Spatial and temporal patterns in nutrient concentrations and periphyton in the Hurunui River

January to May 2015

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Prepared by:
Cathy Kilroy
Janine Wech

For any information regarding this report please contact:

Cathy Kilroy
Freshwater Ecologist

+64-3-343 7883
cathy.kilroy@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd
PO Box 8602
Riccarton
Christchurch 8011

Phone +64 3 348 8987

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	Reviewed by:	Clive Howard-Williams
	Formatting checked by:	Tracy Webster
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Executive summary

The ~100 km of the Hurunui River downstream of Lake Sumner spans a wide gradient of water quality from near pristine low-nutrient waters to mesotrophic conditions at State Highway 1 (SH1). Water nutrient concentrations and nuisance periphyton, especially the potentially toxic cyanobacterium *Phormidium*, have been issues in the lower reaches of the river since the late 1990s. Their management was addressed in the Hurunui and Waiau River Regional Plan (HWRRP), which specifies limits for periphyton biomass and cover, annual load limits for dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP), and concentration limits for DRP.

In 2014, Ngai Tahu Forest Estates Ltd. (NTFE) applied for resource consents related to changing the land use of Balmoral Forest from forestry / dryland farming to irrigated farming /dairying. The application was supported by limited periphyton nutrient limitation investigations in 2014. The outcome was that consent was granted for a limited development only, because of (a) concerns about effects on the Hurunui River and (b) inherent limitations under the requirements of the HWRRP.

Subsequent to the decision, NTFE asked NIWA to undertake a more comprehensive investigation of water quality, periphyton nutrient limitation and growth rates in the middle reaches of the Hurunui River. The aim was to provide information about relationships between nutrient concentrations and periphyton in different parts of the river, which has general relevance in the context of the HWRRP and in particular the extent to which periphyton growth and biomass accumulation can be controlled by managing nutrient concentrations. The study ran from January to May 2015 and included five main components: water quality, periphyton accrual rate trials, nutrient limitation assays, in-river periphyton surveys of cover and biomass, and sediment sources of phosphorus.

Four sites on the Hurunui main stem were included in the study: from upstream to downstream, Mandamus (at the flow recorder), State Highway 7 (SH7, upstream of the Waitohi confluence), Balmoral (upstream of the Dry Stream confluence) and Gorge (15 km upstream of the flow recorder at SH1). Hydrological conditions during the study included a long period of unusually low flows in the lower river, and a series of three small freshes (up to ~110 m^3/s, or ~ 2.9 x median flow at SH1) in March and April.

In the **water quality** study, analysis of samples from fifteen approximately weekly collections confirmed a strong gradient in DIN from Mandamus to Gorge (medians of 0.003 to 0.340 mg/L at Mandamus and Gorge), and a slight gradient in DRP (0.0006 to 0.0011 mg/L at Mandamus and Gorge). Both DIN and DRP at Gorge were unusually low compared to concentrations measured in the lower river in previous years. DIN at Gorge was negatively correlated with flow. Water temperature was coolest at Mandamus and was similar at the three downstream sites (approximately 1 °C warmer on average than at Mandamus).

Two 6-week **periphyton accrual rate trials** were conducted at each site. In Experiment 1 (6 January to 16 February) periphyton chlorophyll *a* accrual on artificial substrates was fastest at Gorge, followed by SH7, Balmoral and Mandamus. Lowest growth rates (assumed to be maximum accrual rates) at Balmoral were attributed to invertebrate gazing. In Experiment 2 (24 February to 20 April) growth rates increased in a downstream direction, as expected, and indicated definite P-limitation at Mandamus and slight P-limitation at Gorge.

A series of **nutrient limitation assays** was conducted between January and May. Periphyton was grown for 2 – 3 weeks on surfaces receiving either additional N, P, both N and P, or no additions

(controls). Higher growth in response to an added nutrient compared to the control indicated that that nutrient limited periphyton growth at that site. Periphyton growth at Mandamus was N-limited or co-limited by both N and P; at SH7 growth was usually co-limited by both N and P. Balmoral and Gorge were primarily P-limited, with possible secondary N-limitation at Balmoral in February. P-limitation was weakest at Gorge. The periphyton taxa *Gomphoneis minuta* var. *cassieae* (a stalked diatom) and *Cymbella kappii* (a mucilage-producing diatom) responded positively to P additions. *C. kappii* may also benefit from additional N. *Phormidium* showed no consistent response to either N or P.

In-river periphyton was monitored in nine surveys from January to May. Biomass and cover were lowest at Mandamus, where the community was limited by very low N and P, but at the same time resisted removal by high flows. At SH7, the periphyton was dominated by the bloom forming diatom *Didymosphenia geminata* (didymo). Biomass potential at SH7 may have become uncoupled from nutrient concentration in the overlying water because small diatom taxa could attain very high densities by utilising recycled nutrients within didymo mats. Cover at SH7 was relatively resistant to high flows. Low cover and biomass at Balmoral was likely a result of the mobile bed sediments at the site, but moderate cover by *Phormidium* was observed in higher velocity areas. Biomass (as chlorophyll *a*) was highest at Gorge, but was very responsive to removal by high flows. There was almost complete turnover of taxonomic composition of periphyton from upstream to downstream, suggesting strong nutrient control of community composition. Between-site variation in periphyton taxa was partly consistent with the taxonomic responses observed on nutrient limitation assays.

An **alternative source of phosphorus** was investigated because *Phormidium* forms blooms in the lower Hurunui, despite very low water column DRP concentrations. It has recently been proposed that *Phormidium* can thrive independently of DRP because fine-sediment-bound P is made available for growth by processes within the algal mat. We determined sedimentation rates (using sediment traps) at each site, and measured concentrations of potentially biologically available P bound to sediment. We found a strong downstream gradient of increasing sediment-bound P. The rate of deposition of loosely bound P on fine sediment (< 63 μm) at Gorge was over 13 times greater at Gorge than at Mandamus, compared to a ratio of less than 2 for DRP concentration. The results were consistent with the hypothesis that high cover by *Phormidium* at Gorge could be sustained by P from sediment.

The study results were used to address five questions:

1. *Which parts of the Hurunui River are saturated in terms of [nutrient supplies for] periphyton growth and which parts are not?*

   Nutrient-saturated periphyton growth implies that nutrient supplies are sufficient to sustain maximum growth rates. From January to April 2015 accrual rate experiments indicated that nutrient supplies were insufficient for maximum periphyton growth at all sites, but the degree of non-saturation varied between sites.

2. *How significant is the growth rate of periphyton to mass accumulation over time? [Are rapid growth rates always associated with high biomass and, conversely, are slow growth rates always associated with low biomass?]*

   Rapid accrual was generally associated with highest chlorophyll *a*, but there were exceptions. Despite relatively high growth rates, other factors can influence periphyton accrual, leading to

low biomass (e.g., invertebrate grazing at Balmoral), and despite low growth rates, biomass can attain high levels (e.g., through nutrient recycling within didymo mats, at SH7).

3. *Is the lower Hurunui River phosphorus limited (as stated in the HWRRP)?*

   Yes, but P-limitation was slight in the lower Hurunui and did not preclude high biomass under the low flow conditions during the study.

4. *Is it possible to manage periphyton growth by retaining phosphorus concentrations at their current levels, while allowing for a modest increase in nitrogen (as stated in the HWRRP)?*

   The studies from January to May 2015 provided some support for the proposal that modest increases in DIN in the middle reaches of the Hurunui will not lead to increased biomass provided DRP does not increase. However, the potential response of *Phormidium* to increased DIN concentrations is unclear. *Phormidium* appeared to grow randomly on all nutrient limitation assay treatments, with no consistent response to either N or P.

5. *To what extent can we "control periphyton growth and biomass accumulation" by managing nutrient concentrations?*

   Major differences in biomass and strong turnover of periphyton taxonomic composition along a strong gradient of increasing DIN and a more muted gradient in DRP indicate that nutrients play a large role in determining periphyton composition, growth and biomass in the Hurunui River.

   The outcomes of any given degree of nutrient reduction are almost impossible to predict, although the community assays in 2015 did provide some insight into periphyton taxa that respond to added N and P in the Hurunui (and therefore might decline in the lower Hurunui if nutrient concentrations decline). However, features of the Hurunui could mean that the consequences of reducing nutrients may not result in the desired outcomes (i.e., reduced periphyton cover and biomass). For example:

   - The presence of didymo in the upper Hurunui means that reducing nutrient concentrations downstream could make the downstream reaches more suitable for didymo.
   - Reducing DRP concentration may be ineffective if periphyton (*Phormidium* in particular) is sustained by sediment-sourced P. The gradient of fine-sediment-bound phosphorus identified in the Hurunui could cancel out reductions in DRP in the lower River (if it is not already doing so in periods such as January to May 2015, when water column DRP was very low).

Further discussion of conclusions is provided in Section 9 of this report.

1 Introduction

1.1 Background

Water quality and nuisance periphyton have been highlighted as issues in the lower reaches of the Hurunui River since the late 1990s. Consequently, the Hurunui has been the subject of a series of investigations to determine river values, effects of flow alterations, and sources of nutrients (e.g., Mosley 2002, Ausseil 2010). These studies collectively contributed to the Hurunui River catchment (along with the adjacent catchment of the Waiau River) becoming the first river in Canterbury for which a regional plan has been developed and implemented. Implementation of the Hurunui and Waiau River Regional Plan (HWRRP) followed a collaborative process, including recommendations from the community through a zone committee which was organised as part of the Canterbury Water Management Strategy. The HWRRP became operational in December 2013 (Environment Canterbury 2013). The purpose of the HWRRP is to set out a framework for managing water use and land use in the two catchments, which permits maintenance of the cultural, ecological and recreational values of the rivers, while also allowing for sustainable expansion of productive land use in the catchment, including additional irrigation. One focus of the HWRRP in relation to the Hurunui River is control of nutrient inputs to the river as a means of mitigating and managing adverse effects on the river. The primary adverse ecological effect considered in developing the HWRRP nutrient limits was proliferations of periphyton, including potentially toxic cyanobacteria.

The ~100 km of the Hurunui River downstream of Lake Sumner spans a wide gradient of water quality from near pristine low-nutrient waters at the flow recording site at Mandamus, approximately 40 km downstream of the lake, to mesotrophic conditions at State Highway 1 (SH1) (according to the classification based on TN and TP concentrations, Dodds et al. 1998), some 50 km farther downstream. Data showing this downstream trend have been sourced mainly from the monitoring sites at Mandamus and SH1. These two sites have been monitored by NIWA as part of the National River Water Quality Network (NRWQN) since 1989 (Davies-Colley et al. 2010). Data on water quality at SH1 and in major tributaries have also been collected by Environment Canterbury (ECan). Nutrient concentration data from Mandamus and SH1 were the primary source of information for setting the limits and standards for river health specified in the HWRRP. Additional investigation showed that the higher nutrient concentrations at SH1 (compared to Mandamus) originate mainly from the tributaries Dry Stream, Pahau River, and St Leonards Drain, with a smaller contribution from the Waitohi River, farther upstream (Ausseil 2010).

The management approach taken by the HWRRP was to specify annual load limits for dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP), measured at Mandamus, and SH1. The loads have been set based on the assumption that periphyton growth in the river is mainly phosphorus limited. Thus the annual limit for DIN allows for moderate additions to the current load (e.g., up to 25% increase), while the annual limit for DRP is similar to the current load.

Both phosphorus and nitrogen limitation of periphyton have been demonstrated upstream of the main tributary nutrient inputs to the Hurunui River. In March-April 2014, NIWA carried out nutrient limitation assays for Ngai Tahu Forest Estates Ltd. (using nutrient diffusing substrates, NDS), which showed N and secondary P limitation at Mandamus, P and secondary N limitation at SH7 (~20 km downstream) and P limitation at a site just upstream of the Dry Stream confluence (called “Balmoral”) (Kilroy and Wech 2014). These assays supplemented ECan results in 2008 and 2009 at SH7, which showed similar results (Wilks 2008, 2009). All assays were carried out in late summer

(February – March). Annual fluctuations in nutrient concentrations (especially of N) mean that the highest risk of a switch to N-limitation is in late summer, when N levels tend to be at their lowest. In contrast, NDS assays carried out by ECan at SH1 in 2008 and 2009 generally indicated neither N nor P limitation of periphyton growth: in other words, there was equal growth on all the nutrient treatments and the controls (Wilks 2008, 2009). These assays at SH1 also indicated very rapid periphyton growth compared to that at SH7. More recent surveys of periphyton cover and biomass (*in situ*) at SH1, have confirmed high periphyton cover and biomass compared to many other rivers in Canterbury (ECan unpublished data).

Information about the assumed consequences of changing nutrient concentrations – i.e., in-river primary production, as periphyton – has been confined to the NRWQN monthly visual surveys of periphyton cover at Mandamus and SH1, and more recent monitoring at SH1 by ECan. The NRWQN record is long (from 1989 to the present) but the data are acknowledged to be approximate (J. Quinn, NIWA, pers. comm.). The data resolution over time is also too coarse to gain more than an approximate understanding of periphyton growth rates. To our knowledge, no more detailed data exist on periphyton cover, biomass or accrual rates in the 50 km between Mandamus and SH1. Thus the nature of the relationships between nutrient concentrations and periphyton in different parts of the river is not known. The studies described in this report were intended to address this gap in knowledge.

1.2 Context of this investigation

In 2014, Ngai Tahu Forest Estates Ltd. (NTFE) applied for resource consents related to changing the land use of Balmoral Forest, near Culverden, from forestry and dryland farming to irrigated farming and dairying. The application was supported by a limited examination of periphyton nutrient limitation downstream of Balmoral Forest. During the hearing of this application, consideration was given to the reliability of the measurement of load limits contained in the HWRRP and relevance in managing water quality (periphyton) outcomes. The outcome of the application was that consent was granted for "a reduced development involving dryland farming and/or a limited dairying operation with a reduced or scaled back water take and limited irrigation area on Balmoral", rather than the preferred "highly developed farming proposal" (RMA decision 2014). The limits to the consent granted were partly due to concerns about the effect of the land-use change on the ecological health of the Hurunui River and partly due to inherent limitations on the grant of consents permitted under the HWRRP (e.g., in relation to allocation of the contribution to nutrient loads in relation to other consents).

NTFE subsequently asked NIWA to undertake a more comprehensive investigation of periphyton nutrient limitation and growth rates in the middle reaches of the Hurunui River and to establish baseline water quality information prior to the commencement of intensive development on the Balmoral property. NTFE anticipate that components of this study may need to be repeated during subsequent reviews of consent conditions. The investigation is also intended to have more general relevance in the context of the HWRRP and in particular the extent to which periphyton growth and biomass accumulation can be controlled by managing nutrient concentrations.

1.3 Project objectives

The brief from NTFE included a series of questions:

1. Which parts of the Hurunui River are saturated in terms of periphyton growth rates and which parts are not?

2. How significant is the growth rate of periphyton to mass accumulation over time? [In other words, are rapid growth rates always associated with high biomass and, conversely, are slow growth rates always associated with low biomass?]

3. Is the lower Hurunui River phosphorus limited (as stated in the HWRRP)?

4. Is it possible to manage periphyton growth by retaining phosphorus concentrations at their current levels, while allowing for a modest increase in nitrogen (as stated in the HWRRP)?

5. To what extent can we "control periphyton growth and biomass accumulation" by managing nutrient concentrations?

To provide information to address these question we conducted a programme of monitoring and experiments over the summer of 2014-2015, comprising five main components:

1. Collection and analysis of water samples to obtain a detailed record of nutrient concentrations in the river over space and time (this part of the study was required for interpretation of the other components);

2. In-river investigations to determine accrual rates of periphyton at four sites representing different reaches of the river;

3. In-river assays to determine the limiting nutrient, and changes over time;

4. Surveys of natural periphyton cover and biomass, to capture natural changes under the variable physical conditions at each site;

5. Sample collection and analyses to investigate alternative sources of phosphorus that might contribute to periphyton growth; in particular, phosphorus potentially available from fine sediment in the bed and water column. This component was added to the study following a review of the proposal by Dr Susie Wood (Cawthron Institute), in January 2015.

In this report we present the results of all five components, which were carried out at four sites in the Hurunui River from January to May 2015. The report is structured as follows. Following a description of the study sites, the methods, results and preliminary discussion of each study component are presented as separate sections (spatial and temporal variation in nutrient concentrations, periphyton accrual rates, nutrient limitation studies, in-river periphyton dynamics and physical habitat, alternative sources of P). In the first four of these sections, an account of flows in relation to sample collection is included, and the effects of flows on the results are discussed. The results of the periphyton studies are also interpreted with reference to the results of the nutrient study. In the final sections, all of the results are then discussed together, with conclusions focusing on the questions that prompted the study.

2 Study sites and timing

Four sites on the Hurunui main stem were selected for the project. The sites spanned a gradient of nutrient concentrations, ranging from near-pristine conditions downstream from the river outlet from Lake Sumner, to the high-nutrient conditions in the lower river. We included two sites in the middle reaches, for which data on periphyton are generally lacking.

2.1 Site locations

The locations of the four study sites are shown in Figure 2-1 and the surveyed reaches at each site in Figure 2-2. Notes follow on each site, from upstream to downstream. At all sites the growth rate trials, nutrient diffusing substrate assays and water sample collections were carried out upstream of the area surveyed and sampled for *in situ* periphyton cover and biomass.

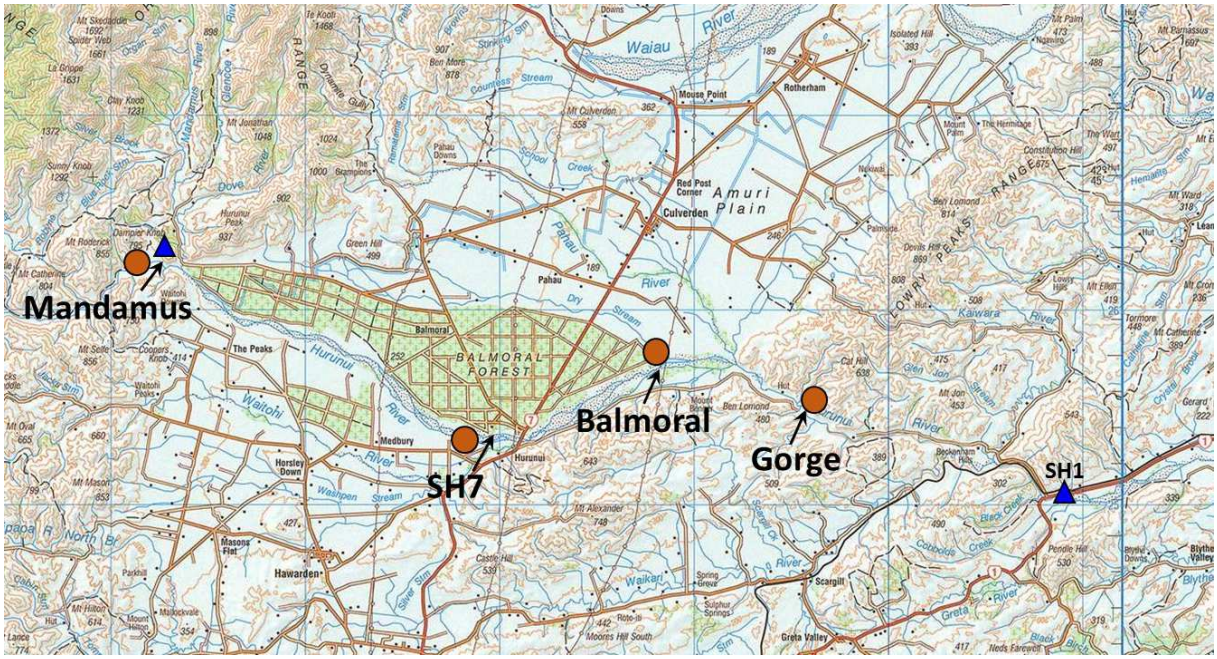

Figure 2-1: Nutrient and periphyton monitoring sites (brown circles) and flow recorders (blue triangles) on the Hurunui River. The black arrows indicate the exact location of each site.

2.1.1 Mandamus

Mandamus is approximately 40 km downstream of Lake Sumner and 1 km upstream of the confluence with the Mandamus River. Access is from Tekoa Road on the north side of the Mandamus River bridge. Mandamus is the site of a NIWA-operated water level recording site and is also part of the NRWQN. For the present project, the survey area was a reach approximately 50 m long 100-150 m downstream of the water-level recorder and staff gauge. The site is in a narrow gorge, but the approximately north-south orientation means that the river bed is unshaded for most of the day. Bed sediments at this site are dominated by boulders.

2.1.2 State Highway 7 (SH7)

The SH7 site is about 1.5 km upstream of the road bridge. The Mandamus River is the only major tributary entering the Hurunui River between Mandamus and the SH7 site. Accessible sites on the

true left of the main stem of the river in this area were all fast flowing with a steep gradient around the water's edge, and were unsafe for surveys and experiments. We therefore located the site in a permanent side braid on the true left of the main stem, which provided a larger suitable survey area. Periphyton cover was similar to that observed at the main stem sites that were checked. The site is accessed from Balmoral Station Road by turning off west of the Balmoral camping ground, and then following the track beside the stopbank in an upstream direction. Bed substrate at the site is a mixture of cobbles and gravels, and the site is unshaded.

2.1.3 Balmoral

This site is approximately 8 km downstream of the SH7 road bridge and about 1 km upstream of the confluence with Dry Stream. The Waitohi River enters the Hurunui River just downstream of the bridge. The site is accessed via Blacks Road, through Balmoral Forest property, then across about 500 m of scrub to the river bed. The survey area was located in the river main stem (true left bank) and is unshaded, with bed substrate similar to that at the SH7 site.

2.1.4 Gorge

For logistical reasons, and to coordinate with a parallel project investigating nutrient dynamics, the most downstream site was located 15 upstream of the NRWQN site at State Highway 1 (SH1) in the gorged area of the lower Hurunui River. Dry Stream, Pahau River and St Leonards Drain join the Hurunui River between the sites at Balmoral and Gorge. Previous studies on water quality identified that nutrient concentrations at the swing bridge 7 km downstream from the Gorge site were not different from those measured in the Hurunui at SH1 (Ausseil 2010). Only minor hill-fed tributaries join the Hurunui between the Gorge and the swing bridge, and we therefore assumed that nutrient concentrations at the Gorge and SH1 would also be similar. The Gorge site is accessed via Hurunui Bluffs Road, through Mount Benger and Glenburn Stations, and is just downstream of the Glenburn irrigation water intake. The visual estimate transects were set up ~200 m downstream of the experiment locations where there was more space in water of a suitable depth. The site is gorged on the east bank, but north – south orientation means that the whole site is unshaded for most of the day. Bed substrate composition is similar to that at SH7 and Balmoral.

2.2 Timing

We proposed to begin the study in November 2014, and estimated that flows of less than 30 m^3/s (at Hurunui @ Mandamus) would be low enough to allow safe access. A consideration was that deploying experiments too soon on the falling limb of a flood would run the risk of exposing the substrata within less than 7 days (the proposed interval between site visits). Therefore, a start in higher flows might be possible if flows appeared to be receding at a slow rate.

All site locations were confirmed during a preliminary site visit on 4 November 2014. The study could not be started at that time because flows were too high (90 m^3/s). An attempt was made to start the first trials on 19 November 2014. At that stage flow at Mandamus was about 55 m^3/s, but was under a steady decline. However, flows rose quickly during the day, and the project start was deferred.

Flows remained high for the rest of November 2014 and remained > 30 m^3/s for most of December. The project was eventually started in early January. The first growth rate trial was installed on 5 January 2015 and the first NDS assays were deployed on 6 January. Details of timing of sample and data collection are provided in the accounts of the five studies.

Figure 2-2: The study reaches at the four sites in the Hurunui River. (a) Hurunui at Mandamus, looking upstream; (b) Hurunui at SH7, side braid, looking downstream; (c) Hurunui at Balmoral, looking upstream; (d) Hurunui at Gorge, looking downstream.

3 River flows and water quality

The aim of the project was to gain a better understanding of the relationships between nutrients, flows and periphyton along a gradient of nutrient concentrations in the Hurunui River. A record of nutrient concentrations was required, which was at least as detailed as the periphyton data we were collecting. While dissolved nutrients (DRP and DIN) are likely to be the main water chemistry variables influencing periphyton, other aspects of water quality and water chemistry may also contribute. Other information collected included total dissolved phosphorus (TDP), total suspended solids (TSS), major cations (metals), and water temperature. Both TDP and TSS may indicate availability of nutrients in insoluble forms that could be used by periphyton under certain conditions (Wood et al. 2014). Major cation concentrations may influence algal community composition, which in turn could affect potential biomass and the occurrence of nuisance growths (e.g., Porter-Goff et al. 2013). Water temperature controls growth rates in algae, and also determines the structure and composition of aquatic communities (Dallas 2008). Most taxa (including periphyton species) have a narrow range of temperatures at which growth is optimal, and a wider range which is tolerated. Differences in water temperature might therefore contribute to observed differences between sites in both periphyton biomass and periphyton community composition.

3.1 Methods

3.1.1 River flows

Flow data were available from the flow recorders at Mandamus and SH1. Mean and maximum daily flows were extracted from the record of instantaneous flows. Maximum daily flows were also extracted because the daily maxima allow identification of short-duration high flows that may have affected water chemistry and/or periphyton cover, but would not show up clearly in a plot of mean daily flows. Data from the sites at Mandamus, SH7 and Balmoral were linked to the flow record at Mandamus, and the site at Gorge was linked to the flow record at SH1.

3.1.2 Water sample collection and analysis

Water samples were collected at each site approximately weekly from 5 January 2015 to 5 May 2015 (15 site visits). Collections were made in flowing water at least 0.3 m deep and at least 3 m from the water's edge, and upstream of the accrual trials, NDS trials and visual estimate transects. The aim was to collect samples that were representative of the water in contact with the periphyton we were measuring.

Samples for analysis of dissolved nutrients were collected by filtering water through 0.45 µm cellulose acetate filters into clean plastic bottles. Samples were stored on ice and transferred within 12 h to a freezer until analysis. Water samples were analysed for nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), dissolved reactive phosphorus (DRP) and total dissolved phosphorus (TDP)[1] concentrations at the NIWA Laboratories in Hamilton, using standard techniques. The stated laboratory detection limits were 1 mg/m^3 (i.e. 0.001 mg/L) for all three analytes. For DRP, in particular, we report concentrations to the nearest 0.1 mg/m^3 because in our experience the laboratory procedure successfully picks up these lower concentrations. Dissolved inorganic nitrogen (DIN) is the sum of NO_3-N and NH_4-N. Total nitrogen and phosphorus (TN, TP) were not measured as part of this contract, but samples were collected and stored for future analysis if needed.

[1] Total dissolved phosphorus is determined from a filtered samples but the analysis includes a digestion step which picks up phosphorus bound to organic molecules, which may also be available for algal uptake.

From 20 January onwards, on each site visit, we also collected a 1-litre unfiltered water sample for analysis of total and volatile (i.e., organic) suspended material. These samples were collected in flowing water up to 0.5 m deep at about one third to one half of the depth above the river bed. In the laboratory samples were filtered through pre-ashed, pre-weighed GFF filters, dried at 105 °C for 24 h, weighed, then ashed at 400 °C for 4 h (to burn off all the organic material) and re-weighed.

Additional water samples were collected on alternate survey dates at each site, for analysis of total iron (Fe), total manganese (Mn), dissolved iron (Fe), and dissolved major cations (calcium, Ca; magnesium, Mg; potassium, K; sodium, Na). For dissolved metals, samples were filtered (as for nutrients) into plastic bottles containing acid preservative. For total metals, unfiltered samples were transferred into plastic bottles containing acid preservative. All water samples for metals were refrigerated until analysis. Samples were analysed for metal concentrations at Hill Laboratories, Hamilton, using standard techniques. Detection limits were dissolved Fe, Mg and Na, 0.03 mg/L; total Fe, 0.021 mg/L, total Mn, 0.00053 mg/L; dissolved Ca, K, 0.05 mg/L.[2]

On each site visit we measured water pH, conductivity, temperature, and dissolved oxygen (DO) at each site, using a hand-held meter. The time of day was recorded for each such set of "spot" measurements.

3.1.3 Water temperature

We deployed Hobo water temperature loggers (Onset Corporation, Bourne, MA, USA) in flowing water at each site to record temperature at 30 min intervals. Loggers were deployed at the Gorge and Mandamus on 6 January 2015, and at SH7 on 20 January 2015 and at Balmoral on 10 February. Because these loggers are vulnerable to loss during floods, they were retrieved and downloaded from time to time, to ensure that we would have data from all of the sites for at least part of the survey period.

3.2 Data analysis

The nutrient data were plotted over time to illustrate differences between sites. Paired T-tests were performed to determine whether the variables differed significantly between sites. Nutrient concentrations (and other water quality variables) may be influenced by flow volume magnitude. Relationships with flow were checked by plotting variables of interest against flow magnitude (mean daily flow) on the day of each survey.

Data on TSS and metal concentrations are presented in tabular form summarising all data at each site. Non-parametric two-sample Kolmogorov-Smirnoff (K-S) tests were used to determine significance of differences among sites, with a probability $P < 0.05$ (of the difference being attributable to chance alone) taken as significant.

All the available water temperature data at each site were plotted as mean and maximum daily temperature over the whole monitoring period. Detailed differences between the sites are shown in plots of the 30-minute time series for periods when data were available for all sites.

[2] The additional samples were collected opportunistically during the project for a separately funded study which is looking at the distribution of *Didymosphenia geminata* in the South Island in relation to various aspects of water chemistry. The data are presented in this report because they may be relevant to distributions of algae in the Hurunui.

3.3 Results and discussion

3.3.1 River flows

The first surveys were carried out 44 days after a large flood that peaked at 193 and 160 m^3/s at Mandamus and SH1 respectively. The period of sample collection then spanned a long period of low flows, interrupted by small freshes in early March and early April (Figure 3-1). The plots of mean and daily flows at both Mandamus and SH1 are illustrated to show subtle differences in flow conditions between the two sites. Although base flows at SH1 are higher than those at Mandamus (e.g., long term median flows of 37 and 48 m^3/s respectively), peak discharge at Mandamus during the same flow event can be higher than that at SH1 some 50 km downstream. The dates and magnitudes of peak flows (m^3/s at Mandamus and SH1, respectively) of four events relevant to the study were 31 December/1 January (63, 57), 6 March (101, 80), 13 April (83, 96), 28 April (94, 115).

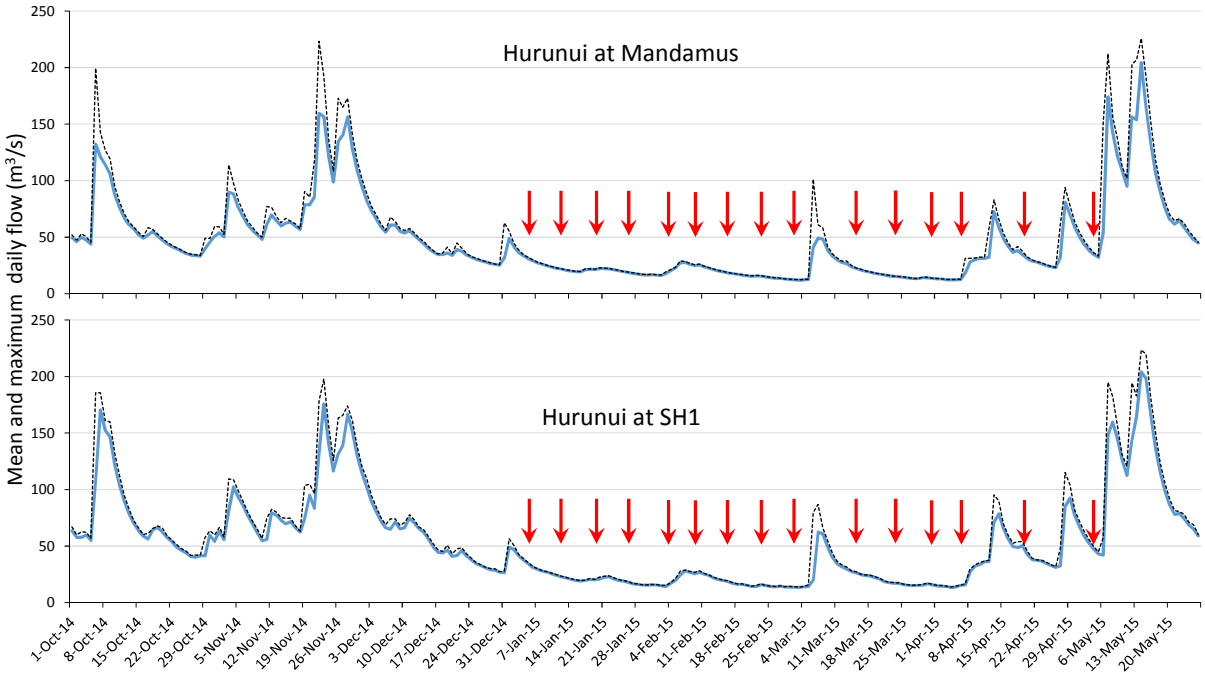

Figure 3-1: Discharge measured at Madamus and SH1 from October 2014 to May 2015. Maximum daily flows (dashed black line) are shown as well as mean daily flows (blue line), to highlight short-lived peak flows. The red arrows show days when water samples and water quality data were collected.

In the context of other years, the flood-free period from January to March 2015 does not appear to be unusual. For example there were extended flood-free periods in 2007, 2008, 2013 and 2014, although the timing was later (Figure 3-2). However, 2015 was unusual in that flows at Mandamus and SH1 were similar, reflecting a lack of, or very low, runoff between the two sites. The most recent occurrence of prolonged low flows that were similar at the two sites was in 2001.

3.3.2 Water quality and nutrients

Values of the main water quality variables measured during the study are summarised in Table 3-1. Water conductivity increased in a downstream direction, from a baseline at Mandamus which was similar to the median for all South Island sites in the NRWQN (72 µS/cm), to about 90 µS/cm at the Gorge, which is still a relatively low value. At low flows (< 23 m^3/s at either site), total suspended solids (TSS) were generally < 1.3 mg/L (which is low in the context of other rivers). Dissolved cations generally increased in a downstream direction.

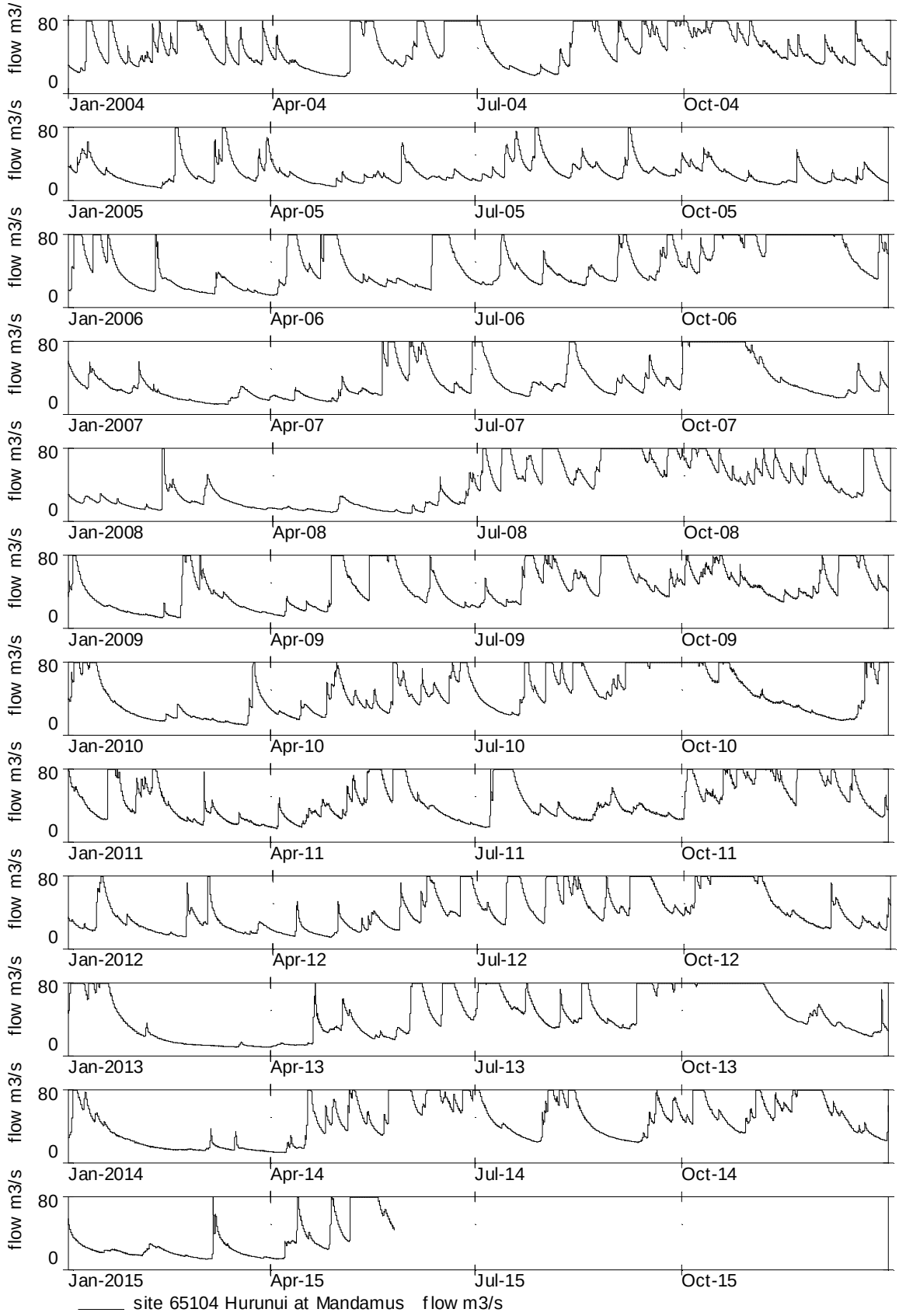

Figure 3-2: Discharge (m^3/s) at Hurunui @ Mandamus from 2004 to 2015. Flows have been truncated at 80 m^3/s so that the lower flows can be seen more clearly.

Table 3-1: Summary values of water quality and water chemistry variables measured at four sites in the Hurunui River, January to May 2015. Median values are highlighted in grey to facilitate comparisons among sites. TSS = total suspended solids. Different superscript letters next to mean values indicate significant differences between sites (using statistical tests, see Section 3.2). No superscript letters means that there was no difference among sites.

		Mandamus			SH7			Balmoral			Gorge		
Variable	**units**	**Median**	**Mean**	**S.D.**	**Median**	**Mean**	**S.D.**	**Median**	**Mean**	**S.D.**	**Median**	**Mean**	**S.D.**
General water quality													
Conductivity	µS/cm	72.6	72.9^{a}	4.0	75.0	73.6^{a}	7.7	80.4	78.8^{b}	5.6	93.8	93.6^{c}	7.6
pH		8.03	7.99	0.41	8.06	8.03	0.32	8.05	8.12	0.53	7.88	7.91	0.32
TSS (at low flows)	mg/L	1.35	1.53	0.90	1.30	1.60	0.98	1.07	1.50	0.97	1.20	1.49	0.85
Dissolved nutrients													
NO_3-N	mg/L	0.001	0.003^{a}	0.004	0.009	0.012^{b}	0.010	0.046	0.046^{c}	0.012	0.341	0.369^{d}	0.117
NH_4-N	mg/L	0.002	0.002^{a}	0.001	0.003	0.003^{ab}	0.001	0.004	0.004^{bc}	0.002	0.005	0.005^{c}	0.001
DRP	mg/L	0.0006	0.0006^{a}	0.0002	0.0009	0.0008^{b}	0.0002	0.0006	0.0007^{ab}	0.0002	0.0010	0.0011^{c}	0.0003
TDP	mg/L	0.0016	0.0016^{ab}	0.0006	0.0016	0.0018^{ab}	0.0004	0.0015	0.0015^{b}	0.0003	0.0020	0.0022^{a}	0.0006
Metals													
Total Fe	mg/L	0.026	0.031	0.01	0.032	0.047	0.05	0.031	0.030	0.02	0.049	0.046	0.03
Total Mn	mg/L	0.0012	0.0011	0.0003	0.0009	0.0011	0.0008	0.0011	0.0010	0.0004	0.0014	0.0013	0.0004
Dissolved major cations													
Ca	mg/L	9.90	10.01^{a}	0.45	10.30	10.17^{a}	0.36	10.80	10.77^{a}	0.59	11.90	12.09^{b}	0.88
Mg	mg/L	0.95	0.97^{a}	0.06	1.04	1.04^{a}	0.05	1.13	1.15^{b}	0.08	1.68	1.64^{c}	0.19
K	mg/L	0.48	0.47^{a}	0.03	0.57	0.56^{b}	0.04	0.58	0.58^{b}	0.04	0.66	0.67^{c}	0.05
Na	mg/L	2.80	2.76^{a}	0.23	2.90	2.91^{a}	0.20	3.10	3.11^{a}	0.30	4.00	4.07^{b}	0.50

Nitrate-nitrogen concentrations increased in a downstream direction by over two orders of magnitude, from 0.003 mg/L at Mandamus to 0.365 mg/L at the Gorge (median values, Table 3-1). DIN at Gorge was approximately four times the median value for all South Island sites in the NRWQN.

DRP concentrations did not differ between Balmoral and either Mandamus or SH7, but DRP was significantly higher at SH7 than at Mandamus and was higher at the Gorge than at all other sites (paired T-tests, $p < 0.01$). TDP concentrations were ~2 times DRP concentrations on average and did not differ between sites except that concentrations at the Gorge were significantly (but only slightly) higher than at Balmoral (Table 3-1).

3.3.3 DRP and DIN over time and in relation to flow

DRP and DIN are the primary variables of interest, and these were examined over time and in relation to mean daily flow on the date of sampling. We also considered total dissolved phosphorus (TDP) in relation to flow. In earlier studies it has been found that where DRP concentrations are low – within the range found in the Hurunui River in this present study – the phosphorus available to algae for uptake may be better reflected by TDP than DRP (Kilroy and Bothwell 2012). Thus, TDP may also provide a better measure of available phosphorus in the water column at different flows.

At Mandamus, DRP declined over the monitoring period (Figure 3-3a), and DIN increased slightly (Figure 3-3b). DIN was < 0.006 mg/L when flows were less than 30 m^3/s, except on 1 April 2015 when DIN was 0.012 mg/L in a low flow period. Neither DRP nor DIN were correlated with flow over the limited range of flows sampled.

At SH7, the lowest DRP concentrations were recorded in March and April, but there was no clear trend over time. DIN remained consistent over time. At Balmoral, neither DIN nor DRP showed a clear trend up or down over time. As at Mandamus, neither DRP nor DIN was clearly related to flow at SH7 and Balmoral (Figure 3-3, Figure 3-4).

At Gorge, DRP fluctuated over time and was not correlated with flow. DIN at Gorge increased over time until mid-April (Figure 3-3b) and there was a negative relationship between DIN and flow (Figure 3-4, centre of bottom row). The relationship between DIN and flow also held when we included an additional sample in the dataset, which was taken when the river flow was 60 m^3/s at SH1 on 19 November. DIN on 19 November (0.155 mg/L) was the lowest recorded at the Gorge, but was still considerably higher than any concentrations recorded at Balmoral, SH7 or Mandamus.

At Mandamus, Balmoral and Gorge (but not SH7), TDP was generally positively correlated with flow. At Mandamus, one sample taken at low flow on 16 February had the second highest TDP recorded in programme at any site, and was an outlier to the relationship with flow (arrowed on Figure 3-4, top row, right). We have no explanation for this outlier, and it may mean that there is no real relationship between TDP and flow at that site.

At Balmoral, the sample collected on 20 April was an outlier in the relationship between TDP and flow (arrowed on Figure 3-4, third row, right). Flow on 20 April was the highest recorded in the programme and the sample was collected at the end of 10 days of elevated flows.

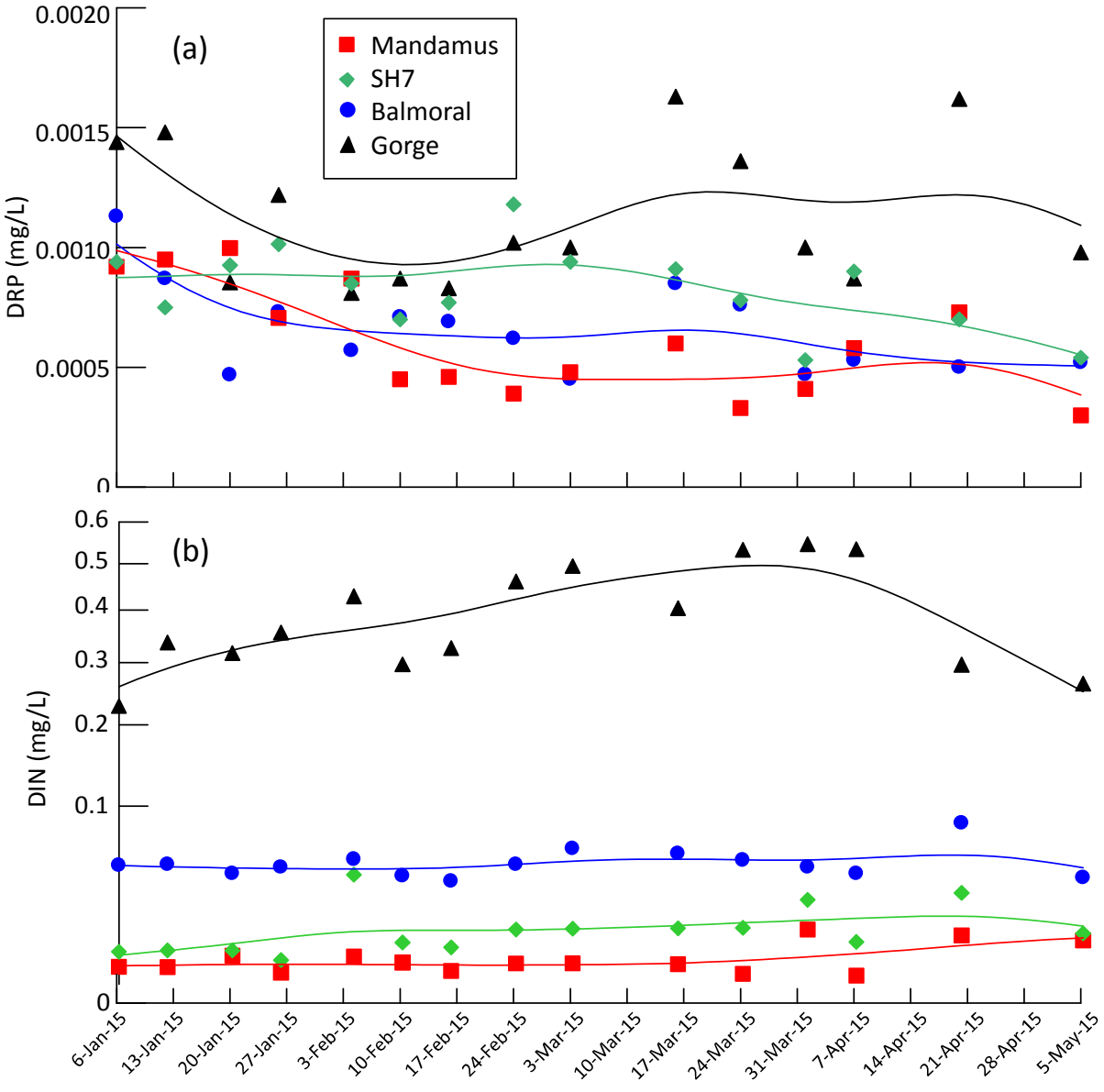

Figure 3-3: Dissolved reactive phosphorus (DRP) and dissolved inorganic nitrogen (DIN) concentrations at four sites in the Hurunui River, plotted over time. General trends in the data over time, and differences among sites are highlighted by distance-weighted least squares (DWLS) smoothing lines. DWLS produces a smooth by running along the x values and finding predicted values from a weighted average of nearby y values (Tension 0.5). The DIN data are plotted on a square-root scale, which shows lower values more clearly.

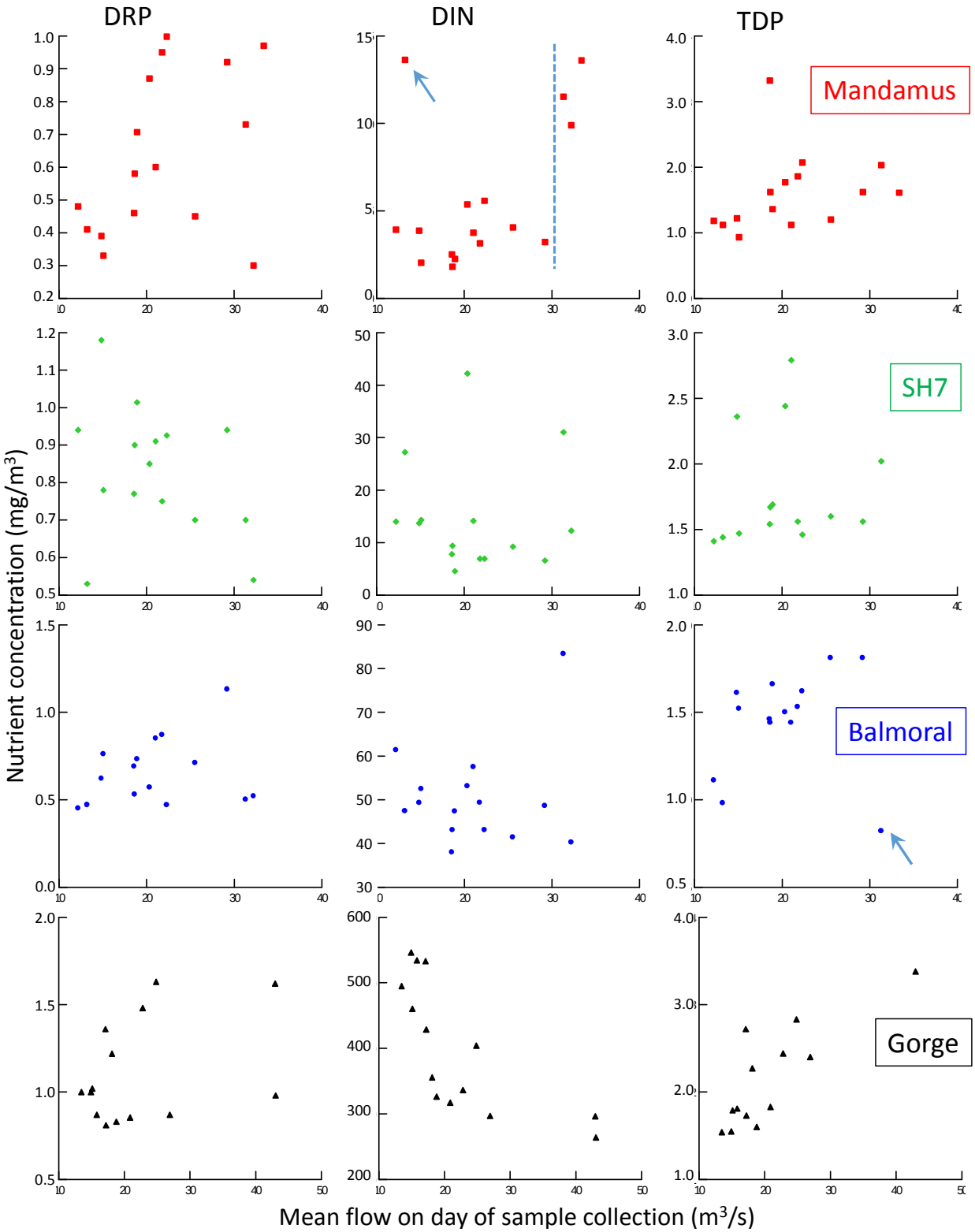

Figure 3-4: DRP, DIN and TDP plotted against mean flow on the day of sample collection at the four sites in the Hurunui River. Flow data for Mandamus, SH7 and Balmoral are from Hurunui @ Mandamus; flow data for Gorge are from Hurunui @ SH1. The blue line on DIN at Mandamus (top row) shows a possible threshold of about 30 m^3/s at Mandamus, above which DIN concentrations were always > 0.01 mg/L. Outliers arrowed on this plot and TDN at Balmoral are discussed in the text. Note that concentration units on these plots are in mg/m^3 to aid readability. Divide by 1000 to get mg/L.

3.3.4 Water temperature

A water temperature record (30 minute intervals) was obtained for the entire monitoring period at Mandamus, and from 20 January to 5 May at SH7. A logger at was deployed at Balmoral on 10 February and downloaded on 16 March. That logger was subsequently lost during the 13 April flood. At Gorge water temperature at the irrigation intake was logged from 6 January to 24 February. Because we suspected that water temperature might be unrepresentative in the slower flowing water (shown by the black dashed line in Figure 3-5), we installed a second logger on the NDS tray on 10 February and downloaded data on 2 March. That logger at the Gorge became inaccessible after the 13 April flood. New loggers were deployed at all four sites on 20 April, attached to the NDS trays. All four loggers were retrieved on 5 May. Simultaneous data from the four sites are available from 10 February to 2 March and from 20 April to 5 May 2015.

From 20 January to 5 May 2015, mean daily temperature was on average 0.9 °C higher at SH7 than at Mandamus, and mean daily temperatures at Balmoral and Gorge were slightly higher than at SH7 and similar to one another (Figure 3-6). The difference between Mandamus and the three sites farther downstream was accentuated with maximum daily temperature, with an average difference of >2.3 °C between Mandamus and SH7 (excluding the high flows in March and April).

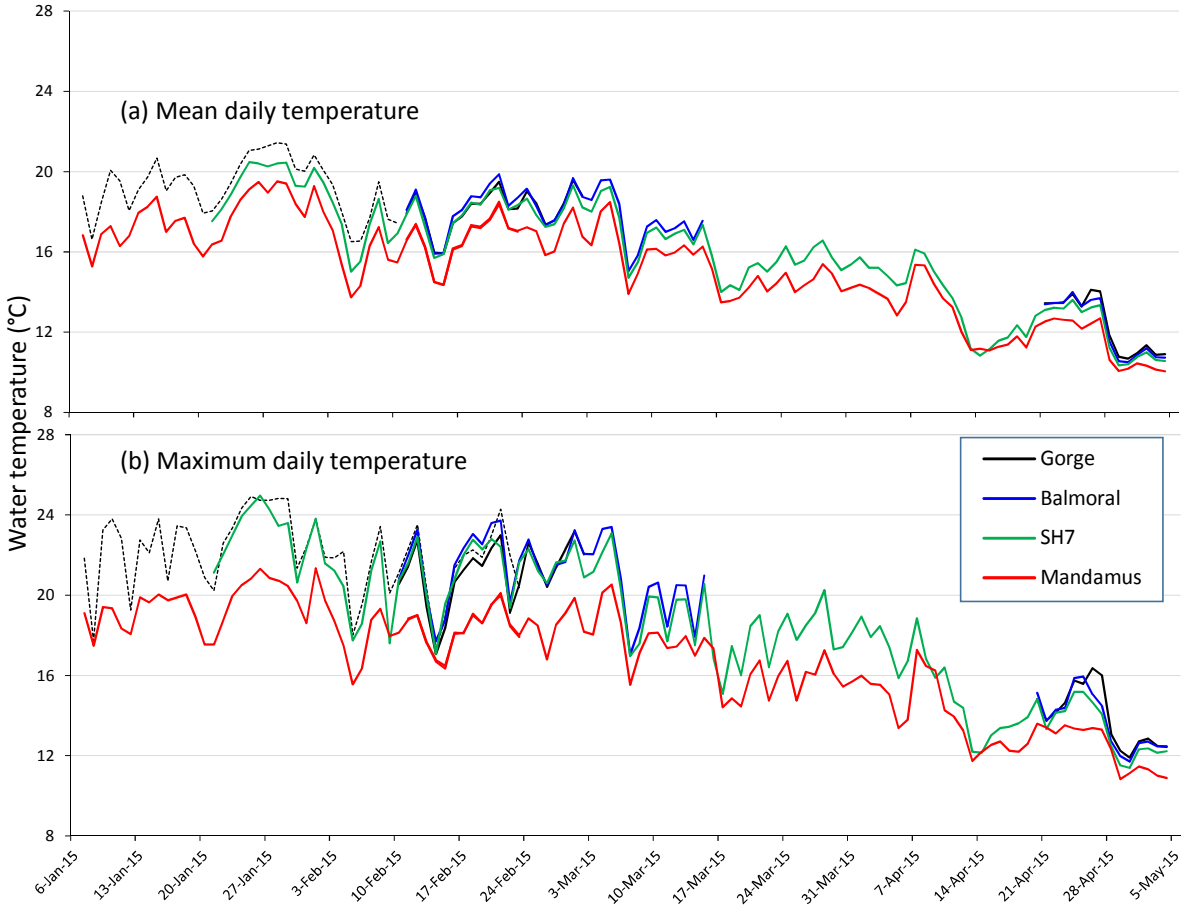

Figure 3-5: Summary of temperature data collected at four sites on the Hurunui River between Janaury and May 2015. All loggers were located in flowing water. The black dashed line is the record from the Gorge site at the irrigation intake. As river flows receded, water flow past the logger became very slow, and the water temperature was likely higher than in the main river. A second logger was therefore deployed on an NDS tray in faster flows.

The shorter time series from Balmoral and Gorge indicated that mean temperatures were similar to each other in February, except that the daily maximum was higher at Balmoral during mid-February. Lower mean daily water temperature at Mandamus than at the other sites was driven by more muted diurnal variation and lower daily maxima in the warmer months (Figure 3-6a), and generally lower minima and maxima later in the season (Figure 3-6b).

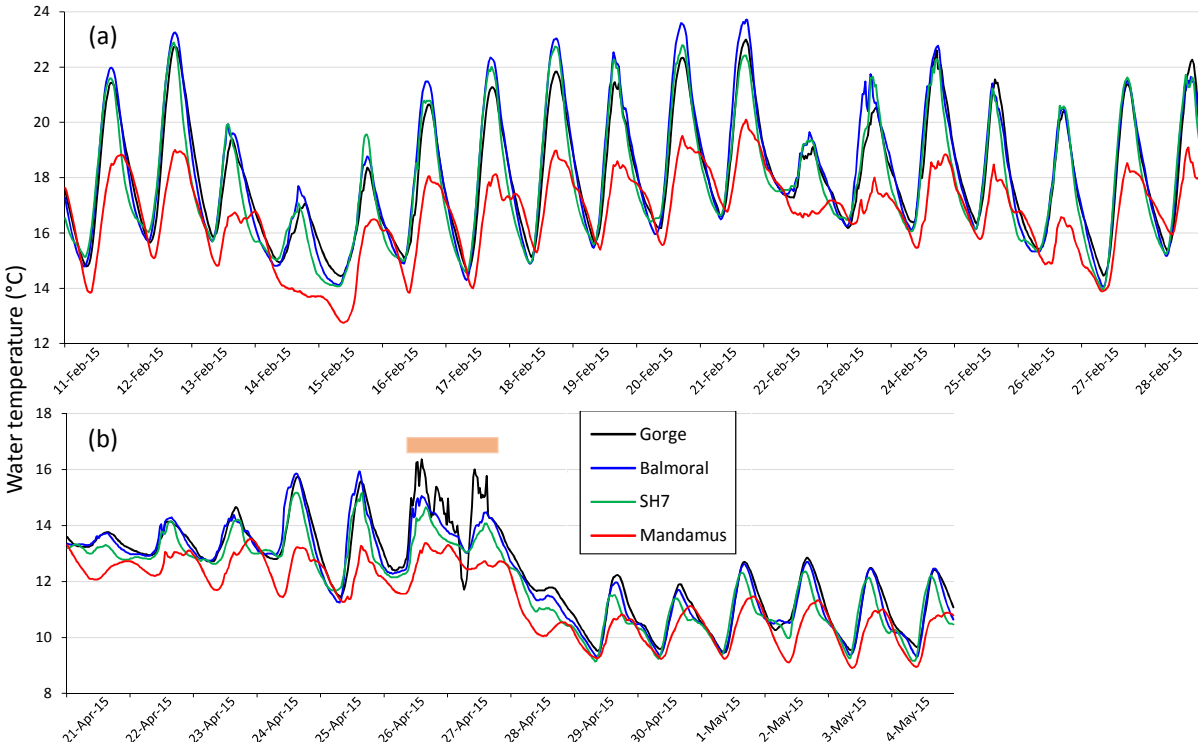

Figure 3-6: Water temperature logged every 30 minutes at four sites in the Hurunui River in February (a) and late April to May (b). The y axis is at the same scale in the two plots, but the temperature range differs. The orange bar on (b) indicates a short period where the logger at the Gorge was out of the water. All loggers were deployed on NDS trays in flowing water.

The temperature difference between Mandamus and the three downstream sites may be biologically significant. In streamside channel experiments, periphyton biomass increased and community composition changed when water temperature was artificially raised by 1.4 °C (Piggot et al. 2011).

3.3.5 Nutrient concentrations in 2014-15 compared with NRWQN data

The data collected in this study indicated that DRP concentrations at the four sites in the Hurunui River in 2014-15 were very low. In comparison with long term mean values in the NRWQN, the overall mean at all four sites (0.0008 ± 0.0003 (s.d.) mg/L) would have been 3rd lowest of all 77 sites.

Three NRWQN data points collected during the period of the present study were available at the time of writing. Comparing DRP and DIN data with the values we recorded over the same period (January to March 2015) shows reasonable correspondence at Mandamus, but mean DIN measured at the Gorge was 35% higher than the NRWQN data from SH1 (Table 3-2). The discrepancy can be explained by referring to the river flows on the NRWQN sample collection dates, and the negative relationship between DIN and flow at the Gorge (Figure 3-4). The NRWQN samples were collected on 14 January, 11 February and 11 March. All three samples missed the very low flows (and higher DIN concentrations) that were sampled weekly during the present study. The NRWQN samples therefore returned lower concentrations of DIN.

Table 3-2: Mean DRP and DIN recorded in the NRWQN database from January to March 2015, and in the present study over the same period. The average of mean flow at the time of sample collection is also shown.

		Number of samples	Mean flow	DRP (mg/L)		DIN (mg/L)	
				mean	s.d	mean	s.d
Mandamus	WQN	3	24.8	0.0005	0.0004	0.0048	0.0052
	This study	11	20.0	0.0007	0.0003	0.0044	0.0031
Gorge	WQN(SH1)	3	32.4	0.0008	0.0007	0.281	0.021
	This study	11	20.6	0.0011	0.0003	0.380	0.092

The load and concentration limits in the HWRRP were based on data from 2005 to 2011. To place the data from January to May 2015 in this context, we compared NRWQN data in summer (from December to April[3]) at Mandamus and SH1 from 2003-04 to 2014-15. In view of possible relationships between flows and nutrient concentration, we considered only samples that were collected at flows below the maximum sampled during the current monitoring programme (i.e., up to 33.5 m^3/s at Mandamus and 43 m^3/s at SH1). The comparison confirmed that DRP was low at both Mandamus and SH1 in summer 2015 compared with DRP in most of the past 12 years (Figure 3-7). Median DIN was low at Mandamus in 2015, but one higher value (0.011 mg/L) was recorded in March 2015. DIN at SH1 was lower than in any year since 2004. Even taking into account the possibly biased sampling in the NRWQN from January to March 2015 (i.e., no samples taken at the lowest flows, as discussed above) the mean value recorded from weekly samples in the present study would still have been low compared to most years since 2004.

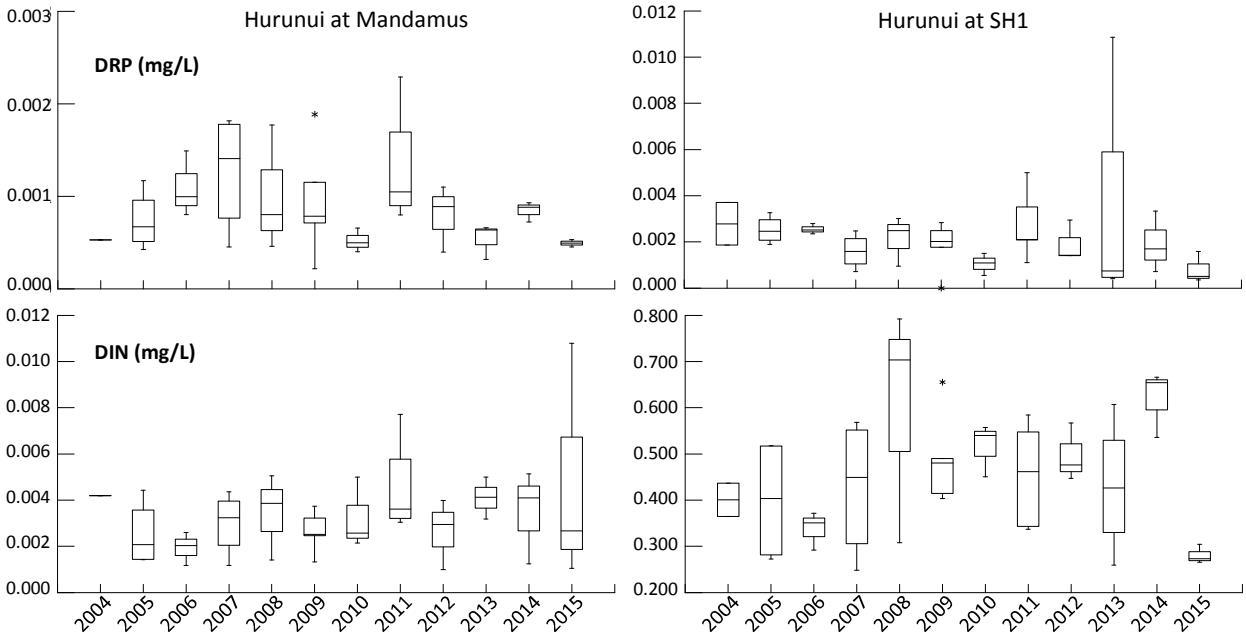

Figure 3-7: Box plots of DRP and DIN from the NRWQN dataset over summer low flows (December to April) each year since 2004. Data are included only for occasions when flow was less than the maximum recorded flow in the 2015 study in the Hurunui. Therefore the comparison does not include nutrient values influenced by very high flows.

[3] At the time of writing, NRWQN data were available up to March 2015.

4 Periphyton accrual rate investigations

4.1 Introduction

In equivalent physical conditions (light, water velocity, water depth, temperature, colonising surface), and with no biomass loss (e.g., from invertebrate grazing), growth rates of periphyton are expected to be controlled by (and therefore proportional to) availability of the nutrient in most limited supply. This hypothesis was tested in the Hurunui River by conducting trials to determine accrual rates of periphyton at four sites along a gradient of nutrient concentrations. The term "accrual" is used rather than "growth" because, in the natural setting of a river, periphyton growth is offset by biomass losses through, for example, grazing by invertebrates, and natural sloughing.

As in previous comparisons of accrual rates (Biggs 1990), we used artificial substrates as growth surfaces to ensure consistency among sites. Two trials (Experiments 1 and 2) were conducted in which we tracked periphyton accrual weekly from 5 January to 16 February and from 24 February to 20 April 2015.

Because periphyton growing on artificial substrates do not necessarily reflect community composition and biomass accrual on natural substrates (i.e., cobbles), we also ran a parallel trial at each site alongside Experiment 1, using an array of natural cobbles. We found that periphyton accrual on the cobbles was much more variable than on the artificial surfaces, and the high variability limited ability to detect patterns in the data. The following account therefore refers to the trials on artificial surfaces only.

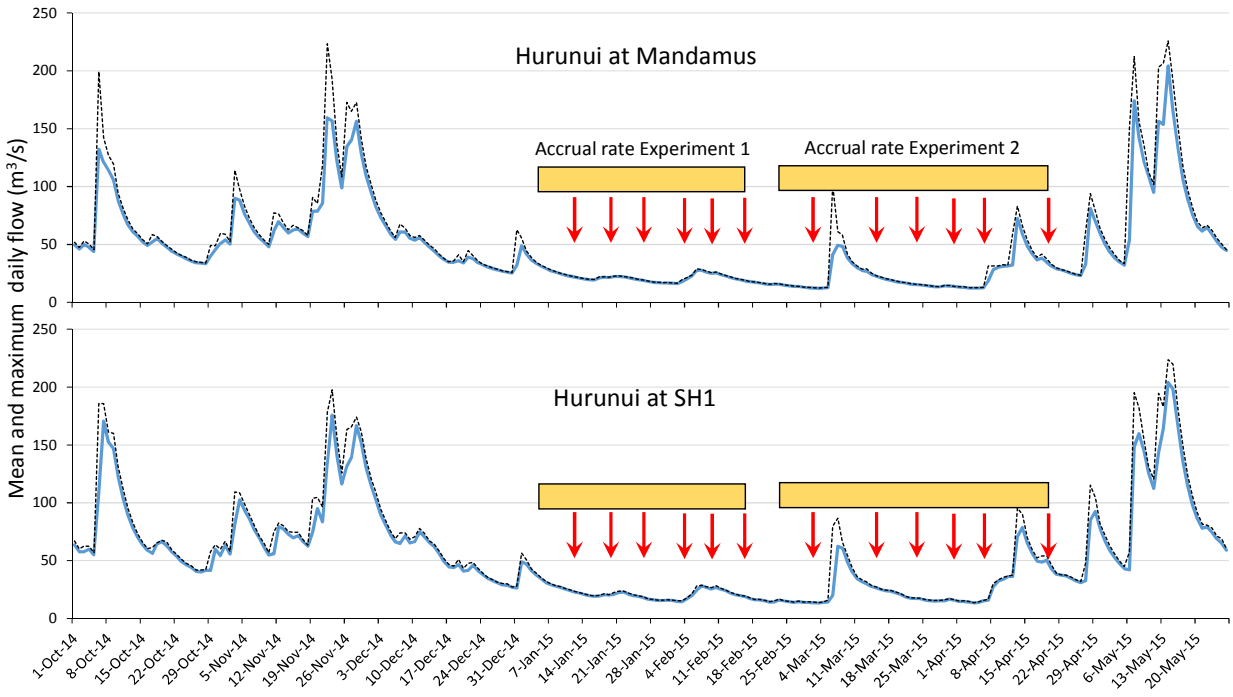

Figure 4-1: Discharge at Mandamus and at SH1 showing timing of two periphyton accrual trials (yellow bars). Red arrows indicate sample collection times (six collections in each experiment).

4.2 Methods

4.2.1 Field and laboratory procedures

The growing surface used in both trials was flexible plastic sheeting with an embedded coating of fine fibres which provided a felt-like surface. Periphyton colonisation has been found to be rapid on this material, and it has used successfully in previous experiments (e.g., Biggs and Thomsen 1995). Squares of the sheeting (5 x 5 cm) were attached to concrete pavers 230 x 170 x 6 cm in a regular array of 12 squares, using household waterproof adhesive (Figure 4-2). All the pavers had been immersed in river water for several weeks and were thoroughly scrubbed and dried prior to use in the Hurunui. This ensured that any leaching from the concrete that might affect periphyton was negligible. Four pavers were prepared per site, and each paver was treated as a replicate at that site.

At the site, pavers were positioned horizontally, set into the natural river substrate so that they were more or less level with the bed, in water 25-40 cm deep, with water velocity of 0.5 – 0.7 m/s. Relatively fast-flowing water was required to prevent sediment building up on the colonising surfaces. Water velocity and depth were measured at the downstream end of each paver at the time of deployment and each time samples were collected. As flow receded during parts of both trials, it was necessary to occasionally relocate pavers at Mandamus, Balmoral and Gorge to ensure that the target depths and velocities were maintained. No relocation was necessary at SH7.

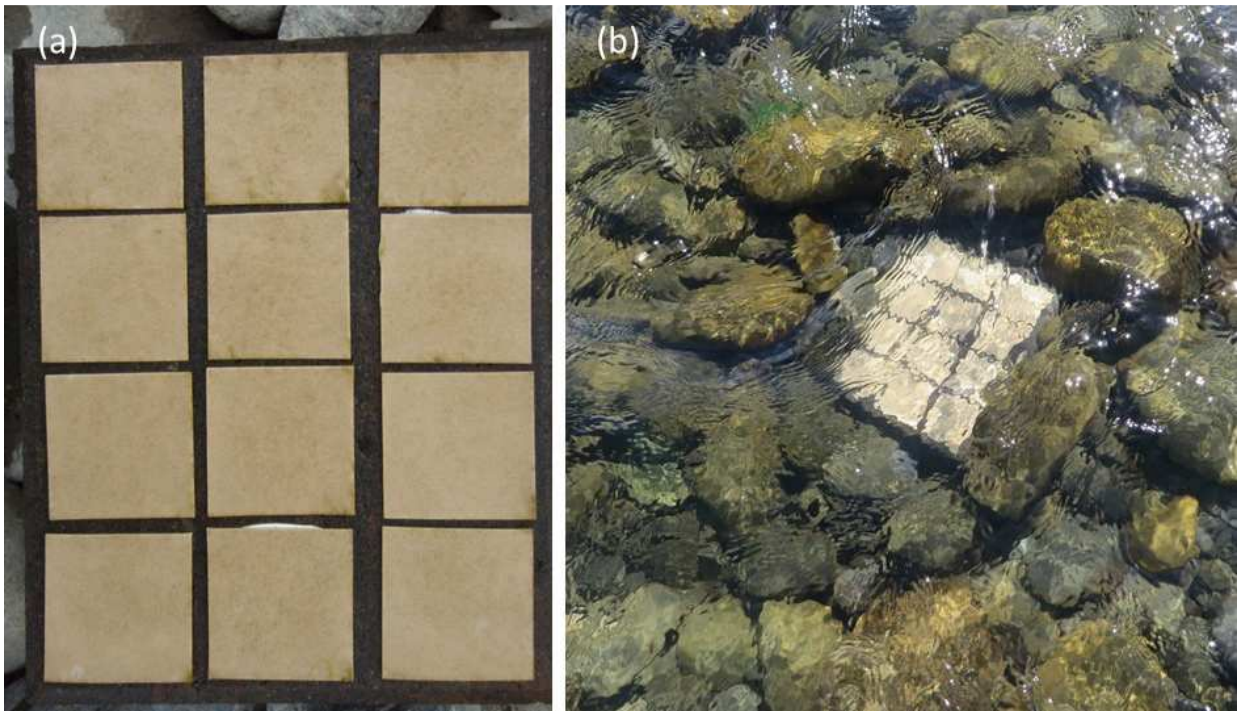

Figure 4-2: Concrete paver with attached growing surfaces, used for the accrual rate trials. (a) growing surfaces with a thin coating of periphyton after 1 week in the river; (b) paver *in-situ* on the river bed.

Samples were collected weekly. Collection commenced after one week because visible periphyton was already present on the surfaces (Figure 4-2a). Pavers were carefully lifted from the water and photographed before removing two plastic squares. The paver was then replaced in the river. A random numbering system was used to pre-select squares for sampling. The two squares were

placed into a labelled container half filled with river water. Samples were kept in a chilly bin on ice and returned to the laboratory within 12 h. This system provided for a 6-week trial (6 weekly sampling occasions until all the squares were used up). Previous trials showed that maximum periphyton biomass on artificial substrates was often attained within 4 weeks (Biggs 1988).

In the laboratory, periphyton on the two squares from each sites was brushed and rinsed off into the container using a soft toothbrush. The resulting algal slurries were then analysed for chlorophyll *a* concentration using the hot ethanol extraction method as described by Biggs and Kilroy (2000). Samples were also analysed for ash-free dry mass (AFDM), which is an alternative measure of periphyton biomass. Chlorophyll *a* concentration reflects the amount of live algae in a sample (because all algae contain this pigment); AFDM reflects the total amount of organic matter in the sample, which includes non-cellular material such as carbohydrate exudates and stalk material. AFDM also includes other organisms in the sample, such as invertebrates. Visible animals were picked out of the sample before analysis. For AFDM, subsamples of the original slurry were filtered onto pre-weighed glass-fibre filters, dried at 105 °C for 24 h, weighed, then ashed at 400 °C for 4 h and re-weighed. In addition to AFDM (dry weight – ashed weight) we calculated the proportion of inorganic material in each sample as 1 – (AFDM / dry weight), which indicates the proportion of inorganic particles within or settled on each sample.

4.2.2 Data analysis

Chlorophyll *a* and AFDM data at each sites were plotted over time to show averaged rates of accrual at each site (over all four replicates). Repeated measures ANOVA (with site as the factor) (RM-ANOVA) was used to identify statistically different periphyton biomass (as chlorophyll *a* and AFDM) among the sites

Data series of chlorophyll *a* can also be used to estimate periphyton growth rates for comparison with theoretical maximum specific growth rate (which takes water temperature into account), as an indication of the degree of growth limitation occurring at a site (Bothwell 1988, Biggs 1990). For these calculations we assumed that losses of periphyton from invertebrate grazing and detachment caused by flow fluctuations were low. Because individual pavers at each site were unavoidably positioned in different physical conditions (i.e., variable water depths and velocities) the calculations were performed on chlorophyll *a* data from pairs of successive samples from the same paver, rather than on averaged data at a site. The calculations were performed for periods over which flows were steady or receding, when losses due to flow fluctuations would have been minimal, and when the slope of the accrual trajectory was positive. Data from the first week of incubation were not included because biomass accrual at this stage would reflect colonisation as well as growth (Biggs 1990).

The net accrual rate was calculated from the following equation:

$$B = a \exp(kT)$$

where:

B is biomass (in chlorophyll *a*, mg/m^2) at the end of the period considered (T days),

a is biomass (in chlorophyll *a*, mg/m^2) at the start of the period considered (i.e., when $T = 0$)

k is the net accrual rate during the exponential growth phase.

Re-arranging the equation, net accrual rate $k = [\text{Log}_e (B/a)]/T$

Net accrual rate *k* is converted to a specific growth rate μ (i.e., cell divisions per day) by correcting for the log transformation:

$$\mu = k/0.693$$

The **relative specific growth** rate was then calculated as: μ/μ_{max}, where μ_{max} is the maximum specific growth rate (as number of cell divisions per day) for nutrient-saturated algae, calculated using the model in Bothwell (1988) (based on P-limiting conditions):

$$\mu_{max} = 0.189 + 0.0278\ t$$

where: *t* = mean temperature (°C) over the accrual period. Where temperature records were not available at a site, we estimated temperature using the relationships established during the periods when records were available from all sites.

According to Bothwell (1988) a relative specific growth rate $\mu:\mu_{max}$ of <0.3 indicates that growth is limited by low nutrient (P) concentrations, 0.3-0.8 indicates slight nutrient deficiency, >0.8 indicates that growth is not limited by nutrients (assuming no other factors are limiting) (Bothwell 1989, Biggs 1990). As noted above, these specific growth rate estimates assume no losses from cell emigration, cell death or invertebrate grazing. This assumption is probably unrealistic for these trials in the Hurunui River, but maximum accrual rates may approximate maximum growth rates. The calculations provide an alternative means of comparing accrual rates among sites.

4.3 Results and discussion

4.3.1 Experiment 1: 5 January to 16 February 2015

River flows were receding or stable throughout most of the experiment, with a period of slightly elevated flow around 4 – 10 February (Figure 4-1). Chlorophyll *a* accrued fastest on the artificial substrata at Gorge, followed by SH7, Balmoral and Mandamus. Mean chlorophyll *a* at each site differed significantly from that at all other sites (Table 4-1). Maximum chlorophyll *a* averaged across all four pavers occurred on the final sampling occasion at SH7, Balmoral and Gorge (Table 4-2). At Mandamus, chlorophyll *a* remained low (< 4 mg/m^2) throughout the experiment.

The pattern of biomass measured as AFDM differed from that of chlorophyll *a*. Biomass over time was significantly lower at Mandamus than at Gorge (Table 4-1) but did not differ from that at the other three sites, which also did not differ from each other (Table 4-1). The rate of increase over time was slower than for chlorophyll *a*, following initial growth in the first week of the experiment (Figure 4-3).

Relative specific growth rates (see explanation above) were calculated using chlorophyll *a* data only. The fastest accrual rates and relative specific growth rates were recorded at the beginning of the period at Gorge, Balmoral and SH7 on at least two of the four pavers, but at Mandamus, the highest relative specific growth rates did not occur until the third period (20 January to 26 January) or later (Table 4-3). The maximum relative specific growth rate recorded on each paver at the four sites indicated P-depleted growth rates at Balmoral ($\mu:\mu_{max}$ <0.3), with slightly less severe P-limitation at Mandamus, then SH7 and Gorge (Table 4-3). Overall mean $\mu:\mu_{max}$ of the four highest values at each site showed slightly higher mean $\mu:\mu_{max}$ at SH7 than at Gorge (Table 4-2).

4.3.2 Experiment 2: 24 February to 20 April 2015

The second accrual rate trial was interrupted by high flows on 6-10 March, between the first and second sample collection, and on 10-17 April, after the fifth sample collection. The results of the first sample collection are not reported, because the trial was effectively re-started on 16 March. The samples used in the analysis were the second to fifth collections, between days 20 and 42, when flows were receding (Figure 4-1).

Biomass as chlorophyll *a* was significantly lower at Mandamus than at all other sites (Table 4-1) but did not differ among the other three sites (Table 4-1). AFDM was variable within sites and did not differ statistically between sites.

Maximum chlorophyll *a* and AFDM were attained on the final collection date (day 42) in most cases (Table 4-2) with maximum relative specific growth rates occurring earlier (Table 4-3). Rates at Balmoral were clearly higher than in Experiment 1 (Table 4-2, Table 4-3). The highest rate across both experiments was recorded at the Gorge (paver 4, Table 4-3); at 0.49, this indicates very slight P-limitation. Maximum relative specific growth rates ($\mu:\mu_{max}$) at each site were in the order (lowest to highest): Mandamus, SH7, Balmoral and Gorge (Table 4-3).

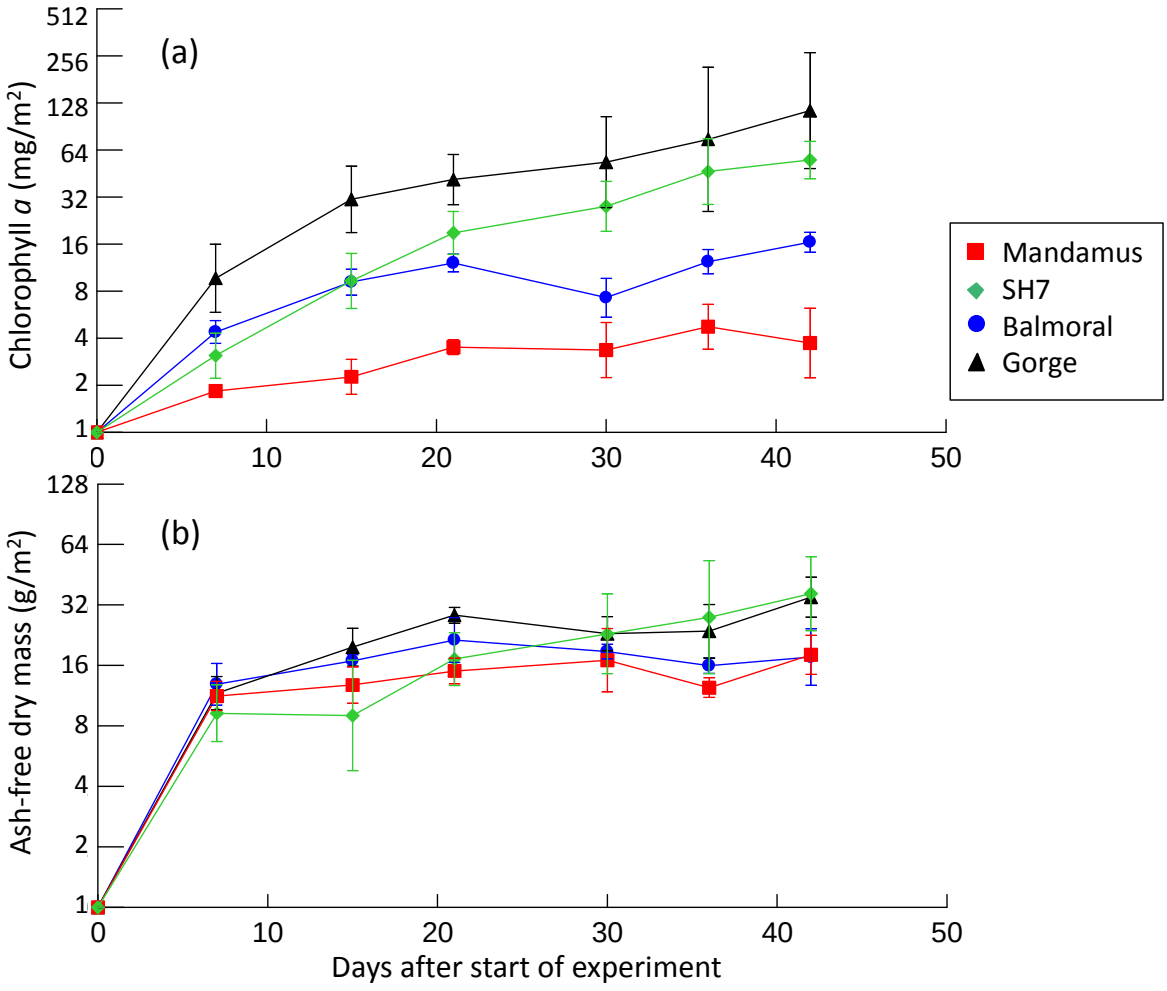

Figure 4-3: Accrual rate Experiment 1: mean chlorophyll *a* (a) and ash-free dry mass (b) measured from periphyton growth on artificial substrates. Biomass data (vertical axis scale) are plotted on a $\log_2$ scale. The trial ran from 5 January to 16 February 2015. n = 4. Error bars are standard deviations.

Table 4-1: Summary statistics from repeated measures ANOVAs to test for differences between sites in periphyton biomass in the accrual trials. Results are shown for the difference between sites and the site x time interaction. A significant interaction indicates that patterns of biomass over time differed between the two sites. Significant differences have P < 0.05 (corrected for multiple tests), shown in bold type.

		Chlorophyll *a*			ADFM		
Comparison between:		Site effect		Site x time	Site effect		Site x time
Site 1	Site 2	F-ratio	P	P	F-ratio	P	P
Experiment 1							
Mandamus	SH7	177.35	**0.00**	**0.00**	5.06	0.39	**0.00**
Mandamus	Balmoral	295.57	**0.00**	0.11	3.95	0.56	0.46
Mandamus	Gorge	170.87	**0.00**	0.42	5.43	**0.01**	**0.02**
SH7	Balmoral	24.09	**0.02**	**0.00**	1.71	1.00	**0.00**
SH7	Gorge	12.70	0.07	1.00	0.49	1.00	1.00
Balmoral	Gorge	55.29	**0.00**	0.10	4.26	0.05	**0.01**
Experiment 2							
Mandamus	SH7	29.97	**0.01**	**0.00**	7.30	0.21	0.12
Mandamus	Balmoral	33.73	**0.01**	**0.00**	1.81	1.00	0.08
Mandamus	Gorge	45.72	**0.01**	**0.00**	2.04	1.00	0.22
SH7	Balmoral	0.73	1.00	1.00	4.96	0.41	0.87
SH7	Gorge	1.47	1.00	1.00	3.87	0.58	0.70
Balmoral	Gorge	6.82	0.24	**0.00**	0.12	1.00	3.00

Table 4-2: Accrual rate trials: maximum biomass at each site, and mean relative specific growth rate for each site. Mean relative specific growth rate ($\mu:\mu_{max}$) was derived from the maximum four values calculated from individual pavers. $\mu:\mu_{max}$ < 0.3 indicates P limitation, 0. 3 – 0.8 indicates slight P deficiency, >0.8 indicates P replete conditions. Mean nutrient concentrations at each site during the trials are shown for reference.

	Maximum mean biomass				Mean $\mu:\mu_{max}$	Mean nutrient concentrations (mg/L)	
	Chl. *A* (mg/m²)	Day	AFDM (g/m²)	Day		DRP ± s.d.	DIN ± s.d.
Experiment 1							
Mandamus	3.9	36	17.3	42	0.33	0.0008 ± 0.0002	0.0037 ± 0.0013
SH7	55.3	42	37.2	42	0.40	0.0008 ± 0.0001	0.0070 ± 0.0015
Balmoral	15.5	42	20.8	21	0.23	0.0007 ± 0.0002	0.0458 ± 0.0052
Gorge	138.1	42	34.5	42	0.38	0.0011 ± 0.0003	0.327 ± 0.0606
Experiment 2							
Mandamus	6.0	42	19.6	28	0.08	0.0005 ± 0.0001	0.0058 ± 0.0053
SH7	92.6	42	58.3	42	0.31	0.0008 ± 0.0002	0.0174 ± 0.0065
Balmoral	74.1	42	30.1	42	0.38	0.0006 ± 0.0002	0.0547 ± 0.0061
Gorge	110.8	36	28.1	42	0.41	0.0012 ± 0.0003	0.494 ± 0.0641

Table 4-3: Maximum relative specific growth rate for chlorophyll *a* recorded for each paver in the two trials. Relative specific growth rates ($\mu:\mu_{max}$) were calculated over periods of ~1 week, between sample collections. The day at the end of the period of maximum growth is the number of days since the start of each trial. $\mu:\mu_{max}$ < 0.3 indicates P limitation, 0. 3 – 0.8 indicates slight P deficiency, >0.8 indicates P replete conditions. Water velocity values shown are means for the paver over the whole trial.

Site	Paver	Experiment 1 Maximum $\mu:\mu_{max}$	Experiment 1 Day at end of max growth	Experiment 1 Water vel. (m/s)	Experiment 2 Maximum $\mu:\mu_{max}$	Experiment 2 Day at end of max growth	Experiment 2 Water vel. (m/s)
Mandamus	1	0.32	21	0.26	0.00	22	0.46
	2	0.38	21	0.29	0.07	22	0.54
	3	0.39	36	0.26	0.08	28	0.65
	4	0.22	21	0.21	0.18	28	0.34
SH7	1	0.44	15	0.61	0.33	36	0.45
	2	0.40	21	0.59	0.26	28	0.56
	3	0.37	15	0.60	0.40	36	0.35
	4	0.38	15	0.69	0.23	28	0.39
Balmoral	1	0.21	15	0.55	0.34	28	0.28
	2	0.18	15	0.41	0.36	36	0.33
	3	0.21	15	0.47	0.37	28	0.50
	4	0.30	15	0.43	0.45	28	0.51
Gorge	1	0.29	36	0.56	0.38	28	0.11
	2	0.35	15	0.44	0.34	28	0.19
	3	0.40	42	0.46	0.41	28	0.40
	4	0.48	15	0.62	0.49	36	0.47

4.3.3 Factors influencing accrual rates

All other conditions being equal, the rate of accrual of periphyton should be proportional to availability of the limiting resource. However, in a river, losses of biomass through grazing and sloughing cannot be avoided. Therefore the accrual rates, here interpreted as growth rates for the highest values recorded, may not represent maximum growth. Furthermore, other physical factors influence growth rates in algae, particularly water velocity.

Water velocity is a key determinant of nutrient uptake rates because velocity controls the rate of delivery of nutrients to the cell surface (Larned et al. 2004), provided velocities are below the threshold that causes algae to be removed through shear stresses. In low-nutrient waters, control of uptake by water velocity is a so-called “subsidy” effect: in fast flowing areas, algae can obtain enough nutrients to grow, even when background concentrations are low. This explains the pattern in riffle areas of rivers of high algal biomass, which generally comprises algae adapted to withstand high velocities. In the accrual rate experiments, the most rapid relative specific growth rates were not correlated with mean water velocity over the whole accrual period. There was some evidence of a link between faster water velocity and maximum chlorophyll *a* at the Gorge site, but the relationship was seen more clearly using data from periphyton growing on the river bed (see Section 6.2.4).

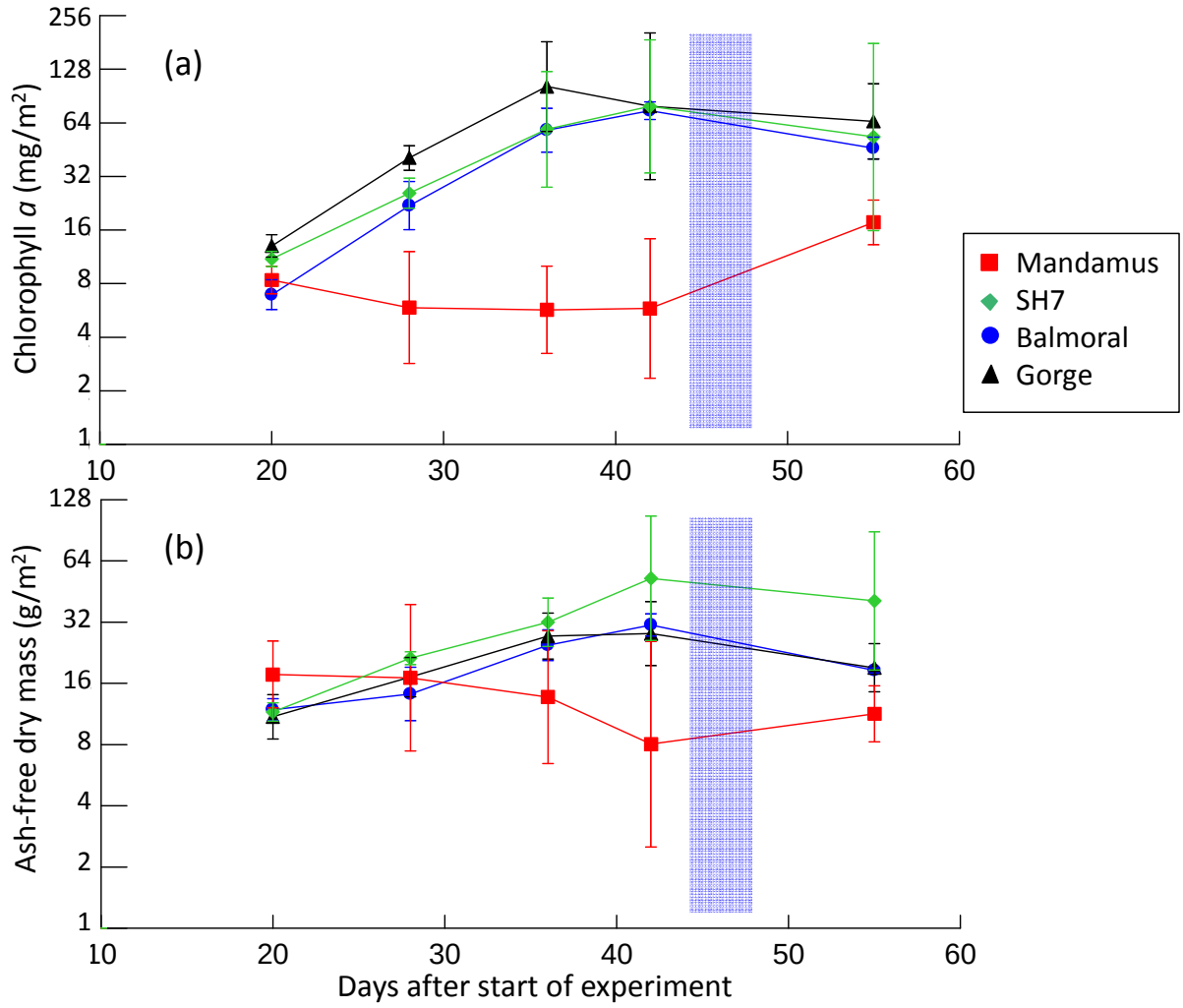

Figure 4-4: Accrual rate Experiment 2: mean chlorophyll *a* (a) and ash-free dry mass (b) measured from periphyton growth on artificial substrates. Biomass data (vertical axis scale) are plotted on a $\log_2$ scale. The trial ran from 24 February to 20 April 2015. n = 4. Error bars are standard deviations. The blue bar between days 40 and 50 indicates a period of high flows that curtailed accrual at SH7, Balmoral and Gorge, but appeared to stimulate accrual at Mandamus.

Differences in accrual patterns over an equivalent period in the two experiments in the Hurunui River illustrate that periphyton development can be very variable. After 42 days of accrual, both mean chlorophyll *a* and mean AFDM (averaged across the four pavers) did not differ between the two experiments at Mandamus, SH7 and Gorge (two-sample T-tests at each site, $P > 0.05$), but were significantly higher at Balmoral in Experiment 2 than in Experiment 1 (two-sample T-test, $P < 0.01$). This is seen clearly in photographs of sample tiles on the two dates: in February periphyton cover comprised a thin film, whereas in April, cover included film, mats, green filaments and didymo (Figure 4-5). Each of the four sites had a distinctive appearance on 16 February (Figure 4-5, top row). However, on 7 April, only Mandamus stood out as being different. At the other three sites, cover included thin films, green filaments and didymo, in different proportions. *Phormidium* was still present on the tiles at Gorge, but as patchy cover (e.g., top left had corner of top square in Figure 4-5) rather than almost 100 % cover in February.

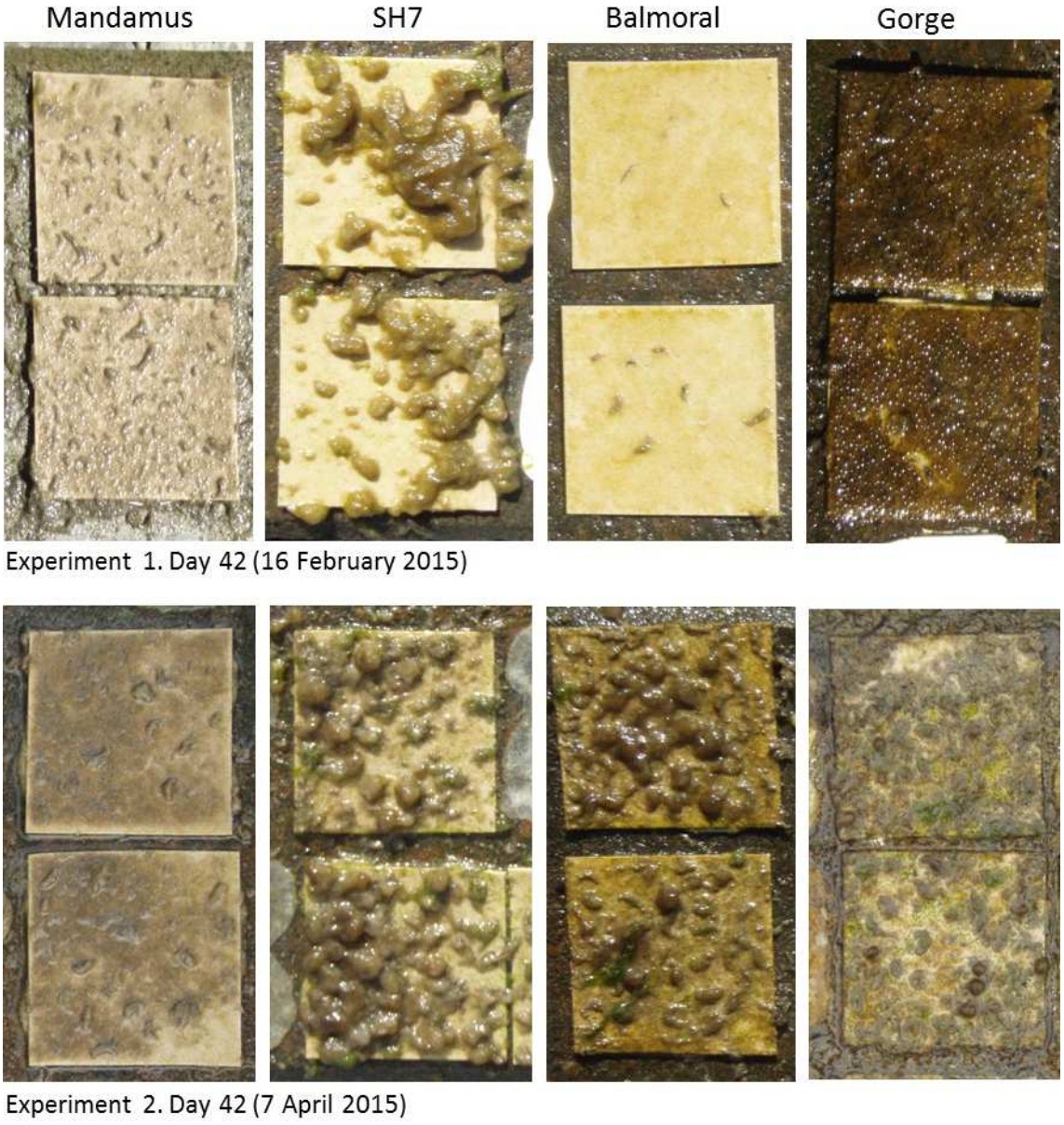

Figure 4-5: Periphyton growth on tiles on Day 42 of the two accrual rate experiments at four sites in the Hurunui River. Periphyton biomass did not differ between the two dates, except at Balmoral, where biomass as chlorophyll *a* and AFDM were, respectively, approximately 5 and 2 times greater in April than in February.

Examination of the photographs taken on each sample collection date highlighted how the appearance of periphyton varied over time and also provide clues as to what was driving biomass changes. Three examples are shown in Appendix A. They show that in Experiment 1:

(a) accrual at SH7 initially included green filamentous algae, but much of this was lost before the end of the trial;

(b) slow or negative accrual at Balmoral after the second sample collection was probably caused by heavy invertebrate grazing;

(c) initial accrual at Gorge was largely inorganic material (>90% inorganic). Once some of this material was lost, accrual of chlorophyll *a* (mainly as *Phormidium*) accelerated.

Periphyton cover on the pavers at Mandamus was almost constantly dominated by brown silty material (not illustrated in Appendix A). The presence of silt was confirmed by consistently low organic content (14 ± 4%). High silt deposition was attributable to low water velocities near the stream bed, even though water column velocity measured at 0.6 of the depth at Mandamus was similar to that at the other sites. The boulder-dominated irregular bed substrate at Mandamus created many areas of quiet water near the bed in the lee of boulders, which could not be avoided. Therefore the environment at Mandamus was rather different from that at the other sites.

In summary, results in accrual rate Experiment 1 were complicated by invertebrate grazing on the substrates at Balmoral, which curtailed periphyton development at that site. The results of Experiment 2 generally supported our initial hypothesis that periphyton growth rates (here inferred from accrual rates) would increase along a gradient of increasing nutrient concentrations: the maximum relative specific growth rate increased in a downstream direction, and there was a strong downriver increase in DIN. The results indicated definite P-limitation at Mandamus to very slight P-limitation at Gorge. The downriver gradient of DRP was not completely consistent with the growth rate results because DRP was slightly higher at SH7 than at Balmoral. However, the uncertainties of sample collection and biomass measurement likely mean that there was no meaningful difference in growth rates between these two sites.

Refer to Section 8 for further discussion on the patterns in and implications of the accrual experiments.

5 Nutrient limitation assays

Growth limitation refers to suboptimal rates of cell division and growth when an essential resource is in short supply. A variety of resources can limit growth including light, major nutrients and trace nutrients. In theory, algal growth can be limited by only one resource at a time. When that resource is supplied, then another may become limiting (Larned 2010). In unshaded river sites, the resources most often limiting algal growth are the major cell nutrients, nitrogen (N) and phosphorus (P). The identity of the limiting nutrient can be inferred from ambient concentrations, and from the ratio of N to P. Based on research in the 1950s on marine phytoplankton these two elements maintain a ratio in cells of about 7:1 (by weight) (Redfield 1958). The so-called Redfield ratio applied to DRP and DIN concentrations (the N : P ratio) in river water is taken to represent an approximate threshold between P limitation and N limitation. In practice the threshold of N : P that distinguishes between N and P limitation is vague because different algae respond differently to nutrients. As a general rule, ratios between say 20 and 4 are inconclusive (Francouer et al. 1999). Thus N : P > 20 usually indicates P limitation, and N : P < 4, N-limitation. The range of uncertainty could be even broader (Keck and Lepori 2012). DIN and DRP concentrations also need to be taken into consideration but, again, "rules" for defining whether DIN or DRP are limiting have proved elusive, particularly for DRP (Keck and Lepori 2012).

Concentrations of DIN and DRP measured at the four sites in the Hurunui River from January to May 2015 suggest that the limiting nutrient differs among sites. We carried out a series of nutrient limitation assays at each site over time to investigate spatial and temporal variation in nutrient limitation.

5.1 Methods

5.1.1 Field and laboratory procedures

Nitrogen and phosphorus limitation in periphyton has been assessed using a range of methods, the simplest of which are based on the principle of diffusion of nutrients from an agar medium through a substrate suitable for periphyton growth. We used the steel tray method described in Biggs and Kilroy (2000), which was designed for use in larger rivers. The steel trays are 0.6 x 0.4 x 0.2 m, with an internal frame that holds 20 reservoirs (400 ml jars). Five jars per tray were filled with 2% agar amended with either nitrogen (as 0.5 molar sodium nitrate, $NaNO_3$, N treatment), phosphorus (as 0.5 molar trisodium orthophosphate, $Na_3PO_4.12H_2O$, P treatment), or both nitrogen and phosphorus (N+P treatment). Five jars held unamended agar, as controls. The five jars for each treatment were arranged longitudinally; i.e., 4 lines each of five jars (Figure 5-2). Growing surfaces (hardened, ashless filter papers) were secured over tops of the open jars in direct contact with the agar. A lid with holes drilled to fit exactly over the jar openings was secured on top of the box. A 15 mm ridge running between each line of growing surfaces helped prevent diffusion of nutrients across to adjacent treatments.

At each site, a depression with a level base was cleared on the riverbed, and the tray was placed in the depression with the current running parallel to the lines of growing surfaces. Rocks and gravel were arranged around the trays so that the top of the tray was more or less level with the river bed. This helped stabilise the tray in the river bed and reduced the risk of damage and loss during high flows. At the time of deployment we measured water velocity at each tray. The target exposure time was 14 days. Any exposure of more than about 20 days risks loss of filter papers, which tend to become more fragile over time. The NDS samples were collected by removing the top lid from the

metal tray, and transferring the filter papers (after trimming to the size of the exposed area) to pre-labelled vials. Samples were stored on ice and frozen until analysis for chlorophyll *a* using the ethanol extraction method (Biggs and Kilroy 2000).

Where possible, we redeployed the same tray for a second assay, to try to obtain as much information as possible at each site. In previous trials, the nutrient reservoirs have been shown to continue to release nutrients for at least one month (Wilks 2008). The second deployment (the "B" assay) provided back-up data, but we consider the first deployment (the "A" assay) to be the more reliable result.

5.1.2 Data analysis and interpretation

Chlorophyll *a* data were log-transformed prior to analysis to achieve a normal distribution of data. Data from each site were analysed separately, using two-way analysis of variance (ANOVA) with treatment as factor. Probability (P) < 0.05 signified significance of the N-treatment, P-treatment, or the interaction between the two (N x P interaction). In a two-way ANOVA all the data from samples receiving either N or P are first compared with the treatments not receiving N or P. A significant N x P effect indicates different effects of N or P within the two main groups. There are eight possible outcomes and we interpreted the results following Tank and Dodds (2003) (Table 5-1). For each site, mean DRP and DIN nutrient concentrations applicable to the period of each assay were calculated, and the N : P ratio calculated as DIN/DRP.

Table 5-1: Interpretation of the eight possible outcomes of a 2-way ANOVA on data from the NDS assays. <0.05 indicates a significant effect of either N, P or N x P. NOTE: The interpretations assume that mean chlorophyll *a* in the treatments with nutrient additions are not less than that in the control. This is normally the case, but exceptions have been reported (e.g., see Francouer et al. 1999). Table adapted from Tank and Dodds (2003).

Interpretation	Effect of P	Effect of N	NxP interaction
P limitation	<0.05		
N limitation		<0.05	
Co-limitation by N and P			<0.05
Co-limitation by N and P	<0.05	<0.05	
Co-limitation by N and P	<0.05	<0.05	<0.05
Primary P limitation, secondary N limitation	<0.05		<0.05
Primary N limitation, secondary P limitation		<0.05	<0.05
No nutrient limitation			

5.1.3 Periphyton community composition

Nutrient diffusing substrate assays almost invariably report periphyton responses only in terms of chlorophyll *a*, even though the response may be stimulation of growth in some taxa and not others. Because NDS assays run for a relatively short period (2-3 weeks), the response measured is often in the growth of early successional stages of periphyton (see Biggs 1996). Mature communities would develop only if growth was extremely rapid, and conditions were optimal (i.e., no constraints on algal growth from nutrients, light, temperature or other potentially limiting resource). Nevertheless, community composition differences even at early stages of succession may indicate how communities might subsequently develop at that site, if the nutrient treatment were maintained.

To obtain a preliminary picture of community differences among sites and in response to the nutrient treatment, subsamples of algae on each NDS treatment were collected from all NDS trays from the first "B" treatment onwards. Subsampling was done by cutting off one quarter of one of the filter paper growing surfaces (randomly selected) in each treatment. Subsamples were stored frozen until analysis. Samples from two NDS assays were analysed: run B of assay 1 (14-day incubation collected on 4 February 2015) and run A of assay 3 (20-day incubation collected on 20 April), representing mid-summer and autumn conditions.

For analysis, periphyton was brushed off the filter papers and made up to a known volume in tap water. Measured subsamples of the algal suspension were pipetted into an Utermöhl chamber (a settling chamber with a glass base), and the algae were allowed to settle for 10-15 min. Counts were made of at least 400 cells in each sample, in a known number of fields, at a magnification of 400 x. This quantitative count enabled estimation of numbers of cells of each taxon per unit area of growing surface. Periphyton taxa were identified to the lowest level possible. This was generally to genus level for green algae, cyanobacteria and some diatoms, and to species level for distinctive diatoms. We also calculated biovolume data by multiplying the counts by the average volume of cells in each taxon, with volume calculated from measurements of 10-12 cells of each taxon, using an ocular micrometer. The biovolumes of common algal taxa in rivers range from < 5 to >50,000 μm^3 per cell. Because very small cells can be extremely abundant compared to large cells, biovolumes provide an alternative picture of the contribution of each taxon to the community.

Densities of the more common taxa were compared individually, in particular to determine whether there were indications of growth stimulation by either N or P. Relative abundancesof the most common taxa, by both numerical density and biovolume, were plotted on composite bar graphs to visualise differences between sites and treatments.

Taxonomic composition data were also analysed using non-metric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM) to determine whether algal assemblages were more characteristic of sites or treatments. In NMDS 2-dimensional plots, samples are positioned so that closely-related samples plot close together and less closely-related samples are more widely separated. ANOSIM uses a re-sampling procedure to determine whether two sets of samples differ significantly (i.e., when the probability of a difference due to chance is less than 5%, or $P < 0.05$). To reduce "noise" in the data, taxa detected in only one of the 16 samples in each assay were not included.

5.2 Results and discussion

5.2.1 Assay performance

Three sets of NDS assays were deployed, starting on 6 January, 24 February and 1 April (Figure 5-1).

The 6 January deployment (the A assay) was retrieved successfully after 14 days, and a second deployment (B) was also successful. The 24 February A assay was affected by the flood on 6 March (peak of >80 m^3/s at Mandamus). The tray at Mandamus was overturned, all growing surfaces were lost and some nutrient reservoirs were damaged; the tray at SH7 withstood the flood and provided usable data; the tray at Balmoral was lost; the tray at the Gorge was undamaged, but some growing surfaces were lost. The trays at SH7 and Gorge were redeployed (B assay) and gave usable results. The third deployment on 1 April was also flood-affected but all trays were intact on collection of the A assay on 20 April, although growing surfaces were lost at Balmoral and Gorge. All trays were redeployed. In the B assay, only the results from SH7 are considered reliable as it was highly likely

that receding flows during the 2-week deployment exposed the growing surface to air at Mandamus, Balmoral and Gorge. Exposure was confirmed at Gorge by the temperature record, which indicated that the logger was out of the water (Figure 3-6).

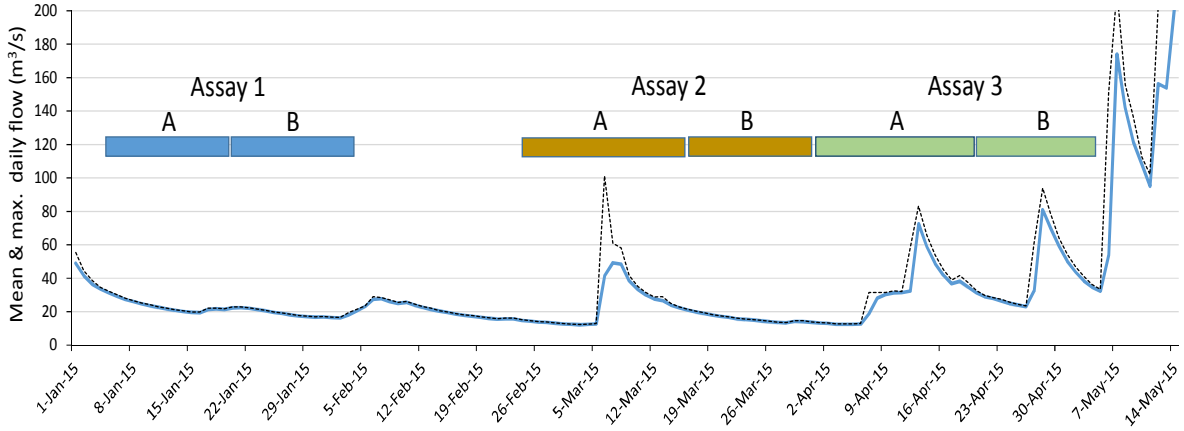

Figure 5-1: Deployment periods of nutrient diffusing substrate assays (coloured bars) overplotted on the flow record for Hurunui @ Mandamus. Only Assay 1 was not flood-affected, but usable results were obtained from some sites from all three assays. B assays refer to redeployments of the nutrient diffusing trays using the same nutrient reservoirs, with new growing surfaces.

5.2.2 Nutrient limitation

Plots of mean chlorophyll *a* in each treatment in all assays are shown in Appendix B, and results are summarised in Table 5-2.

At Mandamus there was clear N-limitation in early January, in agreement with an N : P ratio of 4 and very low DIN over the period of the A assay. The B assay (20 January to 4 February) indicated co-limitation by both N and P, which was consistent with slightly lower DRP at that stage and an N : P ratio of 5 (Table 5-2). The A assay in the third deployment (1-20 April) also indicated clear co-limitation.

SH7 was the only site where we obtained good results for all six assays. Co-limitation of periphyton growth (as chlorophyll *a*) was indicated in all assays except for the A assay in the third deployment, which indicated P-limitation. Very low DRP and an N : P ratio of 32 was consistent with the assay result on that occasion. Co-limitation indicated by the B assay was unexpected given that the nutrient concentrations and N : P ratio were similar to those in the A assay. However, the P effect was much stronger than the N effect (indicated by the high F-ratio for the P effect) (Table 5-2). Therefore the result bordered on primary P limitation with secondary N-limitation.

At Balmoral, N : P ratios of 57 to 123 and low concentrations of DRP (<0.0013 mg/L) indicated that periphyton should be P-limited. The NDS assay results reflected this except that in the first B assay significantly higher chlorophyll *a* on the N + P treatment than the P treatment, and no response to N when added alone, suggested secondary N limitation at that time.

A much higher N : P ratio at the Gorge, compared to the other three sites, in conjunction with relatively low DRP concentrations (> 0.002 mg/L) suggested that periphyton should be P-limited at this site. The NDS assay results were consistent with the N : P ratios, except that the 2-way ANOVA result for the first B assay also suggested secondary N-limitation. Examination of the raw data (Appendix B) shows that the significant interaction term was caused by significantly lower chlorophyll

Figure 5-2: Example of NDS assay after two weeks incubation in the Hurunui River at SH7. Treatments are from bottom to top: N, P, N+P, Control. In this case the appearance of the assay suggests co-limitation of periphyton growth by both N and P at this site because of densest cover on the N+P treatment, and visually similar cover on the other three treatment rows. Note the patch of *Phormidium* growing over the left-hand replicate in the P treatment.

a on the N-treatments than on the control. Apparent inhibition of growth on an NDS treatment compared to the control has been recorded in previous studies (e.g., Francoeur et al. 1999, Tank and Dodds 2003). However, no satisfactory explanation has been found. Potential explanations includedifferential rates of invertebrate grazing, the effect of different water velocities over different parts of the tray, or sloughing of algae from the treatment showing inhibition. There was no evidence for velocity effects or sloughing; lack of obvious colonisation by grazers at Gorge indicated that grazing was not responsible for the difference. At this stage apparent inhibition of chlorophyll *a* on the N-treatment remains unexplained. We did not see this pattern on any other assay.

The strength of P-limitation at the two sites where periphyton was primarily P-limited (Balmoral and Gorge) can be compared by looking at the relative biomass on treatments supplying P compared to the control treatment. Between-site differences were clearest for assay 1A. Chorophyll *a* at Balmoral increased 5.8-fold in response to P compared to 1.9-fold at Gorge (see Appendix B).

In summary, nutrient limitation patterns from NDS assays over space and time in the Hurunui River from January to May 2015 largely reflected the patterns expected from DRP and DIN concentrations and N : P ratios in the overlying water. When DRP concentrations were slightly elevated at Mandamus at the beginning of January, periphyton growth at Mandamus was N-limited. This transitioned to co-limitation by both N and P by late January, which we assume was maintained until April. Slightly higher DIN at SH7 (compared to Mandamus) led to co-limitation there from the beginning of January, and a possible transition to P-limitation by April, possibly driven by lower DRP at that time. Balmoral and Gorge were primarily P-limited, with possible secondary N-limitation at Balmoral in February, and generally stronger P-limitation than at Gorge.

Table 5-2: Summary results of all nutrient diffusing substrate assays, Hurunui River, January - May 2015. Ambient nutrient concentrations in mg/L. Results in grey type indicate assays in which replicates were lost or spoiled for the stated reason; therefore the results are uncertain. NS = non-significant result. "A" and "B" dates refer to the first and second deployments, respectively, of the same nutrient reservoirs.

Site	" A" Dates	Effect	F-ratio	P	Result	DRP	DIN	N:P	"B" Dates	F-ratio	P	result	DRP	DIN	N:P
Mandamus	6 Jan-20 Jan	P	0.1	0.79	N-limited	0.0010	0.004	4	20 Jan-4 Feb	25.5	0.00	Co-limited	0.0009	0.004	5
		N	51.0	0.00						146.5	0.00				
		N*P	1.7	0.21						22.5	0.00				
	24 Feb-16 Mar		Assay lost			0.0006	0.006	10	16 Mar-1 Apr	No assay			0.0005	0.007	14
	1 Apr-20 Apr	P	19.2	0.00	Co-limited	0.0006	0.009	16	20 Apr-5 May	3.8	0.07	NS	0.0005	0.011	21
		N	31.6	0.00						3.4	0.08	(exposure			
		N*P	5.6	0.03						4.0	0.06	to air?)			
SH7	6 Jan-20 Jan	P	22.8	0.00	Co-limited	0.0009	0.007	8	20 Jan-4 Feb	12.0	0.00	Co-limited	0.0009	0.018	19
		N	10.6	0.01						31.6	0.00				
		N*P	8.3	0.01						16.3	0.00				
	24 Feb-16 Mar	P	62.6	0.00	Co-limited	0.0010	0.014	14	16 Mar-1 Apr	32.1	0.00	Co-limited	0.0007	0.019	25
		N	11.9	0.00						8.7	0.01				
		N*P	11.5	0.00						10.2	0.01				
	1 Apr-20 Apr	P	66.5	0.00	P-limited	0.0007	0.023	32	20 Apr-5 May	78.3	0.00	Co-limited	0.0006	0.022	35
		N	2.4	0.14						7.6	0.01				
		N*P	0.9	0.37						2.0	0.17				
Balmoral	6 Jan-20 Jan	P	52.0	0.00	P-limited	0.0008	0.047	57	20 Jan-4 Feb	29.7	0.00	Primary P	0.0006	0.048	81
		N	1.0	0.34						3.9	0.07	Secondary N			
		N*P	1.9	0.19						7.2	0.02				
	24 Feb-16 Mar		Assay lost			0.0006	0.056	88	16 Mar-1 Apr	no assay			0.0007	0.052	76
	1 Apr-20 Apr	P	24.1	0.00	P-limited	0.0005	0.058	116	20 Apr-5 May	0.7	0.40	NS	0.0005	0.063	123
		N	0.9	0.37						0.0	0.95	(exposure			
		N*P	0.6	0.44						1.3	0.27	to air)			
Gorge	6 Jan-20 Jan	P	20.2	0.00	P-limited	0.0013	0.294	234	20 Jan-4 Feb	35.4	0.00	P-limited	0.0010	0.367	382
		N	0.9	0.36						1.3	0.26	note: N-response			
		N*P	0.8	0.37						8.4	0.01	< control			
	24 Feb-16 Mar	P	3.1	0.12	NS	0.0012	0.453	372	16 Mar-1 Apr	39.3	0.00	P-limited	0.0013	0.494	372
		N	0.4	0.52	(papers lost					3.6	0.08				
		N*P	0.3	0.60	in flood)					0.9	0.36				
	1 Apr-20 Apr	P	88.6	0.00	Co-limited?	0.0012	0.459	394	20 Apr-5 May	6.0	0.03	P-limited	0.0013	0.415	334
		N	46.6	0.00	(papers lost)					0.0	0.84	(exposure			
		N*P	0.3	0.61	in flood)					0.4	0.53	to air)			

5.2.3 Periphyton community composition

February sample collection: Fifty periphyton taxa were recorded from the 16 samples (see Appendix C). Five of these taxa were recorded in all samples and a further two in 15 of the 16 samples. Several distinctive taxa showed clear patterns among sites and treatments. The mucilage-producing diatom *Cymbella kappii* was common in all treatments at the Gorge and in the N, P and N+P treatments at Balmoral, but present only at low densities in the control treatment at Balmoral and in all treatments at SH7 and Mandamus. In contrast, the filamentous diatom *Diatoma tenuis* responded strongly to added N at Mandamus and SH7, but was present at much lower densities at Balmoral and Gorge, even though ambient N was higher at those sites. The stalked diatom *Gomphoneis minuta* var. *cassieae* was present in low densities in the control treatments, except at Mandamus where it was not observed in the control, but appeared to respond to added P at all sites. *Phormidium* was recorded mainly at Balmoral and Gorge, but was present on the N+P treatment at SH7 (Figure 5-3).

April sample collection: The same suite of periphyton taxa was recorded in April as in February with minor variations (see Appendix C), although overall biomass was much higher (see Appendix B). *Cymbella kappii* was again abundant at the Gorge on all treatments, and was recorded at Balmoral and SH7 on the P and N+P treatments only. *Gomphoneis* was abundant at the Gorge and on the treatments with added P at Mandamus, SH7 and Balmoral. In April *Phormidium* grew in significant amounts on the NDS substrata only at Balmoral and Gorge (Figure 5-3).

Across the two dates, *Phormidium* appeared to grow randomly on all treatments, with no consistent response to either N or P.

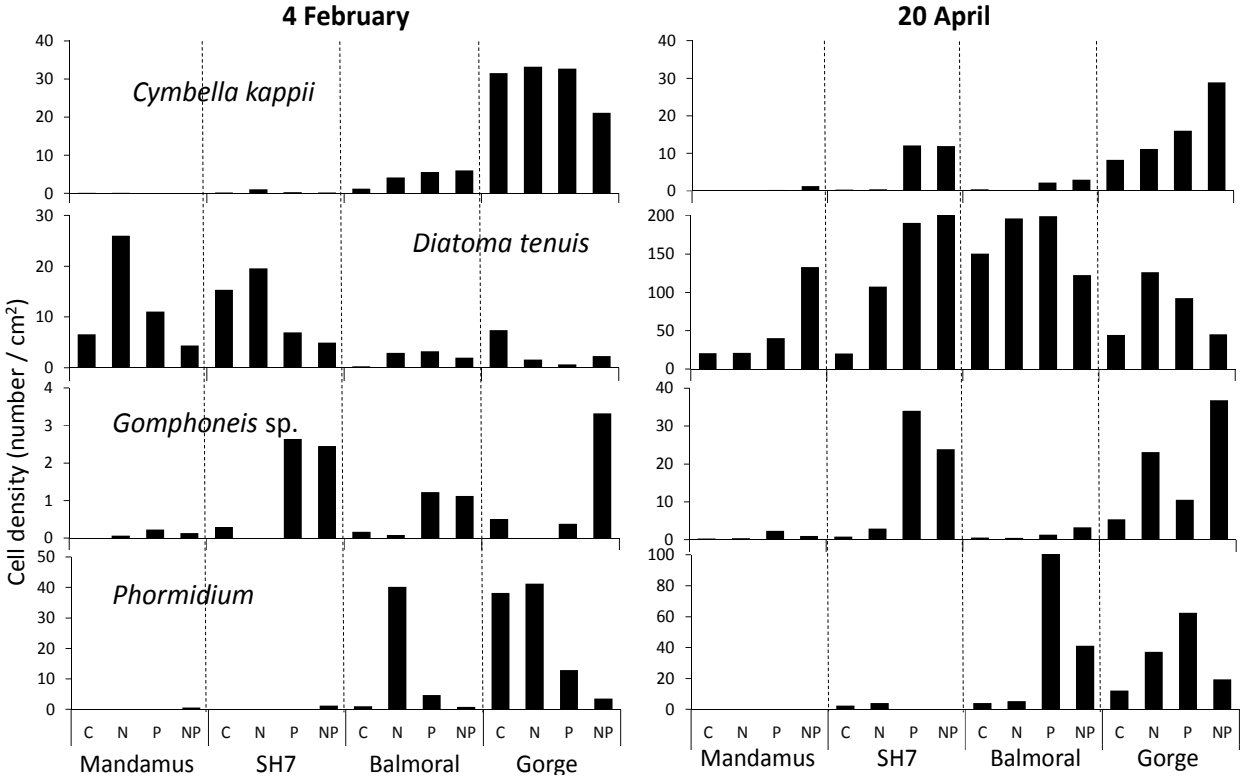

Figure 5-3: Densities of four common algal taxa determined from samples from nutrient-diffusing substrate growing surfaces on 4 February and 20 April. One sample was analysed in each treatment on each date. Dashed vertical lines separate the sites, arranged from upstream to downstream. Note the different scales on the y-axes.

A discrepancy in community composition between the two dates was seen in *Diatoma tenuis*. In the February samples, *D. tenuis* occurred in highest abundance at the upstream sites, Mandamus and SH7. In April, overall abundance of this taxon was much higher than in February, with highest abundance at Balmoral (on all treatments). This contrasting pattern between dates was probably due to the different hydrological conditions leading up to sample collection. The February collection followed two weeks of low flows; the April collection was seven days after a high flow. In the Ohau river, *D. tenuis* was observed to be abundant at early successional stages, especially in the presence of added N, P and N+P. However, after 6-9 weeks of growth in the Ohau River, *D. tenuis* almost completely disappeared except in treatments with no nutrient additions (Kilroy and Larned, unpublished data). In the Hurunui River in February, we assume that *D. tenuis* had been largely outcompeted by other taxa at the two downstream sites. In contrast, in April, the high flow on 13 April may have "re-set" growth on the substrates to some extent, and stimulated rapid growth of *D. tenuis*, especially where some additional nutrients were available.

Community composition across treatments was examined in detail for the February samples. It should be noted that Figure 5-4 and Figure 5-5 show the results from the same single sample in each treatment. Therefore the patterns shown are indicative only. The twelve taxa that were numerically most common made up over 85% of the entire count. The relative abundances of these taxa (Figure 5-4) illustrate the turnover of taxa in a downriver direction. They also show the site-specific response to added N and P together at each site. Except at Gorge, the N+P treatments were dominated by high abundance of a taxon absent or uncommon in all the other treatments. In contrast, adding either N or P alone resulted in shifts in abundance of taxa already present in the control.

Plots of relative abundances by biovolume provided a different picture (Figure 5-5). Biovolume takes into account the size of cells so that large cells that occur in low numbers make up a much higher proportion of the samples than their density suggests. Didymo cells in particular are at least 1000 x larger in volume than common small taxa such as D. tenuis, *Encyonema minutum* or *Achnanthidium minutissimum*. Cells of filamentous green algae also tend to be large compared to many diatom taxa. Biovolume data highlighted several patterns.

- Didymo cells occurred on the substrates only at Mandamus and SH7.
- *Gomphoneis minuta* var. *cassieae* generally responded positively to P additions at each site.
- *Oedogonium* did not occur on any treatments at the Gorge, even though there appeared to be a response to the N+P treatment at Mandamus and Balmoral.
- *Mougeotia* sp. may require some nutrient availability, but may not thrive in high nutrients as indicated by its absence from the Mandamus samples except with added N, its abundance at SH7 except with added N+P, its sparse occurrence at Balmoral (all treatments) and its absence from the Gorge samples.
- *Cymbella kappii* may benefit from extra N in the overlying river water, at least in this early stage of development, in view of its abundance on all treatments at Balmoral and Gorge, and general low abundance upstream.

These results illustrate how the interplay between the effects of flows and nutrients may affect algal community composition.

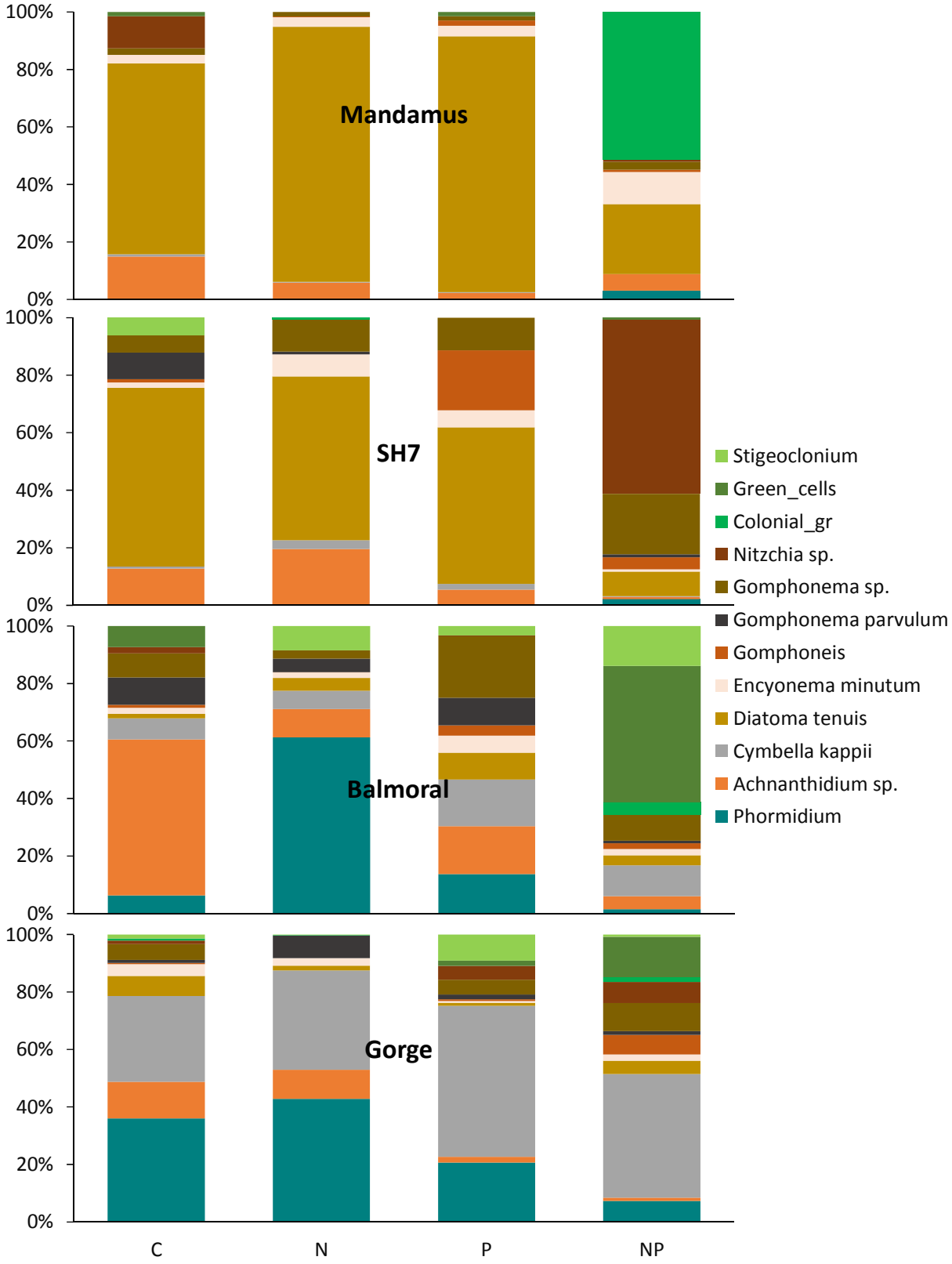

Figure 5-4: Relative abundance (%) of the 12 most common taxa in samples from the four nutrient diffusing substrate treatments. Samples were collected on 16 February 2015. Green shades indicate green algae; brown, orange and grey shades indicate diatoms; teal shading indicates Cyanobacteria. Note that only one sample per treatment was analysed. Therefore these results are indicative only.

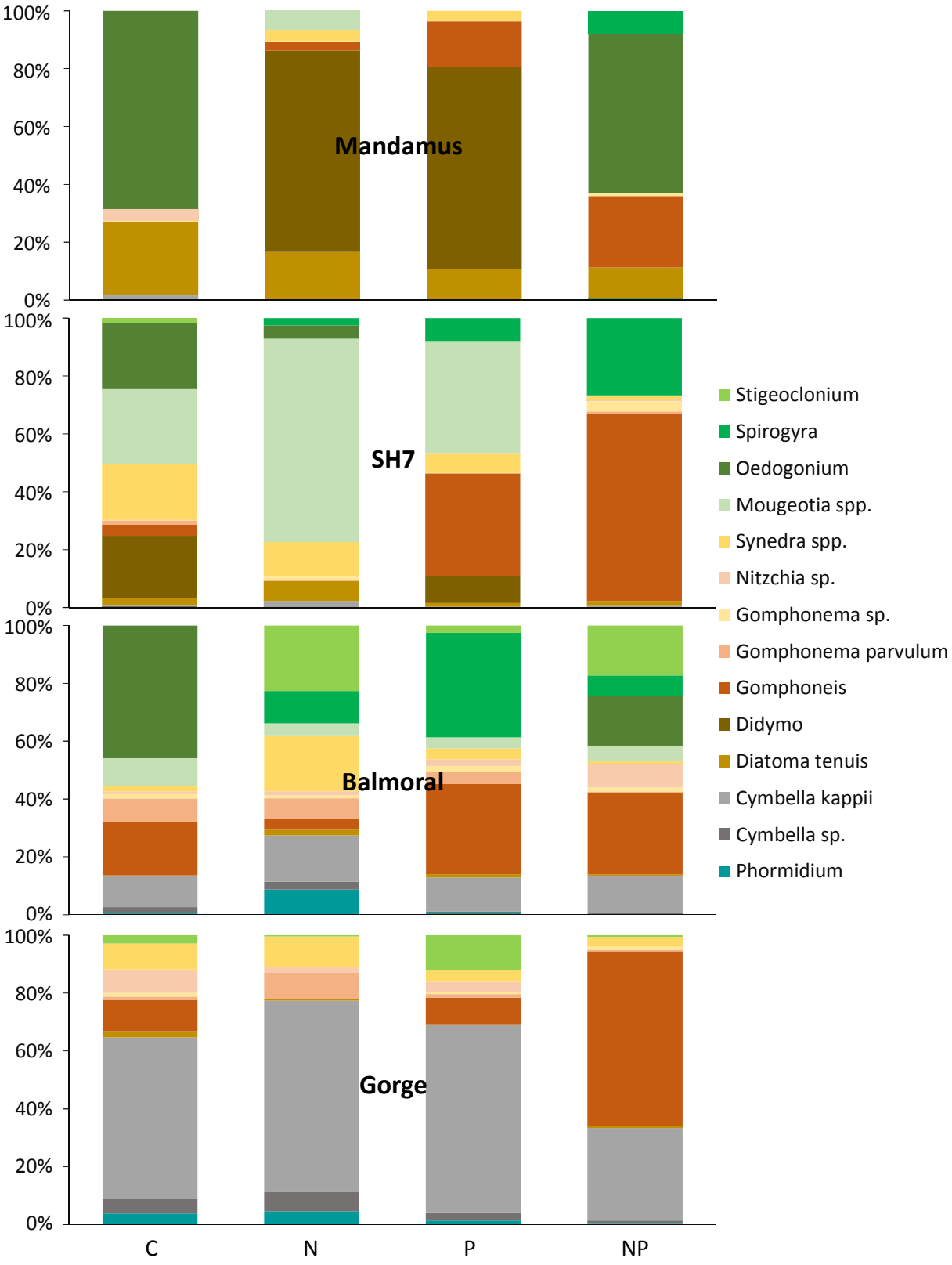

Figure 5-5: Relative abundance (%) by biovolume of the 14 most common taxa in samples from the four nutrient diffusing substrate treatments. Samples were collected on 16 February 2015. Green shades indicate green algae; brown, orange and grey shades indicate diatoms; teal shading indicates Cyanobacteria. Note that only one sample per treatment was analysed. Therefore these results are indicative only..

An NMDS plot generated using all taxa occurring in more than one sample confirmed clear separation between the sites, with a downriver gradient of species composition (Figure 5-6a). Communities at all sites were statistically different from those at the other sites (ANOSIM, $P < 0.05$) except for Balmoral and Gorge (ANOSIM, $P = 0.11$). There was high variability among treatments across sites (Figure 5-6b). However, note that the community growing on the N+P treatment at SH7 was more similar to the communities at Balmoral and Gorge than those on the other treatments at SH7, suggesting convergence of community composition at that site with the communities with higher ambient nutrient concentrations downstream.

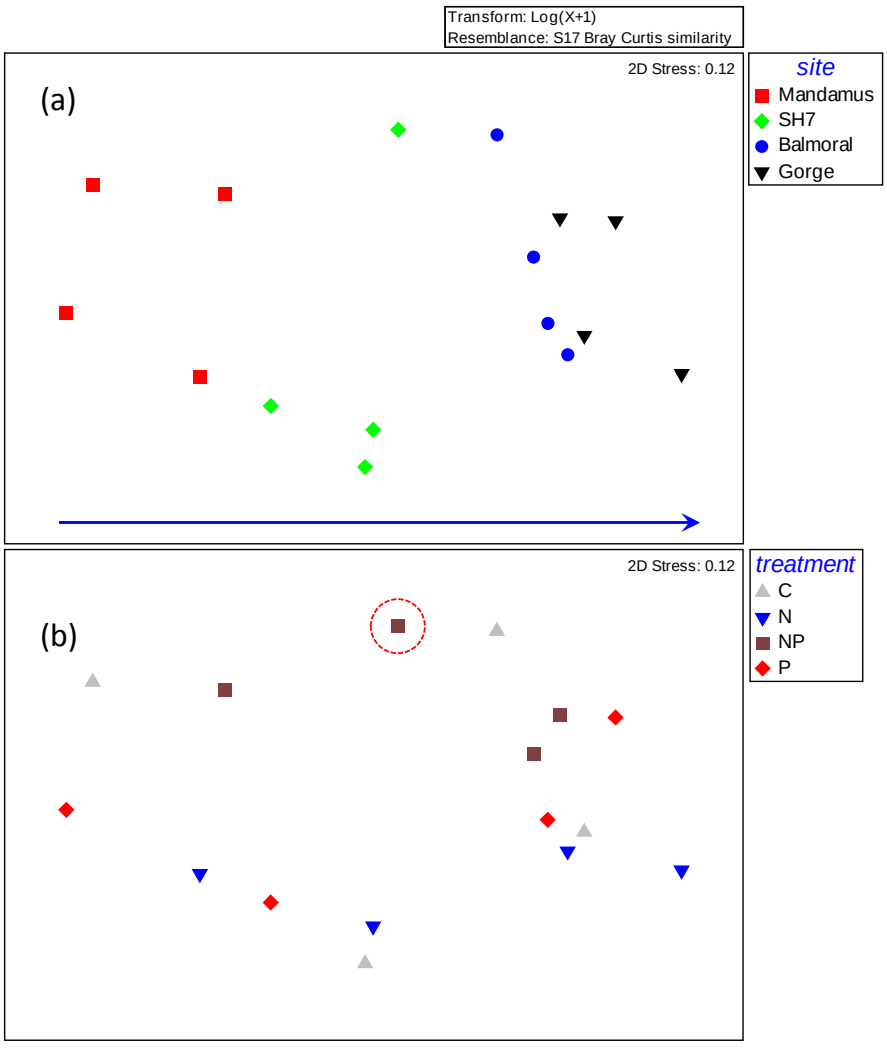

Figure 5-6: Non-metric multi-dimensional scaling plots of periphyton community composition in samples from NDS assays in the Hurunui River. Each data point represents one sample with multiple taxa, from the February survey. Data were log-transformed to down-weight the effect of very abundant taxa. The similarity of samples is indicated by how close together they are on the plots. Plot (a) shows that the samples from each site cluster together with a composition shift down the river (blue arrow). In (b) samples separated by treatment show possible convergence of the N+P sample at SH7 (circled point) with samples from farther downstream.

6 In-river periphyton

6.1 Methods

Regardless of growth rates and nutrient limitation, from a river management perspective the amount of periphyton growing on the river bed is the most relevant metric. Therefore during the course of the experiments described in Sections 4 and 5, we carried out fortnightly surveys of periphyton cover and biomass in the river at each site.

6.1.1 Survey locations

The surveys commenced on 6 January 2015, when 25 fixed survey points arranged along five transects were set out at each site. Each survey point was marked with a painted rock placed on the river bed. We favour fixed survey points for the following reasons: a) viewing the same area on every survey reduces variability in percentage cover estimates introduced by surveying different areas of river bed on each occasion; b) choice of viewing areas is not subjective and should be free of bias introduced by the operator; c) fixed survey points helps ensure that surveyed areas are not affected by trampling during the previous survey. Reason c) is especially important when surveys are conducted at closely spaced time intervals (e.g., up to 14 days).

Transects were defined from the bank by measuring the distance from a marker on the bank to each rock marker in the river. All the distances were recorded and subsequently included on the field sheets for each site. This meant that the positions of the transect rocks could be checked, and they could be moved back into position if they had shifted during flood events. All viewing points were in wadeable areas (up to about 0.7 m deep and water velocity up to 1.2 m/s).

At SH7, Balmoral and Gorge, the surveyed area transitioned from faster-flowing shallower water downstream to slower flowing water upstream. At Mandamus the reach was more uniform. The reaches surveyed generally met the definition of a run (i.e., areas with smoothly flowing water with variable water velocity), but we wanted to ensure that we covered a range of water velocities. It is standard practice in New Zealand to conduct periphyton surveys in runs.

The first survey in the programme was conducted on 5 January 2015. Surveys continued approximately every fortnight until 5 May 2015 (Figure 6-1). Fortnightly surveys are frequent enough to detect the effects of significant flow events between surveys.

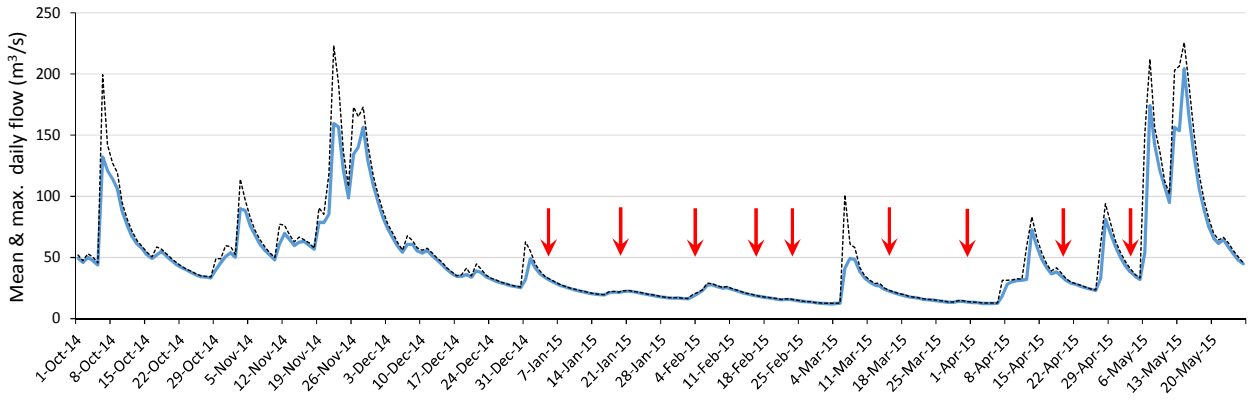

Figure 6-1: Times of periphyton cover surveys (red arrows) overplotted on the flow record for Hurunui @ Mandamus. Surveys commenced 6 weeks after a flood peak of about 200 m^3/s. The series was interrupted by three small flood events (maximum peak of 100 m^3/s).

6.1.2 Visual assessments of periphyton

Visual estimates were conducted through an underwater viewer (Nuova Rade, Genova, Italy) with a diameter of 350 mm. The glass viewing circle was marked into quarters to aid in estimates of percentage cover. All views were located so that the painted marker rock was just out of view at the middle of the downstream side of the area assessed. The size of the area viewed varied with depth, but the difference was small over the range of depths considered and this would not affect the estimates of mean cover. Percentage cover of periphyton within each view was estimated, usually to the nearest 5%, in nine categories. These were:

- No algae – rocks have no green/brown colour and are not slimy/slippery to touch;
- Film – rocks are slimy/slippery and have a visible coating of algae, < 1 mm thick;
- Sludge – loose, unconsolidated, non-filamentous algae often found in slower flowing areas (mostly mucilaginous diatoms, often mixed with fine sediment);
- Mats – more consolidated layers of algae from about 2 mm thick, mostly diatoms but also includes red algae;
- *Phormidium* – distinctive black, dark brown or greenish shiny or mottled mats;
- Didymo – characteristic thin to very thick, wool-like mats with whitish stalks underneath and brown cells at the surface;
- Fils_green – bright green filamentous algae, short or long filaments, sometimes overgrowing other algae;
- Fils_other – other filamentous algae, generally brown; includes filamentous diatoms and green algae with diatom epiphytes
- Macrophytes – vascular plants rooted in the river bed.

The raw data from 25 views were averaged to obtain mean percentage cover of each periphyton category on each survey. Percentage cover of the different algal categories were plotted and results compared across sites.

6.1.3 Physical measurements

Water velocity and depth were measured at each viewing point on three occasions (6 January, 4 February and 16 March 2015). These measurements allowed a comparison of physical conditions between sites (including within-site variability), to aid interpretation of differences in periphyton cover between and within sites. On two occasions we also assessed cover of the river bed by substrate particles of different sizes ranging from boulders (> 250 mm across, to sand and silt).

6.1.4 Periphyton biomass

At the time of each survey, from 20 January onwards, samples were collected to obtain an estimate of mean chlorophyll *a* at each site. Three to five rocks were collected randomly from each of three areas between the transects. The areas were between transects 1 and 2, just downstream of transect 3 and between transects 4 and 5. Periphyton was sampled from each rock by placing a lid of known diameter over an area with periphyton cover representative of the rock, scrubbing off all algae from around the lid, then brushing / scraping the algae from beneath the lid into a container. Samples

from rocks from each area were pooled into a single container, kept on ice, followed by storage at -20 °C until analysis. Samples were analysed for chlorophyll *a* and AFDM as described in Section 4.2.1.

6.1.5 Periphyton community composition

The visual assessments of % cover provided an approximation of community composition differences among sites. For a more detailed picture of community characteristic at each site, composite samples from each site were analysed using the method described in Section 5.1.3. Samples collected on 20 January, 16 February, 16 March, 1 April and 5 May covered communities at various stages of development following high flows.

6.1.6 Data analysis

The percentage cover and biomass data were first plotted over time to enable visualisation of differences among sites. The responses of periphyton at each site to flow fluctuations were assessed by plotting biomass and percentage change in biomass against the maximum flow preceding the survey. When high flows occurred we aimed to carry out the next survey as soon as possible, which was always less than nine days since the flood peak.

Water velocity and substrate composition were compared among sites to assess similarities and differences. Within sites, water velocity and substrate composition may strongly influence the distribution of periphyton cover over the river bed (Biggs and Hickey 1994). Such relationships are of particular interest for the nuisance taxa *Phormidium* and didymo, as part of understanding variation in cover by these taxa among sites and over time. Relationships between water velocity, substrate composition and % cover were examined on the four occasions water velocity was measured at each viewing point. For these analyses, substrate composition was summarised as % coarse bed material (boulders + large cobbles), and % fine material (% sand + gravel). Relationships were identified from correlation analyses of percentage cover by the different categories of periphyton against each physical variable.

The community composition count data were converted to % abundance in each sample. In addition, we calculated biovolume data by multiplying the counts by the average volume of cells in each taxon. These volumes were calculated from measurements of 10-12 cells of each taxon in the samples, using an ocular micrometer. The biovolumes of common algal taxa in rivers range from < 5 to >50,000 μm^3 per cell. Because very small cells can be extremely abundant compared to large cells biovolumes provide an alternative picture of the contribution of each taxon to the community. Community composition data are presented graphically. We also used NMDS and ANOSIM to examine differences between sites and over time (see Section 5.1.3 for details).

6.2 Results and discussion

6.2.1 Physical characteristics

Mean water depth at the viewing points on the transects varied among sites but was within a fairly small range as a result of limiting depth to 0.6 m. Mean water velocity was similar at SH7, Balmoral and Gorge (mean of about 0.6 m/s) but was lower at Mandamus (Table 6-1). Bed substrate composition at the four sites showed a typical pattern in rivers of progressive downstream fining (Table 6-1). The high proportion of boulders on the river bed at Mandamus led to distinctive habitat conditions at that site compared to the three site farther downstream. Although mid water-column water velocities could be high at Mandamus, velocity at the river bed was frequently low because of the sheltering effect of the large substrate particles (see Section 4.3.3).

Table 6-1: Mean water depth and velocity and substrate composition measured at the four monitoring sites. Means were calculated from measurements on four (depth and velocity) and two (substrate) occasions. Water velocity was measured at 0.6 depth (i.e., just above midway up the water column).

	Water depth (cm)		Water velocity (m)		Bed substrate composition (%)					
Site	Mean	s.d.	Mean	s.d.	Boulders (> 25 cm)	Large cobbles (12–25 cm)	Small cobbles (6–12 cm)	Gravel (0.2–6 cm)	Sand (<0.2 cm)	Silt
Mandamus	27.3	12.2	0.41	0.27	62.3	11.5	8.4	12.5	5.2	0.1
SH7	27.3	5.0	0.63	0.20	17.2	22.7	25.5	32.7	1.8	0.1
Balmoral	26.7	11.6	0.57	0.26	8.4	28.5	33.2	28.6	1.2	0.0
Gorge	34.0	11.6	0.58	0.25	8.1	23.6	21.6	30.1	16.6	0.0

6.2.2 Periphyton cover and biomass over time

The first survey was carried out about 6 weeks after a 200 m^3/s flood in late November. By this time, total cover by periphyton excluding thin films exceeded 20% at Mandamus and SH7, and 40% at Gorge, but was still low at Balmoral. Maximum cover occurred in late January/early February at Mandamus, SH7 and Gorge. There was a second peak in cover at the Gorge in early April, and this coincided with peak cover at Balmoral. Cover was stable at Mandamus and SH7 from mid-February to May. On the final survey on 5 May, total % cover by periphyton other than thin films was less than 10% at the Gorge and Balmoral, but more than 25% at SH7 and Mandamus (Figure 6-2).

Periphyton cover at Balmoral and Gorge was better reflected by biomass measured as chlorophyll *a* than ADFM (Figure 6-3). Chlorophyll *a* was highest at Gorge on most survey dates. Between January and 24 February mean chlorophyll *a* at the Gorge increased to a maximum of 250 mg/m^2 before most cover was removed by the high flow event on 6 March (maximum flow of 80 m^3/s at SH1). A second peak of 158 mg/m^2 was recorded on 1 April before removal by the 13 April flow event. At Balmoral, chlorophyll *a* reflected the cover estimates, with highest biomass in April (Figure 6-3a). Cover at Mandamus and SH7 was best represented by biomass measured as AFDM. AFDM was higher at SH7 than at all other sites except on 20 January and 5 May (Figure 6-3b).

Changes in the relationship between biomass and cover along the river were reflected in changes in broad community composition as detected by the visual assessments. The dominant cover at Mandamus was didymo mats. Our field observations indicated that the didymo mats recorded were not healthy (i.e., the mats appeared to be mostly stalk material with few cells). As the season progressed, these mats became more “sludge” like. The high flows in March and April had little effect on the initially low cover. Change in cover over time at Mandamus also reflected declining water level, and the addition of viewing areas farther out into the river than the areas defined on 5 January. These observations were consistent with low chlorophyll *a* concentrations for the whole season. The slight increase in chlorophyll *a* on the final sampling occasion (May) was caused by higher cover of periphyton mats that were not didymo.

At SH7, cover was predominantly didymo, though low cover by *Phormidium* was observed during all surveys. *Phormidium* occurred only on the downstream transects, which had faster water velocities than upstream (see below). Didymo at SH7 comprised healthy-looking mats with increasing cover up to mid-February and reduced cover following the high flow event on 6 March.

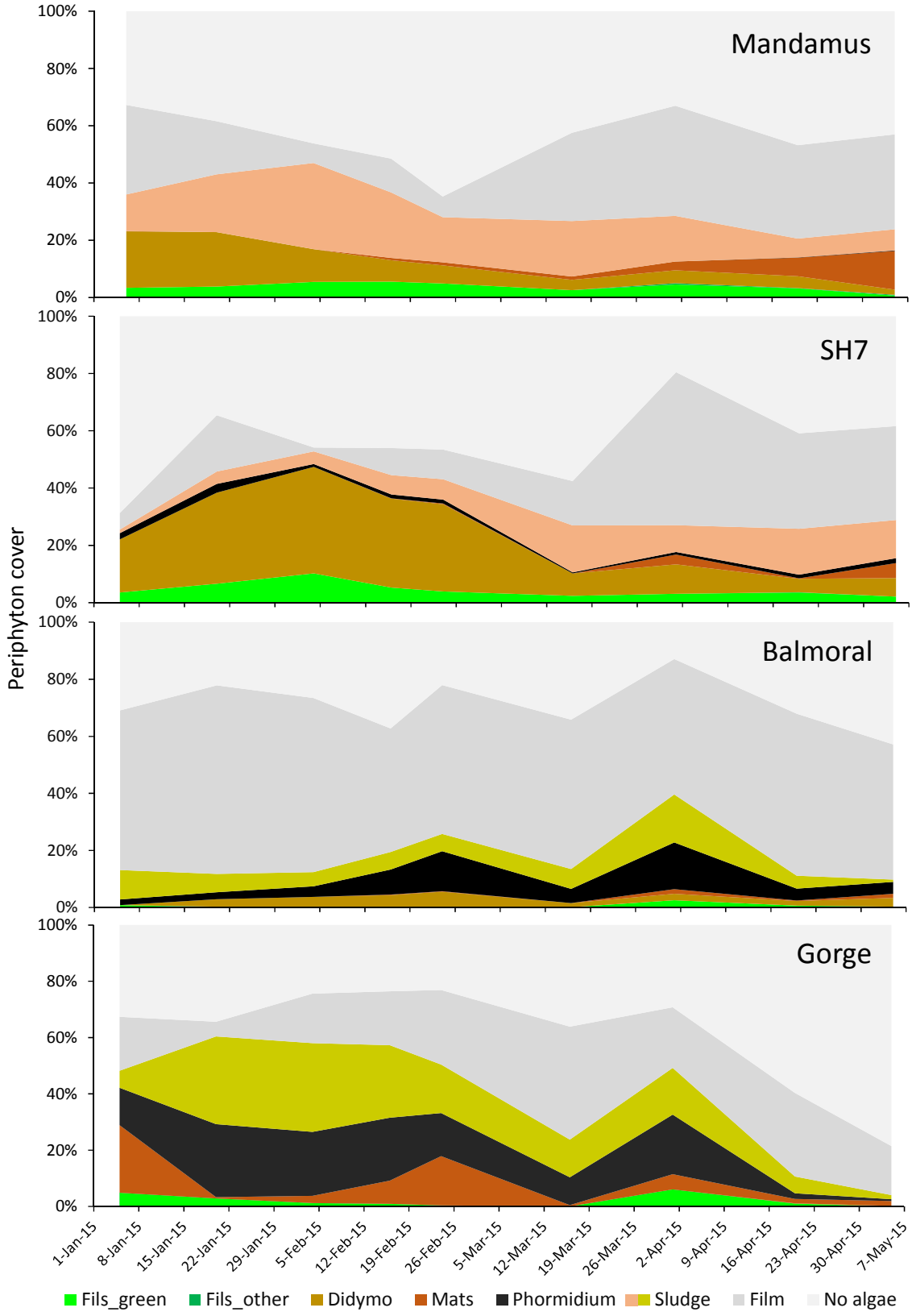

Figure 6-2: Mean percentage cover of periphyton in eight categories recorded at four sites in the Hurunui River, January to May 2015. Percentage cover was the mean of visual estimates of cover in 25 views at each site. The category "sludge" is shown as two different colours because at Mandamus / SH7 sludge was mainly dead and loose didymo, and at Balmoral / Gorge sludge was mainly remnants of *Phormidium* mats and other loose brown algal material.

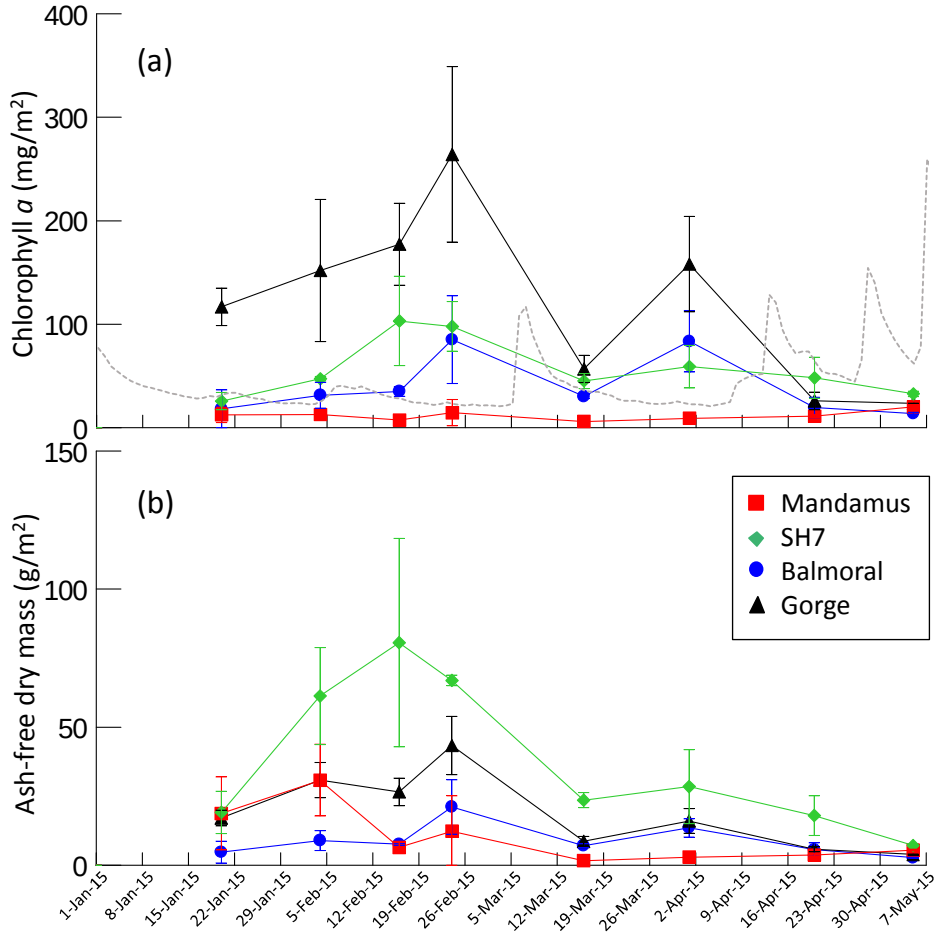

Figure 6-3: Mean chlorophyll *a* (a) and ash-free dry mass (b) determined from periphyton samples collected in the survey areas at each site. The shape of the hydrograph (Hurunui at Mandamus) is overplotted in grey on (a) to show the timing of high flows during the survey period. Error bars are standard errors.

Cover at Balmoral was predominantly thin film for all surveys, but low cover by didymo, as scattered small colonies, was present throughout the season. Declining water levels through January and February left some of the fixed viewing areas defined on 5 January exposed to air. Therefore new viewing areas were defined farther into the river, in faster flowing water. Higher cover by *Phormidium* was observed in these new views. However, *Phormidium* cover was also observed to be increasing within the original views. For example, cover increased from 10% to 25% on view 10 between 5 January and 24 February. Therefore *Phormidium* cover of up to 15% was attributable to increasing cover between surveys as well as recording new areas with *Phormidium.*

At the Gorge, the dominant cover visible was *Phormidium*. Cover reported as "sludge" was often the loose remains of *Phormidium* mats that had peeled off the substratum. During the flood-free period in January and February, we noted a pattern of development of *Phormidium* mats from initial cover by reddish-brown mats of mats other algae, followed by a decline in *Phormidium* cover (presumably through natural sloughing) and re-establishment of the brown mats.

Cover by green filamentous algae was recorded at all four sites, although the type of green alga present varied from site to site (see Section 6.2.5.).

6.2.3 Periphyton in relation to river flows

Three significant high flow events occurred during the survey period (see Section 3.3.1), and the surveys were preceded by a minor fresh on 1 January (peak flow of 63 and 57 m^3/s at Mandamus and SH1 respectively). The general effects of the high flows are seen in the time series plots (Figure 6-2, Figure 6-3) but the effects of different flow events and variations between sites are clearer when biomass and biomass changes are plotted directly against maximum flows in the period between surveys (Figure 6-4).

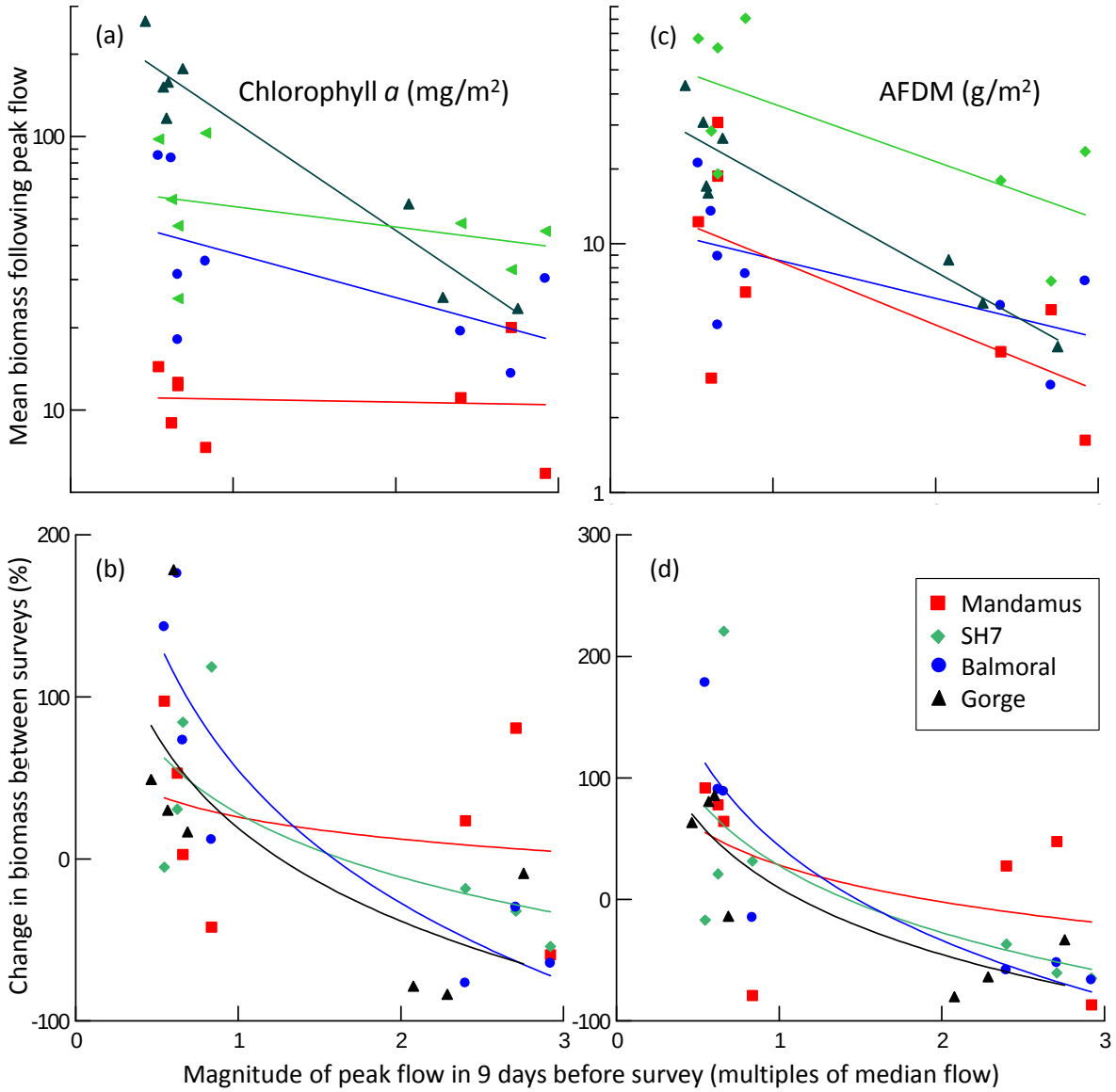

Figure 6-4: Periphyton biomass (a, c) and % change in periphyon biomass (b, d) plotted against peak flow in the 9 days prior to each survey. Peak flow is shown as multiples of the median flow. Flows from Hurunui at Mandamus were used for Mandamus, SH7 and Balmoral; flows from Hurunui at SH1 were used for Gorge. Biomass as (a) chlorophyll *a* and (b) AFDM are plotted on a $\log_{10}$ scale to show the log-linear relationship with 9-day peak flow. Log smoothing lines are fitted on (b) and (d).

Removal of chlorophyll *a* by floods was more effective at Gorge than at the other three sites. Chlorophyll *a* attained high concentrations after low flows, but was consistently lower following high flows, in proportion to the size of the preceding peak flow (Figure 6-4a). For management purposes, a relevant question is "what size flow would be required to remove all periphyton?" Technically this cannot be predicted because it requires extrapolation beyond the range of the data on the plot. However, with the caveats that (a) this is an estimate only, and (b) this applies only to the period of data collection, extrapolating the fitted line on Figure 6-4a to attain chlorophyll *a* of 10 mg/m^2 (equivalent to 100% cover by thin films, Kilroy et al. 2013), peak flow in the past 9 days would have been approximately **3.7 x median flow.**

The relative slopes of the fitted lines on Figure 6-4a indicate that flood removal of periphyton chlorophyll *a* was next most effective at Balmoral. Shallow slopes of the relationships at SH7 and Mandamus show that over the range of flows experienced from January to May 2015, chlorophyll *a* reduction following high flows was low at SH7 and there was no reduction at Mandamus (albeit from very low starting levels).

Plots of percentage change in chlorophyll *a* against 9-day peak flow highlight lower percentage removal and also lower maximum accrual in low flows (left hand side of the plot) at Mandamus and SH7 compared to Balmoral and Gorge (Figure 6-4b). This plot also shows that there could be considerable accrual between successive surveys. For example, biomass at Balmoral and Gorge increased by almost 200% of the initial value (i.e., almost tripling the biomass) between one pair of successive surveys on 16 March and 1 April.

In the same way as chlorophyll *a*, AFDM at Gorge (black plot on Figure 6-4c) can be extrapolated, as above, to determine the flood size required to attain a very low AFDM of 2 g/m^2. The required peak flow in the last 9 days was again 3.7 x median flow. The same result for chlorophyll *a* and AFDM confirms that the two biomass measures were equivalent at Gorge (see Section 8.6). Despite discrepancies between chlorophyll *a* and AFDM at Mandamus and SH7 (compare Figure 6-4a,c), the pattern of accrual and removal of AFDM across sites was similar to that for chlorophyll *a* (compare Figure 6-4b and d).

The plots in Figure 6-5 summarise the data shown in Figure 6-2 in a different way, emphasising differences in cover by various periphyton categories linked to changes in preceding flows rather than changes over time. Patterns include:

- a general decline in cover by filamentous green algae as preceding peak flows increased (except at Mandamus);
- increases in coverage by sludge following high flows at Mandamus and SH7, because sludge includes remnants of didymo;
- generally low cover by didymo at SH7 following high flows, although some cover remains;
- low cover by all categories of cover at Gorge following the largest flood size, compared to significant cover remaining at Balmoral following an equivalent event.

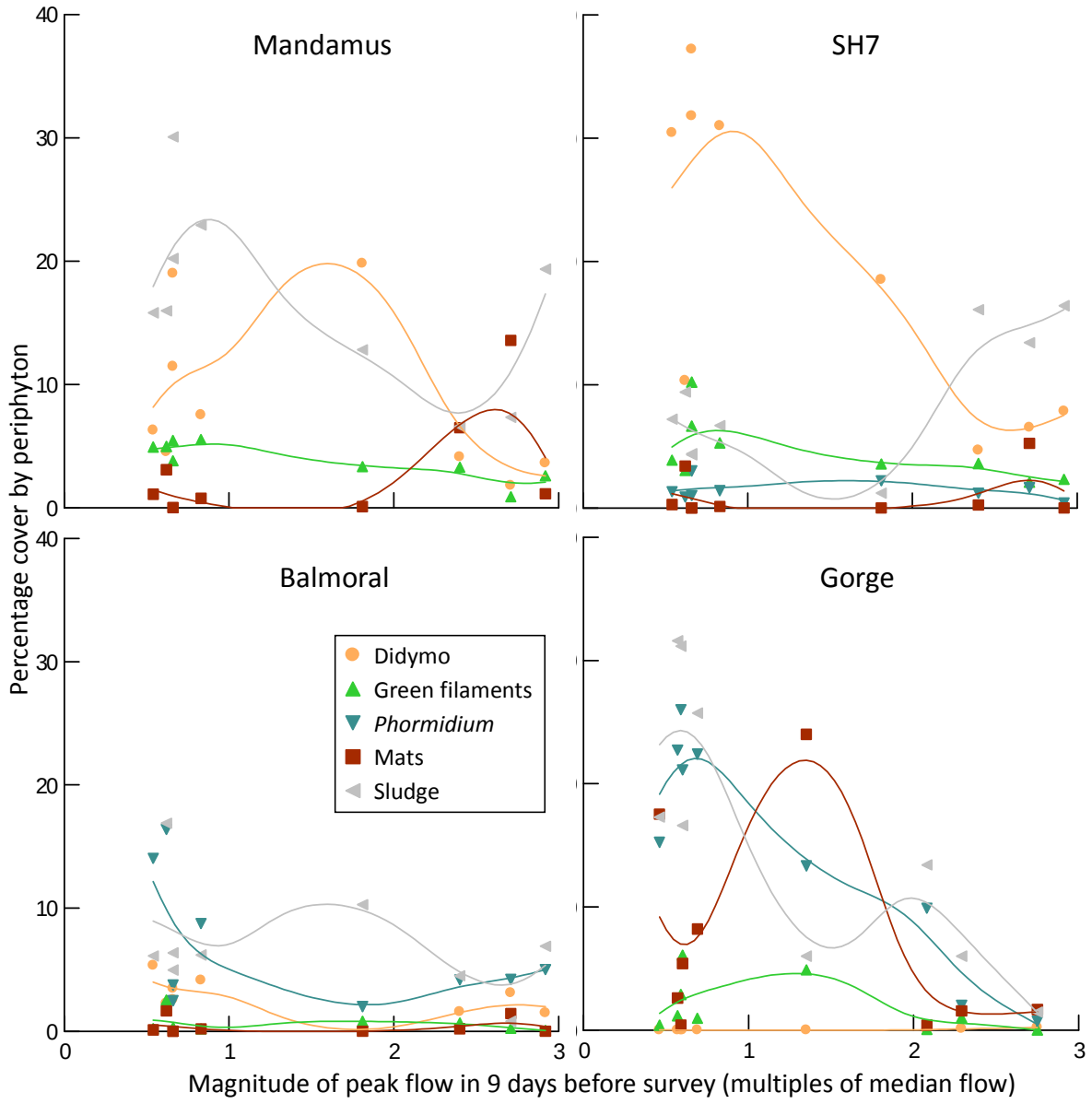

Figure 6-5: Percentage cover by different categories of periphyton cover plotted against peak flow in the 9 days prior to each survey. Peak flow is shown as multiples of the median flow. Flows from Hurunui at Mandamus were used for Mandamus, SH7 and Balmoral; flows from Hurunui at SH1 were used for Gorge. DWLS smoothing lines are fitted for each cover category, with tension of 0.5 (refer to Figure 3-3 for explanation).

6.2.4 Comparison with guidelines

Compliance of each site with a range of periphyton guidelines is summarised in Table 6-2. Chlorophyll *a* at Gorge exceeded the HWRRP threshold of 120 mg/m^2 on four of the eight surveys. The threshold was not exceeded at Mandamus, SH7 or Balmoral. The HWRRP threshold for cover by filamentous green algae (20%) was not exceeded at any site. Compliance with the HWRRP guidelines cannot be determined without more data. The wording of the HWRRP periphyton guideline for the Hurunui is:

> "The 95th percentile of monthly periphyton biomass measurements in the mainstem of the Hurunui River shall not exceed 120 mg/m^2 chlorophyll *a* or 20% cover of filamentous algae more than 2 centimetres long."

Because the spatial context for applying the guideline is the "mainstem of the Hurunui River", compliance with the guideline could be interpreted in different ways. Normally, guidelines are tested by selecting representative sites, then conducting a regular monitoring programme at those sites, and extrapolating the results to the wider area represented by the site. Thus, in the National Policy Statement for Freshwater Management (NPS-FM, NZ Government 2014) the approach is to require councils to identify Freshwater Management Units, defined as: "the water body, multiple water bodies or any part of a water body determined by the regional council as the appropriate spatial scale for setting freshwater objectives and limits and for freshwater accounting and management purposes".

Table 6-2: Summary of compliance of four sites on the Hurunui River to guidelines for periphyton, set to protect a range of instream values. The value relevant to the guideline is shown for each site. If the guideline was exceeded/breached more than once, the maximum value is shown, with the number of breaches in parentheses. Green cells indicate that the guideline was met; red cells indicate a breach; pink cell indicate where a nominal breach occurred, but the guideline requires a longer time series to confirm the breach.

				Hurunui River, January – May 2015			
Guideline for protection of:	**Periphyton metric**	**Threshold**	**units**	**Mandamus**	**SH7**	**Balmoral**	**Gorge**
HWRRP (Environment Canterbury 2013)							
Ecological values	95th percentile, monthly cover by filaments	20	%	5.5	10.2	2.5	6
Hurunui River main stem	95th percentile, monthly chlorophyll *a*	120	mg/m²	20.1	103	85.0	264 (4)
New Zealand Periphyton Guideline (Biggs 2000)							
Aesthetics / recreation values	Max. cover, mats	60	%	41.5	42.6	37.2	57.6
	Max. cover, filaments	30	%	5.5	10.2	2.5	6
	Max. AFDM	35	g/m²	30.7	80.6 (3)	21	43.3
	Max. chlorophyll *a*	120	mg/m²	20.1	103	85.0	264 (4)
Benthic biodiversity	Mean monthly chl *a*	15	mg/m²	11.5	57.3	39.5	122
Trout habitat/angling	Max. chlorophyll *a*	50	mg/m²	20.1	103	85.0	264 (4)
	Max. AFDM	35	g/m²	30.7	80.6 (3)	21	43.3
	Max. chl *a* (mats)	200	mg/m²	20.1	103	85.0	264 (1)
	Max. chl *a* (filaments)	120	mg/m²	20.1	103	85.0	264 (4)
New Zealand Guideline for Cyanobacteria (Wood et al. 2009)							
Human/animal health: alert	Max. cover, *Phormid.*	20	%	0.2	2.2	16.4	22.7
Human/animal health: action	Max. cover, *Phormid.*	50	%	0.2	2.2	16.4	22.7
Periphyton NOF, NPS-FM (NZ Government 2014)							
Ecosystem health of rivers							
Band A, negligible impact	> 8% exceedance (1 of 12), chlorophyll *a* Based on monthly samples, with min. data series of 3 years	<50	mg/m²	9 of 9	6 of 9	7 of 9	3 of 9
Band B, low impact		50–120	mg/m²	0 of 9	3 of 9	2 of 9	6 of 9
Band C, moderate impact		120–200	mg/m²	0 of 9	0 of 9	0 of 9	4 of 9
Band D, below "bottom line"		>200	mg/m²	0 of 9	0 of 9	0 of 9	1 of 9

As it stands, the HWRRP periphyton guideline could be interpreted as applying to average conditions in the entire ~100 km from Mandamus to SH1. However, it seems more likely that the intention of the guideline was that it should be applied to representative sites in different parts of the river, corresponding to known nutrient concentration differences. In NPS-FM terms, the river would

comprise several freshwater management units, each to be monitored separately. The present summer monitoring programme indicated potential for a breach of the guideline at Gorge, but not at the three upstream sites. It should be remembered that the 2015 study was conducted during a period of unusually low flows in the lower Hurunui (represented by Gorge). Strong dependence of periphyton on flows at this site could translate to lower overall biomass in years with "normal" flows.

In terms of other guidelines, chlorophyll *a* at Balmoral and SH7 exceeded 50 mg/m^2 on two and three occasions, respectively. This concentration is the guideline for maintenance of instream biodiversity (based on relationships between stream invertebrates and periphyton chlorophyll *a*) in the 2000 MfE guideline (Biggs 2000). The Biggs (2000) guideline also included a threshold of 35 g/m^2 of ash-free dry mass for maintenance of trout habitat and angling values in rivers. That threshold was breached three times at SH7 and once at Gorge.

The threshold value defining the periphyton NOF bottom line of 200 mg/m^2 was exceeded once at Gorge, in mid-February. This cannot be interpreted as a breach of the bottom line because the metric is > 8% of exceedances, based on monthly monitoring over a time series of at least 3 years. However, it provides a warning that there is potential to breach the national bottom line at Gorge.

6.2.5 Within-site patterns in periphyton cover

Scatter plots of percentage cover versus water velocity and substrate composition showed site-specific patterns. At Mandamus, there tended to be higher cover by thin films in faster water velocities. Sludge at Mandamus, Balmoral and Gorge was concentrated in slower water velocities near the water's edge. At SH7, cover by didymo was highest on coarse substrate, and the low cover by *Phormidium* at that site was concentrated in higher water velocities (Figure 6-6).

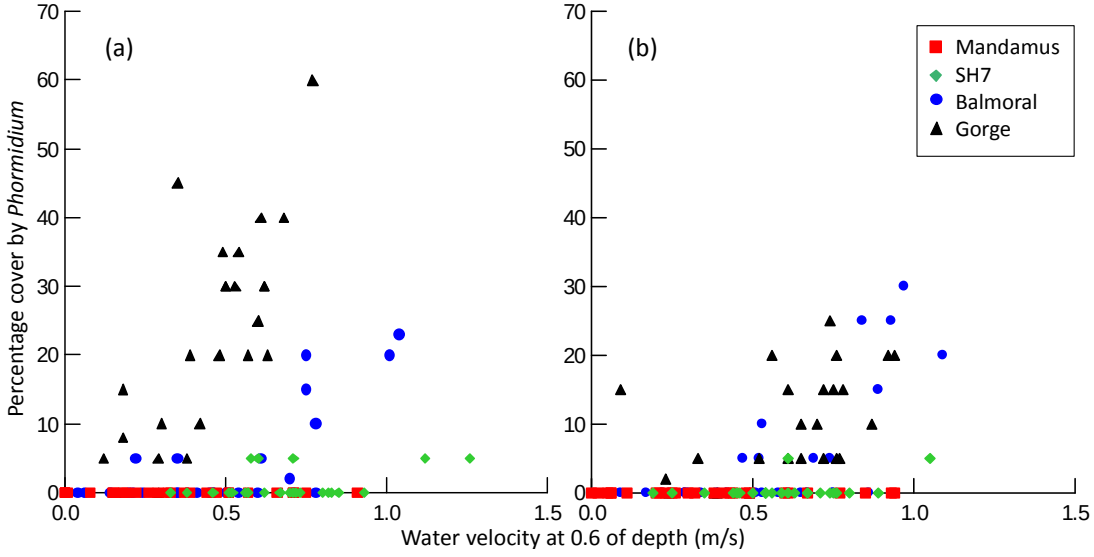

Figure 6-6: Percentage cover by *Phormidium* vs. water velocity at each view on (a) 4 February and (b) 16 March. Note the positive relationship between % cover and water velocity at Balmoral and Gorge.

At both Balmoral and Gorge, the clearest pattern was a positive correlation between *Phormidium* cover and water velocity. When *Phormidium* cover was high at Balmoral and Gorge on 4 February, water velocity explained 40% and 56%, respectively, of the variation in *Phormidium* cover. The relationship differed between sites, with *Phormidium* at Balmoral occurring in mainly higher water

velocities than the range of water velocity recorded at Gorge (Figure 6-6a). On 16 March, *Phormidium* cover was lower following the high flows on 5-10 March. The positive relationship between cover and water velocity was still clear, but the relationship did not differ between sites (Figure 6-6b). This pattern may be a result of the subsidy effect discussed in Section 4.3.3, in which high water velocities permit faster delivery of nutrients to cells, thereby enhancing growth.

6.2.6 Periphyton community composition

A total of 67 periphyton taxa was identified from composite samples collected monthly at each site, of which 51 were diatoms. Refer to Appendix C for a full list of taxa. Sixteen of the taxa accounted for at least 83% of the community at the four sites (by cell count), and their proportions at each site differed. The dominant taxon (percentage abundance, by cell count) at Mandamus was the small filamentous diatom *Diatoma tenuis*, at SH7 *Achnanthidium minutissimum* was dominant, and *Phormidium* dominated at both Balmoral and Gorge (Figure 6-7).

Of the green algae, only *Stigeoclonium* was included in the 16 most common taxa by cell densities. Because cell size of green filamentous algae is large compared to many diatoms green algae were much more prominent in the community than suggested by Figure 6-7; this is illustrated in Figure 6-8, which shows proportions of the 10 most common taxa when determined using biovolume.

Taxa characteristic of Mandamus were the cyanobacterium *Calothrix* and the red filamentous alga, *Audouinella* (Figure 6-7). The green filamentous algae *Cladophora* and *Oedogonium* were conspicuous in the samples and dominated cell biovolume (Figure 6-8). The communities at SH7 were visibly dominated by didymo cells and stalks. Using count data didymo was the seventh most abundant taxon, but because didymo cells are so large, they dominated community biovolume (Figure 6-8).

Phormidium was numerically dominant at Balmoral, but didymo cells occupied most biovolume. Balmoral shared several other taxa with the two upstream sites (Figure 6-7, Figure 6-8). At the Gorge, *Phormidium* was the dominant taxon by cell density and biovolume. Here, the diatoms *Rossithidium linearis* and *Cymbella kappii* were also abundant in all samples. These two taxa were rare at other three sites. The filamentous green alga *Stigeoclonium* was most abundant at Gorge. *Stigeoclonium* forms proliferations in high nutrient waters (John et al. 2000), possibly in response to both N and P.

The question arises: what was driving the downriver community differences: gradients in nitrogen, phosphorus, both N and P, or some other factor, or combination of factors? For some individual taxa, the analyses of community composition on the nutrient diffusing assays provide clues (Section 5.2.3). For example, the stalked diatom *Gomphoneis* showed a consistent positive response to P additions in cell densities, and also a gradient in abundance going downriver (Figure 6-8). From this we infer that slight P enrichment favours *Gomphoneis*. This taxon can form large proliferations that have been mistaken for didymo. However the blooms tend to be more transient than those of didymo and, to our knowledge, have never been considered to be a “nuisance”.

The mucilage-producing diatom *Cymbella kappii* showed a different pattern in that it grew on all NDS assay treatments at Balmoral and Gorge, but was rare at the two upstream sites, and the pattern was the same in the river samples. This is more suggestive of a positive response to the downriver gradient of N in the Hurunui River, as well as to P. Between-species interactions may also play a part. For example, *C. kappii* was abundant in the Lower Waiau River, Southland, up to the arrival of didymo in the river in 2004. Since then, the periphyton has been dominated by didymo, and *C. kappii* is rarely encountered in samples (Kilroy et al. 2009).

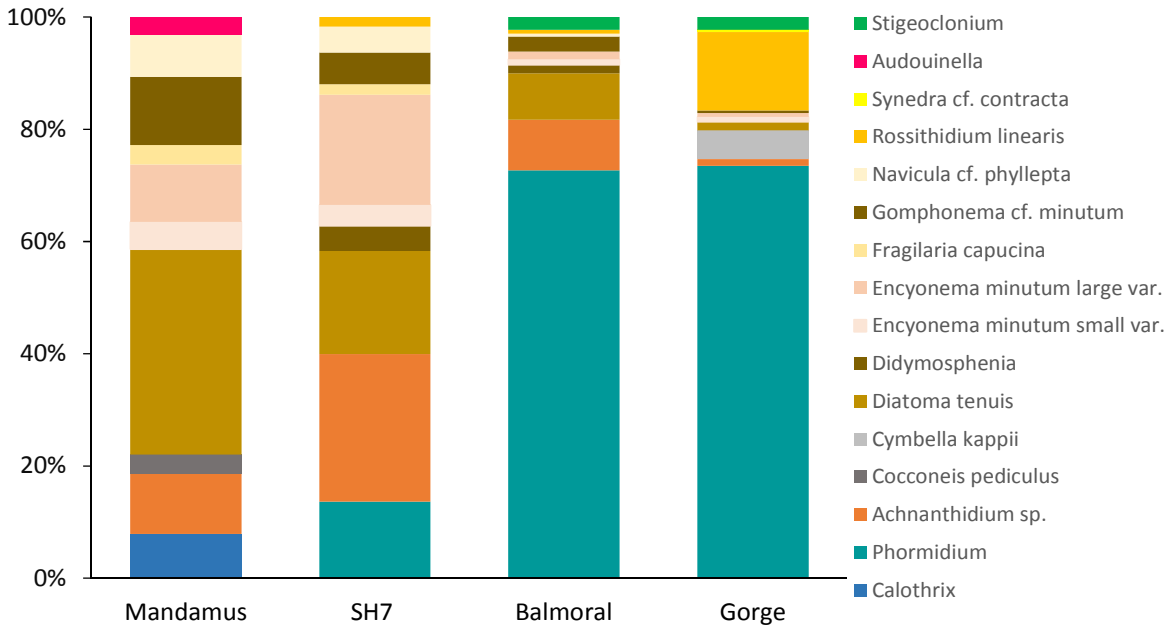

Figure 6-7: Relative abundance (%) of the 16 most common taxa by cell counts in composite samples from the river bed at each site. Each bar shows average relative abundances over the whole season (monthly samples from January to May, n = 5). Green shading indicates green algae; brown, yellow, orange and grey shading indicate diatoms; teal and blue shading indicate Cyanobacteria.

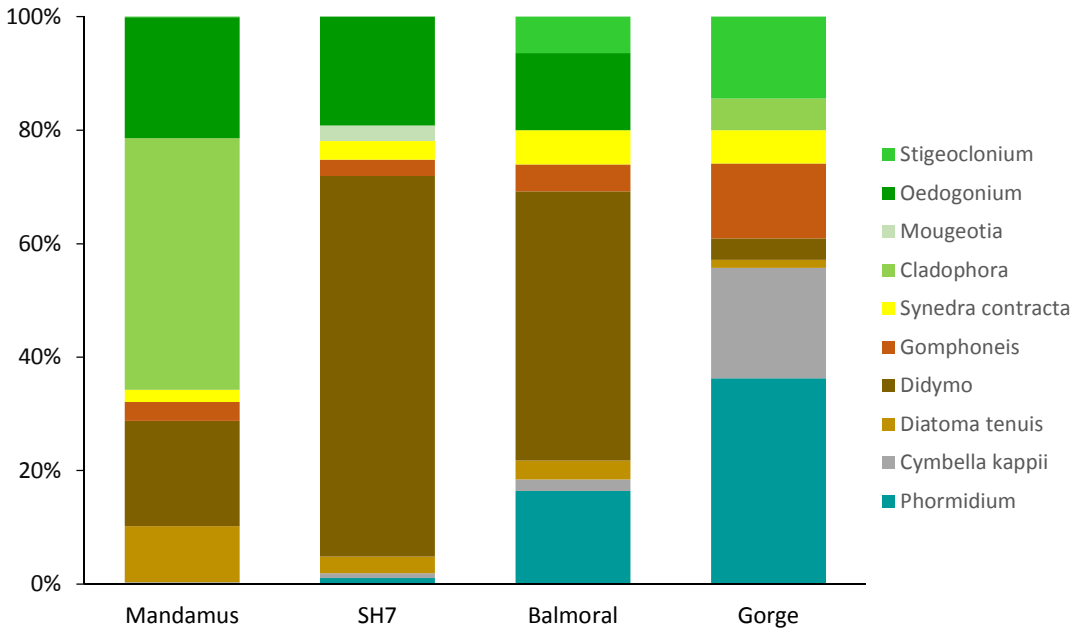

Figure 6-8: Relative abundance (%) of the 10 most common taxa by biovolume in composite samples from the river bed at each site. Refer to Figure 6-7 for explanatory notes.

Nutrient – abundance relationships are not necessarily linear. An example may be the small mucilage-rich diatom taxon *Encyonema minutum*. No clear patterns were discernible from the NDS or river data, except that in-river abundance was highest at SH7. High densities were observed in both N and P treatments on the NDS. In recent experiments in the Ohau River *E. minutum* cell

densities responded positively to slight N-enrichment (unpublished NIWA data), and the taxon is generally considered to indicate mesotrophic conditions (Kelly et al. 2008). Further taxonomic analyses (e.g., from NDS) may be informative.

In summary, both cell densities and biovolumes showed strong downriver turnover in periphyton community composition. Communities at Mandamus shared only a few taxa with those at Gorge. This gradient was reflected in the multivariate analysis of the data (Figure 6-9a). Communities at each site retained their distinctive character over the whole season and differed significantly from one another (ANOSIM, $P < 0.01$ for all pairwise comparisons). Community composition varied from survey to survey, but shifts over time occurred within sites, not in the whole river (Figure 6-9b).

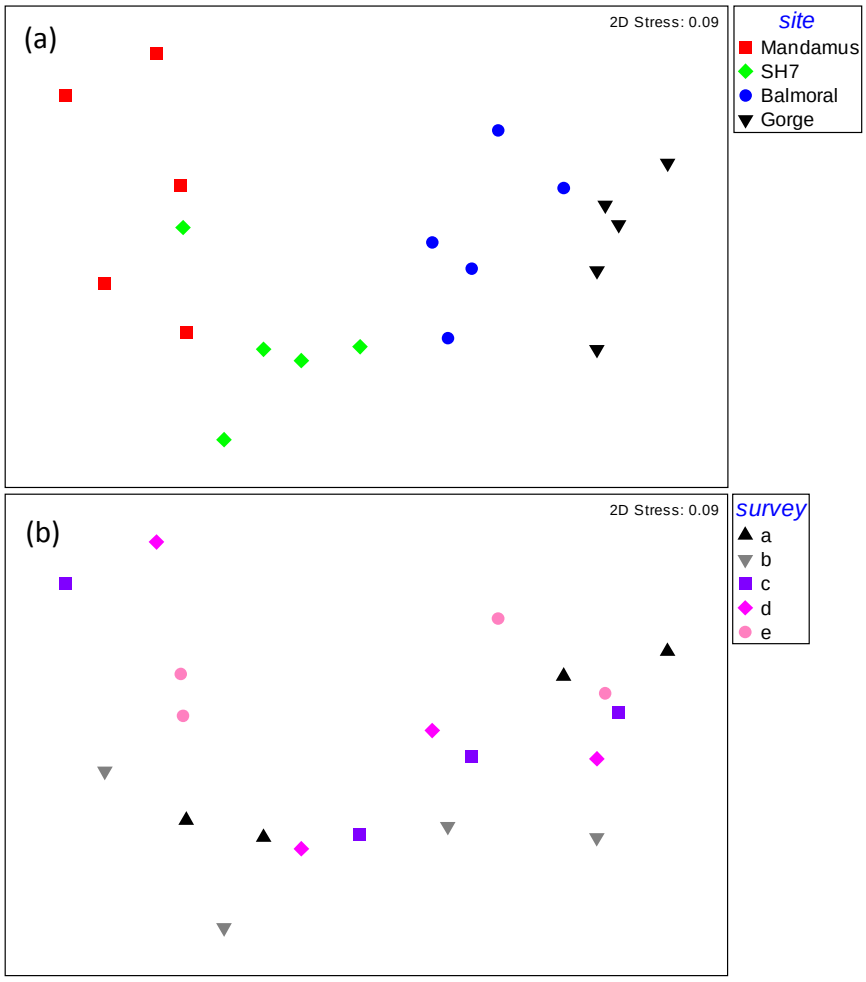

Figure 6-9: Non-metric multi-dimensional scaling plots of periphyton community composition in samples from the river bed at four sites in the Hurunui River. Each data point represents one sample with multiple taxa. Data are relative abundance by cell density, square-root-transformed to downweight the effect of very common taxa. The similarity of samples is indicated by how close together they are on the plots. Plot (a) shows that the samples from each site cluster together with a composition shift down the river. In (b) samples are separated by survey, with a to e representing January to May.

7 Alternative source of phosphorus

7.1 Background

The results of the surveys in summer 2015, and previous surveys by ECan, indicated high periphyton cover and biomass in the lower reaches of the Hurunui River, which from time to time breached the standards set in the HWRRP and other national standards (Table 6-2). The periphyton taxon of most concern is the cyanobacterium *Phormidium*. *Phormidium* is widespread and common in New Zealand Rivers (Biggs and Kilroy 2000) and mats have been observed in a wide range of habitats ranging from pristine headwater streams to low enriched lowland rivers. *Phormidium* has emerged as a serious nuisance alga over the past decade because this cyanobacterium produces neurotoxins that are harmful to mammals (Heath et al. 2011). Guidelines for monitoring and reporting *Phormidium* cover in rivers, especially at locations popular for recreation, were released in 2008 (Wood et al. 2008).

The drivers of *Phormidium* proliferations in rivers are under investigation and the consensus to date is that proliferations are favoured by long periods of low, steady flows, relatively warm water temperatures, and elevated concentrations of nitrogen (Heath et al. 2011, 2015). The distribution of *Phormidium* does not appear to be correlated with concentrations of P in the water column, although TP concentrations are the best predictor of cyanobacterial blooms in lakes (Downing et al. 2001). Recent research by the Cawthron Institute has demonstrated that conditions may exist within *Phormidium* mats that allow this cyanobacterium to acquire P from fine sediment (Wood et al. 2014). If *Phormidium* can utilise the phosphorus adsorbed onto fine sediment (which, under most circumstances, is unavailable to periphyton) then the presence of sufficient P adsorbed onto fine sediment in the Hurunui River could lead to maintenance of *Phormidium* proliferations, even when DRP concentrations in the water column are very low. Acquisition of P by periphyton from fine particles has also been demonstrated in the Manawatu River. Periphyton was shown to be benefitting from P bound to particles discharged from a wastewater treatment plant, after removal of DRP from the discharge (Hamill 2013). DRP release from sediment can be mediated by high pH within and around periphyton mats (Eckert et al. 1997, and see Appendix A in Gibbs and Norton 2013).

The implication of a potential link between *Phormidium* proliferations and sediment-sourced P is that the HWRRP focus on managing DRP concentrations and loads in the Hurunui River may not achieve the desired outcome of minimising *Phormidium* blooms. We therefore carried out a preliminary investigation at the four study sites in the Hurunui River to determine whether *Phormidum* proliferations may be favoured in the lower reaches by higher availability of P from sediment sources. We included this study in the project following a review of the project proposal by Dr Susie Wood, Cawthron Institute, who suggested that an investigation into sediment-sourced P would be a useful addition to the study.

7.2 Methods

Our investigation comprised collection of sediment samples, determination of the proportion of fine material (< 63 µm) at each site, and analysis of selected samples for various forms of P. To allow comparison with previous research, we followed procedures described by Wood et al. (2008). We collected sediment using sediment traps and from direct collection of resuspendible material from the stream bed.

7.2.1 Sediment traps

Sediment traps comprise open containers filled with clean coarse sediment (gravel or small cobbles) which are buried in the river bed, flush with the surface, and left for a known time. During deployment, suspended material from the water column becomes incorporated into the clean gravel matrix in the container, and can be quantified at the end of the deployment. Traps were deployed at each site on 10 February and collected on 16 February.

Each trap was a lidded 2.3 L plastic pail 150 mm high with a 160 mm-diameter opening. Dry pebbles (diameter >10 – 40 mm) were sourced from the river bank at each site and were thoroughly washed and scrubbed in river water to remove all loose material. Each pail was filled to the brim with clean pebbles and the lid secured. The pails were positioned in depressions excavated in the river bed at each site, in areas with similar water depth and velocity (targets of 0.25 – 0.4 m and 0.7 m/s, respectively) where *Phormidium* cover was present. Once the pails were in position, the lids were removed carefully. Painted rocks were used to mark the location of each trap (Figure 7-1).

Figure 7-1: Sediment trap *in situ* in the Hurunui River. The red-painted rocks marked trap locations.

Before removal on 16 February, the lids were replaced on each pail for transport to the laboratory. In the laboratory, all the initial pebbles (i.e., > 10 mm diameter) were removed from the pails and rinsed in minimal water into a collecting tray, along with all remaining trapped sediment. The trapped material was separated into five size fractions (>500, 250-500, 125-250, 63-125, and <63 µm) through a wet sieving tower. The smallest fraction was allowed to settle out in a bucket for 1-2 days before the overlying water was siphoned off. Each fraction was dried at 105 °C to a constant weight and then weighed. Weights were converted to a settling rate (g/day).

Samples from the three finest fractions were analysed for P content at three levels of bioavailability. For this analysis we followed the method used by Wood et al. (2014), which was based on procedures described by Lukkari et al. (2007). Briefly, we followed the first three steps in the five-step procedure described by Lukkari et al. (2007). For each sample we started with about 0.5 g of dry sediment (weighed to the nearest 0.1 mg). In step 1, loosely adsorbed P was extracted in 0.46 M NaCl. In step 2, the residue from the first step was extracted in 0.11 M sodium dithionate ($Na_2S_2O_4$) in

0.11 M sodium bicarbonate ($NaHCO_3$) buffer (pH 7) to remove the redox-sensitive fraction of P bound to hydrated oxides (mostly Fe). In step 3, the residue from step 2 was extracted in 0.1 M NaOH which solubilises P bound to Fe and Al oxides, as well as some organic P. Extractions were carried out at room temperature, on an orbital shaker table (400 rpm). After each extraction and rinsing step, samples were centrifuged at 4000 rpm before pouring off the supernatant, which was then filtered through 0.45 µm cellulose acetate filters prior to analysis for dissolved reactive phosphorus (DRP). After the step 2 extraction, an additional procedure was to bubble compressed air through the solution remove sodium dithionate, which interferes with the process for analysing DRP.

7.2.2 Resuspendible sediment

Samples of resuspendible material were collected from SH7, Balmoral and Gorge on 10 February, using the Quorer method (Clapcott et al. 2011). Coarse substrate precluded collection of Quorer samples at Mandamus. The Quorer method provides a sample of fine sediment representing the material deposited in the river during the most recent bed-disturbing flood. We sampled areas with water depth of 0.25 to 0.35 m, and water velocity of ~0.5 m/s. Algae was cleared from the area of river bed to be sampled, and a 0.6-m high pipe with a diameter of 0.3 m was driven in to the bed. The bed material at the bottom of the pipe was stirred to a depth of about 10 cm using a metal spatula, for 15 seconds. A 1-litre sample of the water within the pipe was collected immediately after stirring. Stirred water depth was measured at five points to enable calculation of total water volume within the pipe. Three or four areas were sampled per site to provide site averages.

In the laboratory, dry weights of suspended sediment in five size fractions was determined as for the sediment trap samples. The weights were converted to weights per unit area of river bed by multiplying up the volume of the sample to the water volume within the pipe and the area of river bed enclosed by the pipe. The original intention was to determine P content in the Quorer samples. However, this was not possible because the samples were too small.

7.3 Results and discussion

Sediment from three traps was analysed at each site except at Mandamus, where only two traps were deployed because of lack of suitable locations among the coarse substrate. The largest fraction (>500 µm) is excluded from the following discussion because some samples contained many fragments of didymo that could not be separated from the inorganic part of the sample.

Flow in the Hurunui River was receding throughout the 6-day sediment trap deployment. Therefore the results reflect “baseline” sediment movement rather than sediment mobilisation caused by changes in flow. Baseline sediment movement is likely to be most relevant to periphyton accrual.

The sediment traps at Gorge accumulated most sediment, on average, although variability among replicates meant that the difference between Gorge and Balmoral was not statistically significant (two-sample T-tests, P>0.05). The traps at SH7 accumulated the lowest quantities of all size fractions except for the finest fraction (< 63 µm), for which the sedimentation rate did not differ among sites (Figure 7-2a). The smallest-sized sediment fraction is likely to be most relevant as a potential source of P for algae, because fine particles would become trapped more easily within a periphyton matrix.

The average sedimentation rate for the smallest fraction, of 15 g m^{-2} day^{-1}, was similar to that recorded at one site in the lower Maitai River, Nelson, in February 2014 (Wood et al. 2015) but was high in the context of other studies. For example Wood et al. (2014) reported rates ranging from 2 to 5 g m^{-2} day^{-1} at three sites in the Mangatainoka River, Manawatu catchment.

In contrast to the sediment traps, the Quorer samples showed highest resuspendible sediment at SH7 and lowest at the Gorge site (Figure 7-2b). This suggests that fine sediment is being continuously flushed through the system at the Gorge (hence the higher deposition rates). Farther upstream, a higher proportion of fine sediment remains trapped below the generally coarser substrate (see Table 6-1), with most mobilisation occurring during high flow events. It is beyond the scope of this report to investigate this further.

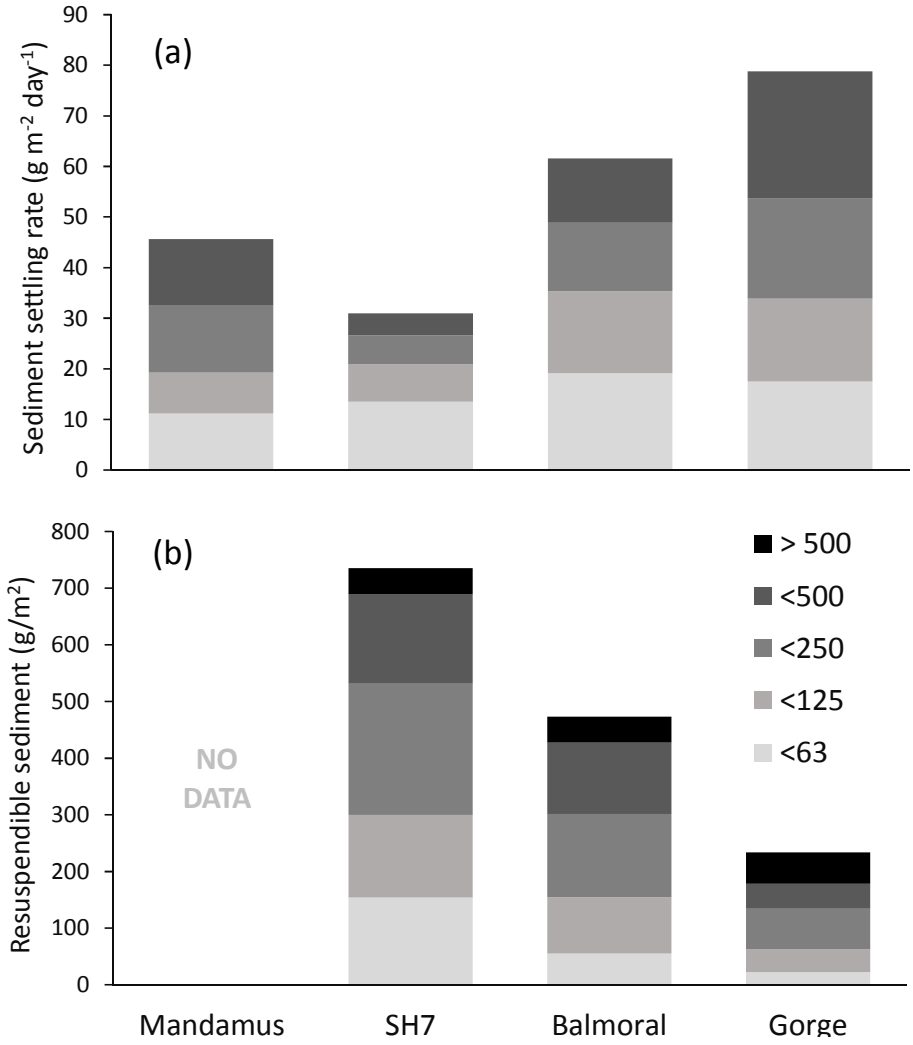

Figure 7-2: Sedimentation rates (a) and resuspendible sediment (b) at four sites in the Hurunui River in February 2015. Data are means from three samples at SH7, Balmoral and Gorge. At Mandamus sedimentation was determined from two samples and resuspendible sediment was not sampled because of coarse substrate. Data for the >500 µm fraction are omitted in (a) because some samples contained didymo stalks. Note that units on (a) are rates of settlement per day, and on (b) quantity re-suspended per unit area. Units for the key are µm.

The P extractions showed increasing sediment-bound phosphorus in a downstream direction. The gradient was particularly strong for loosely bound P on the smallest sediment size fraction (< 63 µm), for which concentrations (as µg/g dry sediment) approximately doubled at each successive site, so that concentrations at Gorge were eight times those at Mandamus (Figure 7-3a). Converting the

concentrations into a flux using the sedimentation rates further accentuated the gradient. The rates of deposition of loosely bound P on sediment < 63 µm at Gorge were 13.3, 5.5 and 2.3 times greater at Gorge than at Mandamus, SH7 and Balmoral, respectively (Figure 7-3b).

Concentrations of loosely bound DRP measured in the Hurunui River samples were low compared to those measured in previous studies. In the Mangatainoka River, loosely bound P on the <63 µm fraction ranged from 40 to 120 mg P/g dry sediment, and in the Maitai River the range was 50 to 160 mg P/g dry sediment (Wood et al. 2014, 2015). In contrast, the range in the Hurunui River in February 2015 was 6 to 51 mg P/g dry sediment (Mandamus and Gorge respectively).

In relation to the Mangatainoka River, a reason for the difference may be that DRP concentrations in the Mangatainoka are consistently higher than those in the Hurunui. For example, at the time of the Wood et al. (2014) study, mean DRP concentration was 0.019 mg/L, over 15 times higher than mean DRP in the Hurunui at the Gorge in 2015 (Table 3-2). Presumably the quantity of P adsorbed onto sediment particles is partly a function of the DRP concentration of DRP in the overlying water. This suggests that the sediment-adsorbed P at Gorge was actually very high relative to ambient concentrations in the water.

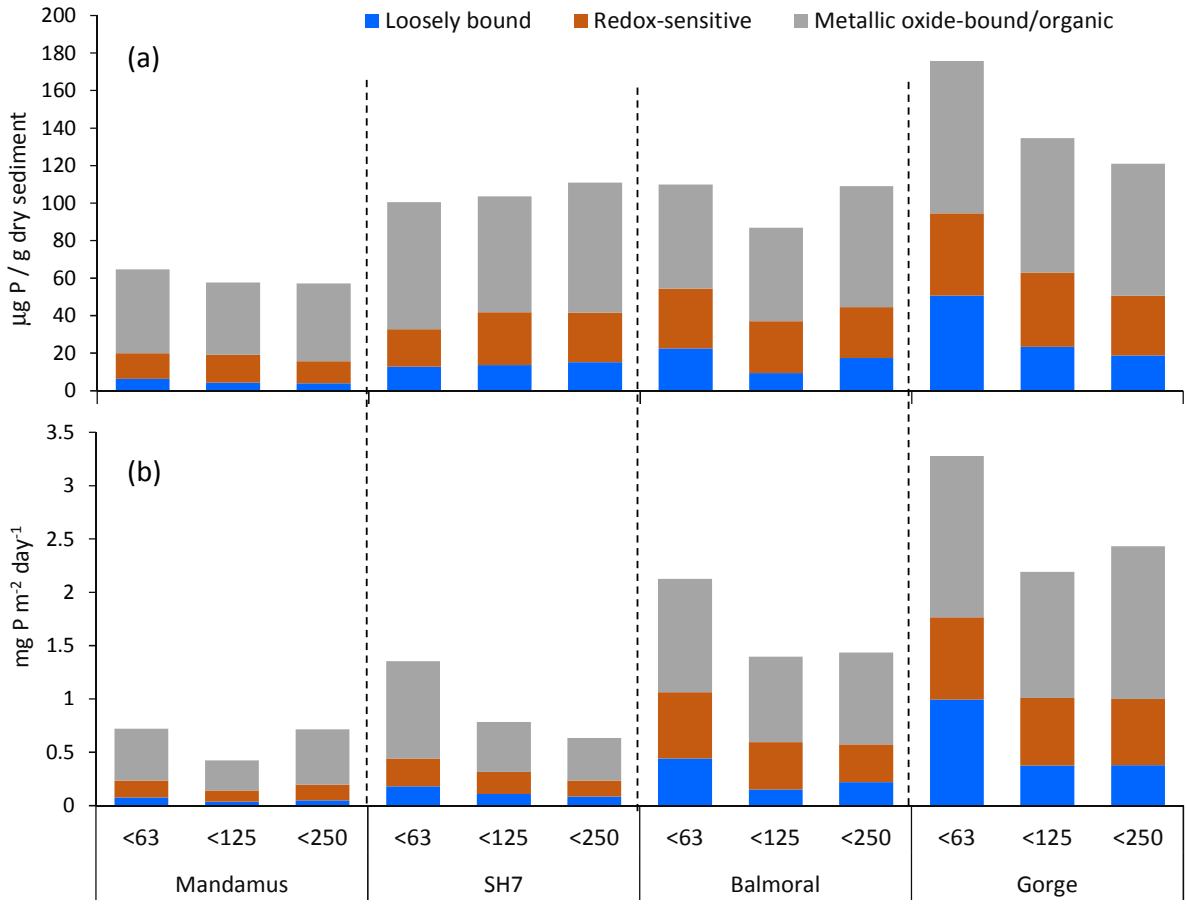

Figure 7-3: Mean concentrations (a) and daily flux (b) of three fractions of sediment-bound P at four sites in the Hurunui River. P fractions were determined for three sizes classes of fine sediment collected in sediment traps from 10 - 16 February 2015. Size classes are in µm. Values are means of two (Mandamus) or three (SH7, Balmoral and Gorge) replicate traps. Flux was calculated by multiplying the concentration by the sedimentation rates shown in Figure 6.2.

7.3.1 Implications of sediment-bound phosphorus

Wood et al. (2014) described a process that suggested that *Phormidium* mats may proliferate in waters with very low DRP concentrations because the cells can obtain sufficient P for growth from fine sediment trapped within the mats. The implication is that if sediment particles carry enough P are constantly being delivered to the river-bed surface, and conditions exist for the bound P to be solubilised, then DRP concentrations in the overlying water are immaterial to *Phormidium* mat development. Conditions facilitating release of DRP from sediment-bound P include elevated pH to at least pH 9.0. Such pH levels can be attained within periphyton mats through the process of carbon uptake during photosynthesis (Wood et al. 2014). We did not measure within-mat pH during the present study although mean pH in the overlying water was around 8 over the period of the study (Table 3-1). These spot measurements would have partly reflected the time of day. In particular the mean of 7.9 at Gorge was pH generally around 9-10 a.m. Maximum pH later in the day would have been higher and pH >9 within periphyton mats would seem feasible. Water-column DRP concentrations were extremely low in the Hurunui River during the study period, at 0.0008 mg/L (average across all sites for the month before sediment trap collection). Analyses of trapped sediment in the Hurunui River in February 2015 indicated that the conditions for sediment-sourced P to sustain *Phormidium* mats were most favourable at the downstream site (Gorge) and least favourable upstream at Mandamus. Therefore, in theory, the process described by Wood et al. (2014) was feasible. However, further research is needed to establish definitively that *Phormidium* (or other periphyton) takes up P from sediment-bound sources.

If the hypothesised process is a significant factor favouring *Phormidium* proliferations then we would expect that *Phormidium* growth would be limited in waters with low DRP concentrations, where P is not available in sediment. The study on the Hurunui River cannot demonstrate this because low sediment P availability at Mandamus and SH7 was accompanied by very low DIN concentrations (means of 0.0038 and 0.0129 mg/L DIN, respectively, over the study period), which may also have limited *Phormidium* growth.

Circumstantial evidence supporting the sediment-P hypothesis has just become available from an experiment carried out in streamside channels fed by water from the Kowai River, Canterbury. Ambient concentrations of DIN and DRP during the experiment were ~0.06 and 0.0011 mg/L, respectively. Additions of P to concentrations of ~0.003 mg/L resulted in significantly higher *Phormidium* in the periphyton community compared to the control treatment (Two-sample T-test, $P < 0.01$, $n = 12$). This result suggested that *Phormidium* growth was P-limited in the control treatments, which had DRP concentrations similar to those recorded in the Hurunui at Gorge in 2015. From this it is possible to infer that *Phormidium* growth at the Gorge would have been P-limited unless some additional P was available. In the Kowai experiment, additions of DIN up to 2 mg/L had no effect on *Phormidium* growth (unpublished NIWA data).

A further consideration is that N and P are not necessarily the only potential limiting nutrients for *Phormidium* in the Hurunui River. Experimental studies have shown that *Phormidium* growth is suppressed at iron (Fe) concentrations of 0.04 mg/L, but not at 0.8 mg/L (Harland et al. 2013). Cyanobacteria in general have higher requirements for Fe than eukaryotic algae (i.e., most other algae, such as diatoms and green filamentous chlorophytes) (see review in Molot et al. 2010). In rivers, the process for acquiring P proposed by Wood et al. (2014) is partly tied to the transformation of Fe from insoluble to soluble forms (e.g., Eckert et al. 1997). Soluble Fe and P may be released from insoluble forms bound to fine sediment particles incorporated into periphyton mats, under specific pH conditions, which can occur within the mats. Therefore this mechanism might also make Fe in

sediments, as well as P, more available to cyanobacteria. Data from January to April showed very low concentrations of total Fe at all sites in the Hurunui River (Table 3-1). In comparison, concentrations in the Kowai and Opuha Rivers were up to ten times higher (0.17 and 0.30 mg/L respectively). However, across all Hurunui River sites, total Fe was positively and significantly correlated with total suspended solids (R = 0.7), indicating the potential availability Fe from fine sediment.

In conclusion, sedimentation rates and concentrations of potentially biologically available P bound to sediment in the Hurunui River in February 2015 were consistent with the hypothesis that high cover by *Phormidium* particularly at the Gorge might have been sustained by P sourced from sediment. The significance of the process remains to be confirmed. Relatively high quantities of sediment-bound P at the Gorge, compared to very low concentrations in the overlying water, suggest either continued sediment-bound P inputs from upstream, or a legacy effect of past activities when P inputs from the upstream catchment were higher (Jarvie et al. 2013), or a combination.

8 Controllers of periphyton in the Hurunui River, summer 2015

The combined data from the flow record, water chemistry and nutrient analyses, periphyton accrual trials, nutrient diffusing substrate assays and in-river periphyton surveys are used below to assess the factors at each site that were controlling periphyton standing crop. This includes consideration of drivers of the dominant taxa at each site.

8.1 Mandamus

Periphyton cover and biomass remained low from January to May 2015, and were well within all periphyton guidelines. Low rates of accrual of chlorophyll *a* during both accrual trials was consistent with minimal change in cover on the river bed. Given the extremely low concentrations of both DIN and DRP at Mandamus, and NDS assays indicating either N-limitation or co-limitation by both N and P, low periphyton standing crop and low accrual rates were expected.

From time to time, surveys in the NRWQN have reported high cover by didymo at Mandamus. Stalk-dominated didymo blooms form only when DRP concentrations are less than about 0.002 mg/L (Kilroy and Bothwell 2012), therefore we expected to see much more didymo at this site. However, recent experiments in the Ohau River have shown that didymo mat formation and cell division are suppressed when DIN concentrations fall below about 0.01 mg/L (Kilroy and Larned, in prep.). Mean DIN of 0.005 mg/L at Mandamus (Table 3-1) explains why didymo cover was so low, and why the cover present appeared to be unhealthy.

Low periphyton cover meant that the effects of high flows were muted, because much of the cover was growing attached to large stable substrata. Large stable substrata are often associated with persistent periphyton cover, comprising taxa well adapted to withstanding the shear stresses of high water velocities while taking advantage of increased delivery of nutrients in higher water velocities (Larned et al. 2004). In this respect, some of the taxa present at Mandamus were expected. The dominant green alga at the site was *Cladophora*, which attaches strongly to substrata. Mean percentage cover of *Cladophora* was stable over time, with percentage cover at individual survey points remaining the same (i.e., little evidence of growth). *Cladophora* is well known as a nuisance alga in rivers (e.g., Frossard et al. 2014), but at Mandamus the species appeared to be just surviving. The second most abundant green filamentous alga was *Oedogonium*, a genus that tends to do well in stable habitats (Novis 2003). Its ability to attach to the substratum using a holdfast may explain its presence at Mandamus. The prominent genus of Cyanobacteria at Mandamus was *Calothrix*, a nitrogen-fixer characteristic of low-DIN environments, which forms firmly attached colonies on rocks. *Phormidium* was not detected in any samples from the river, but was present in low abundance on the N+P NDS treatment in February, which could indicate that *Phormidium* would establish at Mandamus given sufficient nutrients.

The periphyton mats that dominated cover on 5 May appeared to comprise mostly the small filamentous diatom *Diatoma tenuis*. This species is considered to be indicative of good water quality. For example in the UK-developed trophic diatom index (TDI), *D. tenuis* has a score of 1 on a scale of 1 to 5 from low to high tolerance of high nutrient waters (Kelly et al. 2008).

8.2 SH7

Despite low DIN and DRP, cover and biomass at SH7 was relatively high at the start of the surveys in January and maintained high levels during the period of stable flows up to early March. Cover was

removed during the flood event on 10 March and stayed low, but remained very visible for the rest of the study.

In contrast to Mandamus, DIN at SH7, although low, exceeded the suggested threshold required for didymo blooms, and DRP was within the range favouring blooms. Therefore didymo blooms were predictable at SH7. Cover was modest compared to that reported from other rivers. For example, mean didymo % cover at two sites in the Opuha River, with similar water depths and velocities as SH7, exceeded 60% in January – February 2015 (Kilroy and Measures 2015), compared to about 30% at SH7. A possible explanation for the failure of full scale didymo blooms to develop at SH7 may be that flows were variable during January and February, even though there were no major floods. In comparison, the regulated flows in the Opuha River were receding over the period of prolific didymo cover.

Cover by filamentous green algae peaked at SH7 on 4 February at > 10% (Figure 8-1). This was the highest cover by filamentous algae recorded over the whole season at all four sites. Green filamentous algae were also present in all samples from the NDS treatments at SH7 on 4 February; therefore we assume that overall stable conditions at SH7 favoured growth of green filamentous algae at that time. The dominant filamentous taxa were *Mougeotia* sp. and *Oedogonium* sp. *Mougeotia* does not attach to the substrate and would therefore have detached easily even with a small increase in flow. *Mougeotia* species occur across a wide range of nutrient concentrations (Biggs and Kilroy 2000, Hart et al 2013).

Figure 8-1: Obvious cover at SH7 by filamentous green algae on 10 February 2015. Peak filamentous green algae cover on the stream bed was recorded on 2 February. The dominant taxa were *Mougeotia* and *Oedogonium* sp.

The series of small floods in March and April ensured that periphyton cover remained relatively low over that period. The taxonomic analyses indicated that the minor peaks in cover by periphyton mats on 1 April and 5 May probably comprised a mixture of *Diatoma tenuis* and *Encyonema minutum*.

Biomass at SH7 was surprisingly high, particularly as AFDM, despite consistent indication of co-limitation of periphyton growth by both DIN and DRP in the NDS assays. The main reason for the high biomass is likely to be the dominance of didymo in the periphyton. In addition to contributing directly to periphyton biomass through cells and stalk material, the stalks exuded by didymo cells provide additional habitat for small epiphytic diatoms such as *Achnanthidium minutissimum* (Gillis et al. 2014). High concentrations of dissolved nutrients, particularly DRP and NH_4-N, have been measured in water squeezed from didymo mats (NIWA unpublished data). Presumably these nutrients originate through internal recycling from trapped algae, detritus and sediment. While such nutrients do not necessarily benefit didymo cells, which are generally located at the surface of the mat, they should favour the growth of small diatoms below the surface. Algal growth within didymo mats explains the very high proportions (almost 50%) of *A. minutissimum* in the community on 16 February, when biomass was highest.

Patterns of accrual of periphyton on artificial substrates at SH7 reflected biomass in the river, at least in terms of the visible appearance of periphyton on the substrates towards the end of Experiment 1 (see Appendix A). In contrast, the periphyton that accumulated over 2 weeks in the NDS assays evidently represented pioneer colonisation only: didymo cells were present, but in very low abundance, and *Diatoma tenuis* was numerically dominant.

In summary, the patterns of periphyton biomass and cover at SH7 indicated that didymo cover had been developing since the large floods in early December 2014. The mats developed higher chlorophyll *a* than expected from ambient nutrient concentrations and nutrient limitation at the site because of gradual colonisation of the didymo stalk matrix by small attached diatoms. High densities of small diatoms were likely sustained by recycled nutrients within the mats. These processes later in community succession determined the eventual biomass and community composition at SH7. Thus periphyton biomass potential was high despite low nutrient availability in the overlying water.

8.3 Balmoral

At Balmoral, low biomass in the river and low accrual rates, particularly in Experiment 1, would not have been predicted from the general physico-chemical characteristics of the site. Both DIN and DRP concentrations were in the range thought to favour didymo proliferations. Evidence for P-limitation of periphyton growth in the NDS assays was consistent with suitable conditions for didymo blooms. Although the bed substrate was on average smaller than that at SH7, substrate composition was in the range recorded in other rivers with persistent didymo proliferations (e.g., Lower Waiau River, Southland, Kilroy and Wech 2014b). No other water chemistry variables were outside the range associated with didymo (Kilroy and Unwin 2013). Yet didymo occurred only as scattered small colonies in the river.

The potential for high didymo biomass at Balmoral was clear in accrual Experiment 2, in which very obvious didymo cover developed on all the pavers. Photographs taken on the fifth and sixth sampling occasions in Experiment 2 provided one explanation for the much lower didymo cover Balmoral than SH7. Didymo was obvious at both sites on 7 April. However, on 20 April, after the high flow on 15 April, didymo was still on the substrates at SH7 (albeit silt-laden), but the cover had been almost completely scoured off at Balmoral (Figure 8-2). Thus, the same flow event had different consequences at the two sites. The difference could be caused by more effective scouring at Balmoral than at SH7, perhaps caused by higher water velocities or more movement of abrasive bed material. Alternatively, some other environmental difference between the sites led to weaker didymo mats at Balmoral than SH7. Whatever the mechanism, conditions at Balmoral were less

suitable for didymo than at SH7. Observations at the Balmoral reach during the study suggest that the first explanation (more effective scouring) was more likely. Balmoral was the only site where NDS trays were washed away during high flows (on two occasions).

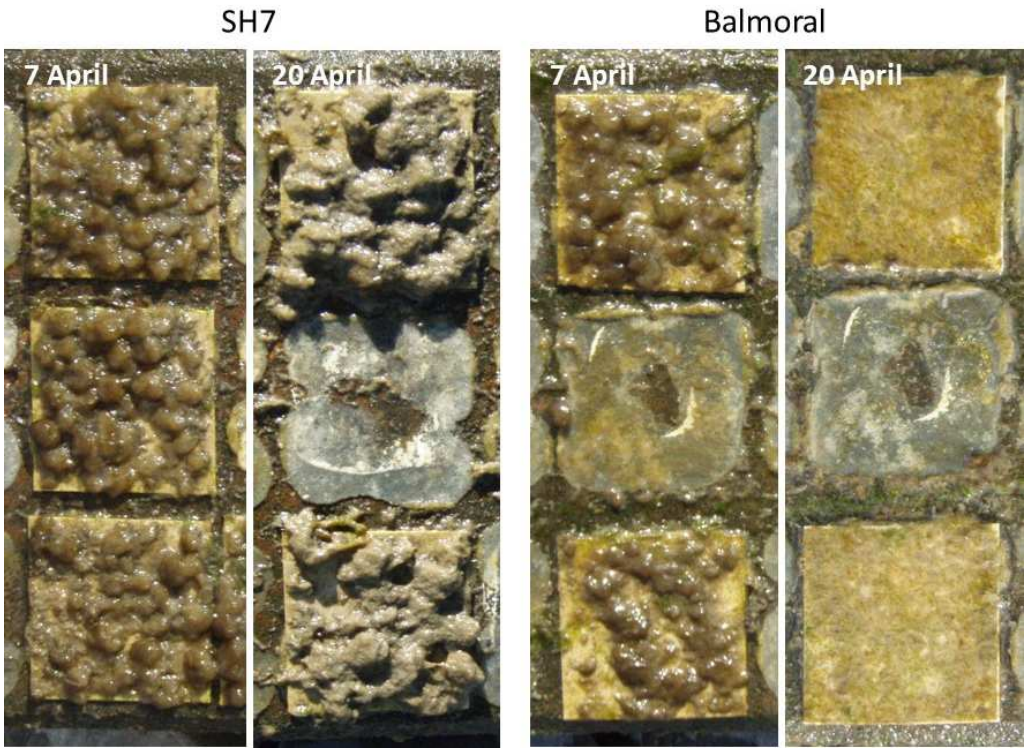

Figure 8-2: Didymo cover on substrates at SH7 (left) and Balmoral (right) on the last two sampling dates of accrual rate Experiment 2. A flood peaking at 72 m^3/s occurred on 15 April, which scoured off virtually all of the didymo at Balmoral but not at SH7.

The appearance of periphyton in the first accrual rate trial (see Appendix A) confirmed that extensive invertebrate grazing was limiting periphyton accrual at Balmoral. From March onwards, faster accrual in the river and on the artificial substrates in Experiment 2, indicated that grazing pressure was lower. An explanation for the difference between the two periods was that the high flows from 10 to 15 March depleted the invertebrate community. Delayed recovery of invertebrate communities following the floods thus allowed rapid periphyton regrowth (Steinman 1996). However, the explanation for such high invertebrate grazing in the first place is not clear. Potential reasons include more suitable riparian vegetation for the adult stages of aquatic insects (than at other sites), or better protection in the river from the damaging effects of UV for the aquatic stages (Bothwell et al. 1994). Whatever the explanation, we assume that invertebrate grazing is a persistent controller of periphyton biomass at Balmoral during low flows because we observed very low cover by periphyton during the NDS assays in 2014 (Kilroy and Wech 2014a).

With reduced impact of invertebrates, the accrual rates recorded in Experiment 2 were more representative of the potential for periphyton growth at Balmoral. Relative specific growth rates and NDS assays both indicated strong P-limitation. The limited community composition data from the NDS assays did not indicate that any individual periphyton taxa responded to addition of N without simultaneous addition of P, except that the response by *Phormidium* was unclear. At this stage, the conclusion is that some additional N at Balmoral may have little effect on periphyton, at least in the short term. However the response by *Phormidium* requires further investigation. The correlation between *Phormidium* cover and water velocity at Balmoral may indicate that, currently, *Phormidium*

can acquire sufficient N at this site only where water velocities are high enough (see comments below).

8.4 Gorge

High chlorophyll *a* recorded at Gorge was attributable to the domination of periphyton cover by *Phormidium*. *Phormidium* may have higher areal content of chlorophyll *a* than other algae with estimates ranging from 380 to 600 mg/m^2 compared to 200 to 500 mg/m^2 for filamentous green algae (Hart et al. 2013, Kilroy et al. 2013). High chlorophyll *a* was reflected by higher chlorophyll *a* accrual rates. In addition, the maximum relative specific growth rate for an individual paver was recorded at Gorge in both accrual rate experiments (Table 4-3).

As discussed in Section 7, the factors thought to promote *Phormidium* proliferations are under debate, and generally based on correlative studies. Therefore, whether high *Phormidium* cover in the lower Hurunui River is attributable to high DIN, to acquisition of P from sediments (Section 7), to both, or whether other factors (such as Fe) also contribute, is uncertain. Water temperature has been suggested as a potentially important factor in the development of blooms (Heath et al. 2011). However, water temperatures at Gorge were similar to those at Balmoral and SH7 and it is unlikely that temperature played a major part in the gradient in *Phormidium* cover along the three sites. The occurrence of *Phormidium* at lower water velocities at Gorge compared to Balmoral could indicate a response to DIN (which is the main difference between the sites in terms of water chemistry, and about 10 times higher at Gorge), as discussed in Section 4.3.3. Investigations into the drivers of *Phormidium* in Canterbury Rivers are underway (e.g. ECAN-funded PhD study by Tara McAllister, University of Canterbury), and the outcomes of these studies may help to explain the dominance of *Phormidium* in the lower Hurunui.

The general physico-chemical conditions at Gorge from January to May 2015 also suggested suitable conditions for didymo, although DIN concentrations were at the top of the range in which didymo blooms have been observed (Kilroy and Bothwell 2012). Very low cover by didymo was recorded in the river at the Gorge on the last survey on 5 May though cover was obvious on the accrual rate substrates. Marginal habitat for didymo at Gorge is consistent with the occasional records of didymo cover at SH1 in the NRWQN.

There was evidence of invertebrate grazing on some of the accrual rate substrates at Gorge, but the effect was patchy compared to the consistent signs of invertebrate on all four pavers at Balmoral in accrual Experiment 1. At the end of accrual Experiment 1 at Gorge three of the four pavers had almost 100% cover by *Phormidium*; there had been evidence of invertebrate grazing only on paver 2, which had little visible *Phormidium*. A recent study at Cawthron indicated that *Phormidium* may have direct negative (i.e., lethal) effects on invertebrates (Bridge 2013), therefore it is possible that the dominance of *Phormidium* on the river bed at Gorge had already led to reduced invertebrate densities, thereby reducing the potential for grazer control of periphyton. Grazing was more evident during Experiment 2 than Experiment 1, even though large *Phormidium* patches developed on three of the four pavers.

Finally, while chlorophyll *a* biomass reached the highest levels of all four sites at the Gorge, biomass was also removed most readily by high flow events (see Section 6.2.4). The strong effect of flows on biomass is largely attributable to finer substrate at this site, which would mobilise more easily and promote abrasion during elevated flows.

Probable and potential drivers of periphyton biomass at the four sites are summarised in Table 8-1.

Table 8-1: Summary of the effects of the range of potential controlling factors on periphyton biomass at four Hurunui River sites. More information refers to the sections in the report that provide more details about each factor.

	Effect of potential controlling factor						
Site	**Flow variability/floods**	**Local stability**	**Water temperature**	**DRP**	**DIN**	**Invertebrate grazers**	**Other factors**
Mandamus	Scouring effect of floods reduced because of coarse substrate.	High substrate stability favours persistent periphyton.	Lowest water temperature - may slow periphyton growth, but effect probably small.	Low DRP, favours didymo blooms, but reduces suitability for other taxa such as such as *Gomphoneis*, *Cymbella*, possibly *Phormidium*	Very low DIN suppresses didymo blooms; favours N-fixing cyanobacteria, and some diatom taxa. Possibly excludes *Phormidium* and other taxa *(Cymbella?)*	Not observed to have a large effect at this site	Fine silt deposition may restrict algal growth in areas of slower water velocity between and downstream of large boulders.
SH7	Moderate effect of floods; probably limits extent of didymo blooms compared to other rivers.	Moderate substrate stability. Some large stable substrate allows persistence of *Phormidium* in fast flowing areas.	Possibly slightly faster growth rates than Mandamus, but the effect is expected to be small.	Low DRP, favours didymo blooms, but reduces suitability for other taxa such as *Gomphoneis*, *Cymbella*, possibly *Phormidium.*	Low DIN is sufficient to support didymo and some green algae. Low cover by *Phormidium* only where water velocity is highest suggests just sufficient DIN (?) uptake in those areas,	Not observed to have a large effect at this site	Once didymo mats are established, internal nutrient cycling (including from sediment?) may support many small diatoms within the mats.
Balmoral	Effective removal of periphyton by high flows except on boulders in fast-flowing water.	Reach is unstable (e.g, bed sediment movement, periphyton removal) = frequent disturbances. *Phormidium* in fast-flowing areas.	No significant difference from SH7	Low DRP, favours didymo blooms, but reduces suitability for other taxa such as *Gomphoneis*, possibly *Cymbella* and *Phormidiu*m	Relatively low DIN (0.04 - 0.06 mg/L) should favour didymo blooms. Possibly not sufficient for broad cover by *Phormidium* (uncertain), but sufficient for *Cymbella.*	Significant effect of grazers on periphyton, Jan-Feb; grazing less evident in Mar - April, following flood.	
Gorge	High flows effective at removing periphyton (in the study reach).	Reach stable, but exposed areas of sand /small gravel throughout the site would promote abrasion of algae during high flows.	No significant difference from SH7	Slightly higher DRP than upstream sites, but low enough to favour didymo blooms. Taxa responding to P upstream [*Gomphoneis* (stalked), *Cymbella (?)*, *Encyonema* (mucilage producing)] were common in river community.	DIN (approx. 0.3 - 0.4 mg/L) may be too high for didymo. Clearly sufficient for *Cymbella* and *Phormidium.*	Grazing effects inconsistent compared to Balmoral. High *Phormidium* cover could have negative effect on invert. grazing.	Evidence for potential source of additional P in fine sediment. Many mucilage producing diatoms already present at this site could benefit as well as *Phormidium*?

8.5 Use of ash-free dry mass data

In this study, we quantified periphyton biomass by determining both chlorophyll *a* and ash-free dry mass (AFDM) on samples from the river and in the accrual rate trials (Section 4). A brief discussion follows on why AFDM was considered, and why it has proved informative.

Most current New Zealand periphyton guidelines specify periphyton measured as chlorophyll *a*. Chlorophyll *a* is internationally accepted as an appropriate surrogate for periphyton biomass because this pigment (used in photosynthesis) is present in all algae. Ash-free dry mass (AFDM) is sometimes used as an additional or alternative measure of periphyton biomass. AFDM estimates the total amount of organic material in a sample, which may include mass from non-algae sources (e.g., invertebrates, mucilage, organic detritus). AFDM has been included in previous periphyton guidelines (Biggs 2000). Chlorophyll *a* and AFDM are often closely correlated, even though they are measuring different components of periphyton; therefore it is not usually informative to measure both.

One scenario where chlorophyll *a* and AFDM might not correspond well is when the periphyton community comprises a high proportion of non-living organic material, such as the stalks and mucilage of diatoms. Didymo stalks are an extreme example of this. Didymo has been present in the upper Hurunui River since about 2008, sometimes in high biomass (according to NRWQN visual estimates at Mandamus). AFDM was measured in the present study because it was suspected that chlorophyll *a* might not accurately represent the visual impact of didymo blooms. If this was the case, then we would expect that the relationship between chlorophyll *a* and AFDM would differ between sites with little or no didymo (Balmoral and Gorge) and sites where didymo dominates the periphyton (Mandamus and SH7). This proved to be the case (Figure 8-3). In this study, data on AFDM therefore enabled quantification of biomass at Mandamus and SH7 that reflected the cover estimated visually (Section 6.2.2).

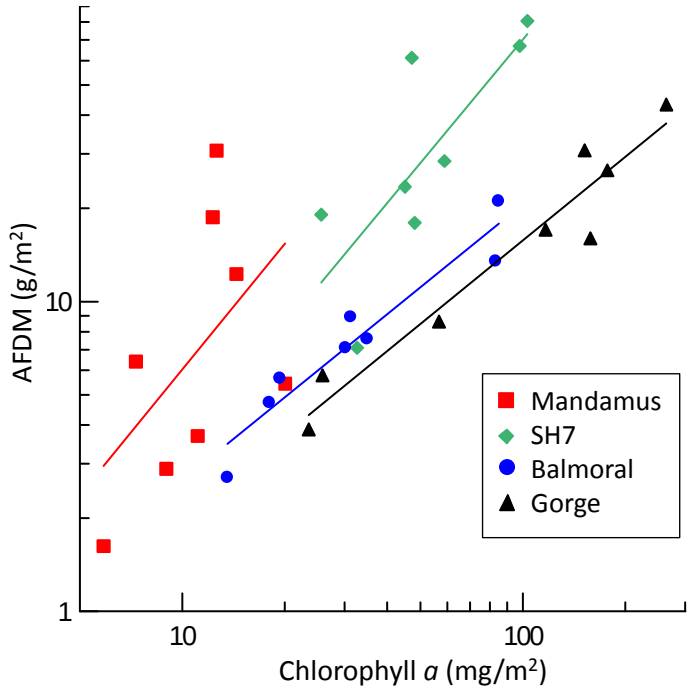

Figure 8-3: Mean ash-free dry mass (AFDM) plotted against mean chlorophyll *a* at each site. Note the close correlation between AFDM and chlorophyll *a* at Balmoral and Gorge, with only a small difference between sites. In contrast, the two metrics correspond poorly at Mandamus and SH7, and form a separate relationship, with higher AFDM relative to chlorophyll *a*.

In summary, AFDM is not a better (or worse) measure of periphyton biomass than chlorophyll *a*; it is a different measure. Contrasts between chlorophyll *a*-based biomass and AFDM-based biomass at the four sites, and the different relationships to visual cover across sites, highlight the importance of visual observations of cover as well as chlorophyll *a* for determining whether periphyton exceeds undesirable levels.

9 Conclusions

9.1 Questions from the brief

The following conclusions are framed as responses to the questions posed in the original study brief.

1. **Which parts of the Hurunui River are saturated in terms of [nutrient supplies for] periphyton growth rates and which parts are not?**

   When more than sufficient nutrient supplies are available to sustain maximum growth rates in periphyton, growth rates should approach the maximum possible. Both the accrual rate trials and nutrient limitation assays indicated that from January to April 2015 nutrient supplies were insufficient for maximum periphyton growth at any site. The degree of non-saturation varied between sites. In the second accrual rate trial, maximum relative specific growth rates at each site indicated strongest P-limitation at Mandamus and weakest at Gorge (Section 4.3.2). The assays showed that nitrogen limitation operated at Mandamus at the start of the season, transitioning to co-limitation by both N and P. Co-limitation dominated at SH7, P-limitation with possible occasional co-limitation at Balmoral, and consistent P-limitation at the most downstream site, Gorge (Table 5-2). The first assay also showed that P-limitation at the Gorge was weaker than at Balmoral. Weak P-limitation at Gorge did not preclude high biomass, which exceeded several periphyton guidelines at this site (Table 6-2). Furthermore, at SH7, biomass (as both chlorophyll *a* and AFDM) attained higher levels than expected given consistent co-limitation of N and P indicated by the nutrient assays. We attribute this to recycled nutrients (possibly from trapped sediment) within didymo mats stimulating high densities of small attached diatoms.

2. **How significant is the growth rate of periphyton to mass accumulation over time? [In other words, are rapid growth rates always associated with high biomass and, conversely, are slow growth rates always associated with low biomass?]**

   Accrual rates on artificial substrates generally reflected biomass accumulation in the river: the sites where accrual was fastest also had highest chlorophyll *a*, particularly during the longest low flow period (January to February). However, the relative specific growth rates determined in the accrual rate trials over this period (Experiment 1) were clearly not maximum at Balmoral, where there was evidence of heavy invertebrate grazing. The maximum relative specific growth rate at Balmoral may have been approximated by the maximum reported in the second low flow period (mid-March to early April, Experiment 2). SH7 was an outlier in the pattern. Although mean relative specific growth rates at SH7 in Experiment 2 were low (Table 4-2), chlorophyll *a* had attained the same level as that at Balmoral and Gorge by early April, possibly because biomass losses were lower. Didymo-dominated periphyton at SH7 seems to be a case where the slow growth rates did not lead to low biomass (see Section 8.3).

   In summary, despite relatively high growth rates, separate loss processes can lead to low biomass (e.g., invertebrate grazing at Balmoral), and despite low growth rates, biomass can attain high levels (e.g., through nutrient recycling within persistent didymo mats, at SH7).

3. **Is the lower Hurunui River phosphorus limited (as stated in the HWRRP)?**

   The studies from January to May 2015 indicated that periphyton growth at the site in the lower Hurunui River (Gorge) was phosphorus limited over that period. Additional comments

are: i) P-limitation was slight; ii) only slight P-limitation was detected in spite of very low ambient DRP over the study period, and N to P ratios of 230 to 380; iii) slight P-limitation did not preclude high biomass.

4. Is it possible to manage periphyton growth by retaining phosphorus concentrations at their current levels, while allowing for a modest increase in nitrogen (as stated in the HWRRP)?

The "baselines" to which this question applies need to be clarified. From January to March 2015 both phosphorus (DRP) and nitrogen (DIN) concentrations were low in the lower Hurunui River (SH1) compared to most of the previous 10 years (Figure 3-7). This was particularly the case for DIN. Therefore any discussion of the effects of additional DIN based on the 2015 data might be describing what would occur assuming DIN concentrations return to those more typical in recent years. DIN in the lower Hurunui is not relevant if the "current levels" referred to apply to sites upstream of the Pahau and Dry Stream confluence (i.e., Balmoral and upstream) because DIN concentrations at Mandamus were within the typical range of the past 10 years.

In this study, the effect of increasing N at Balmoral and SH7, can be partly inferred from the results of the nutrient limitation assays and from the in-river surveys, and with reference to periphyton community composition. Two of the assays at Balmoral showed that additional DIN did not affect chlorophyll *a*, at least for the first 2 – 3 weeks of accrual. A third assay indicating secondary N-limitation suggested that additional N might increase chlorophyll *a* if DRP also increased slightly. In addition, in two assays (4 February and 20 April), the strongest shifts in community composition occurred when both N and P were added together. No taxa were identified as consistently responding to N additions across all sites, although a larger number of samples would need to be analysed to have more confidence in this conclusion. Overall, there is some support for the proposal that a modest increase in nitrogen should have little effect on biomass or community composition. A caveat is that it is likely that even a very small increase in DRP would promote changes.

However, the dominance of *Phormidium* at Gorge compared to the sites farther upstream must be a concern. DIN concentration is the most obvious difference between sites. To date, involvement of DIN in promoting *Phormidium* blooms has been demonstrated only from correlative studies, as far as we are aware. The fact that *Phormidium* grows well at Balmoral (and to a lesser extent at SH7) only in areas of high water velocities suggests that some aspect of water chemistry is involved, and DIN is the most obvious contender. The only way to resolve this question would be to conduct an in-river nutrient amendment experiment with a duration of up to 6 weeks. We know from the 2015 accrual rate trials that 6 weeks is sufficient for development of mature periphyton communities (provided river flows are stable). We have set out the additional experiment requirements in Section 0.

In summary, the studies from January to May 2015 provided some support for the proposal that modest increases in DIN in the middle reaches of the Hurunui (represented by sites at SH7 and Balmoral) will not lead to increased biomass provided DRP does not increase. However, the potential response of *Phormidium* to increased DIN concentrations is unclear.

- *Phormidium* appears to thrive at DIN concentrations typically found at the Gorge (0.341 mg/L) despite low DRP concentrations (0.0010 mg/L). *Phormidium* did not

respond to additional N at these in-stream concentrations (at least over the 2-3 week time-frame of the nutrient limitation assays;

- *Phormidium* growth was lower at Balmoral where DIN concentrations were low (0.046 mg/L), except in higher water velocities. At these low DIN concentrations, there was some evidence that *Phormidium* responded to additional N. However, responses to N and P were inconsistent across assays.

5. To what extent can we "control periphyton growth and biomass accumulation" by managing nutrient concentrations?

Major differences in biomass and strong turnover of periphyton taxonomic composition along a strong gradient of increasing DIN and a more muted gradient in DRP indicates that nutrients play a large role in determining periphyton community composition, growth and biomass in the Hurunui River. The corollary to this is that managing or reducing nutrient inputs in the lower river reaches (represented in this study by Gorge) would be expected to alter periphyton communities there to become more similar to those upstream. The outcomes of any given degree of nutrient reduction are almost impossible to predict, although the community assays in 2015 did provide some insight into periphyton taxa that respond to added N and P in the Hurunui (and therefore might decline in the lower Hurunui if nutrient concentrations decline). However, features of the Hurunui could mean that the consequences of reducing nutrients may not result in the desired outcomes (i.e., reduced periphyton cover and biomass). For example:

- The presence of didymo in the upper Hurunui means that reducing nutrient concentrations downstream could make the downstream reaches more suitable for didymo.
- The gradient of fine-sediment-bound phosphorus identified in the Hurunui could cancel out reductions in DRP in the lower River (if it is not already doing so in periods such as January to May 2015, when water column DRP was very low). The sources of the high levels of sediment P in the lower Hurunui at the Gorge are unknown.

9.2 Potential further studies

It is clear from the preceding section that the comprehensive studies in the Hurunui River in summer 2015 have provided some information to assist in answering the questions posed in the study brief. However the effects of increasing DIN in the middle reaches of the Hurunui (as a result of land-use changes) are still unclear. The question of what is driving persistent *Phormidium* blooms in the lower Hurunui River (DIN or DRP) also remains unresolved.

The first suggested further study (**Study 1**) comprises longer term instream DIN addition experiments at least two sites in the middle reaches of the Hurunui River. The experiments could comprise additions of N by gradual dissolution of solid chemical from reservoirs (e.g., perforated jars) onto small artificial substrates (e.g., those used in the accrual rate trials), with regular (e.g., weekly) replacement of the reservoirs. Control substrates would be located upstream. The exact concentration of the nutrient release cannot be controlled exactly in such a method, but it can be set to be within a target range, and the concentrations can be measured. Such a design has the advantage of being uncomplicated and robust, compared to more precise (but complex and delicate) instream or stream-side channels. Ideally the experiment would be conducted at both SH7 and Balmoral (or similar sites, with an existing gradient of DIN).

Study 1 could be expanded to address the issue of whether *Phormidium* in the lower river (i.e. Gorge) is driven mainly by DIN, DRP, or both, by including additions of P only and also N+P. We showed in the present study that sediment-bound P is lower at SH7 and Balmoral than at Gorge. Therefore if *Phormidium* at the Gorge is primarily responding to this source of P, we would expect *Phormidium* at SH7 and Balmoral to respond positively to additional P in the overlying water.

Because nutrient concentrations fluctuate over time it would also be possible to obtain more insight into any general consequences of nutrient changes for periphyton from further analyses of nutrients and periphyton from the middle reaches of the river (i.e., represented by SH7 and Balmoral), particularly in years with different hydrological conditions from those in early 2015. Intensive monitoring of nitrate-N (e.g., similar to the programme currently underway at the Gorge, run by Lincoln Agritech) would be very useful to link to such analyses.

Study 2 would be a regular programme of nutrient and periphyton monitoring at site(s) in the middle reaches of the river. Periphyton monitoring would focus on biomass and community composition attained after low flows and therefore need not be intensive (e.g., up to three surveys over the summer), and an accompanying fine-scale record of nitrate concentrations would greatly aid interpretation.

Further questions arising from the study in 2015 relate to the source of *Phormidium* colonisation in the lower river. Regrowth clearly occurred rapidly in early 2015 following the large floods in November-December 2014. It is likely that once *Phormidium* becomes abundant in a river, and conditions favour rapid growth from time to time, then the small cells become lodged in refuges throughout the bed. Sampling following major floods could confirm this

10 Acknowledgements

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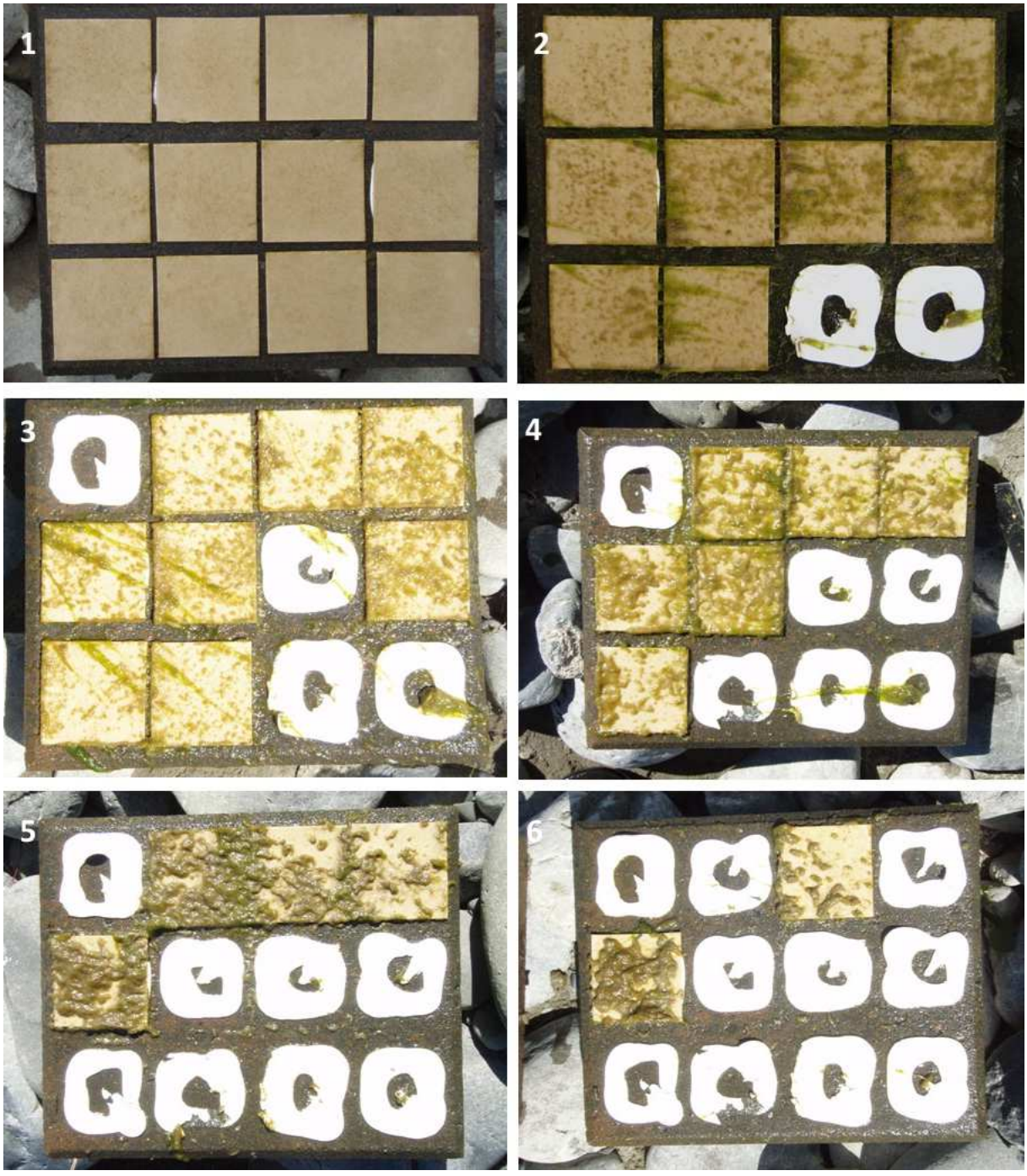
Appendix A Examples of periphyton accrual

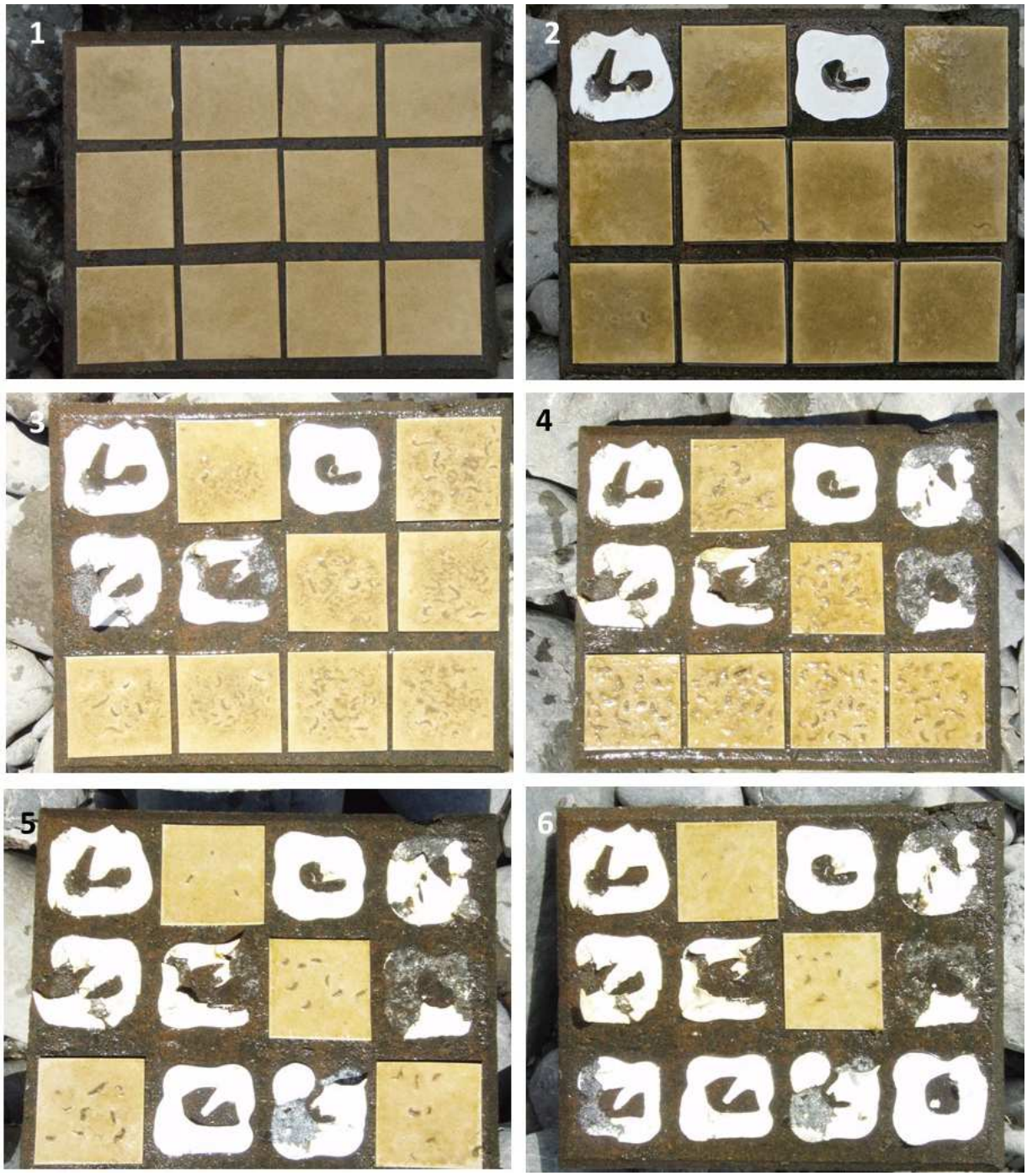
SH7, Experiment 1, Paver 3

By week 2, cover by green filaments and didymo was developing. Accrual was fastest between weeks 1 and 2. Didymo cover continued to increase, but between weeks 4 and 5, much of the green filamentous algae disappeared, possibly due to the very minor increase in flow between those two sample collections (see Figure 4-1).



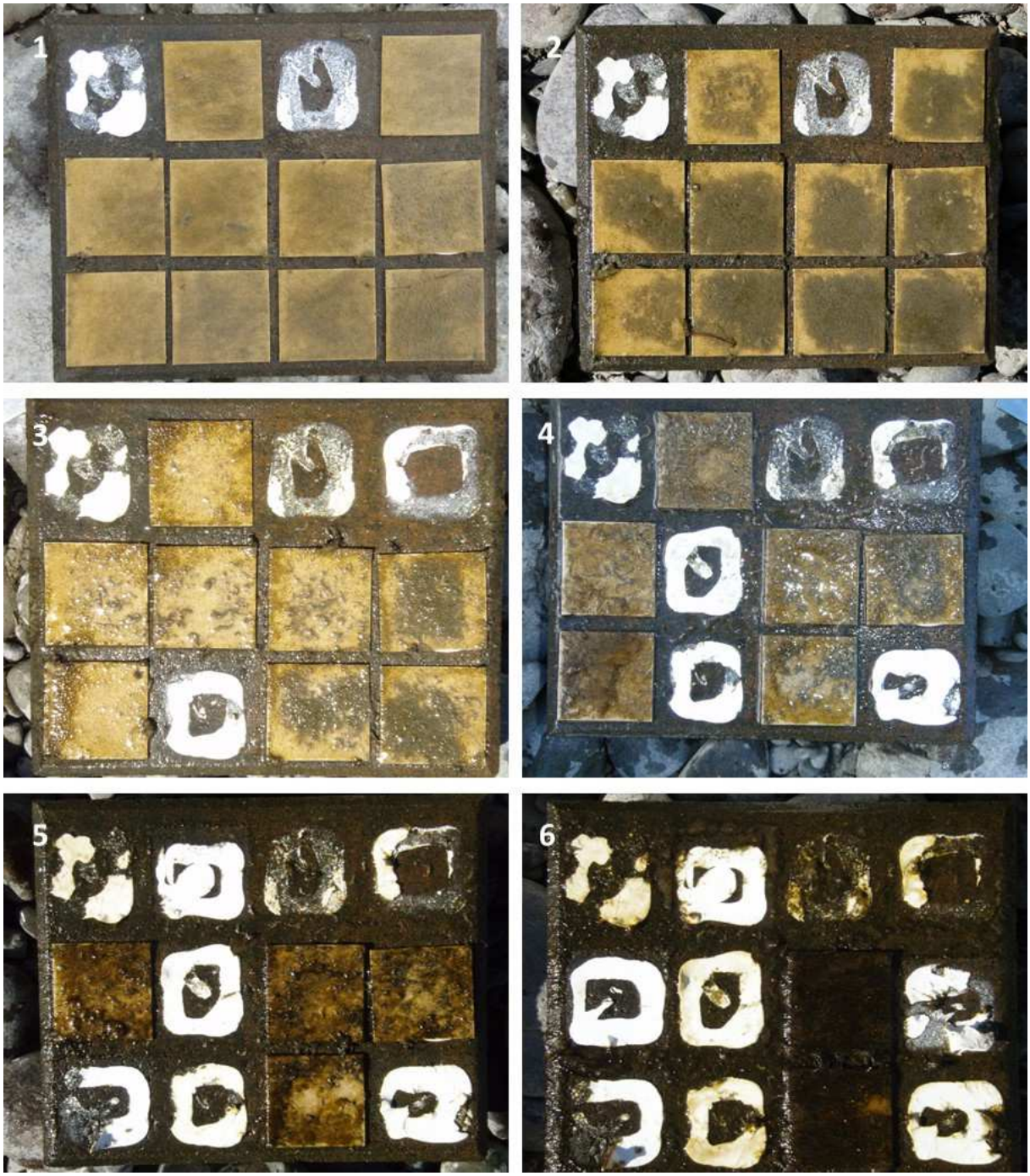
Balmoral, Experiment 1, Paver 1

Accrual was fastest between weeks 1 and 2. After this, the photos show evidence of invertebrate grazing (the piles of fine particulate matter), which curtailed accrual of chlorophyll *a* (see Figure 4-3).



Gorge, Experiment 1, Paver 1

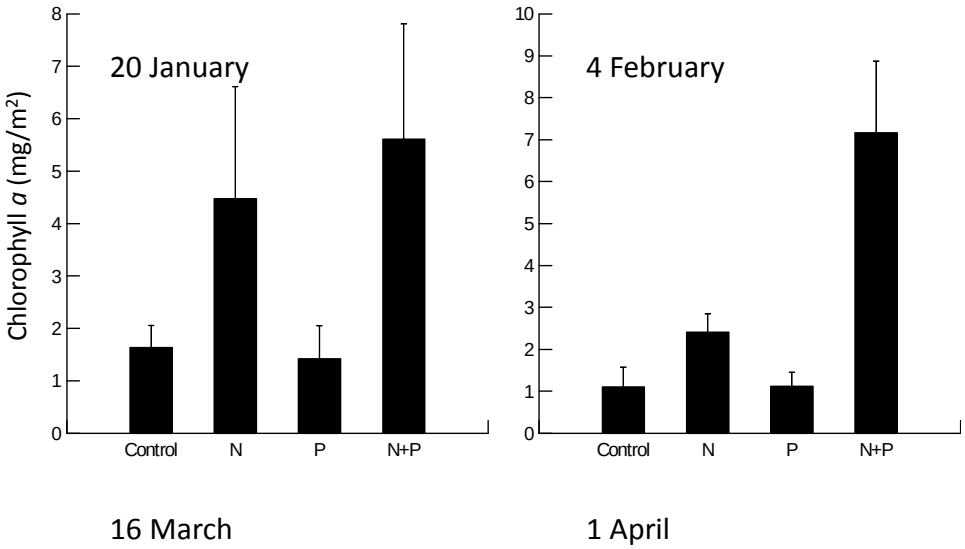
Accrual was fastest between weeks 4 and 5. Accrual up until week 2 was largely inorganic material (sediment). This was evident visually and was confirmed from the ash-free dry mass data which indicated organic content of less than 10%. For comparison, organic content at SH7 on paver 3 was almost 50% in weeks 1 and 2, and was over 30% at Balmoral on paver 1. After some loss of inorganic material between weeks 2 and 3, chlorophyll *a* increased slowly. By week 6, there was almost 100% cover by *Phormidium*. At this stage organic content was 40%.



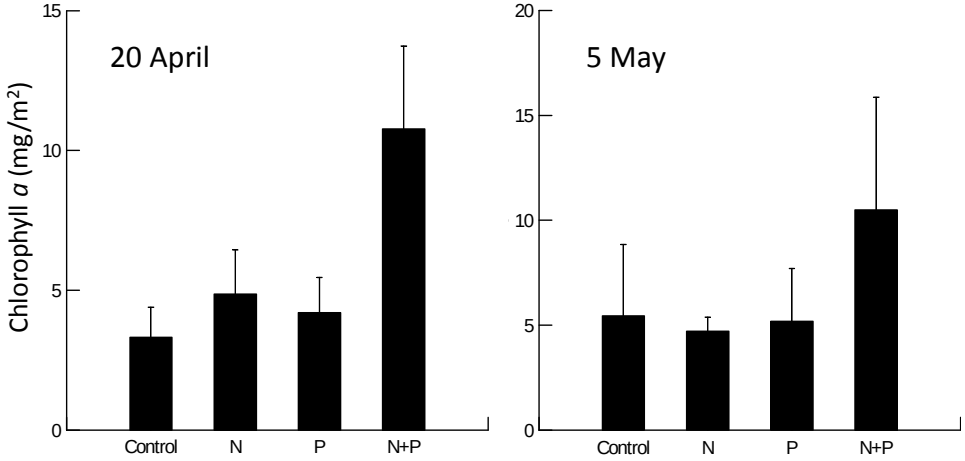
Appendix B NDS trials: biomass plots for all assays

The following plots show mean chlorophyll *a* for each treatment for separate assays at the four sites. Error bars are standard deviations. The standard number of replicates was five. Numbers above the bars indicate numbers in cases when the number of replicates was lower because growing surfaces were lost. For statistics for each test, refer to Table 5-2. Sites are from upstream to downstream. Note that the scales on the vertical axes are different in each plot.

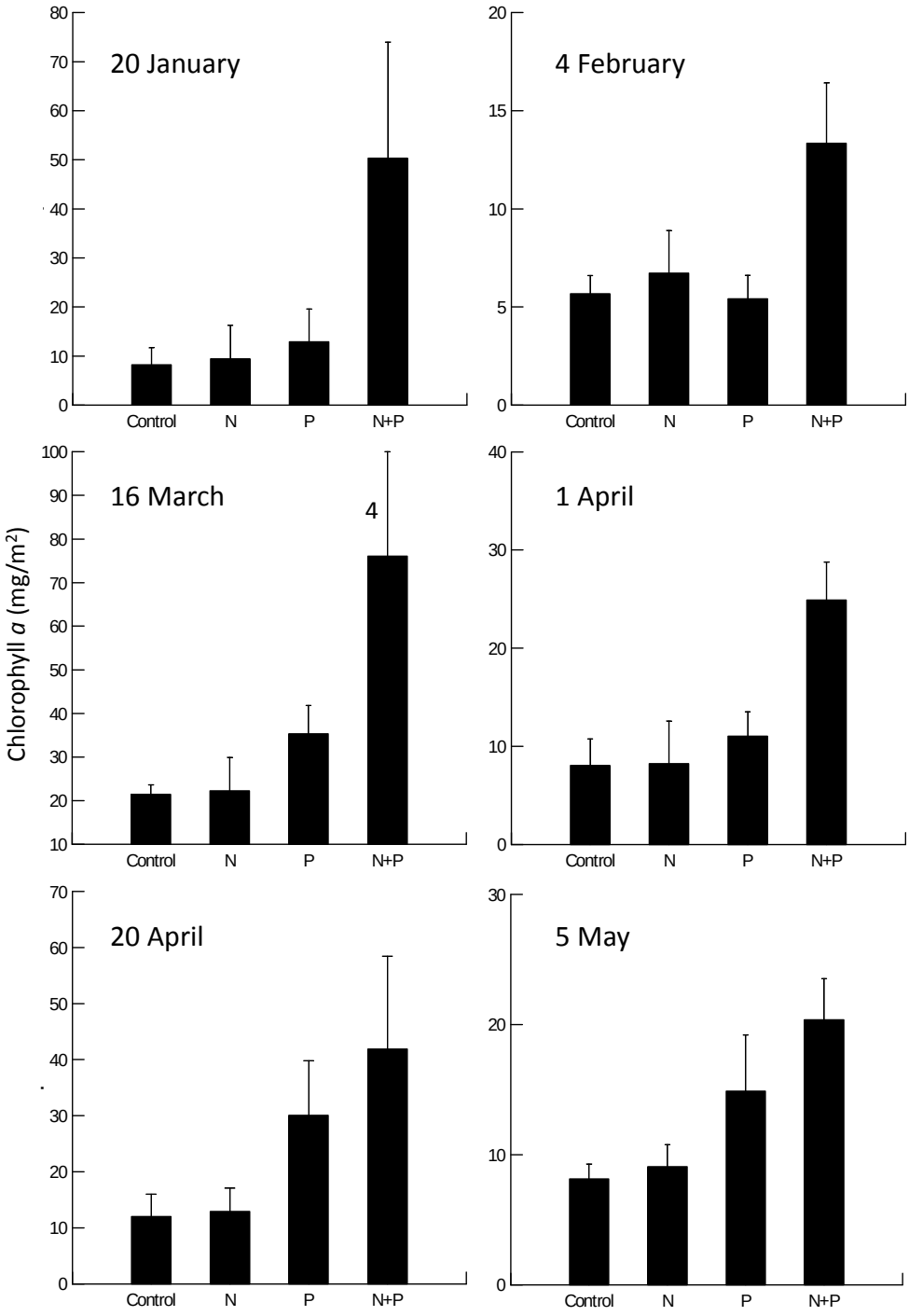
Mandamus



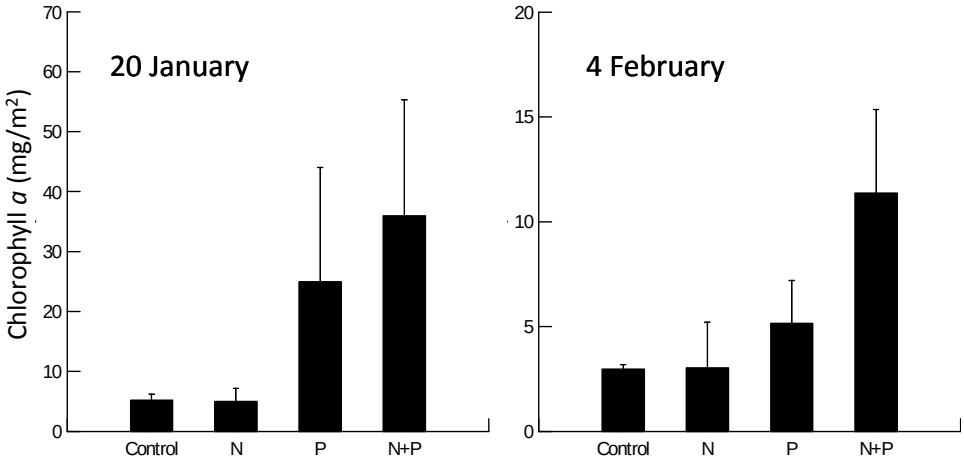
Flood affected



SH7

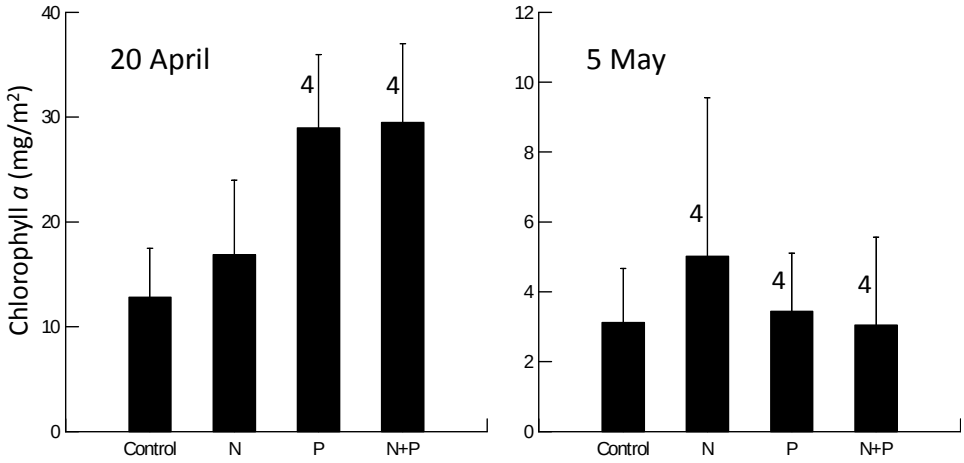

20 January
4 February
16 March
1 April
20 April
5 May
Control
N
P
N+P
Chlorophyll a (mg/m²)

Balmoral

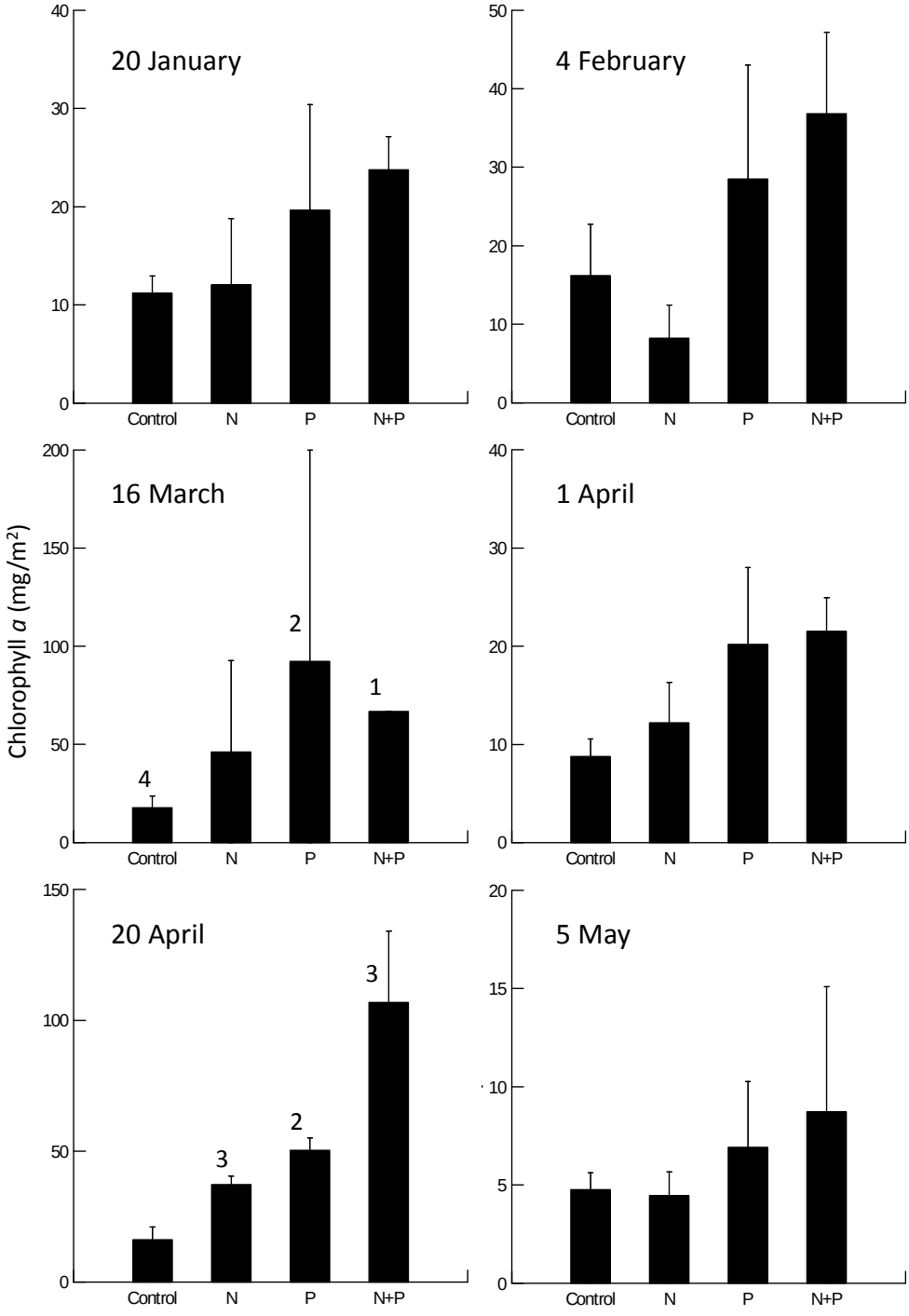


16 March 1 April

Flood affected



Gorge


20 January
4 February
16 March
1 April
20 April
5 May
Chlorophyll a (mg/m²)
Control
N
P
N+P
4
2
1
3
2
3

Appendix C List of periphyton taxa identified from NDS growing surfaces and from the river bed at each site

All data are percentages of communities, using cell counts. The NDS columns show the mean percentage of each taxon calculated across all four nutrient treatments, on two occasions. The river columns show mean percentages from five samples at each site, collected monthly from January to May 2015. Rare taxa, with relative abundance of less than 0.05% are shown as <0.05.

	NDS , February				NDS, April				River, average from January to May			
Taxon	**Mandamus**	**SH7**	**Balmoral**	**Gorge**	**Mandamus**	**SH7**	**Balmoral**	**Gorge**	**Mandamus**	**SH7**	**Balmoral**	**Gorge**
Cyanobacteria												
Calothrix	0.9								6.6			
colonial Cyanobacterium									0.4			
Leptolyngbya				1.4				0.4				
Merismopedia				<0.05		<0.05						<0.05
Nostoc									0.7			
Phormidium	0.7	0.7	23.9	26.9		0.7	25.0	13.4		12.5	68.7	72.1
Diatoms												
Achnanthes oblongella							0.1	<0.05				
Achnanthidium minutissimum	5.7	6.5	12.1	7.0	16.1	3.9	1.5	4.7	9.0	24.1	8.5	1.2
Aulacoseira					0.4							
Brachysira											<0.05	<0.05
Cyclostephanos sp. (cf.)	0.2	<0.05		<0.05		<0.05			0.6	0.6	0.1	
Cocconeis pediculus	0.5								2.8	0.2		
Cocconeis placentula	0.4		<0.05	<0.05		<0.05		0.1	0.7		<0.05	<0.05
Cymbella cf. cistula		0.1	0.3	0.9				0.1			<0.05	0.2
Cymbella kappii	0.2	1.0	8.7	33.3	0.9	2.6	0.5	6.6	0.1	0.5	0.5	5.1
Cymbella naviculiformis							<0.05					
Diatoma hiemale							0.1		0.1	0.2		
Diatoma tenuis	60.4	27.4	4.3	3.3	39.5	58.6	57.1	31.7	30.5	16.9	7.8	1.5
Diatoma vulgare	0.1	0.2		<0.05		0.6	0.1	0.2	0.1			<0.05
Didymosphenia geminata	0.3	0.1			0.2	0.2	0.1	<0.05	1.2	4.1	1.4	<0.05
Diploneis							<0.05			0.1		
Encyonema gracile					0.1							
Encyonema minutum large					4.1	0.1	2.5	4.6	4.1	3.5	1.0	0.9

	NDS , February				NDS, April				River, average from January to May			
Taxon	**Mandamus**	**SH7**	**Balmoral**	**Gorge**	**Mandamus**	**SH7**	**Balmoral**	**Gorge**	**Mandamus**	**SH7**	**Balmoral**	**Gorge**
Encyonema minutum small	4.7	2.5	2.5	2.3	14.2	12.4	3.3	14.6	8.5	18.0	1.3	0.7
Encyonema prostratum									0.1	<0.05		
Epithemia spp.	0.1				0.1	<0.05	0.1	0.1	1.1	0.1	<0.05	<0.05
Eunotia sp.					0.1							
Fragilaria capucina			<0.05	0.1	3.9	1.4	0.6	0.5	2.9	1.8	0.1	
Fragilaria construens	0.2				<0.05	0.1				0.1		
Fragilaria crotonensis				0.4								
Fragilaria vaucheriae	0.9	0.8		0.3	1.3	0.6	0.4	0.1	1.3	1.2	0.2	0.1
Frustulia crassinervia									0.1			
Gomphoneis minuta var. cassieae	0.6	3.2	1.3	1.2	1.9	6.5	0.5	7.8	1.1	0.3	0.2	0.3
Gomphonema cf. *clavatum*	0.2			<0.05	2.2				0.2	<0.05		
Gomphonema parvulum		1.9	4.3	2.9		0.2	0.3	0.7		0.1	<0.05	<0.05
Gomphonema cf. *minutum*	1.8	11.1	8.0	3.9	10.0	4.2	2.0	2.9	10.2	5.1	2.5	0.4
Gomphonema truncatum			0.1									
Melosira varians	0.4			0.1	0.1	<0.05			0.1		0.1	<0.05
Navicula capitatoradiata				0.1			<0.05	0.1	0.1	<0.05		0.1
Navicula cryptocephala												<0.05
Navicula sp. A												<0.05
Navicula lanceolata									0.1	0.1		
Navicula cf. *phyllepta*	0.2	0.1		0.2	0.8	0.3	0.2	0.3	6.2	4.3	0.5	0.2
Navicula margalithii												<0.05
Navicula sp. B							<0.05	<0.05				
Navicula radiosa									0.1			
Navicula rhynchocephala						<0.05					0.1	
Naviculoid (small)	0.1	0.2		0.5	0.5	1.0	1.0	0.5	0.3	0.6	0.4	0.1
Navicula trivialis				<0.05				0.1				<0.05
Nitzschia acicularis	0.1	0.7	<0.05	0.1				<0.05				
Nitzschia s. A (wide)	0.2	0.1	2.5	1.8		<0.05	0.2	0.1		0.1		
Nitzschia cf. *linearis*	1.9	0.6	0.6	1.9	0.2		0.1		0.8	0.3	0.1	
Nitzschia sp. B (long, fine)	2.4	2.5			0.9	0.6		1.8	0.2	0.2	0.1	<0.05
Nitzschia sp. C (narrow)		0.4			0.3	0.3		0.1				

	NDS , February				NDS, April				River, average from January to May			
Taxon	**Mandamus**	**SH7**	**Balmoral**	**Gorge**	**Mandamus**	**SH7**	**Balmoral**	**Gorge**	**Mandamus**	**SH7**	**Balmoral**	**Gorge**
Nitzschia (small spp.)	1.6	20.5	0.2	2.2					0.1	0.1		<0.05
Planothidium lanceolata						0.3	0.2	0.1			<0.05	<0.05
Reimeria			1.4	0.4	0.3	0.2	0.1	0.4	0.2	0.7	0.3	0.3
Rhoicosphenia								<0.05		<0.05		
Rhopalodia novaezelandiae						<0.05			0.6	<0.05	<0.05	
Rossithidium linearis	0.1		<0.05			<0.05	<0.05	0.2	1.8	1.6	0.6	13.7
Surirella sp.											<0.05	
Synedra acus	0.1	4.2	0.2	0.3	0.1	1.0	<0.05	0.4				<0.05
Synedra biceps	0.1	0.3	0.1		0.6	0.2	<0.05		0.3			
Synedra contracta + spp.	0.7	0.6	1.3	1.2	1.3	1.8	3.0	0.9	0.5	0.9	0.5	0.4
Synedra delicatissima		0.4	0.4								0.1	<0.05
Tabellaria flocculosa					0.1				0.1			
Green filamentous algae												
Cladophora									0.9			<0.05
Microspora		1.3										
Mougeotia	0.3	6.1	0.5							0.3		
Oedogonium	0.4	0.7	0.3						2.3	0.7	0.4	
Spirogyra	0.1	1.1	1.1				0.1					
Stigeoclonium		0.9	7.4	2.2		0.1	0.1	5.2	0.2		2.2	2.2
Ulothrix								<0.05				
Zygnema		0.5				1.1						
Green algae, single cells or colonial												
Ankistrodesmus spp. (cf.)	1.5	2.9	0.6	1.4		0.6	0.2	<0.05	0.2	<0.05	0.2	0.1
Cosmarium spp.		0.1	<0.05	0.1						0.1	<0.05	<0.05
Gloeocystis							0.1		0.1		0.1	
LGB	11.6	0.1	1.3	0.5		<0.05	0.6	0.5		0.2	0.1	
Scenedesmus	0.4	0.1	2.2	0.7		<0.05		<0.05		0.1	0.1	0.1
small green	0.4	0.3	14.2	2.3			0.1	0.5			<0.05	0.1
Red algae, filamentous												
Audouinella									2.7			