



Environmental factors that promote *Phormidium* blooms in Canterbury rivers

Summer Scholarship Report

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TITLE: **Environmental factors that promote *Phormidium* blooms in Canterbury rivers**

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Executive Summary

During the past ten years there has been an apparent increase in the prevalence of benthic cyanobacterial blooms in New Zealand rivers (Heath *et al.*, 2011). *Phormidium* appears to be the dominant genus (Heath *et al.*, 2010). *Phormidium* can produce powerful neurotoxins, which pose a risk to human and animal health, and animal toxicosis associated with benthic cyanobacteria has become increasingly prevalent in New Zealand (Hamil, 2001; Wood *et al.*, 2007). Despite this health risk, little is known about the environmental factors that are important in regulating the occurrence of riverine benthic cyanobacterial blooms. In comparison, a breadth of knowledge exists regarding the environmental factors that lead to planktonic algal blooms (Oliver *et al.*, 2012). Hydrological regime (Heath *et al.*, 2011), water temperature (Heath *et al.*, 2013) and nutrient concentrations (Biggs, 2000; Wood & Young, 2012) are all implicated as important environmental factors in regulating benthic cyanobacterial blooms.

In this study, we monitored three sites along the Ashley River/Rakahuri for 10 weeks and also analysed historical data for 10 sites (five with regular *Phormidium* blooms and five without) from the wider Canterbury region. Environmental factors investigated included nutrient concentrations (DRP, DIN, TP, TN, and nitrate), water temperature, depth, river flow, point velocity and substrate composition. The aim of this study was to identify environmental factors that correlate with *Phormidium* percentage cover.

Sites with regular *Phormidium* blooms, with the exception of Temuka at Manse Bridge were dominated by larger substrate (boulder and cobble). Comparatively, sites without *Phormidium* blooms were dominated by smaller substrate (sand/silt, fine gravel and gravel). All sites had low DRP concentrations. There were differences in DIN concentrations but these did not relate to probability of bloom formation. Heath *et al.*, (2011) highlights temperature as an important factor in determining whether *Phormidium* is present or absent. In this study, we found no correlative relationship between *Phormidium* percentage cover and water temperature. Furthermore, *Phormidium* was observed in a range of water temperatures, between 4–20°C. A distinct pattern existed at some sites between flushing flows (3 times median flow) and *Phormidium* percentage cover, with more frequent flushing flows resulting in decreased *Phormidium* percentage cover. However, the general flushing flow rule that three times the median flow is sufficient to remove all *Phormidium* mats was not applicable in all of the Canterbury rivers studied. For example, a large flushing flow of 22 times the median occurred at Pareora at the huts on the 28.01.2011 and did not remove all the *Phormidium*. Like Heath *et al.*, (2013), we found that *Phormidium* had no specific preference for water velocity and depth, but occurred at a range of depths (0.03–0.59 m) and point velocities (0–1.4 ms⁻¹). At present, it appears that water quality is a weak predictor of *Phormidium* blooms. Rather substrate stability and flow may be the most important factors controlling the dynamics of *Phormidium* in Canterbury rivers.

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Section 1 Introduction

Globally, many toxic cyanobacterial blooms resulting in human and animal toxicosis and deaths have been documented (Lawton & Codd, 1991; Edwards *et al.*, 1992, Moreno *et al.*, 2004; Catherine *et al.*, 2013). However, these events are often attributed to planktonic cyanobacterial blooms (Carmichael *et al.*, 2001; Azevedo *et al.*, 2002). Consequently, the environmental factors involved in regulating *planktonic* cyanobacterial blooms have been researched extensively (Oliver *et al.*, 2012). Comparatively, there is a limited understanding of the environmental factors that lead to *benthic* cyanobacterial proliferations (Mez *et al.*, 1998; Heath *et al.*, 2011). During recent decades, there has been an apparent increase in blooms of the benthic cyanobacteria *Phormidium* in New Zealand Rivers (Biggs & Kilroy, 2000; Heath *et al.*, 2010, 2013). Cyanobacterial blooms are often associated with water quality issues. In addition to causing tastes and odours, some species can produce toxic secondary metabolites known as cyanotoxins that can cause acute neurotoxicity (Carmichael, 1992) and pose a health risk to humans and animals (Codd *et al.*, 1999; Falconer & Humpage, 2005; Funari & Testai, 2008). Homoanatoxin-a and anatoxin-a are the most common toxins produced by benthic cyanobacteria in New Zealand (Hamill, 2001; Wood *et al.*, 2007; Heath *et al.*, 2010). Animal and human toxicosis associated with the toxins produced by benthic cyanobacteria have also become increasingly prevalent (Edwards *et al.*, 1992; Mez *et al.*, 1997 Hamill, 2001; Gugger *et al.*, 2005; Wood *et al.*, 2007; Heath *et al.*, 2011).

Hydrological flow regimes are important in regulating the biomass of benthic periphyton communities (Clausen & Biggs, 1997; Biggs & Kilroy, 2000). Clausen and Biggs (1997) found that flows greater than three times the median flow were an important mechanism in determining periphyton biomass within New Zealand Rivers. Consequently, cyanobacteria often proliferate in periods of stable flow (Bowling & Baker, 1996; Sabatar *et al.*, 2003; Heath *et al.*, 2011, 2013).

Heath *et al.*, (2011) estimated the percentage cover of *Phormidium* and measured other physico-chemical factors in two New Zealand Rivers for one year. Water temperature and river flow were identified as main factors regulating the occurrence of *Phormidium*. Furthermore, Heath *et al.*, (2013) created a Habitat Suitability Criteria for *Phormidium* in the Hutt River, which suggested that flushing flows (3 times median) are the most important factor in regulating *Phormidium* cover. However, this research also suggests that in periods of stable flow, other physico-chemical factors, including dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorous (DRP) become important. Fujimoto *et al.*, (1997) highlights how temperature influences *Phormidium* growth. Fujimoto *et al.*, also observed that *Phormidium* was dominant in all seasons in Lake Kasumigaura, suggesting a wide temperature tolerance. Furthermore, Fujimoto *et al.*, (1997) also demonstrated through laboratory and field experiments that *P. tenue* was competitively dominant over eukaryotic algae when the nitrogen: phosphorus ratio was high. Previous studies also highlight the importance of water conductivity and nutrient ratios in regulating benthic cyanobacterial growth (Biggs & Close, 1989; Biggs, 1990; Chételet *et al.*, 1998; Wood & Young 2011, 2012).

In this study, three sites along the Ashley River/Rakahuri were sampled weekly for 10 weeks. Samples of cyanobacterial mat were collected weekly for morphological identification and later toxin analysis. Water samples were also collected weekly for nutrient

analysis. Water temperature, conductivity, substrate composition and periphyton percentage cover, depth and point velocity were measured on each sampling day. The physico-chemical measurements in conjunction with flow data (acquired from Environment Canterbury (ECAN)) and the percentage cover were utilized to identify factors which correlated with *Phormidium* proliferations. Furthermore, as part of this summer project an analysis of historical ECAN data for 10 sites from the wider Canterbury region was undertaken (See Fig. 1). This included flow, temperature, nutrient and periphyton composition data from 2009–2014.

1.1 Research aims

The purpose of this study was to:

- Collate and analyse existing Environment Canterbury river monitoring data, including five sites with persistent *Phormidium* blooms, and five without.
- Identify the environmental factors that are best correlated with *Phormidium* proliferations in the Canterbury region.
- Conduct regular site surveys at three sites along the Ashley River/Rakahuri, to investigate temporal and spatial changes in *Phormidium* cover and relate this to differences in environmental factors.

Section 2 Methods

2.1 Sampling sites

The Ashley River/Rakahuri is located in Northern Canterbury and generally flows south east for 65 km, discharging into the Pacific Ocean at Waikuku Beach (Fig. 1). Three sites (SH1, Loburn, and Gorge) were sampled weekly on the Ashley River/Rakahuri (Fig. 1) between 2 December 2013 and 10 February 2014. Sites were selected primarily due to historical cyanobacterial proliferations and accessibility. For example, the Ashley River at Gorge site generally has low cyanobacterial percentage cover whereas over the last few years both lower sites (Ashley at SH1 and Loburn) have regularly had blooms that required the issuing of public health warnings.

2.2 Site surveys

The monitoring procedure was consistent with the New Zealand Guidelines for Monitoring Cyanobacteria in Recreation Fresh Waters (Wood *et al.*, 2009). Transect surveys were conducted in approximately the same location every week. Transect lengths varied from 6–16 m. Four transects were surveyed at each site on each sampling date. Substrate composition and periphyton percentage cover were estimated at five evenly spaced points along each transect using an underwater viewer. The data from the 20 views were used to calculate the overall average of periphyton cover and substrate composition. The date, site, time, weather, last rainfall and any pertinent comments were also recorded.

2.3 Sampling protocol

2.3.1 *Phormidium* collection

Ten *Phormidium* mat samples were collected at each site where possible. Mat samples were stored in a chilly bin until arrival at the laboratory. In the laboratory, mat samples were homogenised using a sterile rod. Sub-samples (ca. 0.5 g) were preserved with lugols iodine for morphological identification and the remainder was frozen for later toxin/molecular analysis. Analysis of these samples was not undertaken as part of this study.

2.3.2 Environmental parameters measured *in situ*

At each site, pH, water temperature, conductivity and dissolved oxygen were measured using a Hach HQ40d portable meter. Turbidity was measured using a hand-held HACH DR/890 Colorimeter. At each view the point velocity, river flow and depth were evaluated using a Global Water Flow probe.

2.3.3 Water samples

Water samples were collected weekly at all sites to analyse for metals (not analysed as part of this study), dissolved organic carbon (DOC), total nitrogen (TN) and phosphorus (TP), dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP). Samples were also collected for possible later analyses of trace metals and major ions, however these analyses were not conducted as part of this study. Water samples were stored in a chilly bin until arrival at the laboratory. Samples for dissolved nutrient analysis were filtered with GF/C filters and stored in the freezer until further analysis

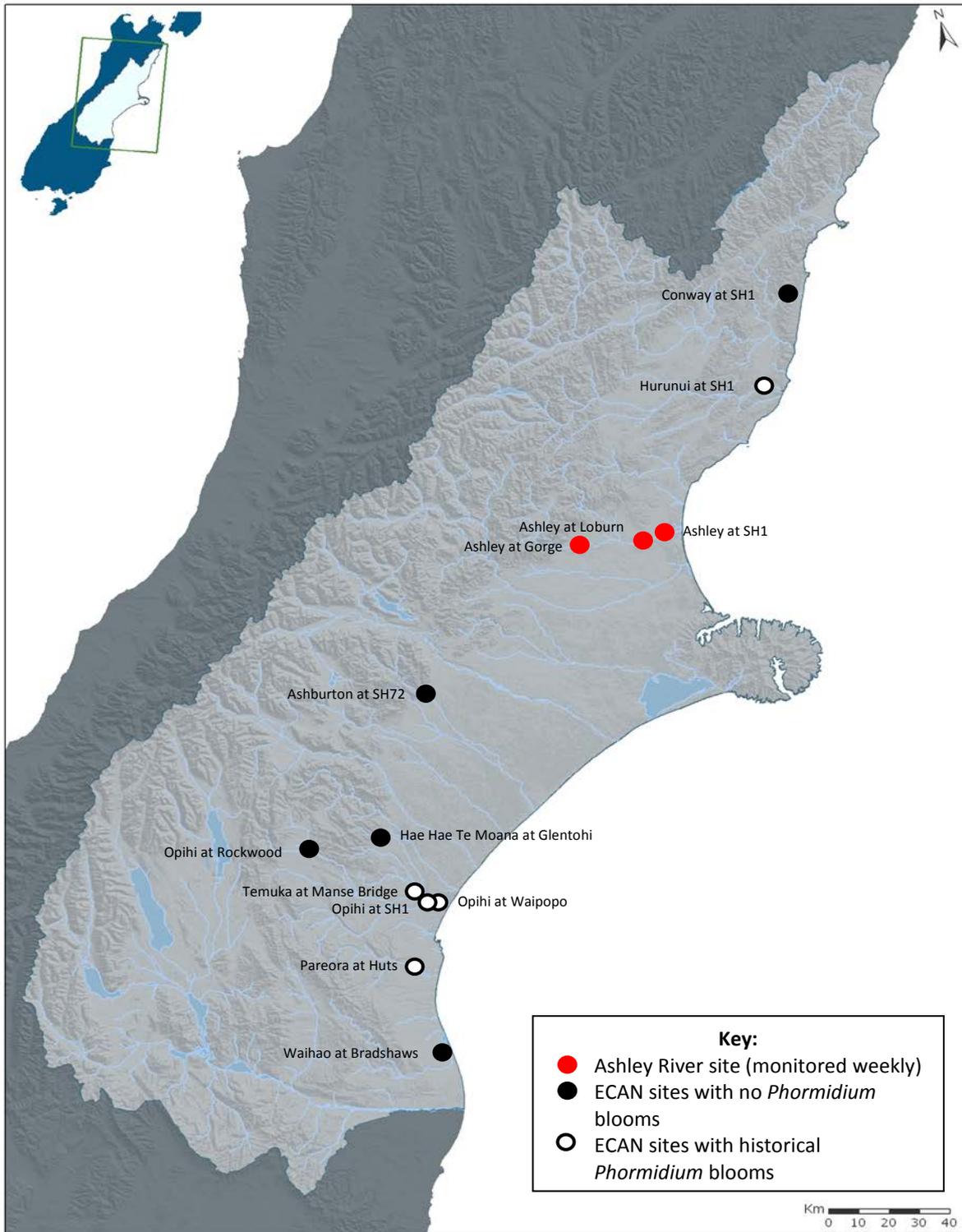


Figure 1. Map of the approximate locations of sampling sites. For site coordinates see Appendix 1. Map originally obtained from ECAN website (<http://ecan.govt.nz/services/online-services/Pages/maps-canterbury-region.aspx#canterbury-general>).

2.4 Analytical methods

Dissolved reactive phosphorus was determined using molybdate colorimetry (Eaton & Franson, 2005). Absorbance was measured using a Hach DR/3900. Nitrate concentrations were analysed using the cadmium reduction method (Hach, 2002). Samples for total phosphorus were digested using the persulphate method, and analysed for dissolved reactive phosphorus (Eaton & Franson, 2005). Total nitrogen was digested as nitrate and analysed using the Hach DR/890.

2.5 Historical Environment Canterbury data

Data from 10 sites was acquired from Environment Canterbury's state of environment and summer recreational health monitoring programmes (Fig. 1). Flow data was also provided by ECAN. Sites were chosen specifically for comparative purposes; five had consistent *Phormidium* issues, generally indicated by the issuing of public health warnings, and five had no *Phormidium* issues. Five of the sites (Hurunui at SH1, Opihi at SH1, Pareora at Huts, Opihi at Waipopo, and Waihao at Bradshaws) are ECAN summer recreation monitoring sites. These sites are monitored weekly from November to March. Cyanobacterial percentage cover is estimated by ECAN staff by bankside observations. When the percentage cover is estimated to be over 20%, transect surveys are usually conducted following the methods in the New Zealand Guidelines for Cyanobacteria in Recreation Fresh Waters (Wood *et al.*, 2009). For the five aforementioned sites some monthly nutrient data was available. The remaining five sites (Conway at SH1, Opihi at Rockwood, Ashburton at SH72, Hae Hae Te Moana at Glentohi, and Temuka at Manse Bridge) were periphyton modelling sites, which have been monitored monthly since July 2011. At these sites, percentage cover of periphyton was estimated with 20 views along four transects. Water temperature, conductivity, DIN and DRP were also available for these sites. Daily medians and long-term median river flow for each site were obtained from ECAN. The 10 chosen sites were revisited and substrate composition was analysed. However, it is important to emphasize that the time of cyanobacterial estimations was not synchronous with substrate composition estimations, which may introduce some inaccuracies to our data set.

Section 3 Results

3.1 Survey of Canterbury rivers

Flow data and historic *Phormidium* percentage cover estimates were provided by ECAN. This was complemented by our own in depth study of three sites on the Ashley River through the summer of 2013–2014. Data collected on dissolved oxygen, conductivity and pH were not analysed as part of this study.

3.1.1 Site characteristics

Summaries of nutrients, *Phormidium* percentage cover and flow for each site are provided below. It is important to note that *Phormidium* did occur at all sites, despite being at low abundance at many.

Ashburton at SH72

The percentage cover of *Phormidium* did not exceed 1.5% and this was recorded in a period of prolonged low flow (Fig.3). This site has a high median flow of 8890 L/s. The majority of the substrate (82%) is small (gravel, fine gravel and sand/silt). Water temperatures are generally between 9 and 15°C. This site has the lowest DIN concentration.

Conway at SH1

Phormidium was only observed once during monitoring with 0.3% cover occurring during a period of low flow (Fig. 4). The median flow for this site is 3151 L/s and the substrate is predominantly small (71% gravel, fine gravel, sand/silt). Boulder and cobble accounted for 29% of the substrate. This site was dominated by brown filamentous algae. This site has a low DIN (0.3 mg/L).

Hae Hae Te Moana at Glentohi

Phormidium has never been recorded in the ECAN surveys at this site (Fig. 5). However, during a site visit (5 February 2014) as part of this study a small amount (ca. <0.01%) of *Phormidium* was observed. The substrate was dominated by gravel and fine gravel (69%). The remaining 31% was cobble. The median flow for this site is 471 L/s. However, during the sampling period flushing flows (>1413 L/s) occurred regularly. The DIN concentration is low (0.1 mg/L).

Opihi at Rockwood

The greatest *Phormidium* percentage cover observed at this site was 4% on the 13 December 2013 and the highest water temperature 19.6°C was also recorded on this date. This increase in *Phormidium* cover also coincided with a period of low flow (Fig. 6). *Phormidium* was not observed during sampling on the 23 November 2011 and between the

19 November 2012 and the 15 April 2013, but was present in small amount (<4%) on all other sampling dates. A flushing flow of 21397 L/s (6.3 times median) occurred within 10 days of sampling on the 23 November 2013. Furthermore, flushing flows occurred within a 10 day period of sampling on the 19 November 2012, 10 January 2012 and the 21 March 2013. This site has a high DIN concentration (1.3 mg/L) and has the highest proportion (40%) of large substrate (boulders and cobble) of all the sites without *Phormidium* issues.

Waihao at Bradshaws Bridge

Small amounts of *Phormidium* (1–2%) were observed from the 1 December 2010 to the 22 December 2010 during a period of low flow (less than the median of 1514 L/s). A substrate survey was not at this site as it is a tidal pool, however, bankside observations indicate that sand/silt are the dominant substrates with some large boulders. *Phormidium* was not observed from 6 January 2011 to the 8 January 2014 where the median flow was exceeded on many occasions (Fig. 7). However, detached *Phormidium* has been observed on multiple occasions. This site has the highest DIN concentration of 1.7 mg/L.

Hurunui at SH1

Percentage cover of *Phormidium* peaked on the 3 February 2012 at 39% during an extended period of low and stable flows. A large flushing flow (365455 L/s, 6.9 times the median) occurred within 10 days of sampling on 7 January 2013 preventing the documentation of any *Phormidium* until the 11 February 2013, when percentage cover was estimated to be 1%. Five days later the cover was estimated at 26%. The median flow for this site is 53300 L/s, which is high compared to the other study sites (Fig. 8). No *Phormidium* was observed from 15 November 2010 to the 27 January 2012, during this period flow exceeded the median on many occasions. The substrate at this site is 59% large (cobbles and boulders) and the DIN concentration is 0.5 mg/L.

Opihi at SH1

During a period of stable flow between 13 December 2011 and 21 February 2012, percentage cover of *Phormidium* increased, peaking at 70% on the 14 February 2012 (Fig. 9), after which, a large flushing event (84210 L/s, 9.5 times median) occurred removing all *Phormidium*. Six other flushing events then occurred between the 28 February 2012 and the 8 February 2013 preventing *Phormidium* cover from exceeding 3%. All flushing flow events appear to correspond with significant reductions of *Phormidium* cover (Fig. 9). This site has a high DIN concentration (0.65 mg/L) and gravel and cobble are the dominant substrate types (44 and 51% respectively).

Opihi at Waipopo

Gravel and cobble were the only substrate type recorded (54 and 46%, respectively). The DIN concentration is 0.7 mg/L. A peak in percentage cover of *Phormidium* (70%) occurred on the 19 February 2013 during a period of relatively stable flows (Fig. 10).

Pareora at Huts

The substrate at this site is dominated by large cobble and boulders (86%). Furthermore, the substrate was fairly embedded in the riverbed. The median river flow at this site is 1432 L/s, which was exceeded on multiple occasions between 2009 and 2014 (Fig. 11). Percentage cover of *Phormidium* peaked (88%) on the 28 January 2011 after a long period of stable flows. A large flushing flow (32568 L/s, 22.7 times median) caused this percentage cover to reduce to 20%. This site has a DIN concentration of 0.6 mg/L.

Temuka at Manse Bridge

Peaks in *Phormidium* cover ($\geq 20\%$) have been recorded at this site on the 26 January 2013 and the 5 March 2013, which coincide with a period of low and stable flows (less than the median flow of 3473 L/s). The majority of the substrate is composed of gravel (76%). This site has the highest DIN concentration (1.7 mg/L). No *Phormidium* was observed on the 28 November 2012 and 17 December 2012 (Fig. 12). A flushing flow occurred within 10 days of each of these sampling days.

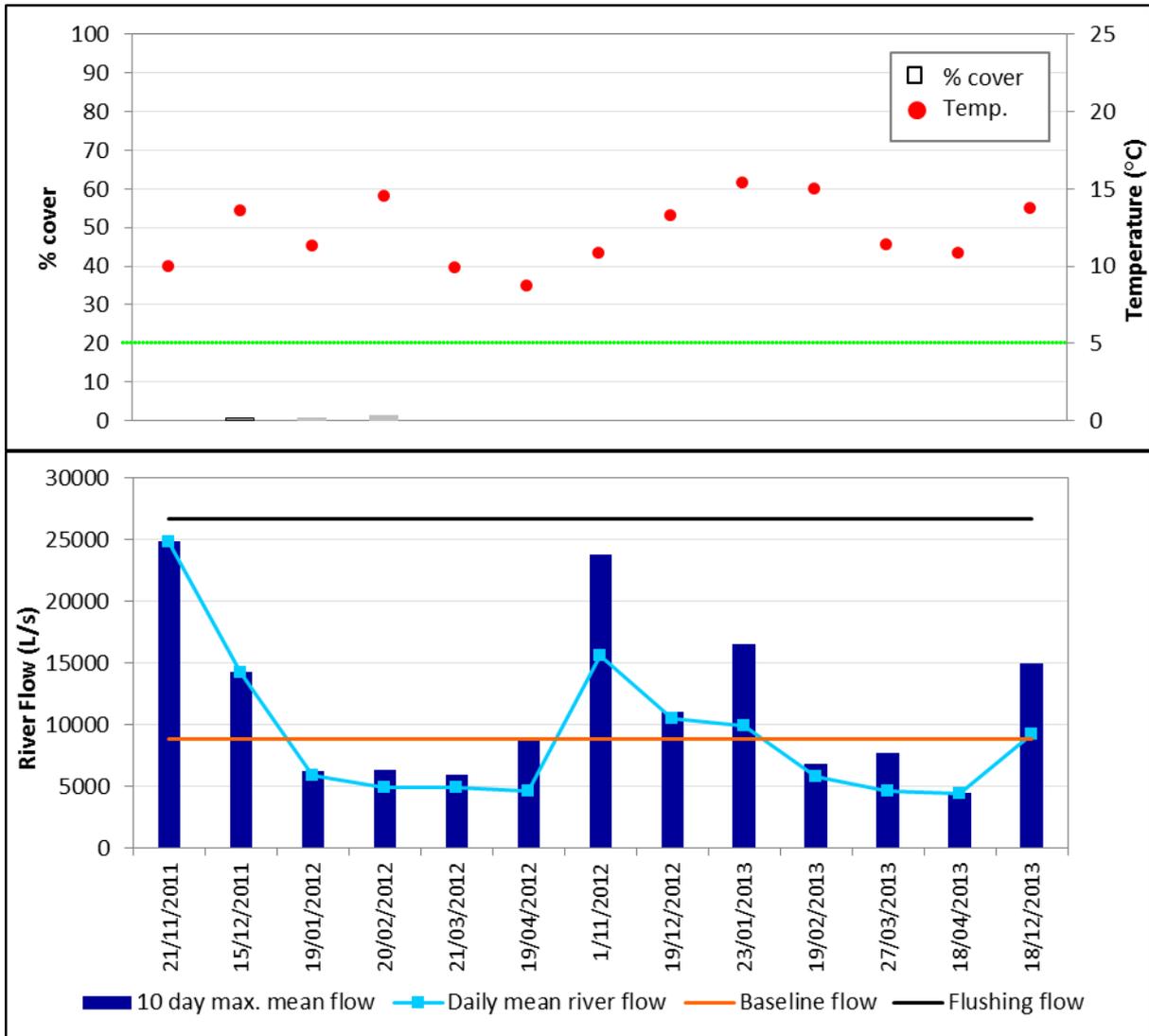


Figure 3. Average *Phormidium* mat cover and temperature for Ashburton River at SH72. Flow data is for the Ashburton River at Mt Somers. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

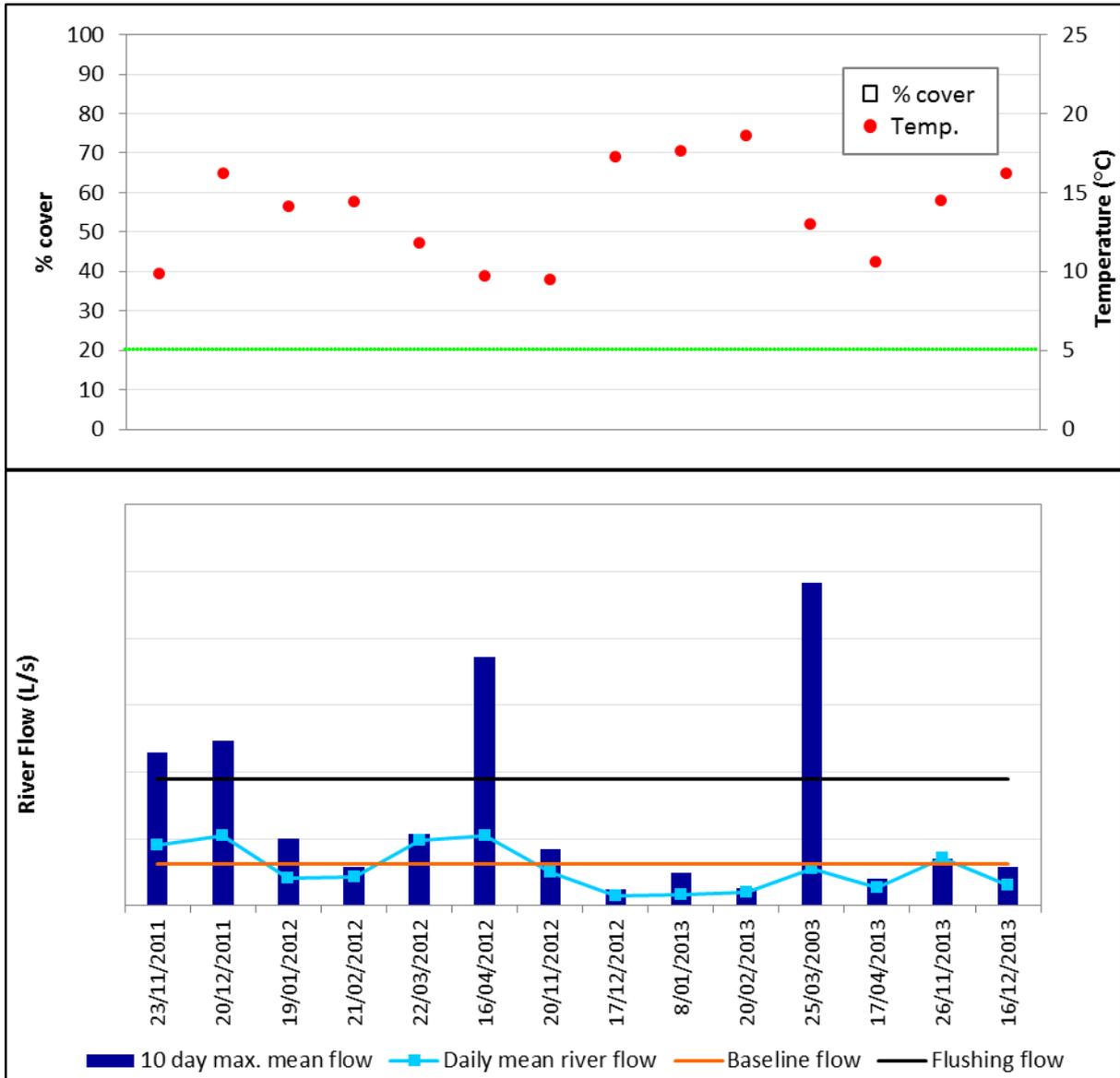


Figure 4. Average *Phormidium* mat cover, temperature and river flow for Conway River at SH1. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

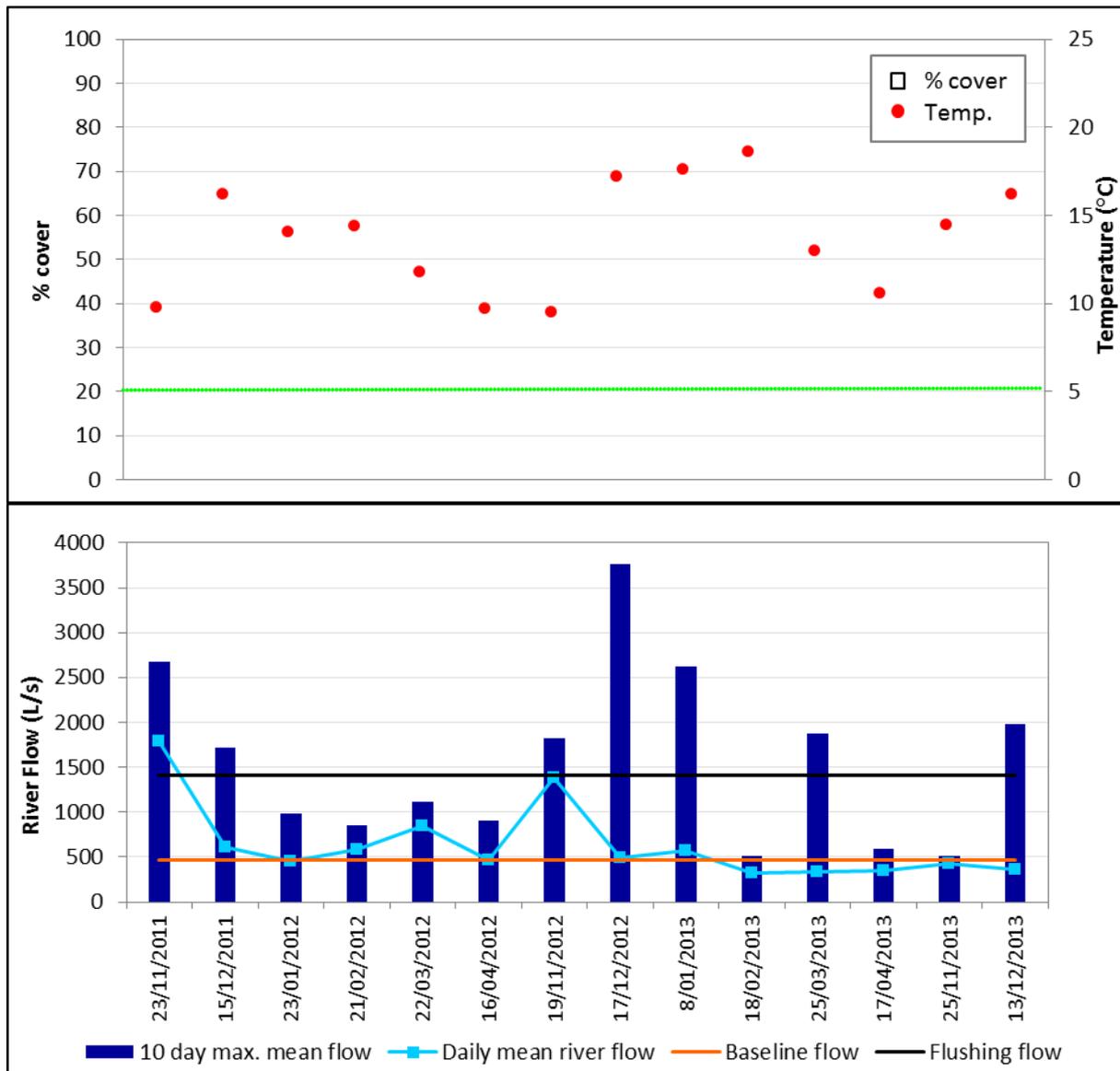


Figure 5. Average *Phormidium* mat cover, temperature and river flow for Hae Hae Te Moana River at Glentohi. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

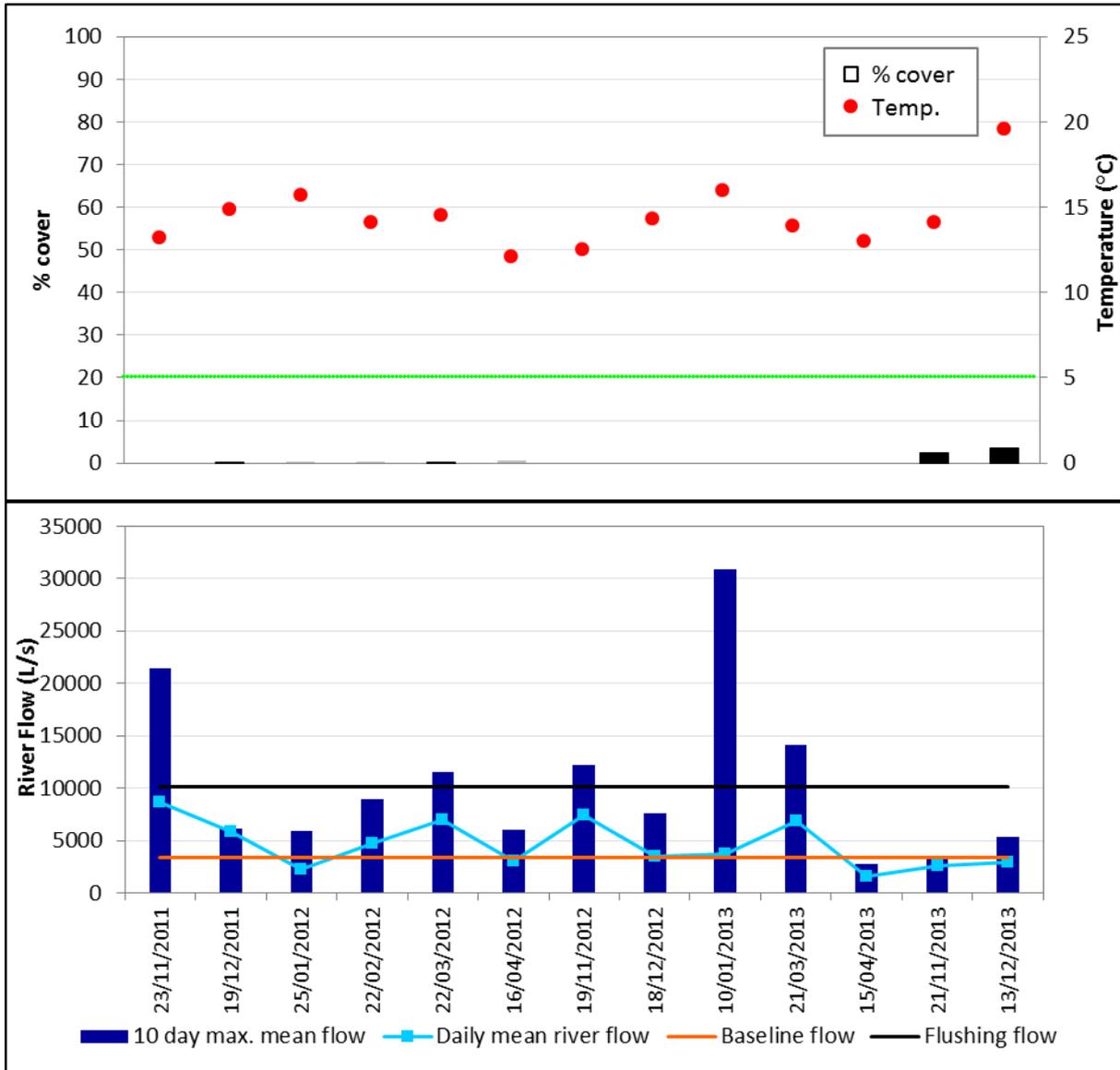


Figure 6. Average *Phormidium* mat cover, temperature and river flow for Opihi River at Rockwood. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

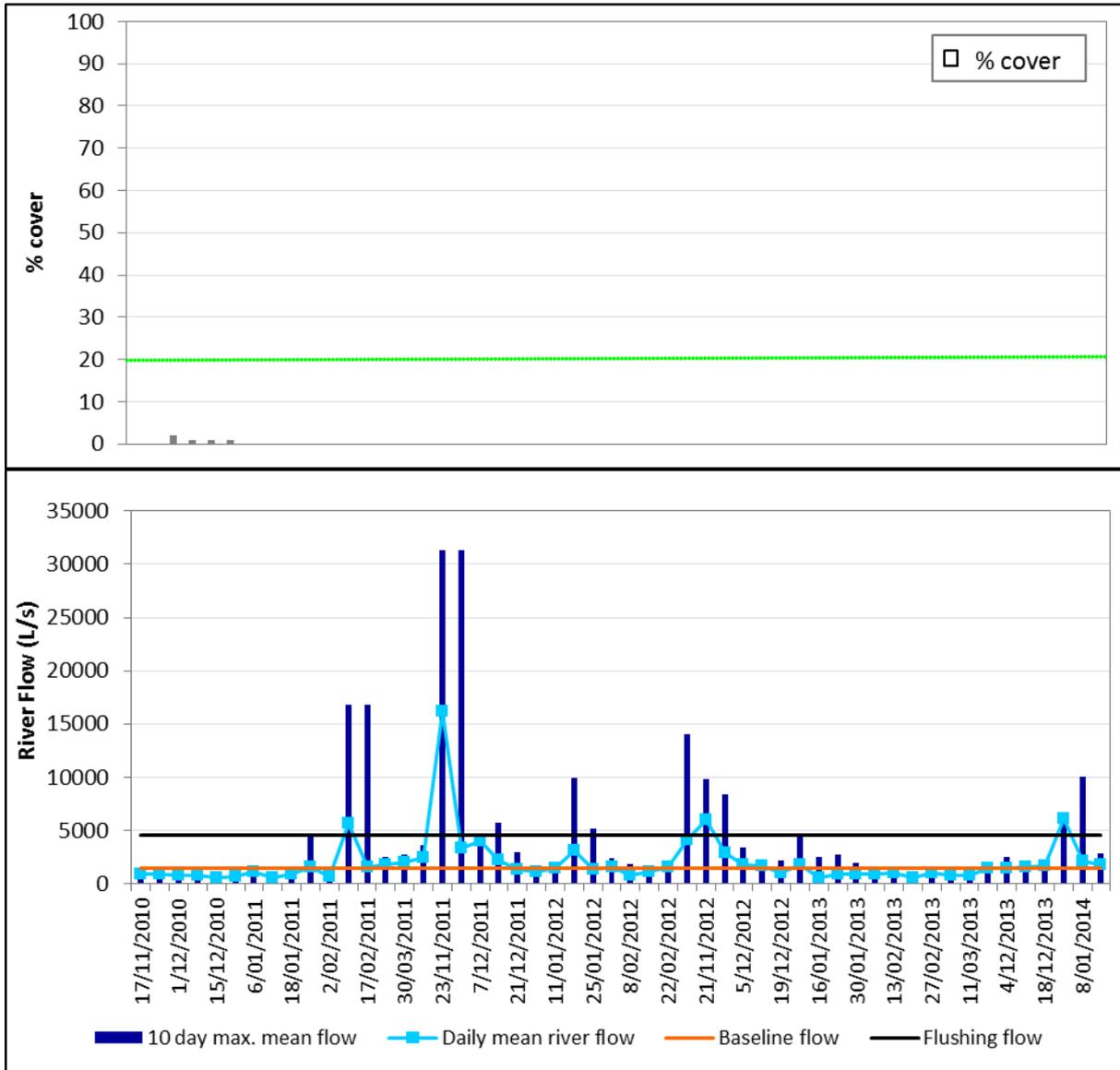


Figure 7. Average *Phormidium* mat cover and river flow for Waihao River at Bradshaws Bridge. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

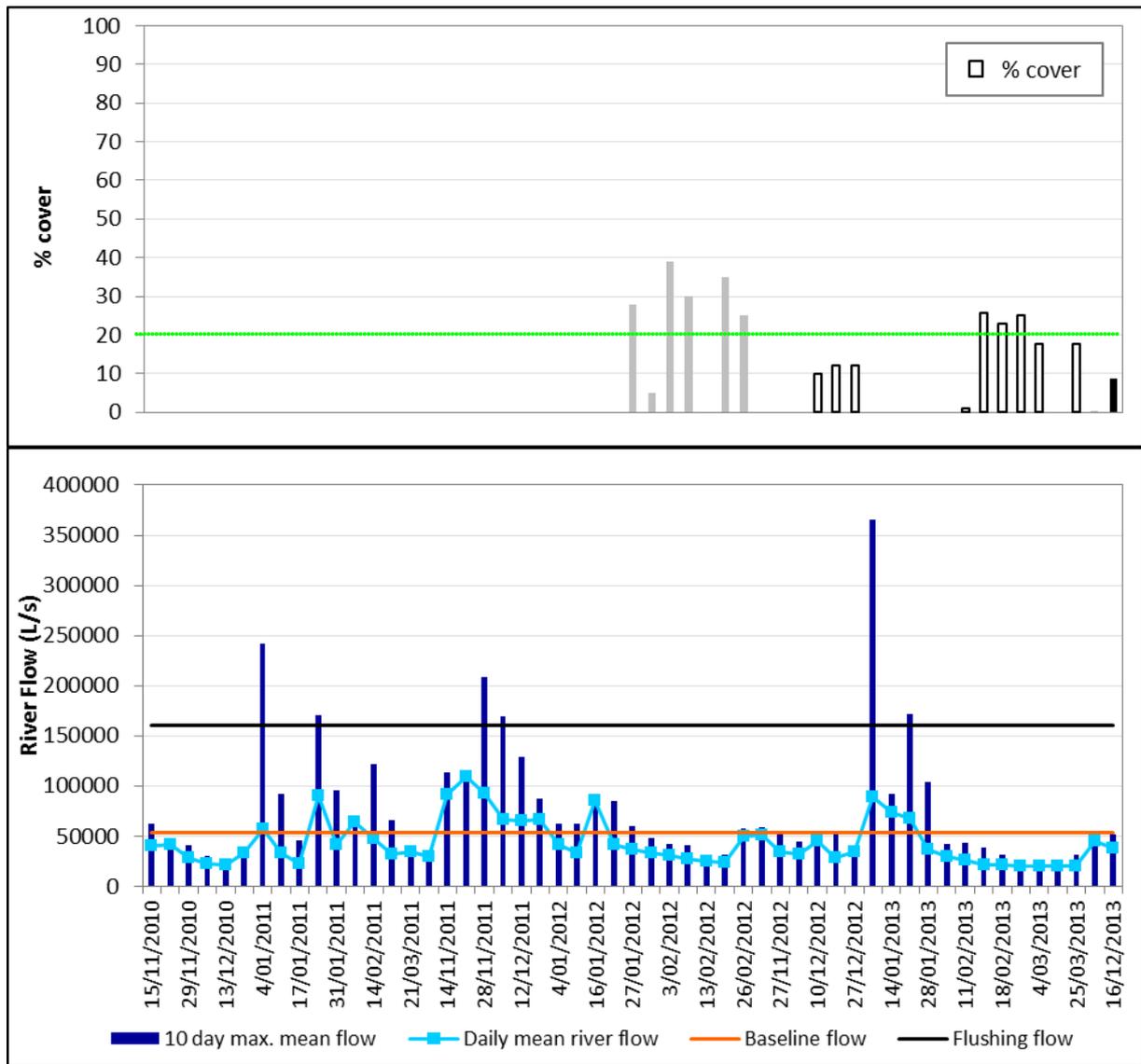


Figure 8. Average *Phormidium* mat cover and river flow for Hurunui River at SH1. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

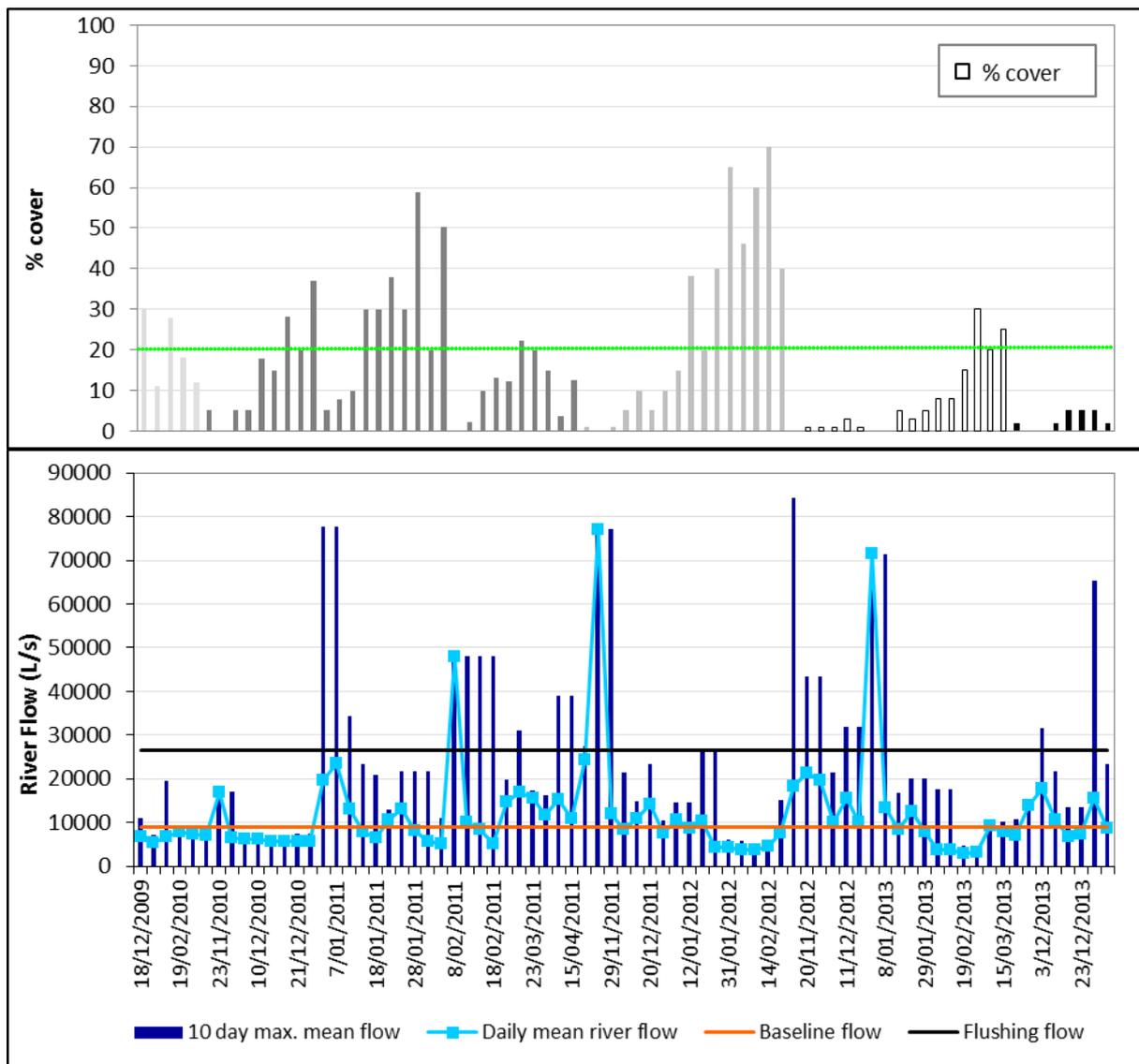


Figure 9. Average *Phormidium* mat cover and river flow for Opihi River at SH1. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

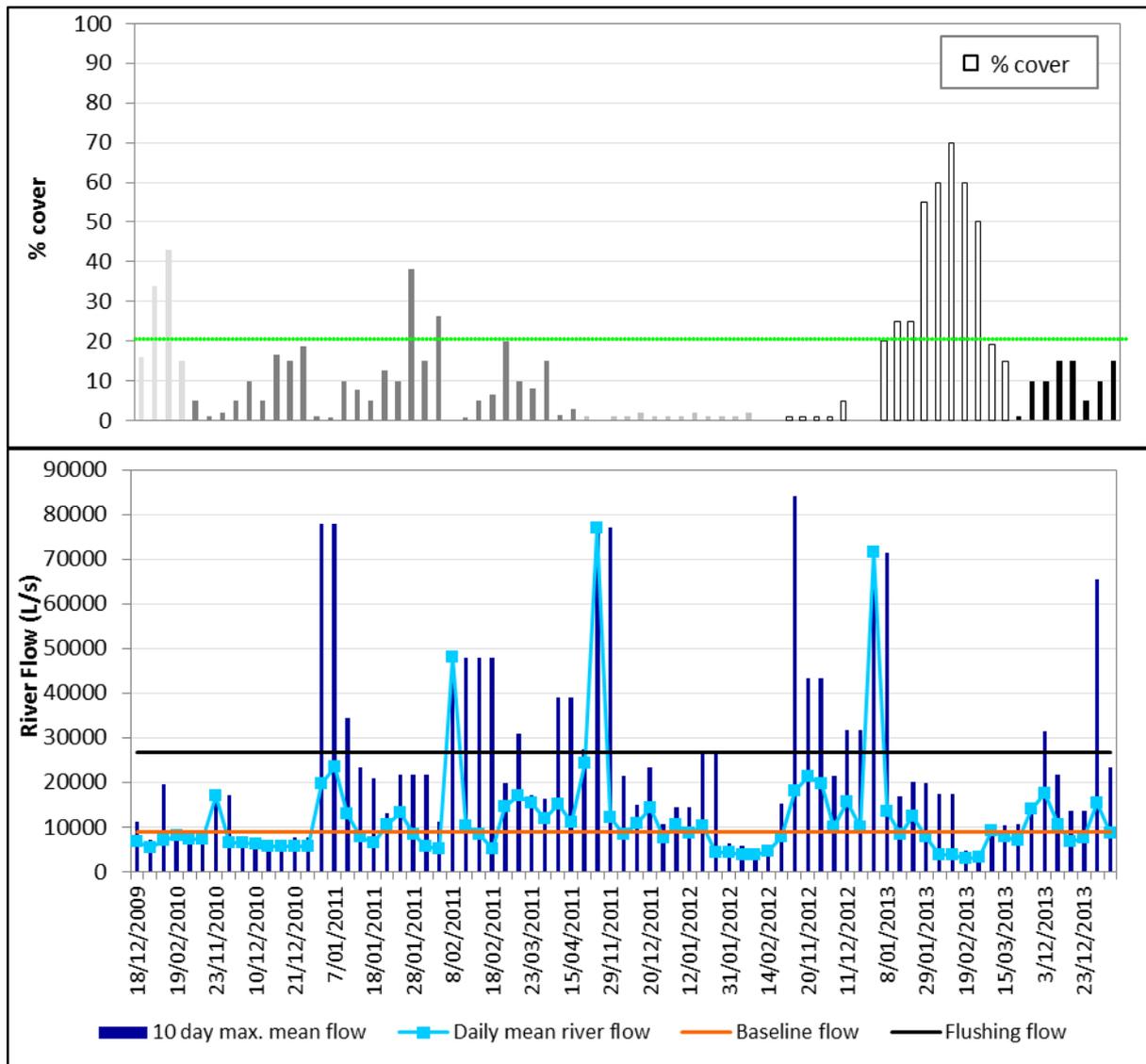


Figure 10. Average *Phormidium* mat cover for Opihi River at Waipopo. Flow data is for the Opihi River at SH1. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

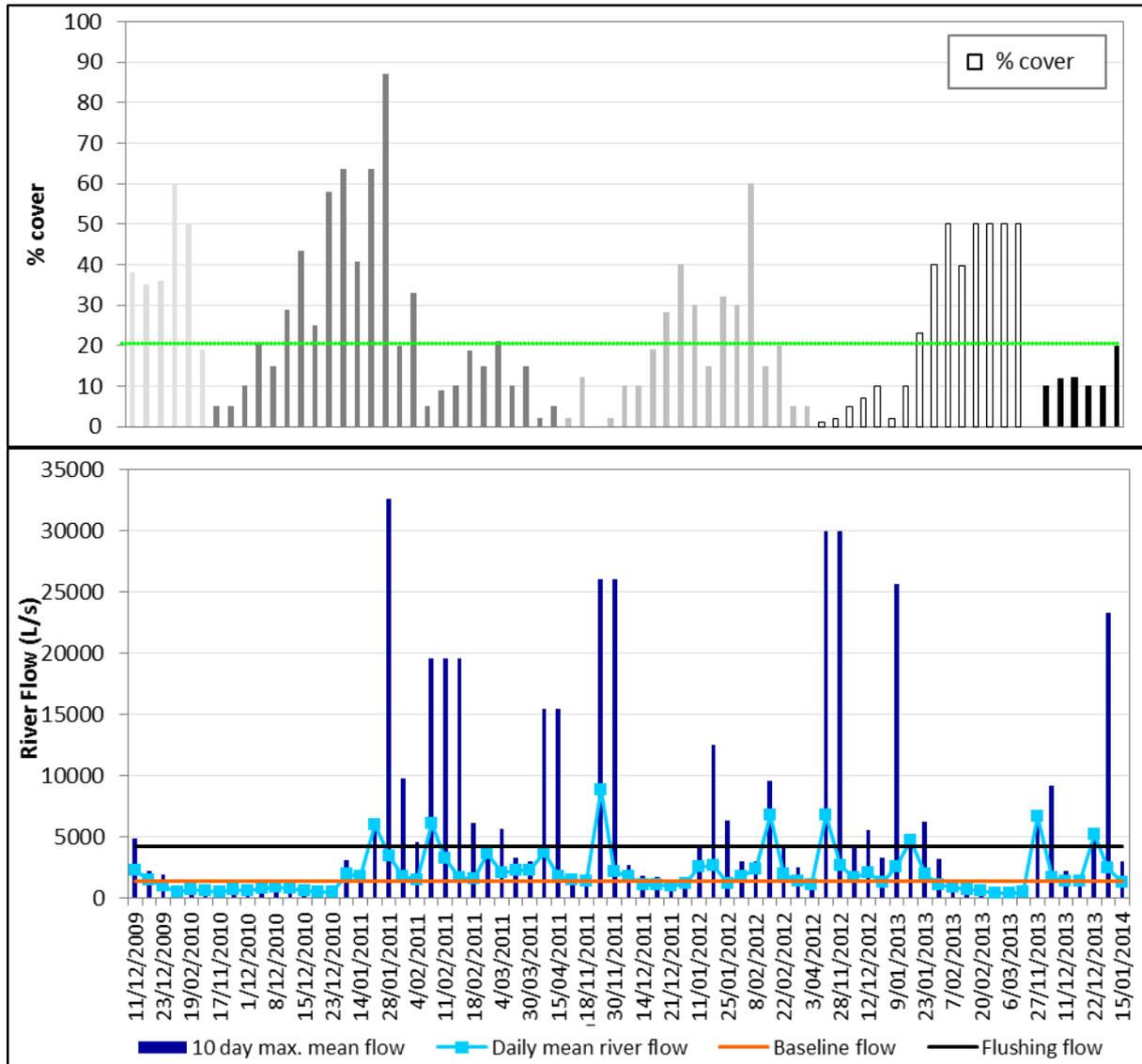


Figure 11. Average *Phormidium* mat cover and river flow for Pareora River at the huts. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

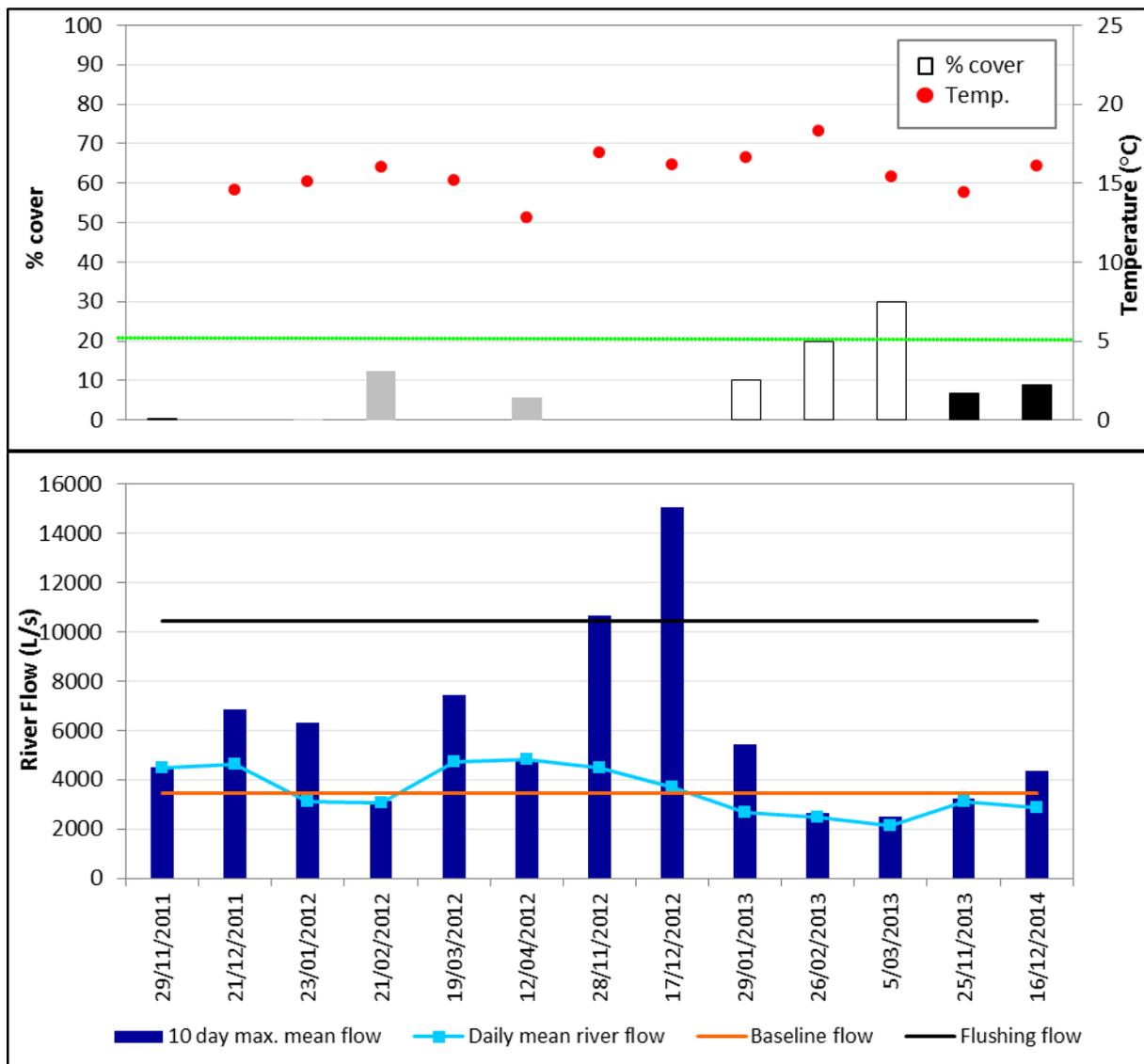


Figure 12. Average *Phormidium* mat cover, temperature and river flow for Temuka River at Manse Bridge. Light grey bars represent November 2009- April 2010, dark grey bars represent November 2010- April 2011, grey bars represent November 2011- April 2012, white bars with black outline represent November 2012- April 2013 and black bars represent November 2013- January 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

3.1.2 Substrate Composition

The dominant substrates for most sites were gravel and cobble. Ashley at the Gorge, Pareora at Huts and Hurunui at SH1 had a larger proportion of boulders (>20%) compared to all other sites (Fig. 2). Interestingly, some of the sites without *Phormidium* problems have a larger proportion (>60%) of gravel, fine gravel and sand/silt, than those with *Phormidium* problems, with the exception of the Temuka at Manse Bridge site. The sites with persistent *Phormidium* problems are dominated (excluding Temuka at Manse Bridge) by larger substrate (<46% boulder/cobble).

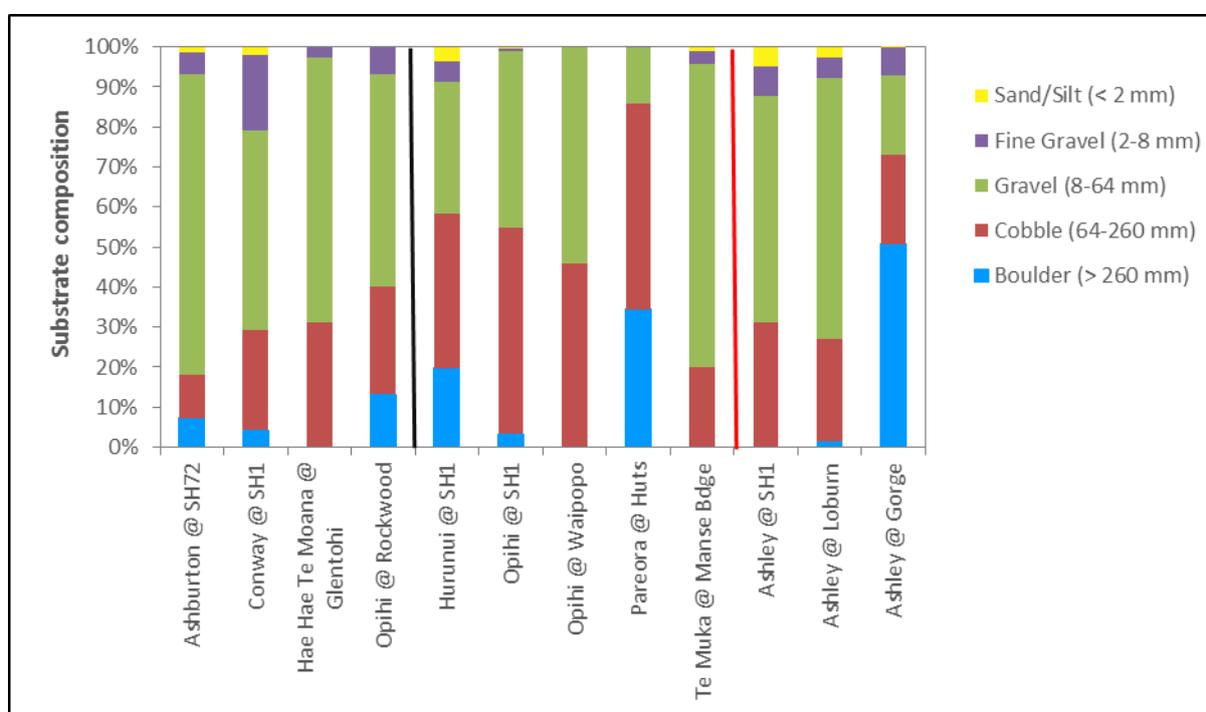


Figure 2. Average percent cover of various substrates at all sampling sites. Values are averages of 20 measurements for all sites, except the three sites along the Ashley River /Rakahuri which are averages of 200 views. The four sites left of the black line indicate sites without historical *Phormidium* proliferations, the five sites directly on the right are sites with historical *Phormidium* proliferations.

3.1.3 Nutrients

The results in Table 1 below are averages calculated from data (2009-2014) provided by ECAN. Results for sites marked with a * are averages from weekly samples between 2013 and 2014 and N +N is only nitrate-N. Highlighted sites are ones with historical cyanobacterial proliferations. There were no significant differences between DRP at sites with and without

Phormidium issues. All are rather low and none exceeded 0.01 mg/L. In contrast DIN concentrations are high at all sites, with the exception of Ashburton at SH72. TN:TP and DIN:DRP ratios (by weight) tended to exceed the value of 10 that is commonly taken to indicate the switch between N and P limitation of growth rate, though all sites along the Ashley River/Rakahuri, where blooms were present, had low TN:TP ratios.

Table 1. Summary of nutrient concentrations for all study sites, including; ratio of total nitrogen to total phosphorus (TN:TP), ratio of dissolved inorganic phosphorus to dissolved reactive phosphorus (DIN:DRP), dissolved inorganic nitrogen (DIN), dissolved reactive phosphorus (DRP), total nitrogen (TN), total phosphorus (TP), nitrate-N and nitrite-N (N + N) and ammoniacal-N (NH₄). The first five unhighlighted sites are those without historical proliferations. All measurements are given in mg/L.

Site	DIN	DRP	TN	TP	N + N	NH ₄	TN:TP	DIN:DRP
Ashburton @ SH72	0.0342	0.0018			0.0289	0.0054		19:1
Conway @ SH1	0.3037	0.0083			0.3998	0.005		36.6:1
Hae Hae Te Moana @ Glentohi	0.1053	0.0037			0.1178	0.0054		28.5:1
Opihi @ Rockwood	1.2637	0.0077			1.4913	0.009		164.1:1
Waihao @ Bradshaws Bridge	0.57	0.0033	0.6942	0.0108	0.5835	0.0094	64.3:1	172:1
Hurunui @ SH1	0.4967	0.0043			0.4917	0.005		115.5:1
Opihi @ SH1	0.65	0.0051	0.6390	0.0081	0.6328	0.0051	78.9:1	151.2:1
Opihi @ Waipopo	0.71	0.0036	0.8075	0.0069	0.6988	0.0109	117:1	127.5:1
Pareora @ Huts	0.59	0.0068	0.89	0.0082	0.5775	0.0098	108.5:1	86.8:1
Temuka @ Manse Bridge	1.7031	0.0087			1.9971	0.0127		195.8:1
Ashley @ SH1*		0.0059	0.2842	0.0365	0.1092		11:1	
Ashley @ Loburn*		0.0073	0.1245	0.0364	0.1220		5.5:1	
Ashley @ Gorge*		0.0053	0.3258	0.0495	0.0431		9:1	

3.1.4 Water temperature

Phormidium was observed in temperatures ranging between 4–20°C (data not shown) in Canterbury rivers. On 26 July 2011, 4% cover of *Phormidium* was recorded at the Rangitata River at a water temperature of 4°C. Furthermore, a bloom of 24% cover was recorded on the 26 June 2012 in the Temuka River at a temperature of 8°C (data not shown). There appears to be no correlation between temperature and *Phormidium* percentage cover (data not shown).

3.2 Ashley River Survey

3.2.1 Ashley River/Rakahuri at SH1

Persistent blooms have been recorded at this site from 2009–2014 (data not shown), despite the substrate being 57% gravel (Fig.2). During this study, two blooms were observed (Fig. 13). The water temperature varied between 15 and 17°C. A flushing flow occurred on the 30 December 2013, which appeared to remove all *Phormidium*, after which the percentage cover increased weekly by approximately 6% under stable flow conditions. This site has a low TN:TP ratio of 11:1 (Table 1).

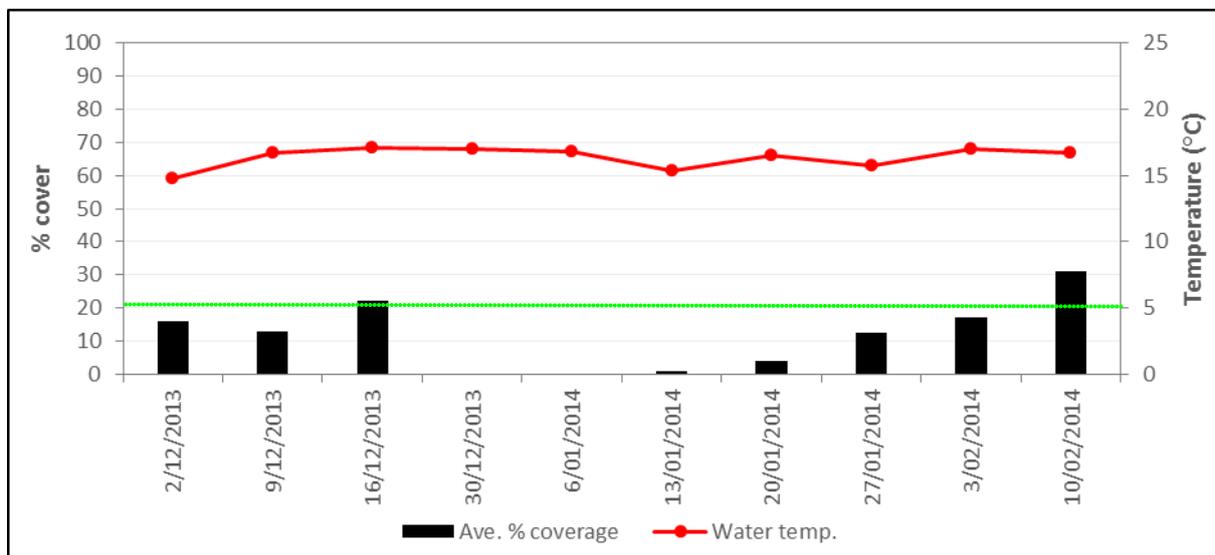


Figure 13. Average weekly *Phormidium* mat cover and water temperature for Ashley River/Rakahuri at SH1 from 2 December 2013 to 10 February 2014. The dashed green line represents 20% coverage indicating a bloom event.

3.2.2 Ashley River/Rakahuri at Loburn

Historically, cyanobacterial proliferations have been documented at this site on many occasions (data not shown). However, during this study cyanobacterial cover did not exceed 13.5% coverage (Fig. 14). This site went dry between the 14 January 2014 and the 20 January 2014. On the 20 January 2014 the survey was conducted on a dry riverbed. A flushing event occurred on the 30 December 2013 and the *Phormidium* percentage cover increased on average by 4.5% in subsequent weeks. This site has a similar substrate composition as Ashley River at SH1, with the dominant substrate being gravel (Fig. 2). This site had an extremely low TN:TP ratio of 6:1 (Table 1).

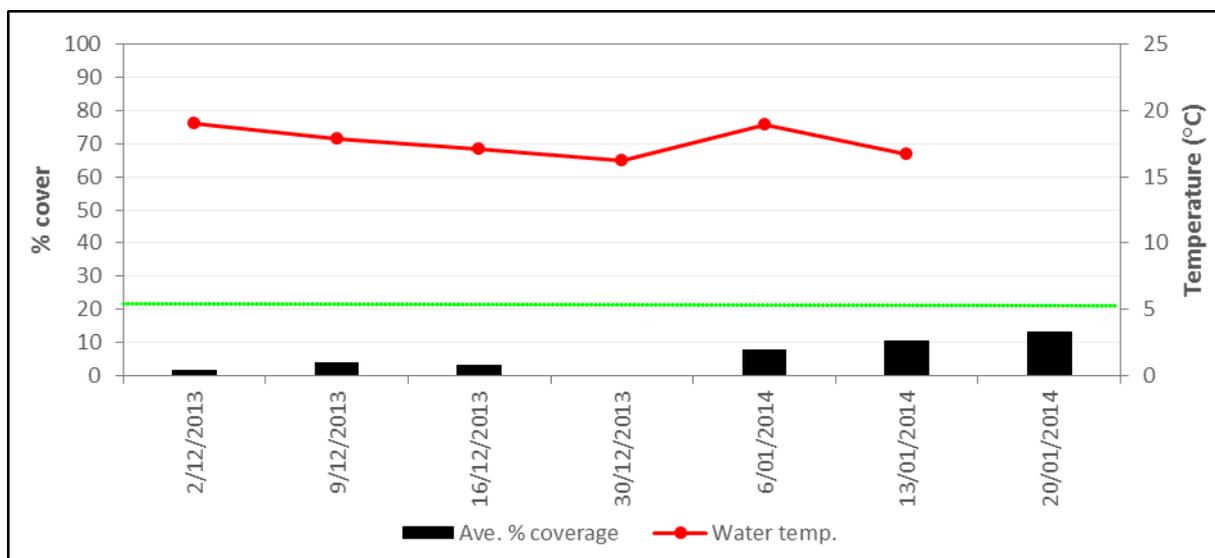


Figure 14. Average weekly *Phormidium* mat cover and water temperature for Ashley River/Rakahuri at Loburn from 2 December 2013 to 20 January 2014. The dashed green line represents 20% coverage indicating a bloom event.

3.2.3 Ashley River/Rakahuri at Gorge

A flushing flow of 403446 L/s (60 times the median flow) occurred on the 30 December 2013, which significantly reduced the *Phormidium* percentage cover (Fig. 15). Preceding this was a period of stable low flow, which allowed *Phormidium* percentage cover to increase by ca. 6% weekly for the next five weeks. Boulders are the dominant substrate type at this site (5%, Fig. 2). This site had a low TN:TP ratio of 9:1 (Table 1).

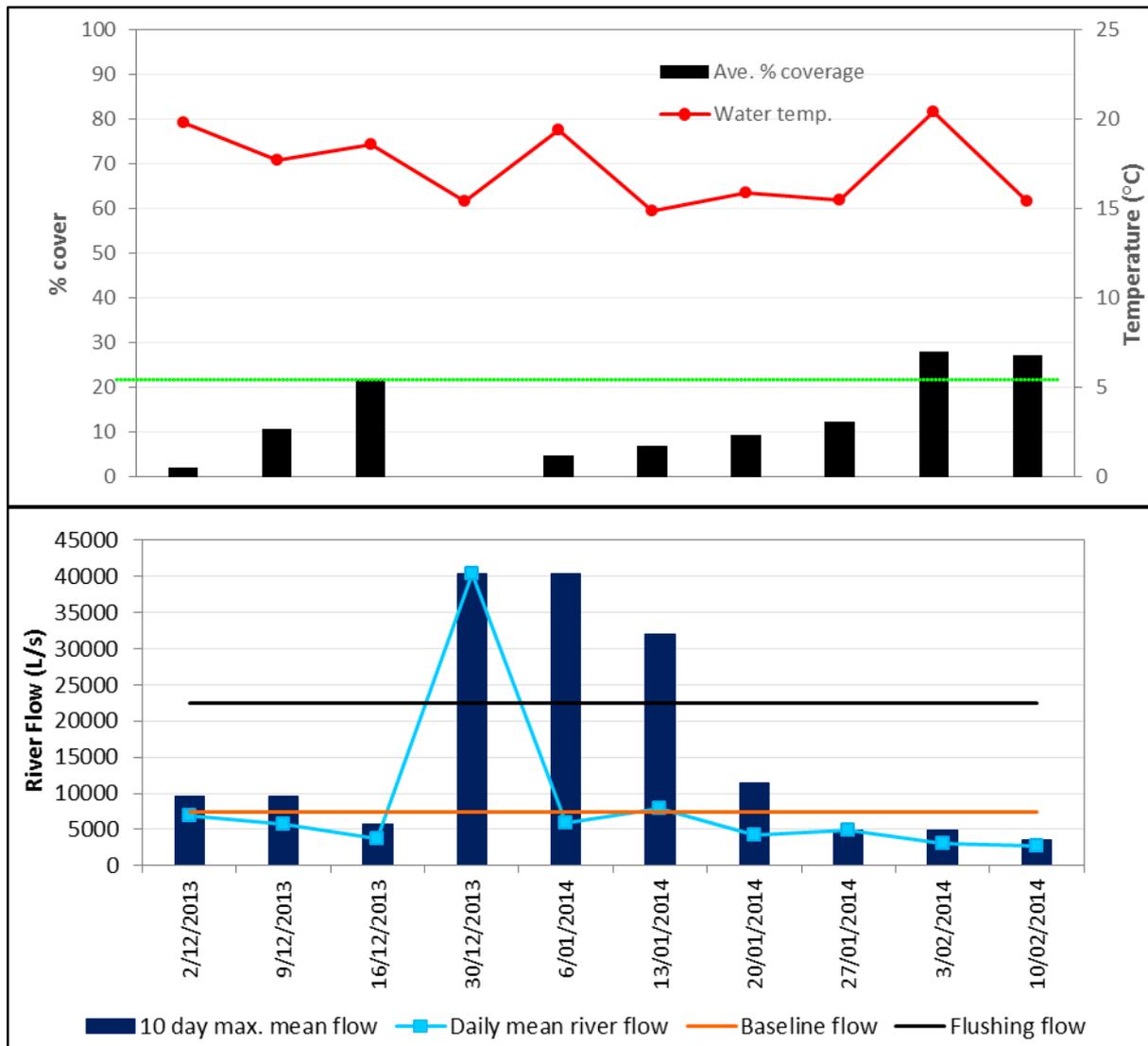


Figure 15. Average weekly *Phormidium* mat cover, water temperature and flow data for Ashley River/Rakahuri at Gorge from 2 December 2013 to 10 February 2014. The dashed green line represents 20% coverage indicating a bloom event. The baseline flow represents the median river flow for the entire record. The flushing flow is three times the baseline flow.

3.2.4 Depth and Point Velocity for Ashley River/Rakahuri

Depth ranged from 0.1 m to 0.9 m (Table 2). Average point velocities were similar at the SH1 and Loburn sites (Table 2). Comparatively, the Gorge site had a higher average velocity of 0.5 ms^{-1} and had the greatest mean depth of 0.29 m.

Table 2. Mean, minimum and maximum depths and point velocity (measured at the riverbed/water interface) for the three Ashley River/Rakahuri sites. Means were calculated from 200 measurements, except for the Loburn site, where means were calculated from 120 measurements.

	Depth (m)			Point velocity (ms^{-1})		
	Mean	Min.	Max	Mean	Min	Max
SH1	0.23	0.1	0.9	0.39	0	0.8
Loburn	0.13	0.03	0.45	0.32	0	0.8
Gorge	0.29	0.1	0.59	0.47	0	1.4

Section 4 Discussion

4.1 Substrate composition and *Phormidium* cover

An increase in the proportion of large sized substrate appeared to correlate with an intensification of *Phormidium* percentage cover. This is consistent with the findings of Heath *et al.*, (2012) who studied *Phormidium* at seven sites in the Hutt River over a 12 month period. Small substrate was the dominant substrate type at Conway at SH1. This, in conjunction with the occurrence of relatively frequent flushes at this site as well as Ashburton at SH72, provides a relatively unstable habitat that may prevent *Phormidium* blooms from occurring.

Heath *et al.*, (2013) also observed that *Phormidium* can proliferate on all substrate types. A similar observation was made in this study where over the study period (November 2013 to January 2014) it was noted as present on all substrate types from bedrock to silt in Canterbury rivers. This infers that substrate size alone may not be a key variable in controlling *Phormidium* blooms, but rather the combination of substrate stability and flow variability. Large boulders may provide refuge for *Phormidium* inoculums during periods of high flow (Murdock & Dodds, 2007). During various site visits of the Canterbury rivers when *Phormidium* was present at low levels (<0.1%) it was observed mostly on large, stable substrate (personal observations).

4.2 Nutrients and *Phormidium* cover

The Canterbury river sites with high *Phormidium* coverage (>20% on multiple occasions) all had low DRP (<0.009 mg/L). This is consistent with studies in other regions of New Zealand (Biggs & Price, 1987; Wood & Young 2012; Heath *et al.*, 2013). However, the sites without *Phormidium* issues also have low DRP, this suggests that this is not the only variable regulating *Phormidium* blooms in Canterbury rivers.

Other studies of New Zealand rivers have demonstrated a correlation between high TN:TP ratios (>15:1) and *Phormidium* blooms (Heath *et al.*, 2011; Heath *et al.*, 2012; Wood & Young, 2012) and it has been hypothesized that *Phormidium* has a competitive advantage over other algae in low phosphorus environments. However, more research is required to elucidate how *Phormidium* obtains enough phosphorus to support its rapid growth. A unique feature of *Phormidium* mats is the occurrence of a thin layer of fine sediment at the substrate/mat interface. Thus one hypothesis is that phosphorus bound to the fine sediment may become accessible to *Phormidium* due to the thick cohesive nature of the mats and the process of photosynthesis (raising pH during the day) and/or respiration (lowering oxygen concentrations at night), which could enhance release of biologically available phosphorus. *Phormidium* filaments are extremely motile and it is proposed that the filaments' capture sediment and then use their motility to stay above the sediment.

With the exception of 'Ashburton at SH72', all sites had DIN elevated above the threshold of 0.1 mg/L suggested by Wood and Young (2012) as being required for *Phormidium* proliferation. *Phormidium* in New Zealand is thought to lack the ability to fix nitrogen (Heath, unpublished data), therefore, requires moderate nitrogen concentrations for rapid

growth. Low DIN concentrations may therefore restrict the growth of *Phormidium* at 'Ashburton at SH72' and 'Hae Hae Te Moana at Glentohi'. Low DIN concentrations may also favour algal species with the ability to fix nitrogen and therefore may explain the lack of *Phormidium* at 'Conway at SH1' and the dominance of other algal species.

Increased nitrate concentrations in a water body is expected to stimulate faster biomass accrual, as a larger concentration gradient will increase the rate of diffusion from the water to the cell surface (Chételat *et al.*, 1999). This may explain the fast biomass accrual observed at some sites. For example, *Phormidium* percentage cover doubled from 30% (1 February 2012) to 60% (8 February 2012) in one week at the 'Pareora River at Huts'. High resolution sampling of nutrient concentrations and growth rate would be required to confirm possible relationships.

4.3 Temperature and *Phormidium* cover

Peaks in periphyton percentage cover have been documented during summer months in New Zealand, when the flow is stable and the temperature is elevated (Biggs & Close, 1989; Biggs, 1990; Heath *et al.*, 2010, 2011). This is also the case in the Canterbury region, with peaks in *Phormidium* cover usually occurring in late summer. Temperature has been identified as being an important factor in regulating the occurrence of *Phormidium* blooms in New Zealand (Heath *et al.*, 2011). However, no clear relationship was found between temperature and *Phormidium* percentage cover in this study because only data from the summer periods were used.

4.4 River flow and *Phormidium* cover

It has been suggested that the frequency of flushing events and duration of stable flow may be more important than the river flow in predicting the occurrence of *Phormidium* (Milne & Watts, 2007, Heath *et al.*, 2011, Wood & Young, 2007, Heath *et al.*, 2012). The general rule implemented within various regional councils around New Zealand is that a flushing flow (usually considered to be three times the median) is adequate to remove *Phormidium* (Clausen & Biggs, 1997, Milne & Watts, 2007).

High flow events clearly did have an impact on *Phormidium* in Canterbury rivers, but the "three times median" rule was not universally applicable. Similarly, Wood and Young (2012) concluded that this rule was not appropriate for all the rivers in the Manawatū-Whanganui region. Flows greater than six times the median were required to reduce the *Phormidium* cover at the 'Pareora at Huts' site. Conversely, at the 'Temuka River at Manse Bridge' site a flow of only two times the median was sufficient to remove all *Phormidium*. This difference may be a function of differences in the stability of the substrate, and therefore the interaction between flow and substrate size. For example, a flushing flow of 23 times the median was recorded at the 'Pareora River at Huts' site. This flushing flow reduced the percentage cover of *Phormidium* from 87% (28 January 2011) to 20% (2 February 2011). This site also had the greatest proportion of bedrock and cobble substrate of all sites (>80%). This suggests that this high river flow may have not been strong enough to mobilize the large substrate and therefore remove all *Phormidium* mats. River-specific flushing flow rules may need to be identified for specific sites, to allow for substrate type and flow characteristics of the site.

4.5 Velocity and depth and *Phormidium* cover

Francoeur and Biggs (2006) highlight the importance of water velocity in removing algal biomass. However, their study also shows that mat-forming species may be more tolerant of higher velocities than other growth forms. An increased water velocity may actually result in more rapid increases in *Phormidium* percentage cover at a site if it reduces the boundary layer surrounding the *Phormidium* mat and allows for faster uptake of nutrients from the surrounding environment. This may explain why *Phormidium* commonly grows in riffles. Heath *et al.*, (2012) document *Phormidium* percentage cover as being greatest at velocities of less than 1.1 ms^{-1} . However, they demonstrated that at a velocity of 2.1 ms^{-1} , *Phormidium* cover still exceeded 20%. Similarly, in this study at the Ashley River at the gorge site, *Phormidium* cover exceeded 20% with an average water velocity of 1.4 ms^{-1} .

Phormidium appears to be efficient in utilizing low light conditions, as it was observed in a variety of depths during this study. Furthermore, Heath *et al.*, (2012) found that *Phormidium* in the Hutt River had no preference for depth. He attributed this finding to clear water, allowing high light penetration. This may also be the case for *Phormidium* in the Ashley River/Rakahuri as turbidity readings did not exceed 10 NTU unless the river was in flood.

Section 5 Conclusions and Further Research

The findings from the current study are preliminary and give an indication of some of the factors that may promote *Phormidium* blooms in the Canterbury region. *Phormidium* was present at all sites sampled, but blooms only formed at some. The frequency of flushing flows appears to be an important environmental parameter controlling bloom formation in Canterbury rivers. All of the rivers with high *Phormidium* bloom frequency had high DIN concentrations ($\geq 0.5 \text{ mgL}^{-1}$) and moderately low DRP concentrations ($\leq 0.01 \text{ mgL}^{-1}$), but some sites meeting these nutrient criteria did not have regular *Phormidium* blooms. The interaction between substrate stability and flow restricts the applicability of a single flushing flow value within all Canterbury rivers. Low DRP concentrations may be required for *Phormidium* to bloom but this requires further testing because low values were observed at all sites. However, the role of DIN is not yet completely understood. Low temperatures do not prevent *Phormidium* growth.

Future research should concurrently measure water quality attributes and cyanobacterial coverage, whilst keeping other environmental factors constant, such as substrate type. Further research is required to discover where *Phormidium* obtains alternative sources of phosphorus, as it does not appear to be the DRP in the water column. This is a phenomena which has recently been discovered in didymo (see Bothwell, Taylor & Kilroy, 2014). Other water quality attributes (dissolved organic carbon, dissolved metals etc.) may be influencing the growth of *Phormidium* and therefore needs to be investigated further. The rate of *Phormidium* growth may also be an important component for predicting the ability of blooms to form at particular sites, which requires further research.

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Appendix

Appendix 1. Site locations

Table A1. Coordinates for all sites.

Site	NZTMX	NZTMY
Ashley at SH1	1574900.9	5208021.7
Ashley at Loburn	1567269.9	5207656.1
Ashley at Gorge	1537076.9	5213274.8
Ashburton at SH72	1481519.4	5170925.3
Conway at SH1	1634279.6	5283617.9
Hae Hae Te Moana at Glentohi	1448658.9	5121784.2
Opihi at Rockwood	1435562.7	5107424.3
Waihao at Bradshaws Bridge	1454091.3	5040401.2
Hurunui at SH1	1608329.3	5250306.6
Opihi at SH1	1462012.9	5097395.5
Opihi at Waipopo	1466881.6	5096730.5
Pareora at Huts	1445305.4	5080570.6
Temuka at Manse Bridge	1461775.2	5099444.2

Appendix 2. Raw data for the Ashley River survey

T1, T2, T3 and T4 represent the averages in benthic cyanobacterial cover for the respective transect. Where cells are empty the parameter was not measured due to equipment malfunction, with the exception of transects on the 30.12.13 where surveys were not conducted due to a flood.

Table A2.1 Environmental parameters measured and the average percentage cover of *Phormidium* for each transect and the total average for Ashley River at SH1.

Date	Temp. (°C)	DO (mg/L)	Cond. (u/S)	pH	Turbidity (NTU)	T1	T2	T3	T4	Overall % cover
02.12.13	14.8	11.18	88.6	7.29	0	2.4	4.7	3	54.4	16.125
09.12.13	16.7	10.11	97	7.53	21.67	15.6	18	9	9.4	13
16.12.13	17.1	8.68	99		4	20	25	28.4	15	22.1
30.12.13	17	9.0	86.1	7.8	44					
06.01.14	16.8	9.4	88	8.1	1	0	0	0	0	0
13.01.14	15.3	9.9	85.6	7.6	0	1.4	1.2	1.8	0	1.1
20.01.14	16.5	9.6	88.1	7.6	0	5.6	4.4	3	2.8	3.95
27.01.14	15.7	9.1	95.4	7.2	7	13.4	15.4	14	8.1	12.725
03.02.14	17	9.61	89		0	15.4	23	14	16	17.1
10.02.14	16.7	8.37	93.2	6.8	0	32	36	33	23.2	31.05

Table A2.2 Environmental parameters measured and the average percentage cover of *Phormidium* for each transect and the total average for Ashley River at Loburn. Site was dry on the 20.01.14 and

Date	Temp. (°C)	DO (mg/L)	Cond. (u/S)	pH	Turbidity (NTU)	T1	T2	T3	T4	Overall % cover
02.12.13	19	11.06	91	7.66	0	0	2.4	0	4	1.6
09.12.13	17.9	10.25	103.1	7.56	14.25	4.5	7	2.6	2.4	4.125
16.12.13	17.1	9.59	106.7	EM	7.3	7.6	2	2.4	2	3.5
30.12.13	16.2	9.13	86.7	7.8						
06.01.14	18.9	9.51	88.6	8.1	1	7.9	10.4	13.4	0	7.925
13.01.14	16.7	9.84	85.8	7.4	0	4.6	12	6	20.4	10.75
20.01.14						25	8.4	8.6	12	13.5

transects were conducted on dry riverbed.

Table A2.2 Environmental parameters measured and the average percentage cover of *Phormidium* for each transect and the total average for Ashley River at the Gorge. Site was dry on the 20.01.14 and transects were conducted on dry riverbed.

Date	Temp. (°C)	DO (mg/L)	Cond. (u/S)	pH	Turbidity (NTU)	T1	T2	T3	T4	Overall % cover
02.12.13	19.8	10.3	77.9	7.99		0.4	4.4	2	2	2.2
09.12.13	17.7	9.25	91.7	7.76	25.25	7	18	5.8	12	10.7
16.12.13	18.6	9.52	96.2		9	26.5	28	22	11	21.875
30.12.13	15.4	9.53	68.8	8.3	20.5					
06.01.14	19.4	9.34	78.4	8.1	1	3.6	5.4	7.2	2.8	4.75
13.01.14	14.9	9.76	76.4	7.8	0	3.4	12	6.2	6	6.9
20.01.14	15.9	9.49	86	7.4	0	16.5	2	12.6	6	9.275
27.01.14	15.5	9.54	95.3	7.4	5	14.6	11.4	11.6	11.8	12.35
03.02.14	22.4	8.74	93.8			31	44	28	9	28
10.02.14	15.4	9.91	97	7.71		41.4	25	32	10.8	27.3

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