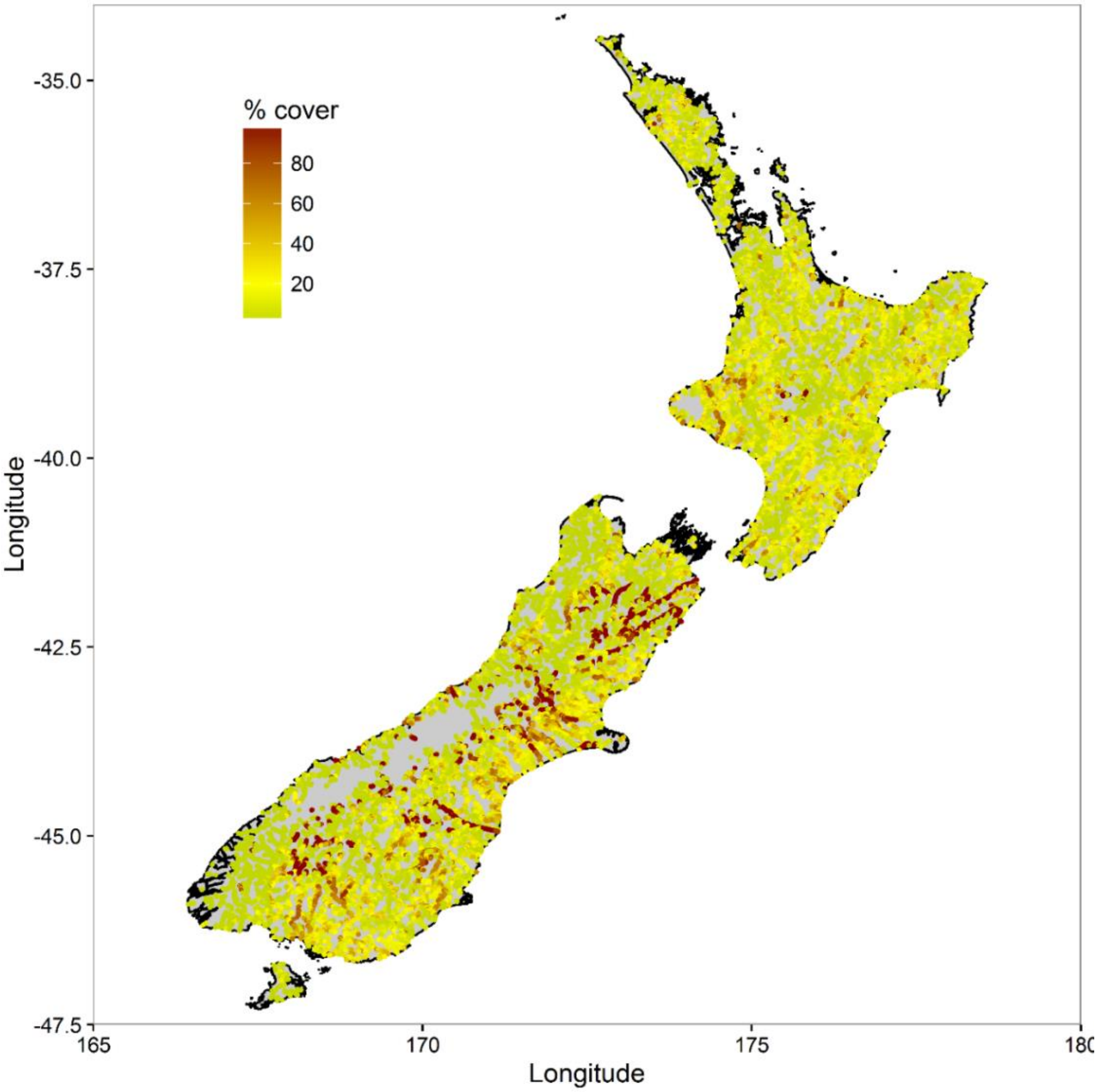

Figure 17. Maximum *Phormidium* cover predicted by the generalised additive model.

4.3.5. Independent model validation

A total of 32 sites were visited (Table 10; more detailed descriptions of each site are given in Appendix 1). Despite *Phormidium* being predicted to occur at all sites it was only observed at 11 sites. At ten of these sites cover was lower than predicted (Table 10). There was only one site (Dove River) where the cover exceeded the levels predicted (Table 10).

Table 10. Rivers visited during independent model validation with predicted and observed *Phormidium* cover.

Name	**Region**	**Predicted *Phormidium (%)***	**Actual *Phormidium* (%)**
Waikaretaheke River	Gisborne	9	0
Motu River	Gisborne	33	1
Manganuku River	Gisborne	0.2	0
Pelorus River	Marlborough	84	0
Rai River	Marlborough	54	0
Mangatoa Stream	Northland	64	1
Te Kao Stream	Northland	59	0
Toronui Stream	Northland	67	0
Te Waitemaunga Stream	Northland	12	10
Otiria River	Northland	17	1
Otautau Stream	Southland	76	0
Waimatuku Stream	Southland	67	0
Alton Burn	Southland	75	0
Aparima River	Southland	85	0
Wairaki River	Southland	4	3
Oreti River	Southland	54	0
Buller River	Tasman	99	3
Moutere River	Tasman	55	30
Pigeon Creek	Tasman	52	0
Stanley Brook	Tasman	76	1
Wairoa River Right Branch	Tasman	64	2
Dove River	Tasman	45	70-80
Abbots Creek	Wellington	56	2
Haast River	West Coast	100	0
Karangarua River	West Coast	100	0
Mahitahi River	West Coast	99	0
Maori River	West Coast	87	0
Ohinetamatea River	West Coast	90	0
Omotumotu Creek	West Coast	89	0
Poerua River	West Coast	100	0
Waitaha River	West Coast	100	0
Waitangitaona River	West Coast	83	0
Wanganui River	West Coast	99	0

Among sites with *Phormidium* there was considerable variability in many parameters including; size of the river, water velocity, depth and size substrate (Figure 18D, F; Appendix 1). Among sites without *Phormidium* there were sites which appeared unsuitable for *Phormidium* blooms to occur, i.e., small streams with soft substrate (e.g., Alton Burn in Figure 18B), or tidal streams (e.g., Toronui Stream). There were also a number of sites where the substrate and general habitat appeared suitable for blooms e.g. Pigeon Creek and Poerua (Figure 18A), but there was no *Phormidium* present.

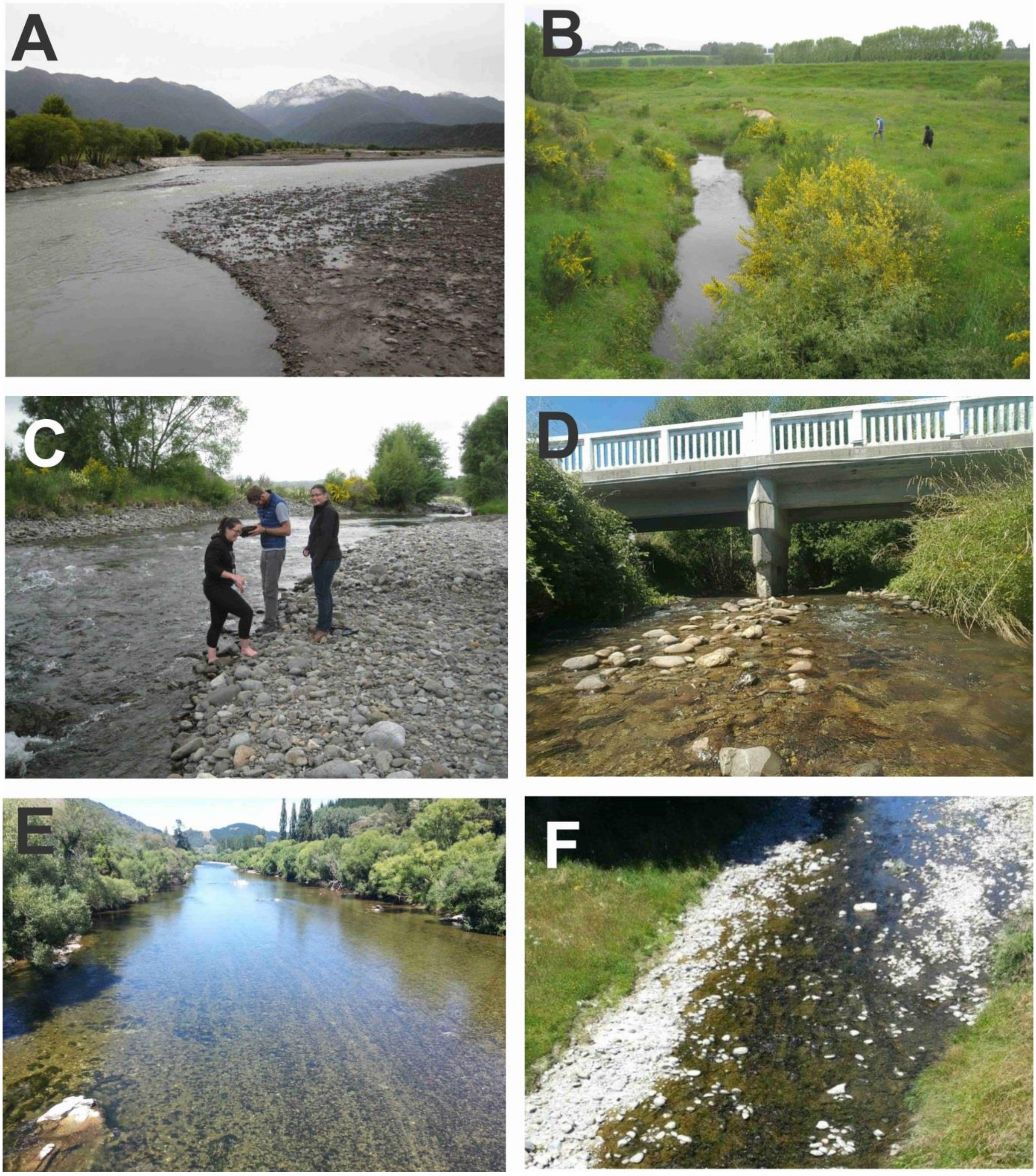

Figure 18. Six of the independent model validation sites. (A) Poerua (West Coast), (B) Alton Burn (Southland), (C) Wairaki (Southland), (D) Stanley Brook (Tasman), (E) Motueka River (Tasman), and (F) Dove River (Tasman). *Phormidium* was present at the sites in (C) and (D; not visible in photographs), and blooms were present at the sites in in (D) and (F; dark brown/black areas on river substrate).

4.4. Discussion

4.4.1. River susceptibility model

The *Phormidium* river susceptibility model performance was satisfactory, enabling us to make national scale predictions. Because the processes determining *Phormidium* mat colonisation, growth/expansion and resetting are complex (see the discussion in other sections of this report for further explanation), some unexplained variation in our models is expected. Furthermore, many of the variables regulating these processes are not represented by the catchment-averaged spatial predictor variables used in the model. For example, concentrations of dissolved nutrients in rivers are influenced by biological assimilation and adsorption-desorption processes; these mechanisms are not captured by the FENZ and land cover variables used in the model.

The monitoring sites used for fitting our *Phormidium* river susceptibility model were reasonably representative of the range of environmental characteristics of New Zealand's rivers when we excluded river segments that we do not expect to be associated with *Phormidium* proliferations, such as small streams, high altitudes sites, and still waters. There was a lack of data for some areas such as the Waikato, Auckland, West Coast and Fiordland regions, but this did not impact the representativeness of the monitoring sites (refer Figure 14).

In general the model predicts high cover in regions where *Phormidium* proliferations are known to occur, e.g. south Canterbury, Taranaki, some rivers in the Manawatu, Wellington and Wairarapa (refer Figure 17). The model predictions conform to our expectations that proliferations generally occur in lowland rivers in regions where water quality is relatively good, and do not occur in slow flowing, nutrient-rich habitats. For example, rivers in the Waikato region which tend to be slower flowing and soft bottomed and have poorer water quality, were not predicted to experience *Phormidium* proliferations. The model predicted high *Phormidium* cover in a number of regions where observed data are lacking (Figure 17), notable areas include: central Otago, inland Marlborough, several rivers on the West Coast of the South Island, central Auckland, and the East Coast of the North Island.

The predictions made using the river susceptibility model provides a description of regional to national scale patterns in rivers which might experience *Phormidium* proliferations. The predictions should be interpreted with caution at a site-scale, as other processes not included in the model, e.g. water column nutrients, can influence *Phormidium* proliferations. Additionally, seasonal and inter-annual variability is not accounted for in our models. However, based on results presented in previous sections it is expected that the maximum *Phormidium* cover values predicted are more likely occur in summer or early autumn. Recent flow and climatic conditions, i.e., time since a flushing flow, will also impact whether a site experiences a proliferation at a given point in time. Despite these caveats the river-scale predictions provide some guidance on where proliferations might occur and could be useful for guiding agencies on monitoring site selection. The models could also be useful for predicting how environmental or climate change might affect the distribution of *Phormidium*, and estimating how the extent of *Phormidium* proliferations has varied with land-use change.

4.4.2. Independent model validation

A single site visit provides only limited information on the likelihood of a site experiencing *Phormidium* blooms. For example, despite targeting our visits in summer, in some regions such as on the West Coast, it was extremely challenging to find periods over the 2016/17 summer when significant rainfall events had not occurred in the 2 to 3 weeks prior to sampling. Additionally, blooms appear to occur only during specific months in some streams, for example, in the Maitai River (Nelson). Without a priori knowledge of when blooms proliferate in different rivers it is not possible to time site visits with the period when blooms are most likely to occur.

Despite this limitation the independent model validation site visits provided a number of valuable observations:

- *Phormidium* occurs, and can bloom, in a wide range of rivers.
- The river susceptibility model could be refined through removing sites affected by salinity intrusions.
- In general the model over-predicts *Phormidium* coverage, for example, a maximum > 90% cover was predicted for eight of the sites we visited. During our intensive studies at sites with known histories of *Phormidium* blooms (Section 2), coverage of > 90% only occurs at a few sites. However, in contrast, the coverage in the Dove River at the time of our site visit was nearly twice that predicted by the model.

Collectively these observations reinforce the caveat that the river susceptibility model should be used at a broad scale to identify regions (or rivers) where *Phormidium* blooms might be prevalent, and that these predictions need to be confirmed through multiple site visits following periods of stable flow.

5. NATIONAL OBSERVED DATA ANALYSIS

5.1. Introduction

The objective of this section was to determine whether the relationships observed between *Phormidium* cover and environmental drivers in the analysis of high frequency (weekly) data in three regions (Section 2) are consistent at a national scale. To do so required us to collate *Phormidium* cover and corresponding environmental data from across New Zealand. The high frequency analysis suggested different hierarchies of importance in physicochemical variables occur between sites. Here we attempt to determine what these hierarchies are at a national scale. The same dataset analysed in Section 4 was used, but in this section we examine temporal rather than spatial variability. The data used for this section was therefore *Phormidium* cover averaged by 'month-time-year' and not the site maxima.

There are a number of challenges with this approach. Firstly, estimating *Phormidium* cover is subjective and there may be inter-user variability and hence sampling error; therefore gathering data from many samplers introduces noise to the data. Additionally, different regional councils record or measure different suites of environmental variables such as TN versus DIN. Finally, sampling is undertaken at varying frequencies by different regional councils. To address these challenges we focused our analysis in this section on a small subset of environmental variables that are measured in most monitoring programmes. To account for the differences in sampling frequency, data were averaged by month-site-year.

5.2. Methods

5.2.1. Phormidium cover and environmental data

Datasets used in this analysis are described in Section 4. To maximise the size of our dataset we focused on the four most commonly recorded environmental variables: DIN, DRP, conductivity and water temperature (Table 11). These were also all identified as important in the analysis of the high frequency (weekly) datasets (Section 2).

Table 11. Sampling effort of data used in the national observed analysis by regional councils, showing number of observations for *Phormidium* cover and four environmental predictors. DIN = dissolved inorganic nitrogen, DRP = dissolve reactive phosphorus. BoPRC = Bay of Plenty regional Council, ECAN = Environment Canterbury, GWRC = Greater Wellington Regional Council, HBRC = Hawkes Bay Regional Council, NCC = Nelson City Council, NRC = Northland Regional Council, ORC = Otago Regional Council, ES = Environment Southland, TDC = Tasman District Council, TRC = Taranaki Regional Council. * data not used in final analysis due to incomplete environmental predictors.

		Number of observations				
Council	Sites	*Phormidium*	DIN	DRP	Conductivity	Temperature
BoPRC*	12	199				
ECAN	163	7,210	3,923	3,923	3,489	7,122
GWRC	66	3,490	1,109	1,109	1,088	1,113
HBRC	63	1,033	998	1,033	904	917
Horizons	63	2,211	2,066	2,068	2,049	2,056
NCC*	26	403	377	377	377	
NRC	36	530	527	527	518	518
ORC	8	39	33	33	33	33
ES	32	138	87	86	78	87
TDC	67	1,385	496	253	719	1
TRC*	33	296				
Total	569	16,934	9,616	9,409	9,255	11,847

Three regional councils did not sample all four environmental variables and so their *Phormidium* data were excluded from further analysis, namely BoPRC, NCC and TRC. Sampling frequency also varied between councils, but was mostly weekly or monthly, thus data was averaged into month-year-site nodes. The final dataset comprised 7,752 observations.

5.2.2. Data analysis

Non-linear trends in *Phormidium* cover in relation to time of the year and environmental variables were explored using generalised additive models (GAM; Hastie & Tibshirani 1990). *Phormidium* cover data were highly right-skewed, over-dispersed and with a large proportion of zeros, thus models were constructed using log-normal errors. Based on initial data exploration (completeness of observations) and our a priori knowledge, four non-collinear predictor variables were included in the model: month of the year (seasonal trends), water temperature (°C), log-transformed DIN and DRP concentrations (mg L^{-1}). Additionally, regional council and site were included as fixed and random effects, respectively. To account for temporal autocorrelation in the time series data, models were fitted using auto-regressive moving average correlation structure of order 1. A stepwise procedure based on the GAIC was used for model selection and were validated by inspecting the deviance residuals. Partial effects plots were used to show the effect of each predictor variable conditional to others in the model. The partial effects of each predictor are displayed as cubic splines showing either negative or positive effects relative to the overall mean of the response variable centred on zero. Analyses were performed with the software R (R Core Team 2014) and GAMs models using the ‘mgcv’ package (Wood 2006).

5.3. Results

5.3.1. Environmental variables

Median DIN concentration in monitored streams ranged between a maximum of 0.778 mg L^{-1} in Southland and a minimum of 0.047 mg L^{-1} in Otago (Figure 19). Median concentrations of DIN fluctuated around 0.3 mg L^{-1} in streams in other regions. Median DRP concentrations were relatively low in monitored streams in most regions, with median values fluctuating around 0.007 mg L^{-1}, a maximum of 0.011 mg L^{-1} observed in Northland and a minimum of 0.002 mg L^{-1} in Hawke’s Bay (Figure 19).

As expected, water temperatures varied considerably across regions, likely reflecting climatic differences. Temperature fluctuated between 0.1 and 29.6°C. Maximum median water temperatures were recorded in Northland (16.6°C) and a minimum median of 11.7 °C was recorded in Canterbury. Conductivity varied markedly among regions (range 0.1–898 µS cm^{-1}), with monitored streams in Otago having a lower conductivity than other regions (Figure 19).

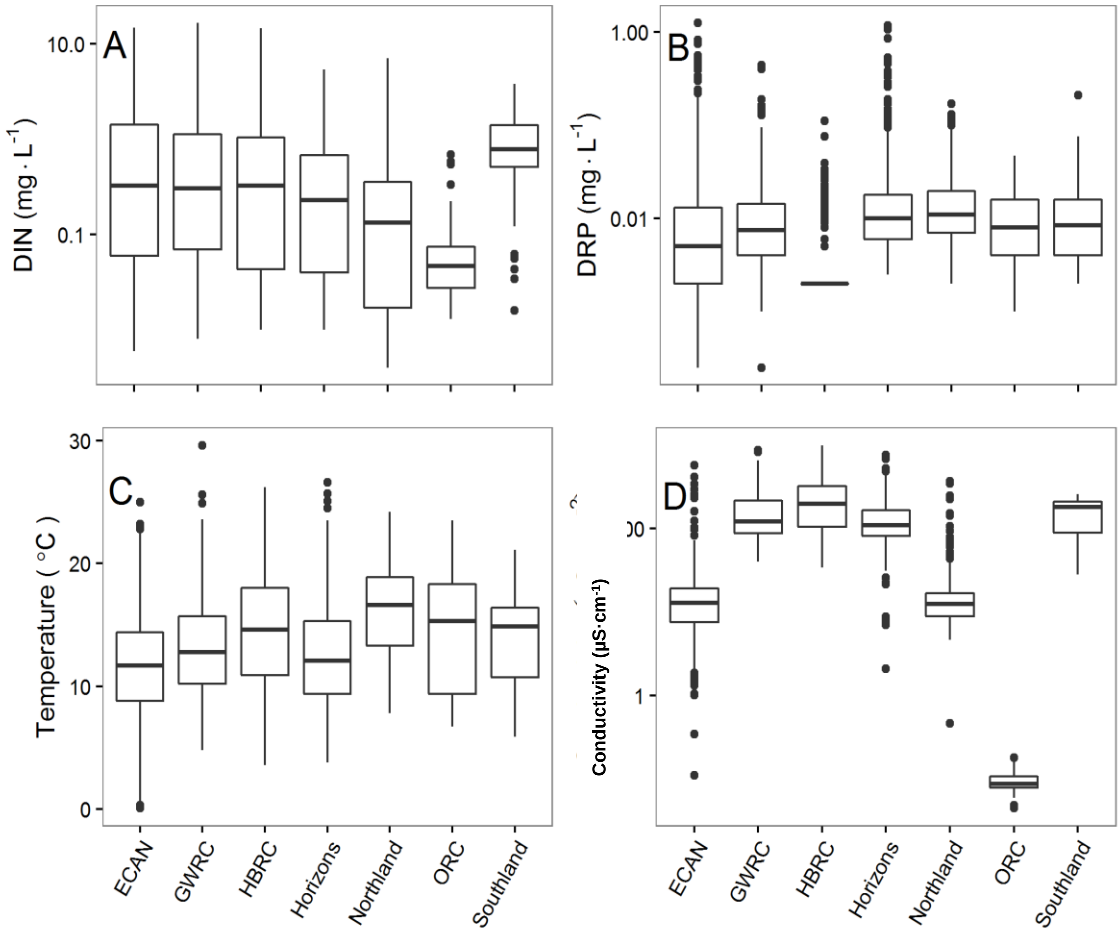

Figure 19. Physiochemical variable by regional council: (A) dissolved inorganic nitrogen (DIN), (B) dissolved reactive phosphorus (DRP), (C) temperature, and (D) conductivity. The y-axis in (A) and (B) are in a log scale to aid in visualisation.

5.3.2. Generalised additive model

The most parsimonious GAM explained only 16% of the total deviance in the *Phormidium* cover data. The final model included month of the year, water temperature, DIN and DRP, but excluded conductivity (Figure 20). Month of the year had a weak but significant seasonal effect ($P < 0.001$, Figure 20), with a positive effect on *Phormidium* cover predicted for the summer months (i.e. November to April; Figure 20a). Water temperatures greater than 14°C had a positive and significant effect ($P < 0.001$), whereas colder temperatures had no effect on *Phormidium* cover (Figure 20b). Both nutrient variables (DIN and DRP concentrations) had the largest relative importance based on the amplitude of the partial effect plots. There was a significant effect of DRP ($P < 0.001$), with predicted negative effects for DRP > 0.006 mg L^{-1} (Figure 20c). The model predicted greater *Phormidium* cover with increasing accrual DIN concentrations up to 0.8 mg L^{-1}, after which the effect remained constant ($P < 0.001$, Figure 20d). Region (regional council) had a strong significant effect with largest positive effect on *Phormidium* cover predicted for Otago and Southland (P < 0.001, Figure 20e). Additionally, there was large spatial variability at the scale of Site within regional councils (Figure 20f).

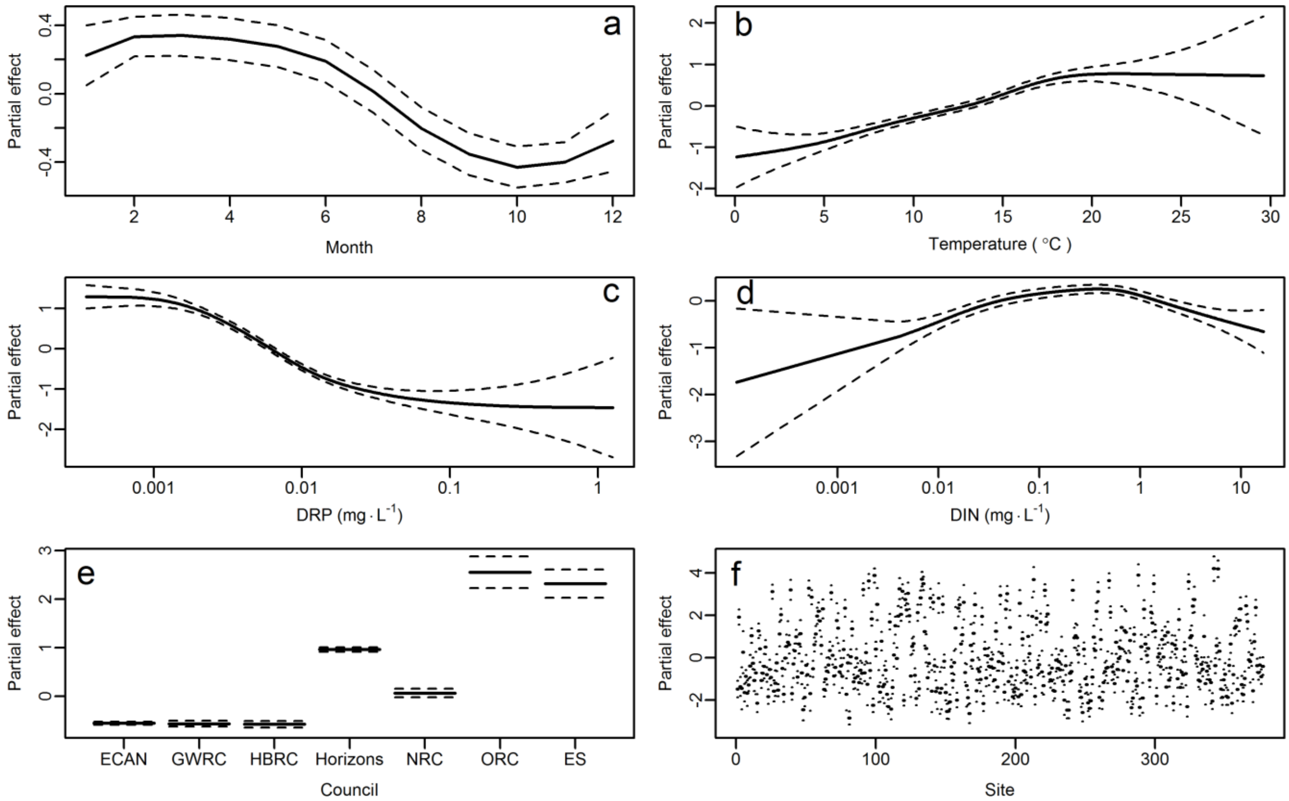

Figure 20. The effects of: (a) season (month of the year), (b) water temperature, (c) dissolved reactive phosphorus (DRP), (d) dissolved inorganic nitrogen (DIN), (e) regional council and (f) site. Solid lines represent cubic splines (± standard error, dashed black lines) fitted using a generalised additive model. See methods for description of *x*-axis partial effect scale.

5.4. Discussion

The GAMs had a low (16%) explanatory power, which is considerably less that observed for other models (Sections 2, 3, and 4) developed as part of this project. There are a number of possible explanations:

- *Phormidium* cover was assessed using several methodologies, including in-river transects and bank side estimates. Both of these methodologies are somewhat subjective and estimates can vary between assessors, this creates ‘noise’ in the data.
- The basic model for control of periphyton biomass in cobble-bed rivers identifies hydrologic disturbance as the primary regulator, whilst nutrients operate within this

by influencing the rate of accrual during stable periods (Biggs 1995; Biggs et al. 2005). Our previous analyses (e.g. Section 2 and 3, and Wood et al. 2016) have also highlighted flow as an important variable in regulating *Phormidium* proliferations. Obtaining flow data for the sites in the national dataset was beyond the scope of this study, but their absence likely reduces the explanatory power of the GAMs.

- Some sites are surveyed/sampled weekly, more commonly the assessments were monthly, and others were sporadic. When monthly or sporadic sampling is undertaken there is a high likelihood of missing periods when *Phormidium* cover is high. This will reduce the effectiveness of the model in identifying meaningful relationships. In our analysis we set a threshold of five observations for inclusion of a site. As datasets improve this threshold should be set much higher, and sites with limited observations excluded.
- As also observed in Section 2, site (or river) frequently emerges as an important predictor, indicating that different variables or river-specific features (e.g., substrate stability) that are not included in the models are likely significant in explaining much of the observed variability. This is discussed further in Section 6.

Despite the low explanatory power of the model, in general the patterns and variables which were significant in the GAM matched those identified in our previous analyses (Sections 2 and 3). The two environmental variables that showed the strongest relationships with *Phormidium* cover were DIN and DRP. There was a predicted increase in *Phormidium* cover with DIN up to 0.8 mg L^{-1}, this was higher than 0.05 mg L^{-1} observed in the high frequency analysis (Section 2) and 0.5 mg L^{-1} observed in the regional monthly analysis (Section 3). Although concentrations above this did not result in increased cover, they did not have a negative effect, and collectively the data in this study continue to support the hypothesis that rivers with slightly elevated DIN are more susceptible to *Phormidium* proliferations. A comparison of DRP concentrations with the weekly analysis suggests similar patterns with lower DRP favouring *Phormidium* proliferations. There doesn't appear to be a lower threshold, instead a near linear pattern with increasing DRP related to a decrease in *Phormidium* cover to an upper concentration of ca. 0.02 mg L^{-1}.

The collation of the datasets used in this study highlighted marked discrepancies in the methods used to sample and survey *Phormidium* (and periphyton) across regions of New Zealand. We are aware of processes in place to create a more unified national approach (e.g. the National Environmental Monitoring and Reporting project) and commend this. Some councils monitored variables that may provide useful data for increasing knowledge on *Phormidium* or periphyton accrual, e.g., trace metals, organic carbon, but these datasets were short in duration and only in select regions, preventing their analysis in this study.

6. DISCUSSION AND RECOMMENDED EXPERIMENTAL STUDIES

To date most studies aimed at identifying variables that play significant roles in controlling proliferation of *Phormidium* in rivers have primarily used observational data. Specifically, regional councils and researchers have collected synoptic information on *Phormidium* cover and potential environmental driver variables (temperature, flow, nutrients, pH etc) and used correlative analysis across multiple sites to identify variables that are consistently associated with proliferations. This approach, as illustrated in Sections 2 and 3, has many advantages; it uses variables that are being routinely monitored by regional authorities, and potentially provides a direct entry point for management interventions. It has the added attraction of being conceptually similar to successful approaches for lakes that link nutrients to algal blooms, (in New Zealand via the Trophic Lakes Index approach (Burns et al. 2000)), although where similar eutrophication relationships have been sought for rivers less success is achieved because of fundamental differences in ecosystem processes (Biggs 2000).

Statistical approaches do, however, have limitations. These limitations particularly apply to the risk that correlations between measured variables may not be causative, rather they may be consequential, or the result of complex aliasing relationships with other variables that are not part of the existing dataset. An example of a consequential relationship could be the conclusion that *Phormidium* proliferations tend to occur at low nitrate concentrations, when in fact low nitrate is due to rapid uptake by *Phormidium* and other algae. A related example of aliasing might be where proliferations appear to be associated with low nitrate concentrations, but this could be due to the frequent co-occurrence in summer of low nitrate, low flow, high temperature, low sediment etc, various elements of which are related to an increased probability of proliferations.

Another key challenge highlighted during the statistical examination (Section 2.3.2, Figure 3), which points to the need for a modified approach, is that when ‘river’ is included as a variable, it emerges as an important predictor, indicating that rivers are behaving somewhat idiosyncratically, or at least in ways that are not determined by variables currently measured.

It is axiomatic in ecological investigations that relationships observed between measured predictor and response variables can point towards possible causative relationships, but that robust experimentation is required to demonstrate causation. Therefore, experimental approaches that control the accrual of *Phormidium* blooms are required to test which of the multiple variables implicated by correlative analysis are in fact causative. We start this section by considering the environmental variables that were shown to be important through varying stages of the accrual cycle to develop an experimental approach. We then provide suggestions for further in river measurements and experiments, followed by mesocosm or streamside channel experiments.

6.1. Using high resolution datasets to estimate accrual cycle parameters

An obstacle to developing clear relationships between environmental variables and changes in *Phormidium* cover is the accrual cycle. Accrual describes a pattern comprising phases from colonisation (or relic population) through exponential growth, to a maximum biomass. Each of these may be controlled by different variables, but we might expect that accrual would follow a logistic growth model, which takes the form of:

$$dN/dT = r \cdot N \cdot [(K-N)/K]$$

where r is the biomass-specific, exponential growth rate, N the biomass and K the carrying capacity (or maximum biomass) for the site and T is time. Reformatting for our application, in terms of cover this becomes

$$C_T = [K \cdot C_0 \cdot e^{(r \cdot T)}]/[K+C_0 \cdot (e^{r \cdot T}-1)]$$

Where C_T and C_0 are cover at time T and 0, K remains the maximum cover and r the exponential rate of cover increase. Cover at any one time thus depends on the time since expansion began, the population at T = 0, the growth rate, and the proximity to carrying capacity. Figure 21 shows the accrual of *Phormidium* cover at two sites that follow the logistic pattern of near-logarithmic increase at low cover rising more slowly as a cover maximum is approached.

Important points from Figure 21 are:

1. Accurate information on the logistic growth cycle requires sampling on near-weekly time bases. Infrequent sampling (e.g. monthly) is unlikely to return a useful estimate of any accrual cycle parameter.
2. The carrying capacity may take several months to be reached and is not always attained in all cycles, but
3. When carrying capacity is reached there is some evidence for a river-specific value to be reached, and that
4. The rate of increase in percent *Phormidium* cover (the slope of lines R1-R3) is reasonably consistent between and within rivers.

Loss processes are not included in this simple explanation, but clearly evident in Figure 21 is that at all stages of the accrual cycle there is a risk of full or partial biomass resetting.

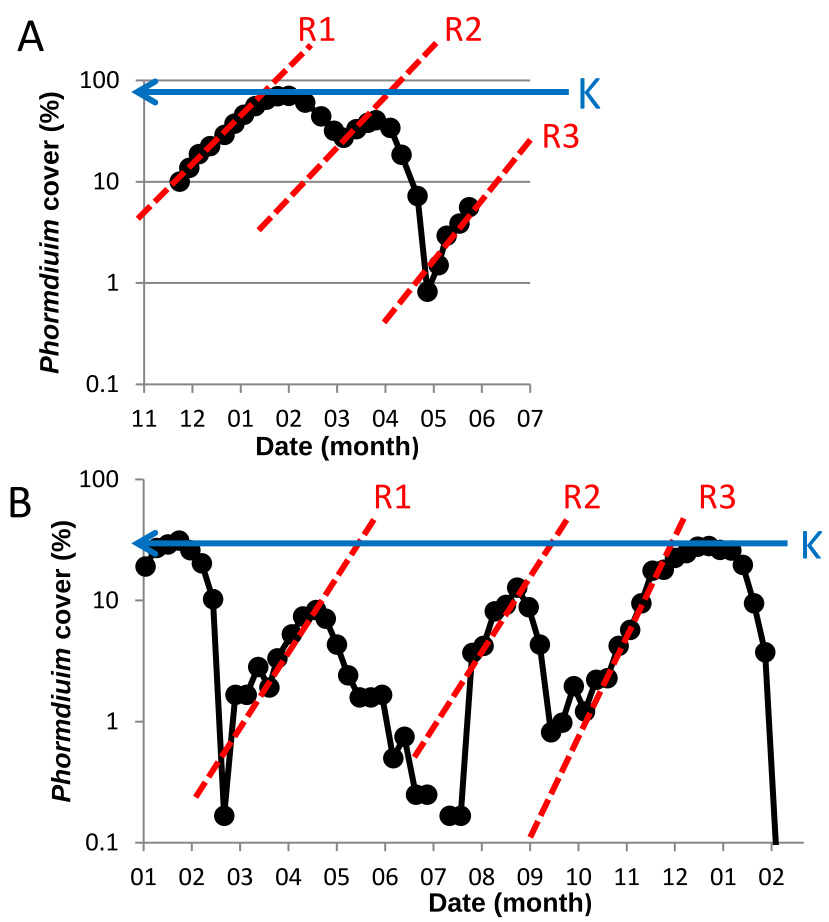

Figure 21. parameters of the accrual phase of the accrual cycle. (A) Opihi River, Canterbury; (B) Makakahi River, Manawatu. In each case the log of cover is plotted so that exponential expansion will appear as a straight line, with initial slope equivalent to growth rate (R), Examples of how weekly datasets on *Phormidium* cover can be used to approximate while curves will flatten off as carrying capacity (K) is approached.

The carrying capacity and the exponential growth rate are clearly useful parameters of the *Phormidium* cover accrual cycle that, if they can be estimated across a range of conditions, represent an alternative way of examining the mechanisms underlying bloom development. The ability to adequately describe the growth elements of accrual cycles depends on sampling on a time base much shorter than that of the cycle itself, but the three high frequency datasets described in Section 2 allow us to investigate carrying capacity and accrual rates.

6.1.1. Carrying capacity

Few of our weekly datasets contained as clear evidence of attainment of carrying capacity as Figure 22. However, differences in the upper limit of observed cover (for example the 90th percentile value in high-frequency data), may reflect variation in the carrying capacity for a given river. The 21 river sites for which high frequency sampling is available showed considerable variation (0-68%) in 90th percentile biomass. However, none of variables measured as part of these projects could be related to it, and no relationship was observed between 90th percentile cover and median concentrations of water column nitrate and DRP (Figure 22). This conclusion needs to be tempered by the fact that visual inspection of the data showed that evidence for an asymptotic, maximum cover was evident in only approximately 15 of the sites.

Site-to-site differences in carrying capacity (e.g. Figure 22) might be expected to be affected rather more by habitat suitability (e.g. slope, flooding regime and substrate stability), than resource availability, and those physical attributes tend not to be routinely measured in monitoring programmes. Thus we consider that there are likely to be substantial differences between rivers in carrying capacity, which will add noise to any nationwide attempt to link low frequency, synoptic environment-cover observations. An improved understanding of the attributes linked to high carrying capacity, which we hypothesise will be linked to flow or substrate-related variables will be a useful strategy for further research into *Phormidium* blooms.

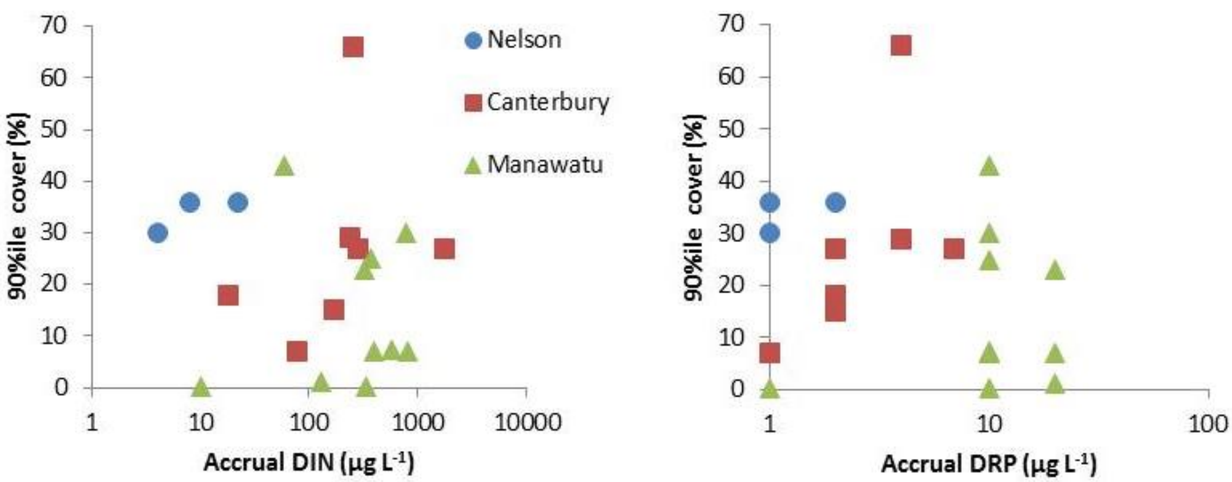

Figure 22. Relationships between 90th percentile *Phormidium* cover in Nelson, Canterbury and Manawatu rivers (see Section 2) and median water column nitrate and dissolved reactive phosphorus (DRP) concentrations. Note logarithmic scale on the X axis.

6.1.2. Accrual rate

In terms of the logistic growth model, nutrient concentrations may more likely affect the rate of accrual (r) than carrying capacity (K). Weekly datasets allow identification of periods of near exponential accrual from low cover, from which estimates of accrual rate can be derived by fitting of the logistic model, and compared to environmental conditions over the accrual period (Figure 23).

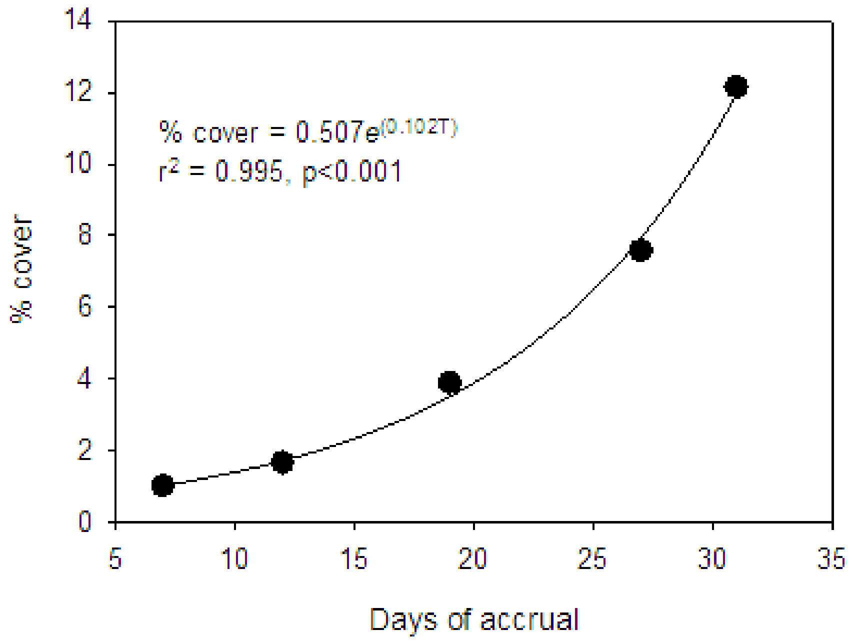

Figure 23. Fitting an exponential growth curve to the early part of an accrual cycle allows an estimate of the mat expansion rate. In this case exponential growth of percent cover was 0.102 d^{-1}, or an approximate doubling of percent cover every seven days.

When estimates of rates of increase in cover were explored to identify whether any measured variable was correlated, temperature emerged as significant (Figure 24). Neither water column DIN (Figure 24), nor DRP (not shown) had any statistical link to expansion rate. Indeed, no measured variable other that temperature was correlated with the residuals of the temperature versus growth rate regression. Stepwise multiple regression including water column nutrients and temperature yielded no increase in predictive power and only temperature was returned as a significant predictor of growth rate. The exponential rise in cover expansion rate with temperature was consistent across all sites and regions and, with a Q_{10} (the increase in rate for a 10°C rise in temperate) of 2.5, is close to what would be expected for a biological process. This observation is consistent with the suggestion by Francoeur et al. (1999) that temperature was the most important determinant of accrual rate for river periphyton in general, and with observations by Heath et al. (2011) that *Phormidium* proliferated in warm water periods, and do not contradict observations of high cover at low temperatures (McAllister et al. 2016; Wood et al. 2017) if these occur after prolonged accumulation periods.

It is important to recognise that this conclusion is tentative, and based on a small dataset. Reliable estimates of growth rate require at least weekly sampling, and a period of several weeks with no disturbance. Figure 24 is based on 31 values from 17 sites. Further confirmation of the absence of a clear effect of nutrients on growth rate requires further such studies, though at present indications are that, for nitrate values of 0.020 to 0.140 mg L^{-1} and DRP of 0.001 to 0.0024 mg L^{-1}, growth rate, like carrying capacity, is not determined by water column nutrient concentration.

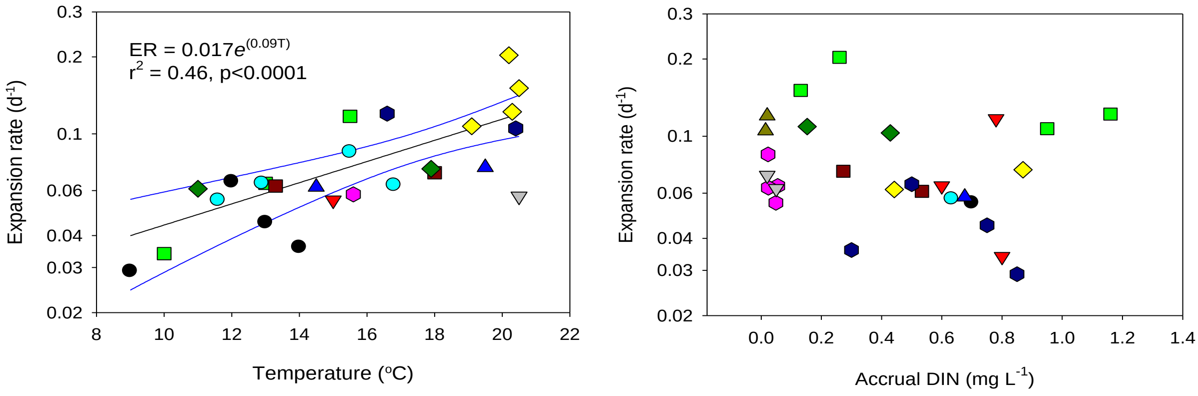

Figure 24. Relationships between (left) accrual period average temperature and (right) accrual period average DIN concentration, and exponential rate of cover increase across rivers and regions. Shapes represent regions (squares Manawatu, circles Nelson, triangles Canterbury) and colours differentiate rivers within regions. For some rivers more than one accrual period could be followed. A significant exponential relationship was evident with rate of increase and temperature but not for DIN.

6.1.3. Resetting

Evident in Figure 21 (Section 6.1.1) is a constant risk of full or partial biomass resetting throughout the accrual cycle. Disturbance occurs either when shear stress exceeds the attachment tenacity of *Phormidium*, or through substrate disturbance. Field observations have been used to relate the flood frequency in New Zealand rivers 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 have sometimes been over-interpreted as indicating that 3 x median flow is a universal 'flushing flow'. This is not the case and we are now aware of species- and river-specific differences in what constitutes a flushing flow. *Phormidium* mats often tolerate higher flows than other periphyton classes (Hart et al. 2013), and studies in both Canterbury (reported in McAllister et al. 2016) and Manawatu-Whanganui regions (Wood et al. 2017) have consistently shown that the magnitude of flow increase over median flow required to remove *Phormidium* mats is not a uniform three times the median flow. Wood et al. (2017) used a quantile regression approach to define the '*Phormidium* flushing flow' for each river as the multiple of median flow at which 80% of measurements of cover were predicted to be below 20%. Their analysis highlighted marked differences between rivers, ranging from 1.2 to 21.5 x median flow in four rivers for which sufficient data were available. Taking a less robust approach to estimating resetting flows, inspection of all high frequency data for a maximum flow (x median) above which cover was always at or very close to zero (e.g. Figure 25), yielded a median of

4.5x, but a range of 0.7–22x. In Figure 25, for example, it appears to be possible to maintain high *Phormidium* cover at higher flow in the Opihi River than the Te Ana a Wai River, with high cover rare above 9x and 2x median flow, respectively. The ranges of critical flow were similar across Canterbury (2-15x) and Manawatu (0.7-22x), and where more than one site per river was examined the critical flow was similar at all sites.

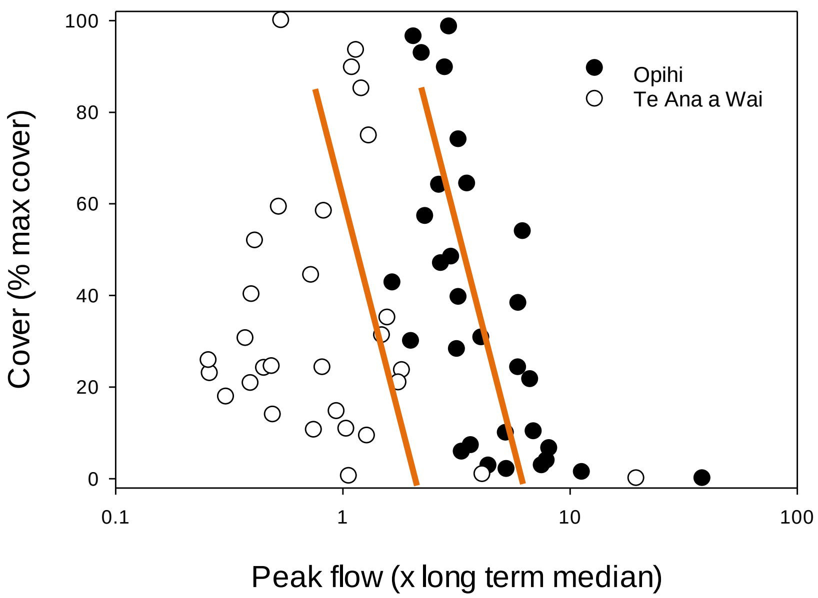

Figure 25. Relationship between peak flow (week prior to observation) and *Phormidium* cover in two Canterbury rivers in the summer of 2015-16. The orange lines are drawn by eye to show how the flow above which cover is reset appears to vary between the two rivers.

6.1.4. River to river variation – additional measurements at sites with high frequency monitoring

Earlier we suggested that the significance of 'river' as a predictive variable in the GAMs analyses (Sections 2 and 5) indicated the individualistic behaviour of waterways. Analysis of high frequency datasets in this section allows hypotheses to be developed that these site-site differences may relate to their carrying capacity and/or vulnerability to flow-driven resetting, which could be correlated. Putative mechanisms underlying such differences could include all or some of the following:

- substrate stability may differ between sites making them differentially vulnerable to flow variations,
- substrate suitability and/or stability may differ within sites making the carrying capacity different between sites,
- relationships between flow and shear stress may vary between or within sites.

Accommodation of hydraulic and stability proxies into descriptors of river reaches is difficult to do accurately, but approximations are possible. The minimum requirement is for knowledge of the substrate size, temperature, mean depth and water surface slope. The last three of these can be used to estimate reach shear stress using an approximation of the DuBoys equation for boundary shear (Baker and Ritter 1975), where

Boundary shear stress = water density x mean depth x slope

Many monitoring programmes already measure temperature, depth and substrate, and an estimate of slope would be a simple extra measurement. With an understanding of shear stress, the size of particle that can be moved can be calculated with reasonable confidence (Giberson & Caissie 1998), and this approach would allow rivers to be assessed in terms of their hydraulic suitability to *Phormidium* blooms.

Other chemical variables outside those measured in the studies reviewed here may also play a role in determining *Phormidium* proliferation. 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 *Phormidium* blooms (Wood et al. 2015a; McAllister 2015). The analysis in this study suggested that conductivity, sometime used as a proxy for nutrient or metal availability, and individual elements (e.g. iron (Wood et al. 2015a)), might be associated with sites where *Phormidium* proliferates. Current datasets are too small to confirm these observations, and only a limited number of councils currently collect these data. While more data may help confirm these relationships, a more informative method would be to individually or synergistically test the impact of these compounds using recirculating channels as outlined below.

6.2. A new technique for in-river experiments

In addition to further measurement at sites with high-frequency (i.e. at least weekly) monitoring, we suggest in-river experiments. From the high resolution data (Section 2 and above), we can identify rivers with high, medium and low DIN that have significant *Phormidium* cover. For example, candidate rivers in the Canterbury region are the Temuka, Opihi, and Orari. In the Manawatu region, Oroua downstream of the sewerage treatment plant, Makakahi and Tokomaru rivers and in Nelson the Avon Terrace and Campground sites on the Maitai River. We have recently developed a technique to inoculate rocks with a constant amount of *Phormidium.* This involves drilling a small hole (ca. 5 mm dia. and 5 mm depth; Figure 26) into the cobble which is then filled with *Phormidium* mat (sourced from the river where the experiment will be undertaken). The rate of expansion from this inoculum can be monitored by repeat photographs followed by image analysis.

This technique specifically examines accrual, rather than colonisation processes (the colonisation phase is standardised). By using a small amount of a mature mat as the inoculum, all of the mix of *Phormidium,* other cyanobacteria, bacteria and algae that make up the mat are introduced together rather than having to colonise from outside. These organisms might be involved in nutrient cycling processes, remembering that nitrogen-fixing bacteria have been identified within *Phormidium* mats. Complete communities may regulate accrual/expansion rates differently to those involved forming the mat as a consequence of sequential or gradual colonisation, and in this way it is possible to examine the controls on mat expansion with no compromise from colonisation. A possible modification to this experiment, that may allow the importance of microbial assemblage development to be assessed would involve using 'pure' laboratory cultures of *Phormidium* that are either completely free of bacteria (axenic) or have been shown to be devoid of bacteria involved in nutrient cycling processes, i.e. nitrogen cycling.

Figure 26. Cobble showing a 5-mm diameter hole which is then filled with *Phormidium* mat. Photo: E Martin, Victoria University.

In recent pilot studies, the mat-inoculated cobbles have been placed in slow, medium and fast flowing sections of the Canterbury rivers, known to support *Phormidium* to determine the response of expansion rate to flow. Expansion rates have been determined by revisiting each cobble every 3-4 days and taking photos for image analysis. Biomass is measured by sacrificing certain rocks at less frequent intervals. Biomass analysis includes measurements of phycoerythrin (a cyanobacterial-specific pigment), and microscopic counts.

Experimental investigations into the role of nutrients in facilitating blooms could involve comparative studies in rivers where *Phormidium* is known to proliferate with those that are seemingly suitable for proliferations to occur (i.e. nutrients are within optimal ranges), but do not. Another alternative would be rivers with nutrient gradients (i.e. Hutt River), where *Phormidium* proliferations do not occur in the upper reaches but are prevalent in lower reaches. The result of a suite of these experiments would help determine the relative contribution of flow and DIN on accrual rates, and confirm our hypothesis that water column DIN has a limited effect on growth rates. If cultures were used as the inoculum, it may provide additional information on variables that affect colonisation. This experiment could be relatively easily up-scaled to a nationwide study, whereby researchers set up the experiment, and regional council (or perhaps citizen scientists) could return at set intervals and take the photographs and samples.

In-river experiments could also be used to assess the role of frequency and intensity of disturbance. Disturbances play an important role in *Phormidium* accrual cycles by resetting the system and initiating new accrual. Thiesen (2015) undertook a preliminary study investigating how relic populations, (i.e. the amount of mat remaining after a flushing flow) affect accrual rates. Increasing knowledge on relic populations would help in predicting how quickly blooms might 'recover' following flushing events. A protocol similar to that used in Thiesen (2015) could be used. Thiesen (2015) undertook a programme of repeat photography and analysed the sizes of natural *Phormidium* patches in four rivers. Increasing the number of rivers and surveying over a longer time span (> 4 weeks) than used in Thiesen (2015) should result in a robust dataset.

In-river experiments could also be used to examine the effects of experimental disturbances on *Phormidium* mats. Using the methods described above, *Phormidium* growth rates in undisturbed control plots could be compared to plots exposed to varying frequencies and intensities of artificially manipulated flow-disturbance events.

Several studies have indicated that fine sediment contributes to *Phormidium* proliferations (e.g., Wood et al. 2016). In addition to *ex situ* channel experiments (described below) this potential relationship could be explored further using in-river/stream experiment. Sediment barriers (i.e. bags filled with clean sand or similar) could be deployed in river/stream and *Phormidium* cover and biomass monitored up and downstream of these. An alternative would be the addition of fine sediment to small river sections where nutrient conditions are suitable but blooms do not occur, or using the *Phormidium* inoculation technique it may be possible to set up an experiment on alternative sides of a river whereby one side receives sediment and the other does not; mat accrual rates could then be monitored.

Grazing macroinvertebrates may have direct or indirect effects on *Phormidium* growth. The filamentous morphology of *Phormidium* may make it less palatable than diatoms or green algae. Preferential consumption of diatoms or green algae may open up new space for *Phormidium* colonisation. Preliminary observations in New

Zealand rivers suggests that at some sites the effect of grazers vary with flow habitat. For example, in pools (slow-flowing habitats) freshwater snails were observed to remove *Phormidium* mats, however, these snails were largely absent in fast-flowing riffles where presumably water velocity make it difficult for the snails to remain attached. Experiments could involve macroinvertebrate exclusion experiments, for example mesh bags could be placed over rocks containing *Phormidium* and these, and controls could be placed in different flow regimes at specific sites.

In some streams or regions there appears to be a relationship between groundwater and *Phormidium*, with blooms occurring in gaining reaches. It is unknown whether this is related to the generally higher levels of nitrate in groundwater, or other differences in water chemistry. Further studies could involve more in-depth analysis of the elemental composition of groundwater, and any compounds of interest could then be assessed using the *ex situ* channel system described below.

6.3. Mesocosm or stream channels

As discussed in earlier sections of this report, several studies have suggested that fine sediment may be involved in promoting *Phormidium* proliferations. We suggest using recirculating channels to show experimentally whether there are correlations between fine sediment and *Phormidium* mat development/growth rates. These experiments may ultimately lead to knowledge on how much sediment is required to enhance growth, which could assist in setting fine sediment limits for river affected by *Phormidium*. A ‘closed’ recirculating system, as opposed to a system fed by natural river water is required to accurately regulate the amount of sediment entering the system.

A suitable system has recently been set up at Cawthron and consists of four sets of four recirculating channels (Figure 27). Experiments to date have involved placing nine cobbles in each channel. To ensure each of the rocks started with the same inoculum, the inoculation technique described above could be used in future experiments.

Figure 27. Recirculating channels experiment at the Cawthron Institute.

The channel set up allows different quantities of fine sediment to be added to each replicate set of channels. *Phormidium* mats could then be assessed visually every 2-3 days, and biomass samples collected at less frequent intervals (ca. weekly). An extension of this experiment could involve adding the same quantity of sediment, but with varying amounts of biologically available phosphorus.

This same experimental set-up could also be used to test the effects of nutrients, or trace metals, and interactive experiments could be undertaken, e.g., the effect of nutrients and sediment, or iron and nitrogen.

One of the limitations with the recirculating channels is that it is not very practical to manipulate flow. NIWA have a larger channel set up near Christchurch (Figure 28), which has been used for a number of periphyton research projects. Recently, PhD student Tara McAllister used the NIWA channels to investigate the effect of flow and nitrate concentrations on *Phormidium* accrual. A number of other iterations or similar experiments could be undertaken including manipulating DRP concentrations, exploring the role of algal competition, and the effect of grazers (i.e. macroinvertebrates). The NIWA channels could also be used to investigate resetting process, both autogenic and due to abrasion or shear stress. Because the water is sourced from and returns to the river, careful consideration has to be given to the return of water post experimentation. This may limit some experiments, such as additions of trace metals, emerging contaminants and herbicides. The river water also has a high sediment load, preventing experiments involving sediment addition being undertaken.

Figure 28. The NIWA flow through channels.

6.4. Understanding process at the mat scale

Previous studies have shown or indicated the importance of within mat processes in influencing *Phormidium* accrual (Wood et al. 2015c; Aristi et al. 2016). Brasell et al. (2015) showed that the microbial communities within these mats are transient and shift during growth phases. Improved knowledge on processes occurring within the mats would assist in understanding how and when water column variables influence different part of the accrual cycle. This would assist in predicting the effectiveness of management actions, e.g. nutrient reductions.

There are a number of well-established biochemical assays and new emerging techniques that could be used to analyse mat samples, and we suggest analysis from samples collected through the accrual cycle. These could include:

- The acetylene-ethylene assay to measure nitrogen fixation.
- The alkaline phosphatase assay to measure the capacity for organic phosphorus to be converted to inorganic forms.
- Measurement of total nitrogen, phosphorous and carbon within the mats to try and establish which, if any, elements are limiting growth. We acknowledge this is likely to be challenging to interpret as the mats contain a mixture of other organisms and inorganic material.
- Genomic techniques, particularly metatranscriptomics, may provide some interesting insights into the functioning of *Phormidium* and entire microbial communities. Metatranscriptomics is the profiling of community-wide gene expression. It is a powerful tool to study functional changes in communities. Metatranscriptomics allows for the identification of biologically active (rather than simply present) organisms in the study environment and assessment of the

functional activity. For example, these techniques could be used to determine when nutrient acquisition genes are upregulated.

6.5. Prioritising future data collection and research

The Ministry for the Environment Benthic Cyanobacteria Working Group met on 2 March 2017 and discussed the experimental options described above. The Group collectively prioritised the following projects to identify and improve our understanding of drivers of *Phormidium* blooms:

- Undertaking the *Phormidium* inoculation experiment described above in a wide range of rivers across New Zealand. This project would need support from regional councils to monitor growth and collect samples.
- Further research on the role of fine sediment. This could involve both in-stream experiments and experiments in the recirculating channels.
- Obtaining more weekly resolution data including corresponding site descriptors such as water depth and slope and substrate. This would involve ensuring that regional councils that are already undertaking weekly surveys do so in a standardised manner, and that additional samples, such as water column nutrients, are also collected.

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8. REFERENCES

APHA 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed., American Public Health Association, Washington, DC.

Aristi I, Clapcott JE, Acuña V, Elosegi A, Mills H, Wood SA, Young R 2016. Forestry influences the abundance of *Phormidium*-dominated biofilms and functioning of a New Zealand river ecosystem. Freshwater and Marine Research In press.

Baker VR, Ritter DF 1975. Competence of rivers to transport coarse bedload material. Bulletin of the Geological Society of America. 86:975-978.

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 1995. The contribution of flood disturbance, catchment geology and land use to the habitat template of periphyton in stream ecosystems. Freshwater Biology 33:419-438.

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

Biggs BJF, Kilroy C 2000. Stream Periphyton Monitoring Manual. Prepared for the New Zealand Ministry for the Environment. 246 pages.

Booker DJ 2013. Spatial and temporal patterns in the frequency of events exceeding three times the median flow (FRE3) across New Zealand. Journal of Hydrology (New Zealand) 52:15-39.

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.

Burns N, Bryers G, Bowman E 2000. Protocol for monitoring trophic levels of New Zealand lakes and reservoirs. Lakes Consulting Client Report: 99/2. 122 p.

Carneiro RL, Alípio ACN, Bisch PM, de Oliveira Azevedo SMF, Pacheco ABF 2011. The inhibitory effect of calcium on *Cylindrospermopsis raciborskii* (cyanobacteria) metabolism. Brazilian Journal of Microbiology 42:1547-1559.

De'ath G. 2007. Boosted trees for ecological modeling and prediction. Ecology 88:243-251.

Elith J, Leathwick J 2014. Boosted Regression Trees for ecological modeling. Vignette for R dismo package version 1.0-5. http://cran.r-project.org/web/packages/dismo/.

Elith J, Leathwick JR, Hastie T 2008. A working guide to boosted regression trees. Journal of Animal Ecology 77:802-813.

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.

Giberson DJ, Caissie D 1988. Stream habitat hydraulics: inter-annual variability in three reaches of Catamaran Brook, New Brunswick. Canadian Journal of Fisheries and Aquatic Sciences 55:485-494.

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.

Hastie T, Tibshirani R 1990. Generalized additive models. Chapman & Hall, London.

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.

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

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 MH, Wood SA, Young RG, Ryan KG 2016. The role of nitrogen and phosphorous in regulating *Phormidium* (cyanobacteria) growth and anatoxin production. FEMS Microbiology Ecology. http://dx.doi.org/10.1093/femsec/fiw021

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.

Landcare Research 2013. Land cover database (v3). https://lris.scinfo.org.nz/layer/404-land-cover-database-lcdb-v33-change/

Leathwick JR, Elith J, Francis MP, Hastie T, Taylor P. 2006. Variation in demersal fish species richness in the oceans surrounding New Zealand: an analysis using boosted regression trees. Marine Ecology Progress Series 321:267-281.

Leathwick J, Snelder T, Chadderton W, Elith J, Julian K, Ferrier D 2011. Use of generalised dissimilarity modelling to improve the biological discrimination of river and stream classifications. Freshwater Biology 56:21-38.

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

McAllister T, Wood SA, Hawes 2016. The rise of toxic benthic *Phormidium* proliferations: a review of their taxonomy, distribution, toxin content and factors regulating prevalence and increased severity. Harmful Algae. 55:282–294

McAllister T, Wood SA, Atalah J, Hawes I (In review). Spatiotemporal dynamics of *Phormidium* accrual and anatoxin concentrations in eight New Zealand rivers with contrasting nutrient and flow regimes. Science of the Total Environment.

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.

Moriasi DN, Arnold JG, Van Liew MW, Bingner RL, Harmel RD, Veith TL 2007. Model evaluation guidelines for systematic quantification of accuracy in watershed simulations. Transactions of the ASABE 50(3): 885-900.

Nash JE, Sutcliffe JV 1970. River flow forecasting through conceptual models part I-A discussion of principles. Journal of hydrology 10(3): 282-290.

Parker DL, Kumar HD, Rai LC, Singh JB 1997. Potassium salts inhibit growth of the cyanobacteria *Microcystis* spp. in pond water and defined media: Implications for control of microcystin-producing aquatic blooms. Applied and Environmental Microbiology 63:2324-2329.

Piñeiro G, Perelman S, Guerschman JP, Paruelo JM 2008. How to evaluate models: observed vs. predicted or predicted vs. observed? Ecological Modelling 216: 316-322.

R Core Team 2014. R: A language and environment for statistical computing. Vienna, Austria. URL http://www.R-project.org/.

Ridgeway G 2013. gbm: Generalized Boosted Regression Models. R package version 2.1. http://cran.r-project.org/web/packages/gbm/.

Rier ST, Kinek KC, Hay SE, Francoeur SN 2016. Polyphosphate plays a vital role in the phosphorus dynamics of stream periphyton. Freshwater Science 35:490-502.

Rigby RA, Stasinopoulos DM 2005. Generalized additive models for location, scale and shape. Journal of the Royal Statistical Society: Series C. 54:507-554.

Sand-Jensen K 1983. Physical and chemical parameters regulating growth of periphytic communities. Pages 63–71 in RG Wetzel (editor). Proceedings of the first International workshop on periphyton of freshwater ecosystems. Junk Publishers, Hague

Schneider SC 2015. Greener rivers in a changing climate? Effects of climate and hydrological regime on benthic algal assemblages in pristine streams. Limnologica 55:21-32.

Seale DB, Boraas ME, Warren GJ. 1987. Effects of sodium and phosphate on growth of cyanobacteria. Water Research 21:625-631.

Snelder TH, Biggs BJF 2002. Multiscale River Environment Classification for water resources management. Journal of the American Water Resources Association 38: 1225-1239.

Snelder TH, Booker DJ, Quinn JM, Kilroy C. 2014. Predicting periphyton cover frequency distributions across New Zealand's rivers. JAWRA Journal of the American Water Resources Association 50: 111-127.

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

Wagenhoff A, Liess A, Pastor A, Clapcott JE, Goodwin EO, Young RG 2017. Thresholds in ecosystem structural and functional responses to agricultural stressors can inform limit setting in streams. Freshwater Science 36: 178-194.

Wood SA, Atalah J, Wagenhoff A, Brown L, Doehring K, Young RG, Hawes I. 2016. Effect of river flow, temperature, and water chemistry on proliferations of the benthic anatoxin-producing cyanobacterium *Phormidium*. Freshwater Science. 36: 63-76.

Wood SA, Bridge B 2014. Preliminary analysis of *Phormidium* abundance in the Maitai River and recommendation for on-going monitoring. Prepared for Nelson City Council. Cawthron Report No. 2537. 16 p.

Wood SA, Hawes I, McBride G, Truman P, Dietrich D 2015a. Advice to inform the development of a benthic cyanobacteria attribute. Prepared for Ministry for the Environment. Cawthron Report No. 2752. 91 p.

Wood SA, Wagenhoff A, Kelly D 2015b. *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, Depree C, Brown L, McAllister T, Hawes I. 2015c. Entrapped sediments as a source of phosphorus in epilithic cyanobacterial proliferations in low nutrient rivers. PloS one 10(10), e0141063.

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.

Wood SN 2006. Generalized Additive Models: An Introduction with R, Chapman and Hall/CRC.

Woods R, Elliott S, Shankar U, Bidwell V, Harris S, Wheeler D, Clothier B, Green S, Hewitt A, Gibb R, Parfitt R. 2006. The CLUES Project: Predicting the effects of land-use on water quality – Stage II. NIWA Client Report MAF05502.

Zuur AF, Ieno EN, Elphick CS 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3-14.

APPENDIX

Appendix 1. Sites visited during independent model validation.

Name	Council	Date	Predicted *Phormidium* (%)	Actual *Phormidium* (%)	Approx. width (m)	Approx. depth m	Substrate type/description	Description/notes
Waikaretaheke	Gisborne	26/12//2017	9	0	5-7	0.2-1.5	Medium cobbles	Some filamentous green and *Nostoc* present
Motu River	Gisborne	29/12/2016	33	1	5-10	0.05	Medium cobbles	Small mat on one rock in riffle. Site surrounded by farmland. Possibly some *Nostoc*.
Manganuku River	Gisborne	29/12/2016	0.2	0	7-10	0.4	Cobbles	Native bush catchment, water temp felt cold, thin black mat present on many cobbles
Pelorus River	Marlborough	27/12/2017	84	0	10	0.3	Cobbles and boulders	Substrate looks suitable for *Phormidium*, but none present. Have had small samples from this area before, but never reports of blooms,
Rai River	Marlborough	27/12/2017	54	0	10	0.3	Cobbles and boulders	Substrate looks suitable for Phormidium, but none present. Have had small samples from this area before, but never reports of blooms,
Mangatoa Stream	Northland	1/12/2016	64	1	-	-	Cobbles	Sample taken. Few mats of *Phormidium.* Dairy farming in catchment. Lot of green filamentous algae
Te Kao Stream	Northland	30/11/2016	59	0	-	-	Small cobbles	Small stream, few riffles, tanin stained.
Toronui Stream	Northland	1/12/2016	67	0	-	-	N/A	Tidal stream, low flow and estuarine habitat. Cape Reinga
Te Waitemaunga stream	Northland	1/12/2016	12	10	-	-	Cobbles	Thick *Phormidium* mats present.
Otiria	Northland	1/12/2016	17	1	-	-	Cobbles	Tanin stained stream, few riffles.
Otautau Stream	Southland	9/12/2016	76	0	3	0.3	Small cobbles	Slow flowing, tanin stained, lots of agriculture, no *Phormidium* present, seems unlikely that it would grow here.
Waimatuku Stream	Southland	9/12/2016	67	0	3	0.3	Embedded substrate, small cobbles.	Very tannin stained water, land-use is agriculture/sheep farming, grassed riparian, no *Phormidium* present, seems unlikely that it would grow here.

Name	Council	Date	Predicted *Phormidium* (%)	Actual *Phormidium* (%)	Approx. width (m)	Approx. depth m	Substrate type/description	Description/notes
Alton Burn	Southland	9/12/2016	75	0	1-2	0.2-0.5	Soft substrate	Slow flowing water, tannin stained, quite a lot of green algae, lots of macroinverts, surrounds by farmland, no *Phormidium* present, seems unlikely that it would grow here.
Aparima River	Southland	9/12/2016	85	0	20-30	0.5+	Gravel	Large cobble-bed river, wide open, no *Phormidium* present but it looks like it would grow here during stable periods perhaps lower flows.
Wairaki River	Southland	9/12/2016	4	3	10	0.2-0.5	Small, medium and large cobbles	*Phormidium* on large boulders. Fast flowing, quite open, no tannin staining.
Oreti	Southland	9/12/2016	54	0	20-30	0.5+	Gravel	Large cobble-bed river, wide open, no *Phormidium* present but it looks like it would grow here during stable periods perhaps lower flows.
Buller River	Tasman	19/02/2017	99	3	20	1	Large cobbles and boulders	River very fast flowing, lots of didymo, *Phormidium o*nly a few select rocks, also some filamentous green algae. Cyanobacteria were also present at base of didymo mats.
Moutere River	Tasman	15/01/2017	55	30	3-5	0.2	Small to medium cobbles	Lots of sediment and filamentous green algae. *Phormidium* only in riffles – not in slow flowing areas upstream
Pigeon Creek	Tasman	29/12/2017	52	0	1-2	0.1	Small to medium cobbles	Very little algae, looks like it could get *Phormidium* if conditions were right. Relatively stable substrate – mixture of shade and open. Catchment in farm and forestry. Flows into Wai-iti River at an area where *Phormidium* blooms are known to occur.
Stanley Brook	Tasman	30/01/2017	76	1	1-2	0.1	Small to medium cobbles	Small-medium patches of *Phormidium* on some cobbles in riffles. *Phormidium* under bridge looks very different – much darker green in colour.
Wairoa River Right Branch	Tasman	2/01/2017	64	2	10	0.3	Large cobbles and bedrock	Reasonable patches of *Phormidium* on bed rock – have been up this river quite bit and never seen blooms.
Dove River	Tasman	15/01/2017	45	70-80	7	0.1-0.2	Small to medium cobbles	Extensive bloom especially in riffles.

Name	Council	Date	Predicted *Phormidium* (%)	Actual *Phormidium* (%)	Approx. width (m)	Approx. depth m	Substrate type/description	Description/notes
Abbots Creek	Wellington	17/01/2017	56	2	1.5-2	0.2	Large cobbles and boulders	Fast flowing with some deep pools in places. Lots of bank vegetation but most of the channel is very open. Land use is predominantly bush – native in upper catchment. Some filamentous greens present. Likely a good spot for *Phormidium* blooms under more favourable weather conditions.
Haast River	West Coast	18-Jan-17	100	0	680	2.5 est.	Gravel and sand	Farmed on left bank for 10 km U/S of bridge. No obvious farming on right bank.
Karangarua River	West Coast	18-Jan-17	100	0	115	3.0	Gravel and sand	Sheep on right bank near bridge but probably no farming U/S. Farms located D/S of bridge.
Mahitahi River	West Coast	18-Jan-17	99	0	115	1.5	Gravel and sand	Confluence with Flagstaff Creek on right bank just U/S of bridge. Farmed up this side river. Farming on left bank of main river but only for about 1 km U/S.
Maori River	West Coast	12-Jan-17	87	0	19	2.5	Silt? Steep banks so bed not visible	Flow uniform across rectangular channel. Flow controlled by swamp and small lake U/S and may not vary much. Area has not been recently farmed. Water is tannin stained.
Ohinetamatea River (Saltwater Creek)	West Coast	18-Jan-17	90	0	42	1.5	Coarse gravel	No farming near bridge. Topo map shows no farms U/S. Farming in area to north of bridge but does not extend any distance U/S.
Omotumotu Creek	West Coast	21-Jan-17	89	0	12	1.0	Silt. Steep banks so bed not visible	Very slow flow in rectangular channel. Tannin stained water. Channel 2 km U/S was similar. Also similar at rail bridge about 60 m D/S. No significant farming U/S with pine trees weeds and regenerating bush.
Poerua River	West Coast	12 and 21-Jan-17	100	0	111	2.5	Gravel and sand. Access to bed cut-off by rip-rap on right bank and scour channel on left bank.	In flood with river bank to bank on 12-Jan. Flood had subsided at time of second inspection on 21-Jan. Farmed near bridge on both banks. Flows through bush at approx 4 km U/S.
Waitaha River	West Coast	21-Jan-17	100	0	132	2	Gravel and sand	No farming near bridge. Topo map shows farms 3 km U/S on right bank.

Name	Council	Date	Predicted *Phormidium* (%)	Actual *Phormidium* (%)	Approx. width (m)	Approx. depth m	Substrate type/description	Description/notes
Waitangitaona River. East braid.	West Coast	19-Jan-17	83	0	10	1.0	Gravel and sand. Weed growing in bed.	Farmed U/S and D/s on right bank. Only a small braid of channel at inspection point. Main channel about 50 m to west. Not easy to cross braid to get to main channel.
Waitangitaona River. Main channel.	West Coast	19-Jan-17	83	0	25	2.0	Gravel and sand. Not much of bed showing. Steep silty banks at bridge.	Farmed U/S and D/s on right bank. Channel is not braided at road bridge. Much larger volume than in east braid. Inspection point is at bridge 550 m from intersection of Whataroa Flat Road and Waitangitaona Road.
Wanganui River	West Coast	19-Jan-17	99	0	500	2.5	River was in flood with water almost bank to bank but gravel beds visible on right bank.	Farming commences 3 km U/S on left bank.
Wanganui River. SH6 Bridge	West Coast	21-Jan-17		0	40	1.5	Gravel and sand	Bridge is 18 km U/S of nominated inspection point. Farming near bridge on right bank but this does not appear to extend far U/S