



Advice on a Monitoring Programme to Assess the Ecological Effects of *Phormidium* on Macroinvertebrate Communities



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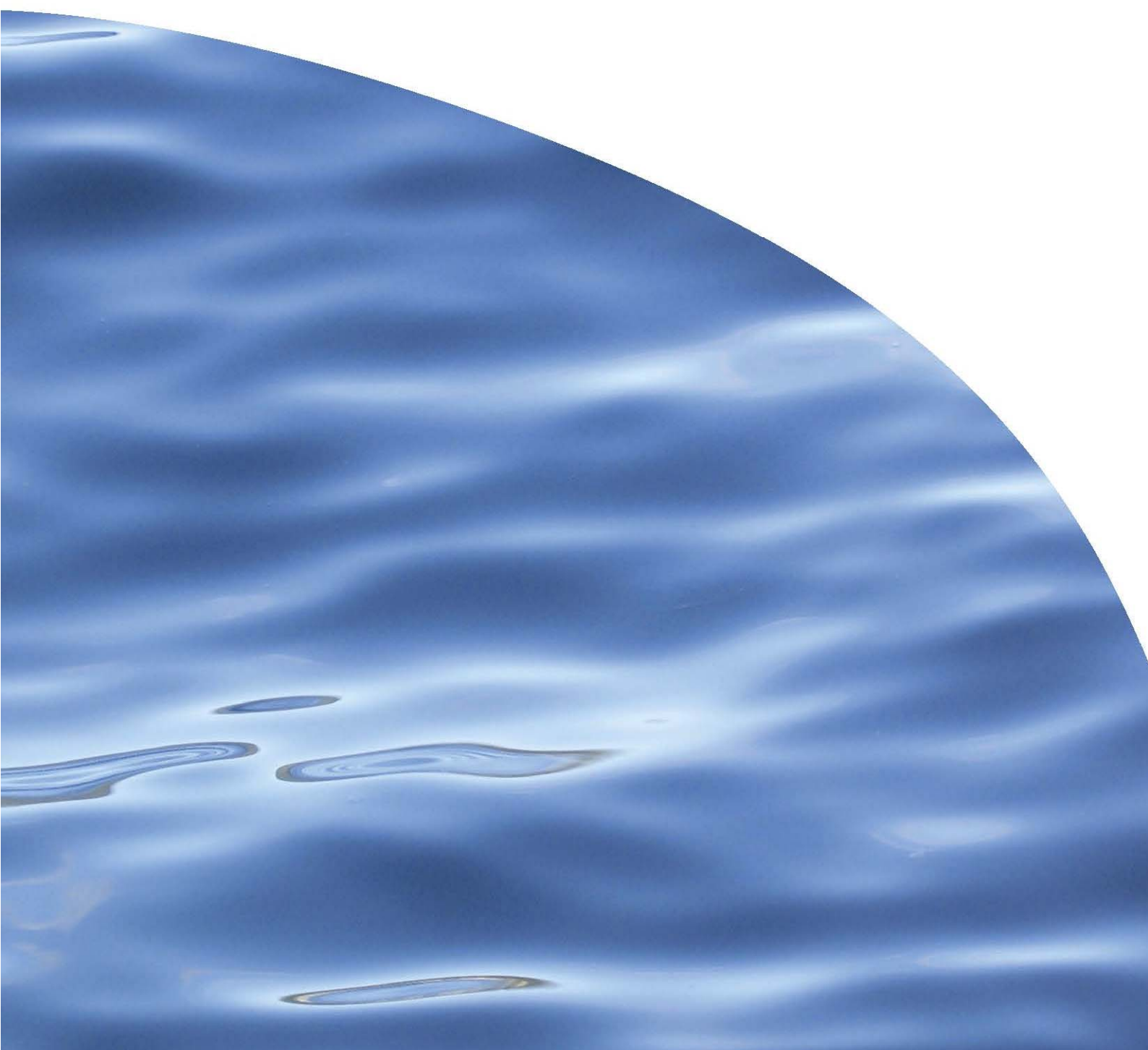
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REPORT NO. 2624

**ADVICE ON A MONITORING PROGRAMME TO
ASSESS THE ECOLOGICAL EFFECTS OF
PHORMIDIUM ON MACROINVERTEBRATE
COMMUNITIES**



ADVICE ON A MONITORING PROGRAMME TO ASSESS THE ECOLOGICAL EFFECTS OF *PHORMIDIUM* ON MACROINVERTEBRATE COMMUNITIES

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EXECUTIVE SUMMARY

Under favourable conditions, *Phormidium* forms expansive black / brown leathery mats that can cover kilometres of river substrate. *Phormidium* produces powerful neuromuscular blocking toxins, which pose a threat to humans and animals. However, the impact of these toxins and *Phormidium* mats on aquatic communities is largely unknown. The aim of this project was to determine the best methodology to investigate how percentage cover of *Phormidium* and toxins impacts macroinvertebrate communities in New Zealand rivers.

The specific objectives of the project were to:

1. Develop a sample design and methodology to determine the effect of *Phormidium* on macroinvertebrate communities.
2. Identify the number of macroinvertebrate samples required to provide scientifically and statistically robust data.
3. Recommend appropriate statistical analyses for assessing the biological data.
4. Identify a selection of potential sampling sites specific to the Horizons Regional Council (Horizons) region and New Zealand-wide.

Statistical analysis was undertaken on a pilot study dataset collected in 2011 from four rivers to assist in answering objectives 1 to 3. The key findings of the analysis were:

- Twelve Surber samples (0.1 m² area, 0.5 mm mesh; six with and six without *Phormidium*) appear adequate to provide a representative sample at the riffle scale.
- The ecological response to *Phormidium* appears site dependent. We found that metrics which summarise macroinvertebrate communities are likely to be the most informative response variables. The most sensitive metrics include; total density (no./m²), Quantitative Macroinvertebrate Community Index (QMCI) score, percentage Ephemeroptera, Plecoptera, Trichoptera (% EPT) abundance, and percentage chironomid abundance.
- A weighted riffle-scale average (based on the percentage cover of *Phormidium* at each site) could be used to examine the relationship between percentage *Phormidium* cover and macroinvertebrate metrics among sites. This approach would allow sample replicates at each site to be pooled, markedly reducing laboratory analysis costs. The riffle-scale data could be analysed using general linear models. A non-linear modelling approach could be selected if appropriate. Potential thresholds in responses curve would be identified using change-point analysis.

Based on statistical assessment and a literature review we recommend that samples are collected from a minimum of 20 and preferably more than 30 sites. Details of sample design and methodology are provided in the report, but in brief we propose the following sampling approach for each site:

1. A whole site assessment of periphyton / *Phormidium* coverage and substrate composition.
2. The collection of 12 Surber macroinvertebrate samples (six taken where *Phormidium* is present and six where *Phormidium* is absent).
3. From each Surber, where *Phormidium* is present, three periphyton samples should be collected and pooled for chlorophyll-a and ash-free dry mass analysis (six samples per site).
4. Ten samples be collected and pooled for toxin analysis.

Potential sampling sites specific to the Horizons region and New Zealand-wide are provided in the report.

TABLE OF CONTENTS

1. INTRODUCTION.....	1
2. SAMPLING DESIGN AND METHODOLOGY	3
2.1. Sample design considerations.....	3
2.2. Sampling methodology.....	4
2.2.1. <i>Periphyton / cyanobacterial coverage and substrate composition</i>	4
2.2.2. <i>Macroinvertebrate and algal biomass (chlorophyll-a and ash-free dry mass)</i>	6
2.2.3. <i>Samples for toxin analysis</i>	8
2.2.4. <i>Samples for microscopic identification of Phormidium</i>	9
3. MACROINVERTEBRATE SAMPLE NUMBERS REQUIRED.....	10
3.1. Analysis of <i>Phormidium</i> / invertebrate data from a pilot study.....	12
3.1.1. <i>Treatment effect — replication at a patch scale</i>	12
3.1.2. <i>Relationships between percent Phormidium cover and community metrics</i>	15
3.1.3. <i>Relationships between toxin concentrations and community metrics</i>	17
3.1.4. <i>Power analysis to determine sample numbers</i>	18
3.1.5. <i>Community composition</i>	20
4. STATISTICAL ANALYSES FOR ASSESSING BIOLOGICAL DATA.....	22
4.1. Macroinvertebrates.....	22
4.2. Periphyton biomass and toxin analysis	23
5. SAMPLE AND PRELIMINARY DATA ANALYSIS.....	24
5.1. Laboratory analysis	24
5.2. Preliminary data analysis — macroinvertebrates	24
6. COST ESTIMATES FOR LABORATORY ANALYSES.....	26
7. POTENTIAL SAMPLING SITES	27
8. REFERENCES.....	29
9. APPENDICES	32

LIST OF FIGURES

Figure 1.	Schematic of set up for undertaking periphyton coverage assessment assuming an 11 m stretch of riffle.	5
Figure 2.	Schematic of set up for undertaking macroinvertebrate sampling.....	6
Figure 3.	Relative precision of five assemblage metrics, calculated as coefficients of variation.....	11
Figure 4.	Boxplots of invertebrate metrics from different <i>Phormidium</i> treatments grouped by site.	14
Figure 5.	Significant linear relationships between percentage <i>Phormidium</i> cover and invertebrate metrics at; A) the patch scale, and B) the riffle-scale.	16
Figure 6.	A) Significant linear relationships between <i>Phormidium</i> toxins and invertebrate metrics at the patch scale, and B) Scatterplots with correlation coefficients at the riffle-scale.	18
Figure 7.	Multi-dimensional scaling ordination of macroinvertebrate community composition in the pilot study.	20
Figure 8.	Cluster analysis of invertebrate community composition in the pilot study.	21

LIST OF TABLES

Table 1.	Recommended field equipment and consumables.....	8
Table 2.	Percentage <i>Phormidium</i> cover and mean invertebrate community metrics from pilot study sites.	13
Table 3.	Nested analysis of variance to test for differences between <i>Phormidium</i> treatments using data from pilot study.	13
Table 4.	Number of Surber samples required to detect significant <i>Phormidium</i> effect at the patch scale for statistical power .80 at the .05 level.	19
Table 5.	Interpretation of Macroinvertebrate Community Index and Quantitative Macroinvertebrate Community Index values from stony riffle streams.....	25
Table 6.	Cost of Cawthron laboratory analyses assuming all 12 samples are analysed separately.....	26
Table 7.	Cost of Cawthron laboratory analyses – assuming all 12 samples are pooled.	26

LIST OF APPENDICES

Appendix 1.	Random number table to select macroinvertebrate sampling sites within lanes.....	32
Appendix 2.	Excerpt taken from Stark <i>et al.</i> (2000) discussing protocol C3 – collection of quantitative sample from a hard-bottomed substrate using a Surber sampler.	33

1. INTRODUCTION

Since 2005 there has been an apparent increase in the distribution and prevalence of the mat-forming benthic cyanobacteria *Phormidium* in some New Zealand rivers. Under favourable conditions, *Phormidium* can form expansive black / brown leathery mats that may cover the entire substrate and stretch for many tens of kilometres throughout a river.

Phormidium produces powerful neuromuscular blocking toxins, collectively known as anatoxins. These toxins pose a threat to humans and animals when consumed or when there is contact with contaminated water. Anatoxins have killed approximately 100 dogs in New Zealand in the last 10 years and resulted in health warnings against any contact with the water having been posted along the banks of many rivers.

To date most *Phormidium* research and monitoring efforts have focused on assessing its distribution, collecting samples for toxin analysis, and exploring physico-chemical drivers of bloom formation (e.g. Heath *et al.* 2011, 2014; Wood & Young 2011, 2012; Wood *et al.* 2014; Wood & Bridge 2014). Recent studies also indicate high toxicity of aqueous *Phormidium* extracts to the mayfly *Deleatidium spp.* (Wood & Bridge, unpublished data). However, the impacts of anatoxins and *Phormidium* mats on aquatic communities are largely unknown.

Macroinvertebrate¹ communities, can be influenced by changes in periphyton² biomass. Macroinvertebrate communities shift from predominately large drift-prone taxa such as mayflies and stoneflies when algal biomass is low, to small and / or non-drifting taxa such a midge larvae (chironomids) and snails when algal biomass is high (Quinn & Hickey 1990; Braccia *et al.* 2014). This change is not desirable for drift-feeding fish, or insectivorous river birds that rely on the availability of large macroinvertebrate prey to grow. Some macroinvertebrates feed directly on periphyton, and periphyton mats can provide a habitat for these organisms to live amongst. It is likely that changes in the coverage and biomass of *Phormidium* mats will affect macroinvertebrate community composition and density.

Horizons Regional Council (Horizons) are interested in investigating how percentage cover of *Phormidium* impacts macroinvertebrate communities, and to use this as a measure of the effect of *Phormidium* on ecological health nationwide.

¹ Macroinvertebrates are defined in this report as invertebrates > 0.5 mm in length.

² Periphyton refers to algae, cyanobacteria, heterotrophic microbes, and detritus that are attached to submerged surfaces in most aquatic ecosystems.

The objectives of this project are to:

1. develop a sample design and methodology to determine the effect of *Phormidium* on macroinvertebrate communities.
2. identify the number of macroinvertebrate and *Phormidium* biomass and toxin samples, required to provide scientifically and statistically robust data.
3. recommend appropriate statistical analyses for assessing the biological data.
4. identify a selection of potential sampling sites specific to the Horizons region and New Zealand wide.

Horizons has set aside budget within the 2014 / 2015 financial year to undertake the monitoring as recommended by this study. They are also working with other regional councils to see if the recommendations can be adopted at a broader scale to obtain a robust national dataset.

2. SAMPLING DESIGN AND METHODOLOGY

The rationale for the approach and selection of number of samples and sites used in the below study design and methodology is given in Sections 3 to 6.

2.1. Sample design considerations

In developing a sample design for this project, thought was given to potential biases that could occur in employing different sampling strategies. It was concluded that a **stratified random sampling design** approach would avoid edge effect bias that may occur if a purely random sampling design was employed, *i.e.* the margins of a river often contain more algae and macroinvertebrates than the middle of a river due to velocity and depth gradients across the river channel.

Samples should be collected from riffle habitat as these are the areas that *Phormidium* mats coverage is highest (Heath *et al.* 2011) and often where macroinvertebrate diversity and densities are highest (Pridmore & Roper 1985).

Phormidium growth, and in fact periphyton in general, is most prevalent over the summer months when extended periods of low river flow are common. During summer low flows die off and subsequent decomposition of high biomasses of periphyton can contribute to low dissolved oxygen and pH levels in rivers. To increase our chances of encountering *Phormidium* and to avoid seasonal influences on algal and macroinvertebrates, we recommend that **sampling should be undertaken during January to March**.

Flow variation can cause large changes in macroinvertebrate community composition. For example, floods more than six times the preceding base flow or greater than three times the median flow normally are sufficient to slough off most algal mats and associated macroinvertebrates from riffle habitat (Biggs & Close 1989; Clausen & Biggs 1997). Large floods (*e.g.* greater than 10 times mean flow or 40% of mean annual maximum flow) will move a substantial portion of the river bed (Clausen & Plew 2004). Floods of these magnitudes will substantially depress macroinvertebrate communities and recovery (recolonisation of the riverbed) will be slow (in the order of months). Smaller floods and freshes (flushing flows) can flush fine sediment, periphyton and other aquatic vegetation. These flows are usually about three to six times the median flow (Biggs & Close 1989; Clausen & Biggs 1997). Smaller freshes may reduce macroinvertebrate abundance to a lesser extent, preferentially flushing taxa associated with algae (periphyton), and recovery is usually faster (in the order of weeks). To account for the confounding influence floods can have on periphyton and macroinvertebrate communities, **sampling should be undertaken at least 14 days after a flood event** capable of removing periphyton from the substrate (*i.e.* approximately three to six times the median flow).

2.2. Sampling methodology

At each site the following assessments need to be undertaken, and samples collected.

Whole site assessments

- Periphyton / cyanobacterial coverage
- Substrate composition

Samples to collect

- Twelve macroinvertebrate Surber (0.1 m² area, 0.5 mm mesh) samples, six taken where *Phormidium* is present and six where *Phormidium* is absent. These are then pooled to give two samples per site (one from Surbers with *Phormidium* and one from Surbers without *Phormidium*).
- Three pooled periphyton samples for chlorophyll-a / ash-free dry mass analysis per Surber. These are collected from only Surbers with *Phormidium*. There should be a total of six samples per site.
- Ten samples (pooled) collected for toxins at each site.
- Cyanobacterial samples for microscopic identification (only required if there is uncertainty about the identification of *Phormidium*).

Further detail on how to undertake the assessments and collection of the various samples are provided in the sections below.

2.2.1. Periphyton / cyanobacterial coverage and substrate composition

The New Zealand Guidelines for Managing Cyanobacteria in Recreational Fresh Waters (Ministry for the Environment and Ministry of Health 2009) provide guidance on performing a site-based assessment of *Phormidium* cover (as outlined below). This method results in a representative value for the given riffle being obtained, e.g. 0%–100% *Phormidium* cover.

Please note: We **strongly** recommend one person should make the measurements and a second record from the river edge to minimise disturbance in the riffle habitat for invertebrates.

1. Measure a 10 m to 20 m long stretch in a riffle. The length is dependant of the river. In a wide river a shorter length (*i.e.* 10 m) is likely to be sufficient to obtain enough area for sampling. Mark the corners using coloured rocks / flags.
2. Mark out four transects in the selected area, these should be evenly spaced (*e.g.* at 2 m, 4 m, 6 m and 8 m; Figure 1).
3. Starting at the downstream end, wade into the stream at right angles to the water's edge. Where possible go out to a depth of approximately 0.6 m. In shallow rivers

the transect may span the entire width. Wading into fast-flowing water can be dangerous and caution is advised.

4. Hold the underwater viewer about 20 cm under the water, more or less on the transect line. The area of view should not be one that has just been walked over. Holding the viewer steady and as vertical as possible, estimate to the nearest 5% of the proportion of the area you see that is occupied by *Phormidium* mats. At the same time estimate the percentage substrate composition. This should be visually assessed based on the following eight categories: vegetation; mud (< 0.2 mm); sand (0.2 mm–2 mm); fine gravel (2 mm–8 mm); gravel (8 mm–64 mm); cobble (64 mm–264 mm); boulder (> 264 mm) and bedrock.
5. If there is any doubt about the identity of mat cover (*i.e.* whether it is *Phormidium*) at any sampling point, take a sample for microscopic identification. Samples should be collected by scraping a 50 cent piece sized clump of mat into 15 mL Falcon tube. Add ca. 5 ml of river water to the tube and 3 to 5 drops Lugol's iodine. Store in the dark for later morphological analysis.
6. Space the underwater viewing points (five per transect) evenly along the transect to a minimum depth of 0.1 m–0.15 m nearest to the water's edge, although this depth will vary according to the type of river.
7. Move upstream to transects 2, 3 and 4, and repeat the above steps.
8. Calculate the average percentage cover per site from the 20 views.

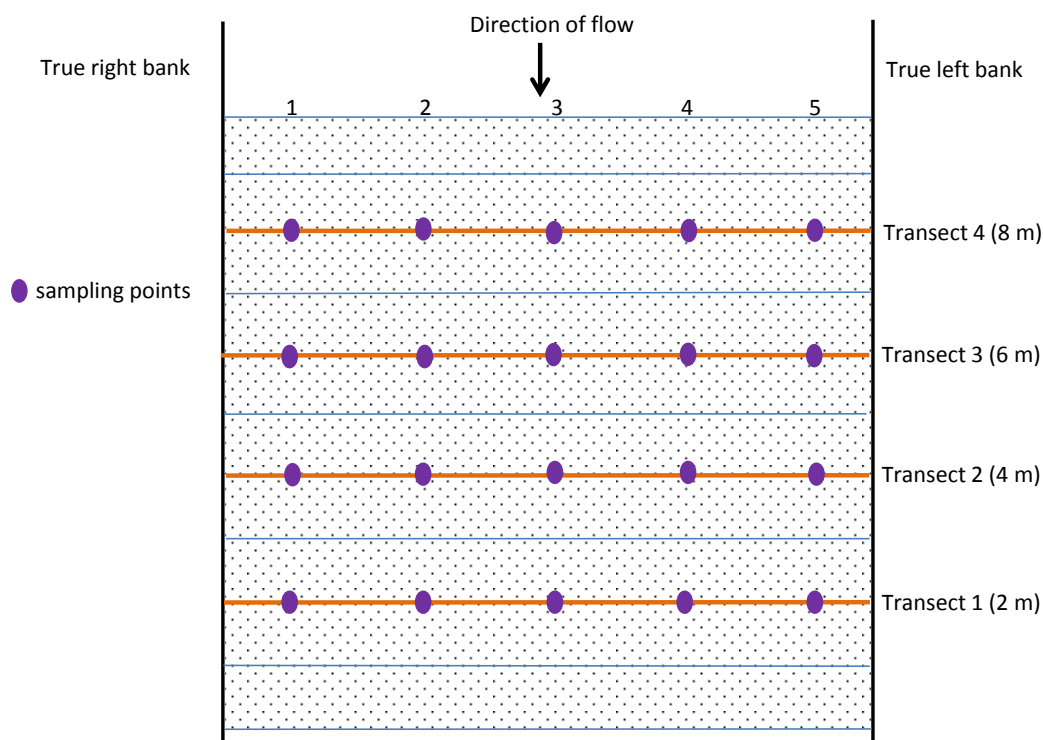


Figure 1. Schematic of set up for undertaking periphyton coverage assessment assuming an 11 m stretch of riffle.

2.2.2. Macroinvertebrate and algal biomass (chlorophyll-a and ash-free dry mass)

When collecting the macroinvertebrate samples it is important to keep in mind that there is a velocity gradient across the river (*i.e.* slow flowing water in the margins and fast in the middle of the channel). This may influence invertebrate community composition and is accounted for in the sampling protocol below.

1. Measure and record the total length of the riffle, and divide the riffle into six lanes (see Figure 2). Use the random number table in Appendix 1 to select six sample locations; one for each lane. Go to the first sample location. Move either **directly** upstream or downstream of this point until you find two areas based on the sampling area of a Surber sampler that; contains less than 5% *Phormidium* cover (= non-mat sample), and contains greater than 50% *Phormidium* cover (= mat sample). Where possible the mat and non-mat sample should have similar velocity and substrate type. The sample should be taken in water depths of ≤ 0.3 m (for safety purposes).

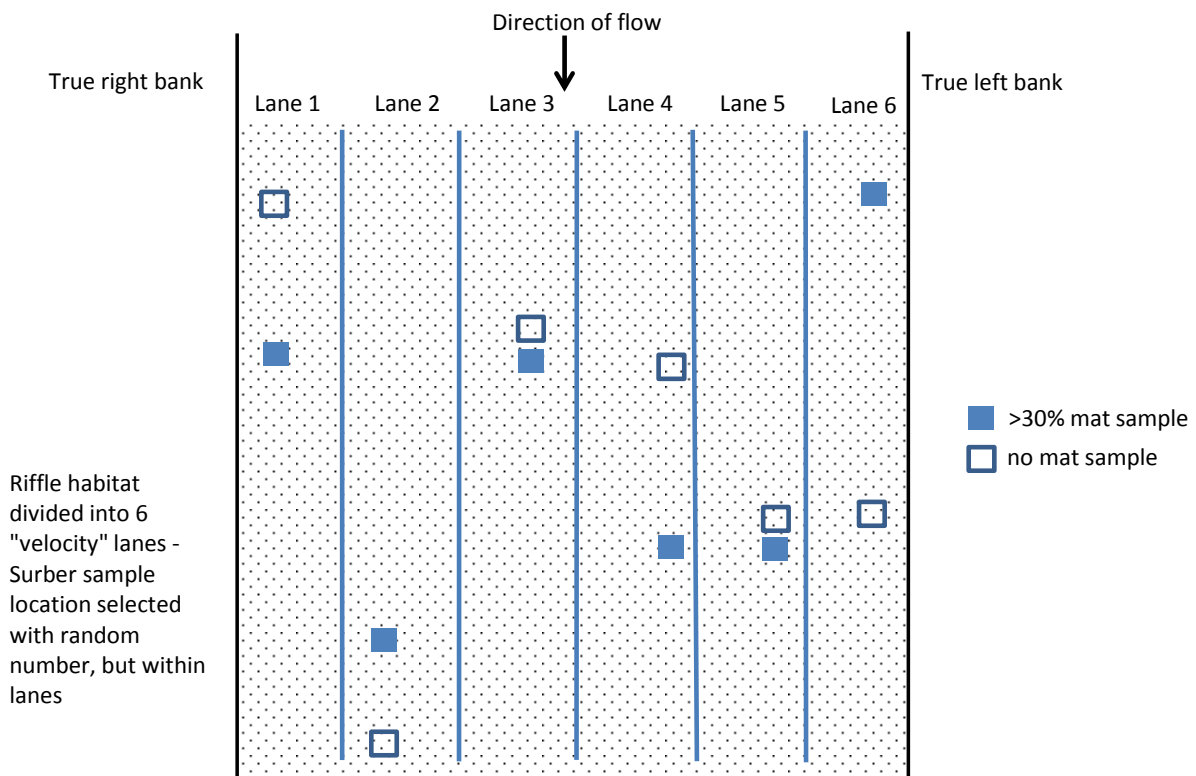


Figure 2. Schematic of set up for undertaking macroinvertebrate sampling. Note that the mat and non-mat samples are always taken either upstream or downstream of each other so they are within a similar velocity / depth / substrate combination. If sampling across the riffle is not possible the six pairs of sample should ideally be collected across a range of different velocities and depths from the margin of the river to as far out as is practical to sample.

2. Record depth and mean column velocity (taken at $0.6 \times$ depth from the water surface) readings at the centre point of where the Surber sample is to be taken.
3. In each sampling location, place a Surber sampler (0.1 m² area, 0.5 mm mesh) on the bottom substrate and record the percentage of *Phormidium* cover.
4. From the macroinvertebrate samples containing only >50% *Phormidium*, select three rocks with *Phormidium* mats and (with Surber sampler still in place), carefully remove them and place into a plastic tray. Take the tray containing rocks to the nearest rivers edge. **Do not** discard the rocks once the following protocol has been completed as they are still part of the macroinvertebrate sample. On each rock define **two** circles on the 'upper' surface by scribing around the mouth of a sampling 50 mL Falcon tube. Using the blunt end of a spatula or knife carefully remove the *Phormidium* from within one of the defined circle areas on each rock and combine in a 50 mL Falcon tube. This tube is for chlorophyll-a. Repeat for ash-free dry mass analysis. The two tubes should be wrapped in tin foil and stored in a cool, dark place, and then frozen (at -20°C) as soon as possible.
5. Ensure all tubes are carefully labelled with date, site and sample number / type.
6. The three rocks used for collection of *Phormidium* samples should be taken back to the person collecting the Surber samples so they can clean the rock surfaces and add any material to the macroinvertebrate sample.
7. While the *Phormidium* scrapings are being collected, the macroinvertebrate Surber samples can also be collected following Protocol C3 of Stark *et al.* (2001; Appendix 2). This method enables macroinvertebrate densities to be calculated.
8. Macroinvertebrate samples should be placed in ~600 mL pottles and preserved with 70% alcohol in the field. Ensure all pottles are carefully labelled on the outside with the date, site and sample number / type, and labelled on the inside of the pottle with the same information using pencil on waterproof paper. The label inside is important as sometimes the outside labelling may rub off in transit, or be accidentally washed off by preservative spillage.

Table 1 provides a list of recommended equipment and consumables that should be taken on each sampling trip. Consumable quantities will depend on the number of planned sampling trips.

Table 1. Recommended field equipment and consumables.

Equipment	Purpose
Tape measure (at least 30 m in length)	Measure out sampling area
Coloured flags / rocks	Mark corners of sampling area
Periphyton viewer	Assessment of <i>Phormidium</i> coverage / substrate size
Random number table (See Appendix 1)	Sampling site selection
Clipboard and field sheets	
Surber sampler (0.1 m ² , 0.5 mm mesh)	Sampling macroinvertebrates
Scrubbing brush, white tray, small hand-held sieve (0.5 mm mesh)	Aid in transfer of macroinvertebrates from net to pottle
Waders	
Field note book and pens/pencils, labels	
Marker pens	Labelling samples
Global positioning system (GPS)	Recording location of sample site (easting and northings or NZTM co-ordinates)
Scissors	
Current meter and wading rod	Measurement of depth and mean column velocity
Metal knife or blunt spatula	Collection of <i>Phormidium</i> samples for chlorophyll-a / ash-free dry mass / toxin analysis
Plastic tray	Sampling rocks with <i>Phormidium</i>
Chilly bin and frozen cooler pads	Sample storage
Consumables	Purpose
Ethanol or isopropyl alcohol (full strength)	Preserve samples in field (70% alcohol: 30% water in sample). Take approx. 420 mL per sample (assuming a full 600 mL pottle)
Plastic pottles (ca. 600 mL)	Storage of macroinvertebrate samples
Falcon Tubes (50 mL), 3 per sample, plus one for measuring area	Storage of chlorophyll-a and toxin samples
Tin foil	Protection of chlorophyll-a samples
Waterproof paper	Labels on inside of pottles containing macroinvertebrate samples
Falcon tube (15 mL), 1 per site	Sample for algal / cyanobacterial taxonomy (if needed)
Lugols	Preserving algal / cyanobacterial samples for taxonomy

2.2.3. Samples for toxin analysis

Randomly select 10 rocks that have not been disturbed during the sampling process. Remove a piece of *Phormidium* mat, about the size of a 20 cent coin, with a spatula / knife from each rock. Combine all 10 samples in a 50 mL Falcon tube. These should be stored in a cool, dark place then then frozen (at -20°C) as soon as possible.

2.2.4. Samples for microscopic identification of Phormidium

Homogenise the sample collected for toxin analysis by shaking. Take a sub-sample (ca. equivalent to 1 mL) and place in a 15 mL Falcon tube. Add approximately 10 mL of river water and 2–3 drops of Lugols until the solution is the colour of weak tea. Store in the dark and at room temperature. Excessive biomass will absorb Lugols, and it is best to check the samples after several days of storage and if necessary further Lugols can be added.

3. MACROINVERTEBRATE SAMPLE NUMBERS REQUIRED

Surber sampling gathers macroinvertebrate data at a patch scale and replicate Surber samples can be collected to provide a riffle-scale measure. However, macroinvertebrate communities vary at the patch scale (Heino *et al.* 2004), and the number of replicate Surber samples required to provide a representative sample at a riffle-scale, can be quite high. The number of replicates required depends on the nature of the response variable. In general, more samples are required to provide a reliable estimate of species density compared to community metrics.

Needham and Usinger (1956) systematically took 100 Surber samples in a 'uniform' riffle in a Californian stream and showed that 73 samples were required to estimate benthic standing crop with 95% confidence intervals within 40% of the mean. Chutter (1972) showed that a sample size of 448 was necessary to estimate numbers of invertebrates within 5% of the mean. Both of these studies showed that two to three samples were enough to collect the most common Ephemeroptera, Plecoptera, Trichoptera, and Diptera taxa. Similarly, Nelson and Scott (1962) demonstrated that 96% of the taxa recovered in 12 Surber samples were identified in the first four samples.

Knowing what the response variable is, and how much it varies, is important. For example, it is possible to predict the change in species richness with increasing sample size. Li and others (2001) demonstrated that multiple macroinvertebrate response metrics adhered to this tenant. Total density was the most variable metric; the coefficient of variation was greater than 50% with five samples, whereas taxon richness, percentage Ephemeroptera, Plecoptera, Trichoptera (%EPT)³, percent dominance and Shannon-Weiner diversity index had coefficients of less than 25% within five samples (Figure 3). Stark (1993) demonstrated that 10 or 11 Surber samples are required to estimate Quantitative Macroinvertebrate Community Index (QMCI)³ values to within $\pm 10\%$ of the combined 12-replicate value (pseudo population estimate); *i.e.* 5 samples pick up a 0.6 QMCI (8.2 MCI) difference whereas 10 samples will pick up a 0.42 difference (5.8 MCI).

³ The percentage of Ephemeroptera (mayflies), Plecoptera (stoneflies) Trichoptera (caddis flies) and MCI and QMCI are all biological indices commonly used to assess macroinvertebrate community health (see Boothroyd and Stark (2000) and Section 5.2).

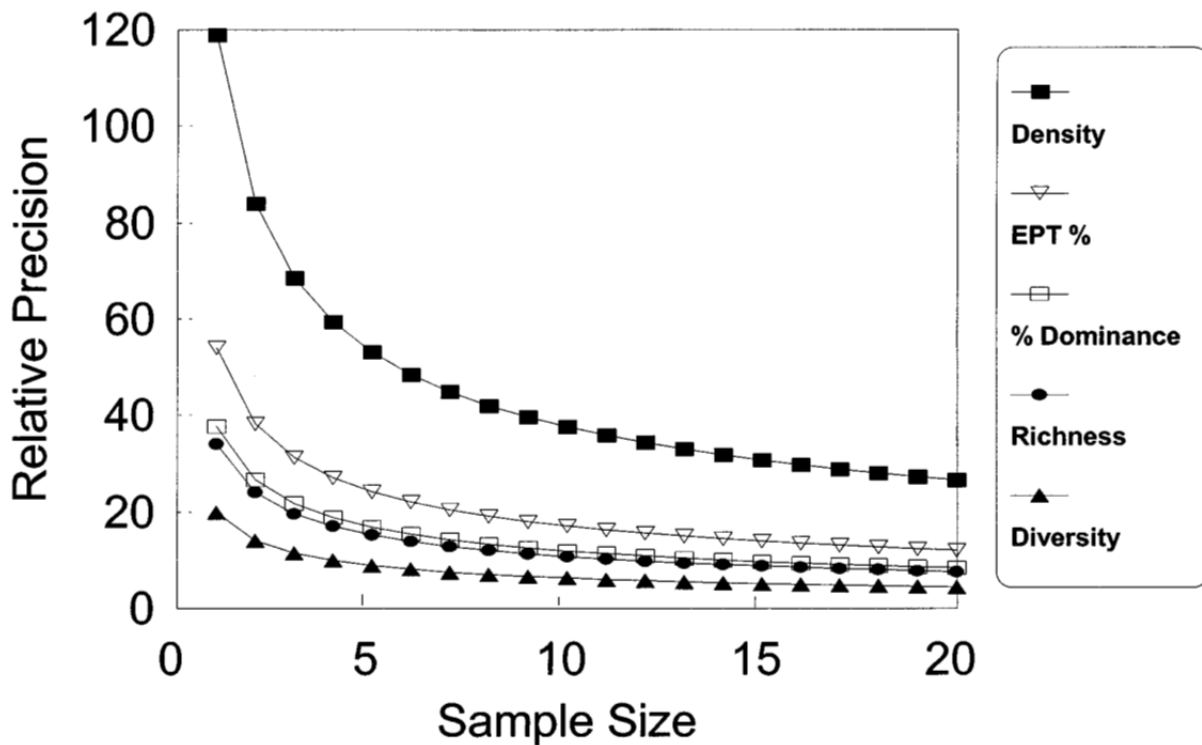


Figure 3. Relative precision (*i.e.* the degree to which assemblage metrics varied) of five assemblage metrics, calculated as coefficients of variation. From Li *et al.* (2001).

It is possible to hypothesise which invertebrate response metrics will be the most informative for assessing the effects of *Phormidium* based on previous studies of periphyton-macroinvertebrate relationships. For example, total macroinvertebrate and chironomid densities declined from 60% to 90% and invertebrate taxa richness declined between 90% and 98% due to shade limiting periphyton biomass (Quinn *et al.* 1997).

Total invertebrate numbers and density were observed to increase significantly with increasing algal biomass (Dudley *et al.* 1986). In this study, insects formed most of the community and were classified according to three categories of macroalgal effects on benthic densities.

1. Negatively affected by macroalgae due to competition for space.
2. Positively affected due to structural habitats created by algae.
3. Positively affected by both macroalgal structure and associated food resources.

In summary, benthic macroinvertebrate distributions are very patchy. More samples are required to gather robust data on species densities compared to community metrics. But invertebrate densities and relative species densities (*e.g.* EPT taxa) are likely to be informative measures of the effects of *Phormidium* at a patch-scale.

We explored two possible approaches:

1. Ensure sufficient replication occurs at the patch scale to provide a representative riffle-scale measure. This could be made logistically achievable by pooling patch-scale measures, although this would only allow for a riffle-scale comparison.
2. Use only community metrics.

These options are further examined below using data from a pilot study.

3.1. Analysis of *Phormidium* /invertebrate data from a pilot study

A pilot study was undertaken by Victoria University Students (supervised by Associate Professor Ken Ryan) and Cawthron Institute (Cawthron) in 2011. The study involved sampling four sites in four rivers; Ashley River (Christchurch), Maitai River (Nelson), Pukuratahi River (Wellington) and Hutt River (Wellington). At each site, the total *Phormidium* coverage was determined and 10 Surber samples (5 with, and 5 without *Phormidium* coverage) were collected.

3.1.1. Treatment effect — replication at a patch scale

The differences in invertebrate communities, found in patches with or without *Phormidium* present at the four river sites, were investigated using the data from the pilot study (Table 2). Analysis of variance (ANOVA) was used to test whether there was a difference in community metrics between treatments nested within river sites (Table 3). Density was *log*-transformed prior to analysis to meet assumptions of normality.

The ANOVA showed a significant difference between sites, with differences between treatment significant for only taxa richness, density, QMCI score and %EPT abundance (Table 3, Figure 4). Contrastingly, there was no difference between treatments for MCI score and %EPT taxa. High variance in treatment effects among sites was evident (Figure 4).

Table 2. Percentage *Phormidium* cover and mean invertebrate community metrics from pilot study sites. Treatment 1 = Surber containing high *Phormidium* cover, 2 = Surber with very low *Phormidium* cover. *N* = 5 Surbers per treatment. MCI = Macroinvertebrate Community Index, QMCI = Quantitative MCI, EPT = Ephemeroptera, Plecoptera, Trichoptera.

Site	Ashley		Pukuratahi		Hutt		Maitai	
	1	2	1	2	1	2	1	2
Ave. % <i>Phormidium</i> cover within Surber	46	1.8	45	1.6	63	3.6	89	1.6
Taxa richness	13.2	8.6	17.2	13.6	16	13	12.6	12.4
Density (no./m ²)	8,020	4,164	2,754	1,574	2,196	2,006	2,742	1,714
MCI sample score	112.7	107.4	129.0	133.0	123.5	116.1	98.5	97.8
QMCI sample score	4.7	5.4	5.9	7.5	6.9	7.6	3.3	3.9
EPT richness	8.8	5.6	11.2	9	10.2	8.8	6.4	5.6
% EPT _{Taxa}	66.0	64.9	64.8	64.7	63.3	68.6	50.8	44.4
% EPT _{Abundance}	78.4	95.6	53.9	89.6	75.7	93.6	34.3	27.0

Table 3. Nested analysis of variance to test for differences between *Phormidium* treatments using data from the pilot study. MCI = Macroinvertebrate Community Index, QMCI = Quantitative MCI, EPT = Ephemeroptera, Plecoptera, Trichoptera.

Metric	Source	Sum-of-squares	df	Mean-square	F-ratio	P
Taxa richness	Treatment	81.225	1	81.225	12.593	0.001
	Site (Treatment)	149.150	6	24.858	3.854	0.005
	Error	206.400	32	6.450		
LogDensity	Treatment	2.287	1	2.287	24.524	0.000
	Site (Treatment)	8.161	6	1.360	14.588	0.000
	Error	2.984	32	0.093		
MCI	Treatment	55.554	1	55.554	0.498	0.485
	Site (Treatment)	6051.896	6	1008.649	9.050	0.000
	Error	3566.618	32	111.457		
QMCI	Treatment	8.510	1	8.510	88.564	0.000
	Site (Treatment)	81.667	6	13.611	141.648	0.000
	Error	82.983	32	2.371		
% EPT _{Taxa}	Treatment	2.839	1	2.839	0.039	0.845
	Site (Treatment)	2557.299	6	426.217	5.860	0.000
	Error	2327.525	32	72.735		
% EPT _{Abundance}	Treatment	2515.268	1	2515.268	42.273	0.000
	Site (Treatment)	22802.748	6	3800.458	63.873	0.000
	Error	1904.010	32	59.500		

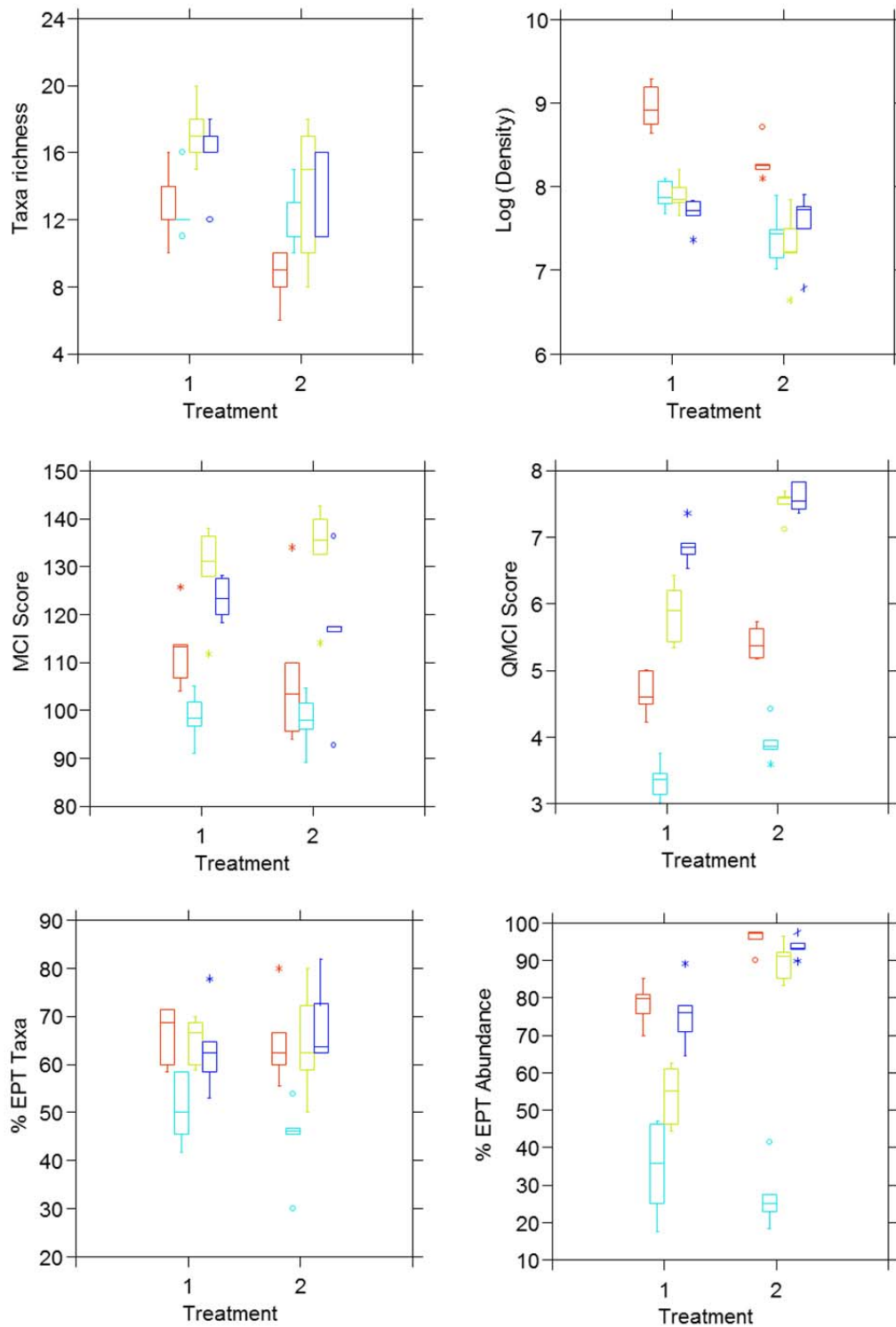


Figure 4. Boxplots of invertebrate metrics from different *Phormidium* treatments (1 = Surber containing high *Phormidium* cover, 2 = Surber with very low *Phormidium* cover) grouped by site. Red = Ashley River, light blue = Maitai River, green = Pukuratahi River and dark blue = Hutt River. MCI = Macroinvertebrate Community Index, QMCI = Quantitative MCI, EPT = Ephemeroptera, Plecoptera, Trichoptera.

3.1.2. Relationships between percent *Phormidium* cover and community metrics

The *Phormidium* treatments in the pilot study were characterised by a gradient of percentage cover ranging from 45% to 99% at different sites, whereas the no *Phormidium* treatment was characterised by less than 5% (Table 2). We explored whether there was a linear relationship between %cover and invertebrate metrics at a patch scale using replicates from all sites. There was a significant positive relationship with increasing *Phormidium* cover and increasing taxa richness ($R^2 = 0.106$, $P = 0.041$), and a significant negative relationship with decreasing QMCI score ($R^2 = 0.154$, $P = 0.012$), and % EPT abundance ($R^2 = 0.156$, $P = 0.012$). High variability within and among sites appears to be a factor weakening these relationships (Figure 5A).

We then weighted the average patch values per treatment by percentage cover of *Phormidium* at the riffle-scale to estimate a riffle-scale invertebrate metric response and explored whether there were any linear relationships. With only four sites, limited inference can be made. However, there was a significant negative relationship with increasing *Phormidium* cover and % EPT taxa ($R^2 = 0.905$, $P = 0.049$) and % EPT abundance ($R^2 = 0.953$, $P = 0.024$), and a significant positive relationship with % chironomid abundance ($R^2 = 0.969$, $P = 0.016$). Maitai River was the only site with greater than 50% *Phormidium* cover at the riffle-scale, and this drives the observed relationships (Figure 5B).

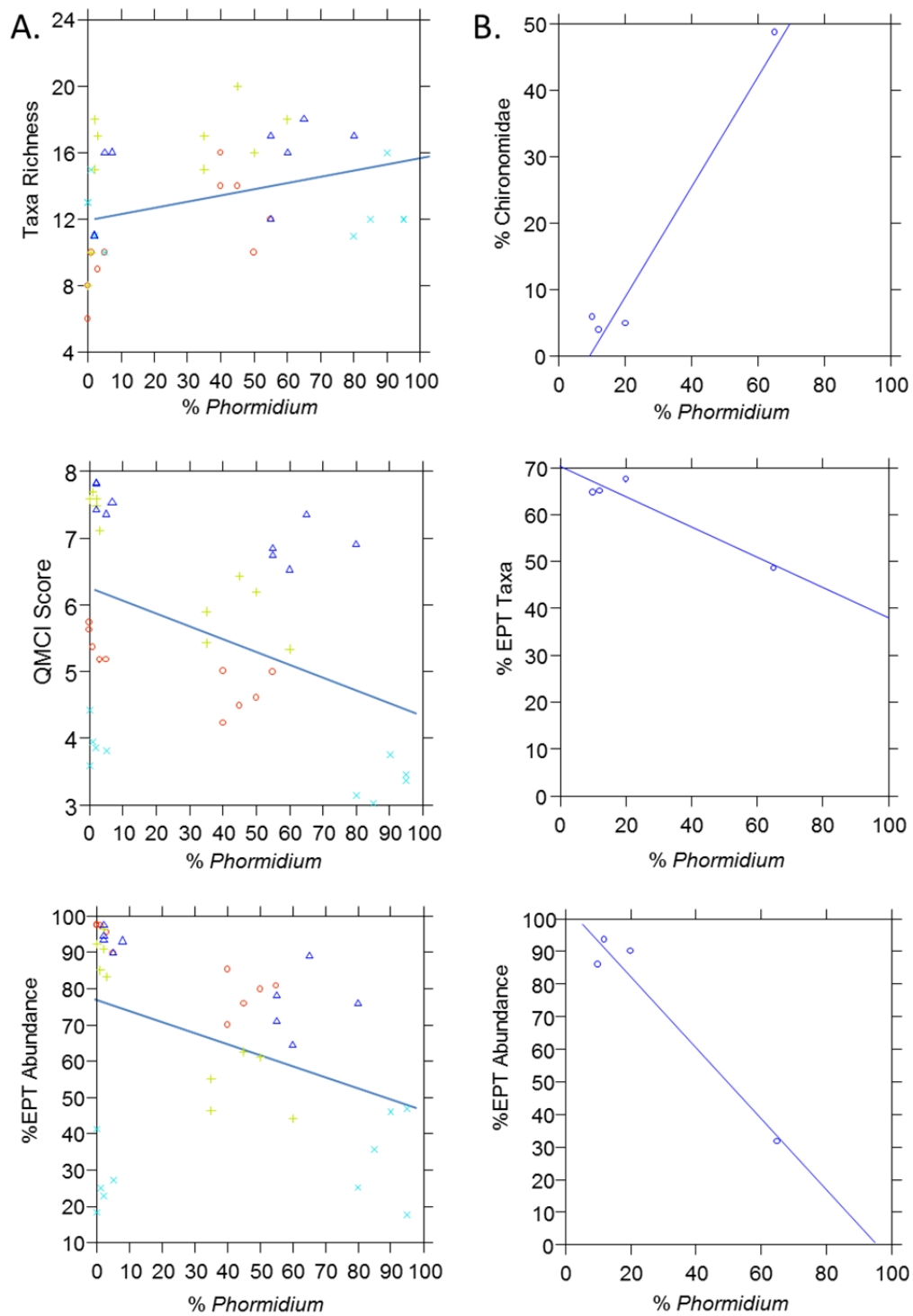


Figure 5. Significant linear relationships between percentage *Phormidium* cover and invertebrate metrics at; A) the patch scale (Ashley River [○], Maitai River [x], Pukuratahi River [+], Hutt River [▲]), and B) the riffle-scale. MCI = macroinvertebrate community index, QMCI = quantitative MCI, EPT = Ephemeroptera, Plecoptera, Trichoptera.

3.1.3. Relationships between toxin concentrations and community metrics

Recent studies have shown a high toxicity of aqueous *Phormidium* extracts to the mayfly *Deleatidium* spp. (Wood & Bridge, unpublished data). When *Phormidium* is healthy, most toxins are contained within their cells. As cells die and lyse (which will continually occur in well-developed mats) these highly water soluble toxins are released into the water. Some macroinvertebrates may also be exposed to the toxins if they feed directly on the mats. In the pilot study the concentrations of toxins in *Phormidium* mats ranged from 17 µg/kg to 582 µg/kg. We log transformed values to meet the assumptions of normality and investigated whether there were any relationships between log-toxin concentration and invertebrate community metrics.

Across all sites, there was a significant negative relationship with increasing toxin concentration and density ($R^2 = 0.277$, $P = 0.012$), and the relative abundance of *Aoteapsyche* ($R^2 = 0.275$, $P = 0.031$). As with *Phormidium* cover, high variability within and among sites appears to be a factor weakening these relationships (Figure 6A). There were no other significant linear relationships, neither was toxin concentration associated with *Phormidium* cover in the pilot study data. Likewise, there were no significant linear correlations between toxin concentration and invertebrate metrics at the riffle-scale, although correlation coefficients were indicative of potential relationships (e.g. Figure 6B).

In the pilot study the mats at all sites surveyed contained toxins. It would be valuable to include sites that have non-toxic mats. It is not easy to predict whether mats will contain toxins and toxin concentrations can change rapidly (Wood & Young 2012). By increasing the number of sites surveyed it is likely that sites with no toxin will be included, and this would enable a more in-depth assessment of the impact of toxins to be investigated.

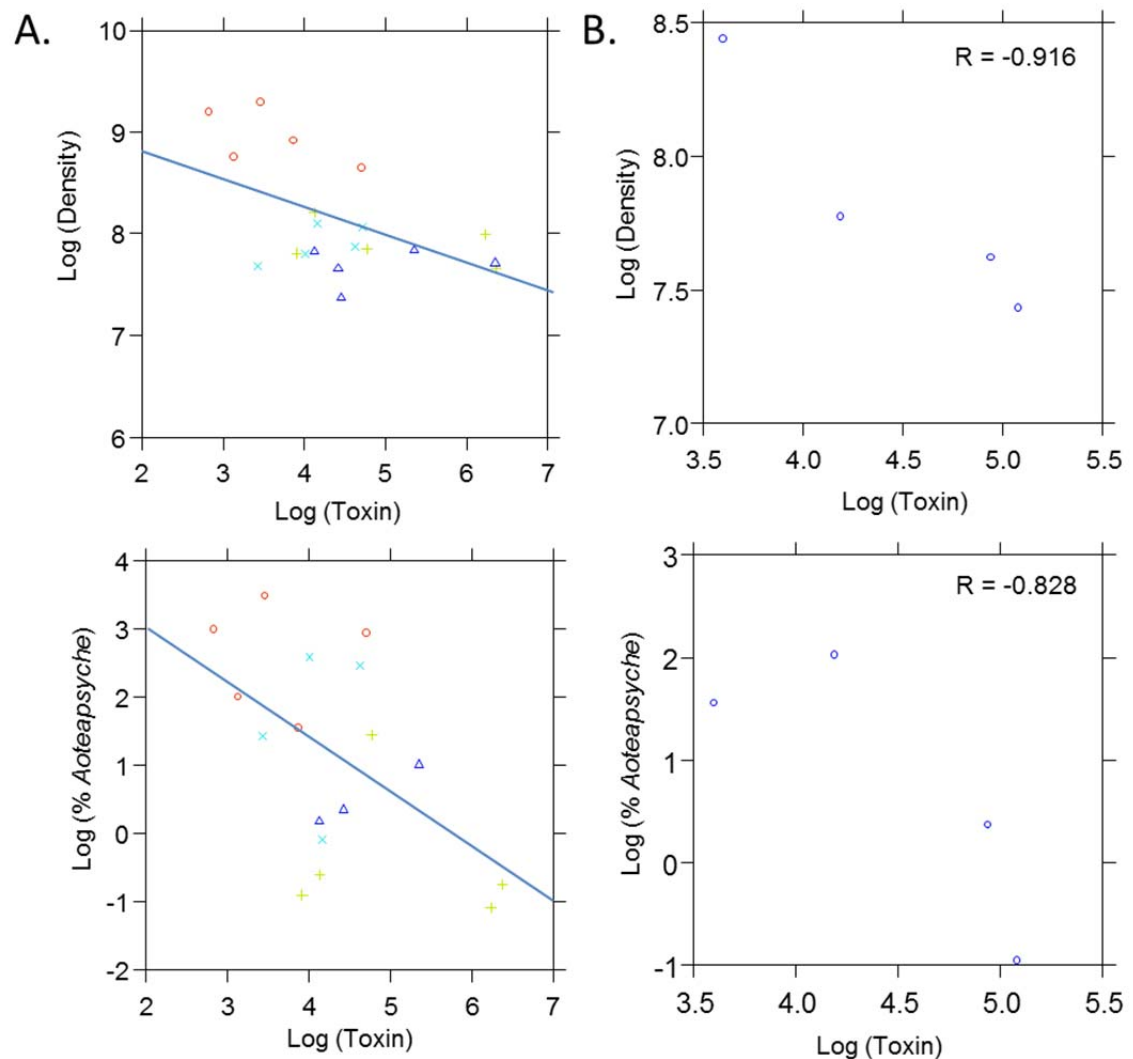


Figure 6. A) Significant linear relationships between *Phormidium* toxins ($\mu\text{g}/\text{kg}$) and invertebrate metrics at the patch scale, and B) Scatterplots with correlation coefficients at the riffle-scale (Ashley River [○], Maitai River [x], Pukuratahi River [+], Hutt River [▲]).

3.1.4. Power analysis to determine sample numbers

The pilot study data was used to investigate how many samples are required per site to statistically differentiate invertebrate metric responses to the *Phormidium* treatment. We also calculated how many samples would be required to determine the response of net-spinning caddis (*Aoteapsyche*) and midge fly larvae (*Chironomidae*) to *Phormidium*. These taxa were included in our analysis as they are strongly associated with higher algal biomass. In other words, we would expect to see these indicator taxa become numerically dominant as algal biomass increases (while the inverse should occur for EPT taxa). Treatment means were examined for all sites, and for each site.

Macroinvertebrate density, QMCI score, %EPT abundance and chironomid density and relative abundance were the most sensitive response variables globally (combining

data from all sites) and treatment effects can be reliably detected using as few as six Surber samples (Table 4). Among the sites the effects of *Phormidium* clearly vary suggesting that the *Phormidium* effect is dependent on other factors, potentially including; environmental setting, water quality and velocity, or other stressors.

Table 4. Number of Surber samples required to detect significant *Phormidium* effect at the patch scale for statistical power .80 at the .05 level. *Phormidium* cover treatment is the difference in average % cover between patches with and without *Phormidium*. MCI = Macroinvertebrate Community Index, QMCI = quantitative MCI, EPT = Ephemeroptera, Plecoptera, Trichoptera.

Site	All rivers				
	combined	Ashley	Pukuratahi	Hutt	Maitai
<i>Phormidium</i> cover treatment (%)	59	42	43	60	87
Taxa richness	13	5	14	12	1,420
Density (no./m ²)	6	5	6	130	6
MCI sample score	265	85	116	30	1010
QMCI sample score	4	5	3	5	6
EPT richness	20	7	29	30	57
%EPT _{Taxa}	3,870	800	>10,000	46	27
%EPT _{Abundance}	5	3	3	4	36
Chironomidae density (no./m ²)	4	3	3	4	4
%chironomidae abundance	4	3	3	4	11
<i>Aoteapsyche</i> density (no./m ²)	8	6	14	240	497
% <i>Aoteapsyche</i> abundance	30	6	24	108	19

We also examined how many samples would be needed to identify significant linear relationships at the riffle-scale. Based on the correlation coefficients between macroinvertebrate metrics (macroinvertebrate density, QMCI, % EPT abundance and chironomid density) and %*Phormidium* cover as few as 10 sites would be needed. Similarly, the correlation coefficients between macroinvertebrate metrics (macroinvertebrate density, %*Aoteapsyche* abundance) and toxin concentration suggested as few as five sites would be needed.

However, so few sites is generally insufficient to identify non-linear relationships, e.g. thresholds of change. As with multiple linear analysis, the general rule of thumb for change point analysis is 10 sites per slope (cf. 10 sites per predictor variable; Harrell 2001). Therefore, 20 sites is the minimum number of sites required to detect a single change point. A recent study showed that 30 sites were sufficient to show a threshold response of benthic invertebrate communities to deposited sediment in agricultural streams (Burdon *et al.* 2013).

3.1.5. Community composition

Using the plot study data the relative composition of invertebrate communities at differing treatments and sites was investigated using multivariate analysis. A Bray-Curtis resemblance matrix based on taxa abundance data standardised by total sample numbers was used in a PERMANOVA (permutational multivariate ANOVA) equivalent of the previous ANOVA. Results showed no significant effect of treatment but a significant difference among sites. Multi-dimensional scaling (MDS) analysis further illustrated the community similarity among treatments and sites (Figure 7). A Cluster analysis also demonstrated as little as 40% similarity among sites. Treatments were not differentiated by a Cluster analysis; however, examination of site-specific treatment response seems warranted (Figure 8).

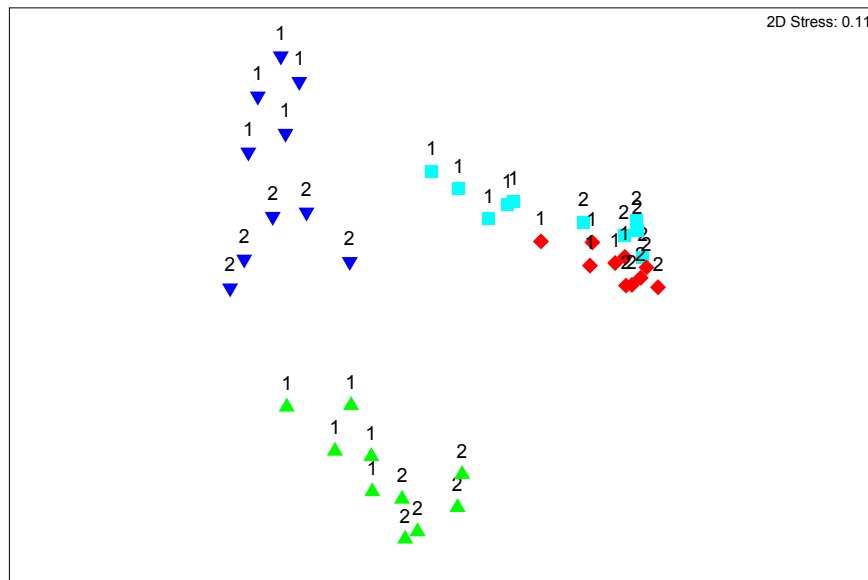


Figure 7. Multi-dimensional scaling ordination of macroinvertebrate community composition in the pilot study. 1 = Surber samples containing high *Phormidium* cover, 2 = Surber samples with very low *Phormidium* cover. Ashley River ▲, Maitai River ▼, Pukuratahi River ■, Hutt River ◆.

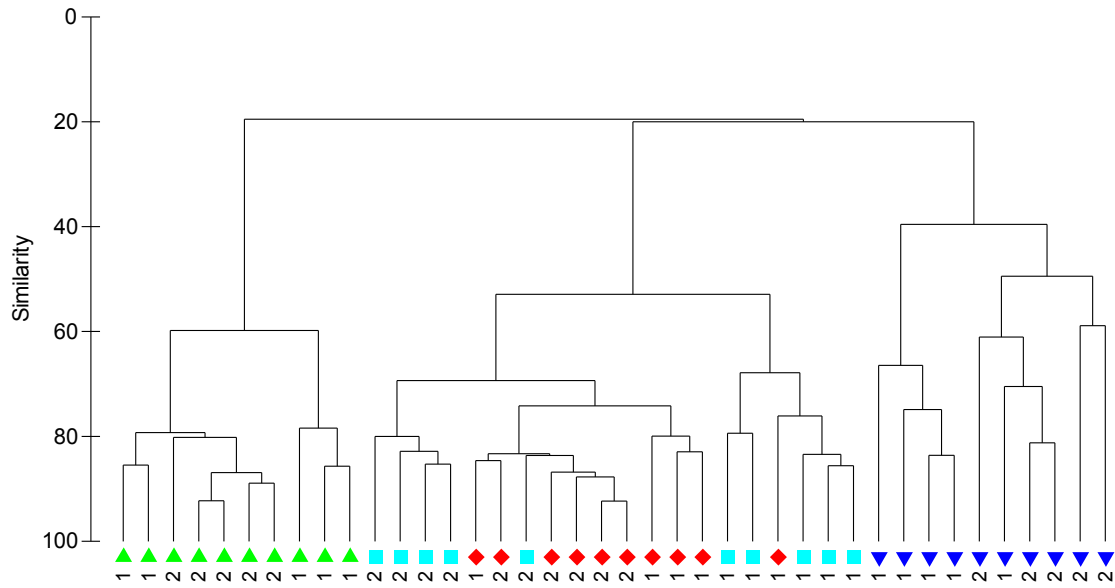


Figure 8. Cluster analysis of invertebrate community composition in the pilot study. 1 = Surber samples containing high *Phormidium* cover, 2 = Surber samples with very low *Phormidium* cover. Ashley River ▲, Maitai River ▼, Pukuratahi River ■, Hutt River ◆.

4. STATISTICAL ANALYSES FOR ASSESSING BIOLOGICAL DATA

4.1. Macroinvertebrates

In summary, the results from Section 2 indicated that:

- The ecological response to *Phormidium* appears site-dependent.
- Metrics that summarise macroinvertebrate communities are likely to be the most informative response variables to assess the effect of *Phormidium*.
- Metrics that appear sensitive to *Phormidium* include total density (no./m²), QMCI score, % EPT abundance, and %chironomid abundance.
- Species densities are likely to be too variable to assess robustly.
- Six Surber (0.1 m² area, 0.5 mm mesh) samples with *Phormidium*, and six without, appear adequate to provide a representative sample at the riffle-scale.
- A riffle-scale average (based on the percentage cover of *Phormidium* at each site) could be used to examine the relationship between percentage *Phormidium* cover and macroinvertebrate metrics among sites. This approach allows sample replicates at each site to be pooled, markedly reducing laboratory analysis costs.
- Based on a review of recent literature, we recommend a minimum of 20 sites (but preferably more than 30 sites) are surveyed.

The assessment of the ecological effects of *Phormidium* on macroinvertebrate communities can be approached at two levels.

1. Patch-scale treatment effect
2. Weighted riffle-scale effect

A patch-scale assessment provides a sensitivity analysis and demonstrates how sensitive in-stream fauna are to *Phormidium*. However, as our pilot analysis shows, the effect of size can be strongly dependent on site-specific factors. Patch-scale effects can be analysed using two-way analysis of variance in a nested design with treatment (fixed factor), site nested within treatment (random factor) and patches as the residual.

A weighted riffle-scale assessment is most appropriate for exploring broad spatial patterns in effects. This would also be most useful for identifying any potential thresholds in responses that could be further used to inform management benchmarks. Another advantage of this method is the six Surbers with, and the six Surbers without *Phormidium* at each site, could be combined into two samples for analysis. This would markedly reduce analysis cost (see Section 3.4). Riffle-scale effect would probably be best analysed using general linear models. However, there may be non-linear

responses (e.g. exponential decay, asymptotic exponential decay, or a four parameter sigmoidal curve) and the most appropriate model would be selected using an information-theoretic approach (Burnham & Anderson 2002). Potential thresholds in the response curve would be identified using change point analysis, e.g. Burdon *et al.* 2013.

Further examination of significant metric responses could be examined using analysis of community composition. This could identify the species responsible for effects observed at the patch or riffle-scale.

4.2. Periphyton biomass and toxin analysis

Periphyton (including *Phormidium*) accrual rate in a river will depend on a number of factors such as time since the last disturbance (e.g. flood), nutrient availability, and daylight hours (Biggs 2000). We recommend below that sampling be undertaken at least 14 days after a flood event to allow time for periphyton mats to establish. However, it is likely that the accrual rate of *Phormidium* mats, and therefore biomass, will differ between rivers, and this may need to be accounted for in the invertebrate / *Phormidium* relationship. Therefore samples for chlorophyll-a and ash-free dry weight should be taken to determine quantitative differences in algal biomass.

We did not have any preliminary data to determine the number of periphyton samples that should be collected; however, Biggs and Kilroy (2000) provide some guidance. They recommend that 10 replicate samples be collected in cases where it has not been possible to collect preliminary samples and / or there is doubt over transferability of data from another site. In the protocol below, we recommend three periphyton samples should be taken per Surber sample, which are then pooled. These only need to be taken from the Surber with *Phormidium*. Therefore at each site a total of six samples (each a composite three sub-samples) are collected. Although this is less than suggested by Biggs and Kilroy (2000), because of the within Surber sampling and pooling, we believe this will be representative of the *Phormidium* biomass at each site.

Recent studies indicate high toxicity of aqueous *Phormidium* extracts to the mayfly *Deleatidium* (Wood & Bridge, unpublished data). Anatoxins have also been shown to affect the fertility and survival of other aquatic animals (e.g. rotifers [Gilbert, 1996a, b] and toads [Rogers *et al.* 2005]). Anatoxins may add other synergistic effects (in addition to just biomass and coverage) on macroinvertebrates and we recommend that this is also assessed in the proposed study. Wood *et al.* (2010) sampled seven rivers and showed spatial variability in anatoxin concentration within 10 m × 10 m grids. Based on these data they recommend that at least 10 *Phormidium* samples be taken per site to collect a sample representative of toxin concentration at a riffle-scale. To reduce costs these samples could then be pooled and a single analysis undertaken to obtain an average toxin concentration at each site.

5. SAMPLE AND PRELIMINARY DATA ANALYSIS

5.1. Laboratory analysis

In the laboratory macroinvertebrate samples would be processed according to protocol P3 from Stark *et al.* (2001). Protocol P3 is a processing methodology where a full count of all animals in the samples is conducted with an option to sub-sample.

Macroinvertebrates should be identified to the lowest possible taxonomic level, sorted into 3 mm body length classes using a 3 mm x 3 mm grid attached to the baseplate of the microscope, then counted and recorded.

The pooled *Phormidium* mat sample from each site, collected for toxin analysis, should be freeze-dried and homogenised. Freeze-dried material (100 mg) would be suspended in 10 mL of Milli-Q water (MQ) containing 0.1 % formic acid and sonicated on ice for 20 minutes. Samples should then be centrifuged ($3,000 \times g$, 10 minutes) and the supernatants analysed for anatoxin-a (ATX), homoanatoxin-a (HTX), dihydroATX and dihydroHTX, using liquid chromatography-mass spectrometry as described in Heath *et al.* (2010).

Periphyton samples would be analysed for chlorophyll-a and ash-free dry mass following the methods of Biggs and Kilroy (2000).

5.2. Preliminary data analysis — macroinvertebrates

The following calculations would be made from the macroinvertebrate results: taxonomic richness, EPT taxonomic richness, densities, EPT densities and the following biotic indices: Macroinvertebrate Community Index (MCI), Quantitative Macroinvertebrate Community Index (QMCI) and %EPT. Percent EPT would be calculated as a proportion of the total taxa present in the samples ($\%EPT_{\text{Taxa}}$) and similarly for density ($\%EPT_{\text{Density}}$).

Macroinvertebrate biomass (mg/m^2) should also be calculated using dry weights (mg) for each taxon based on length:dry weight relationships from the literature (Sample *et al.* 1993; Towers *et al.* 1994). Overall macroinvertebrate biomass (mg/m^2) per sample should be calculated by summing density \times mean dry weight of the 3 mm size classes. Mean dry weight is a weighted average of all the taxa in each size class.

Macroinvertebrate Community Index and QMCI values are calculated according to the method of Stark (1985, 1993). These biotic indices, which were developed for assessing enrichment in stony streams and rivers, rely on prior allocation of scores (between 1 and 10) to macroinvertebrate taxa (usually genera) based upon their tolerance to pollution or fine sediment. Taxa that are characteristic of unpolluted

conditions and / or coarse stony substrates score more highly than taxa that may be found predominantly in polluted conditions or amongst fine organic sediments.

For each sample the scores will be summed (for each taxon present) and then divided by the number of scoring taxa and multiplied by 20 (a scaling factor) to give the MCI value. In theory, MCI values can range between 200 (when all taxa score 10 points each) and 0 (when no taxa are present). However, it is rare to find MCI values greater than 150 and only extremely polluted, sandy / muddy sites or extremely disturbed substrate sites score under 50.

Quantitative MCI values range from 0 to 10. Unlike the MCI, which is based on only the presence or absence of taxa, the QMCI includes percentage community composition to weight the overall index value towards the scores of the dominant taxa.

The interpretation of index values when applied to stony streams throughout New Zealand is given in Table 5.

Table 5. Interpretation of Macroinvertebrate Community Index (MCI) and Quantitative Macroinvertebrate Community Index (QMCI) values from stony riffle streams (adapted from Stark and Maxted 2007).

	MCI	QMCI
Excellent: Clean water	> 120	> 6
Good: Doubtful quality or possible mild pollution	100–120	5–6
Fair: Probable moderate pollution	80–100	4–5
Poor: Probable severe pollution	< 80	< 4

6. COST ESTIMATES FOR LABORATORY ANALYSES

The following tables (Tables 6 and 7) provide an overview of the cost estimates for each method of analyses (separately or pooled). Please note: these costing in Tables 6 and 7 are based on prices as at 1 July 2014 and will be subject to change.

Table 6. Cost of Cawthron laboratory analyses assuming all 12 samples (*i.e.* six non-mat and six mat samples) are analysed separately. Note: all prices are as at 1 July 2014 and GST exclusive.

Laboratory analysis	Per sample cost (\$)	Per site cost (\$)
Macroinvertebrate sample processing	280	3,360
Ash-free dry mass (6 sample per site)	31	186
Chlorophyll-a (6 sample per site)	63	378
Toxin analysis (10 samples pooled)	150	150
TOTAL COST (excl. GST)		4,074

Table 7. Cost of Cawthron laboratory analyses – assuming all 12 samples are pooled (*i.e.* 1 non-mat and 1 mat sample). Note: all prices are as at 1 July 2014 and GST exclusive.

Laboratory analysis	Per sample cost (\$)	Per site cost (\$)
Macroinvertebrate sample processing	555	1,110
Ash-free dry mass (6 sample per site)	31	186
Chlorophyll-a (6 sample per site)	63	378
Toxin analysis (10 samples pooled)	150	150
TOTAL COST (excl. GST)		1,824

7. POTENTIAL SAMPLING SITES

Using the riffle-scale approach suggested multiple sites within a river can be sampled. A single site can also be sampled multiple times, provided there has been sufficient time for the site to recover from the previous sampling. There is no requirement for multiple sites on a river to be sampled or a site to be sampled multiple times, but if this is necessary to obtain enough samples, the statistical analysis will not be affected.

Horizons region

The following sites have been selected as they have had a mean *Phormidium* coverage at each site of greater than 40% on multiple sampling occasions:

- Makakahi River at Hamua
- Manawatu River downstream of the Palmerston North City Council's wastewater treatment plant
- Mangatainoka River at State Highway Two (SH2)
- Oroua River downstream of the Feilding wastewater treatment plant
- Tokomaru River at Horseshoe Bend

The sites listed below are rivers that are known to have experienced cyanobacterial blooms (greater than 20% coverage) during the past 10 years. However, the respective regional councils should be contacted, as sites can change in their propensity to experience blooms. Blooms may also occur in rivers other than those listed.

Bay of Plenty region

- Whakatane River
- Waimana River
- Rangitaiki River

Hawkes Bay region

- Tukutuki River at Patangata
- Tukutuki River at Waipukurau

Wellington region

- Hutt River at Boulcott
- Waipoua River at Masterton Town
- Wainuiomata River
- Waikanae River near SH2

Nelson region

- Maitai River at Avon Terrace
- Maitai River at Campground
- Whakapuka River at Hira
- Whakapuka River at Māori Pa

Tasman region

- Waimea River at Appleby Bridge
- Motueka River at Woodstock

Canterbury region

- Hurunui River at SH1
- Opihi River at SH1
- Opihi River at Waipopo
- Pareora River at Huts
- Temuka River at Manse Bridge
- Ashley River at SH1

Southland region

- Waikia River
- Kuratua River

8. REFERENCES

- Biggs BJF 2000. New Zealand Periphyton Guideline: detecting, monitoring and managing enrichment of streams. Ministry for the Environment, Wellington. 122 p.
- 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 Ministry for the Environment. 226 p.
- Boothroyd I, Stark J 2000. Use of invertebrates in monitoring. In: Collier KJ, Winterbourn MJ ed. *New Zealand stream invertebrates: ecology and implications for management*. Caxton Press, Christchurch. Pp 344-373.
- Braccia A, Eggert SL, King N 2014. Macroinvertebrate colonization dynamics on artificial substrates along an algal resource gradient. *Hydrobiologia* 727: 1-18.
- Burdon FJ, McIntosh AR, Harding JS 2013. Habitat loss drives threshold response of benthic invertebrate communities to deposited sediment in agricultural streams. *Ecological Applications* 23: 1036-1047.
- Burnham KP, Anderson DR 2002. *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach*. Springer, Berlin.
- Chutter FM 1972. Reappraisal of Needham and Usinger's data on the variability of a stream fauna when sampled with a Surber sampler. *Limnology and Oceanography* 17: 139-141.
- Clausen B, Biggs BJF 1997. Relationships between benthic biota and hydrological indices in New Zealand streams. *Freshwater Biology* 38: 327-342.
- Clausen B, Plew D 2004. How high are bed-moving flows in New Zealand rivers? *Journal of Hydrology (NZ)* 43: 19-37.
- Dudley TL, Cooper SD, Hemphil N 1986. Effects of macroalgae on a stream invertebrate community. *Journal of the North American Benthological Society* 5: 93-106.
- Gilbert JJ 1996a. Effect of temperature on the response of planktonic rotifers to a toxic cyanobacterium. *Ecology* 77:1174-80.
- Gilbert JJ 1996b. Effect of food availability on the response of planktonic rotifers to a toxic strain of the cyanobacterium *Anabaena flos-aquae*. *Limnology and Oceanography* 41:1565-72.
- Harrell FE, Jr. 2001. *Regression modelling strategies: with applications to linear models, logistic regression and survival analysis*. Springer-Verlag, New York.
- Heath M, Wood SA, Ryan K 2010. Polyphasic assessment of fresh-water benthic mat forming cyanobacteria in New Zealand. *FEMS Microbiology Ecology*. 73: 95-109.
- Heath MW, Wood SA, Brasell K, Young R, Ryan KG 2014. Development of habitat suitability criteria and in-stream habitat assessment for the benthic cyanobacteria *Phormidium*. *Rivers Research and Applications*. DOI: 10.1002/rra.2722.

- Heath MW, Wood SA, Ryan KG 2011. Spatial and temporal variability in *Phormidium* and associated anatoxin-a and homoanatoxin-a production in two New Zealand rivers. *Aquatic Microbial Ecology* 64: 69-79.
- Heino J, Louhi P, Muotka T 2004. Identifying the scales of variability in stream macroinvertebrate abundance, functional composition and assemblage structure. *Freshwater Biology* 49: 1230-1239.
- Li J, Herlihy A, Gerth W, Kaufmann P, Gregory S, Urquhart S, Larsen DP 2001. Variability in stream macroinvertebrates at multiple spatial scales. *Freshwater Biology* 46: 87-97.
- 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.
- Needham PR, Usinger RL 1956. Variability in the macrofauna of a single riffle in Prosser Creek, California, as indicated by the Surber sampler. *Hilgardia* 14: 383-409.
- Nelson DJ, Scott DC 1962. Role of detritus in the productivity of a rock outcrop community in a piedmont stream. *Limnology and Oceanography* 7: 396-413.
- Pridmore RD, Roper DS 1985. Comparison of the macroinvertebrate faunas of runs and riffles in three New Zealand streams. *New Zealand Journal of Marine and Freshwater Research* 19: 283-291.
- Quinn JM, Cooper AB, Davies-Colley RJ, Rutherford JC, Williamson RB 1997. Land use effects on habitat, water quality, periphyton, and benthic invertebrates in Waikato, New Zealand, hill-country streams. *New Zealand Journal of Marine and Freshwater Research* 31: 579-597.
- Quinn JM, Hickey CW 1990. Characterisation and classification of benthic invertebrate communities in 88 New Zealand rivers in relation to environmental factors. *New Zealand Journal of Marine and Freshwater Research* 24: 387-409.
- Rogers EH, Hunter ES, Moser VC, Phillips PM, Herkovitz J, Muñoz L, Hall LL, Chernoff N 2005. Potential developmental toxicity of anatoxin-a, a cyanobacterial toxin. *Journal of Applied Toxicology* 25:527–234.
- Sample BE, Cooper RJ, Greer RD, Whitmore RC 1993. Estimation of insect biomass by length and width. *The American Midland Naturalist* 129: 234-240.
- Stark JD 1985. A macroinvertebrate community index of water quality for stony streams. *Water & Soil Miscellaneous Publication* 87. Wellington, Ministry of Works and Development. 53 p.
- Stark JD 1993. Performance of the Macroinvertebrate Community Index: effects of sampling method, sample replication, water depth, current velocity and substratum on index values. *New Zealand Journal of Marine and Freshwater Research* 27: 463-478.
- Stark JD, Boothroyd IKG, Harding JS, Maxted JR, Scarsbrook MR 2001. Protocols for sampling macroinvertebrates in wadeable streams. *New Zealand*

- Macroinvertebrate Working Group Report No. 1. Prepared for Ministry for the Environment. Sustainable Management Fund Project No. 5103. 57 p.
- Stark JD, Maxted JR 2007. A biotic index for New Zealand's soft-bottomed streams. *New Zealand Journal of Marine and Freshwater Research* 41: 43-61.
- Towers DJ, Henderson IM, Veltman CJ 1994. Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. *New Zealand Journal of Marine and Freshwater Research* 28: 159-166.
- Wood SA, Heath, MW, Kuhajek J, Ryan KG 2010. Fine-scale spatial variability in anatoxin-a and homoanatoxin-a concentrations in benthic cyanobacteria mats: implication for monitoring and management. *Journal of Applied Microbiology* 109: 2011-2018.
- Wood SA, Young R 2011. Benthic cyanobacteria and toxin production in the Manawatu-Wanganui Region. Prepared for Horizons Regional Council. Cawthron Report No. 1959. 34 p.
- Wood SA, Young RG 2012. Review of benthic cyanobacteria monitoring programme 2012. Prepared for Horizons Regional Council. Cawthron Report No. 2217. 42 p.
- 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, Depree C, Hawes I 2014. Investigating sediment as a source of phosphorus for *Phormidium* blooms. Prepared for Horizons Regional Council. Cawthron Report No. 2576. 33 p.
- 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.

9. APPENDICES

Appendix 1. Random number table to select macroinvertebrate sampling sites within lanes (see Figure 2). The numbers in the table represent a length in metres along a lane (*i.e.* the total length of the riffle being sampled). The table is based on the assumption that the maximum riffle length is 20 m. Ignore numbers greater than the total length measured (*e.g.* if the total riffle length is 13 m, ignore any random number ≥ 14). Note: these tables can be generated in Excel using the function =RANDBETWEEN(1,20).

7	11	12	2	11	12	7	12	16	11	6	9	2	4	20	14	1	12	4	10
18	9	18	10	10	3	5	4	5	10	6	9	1	19	1	14	17	18	4	18
6	3	13	3	2	18	13	9	10	18	15	16	18	18	18	3	2	5	4	13
7	5	10	13	12	4	3	5	7	11	16	3	8	8	19	17	1	9	16	13
13	1	12	19	2	5	15	13	9	20	7	3	2	18	18	1	7	10	10	9
5	4	10	5	18	10	18	2	5	7	13	8	17	8	14	10	13	16	3	4
8	19	16	8	2	1	3	6	14	17	13	1	2	13	6	18	16	17	7	16
11	17	3	9	1	11	5	15	9	15	19	1	11	9	12	19	12	3	14	16
10	11	16	11	15	6	8	14	11	12	6	18	18	7	4	18	18	13	13	17
4	11	15	2	19	20	5	14	20	15	19	6	7	2	4	2	18	16	7	8
5	7	4	20	14	18	6	2	14	1	3	9	14	16	20	2	15	18	10	6
1	17	7	5	8	11	11	11	19	7	6	10	2	4	12	20	6	10	18	1
1	14	1	20	13	12	18	16	3	3	13	1	16	16	11	17	18	16	6	17
1	20	16	6	14	1	3	3	3	13	11	18	3	4	4	10	6	1	4	4
19	8	3	12	15	12	1	12	18	16	4	1	14	1	17	11	1	5	18	8
16	16	7	9	3	6	19	5	3	8	8	18	4	8	2	19	19	14	7	13
17	14	9	20	20	6	7	8	10	12	7	9	1	9	2	18	15	12	13	5
8	19	11	1	3	14	17	10	19	20	2	1	10	8	4	5	7	5	3	11
20	8	10	5	16	13	17	8	14	4	13	11	2	2	11	12	4	4	8	5
5	1	15	11	17	1	1	5	15	16	7	11	6	7	8	13	6	20	8	7

Appendix 2. Excerpt taken from Stark *et al.* (2000) (pages 22–24) discussing protocol C3 – collection of quantitative sample from a hard-bottomed substrate using a Surber sampler. The final page in this appendix can be printed, laminated and used as a field guide if required.

Protocol C3: Hard-bottomed, quantitative

The purpose of quantitative sampling is to estimate densities (usually numbers per square metre) of macroinvertebrates present at a sampling site. Quantitative data, being more costly to obtain, are most suited to compliance monitoring or AEEs where density effects are anticipated. Macroinvertebrate densities are highly variable, both spatially and temporally, frequently in response to flow and substrate conditions. Therefore, isolated density estimates may have limited value unless the flow history and substrate conditions are known, or unless all sampling (say upstream and downstream of a discharge) is undertaken on the same day. In our view, SOE monitoring does not normally warrant the collection of quantitative data and it is likely that densities will show flow-related variation if SOE sampling is spread over several weeks. There are no limits on the metrics and data analyses possible if quantitative data are collected. Quantitative sampling in hard-bottomed streams can be achieved using many different techniques (see review in Merritt & Cummins 1996). Regardless of which sampling device is used in a programme, the same device should be used for all sampling. Different sampling devices may be more or less efficient for sampling some taxa so using more than one sampling method during a study may affect the consistency of the data (Winterbourn 1985). With this in mind, we recommend using a Surber sampler for all quantitative sampling in hard-bottomed streams. The Surber sampler (Surber 1937), a net attached to a grid frame that enables the user to collect a sample over a known area of substrate, is one of the most commonly-used devices for sampling hard-bottomed stream sites both in New Zealand and overseas. While it is an indispensable apparatus for sampling stream invertebrates it does have limitations that users need to be aware of. As with many sampling devices in flowing waters, the Surber sampler relies on stream current to carry animals and detritus into the net. The assumption made when employing the Surber sampler is that, as the substrate is disturbed, organisms and detritus from within the sampling area (and not elsewhere) will all be transported downstream, and retained in the net. This assumption is only valid when certain precautions are taken:

1. Sampling must proceed in an upstream direction, with the Surber placed on an undisturbed patch of streambed. Unlike D-net sampling the operator should not stand upstream of the Surber. Likewise, sampling should not be undertaken downstream of areas where others may be working (The Surber catches drifting organisms as well as benthos).
2. Ideally, the Surber sampler should be used in water no deeper than the top of the frame (*i.e.*, ca. 32 cm for a 0.1 m² Surber). However, sampling can be undertaken in deeper provided that there is a good flow through the net so that backflow does not result in animals being lost around the sides and over the top of the net.
3. The Surber sampler is not effective in low velocity areas (*e.g.*, pools or edge habitats). There must be sufficient current to carry organisms and detritus into the net, without risk of loss from backflow. If necessary, a current can be created by hand.

4. There is an obvious limit to the size of substrate that can be effectively sampled with a Surber sampler, that being the width of the frame (ca. 32 cm). Generally, the Surber sampler works best in gravel and small cobble substrates. Larger cobbles can cause the sampler to lose its seal with the bed, and the sampler can be filled with sand and silt if used in very fine sediments.
5. An effective seal must be formed between the area of streambed to be sampled and the bottom of the Surber frame, otherwise animals may be lost around the base of the sampler. Rubber skirts, foam pads, or lengths of chain can be fitted to improve the seal, but a rolled up towel can also be used. A rubber flap can also be attached beneath the mouth of the sampler to protect the net from abrasion against sharp stones.
6. Care should be taken to prevent the net becoming clogged, as this leads to backflow and loss of animals. If the net begins to balloon out and fill with water it helps to slap the side of the net, or shake it to dislodge the fine detritus that is blocking the mesh. Do not dislodge the sampling frame.
7. Except in bedrock or clay-bottomed streams, the Surber sampler is, in fact, a volume sampler rather than an area sampler. Unfortunately while the area of the sampler is fixed it is much more difficult to ensure that samples are of a uniform volume (and therefore comparable across sites/samples). The only way around this is to sample the substrate to a prescribed depth – usually 5 cm–10 cm. A screwdriver with a mark on the blade can be used as a guide to show when the substrate has been disturbed to the prescribed depth. The depth of sampling should be noted.
8. In addition to sampling to a prescribed depth, the disturbance procedure should be standardised and may involve digging into the streambed with hands (look out for broken glass!), or implements (e.g. handle of scrubbing brush, screwdriver) and brushing larger stones, with a soft-bristled brush. If stones are not scrubbed, some species that strongly adhere to the substrate will be missed. This procedure may damage soft-bodied specimens, but better a damaged specimen than no specimen at all!
9. Finally, be aware that bias can result from different personnel undertaking sampling. Never assume that your staff know what they are doing – provide them with proper instruction.

Protocol C3: Hard-bottomed, quantitative

Requirements:

- Waders or sturdy boots
- Surber sampler (area 0.1 m², 0.5 mm mesh)
- Brush
- White tray
- Sieve or sieve bucket (0.5 mm mesh)
- Plastic screw-top sample containers (600 ml volume)
- Preservative
- Labels and waterproof marker pen, or pencil

Protocol:

1. Ensure that the sampling net is clean.
2. Select a suitable sample reach and habitat (e.g. riffle). Sample beginning at the downstream end of the reach and proceeding across and upstream.
3. Place the sampler on the streambed ensuring a good fit around the perimeter. The sampler should be positioned so that the water current washes dislodged material into the net.
4. Brush material from the upper surface of all cobbles contained within the sample quadrat. Pick up each cobble and, holding it immediately in front of the net mouth, brush all sides of the cobble clean. Repeat for all of the larger substrate elements within the sampler quadrat. Place clean cobbles outside of the sampler quadrat. Disturb the finer substrate remaining within the quadrat to a depth of 5 cm–10 cm. Beware of broken glass and other sharp objects.
5. Remove the sampler from the water, rinse the net several times to concentrate the sample in the bottom of the net (take care not to lose material during this process), and return to the stream bank. Remove and discard large substrate elements that may have entered the net, taking care to remove adhering invertebrates before disposal. Remove sample from collection net either by inverting net into a suitable container, or by removing container attached to end of collection net. Elutriation may also be required (*i.e.* repeated rinsing of sample to separate organic and inorganic fractions).
6. Let the sample settle for a few minutes and decant off excess water via the sieve. Return any macroinvertebrates that are washed out with the water to the sample container. (Tweezers may be useful here).
7. Add preservative. Aim for a preservative concentration in the sample container of 70%–80% (*i.e.* allowing for the water already present). Be generous with preservative for samples containing plant material (leaves, sticks, macrophytes, moss or periphyton).
8. Place a sticky label on the side of the sample container and record the site code / name, date, and replicate number (if applicable) using a permanent marker. Write on the label when it is dry and do not rely on a label on the pottle lid! Place a waterproof label inside the container. Screw the lid on tightly.
9. Note the sample type (e.g. Surber 0.1 m²), collector's name and preservative used on the field datasheet.



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