Understanding the hydrological
and physicochemical drivers of
Phormidium proliferation in the
Hutt and Waipoua rivers

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Abstract

The Hutt and Waipoua rivers are affected annually by proliferations of the potentially toxic benthic cyanobacteria - *Phormidium*. Ingestion of these mats has resulted in numerous dog deaths and is therefore a risk to human health. This has resulted in the establishment of warning signs at many recreational sites on these rivers during summer months. Recent research has concluded that river flow and water column nutrients are two of the primary factors regulating *Phormidium* growth. Proliferations tend to form in rivers where there is slightly elevated water column dissolved inorganic nitrogen, low dissolved reactive phosphorus, and during periods of stable flow. It has been hypothesised that fine sediment may provide a source of phosphorus for *Phormidium*. These mats 'capture' fine sediment suspended in the water column, which becomes incorporated into the mat matrices when motile *Phormidium* filaments move over the sediment. Diffusive boundary layers at the surface of the mats limit the flow of nutrients and gases between mat and bulk river water, creating conditions (for example, lower dissolved oxygen, elevated pH) conducive to the release of phosphorus from sediment.

The aim of this project was to identify why *Phormidium* proliferates in certain parts of the Hutt and Waipoua rivers, as well as investigate the relationship between fine sediment and mat growth. Monitoring of river data was carried out in the Hutt and Waipoua Rivers between November 2014 and May 2015. Over this period, physicochemical and hydrological data was monitored to identify the influencing factors of *Phormidium* abundance. During February 2015, sediment traps were deployed to determine the sedimentation rates in parallel to *Phormidium* cover at each site. The collected fine sediment was fractionated and analysed for biologically available phosphorus. Finally, a manipulative study using stream channel mesocosms was undertaken to provide causative evidence that fine sediment deposition influences *Phormidium* growth. In this three-week study, four mesocosms were deployed containing different fine sediment treatments. Biomass samples were collected at regular intervals to determine total photosynthetic biomass and *Phormidium* specifically.

Phormidium cover during 2014-15 was influenced by water column nitrate-nitrite nitrogen concentrations and sediment deposition. *Phormidium* cover was considerably lower compared to previous years, with a maximum cover of 20.7% occurring in the Hutt River during February 2015. Analysis of historical flow and nutrient data suggests that the annual variation in *Phormidium* proliferation over the summer months was site specific and not generally driven by flow or nutrient concentrations.

It is likely that fine sediment plays a role in providing *Phormidium* mats with phosphorus in the Hutt and Waipoua River. This is shown through phosphorus concentrations within *Phormidium* mat water, which were 200-fold higher than the bulk water column. Maximum values of sedimentation, 272.0 g/m²/day, and biologically available phosphorus (bound to sediment) 1.4 mg P g⁻¹, occurred at sites with the highest *Phormidium* cover, which further confirms this correlation. Furthermore, mesocosm experiments showed that *Phormidium* biomass increased significantly ($p=0.015$) with an increased amount of sediment. However, the maximum biomass of 64.75 mg/m² did not occur in the mesocosm channel with the most sediment added to it. This suggests that a deposition threshold exists due to the attenuation of light.

Findings from this research provide some insights in to management options which may help to mitigate *Phormidium* proliferations in the future. The data indicates that reducing sediment inputs, or resuspension of fine sediment during flood remediation works, would reduce *Phormidium* proliferations. Riparian planting as well as the collaboration with local councils is needed to help reduce diffuse and remaining point sources of sediment and river bed disturbance during flood protection activities. Using a combination of observational and experimental studies, this research has shown that multiple factors influence *Phormidium* proliferation, and has highlighted the key role that fine sediment plays. Suggestions for future studies include in-river experiments to further explore the role of fine sediment and the optimisation of mesocosms, which may also help to investigate finer scale data on causative factors such as sediment thresholds.

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Contents

Abstract	i
Acknowledgements	iii
List of Figures	x
List of Tables	xii
 Chapter 1 : Introduction and background.....	 13
1.1 Introduction.....	13
1.2 Study sites – Waipoua and Hutt Rivers	14
1.2.1 Waipoua River	14
1.2.2 Hutt River	16
1.3 Cyanobacteria, <i>Phormidium</i> and drivers of <i>Phormidium</i> proliferations ...	17
1.4 Monitoring <i>Phormidium</i> proliferations and their occurrence in the Hutt and Waipoua rivers	19
1.5 Synthesis	20
 Chapter 23: Identifying the hydrological and physicochemical variables that influence <i>Phormidium</i> growth in the Hutt and Waipoua rivers	 23
2.1 Introduction.....	23
2.2 Methods	23
2.2.1 Baseline monitoring of river characteristics.....	23
2.2.2 Nutrient and elemental concentrations.....	25
2.2.3 Within mat water collection.....	25
2.2.4 Historical monitoring data in the Hutt and Waipoua rivers	26
2.3 Results.....	26

2.3.1 Periphyton growth	26
2.3.2 Physicochemical characteristics	28
2.3.3 Nutrient and elemental concentrations	30
2.3.4 Within mat water analysis.....	34
2.3.5 Historical data analysis.....	34
2.4 Discussion	38
2.4.1 Physicochemical data and <i>Phormidium</i> cover	38
2.4.2 Nutrient and elemental concentrations and <i>Phormidium</i> cover	40
2.4.3 Within mat water analysis.....	43
2.4.4 Comparisons of historical data	44
 Chapter 3: Does fine sediment provide a source of phosphorus for <i>Phormidium</i> growth in the Hutt and Waipoua rivers?	 49
3.1 Introduction	49
3.2 Methods	50
3.2.1 Sedimentation rates and size fractionation	50
3.2.2 Sequential extraction of biologically available phosphorus.....	52
3.3 Results	53
3.3.1 Sedimentation rates	53
3.3.2 Sequential extraction of biologically available phosphorus.....	55
3.4 Discussion	56
3.4.1 Sediment deposition and river flow	56
3.4.2 Sediment deposition and landuse	57
3.4.3 Sediment deposition and <i>Phormidium</i> cover relationships	58
3.4.4 Sequential extraction of phosphorus	59

Chapter 4: Investigating the relationship between fine sediment deposition and <i>Phormidium</i> growth.....	63
4.1 Introduction.....	63
4.2 Methods.....	63
4.2.1 Mesocosm Set-up.....	64
4.2.2 Baseline Monitoring of water characteristics.....	67
4.2.3 Biomass monitoring	67
4.3 Results.....	72
4.3.1 Physical water characteristics.....	72
4.3.2 Nutrient concentrations.....	74
4.3.3 Biomass	75
4.4 Discussion.....	80
4.4.1 Physicochemical water characteristics.....	80
4.4.2 Nutrient concentrations and <i>Phormidium</i> growth	81
4.4.3 Sediment addition and biomass.....	82
4.4.4 <i>Phormidium</i> removal processes.....	83
4.5 Experiment limitation and suggestions for future development.....	84
4.5.1 Mesocosm set-up	84
4.5.2 Nutrient concentrations.....	85
Chapter 5: Synthesis.....	87
5.1 Identifying the hydrological and physicochemical parameters that influence <i>Phormidium</i> growth in the Hutt and Waipoua Rivers.....	87
5.2 Does fine sediment provide a source of phosphorus for the growth of <i>Phormidium</i> in the Hutt and Waipoua Rivers?	88

5.3 Investigating the relationship between fine sediment and <i>Phormidium</i> growth.....	88
5.4 Mitigation suggestions and future direction	89
References	91
Appendix 1	101
Appendix 2.....	102
Appendix 3	103
Appendix 4	106
Appendix 5	107
Appendix 6	108
Appendix 7	109
Appendix 8	112

List of Figures

Figure 1.1	Location of the Hutt and Waipoua rivers	14
Figure 1.2	<i>Phormidium</i> mats growing on a variety of substrates	17
Figure 2.1	Location of sampling sites in the Hutt and Waipoua rivers	24
Figure 2.2	Boxplots showing average periphyton cover	28
Figure 2.3	Nitrate-nitrite nitrogen concentrations over time	30
Figure 2.4	Dissolved reactive phosphorus concentrations over time	31
Figure 2.5	Boxplots showing average dissolved reactive phosphorus	32
Figure 2.6	Ammoniacal nitrogen concentrations over time.....	33
Figure 2.7	Bar graph showing <i>Phormidium</i> cover over seven years.....	35
Figure 2.8	Flushing flow frequency and <i>Phormidium</i> cover over time	37
Figure 2.9	Scatterplot showing <i>Phormidium</i> cover and flushing flows	38
Figure 3.1	Sediment traps positioned in the river bed	51
Figure 3.2	Bar graph showing average sedimentation rates	54
Figure 3.3	Total BAP bound to three sediment size fractions	55
Figure 3.4	Total BAP bound to three species of phosphorus	56
Figure 4.1	Mesocosm chambers in place at the Cawthron Institute	65
Figure 4.2	Cobble inoculated with <i>Phormidium</i> mats	66
Figure 4.3	Gathering biomass samples from mesocosms chambers	68
Figure 4.4	Nitrate nitrite nitrogen within each mesocosm.....	74
Figure 4.5	Dissolved reactive phosphorus within each mesocosm	75
Figure 4.6	Boxplots showing chlorophyll- <i>a</i> within each mesocosm.....	76
Figure 4.7	Boxplots showing AFDM within each mesocosm.....	77
Figure 4.8	AFDM and inorganic content within each mesocosm	77

Figure 4.9	Bar graph showing autotrophic index within each mesocosm	78
Figure 4.10	Growth rates of <i>Phormidium</i> within each mesocosm chamber	79
Figure 4.11	<i>Phormidium</i> biovolume values in each mesocosm chamber	80
Figure 4.12	Fine sediment covering <i>Phormidium</i> mats	82
Figure 4.13	Oxygen bubbles developing within <i>Phormidium</i> mats	84

List of Tables

Table 2.1	Water quality variables in the Hutt and Waipoua River	29
Table 2.2	Nutrient concentrations from mat water	34
Table 4.1	Physicochemical variables within each mesocosm	73

Chapter 1

Introduction and background

1.1 Introduction

The Hutt and Waipoua rivers are of great recreational, ecological and economic importance to the Wellington Region. However, over the past decade they have been significantly affected by toxic mat-forming benthic cyanobacteria from the genus *Phormidium*, which has adversely influenced the ecological integrity of the rivers. Research has been carried out on *Phormidium* nationally. Recent studies suggest that in some rivers around New Zealand, *Phormidium* is able to out-compete algal species due to physiological advantages that enable them to proliferate in nutrient-depleted environments. This thesis aimed to identify the physicochemical variables that influence cyanobacterial proliferations in the Hutt and Waipoua Rivers and to investigate whether fine sediment might provide a source of phosphorus for the promotion of *Phormidium* proliferation.

The Hutt and Waipoua rivers are located in the Greater Wellington Region of New Zealand (Fig. 1.1) and are popular recreational sites for swimming, fishing and tramping. The Hutt River provides drinking water to the majority of the Wellington Region (Greater Wellington Regional Council, 2016) and both rivers support the surrounding scenic reserves, regional parks and forests (Upper Hutt City Council, 2012). Due to their strong ecological integrity, the Hutt and Waipoua rivers provide essential spawning areas and habitats for many native species of flora and fauna (Forest and Bird, 2014).

Access to the recreational areas supported by the Hutt and Waipoua rivers is restricted by local councils for extended periods during the summer months, when the cyanobacterium *Phormidium* proliferates. *Phormidium* can produce neuromuscular blocking compounds that have killed over 70 dogs since 2005 in New Zealand (Hamill, 2001, Wood et al., 2007, Heath et al., 2010, McAllister et al., 2016). These natural toxins are a threat to humans and animals when consumed,

and therefore their presence in recreational waterways is deemed a significant public health risk (Ministry for the Environment and Ministry of Health, 2009).

Figure 1.1: The Hutt and Waipoua catchment areas are both located in the Greater Wellington Region of New Zealand in the southern area of the North Island. The Hutt River catchment is located in the Upper and Lower Hutt city districts, and the Waipoua River catchment is located in the Masterton District (ArcMap 10.1, 2015).

1.2 Study sites – Waipoua and Hutt Rivers

1.2.1 Waipoua River

The Waipoua River is a steep, gravel-phase river with a relatively stable and narrow single thread channel (Gyopari and McAlister, 2010). With headwaters originating in the eastern slopes of the Tararua Ranges, the river flows for 30 km through the Wairarapa Valley before discharging into the Ruamāhanga River to the east of Masterton (LAWA, 2016). The Waipoua river is the first major western tributary of the Ruamāhanga River, and has a catchment area of 141 km² (Chrystall, 2007).

Dry weather conditions and significant wind erosion has led to low-to-moderate soil fertility within the catchment, which limits its suitability for sustainable pastoral farming (Baker et al., 1994). Landuse in the Waipoua catchment consists mainly of agriculture, with low-intensity sheep and beef farming making up almost 80% of the land area in the catchment (Chrystall, 2007). Surrounding vegetation is predominantly pastoral and species such as willow, which have been introduced for flood and erosion control purposes, are abundant (Greater Wellington Regional Council, 2001a).

Climate in the Waipoua River catchment is characterised by high temperatures of over 30°C in the summer months and relatively cool winter months, when air temperatures decrease to an average of 9°C (Heslop et al., 1996). Increased temperatures during warmer months cause high evaporation levels and water deficits in shallow areas of the river (Greater Wellington Regional Council, 2001a), where low flows can reach less than 1 m³ s⁻¹ and water temperatures can increase to over 20°C (Greater Wellington Regional Council, 2017). During cooler months, rainfall is influenced by the Tararua Ranges through orographic effects. Rainfall in the Tararua Ranges can significantly increase river flow during the winter months, which has reached over 200 m³ s⁻¹ (Greater Wellington Regional Council, 2017).

Erosion and landslides are the main sources of bed load for the Waipoua River. Erosion in the Tararua Ranges is exacerbated by steep slopes composed of fragmented materials such as greywacke bedrock (Adkin, 1949), as well as high rainfall events, which suggests that there may be an increased bedload during the winter months (Heslop et al., 1996). Bank instability, primarily driven by vegetation depletion, is also caused by browsing mammals, which disturb the litter layer and prevent re vegetation (Greater Wellington Regional Council, 2001a). Large bed loads have prompted significant flood protection works, where artificial channel alignment and deepening are used to prevent flooding and protect the surrounding areas (Heslop et al., 1996). Various gravel extraction activities occur annually in the Waipoua River to maintain channel alignment and river flow (Chrystall, 2007).

1.2.2 Hutt River

The Hutt River is a 56-km long aggrading river that encompasses a catchment area of 655 km². The Hutt River's headwaters also begin in the Tararua Ranges, but it flows in a southerly direction parallel to the Wellington fault line, entering the Wellington Harbour at Petone (Greater Wellington Regional Council, 2016).

The upper reaches of the Hutt River catchment are principally native forest and scrublands. Landuse in the lower reaches comprises agricultural, commercial and residential areas (Ballinger et al., 2011). Residential and industrial areas of the Hutt Valley were once densely forested, which stabilised the river and alleviated erosion of bank edges (Greater Wellington Regional Council, 2001a). The intensive settlement and landuse on the river floodplains have altered the vegetation to farmland, scrub and regenerating forest, as well as domestic gardens in the lower reaches of the river (Ballinger et al., 2011).

The Hutt Valley typically receives between 1,100 and 1,300 mm of rain per year (Gyopari, 2015). However, up to 5,000 mm of rain may annually fall on the Tararua Ranges, which significantly contributes to the highly variable flow of the Hutt River. High rainfall, in addition to reduced vegetation cover and industrialisation over the past 100 years, has caused extensive flooding events in the Hutt catchment (Greater Wellington Regional Council, 1996). This has prompted local councils to construct a flood defence system, which includes river re-alignment, gravel extraction and the construction of stop banks (Greater Wellington Regional Council, 2001b).

The Hutt and Waipoua Rivers have similarly high sediment loads, and both experience *Phormidium* proliferations. Due to erosion, rivers originating from the Tararua ranges tend to have higher sediment loads that, in combination with low flows, lead to aggradation. As described above, the Hutt and Waipoua rivers have both been significantly altered and excavated to mitigate the effects that high bed load has on flood risks. The proliferation of *Phormidium* has been a problem for both rivers. Over the past ten years, the percentage cover of *Phormidium* mats has exceeded national guideline warning limits almost annually during summer, which has impacted the river's attraction as a recreational area (Wood et al.,

2007) and may have some negative implications for aquatic organisms (Oberemm et al., 1999).

1.3 Cyanobacteria, *Phormidium* and drivers of *Phormidium* proliferations

Cyanobacteria are photosynthetic micro-organisms that live in a wide range of habitats including freshwater. Benthic cyanobacteria have been observed to grow on a variety of substrates, ranging from bedrock to organic vegetation and wharf piers, and can grow in varied water quality conditions (Fig. 1.2) (Quiblier et al., 2013).

Figure 1.2: *Phormidium* growing on a variety of substrates in the middle reaches of the Hutt River (Photos taken April 2015)

Phormidium is the most common mat-forming benthic cyanobacteria in New Zealand rivers (Biggs and Kilroy, 2000, Heath et al., 2010). *Phormidium* can produce powerful neuromuscular blocking compounds called anatoxins, and the consumption of toxic *Phormidium* mats has resulted in over 70 reported dog deaths since 2005 within Wellington, Tasman, Nelson, Canterbury and the Bay of Plenty regions (Hamill, 2001, Wood et al., 2007, Heath et al., 2010, McAllister et al., 2016). Although there have been no reported human fatalities from anatoxins (Ministry for the Environment and Ministry of Health, 2009), the death of five dogs in the Hutt River in just one year in 2005 stimulated a variety of research to identify the main drivers of *Phormidium* proliferations, and to understand the toxicity of this organism.

Over the past decade, *Phormidium* abundance has noticeably decreased in the Hutt and Waipoua Rivers. During the summers of 2013-14, average *Phormidium* cover in both rivers decreased 5-fold compared to the previous summer (Greater Wellington Regional Council, 2017). Fine scale investigations into the role of nutrients and physical water characteristics in the proliferation of *Phormidium* has not been carried out in the Waipoua River, and in the Hutt River, previous investigations have targeted limited areas within the catchment. These investigations have shown that *Phormidium* tends to proliferate under certain physicochemical conditions in New Zealand rivers. Key drivers of *Phormidium* proliferations in New Zealand include flow, water temperature and nutrients, primarily slightly elevated nitrogen, as *Phormidium* is unable to fix nitrogen, and depleted phosphorus (Heath et al., 2011, Wood and Young, 2012, Heath et al., 2013). Recent investigations in the Hutt River by Heath et al. (2013) showed that an increase in the mean annual low flow had a negligible effect on *Phormidium* proliferations. Studies by Milne and Watts (2007), Heath et al. (2011), Heath et al. (2010), and Wood and Young (2011) suggested that the duration of stable flow and frequency of flushing flows limit the growth of *Phormidium*. Flushing flows, which are here regarded as a three times the median flow rate, may reduce *Phormidium* proliferation through abrasion by mobilised sediments (Biggs et al., 1999). However, investigations in rivers in the Manawatu have shown that at many sites, the flow needed to reduce *Phormidium* cover is much greater than three times the median, and that the stage of growth needs to be taken into account when analysing this relationship (Wood et al., 2016, Wood et al., 2014b).

River sites with the greatest *Phormidium* mat coverage generally have low concentrations of dissolved reactive phosphorus (DRP; ca. $<0.01 \text{ mg L}^{-1}$) and slightly elevated dissolved inorganic nitrogen (DIN; ca. $>0.1 \text{ mg L}^{-1}$) (Wood and Young, 2012, Heath et al., 2013). A possible explanation for the ability of *Phormidium* to proliferate at low DRP concentrations relates to growth structure. Unlike other river periphyton, *Phormidium* mats are thick and cohesive and trap river water in a mucilaginous matrix (Wood et al., 2014a). Well-developed *Phormidium* mats have a fine layer of sediment at the substrate-mat interface, that has been 'captured' by Extracellular Polymeric Substances (EPS; a sticky mucus) secreted by the *Phormidium* mats as the fine sediment is transported

down the river. Recent research carried out on the Mangatainoka River (New Zealand) has demonstrated that the formation of geochemical conditions (e.g., elevated pH and low dissolved oxygen) within mats are very different to the surrounding water column (Wood et al., 2015a). The authors suggest these conditions enable the release of DRP bound to sediments entrapped in the mat and show that the water trapped in the mucilaginous matrix had an average DRP concentration 320 times higher than the surrounding river water (Wood et al., 2015a). Sequential extraction of phosphorus from sediment deposited at sites showed that the concentrations of biological phosphorus were two to four times higher at sites with *Phormidium* proliferations (Wood et al., 2015a). Other variables that may affect the growth of *Phormidium* proliferations include temperature, light intensity, starting inocula and *Phormidium* strain – further research is required to determine the relative importance of these (Heath, 2014).

1.4 Monitoring *Phormidium* proliferations and their occurrence in the Hutt and Waipoua rivers

In the Hutt and Waipoua rivers, *Phormidium* can appear as solitary, free living cells as well as extensive mats when environmental conditions are favourable. A *Phormidium* proliferation, defined as greater than 20% cover of the river bed, triggers the ‘amber mode’ alert in the national guidelines for cyanobacteria (Ministry for the Environment and Ministry of Health, 2009). As *Phormidium* mats grow, oxygen bubbles form in the mat matrix as a result of photosynthesis, causing it to become buoyant. As the attachment strength of *Phormidium* decreases and mats can detach from the river substrate, accumulating as ‘rafts’ at the river’s edge (Quiblier et al., 2013). These *Phormidium* mats can accumulate along the edges of rivers increasing the likelihood of contact with animals and humans. Therefore, the risk posed by *Phormidium* is likely to rise as the number of detached mats increases (Ministry for the Environment and Ministry of Health, 2009).

To mitigate the health risk posed to humans and animals in the Wellington Region, monitoring of *Phormidium* cover is carried out at popular recreational

areas by the Greater Wellington Regional Council (GWRC). This is conducted as a part of the recreational water quality monitoring programme and involves visual estimates of periphyton and cyanobacteria cover on a weekly basis during the summer months; when *Phormidium* proliferations are most likely to occur (Morar and Greenfield, 2014). The recreational monitoring programme follows the alert-level framework set out by the Ministry for the Environment, which identifies three layers of monitoring depending on the appearance and quantity of observed cyanobacteria (Ministry for the Environment and Ministry of Health, 2009). When the cover of cyanobacteria exceeds the amber/alert level threshold of 20% cover, the area is deemed a public health risk, and warning signs are erected advising the public not to go into the water and to be cautious when walking their dogs near the river (Ministry for the Environment and Ministry of Health, 2009).

The cover of potentially toxic cyanobacteria at the sites monitored in the Hutt and Waipoua rivers has exceeded the amber/alert level threshold every year in the last decade, except for the 2013-14 swimming season (Morar and Greenfield, 2014). There were at least three reported dog deaths following contact with *Phormidium* mats in the Hutt River during 2005, 2007, and 2012. One dog died in 2010 in the Waipoua River due to *Phormidium* contact (Ryan and Warr, 2008, Morar and Greenfield, 2012, Morar and Warr, 2011). It is clear that *Phormidium* abundance has adversely impacted the river's attraction as a popular recreational area, both through direct access restrictions and health warnings issued by local councils (Milne and Watts, 2007).

1.5 Synthesis

Further research is required to fully understand why *Phormidium* proliferations occur and how they can be mitigated. This thesis aims to expand knowledge of *Phormidium* proliferation in the Hutt and Waipoua rivers, primarily through exploring the following objectives:

Objective 1: Identify the hydrological and physicochemical variables that influence *Phormidium* growth in the Hutt and Waipoua rivers.

Phormidium growth in the Hutt and Waipoua rivers has varied over the past seven years it has been monitored. To further understand why these changes may have occurred, a multivariable analysis was carried out on a variety of physical and chemical water parameters which may influence *Phormidium* cover. An analysis of annual flow and nutrient data in relation to the history of *Phormidium* proliferations was also undertaken.

Objective 2: Identify whether fine sediment provides a source of phosphorus for *Phormidium* proliferations in the Hutt and Waipoua rivers.

To further understand the relationship between sediment deposition and *Phormidium* growth, sediment traps were deployed over a three-week period to understand the sedimentation regimes in the Hutt and Waipoua rivers. Fractionation of sediment as well as phosphorus speciation was carried out to observe the spatial differences of phosphorus bound sediment in both rivers, as well as identifying the potential for phosphorus utilisation.

Objective 3: Identify whether fine sediment deposition influences the growth of *Phormidium* mats in a controlled environment.

To provide further evidence to suggestions that *Phormidium* mats are able to utilise fine sediment as a source of phosphorus, a manipulative study was carried out using mesocosms. Observations of *Phormidium* mat areas and biomass as a result of different sediment treatments were analysed over a three-week period, under controlled water quality conditions. This experiment was one of the first to investigate the effects of *Phormidium* growth to sediment deposition in a controlled environment setting.

It is apparent that the key variables associated with *Phormidium* growth require further exploration. Investigations to date that have taken place in the Hutt and Waipoua rivers are based on infrequent and fragmentary datasets, which have not included information around the role of fine sediment in *Phormidium* growth.

This study will contribute to existing research by providing a higher resolution dataset containing river characteristics and conditions collected weekly over a seven-month period, when *Phormidium* growth is expected to be at its peak. The study will also provide information on the role of fine sediment to *Phormidium* growth in New Zealand Rivers. Sediment trap studies and phosphorus fractionation will provide information on sediment deposition and its uses for transporting phosphorus to *Phormidium* for growth. Furthermore, a manipulative study using flow through channels was carried out to confirm correlative observations between sediment deposition and *Phormidium* growth. The data collected will also provide information that may aid mitigation efforts in the future.

Chapter 2

Identifying the hydrological and physicochemical variables that influence *Phormidium* growth in the Hutt and Waipoua rivers

2.1 Introduction

This study was one of the first to analyse the hydrological and physicochemical variables in the Hutt and Waipoua rivers on a weekly basis. Previous long term monitoring of these rivers has been carried out monthly, and many parameters have not been analysed on a weekly scale over such a long period. Factors analysed in this chapter provide new insights into how the physical and chemical characteristics of these rivers change on a shorter time scale and whether these changes affect *Phormidium* cover. This has helped improve the understanding of variables that cause *Phormidium* proliferations in the Hutt and Waipoua rivers.

2.2 Methods

2.2.1 Baseline monitoring of river characteristics

Hydrological and physicochemical parameters were assessed in the Hutt and Waipoua rivers weekly between November 2014 and May 2015. Four sites were monitored within the Hutt River and two in the Waipoua River (Fig 2.1). Selection of these sites was based on the history of *Phormidium* proliferations, landuse (forested, urban, residential) (Heath et al., 2013) and the continuity of existing historical datasets. The two sites in the Waipoua River were placed relatively close together, being approximately 1.5 km apart. This was because the river is known to run dry during the summer months and flows through inaccessible

private land in the upper catchment area (pers. Comm. Mark Heath, November 2014). Sites in the Hutt River were approximately 10 km apart.

Water temperature, pH, dissolved oxygen, and specific conductivity were recorded approximately 50 cm below the water surface using a handheld YSI multiprobe (YSI Incorporated, USA). The percentage of *Phormidium* mat cover was determined using the transect method outlined in the New Zealand guidelines for managing cyanobacteria in recreational fresh waters (Ministry for the Environment and Ministry of Health, 2009). At each site, four transects positioned at right angles to the water's edge and extending to a depth of 0.6 m were surveyed. *Phormidium* mats and other algal types were assessed at five points along each transect using a bathyscope (Model 0800, Nuova Rade). The cover of *Phormidium*, filamentous algae, all mats with a thickness greater than three millimetres, diatom mats and fine sediment were obtained by determining the average of 20 data points (5 points × 4 transects) at each site.

Figure 2.1: Map showing the location of the Hutt and Waipoua sampling sites. *Phormidium* coverage at each site is indicated by the size of the red circles. Blue circles represent long term sampling sites monitored by Greater Wellington Regional Council, as a part of the State of the Environment monitoring programme.

2.2.2 Nutrient and elemental concentrations

Water samples (50 mL, 28 replicates) were collected weekly during the seven-month monitoring period. Samples were filtered in the field using 0.45- μm polyethersulfone filters (Sartorius), and stored frozen (-20°C) until analysis. Total nitrate-nitrite nitrogen (NNN; automated cadmium reduction; APHA 2012), total ammoniacal nitrogen ($\text{NH}_4\text{-N}$) phenol/hypochlorite colorimetry, APHA 2012) and dissolved reactive phosphorus (DRP; molybdenum blue colorimetry, APHA 2012) were measured using flow injection analysis at Hills Laboratories, Hamilton. The accredited detection limits were 0.002 g/m^3 , 0.0010 g/m^3 and 0.005 g/m^3 , respectively. Water samples (10 mL) were also taken monthly for analysis of elemental composition; in particular strontium, potassium, sodium, iron and copper, which have been cited as important to cyanobacteria growth previously (Wood et al., 2015b, Seale et al., 1987). These samples were filtered in the field using 0.45- μm polyethersulfone filters (Sartorius), preserved with nitric acid (final concentration 2%) and stored at 4°C until analysis. Elemental analysis was carried out using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, using a Perkin-Elmer SCIEX ELAN DRC II inductively coupled plasma mass spectrometer) at Waikato University, Hamilton. Pearson's r correlation analysis was computed using IBM SPSS Statistics (Version 23) to assess the relationship between the maximum monthly *Phormidium* cover and trace element concentrations. An independent-samples t-test was also carried out using IBM SPSS Statistics (Version 23) to compare trace element concentrations at times with greater than 5% *Phormidium* cover and no *Phormidium* cover conditions.

2.2.3 Within mat water collection

Within *Phormidium* mat water was collected at the sampling sites H02, H03 in the Hutt River and W01 in the Waipoua River on the 10, 17 and 18 April 2015. Due to a lack of *Phormidium* mat abundance over the summer sampling period, within mat water was not collected on any other occasions.

To collect within mat water, 10 rocks covered in thick *Phormidium* mats were taken at random from the river bed and excess water drained off them for 30

seconds. The mat was then scraped off into a beaker and gently squeezed against the side to allow water to drain from the mat. Water samples were filtered using 0.45-µm polyethersulfone filters (Sartorius) and frozen until analysis. The frozen mat water was analysed for total NNN, total NH₄-N and DRP as described above.

2.2.4 Historical monitoring data in the Hutt and Waipoua rivers

Historical river flow, nutrient and *Phormidium* cover data, ranging from 2009 to 2016 (seven years), was obtained from the Greater Wellington Regional Council (GWRC) archives and analysed for long term changes. Flushing flow frequency was determined by calculating the maximum daily flow between November and May, defined by the Greater Wellington Regional Council as the *Phormidium* summer growth period. In this analysis, a flushing flow was defined as a flow event larger than three times the annual median (Milne and Watts, 2007, Wood et al., 2015b, Heath et al., 2013). Monthly concentrations of water column NNN, DRP and NH₄-N from three sites in the Hutt River and one site in the Waipoua River (Fig. 2.1) were assessed for long term trends in the data between the periods of November to May. A Pearson's r regression analysis was carried out using IBM SPSS Statistics (Version 23), to assess the strength of the relationship between annual nutrient concentration *Phormidium* cover.

2.3 Results

2.3.1 Periphyton growth

The highest cover of *Phormidium* was in the middle reaches of the Hutt River (H03), with a maximum cover of 20.7% (Appendix 1, Table 1). This was followed by both sampling sites in the lower Waipoua River, W01 and W02, with maximum covers of 15.3 and 16.8%, respectively. In the Hutt River, *Phormidium* cover appeared to increase downstream until the fourth sampling site, H04, which had an average cover of < 1% (Fig. 2.2).

Phormidium was not as abundant as in previous years and only exceeded the amber alert of the New Zealand guidelines for cyanobacteria in recreational fresh waters (Ministry for the Environment and Ministry of Health, 2009) on one occasion in the Hutt River (24 February 2015; Appendix 2, Fig. 1). Mat cover was highest between 12 January 2015 and 6 April 2015 in both rivers. In the Waipoua River, diatom and green filamentous algae growth were notably lower than in the Hutt River during this period (Appendix 2, Fig. 2).

Overall, green filamentous, diatom and *Phormidium* cover (hereafter referred to as periphyton cover), was generally greater in the Waipoua River than in the Hutt River. The site with the lowest overall periphyton cover throughout the sampling period was H01, located near the headwaters of the Hutt River. This site has the lowest abundance of green filamentous algae, diatoms and *Phormidium*, with a maximum cover of 1.1, 4.8 and 1.2%, respectively. In the Waipoua River, all periphyton cover increased downstream (Fig. 2.2). The maximum cover of green filamentous algae and diatom mats occurred at site W02, the furthest downstream site in the Waipoua River, with values of 13.3 and 20.4%, respectively (Fig. 2.2, Appendix 1, Table 1).

A Pearson's r correlation showed that there was a weak correlation between average *Phormidium* cover, and green filamentous algae in both the Hutt and Waipoua Rivers, $r = 0.25$, $p = 0.009$ and $r = 0.25$, $p = 0.076$, respectively. There was also a weak relationship between fine sediment cover and *Phormidium* cover in the Waipoua River, $r = 0.28$, $p = 0.049$. The relationships between *Phormidium* cover and diatom cover in both rivers, as well as *Phormidium* and fine sediment cover in the Hutt River showed no significant correlations.

Observed fine sediment cover was notably higher at site H04 in the Hutt River, with maximum values of 47.8% cover (Fig. 2.2).

Figure 2.2: Boxplots showing average cover of *Phormidium*, green filamentous algae, diatom material and fine sediment. Cover was estimated weekly between the 19 November 2014 and 25 May 2015 (n=28). Dark lines within the boxes represent the data median, boxes represent the 25th and 75th quartiles, whiskers extend out to the maximum values and circles represent outliers. Sites are shown upstream to downstream from left to right (i.e. H01 is the furthest upstream site in the Hutt River).

2.3.2 Physicochemical characteristics

Water characteristics did not vary markedly between the two rivers. However, there was a notable difference in temperature, specific conductivity and total dissolved solids between sites in the Hutt River (Table 2.1). Water temperature in the Hutt River increased downstream, with the lowest temperature of 9.4°C at H01, and the highest water temperature of 23.1°C at H04 (Table 2.1), both sites contained an average *Phormidium* cover of less than 1%. Differences in temperature between sites in the Waipoua River were negligible. Specific conductivity and total dissolved solids (TDS) both increased downstream in the Hutt River (Table 2.1), where the lowest conductivity (39.8 $\mu\text{S cm}^{-1}$) and TDS (20.0 mg/L) were measured at H01.

The highest values of conductivity and TDS occurred at H04, with values of 117.4 $\mu\text{S cm}^{-1}$ and 80.6 mg/L, respectively (Table 2.1). Differences in pH and dissolved oxygen values were negligible between rivers and sampling sites, with ranges between 6.22 and 8.9, and 83.9 and 137.1%, respectively (Table 2.1). There was no significant relationship between *Phormidium* cover and water column pH, dissolved oxygen, conductivity or TDS.

Temporally, pH and dissolved oxygen increased at all sites in both rivers over the sampling period (Appendix 3, Fig. 1, 2). Conductivity increased until February 2015 (Appendix 3, Fig. 4), when *Phormidium* cover was at a maximum. A reduction in temperatures followed peak values of 23.1 °C in the Hutt River and 19.3 °C in the Waipoua River (Appendix 3, Fig. 3). The TDS concentration did not markedly change over time, although a sharp decrease in TDS was observed during December at all sites, and an increase during March 2015 was recorded at H01 and H02 (Appendix 3, Fig. 5).

Table 2.1: Average, maximum and minimum values of water quality variables (n=28), observed weekly over a 28-week period between 19 November 2014 and 25 May 2015. Values were recorded at the same time each day on a weekly basis using a handheld YSI multiprobe held 30 cm under the water's surface.

	pH			Dissolved Oxygen (%)			Temperature (°C)			Specific Conductivity ($\mu\text{S cm}^{-1}$)			Total Dissolved Solids (mg/L)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
H01	7.6	6.2	8.9	108.4	49.3	137.1	12.0	6.9	16.1	55.8	39.8	110.8	50.38	20.0	55.9
H02	7.5	6.2	8.9	109.9	97.6	129.3	14.8	7.1	20.3	75.4	51.4	109.2	60.65	39.0	75.4
H03	7.5	6.3	8.9	113.0	95.8	128.7	16.2	7.2	22.5	83.6	59.0	108.4	67.43	50.7	77.4
H04	7.3	6.2	8.7	108.8	83.9	126.2	17.2	7.2	23.1	87.0	58.2	117.4	68.1	50.5	80.6
W01	7.5	6.2	9.0	109.4	98.7	130.4	15.0	9.0	18.8	81.3	50.5	101.4	69.4	48.1	84.5
W02	7.6	6.3	8.4	110.5	95.8	137.1	14.9	9.4	19.3	86.8	58.9	103.9	67.7	58.9	79.3

2.3.3 Nutrient and elemental concentrations

Nitrate-nitrite nitrogen

The concentration of water column NNN varied greatly between the two rivers (Fig. 2.3). The lowest concentrations of NNN were in the Hutt River, where values varied between 0.004 mg/L and 0.42 mg/L. Average NNN concentrations in the Waipoua River were 4-fold higher than in the Hutt River, where NNN concentrations varied between 0.18 mg/L and 1.14 mg/L (Appendix 4, Table 1). In general, NNN increased downstream in the Hutt River, but was reduced at H04; the site furthest downstream. NNN concentrations were higher at the upstream site in the Waipoua River (Fig. 2.3).

Figure 2.3: Line graph showing concentrations of nitrate-nitrite nitrogen for each sampling site over time. Surface water samples were collected from each site weekly over a 28-week period, between 19 November 2014 and 25 May 2015 (n=28). Water was analysed for nitrate nitrite nitrogen. Red triangles on the X-axis indicate that a flushing flow has occurred within 7 days of the sample date.

Dissolved inorganic nitrogen (DIN) concentrations, which are the sum of $\text{NH}_4\text{-N}$ and NNN, exceeded 0.1 mg/L at least once at all sites except H01, the site with the lowest *Phormidium* cover (Table 2.1). Water column DIN concentrations greater than 0.01 mg/L have been suggested as the required concentration for a

river to support *Phormidium* proliferations (although there are notable exceptions; (Wood et al., 2015b).

On a temporal scale, NNN concentrations gradually decreased over time in both the Hutt and Waipoua rivers (Fig. 2.3). Concentrations in the Hutt River increased in response to increased flow events, such as the flushing flow event on the 13 April 2015, when NNN concentrations increased 2-fold as a response to flows 10 times greater than the long-term median (Fig. 2.3).

Dissolved reactive phosphorus

Dissolved reactive phosphorus responded abruptly to high flow events, particularly that of the 1 April 2015, where concentrations in the Waipoua River at sites W01 and W02 reached 0.015 and 0.016 mg/L, respectively (Fig. 2.4; Appendix 4 Table 1). On a temporal scale, DRP concentrations did not show any change in both the Hutt and Waipoua rivers (Fig. 2.4).

Figure 2.4: Concentrations of dissolved reactive phosphorus for each sampling site over time. Surface water samples were collected from each site weekly over a 28 week period, between 19 November 2014 and 25 May 2015. Water was analysed for dissolved reactive phosphorus at Hills Laboratories (Hamilton). Red triangles on the X-axis indicate that a flushing flow has occurred within 7 days of the sample date.

In the Hutt River, DRP generally increased downstream, and ranged between 0.002 and 0.01 mg/L (Fig. 2.4). The lowest DRP concentrations occurred at H01 and the highest concentrations were measured at H04, the farthest downstream site in the Hutt River (Fig. 2.5). DRP concentrations in the Waipoua River ranged from 0.001 to 0.01 mg/L. DRP concentrations were generally lower in the downstream site in the Waipoua River, but were similar to the highest concentrations of DRP in the Hutt River (Fig. 2.5).

Figure 2.5: Concentrations of DRP for each sampling site. Surface water samples were collected from each site weekly over a 28-week period, between 19 November 2014 and 25 May 2015 (n=28).

Total ammoniacal nitrogen

Total ammoniacal nitrogen increased downstream in the Hutt River, ranging between 0.005 to 0.023 mg/L (Appendix 4, Table 1). A high rainfall event on the 1 April led to $\text{NH}_4\text{-N}$ concentrations of 0.074 mg/L in the Waipoua River, 8-fold higher than the average concentrations (Fig. 2.6). This was not as evident in the Hutt River sites, where $\text{NH}_4\text{-N}$ concentrations rose to 0.023 mg/L on the 13 April; 3-fold higher than the average. Samples were lower than the $\text{NH}_4\text{-N}$ detection limit of 0.005 mg/L on more than one occasion at all sites except for H04, the site with the greatest $\text{NH}_4\text{-N}$ concentrations, and the Hutt River site that was farthest downstream.

Figure 2.6: Concentrations of total ammoniacal nitrogen for each sampling site over time. The brown line signifies a spike in ammoniacal nitrogen concentrations as a result of high rainfall during April 15. Surface water samples were collected from each site weekly over a 28-week period, between 19 November 2014 and 25 May 2015. Water was analysed for total ammoniacal nitrogen. Red triangles on the X-axis indicate that a flushing flow has occurred within 7 days of the sample date.

Trace elements

Average elemental concentrations were generally highest at sites H01 and H04; the sites located furthest upstream and furthest downstream in the Hutt River, respectively. In general, the sites H02 and H03, which also experienced the highest *Phormidium* cover in the Hutt River, showed lower concentrations of all 20 elements analysed (Appendix 5, Table 1). In the Waipoua River, concentrations of all elements were below those of the Hutt River, with the exception of strontium (Sr) and potassium (K), which had the highest averages at sites W02 and W01, respectively.

Elements cited in previous studies associated with *Phormidium* include; iron, copper (Fe, Cu; Harland et al., 2013), sodium (Na), K and Sr (Na, K, Sr; Wood et al., 2015b). In the present study, there were no significant correlations between the maximum monthly *Phormidium* cover and monthly trace element concentrations. A marked increase in all elements at both H01 and H04 occurred on the 20 February 2015.

There was a significant difference between Fe, $p = 0.03$ and P, $p = 0.01$ at sampling occasions with no *Phormidium* and those with over 5% *Phormidium* cover. These elements were elevated at sites with the lowest *Phormidium* cover.

2.3.4 Within mat water analysis

Concentrations of $\text{NH}_4\text{-N}$ and DRP were between 40 and 257 times higher in the mat water than in the water column on the four sampling occasions (Table 2.2). Nitrate-nitrite nitrogen concentrations were lower in the mat water than in the water column, although the ratio of surface-to-within mat water was less than 1 at all sampling points.

Table 2.2: Nutrient concentrations collected from within mat water and surface water samples. Samples were collected on three separate occasions during February 2015 at three sampling sites only, as a lack of *Phormidium* mat growth limited the amount of within mat water collected.

		Total Ammoniacal Nitrogen (mg/L)			Nitrate-Nitrite Nitrogen (mg/L)			Dissolved Reactive Phosphorus (mg/L)		
Site	Date	Surface Water	Mat Water	Ratio	Surface Water	Mat Water	Ratio	Surface Water	Mat Water	Ratio
H03	10/02/2015	0.003	0.433	173	0.184	0.032	1	0.003	0.652	198
H02	17/02/2015	0.003	0.513	205	0.097	0.036	0	0.004	0.705	172
H03	17/02/2015	0.005	0.202	40	0.200	0.022	0	0.003	0.321	97
W01	18/02/2015	0.006	1.119	186	0.740	0.444	1	0.006	1.619	257

2.3.5 Historical data analysis

Phormidium cover

Analysis of historical *Phormidium* data shows that in both the Hutt and Waipoua rivers, there was a noticeable decrease in *Phormidium* cover at all sites monitored during the 2013-14 and 2014-15 summer growth periods (Fig. 2.7).

Maximum values over the seven-year period occurred in the Waipoua River during 2016 with an average of 20% *Phormidium* cover. This was five times greater than the maximum cover during the sampling period of the present study (Fig. 2.7). The maximum cover in the Hutt River was 76.75% cover in February 2012, four times greater than the maximum cover during 2014-15. The number of weeks a *Phormidium* proliferation was observed (the percentage of cover exceeding 20%) was the greatest in the 2011-12 sampling period, over a period of six weeks in both the Hutt and Waipoua rivers. In contrast to this, *Phormidium* proliferation occurred only during one week of the 2014-15 sampling period associated with this research.

Figure 2.7: Average *Phormidium* coverage over the past seven years. *Phormidium* cover is sampled by the Greater Wellington Regional Council over the summer growth period (November to March) each year. Cover is averaged over seven sites in the Hutt River and sampled at one site in the Waipoua River. Columns with a red outline indicate *Phormidium* cover observed as a part of this study. Data was obtained from the Greater Wellington Regional Council (2017). Error bars are based on the standard error.

Nutrient concentrations

Long term trends in nutrient data since 2009 show that the highest concentrations of NNN during the summer growth period (November to May) occurred in 2010-11, with average concentrations of 0.17 mg/L and 1.00 mg/L, respectively (Appendix 6, Fig. 1). There was negligible variability in NNN

concentrations in the Hutt River, but minimum concentrations occurred in the 2013-14 summer sampling period, with average values of 0.1 mg/L. This differed from the Waipoua River, where there was greater variability. The lowest concentrations were 0.42 mg/L and occurred during the 2011-12 sampling period.

Over a seven-year sampling period, the Hutt and Waipoua Rivers both had similar ranges of DRP during the summer growth period, with minimum values of 0.002 mg/L and maximum concentrations of 0.006 mg/L (Appendix 6, Fig. 2). Minimum values of DRP both occurred during 2015-16 in the Hutt and Waipoua River, whereas maximum values in the Hutt River occurred in the 2011-12 sampling year and in the 2009-10 sampling year in the Waipoua River (Appendix 6, Fig. 2).

Pearson's r correlation analysis of average monthly NNN and *Phormidium* cover shows that there was no significant relationship between these variables in both the Hutt and Waipoua rivers. Throughout the seven years of historical data, the NNN concentrations in the Waipoua River were at least three times the amount of NNN than in the Hutt River (Appendix 6, Fig. 1).

Flushing flows

Flushing flow frequency, which is defined as the number of flow events greater than 3 times the annual median per year in the Hutt and Waipoua rivers, was the lowest during 2014-15, when *Phormidium* cover was depleted (Fig. 2.8). The maximum flushing flow frequency in the Hutt River occurred in the 2011-12 summer growth period, when both the Hutt and Waipoua rivers experienced 33 flushing flow events (Fig. 2.8).

Figure 2.8: Average flushing flow frequency and *Phormidium* cover during the summer growth period (November to March) over the past seven years. Flushing flow, represented by columns is generated using the daily maximum flow and averaged over six sites in the Hutt River and sampled at one site in the Waipoua River. *Phormidium* cover, represented by lines, was observed on a weekly basis over the summer months (November to March). All data was obtained from the Greater Wellington Regional Council (2015). Error bars are based on the standard error.

Flushing flow frequency in the Waipoua River was at a maximum during the 2013-14 summer growth period, with 54 flushing flow events. During this summer, *Phormidium* cover dropped 6-fold compared to the previous year in both the Hutt and Waipoua Rivers, though the frequency of flushing flows in the Hutt River had not changed (Fig. 2.8).

There appears to be no relationship between *Phormidium* cover and flushing flow frequency in both the Hutt Waipoua rivers, which have an r^2 value of 0.06 and 0.09, respectively (Fig. 2.9). However, during the 2013-14 summer growth period, when *Phormidium* cover was at a minimum in the Waipoua River, flushing flow frequency is the greatest over the seven-year sampling period (Fig. 2.8).

Figure 2.9: Scatterplot showing annual average *Phormidium* cover and flushing flow frequency between 2009 and 2016. Flushing flow frequency is generated using the daily maximum flow averaged over six sites in the Hutt River and one site in the Waipoua River. *Phormidium* cover was observed on a weekly basis over the summer months (November to March). Points with a red outline indicate the *Phormidium* concentrations observed in this study. All data was obtained from the Greater Wellington Regional Council (2017).

2.4 Discussion

2.4.1 Physicochemical data and *Phormidium* cover

Phormidium mats are capable of growing successfully in a wide range of temperatures (Wagenhoff et al., 2013, Heath et al., 2011, House and Denison, 2002). However, proliferations are more likely to occur in the summer months, when temperatures are at an annual high (Biggs and Kilroy, 2000).

Historical data of monthly *Phormidium* cover in both the Hutt and Waipoua Rivers (Greater Wellington Regional Council, 2017), confirms that *Phormidium* does not proliferate during winter months. Conversely, no positive relationship exists between *Phormidium* cover and temperature changes. Wagenhoff et al. (2013) found that although *Phormidium* proliferations tend to occur in temperatures above 15 °C, there was no statistical correlation between the average *Phormidium* cover and water temperature. In the present study, all measurements of *Phormidium* cover exceeding 5% occurred in water

temperatures above 15 °C, and the sites with the highest *Phormidium* cover had moderate positive correlations with water temperature. This suggests that warmer water temperature may contribute to the magnitude and frequency of *Phormidium* proliferations in the Hutt and Waipoua rivers.

Wang and Yin (1997) found a strong correlation between surrounding urban landuse and relative conductivity. This may partly explain why average conductivity in the two downstream sites of the Hutt River was similar to the two sites in the Waipoua River, and the spatial variation of conductivity in the Hutt River showed an increase downstream. The downstream sites in the Hutt River and the two sites in the Waipoua River are both predominantly surrounded by urban land, where discharges to the stream could be more direct, and are likely to contain dissolved solutes attributed to industrial wastes. The upstream Hutt River sites, H01 and H02, where *Phormidium* cover was lowest, are located within forested land, where erosion is less prevalent and soils are more stable. It is also possible that saltwater input from the Hutt estuary may be contributing chloride ions to the water, increasing the conductivity at site H04. Trace concentrations of sodium were notably higher at the site H04, which is located 4 km upstream from the Hutt River mouth. This may explain why conductivity was the highest at this site, and suggests that the low *Phormidium* growth at this site may have been due to saltwater mixing. However, there is little understanding on the tolerance of *Phormidium* to water salinity, as well as the degree to which freshwater and saltwater mix in the Hutt Estuary.

The total dissolved solids (TDS) concentration did not markedly change over time, which indicates that soluble minerals capable of influencing *Phormidium* growth were constant. The decrease in TDS at sites H01 and H02 during December 2014 and an increase during March 2015 is likely to have been due to river flow. This is because runoff from point and non-point sources is likely to enter the river in elevated flow conditions, which contribute to the increase in soluble minerals (O'Conner, 1976).

The increase in dissolved oxygen over time may be attributable to the cooling of the water temperature. The present study found a slight decrease in dissolved oxygen in the warmer months and a notable increase in the dissolved oxygen

content at the beginning of April, when water temperatures dropped below 17 °C. The re-aeration of water may also increase the dissolved oxygen content of the water. According to Davies-Colley et al. (2013), re-aeration in faster flowing, more turbulent water is higher than in slower flowing or still water. As flow increased at all sites in both the Hutt and Waipoua rivers at the beginning of April, it is likely that the flow of the river was partly attributable to the increase in water column dissolved oxygen.

2.4.2 Nutrient and elemental concentrations and *Phormidium* cover

Phormidium proliferations are more likely to occur in low DRP (ca. <0.01mg/L) and elevated dissolved inorganic nitrogen (DIN, ca. >0.10 mg/L) water conditions (Wood and Bridge, 2014, Heath et al., 2013). Certain trace elements in the water column may have an impact on *Phormidium* growth, particularly sodium and potassium (Wood et al., 2015b).

Dissolved reactive phosphorus

The water column DRP concentrations in both the Hutt and Waipoua Rivers are relatively low on a national scale (LAWA, 2016). Abundant DRP degrades water quality and is a key driver of eutrophication (Mainstone and Parr, 2002). As a result of New Zealand soils being naturally low in phosphorus, approximately 91% of the annual phosphorus input to waterways is from diffuse agricultural sources (Gillingham and Thorrold, 2000). Runoff, eroding soils and sub-surface drainage are major pathways for fertilizer deposition in New Zealand rivers (Mainstone and Parr, 2002). As approximately 80% of the land in the Waipoua River catchment is pastoral, it is likely that agriculture is the main source of DRP at the W01 and W02 sites.

Other sources of phosphorus may include runoff from sewage treatment works and industrial waste (Mainstone and Parr, 2002). McDowell et al. (2004) noted that large amounts of phosphorus loss to waterways can occur in small areas of a catchment, termed critical source areas. An increase in DRP downstream in the

Hutt River may be attributable to this; in the lower reaches of the river, land surrounding the sites becomes increasingly developed, and the potential for point source contamination is higher. It is likely that the site H04 in the Hutt River is a critical source area, as this site experienced the highest concentrations of DRP and is also surrounded by highly urbanised land. Furthermore, lead concentrations 20 to 60-fold higher at this site suggest that industrial and residential waste is entering the waterway between the sites H03 and H04.

It is evident that surrounding land is a source of phosphorus in the water column at sites in both the Hutt and Waipoua Rivers. However, all monitored sites experienced DRP concentrations below 0.01 mg/L on all sampling occasions, which is generally considered limiting for algae growth (Wilcock et al., 2007). *Phormidium* may have physiological advantages which enable them to outcompete other algae in low-DRP conditions, including the ability to store phosphates due to luxury consumption (Kromkamp, 1987), as well as the facilitation of the release of phosphorus from fine sediments (Wood et al., 2015a, chapter 4, this thesis). This may partly explain why *Phormidium* mats were able to out-compete other algal species at sites with a higher mat cover (Section 2.3.1).

Nitrogen

Dissolved inorganic nitrogen, which is the sum of total $\text{NH}_4\text{-N}$ and NNN , has previously been investigated as a cause of *Phormidium* proliferations. In both the Hutt and Waipoua rivers, sites with the greatest *Phormidium* cover also possessed the highest concentrations of DIN. This is consistent with previous studies around New Zealand, which have suggested that a threshold of DIN greater than 0.10 mg/L is necessary for a proliferation to occur (Wood et al., 2015b Wood and Bridge, 2014, Heath et al., 2013, Wood and Young, 2012). *Phormidium* mats are unable to fix atmospheric nitrogen, so elevated DIN concentrations in the water column are essential for proliferation. Heath (2014) identified that under nitrate limited environments, *Phormidium* cells and growth rates were significantly reduced. NNN concentrations exceeded 0.10 mg/L on all sampling occasions at sites with the greatest *Phormidium* cover, an exception

being the site H04, which is located in the lower reaches of the Hutt River. This site had an average *Phormidium* cover of less than 1%, but DIN concentrations constantly exceeded the threshold suitable for a proliferation to occur. It is likely that *Phormidium* growth was inhibited at this site due to other factors, such as saltwater input, habitat suitability or elevated lead concentrations in the water column.

NNN concentrations in the Hutt River are relatively low on a national scale, but are elevated in the Waipoua River (LAWA, 2016). NNN concentrations are one of the key drivers of eutrophication and are exacerbated by agricultural and industrial sources (McDowell et al., 2009). Furthermore, Heath and Greenfield (2016) identified groundwater as one of the largest inputs of nitrogen in the Hutt River, particularly in the reach upstream of the H03 site, where the groundwater nitrogen concentrations were up to 10-fold higher than in the surface water column. It is likely that the elevated nitrogen concentrations at site H03 (Fig. 2.3) are attributed to the upwelling of groundwater, which provides nutrients to *Phormidium* mats in the early stages of development.

Sources of elevated nitrogen in the Waipoua River are most likely a combination of runoff from surrounding pastoral land and upwelling of groundwater from the confined aquifer system in the Wairarapa Valley Basin. The Waipoua River catchment is surrounded by pastoral land (80%) and, with low to moderate soil fertility, is heavily fertilised for agricultural purposes (Chrystall, 2007). Though sources of nitrogen from the groundwater have not been identified, major spring systems are connected to the Waipoua river, and that the river may gain between 100-200 L/s of groundwater in reaches upstream of the Masterton fault sampling sites (Gyopari and McAlister, 2010). Lower flows and increased pathways for overland flow in this river may be reasons why nitrogen levels were elevated in the Waipoua River, as inputs of nitrogen would have been less diluted than inputs entering the larger Hutt River. This may also be why NNN concentrations responded more abruptly to elevated flows, which is most likely to have been a consequence of runoff from agricultural land after heavy rainfall (Fig. 2.3).

Trace elements

Although certain elements can promote *Phormidium* proliferations, a correlation between trace element concentrations in the water column and mat cover was not found. Harland et al., (2013) found that Cu and Fe limited the growth of cyanobacterial cells. Similarly, in the present study, though Cu was not significantly correlated with *Phormidium* cover, Fe and P were significantly elevated at sites with low *Phormidium* cover (Appendix 5, Table 1). However, caution must be considered when analysing water column trace element concentrations and *Phormidium* cover, as this does not show a causative relationship. It is unlikely that *Phormidium* mats would be affected directly by elemental concentrations in the water column, as concentrations are relatively low compared with studies investigating periphyton response. For example, Harland et al., (2013) identified that changes in cell growth were observed only in unrealistically high concentrations of Cu and Fe, whereas growth was not restricted under realistic concentrations.

Wood et al., (2015a) identified elevated Fe and P concentrations within *Phormidium* mat water, as a result of conditions conducive to the release of these elements from entrapped sediment. In the present study, elements within *Phormidium* mat water were not analysed, and further investigation is needed to understand whether mats in the Hutt River are directly affected by the concentration of trace elements. Growing *Phormidium* mats in a controlled environment such as mesocosm flow channels (Chapter 4) may enable the analysis of a causative relationship between trace elements and *Phormidium* biomass.

2.4.3 Within mat water analysis

Thick, cohesive *Phormidium* mats are able to create an isolated environment conducive to the release of phosphorus from sediments as a result of elevated pH (as a result of daytime photosynthetic activity) and reduced dissolved oxygen (a result of night-time respiration) (Wood et al., 2015a, House and Denison, 2002) (see chapter 4 for further discussion). Elevated DRP concentrations in

Phormidium mat water in the present study suggests that at least some bioavailable phosphorus was released from fine sediment trapped underneath and within *Phormidium* mats, creating conditions optimal for growth. This can be compared to similar research, (Wood et al. 2014a), which found mat-water DRP concentrations 300-fold higher than bulk river water. This theme will be further developed in Chapter 4, where different treatments of fine sediment addition are exposed to *Phormidium* mats in a controlled environmental setting.

NNN concentrations were lower in the mat water than in bulk river water. This may be attributed to the diffusive boundary layer, which restricts the exchange of nutrients between the bulk water and the *Phormidium* mat matrix (Stewart, 2003). The decrease in nitrogen within the mat may be due to the uptake of nutrients by the biofilm in conjunction with the restriction of NNN entering the mat through diffusion. A notable increase in $\text{NH}_4\text{-N}$ in *Phormidium* mat water may be due to the nitrogen-fixing bacteria growing in the mat matrix. Heath (2014) noted that most *Phormidium* species do not possess nitrogen fixing genes and cannot fix atmospheric nitrogen. However, McAllister et. al. (unpub. data) indicated that nitrogen fixing bacteria may be present in some developed *Phormidium* mats. As mat-water in this study was collected from well-developed mats only, it is possible that nitrogen-fixing bacteria were present and able to fix atmospheric nitrogen from inside the *Phormidium* mat. Due to the diffusive boundary layer, $\text{NH}_4\text{-N}$ was released back into the water column slower than it was being fixed, creating a higher concentration inside the mat.

2.4.4 Comparisons of historical data

Of all the rivers monitored throughout the Wellington Region, the Hutt and Waipoua Rivers have experienced the highest *Phormidium* cover over the past seven years (LAWA, 2016). Research on a national scale has suggested that rivers with the most widespread proliferations tend to be on the eastern side of New Zealand, in the rain shadows of mountain ranges (McAllister et al., 2016). This theory supports findings from the Waipoua River, which is sheltered from prevailing winds by the Rimutaka and Tararua Ranges. The Hutt River is located

towards the west of the North Island, but its headwaters are in the Tararua Ranges and the lower reaches are somewhat sheltered by easterly prevailing winds (Ballinger et al., 2011). Seven-year monitoring data of *Phormidium* cover in the Hutt and Waipoua rivers has shown that there was a notable decrease in *Phormidium* cover in both the Hutt and Waipoua River during the 2012-13 summer sampling period, and generally low cover during the 2014-15 sampling period too. However, during the 2015-16 summer growth period, *Phormidium* growth was prevalent.

River flow

Heath and Greenfield (2016) and Heath et al. (2011) found that in the Hutt River, decreased flow had a negligible effect on the cover of *Phormidium* mats, and the frequency of flushing flow events was more important in regulating the extent of *Phormidium* proliferations. An increase in flow velocity can remove algae through elevated shear stress, abrasion from mobilised sediment and grinding action on the cobbles where algae grow (McAllister et al., 2016, Francoeur and Biggs, 2006). Clausen and Biggs (1997) demonstrated that velocities three times the median flow were enough to reduce average periphyton biomass. This has been adopted as the general recommendation for the removal of *Phormidium* mats in New Zealand rivers and is described as a 'flushing flow' (Milne and Watts, 2007). However, Wood et al. (2016) identified that this rule does not always apply, and is relatively site specific. Varying factors such as substrate heterogeneity, the amount of stable substrate and development stage of *Phormidium* mats are all important factors when considering flow as a cause of mat dispersal. Wood et al. (2016) identified flows between 0.7 and 16.5 times the median flow capable of reducing *Phormidium* cover to below 20%.

Analysis of the frequency of flushing flows over the past seven years has shown that no relationship exists between average *Phormidium* growth and the frequency of flushing flows during the summer growth period in both the Hutt and Waipoua rivers. However, flushing flow does appear to play a part in the reduction of *Phormidium* cover in the Waipoua River during the 2013-14 summer

growth period. This may be due to the higher frequency of flushing flow events during this period.

Though flushing flow frequency may play a role in the reduction of *Phormidium* cover in certain years, it does not explain the low cover during the 2014-15 sampling period associated with this study, where there was a decrease in flushing flow events. It is likely that the frequency of flow needed to significantly reduce *Phormidium* mats is site specific and more analysis is needed. Data observed in the Manawatu River (Wood et al., 2016, Wood et al., 2014b) has shown that a flushing flow of three times the long-term median was enough to remove *Phormidium* mats at some sites, but at other sites this was not the case. This may have been occurring in the Hutt River, and an analysis of the intensity of flow to reduce *Phormidium* cover in the future could help to understand the variability of cover in each summer growth period. *Phormidium* accrual cycles also play a role in the reduction of cover as a response to elevated flows. At early stages of development, mats are more strongly adhered to the substrate and may require flow velocities of greater than three times the median flow to reduce *Phormidium* cover. As mats become more developed, oxygen bubbles form underneath the rock, reducing its strength of attachment to the substrate. Therefore, it may be a combination between the timing of flushing flows as well as the frequency, which may cause a reduction of *Phormidium* mats.

Nutrient concentrations

In the Waipoua River, DRP and NNN concentrations follow a similar trend. Nutrients generally decrease until the 2013 and 2014 summer, where both phosphorus and nitrogen become elevated. During this period, *Phormidium* cover also decreased in abundance. *Phormidium* cover showed an increasing trend in the past five years, as NNN and DRP decrease (Appendix 6, Fig. 1, 2). This is contradictory to previous studies that have indicated elevated NNN concentrations are required in the early stages of mat development. (Heath, 2014, Wood and Bridge, 2014).

Increased DRP concentrations may enable other algal species to out-compete *Phormidium*. A doubling of *Phormidium* mats in both the Hutt and Waipoua Rivers during the 2015-16 sampling period corresponds to the lowest concentrations of DRP (Appendix 6, Fig. 2). This indicates that *Phormidium* mats have physiological advantages, such as the luxury uptake and storage of phosphorus (Kromkamp, 1987) as well as the ability to create conditions conducive to the release of phosphorus from trapped sediment (Chapter 4).

Although there is no long term significant relationship between flushing flow events and *Phormidium* cover in the Waipoua River, flushing flows can be a short-term driver of *Phormidium* cover through elevated nutrient concentrations. Higher flow velocities correspond to an increase in nutrient concentrations, as runoff from diffuse sources is more likely (Biggs and Close, 1989). This was seen in the Hutt and Waipoua rivers during the present study, as flushing flow events corresponded with elevations in nutrient concentrations (Chapter 2, Section 2.3.2). During the 2013-14 growth period, when *Phormidium* abundance was low, the frequency of flushing flows was approximately eight times greater than the previous sampling year, when *Phormidium* cover was over double the amount. Therefore, although nutrients were available for growth in the Waipoua River during the 2013-14 summer growth period, *Phormidium* was unable to establish and proliferate as elevated flow prevented mats from establishing.

The growth of *Phormidium* mats during this study period was notably lower than previous years, with an exception of the 2013-14 summer, where average cover was less than 5% in both the Hutt and Waipoua rivers. *Phormidium* cover exceeded the 'amber mode' alert in the national guidelines for cyanobacteria only once over a 28-week monitoring period. A reduction in *Phormidium* cover during this sampling period requires further investigation into site specific flow and nutrient concentrations, as well as *Phormidium* accrual cycles. The higher frequency monitoring (weekly) suggests that temperature and surrounding landuse all influence *Phormidium* cover. Water collected from *Phormidium* mats suggests the utilisation of fine sediment as a source of DRP was occurring.

Chapter 3

Does fine sediment provide a source of phosphorus for *Phormidium* growth in the Hutt and Waipoua rivers?

3.1 Introduction

Sediment deposition in fluvial systems is influenced by particle size and density, as well as flow velocity, surrounding land-use and river channel gradients (Harden, 1993). River maintenance for flood protection activities may also lead to the release of fine sediment causing sediment plumes to form in the water column (Cameron, 2013).

Well-developed *Phormidium* mats commonly have a fine layer of sediment at the substrate and mat interface. Reasons for this may be that suspended sediment becomes trapped by the extracellular polysaccharide layer (Frantz et al., 2015) secreted by *Phormidium* cells and incorporated into the thick mat matrix by motile filaments (Wood et al., 2015a, McAllister et al., 2016). *Phormidium* mats contain a diffusive boundary layer (Stewart, 2003) that restricts the flow of materials from the bulk water into the biofilm and creates an isolated environment within the mat. Recent studies (Wood et al., 2015a, Wood et al., 2014a) have shown that an increase in the pH due to daytime photosynthesis (Dodds, 2003, Boers, 1991) and a reduction of dissolved oxygen due to respiration at night (Stumm and Morgan, 2012) results in the desorption of bioavailable phosphorus from trapped sediments. Collectively, these studies suggest that fine sediment may be a source of phosphorus for *Phormidium* in New Zealand rivers. This chapter summarises results from a sediment trap investigation carried out during February 2015, which coincided with baseline monitoring. Key research objectives associated with this investigation were:

1. To determine if the deposition of fine sediment in the Hutt and Waipoua rivers is related to *Phormidium* abundance by observing sedimentation rates at four sites in the Hutt River and two sites in the Waipoua River. This was completed over a three-week period using sediment traps and size fractionation. Baseline water quality monitoring and observations of *Phormidium* cover abundance were carried out alongside the sediment trap study.
2. To identify a relationship between the bioavailability of sediment bound phosphorus and *Phormidium* abundance using phosphorus speciation. Three forms of biologically available phosphorus were extracted from three sediment sizes collected in the sediment traps. These were: loosely bound, which is available to cyanobacteria under relatively neutral conditions; reductant soluble, which is bound to iron-hydroxide and manganese-oxide compounds and released under low dissolved oxygen conditions; and metallic-oxide bound, which is connected to iron and aluminium oxides (Lukkari et al., 2007, Holtan et al., 1988, Wood et al., 2015a).

3.2 Methods

3.2.1 Sedimentation rates and size fractionation

Nine sediment traps were deployed at each of four sites in the Hutt River and two sites in the Waipoua River (total 54 sediment traps) on the 28 January 2015. Sediment trap deployment and analysis followed the methods of Wood et al. (2014a).

Each sediment trap, consisting of a 2.2-L plastic container (Sistema, New Zealand), was filled with large cobbles at the bottom and, after washing to remove excess fine sediment, traps were filled with small cobbles and gravel closer to the top. Sediment traps were then placed in the bed of the river with the lids on, such that the top of the trap was level with the river bed (Fig. 3.1).

Once all sediment traps had been placed in the riverbed, starting with the downstream traps, the lids were removed and large cobbles were placed around the edge of each trap to keep it in place. One week after deployment, starting downstream, three traps were collected every seven days from each site for three weeks (21 days) on the 11, 18 and 25 of February 2015, coinciding with baseline monitoring. Sediment traps were refrigerated until analysis (4°C).

Figure 3.1: Sediment Trap in position within the river bed. (a) Pink tags were tied to the trap edges to help locate the traps after a week's deployment. Photo taken in the Hutt River, Feb 2015. A schematic showing the position of sediment traps in the riverbed (b) Traps were deployed in sets of three over a three-week period from 11 to 25 February 2015.

To carry out size fractionation, the contents of the sediment traps were firstly washed over 2-mm sediment sieves into 20-L buckets. Cobbles and gravels with a grain size larger than 2 mm were discarded, and the remaining sediment was left in the buckets for five days to allow fine sediment to settle. Water was siphoned out of the buckets, and the remaining gravel and sediment was oven dried at 105°C for 3 days, hand-sieved using decreasing mesh sizes (500 µm, 250 µm, 125 µm, 63 µm, retaining the fraction <63 µm) and weighed.

Sedimentation rates were calculated using the following equation:

Equation 3.1

$$\text{Sedimentation} = \left(\frac{W}{A} \right) / D$$

Where:

- W is the total weight of dried sediment for each size fraction in each sediment trap.
- A is the surface area of the sediment trap,
- D is the number of days each sediment trap was deployed.

Relationships between sedimentation rates and flow velocity were examined using a Pearson's correlation test was carried out using averages of the weekly flow prior to the sampling date. Flow data was obtained from Greater Wellington Regional Council flow gauges (Greater Wellington Regional Council, 2017).

3.2.2 Sequential extraction of biologically available phosphorus

Three 0.5-g samples were taken from a pool of the nine sediment traps collected from each of six sites sampled. The three pooled samples were taken from the three smallest sizes fractionated (3 sediment sizes $\times$ 3 pooled samples from one site $\times$ 6 sites = 54 samples). The smallest grain sizes of 125 μm , 63 μm and <63 μm were analysed as these are the most likely to be incorporated into developed *Phormidium* mats (Wood et al., 2015a). The two larger size fractions of 500 and 250 μm were not analysed for phosphorus content as they are unlikely to be trapped by EPS secreted by *Phormidium* mats (Chapter 1, Section 1.3).

The forms of biologically available phosphorus (BAP) extracted were: loosely bound phosphorus, reductant soluble phosphorus and metallic oxide-bound phosphorus. These forms of phosphorus are considered available for plant growth (Lukkari et al., 2007). As this analysis was carried out to investigate whether phosphorus bound to sediment was being facilitated by *Phormidium* for growth, other forms of phosphorus such as calcium carbonate-bound and apatite-bound are irrelevant as they are considered non-reactive and therefore not bioavailable (Psenner and Pucsko, 1988). The following methods convert these three forms to DRP, which is analysed using molybdenum blue colorimetry (Rice et al., 2012) at Hils Laboratory, Hamilton, with accredited detection limits of 0.001 g/m³.

Extraction of loosely bound phosphorus from each replicate was carried out by adding 40 mL of 0.46-M sodium chloride (NaCl) to 50-mL Falcon tubes containing the sediment, and leaving to extract for one hour at 30°C whilst shaking at 200 RPM. Falcon tubes containing the sediment and NaCl were then centrifuged for 30 min at 3200 ×g, the supernatant liquid was suctioned off and filtered at 0.45 µm. Samples of the supernatant were frozen until analysis of DRP.

Extraction of reductant soluble phosphorus was carried out by adding a 40-mL solution of 0.11-M sodium dithionite (Na₂S₂O₄) to a 50-mL Falcon tube containing the sediment left over from loosely bound phosphorus extraction. This solution was left to extract for 1 hr at 40°C whilst shaking at 200 RPM. Falcon tubes were again centrifuged for 30 min at 3,200 ×g and the supernatant was filtered at 0.45 µm. The supernatant was then bubbled with compressed air to remove the sodium dithionite and filtered at 0.45 µm for a second time to remove precipitate. Samples were frozen until analysis of DRP.

Metallic oxide-bound phosphorus was extracted from the sediment residue from reductant soluble phosphorus by adding 40 mL of 0.1-M sodium hydroxide (NaOH) to a 50-mL Falcon tube and leaving to extract for 18 hr at 40°C with shaking at 200 RPM. Samples were then centrifuged for 30 min at 3,200 ×g and filtered at 0.45 µm. After the pH of these samples was adjusted using hydrochloric acid (HCl), the samples were frozen until analysis.

Total BAP extracted from each grain size sample was calculated to analyse the total amount of phosphorus available to *Phormidium* mats. This was carried out by calculating the sum of DRP, regardless of phosphorus species for each sediment grain size per site.

3.3 Results

3.3.1 Sedimentation rates

The average sedimentation rate in the Hutt River was approximately seven-fold greater than the Waipoua River. The range of sediment deposition in the Hutt River was between 6.6 and 272.0 g/m²/day (Appendix 7, Table 1). The minimum

rate of sedimentation occurred at the furthest upstream site (H01), during the first sampling week, and the maximum in the middle reaches of the Hutt River at Melling Bridge, also during the first sampling week. Sediment deposition in the Waipoua River ranged between 8.13 and 17.15 g/m²/day (Appendix 7, Table 1).

In the Hutt River, sediment deposition decreased with each size fraction (Fig. 3.2). The largest size fraction observed (500 µm) was the most abundant at all sites in both the Hutt and Waipoua rivers. Sediment particles sizes between 125 and 63 µm were the least abundant in the Hutt River, with average deposits of 2.13 g/m²/day. In contrast, the smallest fraction size (< 63 µm) was one of the most abundant size fractions deposited in the Waipoua River, with 3.12 g/m²/day (Appendix 7, Table 1).

Figure 3.2: Average sedimentation rates of three different fraction sizes, monitored at six sites over a three-week period, between the 4 and 20 February 2015. Sedimentation is averaged between three traps per site deployed each week over a three-week period. Sediment traps at site H04 during the first week of deployment (W1) were washed away by vehicles crossing the riverbed, therefore sedimentation data was not observed. Green points represent the river flow based on the weekly average flow prior to the sampling date, data was obtained from Greater Wellington Regional Council flow gauges (Greater Wellington Regional Council, 2017). Error bars are based on the standard error.

Sedimentation decreased over the three weeks in the Hutt River and did not show a notable change in the Waipoua River (Fig. 3.2). Weekly average flow (determined using historical monitoring data, Chapter 2, Section 2.2.4) in the

Hutt River was notably higher, ranging from 4.4 to 1.0 m³/second compared to the Waipoua, which ranged from 0.3 to 0.7 m³/second. There was a strong positive relationship between the sedimentation rate of the three smallest size fractions and average river flow, with r² values of 0.86, 0.89 and 0.97, for size fractions 125, 63 and < 63 µm respectively.

3.3.2 Sequential extraction of biologically available phosphorus

The smallest sediment fraction (< 63 µm) contained the greatest amount of BAP at all sites (Fig. 3.3).

The site that contained the highest BAP concentration was H03 in the middle reaches of the Hutt River, which contained a dry weight sum of 356, 507 and 570 µg P g⁻¹ dw for 125, 63 and < 63 µm size fractions, respectively (Fig. 3.3). Total amounts of BAP increased downstream in the Hutt River, but stayed relatively similar at the two Waipoua River sites. Site H01, the furthest upstream site in the Hutt River, contained the least BAP, with 45, 167 and 272 µg P g⁻¹ dw for 125, 63 and < 63 µm size fractions, respectively (Fig. 3.3).

Figure 3.3: Concentrations of total BAP bound to three different sediment size fractions. This graph shows the sum of the three forms of phosphorus analysed. Sediment was collected over a three-week period between 4 to the 20 February 2015. Error bars are based on the standard error.

Regardless of size fraction, the metallic-oxide bound phosphorus form of sediment was the most abundant at all sampling sites, excluding H01 and H03. This was followed by the reductant soluble and loosely sorbed forms of phosphorus (Fig. 3.4). At the sites H01 and H03 in the Hutt River, metallic-oxide bound phosphorus was the least abundant form.

Figure 3.4: Concentration of three different phosphorus species, for all three size fractions combined, at each sampling site. Sediment was collected over a three week period between 4 to the 20 February 2015. Error bars are based on the standard error.

Loosely bound and reductant soluble phosphorus showed a positive relationship with *Phormidium* cover, an exception of this being site H04. Metallic oxide-bound phosphorus increased downstream with a maximum concentration at H04, the site with the least *Phormidium* cover in the Hutt River.

3.4 Discussion

3.4.1 Sediment deposition and river flow

Sediment deposition in the Hutt River is strongly related to average flow velocity as well as the magnitude of flood events (Wood et. al., 2016; Clausen and Biggs 1997). This was reflected in the present study, which showed that flow in the Hutt River was strongly correlated to the average sedimentation rates of

fractions below 125 μm . This is also demonstrated by the size fractions of sediment deposited. Larger rivers have a greater capacity to transport larger grain sizes, as the shear stress is greater and velocity is generally higher. The 500 μm size fraction comprised over 60% of the total sediment deposited in the Hutt River at all sites, whereas in the Waipoua River this fraction size made up less than 50% of the total sediment (Appendix 7, Table 1). Further research is needed to understand the sources of sediment in the Waipoua River, but investigations based around the Wairarapa Valley show that the average streamflow is notably lower than the Hutt River for several reasons. The average rainfall in the Waipoua River catchment is lower due to orographic rainfall effects (Chrystall, 2007), and during the summer months, the river flow is lost to the groundwater system (Gyopari and McAlister, 2010). It is likely that the lower sediment deposition observed in this river was attributable to the differences in flows between rivers.

3.4.2 Sediment deposition and landuse

Landuse can have a marked effect on runoff and sedimentation characteristics in riverine systems (Harden, 1993, Wood et al., 2016, Allan, 2004). The upper catchment areas of the Hutt and Waipoua rivers are predominantly surrounded by indigenous forest, which contributes to a reduction of runoff due to higher evapotranspiration rates and increased soil porosity. However, the lower parts of both catchments are dominated by pastoral and urban landuses (Ballinger et al., 2011). Both of these may result in an increase in the flow response to precipitation events as surface sealing as well as a lack of evapotranspiration and infiltration leads to a greater amount of overland and return flow following high rainfall (Hundecha and Bárdossy, 2004). Data from this study reflects this as in the Hutt River, sedimentation was greater in the lower reaches and more urbanised areas of the Hutt River (Fig. 3.2). In the upper catchment, where landuse is predominantly forest and less prone to erosion sedimentation rates are much lower. In the Waipoua River this is not as evident as sedimentation is notably lower, even though the sites are surrounded by urban land. The

differences in sediment deposition between the two rivers is most likely due to the differing flow regimes, as a result of the diversity in landuses.

3.4.3 Sediment deposition and *Phormidium* cover relationships

The amount of sediment deposited in the Hutt River followed similar patterns to the spatial distribution of *Phormidium* proliferations. Sedimentation generally increased downstream in the river in all size fractions, an exception of this being site H04, the site with the lowest *Phormidium* cover. The site with the greatest sediment deposition was H03, the site with the highest average *Phormidium* cover. This supports the hypothesis that sediment provides a source of phosphorus for *Phormidium*, and a relationship between increased sedimentation rates and the prevalence of *Phormidium* proliferations (Wood et al., 2014a). Wood and Bridge (2014) identified a relationship between the quantity of deposited sediment and sites with *Phormidium* proliferations in the Maitai River (Nelson, New Zealand). It is likely that, during the three-week deployment period, phosphorus bound sediment was providing more nutrients for growth at the H03 site.

In the Waipoua River, there was not a strong relationship between *Phormidium* abundance and sedimentation. However, the deposition of the smallest size fraction (<63 µm) at both sampling sites in this river made up over 20% of the total deposited sediments, as opposed to only 2 to 6% in the Hutt River. Therefore, it is possible that, although there is less total sediment deposited in the Waipoua River, a greater amount of sediment is available for capture by *Phormidium* mats and therefore the potential of BAP release is greater. This may explain why total sedimentation in the Waipoua River is not consistent with the variation in *Phormidium* abundance.

3.4.4 Sequential extraction of phosphorus

Sediment size and total phosphorus

A common feature of most *Phormidium* mats is a fine layer of sediment between the mat and rock interface (Frantz et al., 2015). These particles may become captured and incorporated into the mat matrix by extracellular polysaccharide (EPS) secretions (Frantz et al., 2015). In this study, the smallest fraction size analysed (<63 µm) contained approximately 45% of the total BAP concentrations at all sites. This is consistent with previous studies, which suggest that the potential for river sediment to retain trace elements is higher in smaller sediment sizes, due to an increased surface area (Horowitz and Elrick, 1987). In the Mangatainoka River, fine sediment below 125 µm had almost double the amount of sediment bound phosphorus than the larger size fractions (Wood et al., 2014a, Wood et al., 2015a). Sediment grain sizes below 63 µm are more likely to be incorporated into a *Phormidium* mat (Wood and Bridge, 2014, Wood et al., 2014a, House and Denison, 2002), and microscopic analysis identified particles separated from the mats were sized between 1-5 µm (Wood et al., 2015a). It is likely that the sediment deposited in the Hutt and Waipoua Rivers is acting as a source of phosphorus for the growth of *Phormidium* mats; this is particularly evident at sites with the highest cover (Fig. 3.2).

In the present study, a positive relationship exists between the amount of BAP bound to fine sediment and *Phormidium* cover, suggesting that the potential for phosphorus uptake by mats at these sites is greater. A notable example of this is at the site H03 in the middle reaches of the Hutt River, which contains the highest average *Phormidium* cover. In all three size fractions monitored, there was an almost 50% increase in total BAP in the 5.4-km reach between H03 and the site directly upstream, H02 (Fig 3.3). According to Ellison and Brett (2006), differing concentrations of BAP may be attributable to surrounding landuse. They demonstrated that, under base flow conditions, the percentage of BAP bound to fine sediment was substantially higher (a mean of 73%) in urbanised streams, compared with agricultural and forested streams (13-29%). In the Waipoua River, though the catchment area is predominantly pastoral land, the sites W01 and W02 are located close to the Masterton township. Furthermore, McDowell et

al. (2016) demonstrated using sediment fingerprinting methods, that the mobilisation of phosphorus from soils was much greater within developed catchments.

Sediment fingerprinting has been used to determine the sources of river sediments in terms of topsoil, subsoil, bank sediments and bed sediments (McDowell et al., 2016). It may be useful in future studies to utilise this method in the Hutt and Waipoua Rivers and determine the sources of sediment deposited at sites with a greater *Phormidium* abundance. The inputs of sediment at sites with a history of more frequent *Phormidium* proliferations could be from different sources than sites experiencing little or no proliferation, which is why they contain differing amounts of BAP. The surrounding landuse of sites with the most *Phormidium* cover is highly urbanised in the Hutt River and predominantly pastoral and urban in the Waipoua River. Site H03 of the Hutt River runs between two large golf courses and Moonshine Valley, an area where major forestry activities are carried out. These landuses are fairly land intensive and are likely to contribute to the sediment load in the Hutt River. The sites with less *Phormidium* cover are predominantly surrounded by forested areas, although site H02 is heavily developed on one side of the river. Different landuses, which contribute different sources of sediment, may partially explain the increase in BAP attached to fine sediment at these sites. This also explains why sites H03, W01 and W02 experienced a higher cover of *Phormidium* mats, and potentially why *Phormidium* is able to proliferate so frequently.

Phosphorus Speciation

In both the Hutt and Waipoua Rivers, the most abundant form of phosphorus was the metallic-oxide bound, which is also the only fraction that doesn't have a positive relationship with *Phormidium* growth. This was consistent with recent studies in the Mangatainoka River, where this form of phosphorus comprised almost half of the phosphorus bound to fine sediment at the majority of monitored sites (Wood et al., 2014a, Wood et al., 2015a).

The greatest amount of metallic-oxide bound phosphorus occurred at H04 in the Hutt River, which also had very low *Phormidium* cover and elevated iron concentrations. This was an exception to the other two species of phosphorus analysed, which were notably lower at H04 than at other sites (Fig 3.4). The reason for this may be due to the concentrations of elements in the water column H04, which may have increased the potential for mineralisation of phosphorus to fine sediments. Elemental analysis carried out monthly during the summer of 2014-15 (Chapter 2, Section 2.3.3) showed that the highest concentration of Fe in the bulk river water were measured at H04. This suggests that, either the capacity for river sediments at this site to retain trace elements is greater (Horowitz and Elrick, 1987), or that there is a higher input of sediment upstream of this site.

The other two forms of phosphorus analysed had a positive relationship with *Phormidium* abundance and, similar to sediment deposition, the greatest amounts of loosely sorbed and reductant soluble phosphorus were at H02 in the Hutt River and the two sites of the Waipoua River, W01 and W02. This suggests that the solubilisation of iron, aluminium and magnesium oxides is taking place within developed *Phormidium* mats at this site, which leads to the mobilisation of phosphorus. However, it must be acknowledged that evidence provided in this chapter is circumstantial, and though a correlation exists between *Phormidium* mat cover and phosphorus-bound sediment deposition, this does not prove a causative relationship.

Chapter 4

Investigating the relationship between fine sediment deposition and *Phormidium* growth

4.1 Introduction

Fine sediment can enter river systems through surface runoff during rainfall events, as well as from bank side soil erosion (Maas, 2010). Fine sediment might contribute to *Phormidium* proliferations (Chapter 3, Wood et al., 2014a, Wood et al., 2015a). Thick, cohesive *Phormidium* mats exclude oxygen produced by photosynthesis, which allows specific geochemical conditions, such as low dissolved oxygen and high pH, to form. These conditions may enable sediment bound phosphorus entrapped within the mats to desorb and become biologically available, in part explaining why *Phormidium* proliferations can occur in rivers with relatively low water column DRP concentrations.

This chapter summarises the results of an experiment carried out at the Cawthron Institute in Nelson during April, 2016. The experiment was one of the first to investigate the causative effects of sediment deposition on *Phormidium* growth in a controlled setting, where a river environment was simulated using mesocosms.

The key research objective addressed in this chapter is:

1. To confirm observations that sediment deposition can enhance *Phormidium* growth in New Zealand Rivers.

4.2 Methods

To analyse the relationship between fine sediment deposition and the development and expansion of *Phormidium* mats, four sets of mesocosms

(Fig. 4.1) were set up and monitored from 7 to 25 of April 2016, at the Cawthron Institute in Nelson. Mesocosms were designed to simulate, as much as possible, a river environment. Using a controlled environment eliminates variables that may change if the experiment were carried out in a river (for example, flow or the presence of macroinvertebrates) and it also enables the manipulation of single or multiple factors.

Mesocosms were originally set up in the Hutt River, but sampling was unsuccessful due to the lack of a suitable site with a steep slope gradient, which prevented the siphon water flow system from working, as well as high flow events, construction works at the sampling site and health and safety concerns. Therefore, this experiment was carried out in Nelson using water from the Maitai River and sediment collected from the Hutt River. The Maitai River, which is the largest in the Nelson Region, experiences *Phormidium* proliferations during the summer months and has been the focus of research over the past three years (Wood et al., 2015b).

The experiment consisted of four sets of four recirculating flow-through channels (Fig. 4.1), hereafter termed mesocosms. Different amounts of fine sediment were added to each mesocosm. Sediment was collected from the lower reaches of the Hutt River at Belmont (41°11'36" S, 174°55'40" E) during March, 2016. Sediment was dried in an oven for 3 days at 150°C and sieved using a sieve shaker (Octagon 200CL, Endecotts) through a mesh size of 63 µm prior to the experiment. Sediment particles with a size greater than 63 µm were discarded. This smaller size fraction (< 63 µm) retained the greatest amount of BAP, and that this sediment size is the most abundant within *Phormidium* mats (Wood et al., 2014a, Wood et al., 2015a, Wood et al., 2015b).

4.2.1 Mesocosm Set-up

Mesocosms consisted of plastic 25-L header tanks (Fig. 4.1, A; Storage Box) with four ball valves (Fig. 4.1, B) that drained into channels made from plastic gutter piping with a width of 10 cm, length of 1 m and depth of 7.5 cm (Fig. 4.1, C).

Plastic, 50-L tanks (Storage Box) were placed at the bottom of the channels to catch the outflow (Fig. 4.1, D).

Figure 4.1: Mesocosm chambers in place at the Cawthron Institute, Nelson. Photo taken April 2016, showing water flowing from header tanks (A) through ball valves (B) to the channels containing inoculated cobbles (C) and flowing into the outflow tanks (D) containing submersible pumps to pump water back to the header tanks.

Water collected at one site in the lower Maitai River (41°16'22" S, 173°17'37" E) was transferred into the header tanks, which flowed into the four channels before discharging into the outflow tanks. The mesocosms were recirculating systems, where water was pumped back into header tanks using submersible pumps (Resun, Model King-4). Black plastic shade cloth was placed over the header and outflow tanks to minimise evaporation as well as limit the increase in water temperature during the day (Fig. 4.1). Constant flow was maintained by adjusting the ball valves as required. Flow discharging from the channels was measured every second day. To account for the effects of evaporation and leakage of water, each mesocosm was topped up with approximately 10 L of river water every other day. Mesocosms were also spiked with nitrate solution on the 4, 11 and 18 April 2016, to mitigate nutrient limitation due to the uptake of nitrate by biological growth.

Cobbles with surface areas between 25 cm² and 120 cm² were collected from the lower Maitai River. To ensure each of the rocks started with the same minimum amount of inoculum, holes with a 5-mm diameter and approximately 5-mm depth were drilled into the upper half of each cobble and filled with a portion of *Phormidium* mat (Fig. 4.2).

Figure 4.2: Cobble showing a 5-mm diameter hole which was filled with a portion of *Phormidium* mat. This was carried out to ensure that each cobble placed in the channels was allocated the same minimum amount *Phormidium* inoculum.

Due to a recent flushing flow event, *Phormidium* mats were not abundant enough to collect in the Maitai River. Therefore, mats were collected in the middle reaches of the Hutt River at Silverstream Bridge (41°08'44" S, 174°59'49" E). Mats were scraped off the tops of approximately 10 *Phormidium* covered cobbles into plastic jars filled with river water and stored on ice until use. Nine inoculated cobbles were placed into each flow channel (9 cobbles per channel × 4 channels per mesocosm × 4 mesocosms = 144 cobbles) with *Phormidium*-filled holes facing into the channel flow to ensure the maximum area for mat growth.

To test the hypothesis that fine sediment enhances *Phormidium* growth, different quantities of fine sediment were added to the header tank of the four mesocosms. The first mesocosm acted as a control and no sediment was added. In the second, third and fourth mesocosms, 10 g, 50 g and 100 g of fine sediment were added to each of the header tanks, respectively. These amounts correspond to 3, 15 and 30 times the normal amount of sediment deposition, respectively, based on sediment trap data collected from the Hutt River during February 2016 (Chapter 3, Section 3.3). After being added to the mesocosm, sediment tended to settle out over time. Therefore, three times a day throughout the three-week sampling period, sediment was manually disturbed by stirring the header and outflow tanks to re-suspend deposited material. Sediment was also mobilised in

the channels by gently disturbing deposits that accumulated around the cobbles, avoiding disturbing sediment on the tops of the cobbles.

4.2.2 Baseline Monitoring of water characteristics

To ensure that each of the mesocosms had equal nutrient concentrations, and to assess if changes in *Phormidium* growth were due to nutrients, water samples (50 mL) for nutrient analysis were collected from the header tank of each mesocosm on the after five days, then every seven days. Trace NNN, NH₄-N and DRP were measured following the protocols mentioned in Chapter 2, Section 2.2.2. The accredited detection limits were 0.001 g/m³, 0.005 g/m³ and 0.001 g/m³, respectively.

Monitoring of physio-chemical variables was carried out to ensure that any changes in *Phormidium* growth between the mesocosms were not attributed to variables other than sediment addition. Every second day during the three-week sampling period, water column pH, dissolved oxygen, conductivity and temperature were recorded in both the header tanks and outflow tanks using a handheld YSI multiprobe (YSI Incorporated, USA). Water temperature was monitored at 15 minute intervals using a waterproof continuous temperature logger (Hobo Pendant, Onset, USA); loggers were placed in each of the outflow tanks. Temperature was logged at five minute intervals throughout the deployment period.

4.2.3 Biomass monitoring

To assess the temporal changes in biomass, and to monitor the growth of *Phormidium* as a response to different sediment treatments, samples were collected on three separate occasions for chlorophyll *a*, ash free dry mass (AFDM), and *Phormidium* biovolume using microscopy. Chlorophyll *a* is used as a proxy for the quantity of phototrophic organisms present in the sample, and AFDM is used as an indicator of the total organic and inorganic material in a sample.

Before sampling, each cobble was assigned a number. After five days, then every seven days, nine rocks were chosen from each set of channels using a random number generator ($9 \text{ rocks/channel} \times 4 \text{ mesocosms} = 36 \text{ rocks per week}$). Biomass was collected by scrubbing the top surface of each chosen cobble and analysing the contents for chlorophyll *a*, AFDM and *Phormidium* biovolume. Each rock was removed from the channel, placed in a plastic tray and scrubbed with a toothbrush in 100 mL of Milli-Q water (Fig 4.3a). After scrubbing, rocks were replaced in the same position within the channels with the purpose of maintaining the flow in each channel for subsequent weeks, but with the drilled hole facing downwards to avoid the cobble being sampled twice. The liquid was transferred to a glass media bottle (Schott Duran, Germany), for 30 seconds and stored on ice until analysis.

Figure 4.3: Gathering biomass samples from channels a) scrubbing the top surface area of the cobble in 100-mL of Milli-Q water, b) collected biomass volume ready for analysis after scrubbing and c) measuring cobble surface areas by wrapping tin foil around the top surface of the cobble and trimming the edges before weighing.

Biomass samples were homogenised for 30 s using an Ultra-turrex disperser (IKA, Malaysia), and divided for analysis of three methods of biomass analysis. A 1.5-mL sub-sample of homogenised tissue was transferred to an Eppendorf tube with one drop (ca. 50 μL) of Lugol's solution for *Phormidium* biovolume analysis; a 10-mL sub-sample was transferred to a 15-mL Falcon tube for AFDM analysis; another 10-mL sub-sample was filtered (GF/C filter, Whatman), and the filter was frozen and kept in an Eppendorf tube until analysis of chlorophyll *a*.

Chlorophyll *a* and AFDM analyses were carried out using a modified method from Biggs and Kilroy (2000). Sub-samples for chlorophyll *a* analysis were thawed and transferred to a 15-mL conical polypropylene tube containing 1.5 mL of 90%

ethanol. After boiling at 78°C for 2 min, samples were wrapped in tin foil and left to extract for 24 hr at 4°C. After extraction, samples were centrifuged (3000 $\times g$), and 1 mL of the supernatant was analysed using a spectrophotometer at wavelengths of 655 and 750 nm (to correct for turbidity). To convert chlorophyll *a* to phaeopigments, 0.1 mL of 0.3-M hydrochloric acid (HCl) was added to the samples, mixed, and analysed again at the same wavelengths. Chlorophyll *a* concentrations were calculated using the following equation:

Equation 4.1

$$\text{Chlorophyll } a \text{ (mg/sample)} = \frac{(\text{absorbance pre HCl} - \text{absorbance post HCl}) \times 28.66 \times \text{sample volume} \times \text{extractant volume}}{\text{Filtered sub-sample volume}}$$

Where:

- *Absorbance pre HCl* and *absorbance post HCl* are subtracted to correct for phaeopigments. Each of these values are also corrected for turbidity by analysing at the 750-nm wavelength and subtracting this from the sample analysed at the 655-nm wavelength.
- The value of 28.66 is the absorption coefficient for chlorophyll *a*.
- The *sample volume* is the amount of water used for scrubbing the rock (0.01 L), and the *extractant volume* was the amount of ethanol used to extract chlorophyll *a* (0.0015L).
- The *filtered sub-sample volume* was the amount of water passed through the filter.
- All volumes are in litres.

A one-way analysis of variance (ANOVA) was carried out using IBM SPSS Statistics (Version 23) between each sediment treatment to analysed significant changes in biomass. This was followed by a Tukey HSD test which allowed observations of significant differences between specific sediment treatments.

Sub-samples for AFDM were thawed, oven dried in a pre-weighed crucible for 24 hrs at 105°C, cooled in a desiccator for 30 min and reweighed. This was followed

by ashing for 4 hr at 400°C. After cooling in a dessicator for 24 hrs, samples were then weighed in their crucibles and the crucible weight was subtracted to give the ash free dry mass value. Three, 2-g subsamples of fine sediment added to the flow channels were also oven dried and ashed using the same methods to determine the proportion of organic material bound to the sediment. AFDM was calculated using the following equation:

Equation 4.2

$$\text{Ash-free dry mass (g/sample)} = \frac{((\text{Crucible weight} + \text{sample after drying}) - (\text{crucible weight} + \text{sample after ashing})) \times \text{sample volume}}{\text{Volume of subsample}}$$

AFDM of the fine sediment added to each channel was determined as a percentage of the subsample weight. This was calculated by dividing the AFDM value by the subsample weight in grams and multiplying this by 100.

The surface area of each sampled cobble was determined by wrapping tin foil around the tops of each cobble and cutting around the edges (Fig. 4.3c; (Bergey and Getty, 2006). Square foil pieces measuring 1 cm², 25 cm², 100 cm² and 225 cm² were weighed to create a standard curve. Each surface area foil collected from the cobbles was then weighed and surface area determined using the standard curve equation:

Equation 4.3

$$x = \frac{(y/0.003)}{0.002}$$

Where:

- The value of x is the rock surface area in cm²
- The value of y is the weight of tin foil covering each rock surface area.
- All measurements are in grams.

AFDM and chlorophyll *a* data was normalised to the area sampled by dividing the sample weight in mg by the area of the sample in m².

A one-way analysis of variance (ANOVA) was conducted to compare effect of adding no sediment, 10 g of sediment, 50 g of sediment and 100 g of sediment to each channel, on AFDM.

Analysis of the autotrophic index determines the proportion of the sample composed of either heterotrophic or autotrophic material and is a measure of the degree of organic enrichment (Kilroy et al., 2008). It can be used as an indicator for stream health and is increased by either the reduction of chlorophyll *a* concentration (fewer autotrophs), or an increase in ash free dry mass (more heterotrophs or autotrophs). Therefore, high values indicate large amounts of non-photosynthetic organic material compared with live plant material; indicating a sample affected by organic pollution. The autotrophic index of each rock was calculated using the following equation:

Equation 4.4

$$\text{Autotrophic Index (AFDM: Chl } a) = \frac{\text{AFDM (mg/m}^2\text{)}}{\text{chlorophyll } a \text{ (mg/m}^2\text{)}}$$

In the laboratory, homogenised Lugols-preserved samples were shaken to resuspend cells/filaments, and a 0.1-mL aliquot of each sample was transferred into separate wells of a 12-well plate (Nunclon, Delta Scientific). Each well was then filled with 1 mL of Milli-Q water and 50 µL of Lugol's iodine solution. The plates were stored in the dark for at least 2 hrs to ensure the contents of each well had settled. *Phormidium* measurements were carried out using an inverted microscope (Olympus, CKX41) at 400× magnification. Images were taken with a Toupcam camera (Toup Tek Photonics), and cell dimensions were measured using Toupview camera control software (Toup Tek Photonics).

Biovolume standardisation was carried out in order to convert each sample result to the area of each rock. This was carried out using the following equation:

Equation 4.5

$$\text{Conversion factor (mm}^3\text{/L)} = \frac{A_{\text{well}} \times \text{DF} \times V_{\text{scrubbed}}}{A_{\text{transect}} \times N_{\text{transect}} \times V_{\text{sample}} \times A_{\text{rock}}} \times 10^9$$

Where:

- A_{well} is the area of the sampling well (346.4 mm²) and A_{transect} is the area of the transect analysed (5.94 mm²)
- A_{rock} is the surface area of each rock sampled
- V_{scrubbed} is the volume of water used to scrub each rock (100 mL) and V_{sample} is the volume of sample settled in each well for taxonomic analysis (0.1 mL)
- DF is the dilution factor used on the homogenised sample (1)
- N_{transect} is the number of transects counted for each channel
- The factor of 10^9 converts calculated biovolume values from μm^3 to mm^3

The size of *Phormidium* mats in each channel was recorded every second day to analyse the differences in growth rates. The surface area of *Phormidium* mats was measured from inside the channels from the same three rocks in each channel over the three-week sampling period (3 cobbles per channel $\times$ 4 channels per mesocosm $\times$ 4 mesocosms = 48 cobbles). The lengths and widths of mats were measured using callipers, and surface areas were determined by multiplying mat length $\times$ width in mm. Photos were also taken of the 12 rocks measured for *Phormidium* mat surface area every other day to visually compare mat size.

4.3 Results

4.3.1 Physical water characteristics

Physicochemical characteristics did not vary markedly between different sediment treatments (Table 4.1). Water temperature in the mesocosms ranged between 7.8 to 26.3°C. The warmest temperatures occurred between the hours

of 1200 and 1800 each day, and each mesocosm maintained an average temperature of ca. 15°C, with daily fluctuations between 13.7 and 19.5 °C (Table 4.1).

Differences in water flow, pH and dissolved oxygen were negligible between mesocosms, with ranges between 0.10 and 0.19 L/s, 8.2 and 8.6, and 91.1 and 101.9%, respectively. Water conductivity had a small amount of variability, with a range between 175 and 280 $\mu\text{S cm}^{-1}$. The control mesocosm had the highest conductivity values (Table 4.1).

Table 4.1 Average, maximum and minimum values of physicochemical variables, observed every two days over a three-week period between 7 and 25 April 2016. Values were recorded in both the header and outflow tank, and averaged.

	pH			Dissolved Oxygen (%)			Temperature (°C)			Conductivity ($\mu\text{S cm}^{-1}$)			Flow (L/s)		
	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
Control	8.5	8.6	8.2	94.9	98.7	91.1	19.5	22.8	13.7	229	280	190	0.12	0.15	0.10
10 g Sediment	8.5	8.6	8.2	96.3	99.1	92.4	18.8	22.4	13.9	224	256	194	0.12	0.19	0.10
50 g Sediment	8.5	8.6	8.3	96.7	101.9	93.4	19.5	23.2	13.9	219	261	175	0.13	0.17	0.10
100 g Sediment	8.5	8.6	8.3	95.8	98.7	91.7	19.8	23.5	13.9	225	269	189	0.13	0.17	0.11

Conductivity and pH increased slightly throughout all mesocosms over the three-week period (refer to Appendix 8, Fig. 1 and 2). Conductivity increased the most in the mesocosm with a treatment of 10 g of sediment, with a range of 89.55 $\mu\text{S/cm}$ over the three-week sampling period. The greatest increase in pH was in the control mesocosm, with a range of 0.4 over three weeks. Dissolved oxygen and flow did not vary over time, although water temperature decreased over the three weeks (Appendix 8, Fig. 3 and 4).

4.3.2 Nutrient concentrations

The total ammoniacal nitrogen concentration of each mesocosm only exceeded the detection limit (0.005 g/m^3) twice throughout the sampling period. This occurred on 11 April in the mesocosms with 10 and 100 g of sediment, which possessed concentrations of 0.009 g/m^3 and 0.007 g/m^3 , respectively.

Nitrate-nitrite nitrogen concentrations decreased markedly over the three weeks (Fig. 4.4). NNN only exceeded 0.1 mg/L , the concentration considered optimal for *Phormidium* growth (Heath et al., 2013, Wood and Bridge, 2014), directly after the mesocosms were spiked with nitrate solution. The control site did not reach 0.1 g/m^3 after the first nitrate spike.

Figure 4.4: Concentration of water column NNN over a three-week time period. Samples were taken on six separate occasions over a three-week period, including after two nitrate spiking events on the 11 and 18 April 2016.

DRP concentrations decreased each week in all mesocosms (Fig. 4.5). Concentrations appear to increase in the mesocosms with 10 and 50 g of sediment after the nitrate spike in the second week. The highest concentration of DRP was 0.003 g/m^3 , which occurred in the control mesocosm.

Figure 4.5 Concentration of DRP over a three-week period. Samples were taken on six separate occasions, including after two nitrate spiking events on the 11 and 18 April 2016.

4.3.3 Biomass

Chlorophyll *a*

Chlorophyll *a* content was used as a proxy for phototrophic biomass in each sample. There were significantly different chlorophyll *a* concentrations between sediment treatments (ANOVA $F = 3.64$, $p = 0.015$, $df = 3$; Fig. 4.6). Post hoc comparisons using the Tukey HSD test indicated that the weekly mean chlorophyll *a* concentration for the mesocosm with a treatment of 0-g sediment was statistically lower than the treatment of 100 g sediment (Mean = 12.80, $p = 0.007$). However, the control mesocosm with no sediment added did not significantly differ from the mesocosms with a treatment of 10 g (Mean = 5.71, $p = 0.460$) and 50 g (Mean = 5.94, $p = 0.432$) of sediment.

The lowest value of chlorophyll *a* was 21.93 mg/m² and occurred during the second week of deployment in the control mesocosm. The highest concentration of 64.75 mg/m² was obtained from the mesocosm with a treatment of 50 g sediment during the second week of deployment. Chlorophyll *a* concentration decreased during the second week of sampling in the control mesocosm, but stayed consistent or increased in the mesocosms with sediment treatments (Fig. 4.6). During the third week, chlorophyll *a* concentrations increased in mesocosms with no sediment and a treatment of 10 g sediment. The mesocosms

with a treatment of 50 and 100 g of sediment experienced a decrease in chlorophyll *a* concentration during the third week (Fig. 4.6).

Figure 4.6: Boxplots showing chlorophyll *a* concentrations per week for different sediment treatments. Chlorophyll *a* data was collected on three separate occasions on the 11, 18 and 25 April, 2016 (N=108).

Ash-Free Dry Mass

The organic content of each sample, determined using AFDM, was highest in the mesocosms with 10 g and 50 g sediment addition treatments (Fig. 4.7). There was no significant effect of treatment on AFDM [$F(3,64) = 1.47$, $p = 0.230$], based on a one-way ANOVA.

Temporal trends show that AFDM values increased notably between the first and third weeks of deployment (Fig. 4.8). Sub-samples of ashed sediment show that the proportion of organic material in the sediment added to the mesocosms was two percent.

Figure 4.7: Ash Free Dry Mass (AFDM) over a three-week period for four different sediment treatments. AFDM data was collected on three separate occasions on the 11, 18 and 25 April, 2016 (N=108).

The inorganic content of each mesocosm gives an indication of how much is comprised of non-living material. The inorganic content of samples increased with the amount of sediment added to the mesocosms (Fig. 4.8). The average inorganic content was greatest in the mesocosm with a treatment of 100 g sediment, with a maximum of 81.4 g/sample. Inorganic content did not differ notably between the second and third weeks of deployment (Fig. 4.8).

Figure 4.8: Ash free dry mass and inorganic content of samples collected from four mesocosms with different sediment treatments. Samples were collected on three separate occasions on the 11, 18 and 25 April, 2016 (N=108).

The autotrophic index calculated using chlorophyll *a* and AFDM showed a notable increase during the third week of deployment (Fig. 4.9). During the second week of deployment, the autotrophic index slightly decreased with the amount of sediment added, with the maximum index of 125.6 in the control mesocosm and minimum index of 91.7 in the mesocosm with a treatment of 100 g sediment.

Figure 4.9 Autotrophic Index for four different sediment treatments using chlorophyll *a* and ash free dry mass data collected over a three-week period. Error bars are based on the standard error.

Visual assessments of *Phormidium* mat areas between sampling intervals show that there was a positive relationship between the amount of sediment added to the mesocosms and the size of the *Phormidium* mat. *Phormidium* mats in the mesocosms with no sediment and 10 g of sediment experienced the greatest mat expansion over the study period (Fig. 4.10).

The size of the *Phormidium* mats increased rapidly during the first week in all sediment treatments except for the mesocosm with an addition of 100 g sediment. *Phormidium* mat growth also responded to the nitrate spike added on the 4, 11 and 18 of April. The growth peaked during the end of the second week, and began to decline during the third week (Fig. 4.10). During the final week of sampling, *Phormidium* mat growth rate decreased in the mesocosms with treatments of 50 and 100-g sediment.

Figure 4.10: Average change in *Phormidium* mat size from visual assessments of mat areas. Mat areas were measured from the same 12 rocks in each mesocosm every other day during the three-week deployment period (N=36). The differences between average mat area measurements was used to determine growth rates.

Biovolume of *Phormidium* cells showed a positive trend between the amount of sediment added to each mesocosm and *Phormidium* cell biovolume during the first week of sampling, but *Phormidium* cell concentrations notably decreased for all channels during the second and third week of sampling (Fig. 4.11).

The maximum biovolume of *Phormidium* cells occurred during the first week in the mesocosm with 50 g of sediment, with an average biovolume of 430 mm³/L. The minimum biovolume occurred in the third week in the control mesocosm with 0 g of added sediment. The average biovolume in this mesocosm was 30.4 mm³/L. The control mesocosm contained the lowest *Phormidium* biovolume throughout the entire sampling period (Fig. 4.11).

Figure 4.11: Average *Phormidium* biovolume values over a three-week deployment period. Samples were collected on three separate occasions on the 11, 18 and 25 April, 2016. *Phormidium* biovolume analysis was carried out on the samples collected from four rocks from each channel during each of the weeks of deployment (N=48). Error bars are based on the standard error.

4.4 Discussion

4.4.1 Physicochemical water characteristics

Physicochemical monitoring of water in the mesocosms was carried out to confirm that changes in *Phormidium* growth were not attributable to variables other than sediment addition. The monitoring results show that physicochemical variables were almost uniform throughout the mesocosms.

The observed increase in pH over time may be the result of an increase in photosynthesising material. Photosynthesis can affect the pH due to the consumption of carbon dioxide, which exists as carbonic acid in water (Schneider and Le Campion-Alsumard, 1999). Although *Phormidium* growth did not appear to increase rapidly over the sample period, the growth of other autotrophic material, such as green filamentous algae, may have contributed to the increase in water column pH.

4.4.2 Nutrient concentrations and *Phormidium* growth

The results indicate that, although the water was spiked with nitrate each week, concentrations were lower than expected. This may have been due to incorrect concentrations of the nitrate solution used to spike each mesocosm. The decrease in water column DRP and NNN concentrations may also have been due to the uptake of nutrients by biofilms, which explains why *Phormidium* mats increased rapidly during the first week of sampling, when nutrients were more readily available. During the colonisation and attachment phase of mat growth, an increase in dissolved nutrients promotes periphyton (including *Phormidium*) growth and may be seen as a prerequisite of a proliferation event (McAllister et al., 2016). Furthermore, as mats develop, nitrogen-fixing bacteria can establish (McAllister et. al. (unpub. data), Heath, 2014). The *Phormidium* inoculum used in this experiment were taken from fully developed mats, so nitrogen fixing bacteria may already have developed and aided the initial growth of *Phormidium* mats in the first week of mesocosm deployment.

However, an increase in biomass may lead to an enhanced uptake of nutrients, which may have caused the mats to become nutrient limited during the second and third week of the study. This was evident in the *Phormidium* biovolume and mat area measurements, as data showed a decrease after the first week of sampling. Growth peaked during the end of the second week and began to decline during the third week. During development, *Phormidium* cells secrete an extracellular polysaccharide (EPS) layer to aid filament motility (Hoiczky, 1998), but this may also capture fine sediment particles suspended in the water column. Therefore, *Phormidium* mats may have begun to capture and utilise the phosphorus from sediment during the second week, when filaments were developed enough to become motile. However, during the final week of sampling, biomass decreased in all sediment treatments, which is further evidence of a nutrient limited environment.

4.4.3 Sediment addition and biomass

The increase in AFDM and chlorophyll *a* in the mesocosms with a treatment of 10 g and 50 g sediment suggest that sediment may have been utilised by *Phormidium* mats in these mesocosms, but treatments of no sediment and 100 g sediment may have limited growth. Recent research has determined that *Phormidium* mats are able to facilitate the release of phosphorus from sediment by creating elevated pH and decreased dissolved oxygen concentrations between the mat and rock interface (Wood et al., 2015a).

Sediment may become trapped by the extracellular polysaccharide layer (Frantz et al., 2015) secreted by *Phormidium* cells, and incorporated into the thick mat matrix by motile filaments (Wood et al., 2015a) as discussed in Chapter 3. This may have occurred in the mesocosms with a treatment of 10g and 50 g sediment, during the first and second weeks of sampling, where growth was more rapid and biomass peaked.

Contrary to this, sediment deposition may have limited *Phormidium* growth in the mesocosm treated with 100 g of sediment by restricting photosynthesis. Although *Phormidium* is known to be versatile and grow on a variety of different substrata (Heath et al., 2013), fine sediment has been identified as an inhibitor of periphyton growth through attenuation of light, in a variety of algal and plant species, (Jones et al., 2012, Izagirre et al., 2009). This may apply to *Phormidium* mats in this experiment, as fine sediment was observed to settle on the tops of *Phormidium* mats in the mesocosm treated with 100 g of sediment (Fig. 4.12).

Figure 4.12: Fine sediment covering *Phormidium* mats growing in the mesocosm with a treatment of 100 g of sediment. Black arrows indicate the edges of the mat. Photos were taken during the second week of deployment on the 16 April, 2016.

It is likely that the increase in AFDM, but not chlorophyll *a*, was due to the growth of heterotrophic material, which may have begun to out-compete autotrophic material during the second and third weeks. Heterotrophic material does not contain chlorophyll *a*, so would not have appeared in the spectrophotometer analysis. This is reinforced by the autotrophic index, which can be used as an indicator of a shift in periphyton populations from autotrophic to heterotrophic (Kilroy et. al., 2008), indicates that the proportion of the sample composed of organic material was approximately three times higher during the third week of deployment, than in the second week (Fig. 4.9). As the autotrophic index is used as an indicator of organic pollution, an increase during the third week of deployment may have been due to the growth of heterotrophic material.

4.4.4 *Phormidium* removal processes

Mat dispersal is a natural process in the *Phormidium* accrual cycle and can occur in rivers through shear stress and abrasion, as well as the disturbance of substrate (Quiblier et al., 2013). However, there was minimal substrate to disturb sediment in the channels, and flow velocity was generally lower than what would be occurring in a natural river system. Therefore, the decrease in biomass is likely to have been due to autogenic detachment of the *Phormidium* mat which can be caused by the development of oxygen as a result of photosynthesis within the mat matrix (Fig. 4.13). Low flowing environments exacerbate detachment, as the development of a thicker boundary layer limits oxygen (McAllister et al., 2016). This makes the mats positively buoyant and can eventually lead to their detachment. However, a lack of *Phormidium* mat debris in the collection tank suggests that the decrease in mat size during the second and third week was due to nutrient limitation.

Figure 4.13: *Phormidium* mats in flow channels containing oxygen bubbles underneath (a) and on top of (b) mats.

It is unlikely that abrasion or shear stress would have contributed to the removal of *Phormidium* mats. The addition of suspended sediment to high flows does not exacerbate removal processes, and that velocity alone is enough to explain the removal of biomass (Francoeur and Biggs, 2006).

4.5 Experiment limitation and suggestions for future development

4.5.1 Mesocosm set-up

Temperature is an important factor in regulating the occurrence or growth of *Phormidium* proliferations (Heath, 2014). Proliferations tend to occur in waters with a temperature greater than 15°C (Wagenhoff et al., 2013, Wood et al., 2016, Wood et al., 2017). This helps to explain why *Phormidium* proliferations occur more frequently during the summer months, between December and March (Wood et al., 2016, Heath et al., 2011). As this experiment was carried out during April, temperatures dropped below 15°C in all mesocosms on every day of deployment. Therefore, temperature may have been below optimal for *Phormidium* to develop thicker mats. It is recommended that future mesocosm experiments investigating *Phormidium* growth responses be carried out between December and March to provide the maximum potential for mat development and growth.

Fine sediment was added to the mesocosms approximately 24 hours after the inoculated cobbles were positioned. Visual observations showed that sediment settled on the tops of cobbles, filling the drilled holes. This may have inhibited the growth of *Phormidium* by preventing light and nutrients from reaching the algae at an early stage of its life cycle. Therefore, the response of *Phormidium* growth may have been clearer if sediment addition was delayed, allowing mats to establish for a longer period of time. Visual assessments of *Phormidium* mat sizes showed that there was a substantial amount of growth within the first 24 hours of placement (Fig. 4.10), before sediment was added to each mesocosm. An establishment period of one week may improve the response of *Phormidium* to sediment addition in future experiments, as mats may have developed motile filaments to grow above the sediment particles. Additionally, adding 10-fold less sediment in the initial stages of the experiment may have aided *Phormidium* growth by allowing a gradual incorporation of sediment into the mats, as well as preventing the sediment from shading the mats and limiting photosynthesis.

Algae growth in this experiment showed similar patterns to previous mesocosm experiments. Izaguirre et al. (2009) showed an initial period of rapid periphyton growth in the first 10 days of sediment exposure, followed by a reduction in biomass after two weeks, which mirrors the growth patterns in this experiment. However, Izaguirre et al. (2009) showed an increase in periphyton biomass in the third week, which was attributed to the adaptation of periphyton to light attenuation, changes in community composition, as well as the vertical migration of cells to the sediment surface. This reflects the findings of Wagenhoff et al. (2013), who determined that motile periphyton communities increased from 12 to 30% under rising sediment conditions. It is possible that the mesocosms used in this experiment were not deployed for long enough, and that future experiments may show a difference in *Phormidium* growth after 30 days of exposure to sediment, to allow time for adaptation and vertical migration of cells.

4.5.2 Nutrient concentrations

In this study, *Phormidium* mats may have been phosphorus and nitrogen limited, which may explain why they did not grow as well as expected. As the mesocosms

required a top up of river water several times a week, it is likely that the low nutrient concentrations were a result of dilution, rather than evaporation.

It was originally expected that the loss of channel water may have been due to evaporation, as water temperatures rose to over 20°C on every day of deployment. However, it became apparent that the decrease in water from the channels was from leakage over the sides. Instead of a concentration of nutrients, which is expected under evaporative conditions, the observed decrease in both phosphorus and nitrogen over time suggests that dilution was taking place. It is suggested that in future mesocosm studies, any river water added to the channels should be spiked with phosphorus and nitrogen to prevent nutrient decreases over time.

Chapter 5

Synthesis

Phormidium proliferations in the Hutt River have been extensively researched over the past decade (McAllister et al., 2016, Heath and Greenfield, 2016). In the Waipoua River, *Phormidium* proliferations are common over the summer, but little research had been undertaken in this river. The main aim of this study was to further investigate the main drivers of *Phormidium* proliferations in these rivers, with an emphasis on establishing whether there was evidence that fine sediment was being utilised by *Phormidium* mats as a source of phosphorus. Weekly sampling over a seven-month period in this study showed that the relationship between physiochemical conditions (flow regimes, sedimentation rates, and nutrient and elemental concentrations) and *Phormidium* abundance varied between the two rivers.

5.1 Identifying the hydrological and physicochemical parameters that influence *Phormidium* growth in the Hutt and Waipoua Rivers.

No significant relationships between the majority of the physicochemical parameters measured in this study and *Phormidium* cover were identified. *Phormidium* seemed more likely to proliferate at all sampling sites when nitrogen concentrations were elevated and phosphorus concentrations were depleted, providing optimal conditions for *Phormidium* to outcompete other algal species. Nitrate nitrite nitrogen showed a linear relationship with *Phormidium* cover in both the Hutt and Waipoua rivers. This was expected as *Phormidium* is unable to fix nitrogen, so NNN is required for mat formation. However, historical data has shown that there was no significant relationship between annual *Phormidium*

proliferations and nutrient concentrations. This suggests that the *Phormidium* response to nutrient concentrations is site specific. It is highly likely that a combination of factors enabled *Phormidium* to out-compete other algae at the sites H03, W01 and W02, where *Phormidium* cover was the most abundant periphyton observed. Fine sediment deposited at these sites was rich in BAP, and a 200-fold increase in DRP within the mat water suggests that phosphorus was released from sediment.

In the Waipoua River, summer nutrient concentrations and low flow do not explain the decrease in *Phormidium* cover during the 2013-14 and 2014-15 growth periods.

5.2 Does fine sediment provide a source of phosphorus for the growth of *Phormidium* in the Hutt and Waipoua Rivers?

Biologically available phosphorus bound to fine sediment increased further down the catchments of both rivers and peaked at sites where proliferations have occurred in the past. This may be attributed to the increase in urbanisation in the lower reaches of each catchment area, where phosphorus inputs to land from industrial and residential waste are higher. This was demonstrated by water column phosphorus concentrations, which also increased downstream. Elevated sediment deposition and BAP, as well as the difference in nutrient concentrations in the water column and within-mat water, correlate with *Phormidium* abundance and suggest that developed mats are able to utilise BAP from fine sediment.

5.3 Investigating the relationship between fine sediment and *Phormidium* growth.

Using mesocosm chambers, an experiment investigating the influence of fine sediment on *Phormidium* growth showed that an increase in sediment deposition increased mat biomass up to a threshold, above which growth was limited. This

suggests that there may be a maximum amount of sedimentation before mat growth is inhibited due to the attenuation of light.

It is likely that existing nitrogen fixing bacteria, nitrogen spiking and a stored source of phosphorus within the cells contributed to the immediate growth of *Phormidium* mats during the first week of mesocosm deployment. However, as data from the Hutt and Waipoua rivers confirmed, nitrogen-limited environments constrain the growth of *Phormidium* mats during the development stage of accrual. Water became nutrient limited during the second and third weeks due to a lack of nutrient replenishment, which partially explains the lack of *Phormidium* growth due to nutrient limitation after the first week of channel deployment. This may also account for limited growth in the control channel, as mats were limited by the absence of phosphorus-bound sediment as well as NNN.

Mat dispersal processes, a natural part of the *Phormidium* life cycle, were likely to have contributed to the loss of biomass during the third week of deployment. Observed oxygen bubble formation reduced the tenacity of mats growing on the rocks, which were more vulnerable to detachment through shear stress.

5.4 Mitigation suggestions and future direction

It is widely known that the input of nutrients to waterways results in eutrophication (Biggs and Kilroy, 2000). Based on the findings of this research, the reduction of nutrient runoff to waterways, as well as the reduction of fine sediment inputs may help to alleviate the proliferation of *Phormidium* in the future. On a national scale, NNN and DRP concentrations are relatively low in both the Hutt and Waipoua rivers, which suggests that it may not be easy to further reduce nutrient inputs to waterways. One of the best options for mitigation may be to focus on decreasing fine sediment mobilisation, which is exacerbated by erosion and disturbance of the river bed resulting from deforestation and flood protection activities in the Hutt and Waipoua rivers. Minimising the addition of phosphorus (for example through addition of fertilisers) may also decrease the amount of BAP available to *Phormidium*. Regulating *Phormidium* proliferations in the future may require riparian planting to strengthen river banks and reduce

sediment input to waterways, as well as collaboration with the Greater Wellington Regional Council to reduce both point and non-point nutrient sources and river bed disturbance during flood protection activities.

Further investigation into sediment sources for the Hutt and Waipoua rivers is needed. For instance, sediment fingerprinting is the comparison of geochemical signatures of stream sediment to likely sources, such as stream banks and topsoil (McDowell et al., 2016). Furthermore, in future summer growth periods, it may be useful to analyse *Phormidium* mat water at other sites with mat growth on multiple sampling occasions. This may provide further information on the relative importance of water column, sediment bound and internal nutrient cycling.

A number of improvements to the flow-through channel experiment (Chapter 4) are suggested. More research is required to fully understand the role of fine sediment as a source of phosphorus, and flow-channel experiments eliminate factors which may vary and affect results. Suggestions for future experiments are:

1. Implement an establishment period to ensure that *Phormidium* mats are developed enough to utilise sediment deposition. This may be up to 24 hours or more.
2. Carry out the experiment earlier in the summer season during warmer temperatures. This may be between December and February.
3. Supplement the water added to the channels with nitrate and phosphorus to prevent nutrient limitation and provide a suitable growth environment for *Phormidium*.
4. Add fine sediment in gradual increments, instead of on one occasion. This may not only simulate a more natural deposition event in a river system, but will likely prevent the attenuation of light and allow time for motile filaments to grow above the sediment, incorporating it into the mat.

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Appendix 1: Periphyton cover descriptive statistics

Table 1: Average, maximum and minimum values of water quality variables, observed weekly over a 28-week period between 19 November 2014 and 25 May 2015. Cover was estimated using the transect method outlined in the New Zealand guidelines for managing cyanobacteria in recreational fresh waters (Ministry for the Environment and Ministry of Health 2009). H01-H04 indicates sites located in the Hutt River, and W01-W02 indicates sites located in the Waipoua River.

	<i>Phormidium</i>			Filamentous green algae			Diatoms			Fine Sediment		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
H01	1.2	0.00	0.07	1.1	0.00	0.10	4.75	0.00	0.38	10.25	0.00	1.72
H02	8.25	0.00	1.77	9.25	0.00	0.82	44.75	0.00	3.09	7.25	0.00	1.08
H03	20.65	0.00	4.06	12	0.00	1.95	11.75	0.00	0.77	15	0.00	3.01
H04	1.15	0.00	0.06	41	0.00	4.40	49.5	0.00	3.34	47.75	0.00	11.95
W01	15.25	0.00	3.68	6.4	0.00	1.00	20	0.00	4.09	16	0.00	4.46
W02	16.75	0.00	4.60	13.25	0.00	1.98	20.35	0.00	5.16	10.75	0.00	3.95

Appendix 2: Temporal variability of periphyton cover

Figure 1: Temporal trends of *Phormidium* cover monitored weekly over a 28-week period between 19 November 2014 and 25 May 2015. The horizontal line represents the 'amber mode' alert for *Phormidium* cover. Red triangles on the X-axis indicate that a flushing flow has occurred within 7 days prior to the sample date.

Figure 2: Temporal trends of periphyton cover monitored weekly over a 28-week period between 19 November 2014 and 25 May 2015. Values were recorded in the Hutt and Waipoua rivers using a handheld YSI multiprobe in direct river flow.

Appendix 3: Temporal variability of baseline monitoring characteristics

Figure 1: Temporal trends of water column pH monitored weekly over a 28 week period between 19 November 2014 and 25 May 2015. Values were recorded using a handheld YSI multiprobe in direct river flow.

Figure 2: Temporal trends of water column dissolved oxygen monitored weekly over a 28-week period between 19 November 2014 and 25 May 2015. Values were recorded using a handheld YSI multiprobe in direct river flow.

Figure 3: Temporal trends of water column temperature monitored weekly over a 28 week period between 19 November 2014 and 25 May 2015. Values were recorded using a handheld YSI multiprobe in direct river flow.

Figure 4: Temporal trends of total conductivity in the water column. Water was monitored weekly over a 28-week period between 19 November 2014 and 25 May 2015. Values were recorded using a handheld YSI multiprobe in direct river flow.

Figure 5: Temporal trends of water column total dissolved solids monitored weekly over a 28-week period between 19 November 2014 and 25 May 2015. Values were recorded using a handheld YSI multiprobe in direct river flow.

Appendix 4: Nutrient concentrations descriptive statistics

Table 1: Average, maximum and minimum values of water column nutrients total ammoniacal nitrogen, nitrate-nitrite nitrogen and dissolved reactive phosphorus. Surface water samples were collected weekly over a 28-week period between 19 November 2014 and 25 May 2015. Water was analysed at Hill Laboratories (Hamilton).

Total Ammoniacal Nitrogen			Nitrate-Nitrite Nitrogen			Dissolved Reactive Phosphorus		
Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
0.023	0.003	0.004	0.063	0.004	0.027	0.005	0.002	0.004
0.023	0.003	0.007	0.280	0.056	0.120	0.008	0.003	0.004
0.022	0.003	0.007	0.420	0.106	0.208	0.009	0.002	0.004
0.021	0.003	0.011	0.390	0.075	0.177	0.010	0.002	0.005
0.074	0.003	0.008	1.140	0.195	0.596	0.015	0.001	0.006
0.079	0.003	0.010	1.050	0.175	0.564	0.016	0.002	0.005

Appendix 5: Trace element concentrations in the water column

Table 1: Average trace element concentrations of the water column over a 7-month period. Samples were collected at monthly intervals between 19 November 2014 and 25 May 2015. Water was analysed at Waikato University.

Element	Average Concentration over a 7-Month Period (µg/L)					
	H01	H02	H03	H04	W01	W02
<i>Aluminium</i>	73.4	33.2	24.8	56.6	24.7	28.9
<i>Arsenic</i>	0.3	0.2	0.2	0.4	0.4	0.3
<i>Boron</i>	14.0	17.0	17.5	19.7	16.2	15.9
<i>Calcium</i>	10497	4949	5147	7341	9266	7668
<i>Chromium</i>	0.6	0.4	0.3	0.8	0.4	0.5
<i>Cobalt</i>	0.1	0.1	0.1	0.1	0.1	0.1
<i>Copper</i>	3.1	0.8	1.6	2.6	2.0	1.9
<i>Iron</i>	67.0	50.1	34.1	90.9	36.2	31.3
<i>Lead</i>	0.4	0.2	0.1	9.3	0.2	0.2
<i>Magnesium</i>	1369	1549	1639	1953	1974	1914
<i>Manganese</i>	4.2	1.7	1.4	4.9	1.8	1.6
<i>Nickel</i>	1.0	0.6	0.8	0.7	0.8	0.7
<i>Phosphorus</i>	17.0	7.9	9.9	15.6	10.0	6.7
<i>Potassium</i>	819	802	850	984	1217	1157
<i>Selenium</i>	0.2	0.2	0.1	0.2	0.3	0.1
<i>Silicon</i>	4575	4882	5317	5717	4608	4493
<i>Sodium</i>	6936	8158	8593	10354	7875	7775
<i>Strontium</i>	56.4	47.3	47.4	52.1	68.4	70.1
<i>Vanadium</i>	0.3	0.3	0.2	0.4	0.3	0.3
<i>Zinc</i>	9.1	2.5	3.7	9.0	3.4	4.0

Appendix 6: Annual variability of historical river characteristics

Figure 1: Bar graph showing average concentrations of nitrate-nitrite nitrogen (NNN) in the Hutt and Waipoua rivers during the summer growth period (November to March) over the past seven years. NNN is monitored on a monthly basis as a part of the Greater Wellington Regional Council State of the Environment monitoring programme. Columns with a red outline indicate the NNN concentrations observed in this study. Data was obtained from the Greater Wellington Regional Council (2016).

Figure 2: Bar graph showing average concentrations of dissolved reactive phosphorus (DRP) in the Hutt and Waipoua rivers during the summer growth period (November to March) over the past seven years. DRP is monitored on a monthly basis as a part of the Greater Wellington Regional Council State of the Environment monitoring programme. Columns with a red outline indicate the DRP concentrations observed in this study. Data was obtained from the Greater Wellington Regional Council (2016).

Appendix 7: Sedimentation rates in the Hutt and Waipoua rivers

Table 1 Table showing the sedimentation rates of each sediment traps deployed over a three-week period between the 4 and 20 February 2015. Sediment traps were set up at six monitoring sites, and coincided with baseline river monitoring. Samples with * indicate that sediment traps were unable to be retrieved from the river.

Site	Date Collected	Week	Sedimentation (g/m ² /day)					Total Sediment Deposition	Average over three weeks
			500-µm	250-µm	125-µm	63-µm	< 63-µm		
EM01	4/02/2015	1	1496.31	21.93	5.21	4.13	4.67	1532.24	386.26
EM01	4/02/2015	1	482.10	8.15	73.45	3.74	5.72	573.16	
EM01	4/02/2015	1	1111.72	143.03	1.58	1.88	1.91	1260.12	
EM01	14/02/2015	2	0.56	0.75	0.51	0.68	0.67	3.18	
EM01	14/02/2015	2	7.06	1.24	0.28	0.42	0.80	9.80	
EM01	14/02/2015	2	3.51	0.90	0.50	0.98	0.96	6.85	
EM01	20/02/2015	3	2.32	0.84	0.36	0.79	0.89	5.20	
EM01	20/02/2015	3	57.77	1.70	1.04	0.79	0.96	62.26	
EM01	20/02/2015	3	19.48	1.51	0.40	1.07	1.07	23.53	
EM02	4/02/2015	1	7.49	10.71	1.31	3.19	4.80	27.51	105.70
EM02	4/02/2015	1	201.34	18.67	6.10	4.55	6.40	237.07	
EM02	4/02/2015	1	339.15	46.81	4.30	4.45	4.51	399.23	
EM02	14/02/2015	2	20.00	1.20	1.07	1.45	1.36	25.08	
EM02	14/02/2015	2	12.54	4.19	0.91	1.31	1.35	20.30	
EM02	14/02/2015	2	10.18	4.18	0.79	0.82	0.62	16.59	
EM02	20/02/2015	3	88.63	3.54	0.63	2.90	3.19	98.89	
EM02	20/02/2015	3	47.28	1.68	5.51	1.10	3.64	59.21	
EM02	20/02/2015	3	54.64	3.89	3.33	2.74	2.87	67.47	

EM03	4/02/2015	1	303.53	58.99	5.75	4.08	6.44	378.79	148.38
EM03	4/02/2015	1	214.31	39.17	6.36	4.40	10.10	274.33	
EM03	4/02/2015	1	106.82	35.31	6.05	4.90	9.82	162.90	
EM03	14/02/2015	2	*	*	*	*	*	0.00	
EM03	14/02/2015	2	*	*	*	*	*	0.00	
EM03	14/02/2015	2	223.55	22.84	5.49	2.43	5.55	259.86	
EM03	20/02/2015	3	58.30	11.92	1.44	2.64	2.78	77.08	
EM03	20/02/2015	3	115.09	9.32	2.72	1.19	2.34	130.65	
EM03	20/02/2015	3	25.71	10.84	10.15	1.33	3.77	51.81	

EM04	4/02/2015	1	*	*	*	*	*	0.00	54.24
EM04	4/02/2015	1	*	*	*	*	*	0.00	
EM04	4/02/2015	1	*	*	*	*	*	0.00	
EM04	14/02/2015	2	5.79	7.09	1.08	2.28	2.89	19.13	
EM04	14/02/2015	2	18.94	1.82	1.26	0.62	0.89	23.53	
EM04	14/02/2015	2	15.22	21.64	1.58	1.50	3.53	43.47	
EM04	20/02/2015	3	25.04	16.04	2.90	1.14	4.38	49.50	
EM04	20/02/2015	3	169.54	175.45	5.02	1.54	1.02	352.56	
EM04	20/02/2015	3	*	*	*	*	*	0.00	

EM05	4/02/2015	1	11.54	3.22	0.45	1.36	2.44	19.01	7.40
EM05	4/02/2015	1	6.59	1.21	0.80	1.73	3.61	13.93	
EM05	4/02/2015	1	2.48	1.05	0.47	3.19	4.70	11.89	
EM05	11/02/2015	2	2.78	6.87	2.75	0.16	1.09	13.64	
EM05	11/02/2015	2	*	*	*	*	*	0.00	
EM05	11/02/2015	2	*	*	*	*	*	0.00	
EM05	18/02/2015	3	0.92	1.82	0.72	1.95	2.71	8.13	
EM05	18/02/2015	3	*	*	*	*	*	0.00	
EM05	18/02/2015	3	*	*	*	*	*	0.00	

EM06	4/02/2015	1	9.20	2.26	1.66	2.96	3.52	19.60	16.10
EM06	4/02/2015	1	10.04	0.91	0.79	1.27	1.26	14.28	
EM06	4/02/2015	1	4.26	0.93	4.42	1.86	2.98	14.45	
EM06	11/02/2015	2	9.73	1.72	1.95	2.07	2.73	18.21	
EM06	11/02/2015	2	2.06	3.28	1.57	0.78	1.34	9.03	
EM06	11/02/2015	2	7.98	4.38	1.31	1.99	2.18	17.84	
EM06	18/02/2015	3	4.01	0.82	1.62	1.49	4.15	12.09	
EM06	18/02/2015	3	4.93	1.85	4.49	5.48	9.15	25.89	
EM06	18/02/2015	3	5.93	1.45	1.13	2.23	2.73	13.47	

Appendix 8: Temporal variability of baseline monitoring data

Figure 1: pH values in the flow channel water column, monitored using a YSI multiprobe. Values were observed every two days over a three week period between 7 and 25 April 2016.

Figure 2: Conductivity values in the flow channel water column, monitored using a YSI multiprobe. Values were observed every two days over a three-week period between 7 and 25 April 2016.

Figure 3: Dissolved oxygen values in the flow channel water column, monitored using a YSI multiprobe. Values were observed every two days over a three week period between 7 and 25 April 2016.

Figure 4: Water velocity in the flow channel water column, monitored using a YSI multiprobe. Values were observed every two days over a three-week period between 7 and 25 April 2016.