Ecological studies on *Didymosphenia geminata*

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Executive Summary

A series of studies on aspects of the ecology of the invasive diatom (alga) *Didymosphenia geminata* was commissioned by Biosecurity New Zealand. This diatom grows attached to rocks in rivers with strong mucilaginous stalks, forming thick mats. The studies had two main aims: (1) to gain a better understanding of likely aesthetic and environmental impacts of *D. geminata* on the river environment; (2) to provide information to support future attempts to control or eradicate this species.

Three separate studies were undertaken based on four surveys at sites in the Mararoa and lower Waiau Rivers between 25 May and 8 August 2005. The studies were: (1) an investigation into the hydraulic habitat preferences of *D. geminata*; (2) a study of short-term temporal changes in *D. geminata* biomass and condition in relation to hydrological changes and water chemistry; (3) a comparison of invertebrate communities from sites affected and unaffected by *D. geminata*, to assess the potential effects of this diatom on higher trophic levels.

Hydraulic habitat study

We aimed to define the hydraulic conditions (water velocities, water depths and derived indices) that are optimum for *D. geminata* growth by sampling its biomass over a broad range of these parameters at three affected sites in the Mararoa and three affected sites in the lower Waiau River, under baseflow conditions.

Near-bed water velocity, mean water column velocity (at 0.6 of depth), water depth, and substrate size were measured at a total of 268 points (average of 45 per site). At each point, % cover and thickness of *D. geminata* were assessed and converted to a visual biovolume index. The periphyton biomass measurements of ash-free dry mass (AFDM – total organic material, mainly stalk material for *D. geminata*) and chlorophyll *a* (indicating the amount of live algal cells in a sample) were also determined from quantitative samples collected at one site in each river.

The results confirmed observations that *D. geminata* will thrive in a wide range of hydraulic conditions, from very slow-moving, shallow waters to beyond the range of depths and velocities that could be safely sampled. At the most downstream site in the Mararoa, and the two upstream sites in the lower Waiau, the visual biovolume index reached maximum values at mean water velocities of approximately 0.5 m/s. AFDM and chlorophyll *a* at two sites greatly exceeded current guidelines for maximum periphyton in rivers to maintain angling and fish habitat values but showed few clear relationships with hydraulic variables.

In contrast to *D. geminata*, other types of algae distinguished during visual assessments showed clear hydraulic preferences. While these distributions may have been influenced by the presence of *D. geminata*, they did highlight the broad hydraulic range of this invasive species.

Since *D. geminata* is reported internationally as being a cold-water species, this mid-winter study may have recorded maximum growth for *D. geminata*. Further observations in warmer temperatures may show up clearer, or different, responses to hydraulic parameters, and possibly different levels of biomass. Therefore a repeat survey in summer is desirable.

Temporal changes in *D. geminata*

Several patterns in *D. geminata* spatial and temporal distributions were identified from the four surveys at six sites in the Mararoa and lower Waiau Rivers.

At the beginning of the study, AFDM in general declined in a downstream direction, but after six weeks with no high flows, AFDM at all sites was similar, and very high compared to typical values in New Zealand. Chlorophyll *a* biomass was initially similar at all sites and tended to increase over the stable period. Small freshes during the stable period did not reduce biomass but may have slowed down biomass accumulation.

It is suggested that a strong downstream nutrient gradient may influence *D. geminata* growth patterns and regeneration. In the more nutrient-rich (particularly for dissolved nitrogen) lower Waiau River biomass accumulation over the period of stable flow from May to July was faster than that in the Mararoa. This suggests a positive response of *D. geminata* to nitrate concentrations. In other words, all other things being equal, rivers with higher nutrient levels will have larger blooms. A consistent temperature difference between rivers may also affect growth, possibly with cooler water favouring AFDM biomass development. Further surveys at different times are necessary to verify both of these suggestions.

Following a moderate-sized flood on 28-29 July, up to 70% of biomass (AFDM) was lost, but considerable growth remained. However, at three sites, AFDM biomass *increased* following the flood, though chlorophyll *a* (live cells) was reduced at all sites. At the sites where the flood had least effect *D. geminata* also showed no hydraulic habitat preferences within the range sampled. These sites may have more stable substrate than the other three sites. This implies that stable areas in the river are more susceptible to extended proliferations over space and time.

At two sites, the best hydrological predictor of biomass was the number of days since the most recent flood greater than 75 – 100 m^3/s, confirming that floods of this magnitude "re-set" biomass levels at these sites. Lack of correlations with hydrological indices at other sites may be because the sites are too far from the flow recorders, i.e. the indices at the flow recorder locations do not reflect conditions at distant sites.

A trial of a "quantitative" visual assessment method during the fourth survey produced an excellent correlation between the index and AFDM biomass. It is suggested that if any future surveys are

considered, use of this method would be an efficient means of maximising the information gained if time or other resources were limited.

Effects on invertebrate communities

Using a replicated design, we compared invertebrate communities (taxonomic composition and abundances) from affected and unaffected reaches of the Mararoa River.

The main difference between *D. geminata*-affected and unaffected samples was that the former contained much higher densities of invertebrates. Community composition also differed. Several invertebrate community indices were significantly lower in the samples from *D. geminata* affected areas, implying higher proportions of invertebrates that are indicative of degraded or polluted conditions. It is suggested that the differences were a result of very high algal biomass rather than *D. geminata per se*. Because *D. geminata* biomass was exceptionally high, the effect was very marked. Although proportions of "desirable" (as fish food) invertebrates, were lower in the D. geminata-affected sites, densities were higher than those in sites free of *D. geminata*. This raises the possibility that the presence of *D. geminata* could be beneficial to fish. Further field studies are needed to verify (or otherwise) this suggestion.

Conclusions

These studies have quantified the observed aesthetic effect of this alga on the Mararoa and lower Waiau river environments. Levels of biomass, breadth of hydraulic habitat, and persistence of growth through high flows were extreme at the sites sampled. *D. geminata* appears to be resistant to floods and capable of growing almost everywhere in rivers where the substrate is not constantly unstable. It is suggested that *D. geminata* itself may be capable of enhancing river-bed stability. This alga also has a clear effect on invertebrate communities, although further work would be needed to determine any flow-on effects to fish.

1. Introduction

The unwanted, invasive Northern Hemisphere diatom *Didymosphenia geminata* has been proliferating in the lower Waiau and Mararoa River system, Southland, since at least October 2004 in the lower Waiau and probably at least three years longer in the Mararoa. This algal species has been reported to form massive and problematic growths in rivers overseas (Kilroy 2004). However, there appear to be virtually no *quantitative* data available internationally either on the size and extent of excessive *D. geminata* growth (spatially or temporally), or on the environmental factors that influence growth. Likewise, the only information on the effects of *D. geminata* on the river environment and food web appears to be qualitative or anecdotal. Both types of information are needed to assist Biosecurity New Zealand and other affected agencies in decision-making related to the response to this incursion. First, some understanding of the likely aesthetic and environmental impacts of *D. geminata* on the river environment is required to help assess the potential overall magnitude of impacts (environmental, economic and social) of this organism on a New Zealand-wide scale. Second, knowledge about the physical and chemical drivers of *D. geminata* growth may be used to support attempts to eradicate this species. For example, if the diatom's growth can be shown to respond positively to, say, low water temperatures, and negatively to higher temperatures, this would suggest that control measures should be timed for warmer conditions, when growth is already reduced.

Biosecurity New Zealand contracted NIWA to design and conduct a series of ecological studies on *D. geminata*. In this report we present the results obtained from four field surveys undertaken in the lower Waiau and Mararoa Rivers between 25 May and 8 August 2005, following a site selection and scoping exercise on 18-19 May. Three aspects of the ecology of *D. geminata* were investigated. First, we considered the hydraulic conditions (water velocity, depth) under which *D. geminata* grows in both rivers, to help predict conditions that might reduce *D. geminata* biomass, or cause it to be displaced. Second, we tracked the biomass of *D. geminata* at six sites over a 10-week period and related this to spatial and temporal variations in hydrological conditions and water chemistry. The aim here was to determine if there were short-term variations in the condition of the alga, which could assist in control measures. Third, we compared invertebrates communities associated with *D. geminata* in the Mararoa River with those in the upper reaches of the Mararoa, where *D. geminata* does not grow. This was to determine whether *D. geminata* proliferations were affecting higher trophic levels in the river.

Below, we first describe the locations of the study sites. Each study is then covered in a separate section, with its own methods, results and discussion. Overall conclusions are presented in a final summary section covering all three studies.

Two supplementary investigations are also discussed. In the first, we trialled a "quantitative" visual assessment procedure, aimed at identifying an acceptable surrogate for laboratory biomass analyses. While the latter are preferred for determining biomass, they are time-consuming and costly. A methodology that produces a good correlation with one or more of the measured biomass parameters would expand the scope for more detailed field studies of *D. geminata* responses to spatial and temporal changes.

In the second supplementary study, we quantified the concentrations of microscopic live cells of *D. geminata* in river water. We assume that transport of such cells is responsible for the downstream spread of the species and any control measures will need to take into account this small fraction of the total population. The ability to detect suspended cells is also important for surveillance and for testing the effectiveness of control measures.

2. Study sites

In a site selection and scoping exercise undertaken on 18-19 May 2005, potentially suitable study locations were examined in the *D. geminata*-affected reaches of the Mararoa and lower Waiau rivers (Figure 1). Although the two rivers are connected, they have different flow regimes and are also very likely to have differences in temperature and water chemistry. Therefore biomass and responses to flows and hydraulic conditions may be expected to differ in the two rivers. Sampling sites were selected according to the following criteria:

- Easy access;
- Primarily comprising stable substrates that provide good habitat for *D. geminata*;
- Including a wide variety of depths and velocities;
- Representative of larger areas of the rivers, which support *D. geminata* growth (in terms of substrate composition, water velocities and water depths).

The selected sites were (from upstream to downstream):

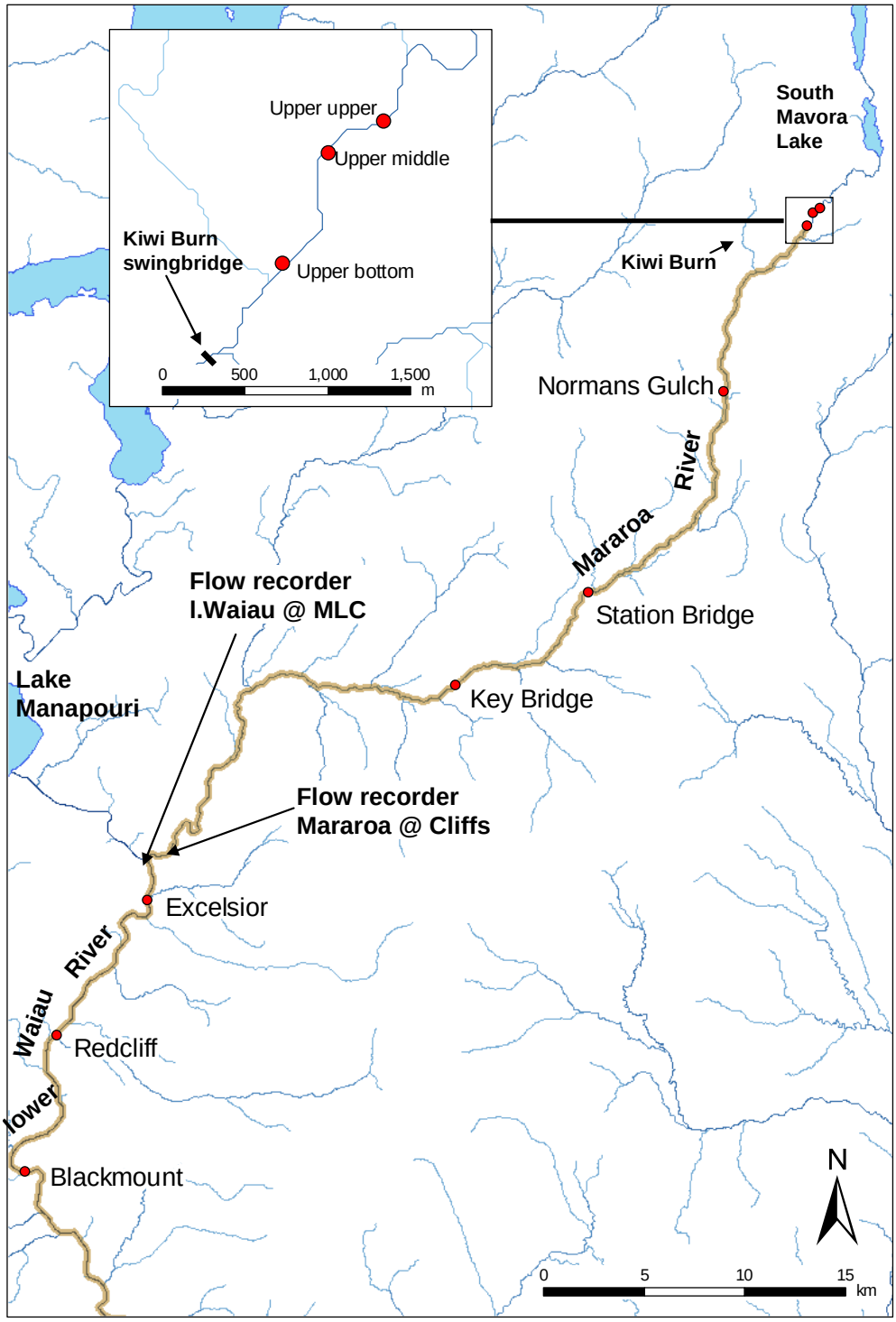

Figure 1. **Map of the Mararoa and lower Waiau Rivers, showing locations of sampling sites and flow recorders. Red dots are sampling sites. The brown colour indicates the stretch of the Mararoa – lower Waiau that is affected by *D. geminata*. Inset map shows locations of the three unaffected sites sampled for the invertebrate comparison.**

Mararoa River

Norman's Gulch

Station Bridge (upstream of bridge)

Key Bridge

lower Waiau River

Excelsior (downstream of creek confluence)

Redcliff (just upstream of creek confluence)

Blackmount (access through Rayonier forest)

For the invertebrate study, a further three sites were selected in the Mararoa River, upstream of the Kiwi Burn bridge (Figure 1), which marks the approximate upstream extent of the *D. geminata* infestation. The section of the Mararoa River immediately below South Mavora Lake is confined and steep. Therefore locations for suitable unaffected sites were limited to the ~6 kilometres upstream of the Kiwi Burn. As a result, the three upper Mararoa sites are located much closer together than the sites in the lower reaches. Nevertheless, at 300 – 700 m apart, the sites may still be considered to be spatially independent of each other at the scale relevant to invertebrates.

3. Hydraulic habitat of *Didymosphenia geminata*

3.1. Introduction

Hydraulic conditions – that is, water velocity, depth and turbulence – are recognized as major influences on the spatial distribution of benthic algae in gravel-bed rivers and streams (Biggs 1996; Stevenson 1996). Hydraulic factors can determine not only where particular taxa may grow, but also the potential biomass of individual taxa and communities. For example, high water velocities may preclude establishment of certain benthic algae, because the drag of the current exceeds the resistance of those algae to being swept away. Conversely, in very low water velocities, some algal species may be unable to thrive because dissolved nutrients do not diffuse through the cell walls rapidly enough to maintain cell metabolism and growth (Biggs et al. 1998).

Observations of *Didymosphenia* colonies in the lower Waiau and Mararoa Rivers suggest that this alga responds positively to a broad range of hydraulic conditions, forming exceptionally high biomass in both low and high water velocities, in water depths up to 1 m or more. *D. geminata*'s growth habit – production of robust polysaccharide stalks that attach strongly to the substrate – also suggests a broad

tolerance to hydraulic conditions. Indeed, this characteristic is likely to be an important contributor to the invasiveness of the species. The aim of this study was to define the hydraulic conditions in which *D. geminata* grows in the lower Waiau and Mararoa Rivers, including identification, if possible, of optimum and limiting conditions. The approach taken was that used by Biggs and Hickey (1994) who related periphyton biomass to hydraulic factors – water depth, water velocity and derived indices – in the Ohau River. To maximise the likelihood of detecting patterns in *D. geminata* spatial distribution, biomass was both assessed visually and determined quantitatively. One hypothesis that we wished to explore was that while visual coverage is known to be broad, more precise measures of suitability of hydraulic conditions might be picked up through changes in measured biomass.

This study was designed to contribute to the following information required by Biosecurity New Zealand on both the environmental effects, and growth patterns of *D. geminata*:

1. Environmental/aesthetic impacts: quantification of spatial distribution and biomass *in situ* was expected to confirm the visual impression that *D. geminata* grows prolifically in a much wider range of hydraulic conditions than many other algal species;

2. Growth patterns for application to control/eradication measures: identification of reach-scale parameters that influence the spatial distribution of *D. geminata* may contribute to determination of the success rate of response measures.

Field collections were undertaken on 25 – 27 May 2005, at the six *D. geminata*-affected sites in the lower Waiau and Mararoa Rivers (Figure 1). On the days of sampling, the flow in the lower Waiau River was at its minimum level (16 m^3/s, measured at the Mararoa Lake Control flow recording site), and the flow in the Mararoa River, was between the median and lower quartile of flow (~20 – 25 m^3/s, measured at Mararoa at Cliffs flow recording site). The most recent significant freshes had occurred 18 days and 73 days previously (~78 and 120 m^3/s respectively in the Mararoa, and ~88 and >300 m^3/s in the lower Waiau).

3.2. Methods

3.2.1. Field collections

Each site encompassed a river reach up to 300 m long. Within each reach, a matrix of water velocities vs. water depth was sampled, covering up to 10 (or more) categories

of each parameter (0.1 m/s velocity intervals and 10 cm depth intervals). Sampling points (defined as a single stone on the river bed) were selected using a wading rod and electronic current meter (FlowMate®). Ten velocity and ten depth bands theoretically would yield 100 sampling points at each site. In fact, many of the possible depth – velocity combinations were not represented. For example, in a gently-sloping reach, very high velocities will rarely be encountered in very shallow water. In addition, it was not possible to sample in depths > 0.8 m at some sites. On average, 45 points were sampled at each site.

The sampling procedure at each sampling point was as follows:

- Water depth (m) was measured using a wading rod;

- Water velocity (m/s) was measured just above the stream substrate (lowest possible point on velocity meter) and at a depth of 0.6 of the total depth. Near-bed velocity indicates the flows more closely impinging on the sampled community. Velocity at 0.6 depth is universally used to represent the mean flow at the point of measurement, averaged over the whole water column, and is used for hydraulic habitat modelling. The Froude (*Fr*) number was calculated for each sample. This dimensionless index identifies the critical point (*Fr* = 1) when smooth-flowing water on which ripples can travel upstream has enough energy to become super-critical, with all ripples being swept downstream:

  $$Fr = v/(gd)^{0.5}$$

  where *v* is water velocity (0.6 depth), g is acceleration due to gravity, and *d* is water depth. *Fr* therefore integrates values of water depth and velocity into a single index that describes the relative force exerted by the water.

- The stone representing the sampling point was retrieved, and a visual assessment made of the percentage cover of the surface by *D. geminata*. The average thickness of the mat was also estimated and assigned a thickness score from 0 to 5, as follows: 0, none; 1, thin (<1 mm thick); 2, medium (1-5 mm); 3, thick (6-15 mm); 4, very thick (16 – 30 mm); 5, extremely thick (> 30 mm).

- The percentage cover of other algae on the stone was assessed, placing these growths into broad categories based on colour and thickness. The categories used were: green filamentous algae (gf); thick brown/black mats (tm); thin brown/black film (tbf); slimy diatom mats, mainly *Cymbella* (cym).

- A quantitative sample of periphyton was collected by scraping/brushing the algae circumscribed by a 62 mm diameter circle placed over the centre of the exposed (upper) surface of the stone.

- Dimensions were recorded for each stone and exposed surface area was estimated using the formula:

$$\text{Stone surface area } (m^2) = 1.59 + 0.811\ (xy + yz + xz)$$

where x, y and z are the length, breadth and depth of the stone (m), respectively (Biggs and Kilroy 2000, p. 82).

All samples were stored on ice and in the dark immediately after collection, and frozen within 8 hrs for transport to the NIWA laboratories, Christchurch.

3.2.2. Laboratory procedures

Samples from one site in the Mararoa (Key Bridge) and one in the lower Waiau (Excelsior) were analysed for chlorophyll *a* and ash-free dry mass (AFDM) using the methods described in Biggs and Kilroy (2000). Chlorophyll *a* (mg/m^2) is a measure of the amount of live algal material in the sample. AFDM (g/m^2) is a measure of the total organic content of a sample, including cell contents, *D. geminata* stalk material, and also any other algae or small organisms trapped within the mat.

Each sample was homogenised using a hand blender and made up to a known volume. Duplicate subsamples of known volume were filtered through glass fibre filters. For AFDM, one of the duplicates (a preweighed filter plus filtered sample) was dried at 105°C for 24 h, reweighed, then ashed at 400°C for 4 h and weighed for a final time. Chlorophyll *a* was extracted from the second filter-plus-sample using boiling ethanol, and concentrations of chlorophyll *a* were read spectrophotometrically at 663 nm, including acidification to correct for phaeophytins. In each case, we calculated quantities per m^2 of stone surface, based on the area of the sampling circle (0.00302 m^2).

3.3. Data analysis

Analysis of variance (ANOVA) was first used to check for significant differences among sites in the physical variables. The significance threshold was taken as a probability of <5% that differences were due to chance ($P < 0.05$).

For all sampling points, the visually assessed % coverage of *D. geminata* was multiplied by the thickness score to produce a visual biovolume index (range 0 – 500). Stones on which some of the volume was attributed to algae other than *D. geminata* were distinguished to enable a comparison of the hydraulic habitat range of *D. geminata* with that of the other algal groups. The biovolume index for *D. geminata* was then plotted against hydraulic factors (water depth, water velocity and Froude number) with trends defined by locally weighted average smoothing (tension of 0.8). Linear regression was used to determine the significance of any linear relationships. The same procedure was used for the measured biomass values (AFDM and chlorophyll *a*) at Excelsior and Key Bridge. Biomass values were log-transformed to meet the requirement for a normal distribution of variables. Measured biomass values were also regressed against biovolume index, to determine whether the visually-assessed index could be used as a surrogate for measured AFDM and chlorophyll *a*. Sampling points with 50% or more cover by other algal groups were omitted from the regression. To check whether any of the other algal groups recognized visually showed hydraulic habitat patterns, percentage cover by these groups was plotted against the four hydraulic variables.

3.4. Results

3.4.1. Field observations

At almost all of the 268 sampling points, the visual assessment included at least some cover by *D. geminata*. On approximately 50% of stones, recorded *D. geminata* cover was 100%, though thickness varied. Only 4% of samples had 100% cover of algae other than *D. geminata*: this was always a slimy diatom growth, which occurred throughout both rivers, except at the most upstream site, Norman's Gulch. Microscopic examination of some of these samples confirmed that these mats comprised mainly the mucilage-producing diatom *Cymbella kappii*. In the lower Waiau River, thin and thick black slimy mats of cyanobacteria were observed on many stones, with coverage up to 80%. About 50% of stones from Blackmount, the farthest downstream site, were affected. Green filamentous algae were present at all sites, but generally covered very small areas (1 – 2%), usually overlaying 100% cover of *D. geminata*. The highest coverage by green filaments was at Blackmount, where it was recorded on about 30% of stones, with a maximum coverage of 20%.

Sampling thus confirmed that *D. geminata* dominated the periphyton at all sites. It was also noted that *D. geminata* mats extended into areas that were too swift and deep to sample safely, indicating that it may not be possible to define an upper hydraulic limit for establishment and growth from this survey.

3.4.2. Physical environment

ANOVAs run on individual hydraulic parameters confirmed that the rivers and sites were reasonably homogenous in terms of their physical characteristics, with the exception of Normans Gulch, at which shallower water and higher Froude number reflected the slightly steeper gradient that might be expected at the most upstream site. This implies that any major between-site differences may be attributed to factors other than hydraulic habitat. For details of the ANOVAs refer to Appendix A (Figure A1).

3.4.3. Biovolume and biomass relationships with physical variables

The visual biovolume data suggested that at the time of the survey, *D. geminata* tended to form the thickest and most complete cover at water velocities of around 0.5 m/s (measured at 0.6 depth) or around 0.4 m/s (measured near the bed). However, this relationship was site-specific.

Scatter plots of the *D. geminata* visual biovolume index (three sites combined) versus the four hydraulic parameters (depth, bed velocity, 0.6 velocity, *Fr*) showed a broad distribution of *D. geminata* cover in both rivers. Although the scatter of points in all cases was too variable to indicate any significant relationships, the weighted smoothing lines do suggest some trends. In the Mararoa River, while there was a suggestion of a water depth limit to very thick cover (Figure 2a), no limit appeared to be reached with respect to water velocity or Froude number (Figure 2b,c,d). Indeed, high scores were recorded at the highest values of *Fr* in the whole survey. These were also the only supercritical values recorded ($Fr > 1$, Figure 2d). In the lower Waiau, on the other hand, while depth appeared to have no limiting effect (and possibly a positive effect, Figure 2e), high values of both velocity measures, and *Fr* were associated with lower cover and there was a *suggestion* of an optimum at bed and 0.6 velocities of ~0.4 and 0.5 m/s respectively, and *Fr* of ~0.25.

When the points for individual sites were separated, the pattern of an optimum mean water velocity of 0.5 m/s emerged more clearly at three sites: Excelsior and Redcliff in the lower Waiau (Figure 3f,g), and Key Bridge in the Mararoa (Figure 3b,c). At the other three sites there were no clear velocity optima. Thus in both rivers, site-specific relationships were obscured when the data were combined for the whole river.

AFDM and chlorophyll *a* measures at Key Bridge and Excelsior did not strongly reflect the patterns shown by the visual index and did not reveal any clearer relationships with hydraulic variables than the visual index. For example, compare the plots of AFDM and chlorophyll *a* versus 0.6 velocity at these sites (Figure 4), with

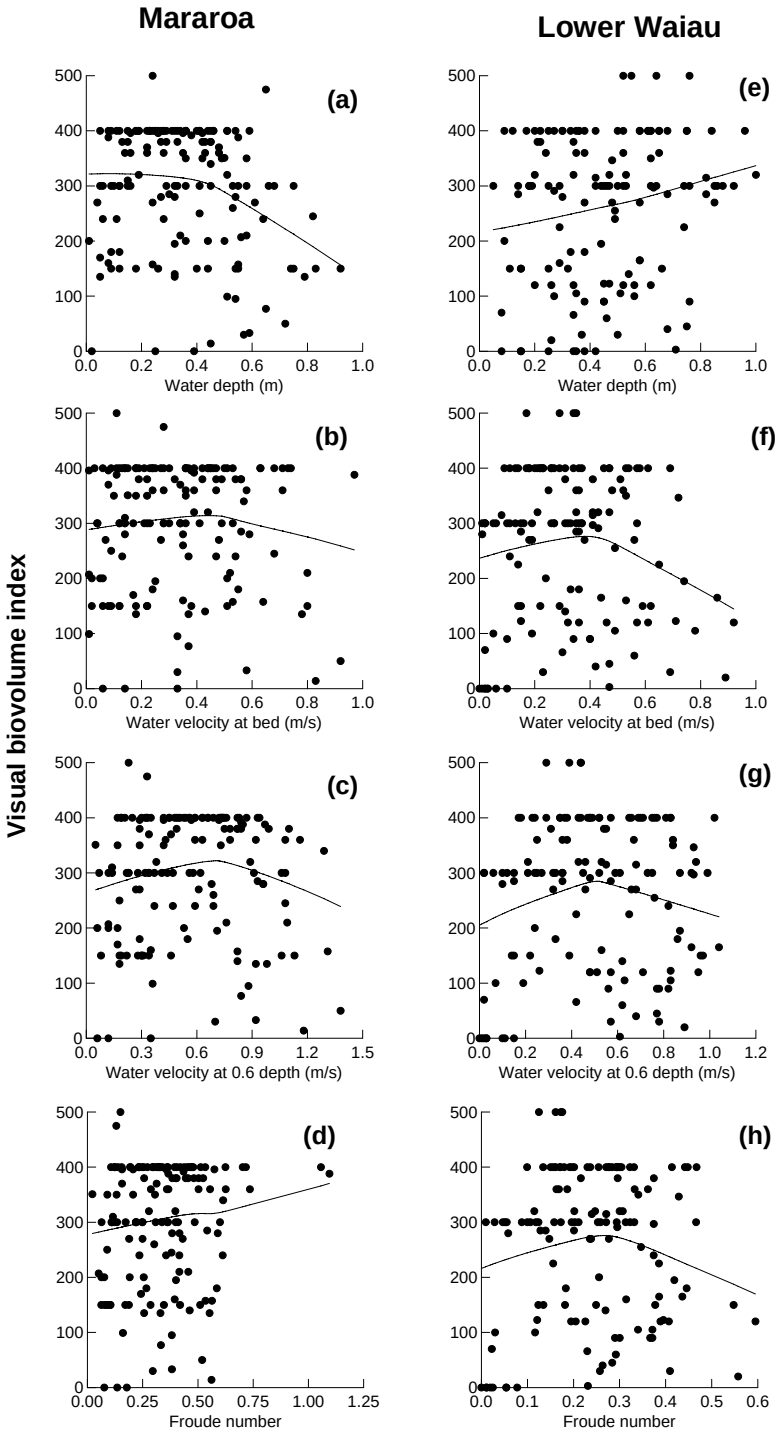

Figure 2: **Visual biovolume index plotted against four hydraulic habitat parameters, in the Mararoa and lower Waiau Rivers. Points from three sites in each river are combined. Lines are locally weighed average smoothing lines (*x*-values predicted from local *y*-values).**

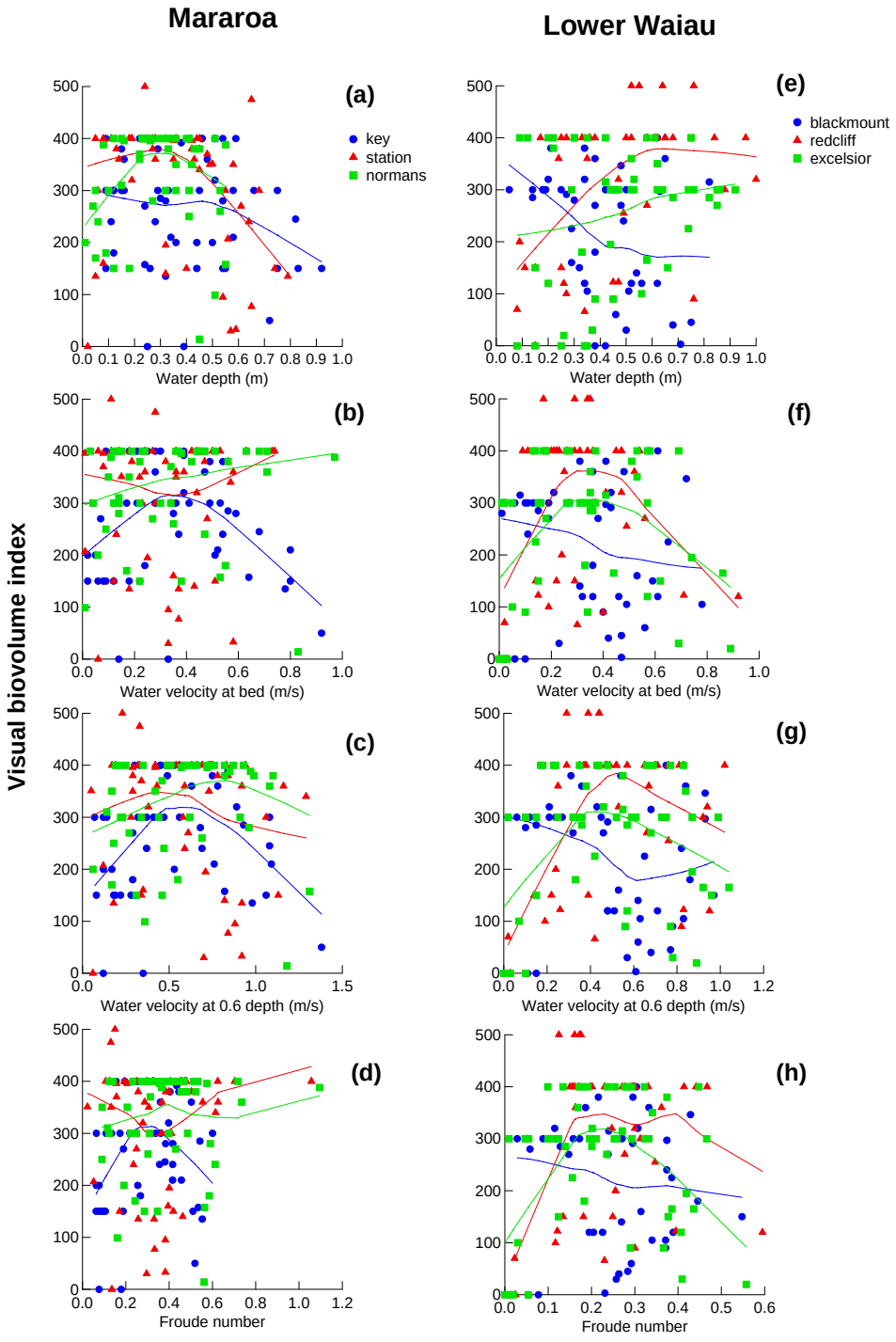

Figure 3: **As Figure 2, but with individual sites overplotted and smoothing lines fitted for each site.**

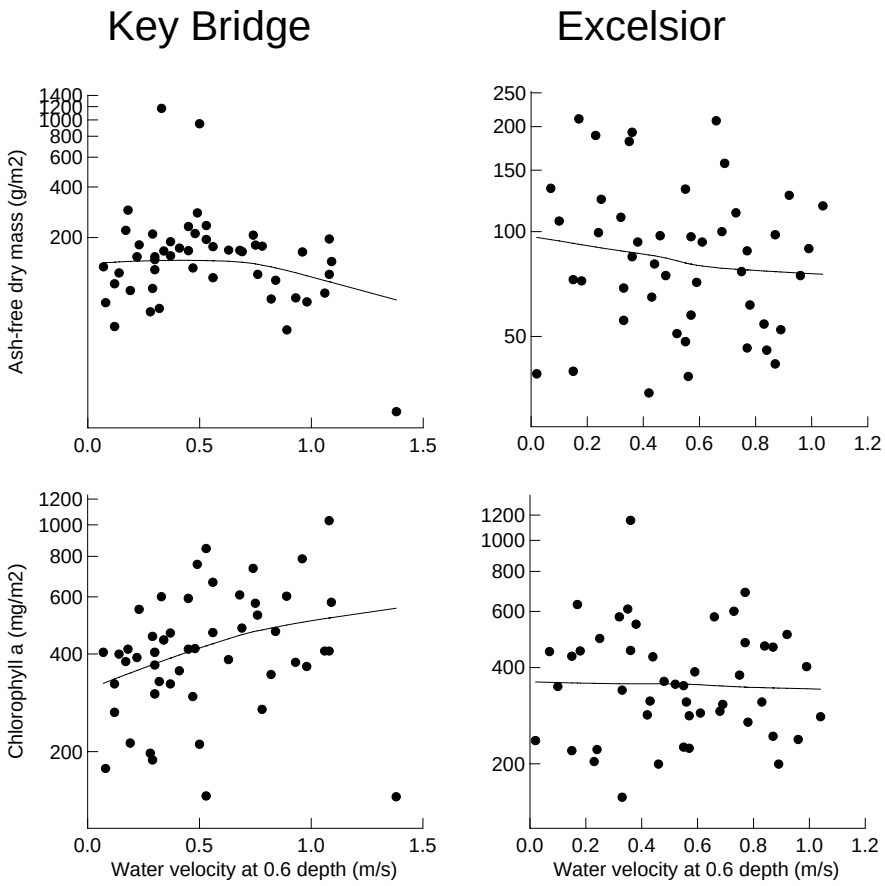

Figure 4: **Relationship between mean water column velocity (0.6 velocity) and quantitaive biomass values (AFDM and chlorophyll *a*) at Key Bridge (Mararoa) and Excelsior (lower Waiau). Lines as in Figure 2.**

Figure 3c,g). For plots of AFDM and chlorophyll *a* versus all parameters, refer to Appendix A, Figures A2, A3.

The visual biovolume index was significantly correlated with AFDM at both sites, and with chlorophyll *a*, at Excelsior only. However, the variation in measured AFDM or chlorophyll *a* explained by the visual index was small (R^2 values shown in Appendix A, Figure A4).

Stone surface area may also be relevant because of the relationship between stone size and probability of being moved in high flows. In other words, large stones are more stable than small stones and may be expected to develop higher periphyton biomass, on average, because of less exposure to physical abrasion as a result of stone movement. Regression of the visual biovolume index, AFDM and chlorophyll *a*

versus stone surface area on a river-wide and site-wide basis showed no significant relationships and no sign of trends (data not shown). It was therefore assumed that stone size (within the range sampled) was not an important influence on *D. geminata* cover at the study sites.

3.4.4. Other algae

Overall, hydraulic habitat preferences for the other algal groups identified visually were much better defined than those for *D. geminata*, even when the relationships were developed for the whole river (data from three sites combined). In the example shown for 0.6 velocity (Figure 5), *Cymbella* slime (red spots) clearly grew only in the lower velocity areas of both rivers. While green filaments (turquoise squares) were distributed across the whole range of velocities, at high velocities the coverage was invariably very low. In the lower Waiau River, thick mats (blue triangles) were generally confined to the mid range of water velocities and *Fr*, while thin black/brown films (green triangles) were more broadly distributed. It was also evident that neither thick mats nor thin films were recorded in very shallow water (<0.2 m depth). Thick mats were not recorded in water with mean water column velocity (velocity at 0.6 depth) <0.3 m/s, or *Fr* < 0.1. Refer to Appendix 1, Figure A5, for the complete set of relationships.

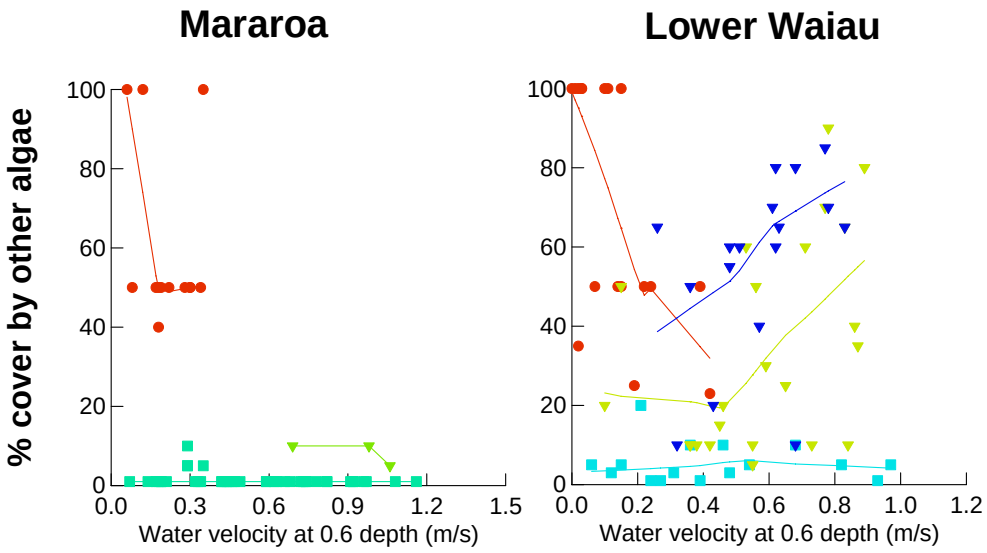

Figure 5: **Percentage cover of sampling points (stones) by algae other than *D. geminata* plotted against 0.6 velocity in each river (combined data from 3 sites). ● slimy diatom mats (*Cymbella*); ■, ■ green filaments; ▼ thick black/brown mats; ▼ thin black film. Lines as in Figure 2.**

3.5. Discussion

The most striking feature of the above results was the very broad range of hydraulic habitat conditions under which *D. geminata* attained high biomass in both the Mararoa and lower Waiau Rivers. Superimposed on this, at some sites, was a pattern of optimum cover (measured using a visually assessed index) at water velocities of around 0.5 m/s. It is difficult to determine why this relationship was apparent at only three of the six sites sampled (Key Bridge, Excelsior and Redcliff). A comparison of physical characteristics among sites indicated that the most upstream site – Normans Gulch – differed from most other sites in Froude number, and also tended to have shallower water and smaller substrate, indicating a slightly steeper gradient at that site. Why this should influence any optimum velocity for *D. geminata* growth is unclear, and it is likely that some other factor was involved. The absence of the pattern at Station Bridge and Blackmount is likewise difficult to explain with the information currently available.

Even at those sites with an apparent optimum water velocity for *D. geminata* visually assessed cover, there was considerable growth over the whole range of hydraulic habitat sampled, including in shallow, slow-moving water. This also applied to AFDM and chlorophyll *a*, which were measured at Key Bridge and Excelsior. Our hypothesis that visual cover may have obscured patterns of reducing (or increasing) habitat suitability (as indicated by the quantitative measures) was not supported by the data: no consistent clearer relationships were evident from the measured parameters (AFDM and chlorophyll *a*) than from the visual index, when compared site by site.

The measured biomass parameters at two sites were only weakly correlated with the visually assessed index. One possible explanation considered for the absence of strong relationships was that assessment of the cover of *D. geminata* over a whole stone includes areas that are exposed to a different microhabitat than that at the top of the stone (from where the quantitative sample was taken). If this was the case, then we would expect that the quantitative samples would show much clearer correlations with hydraulic factors (assuming that *D. geminata* is responding to these factors), than the visual estimates. As indicated above, no such clear pattern was evident.

The data also suggested other river- and site-specific responses to the hydraulic parameters.

Water depth. In the Mararoa River, the visual index in general declined with water depth, particularly in the range >0.6 m (Figure 4a). In the lower Waiau, there was a slight tendency for the visual index to increase with depth (and also chlorophyll *a* at Excelsior). The difference between the rivers may be a result of more areas of slow-

moving deep water in the lower Waiau than in the Mararoa River as a result of the lower overall gradient in the downstream reaches of this river system.

Water velocity. Although there was a peak for the visual index at a water velocity around 0.5 m/s at three sites, this was not necessarily reflected by the measured biomass data (as indicated above). Thus, at Key Bridge (Mararoa – Appendix A, Figure A2), chlorophyll *a* increased with velocity across the whole range, which suggests denser cell concentrations at the higher velocities. In contrast, in the lower Waiau (Excelsior), both visual index and chlorophyll *a* levels tended to be lower at bed velocities >0.6 m/s (Appendix 1, Figure A3). There was no correlation between velocity and AFDM at either site. A possible explanation might be that increased nutrient flux at higher velocities may lead to higher cell concentrations in the Mararoa River, because nutrients are limiting at lower velocities. In the more nutrient-rich waters of the lower Waiau (see Section 4 below) nutrients may not be limiting at any velocity, and a slight decrease in chlorophyll *a* at high velocities may be a direct response to hydraulic factors. Biggs et al. (1998) reported this type of relationship for benthic stalked diatom communities in the Mataura River: both AFDM and chlorophyll *a* increased with near-bed velocity in one reach, but there was no relationship in a more enriched reach.

Froude number. Since *Fr* combines both depth and velocity, it may be expected to be a better descriptor of hydraulic habitat than depth or velocity alone. Biggs and Hickey (1994) found that *F*r was the best predictor of AFDM of a benthic diatom community in the Ohau River, with AFDM increasing with *Fr*. They found no correlation with chlorophyll *a*. In the present study, Froude number was a better predictor of visual index in the lower Waiau River than in the Mararoa (Figure 2d,h), but the index tended to *decline* at high Fr values, rather than increase. This was also the case for AFDM and chlorophyll *a* at Excelsior, though neither trend was significant (Appendix 1, Figure A3d,h). Other studies have found such a negative relationship between biomass and current velocity (e.g., Bourassa and Cattaneo 1998). A slight decline was evident from the Key Bridge AFDM data only, while the trend for chlorophyll *a* was upwards.

Consideration of the results for other algal groups distinguished in the visual assessments helps to place the results for *D. geminata* in perspective. All four groups show relatively clear relationships with the four hydraulic parameters (Appendix A, Figure A5). It is possible that their distributions were being influenced by the presence of *D. geminata*, for example, through competitive exclusion in certain conditions. Nevertheless, they highlight the remarkable lack of such clear relationships for *D. geminata*.

It should be stressed that our measurements of *D. geminata* AFDM and chlorophyll *a* are up to ***an order of magnitude higher*** than those reported in similar studies (Table 1). Such large biomass values and extensive cover may lead to processes that differ from those in periphyton of more "normal" proportions. For example, very thick, persistent cover may have a positive feedback effect of reducing the ability of water flows to move stream bed particles (because they are coated with a continuous layer of algae), thus enabling cover to expand into and persist in even more extreme hydraulic conditions.

Table 1: Minimum and maximum biomass values for periphyton cover in the present study compared with values recorded in similar previous studies.

		AFDM (g/m^2)		Chlorophyll *a* (mg/m^2)	
Study	**River**	**min.**	**max.**	**min.**	**max.**
This study	Mararoa River	18	1171	145	1029
This study	lower Waiau River	34	210	157	1155
Biggs and Hickey (1994)	Ohau River	10	63	~2	~55
Bourassa and Cattaneo (1998)	Quebec Streams	2.4	22.6	5.1	54.6
Biggs et al. (1998)	Mataura River Waiau River	~2.5 -	45 -	- ~0.3	- ~200

Although this study identified an optimum water velocity of around 0.5 m/s for visually assessed cover by *D. geminata* at some sites, the results in general confirmed observations that *D. geminata* will thrive in a wide range of hydraulic conditions. In both rivers, a decline in cover and biomass appeared to be starting as the limit of safe sampling was approached, but the effect was subtle within the range sampled. *D. geminata* colonies were observed to be consistently absent in only very fast-flowing water where the substrate is likely to be continually mobile. In other words, it seems that *D. geminata* is capable of growing almost everywhere in rivers where the substrate is not constantly unstable.

In relation to control measures, if flow manipulation is to be considered as part of a strategy to reduce biomass, then only extremely high flows capable of moving the substrate in a substantial proportion of the river are likely to be effective in reducing overall biomass. Rough estimates of the flows required to *start* reducing *D. geminata* biomass were made following a programme of visual monitoring over the summer of 2004 – 2005 at three sites in the lower Waiau (Kilroy et al. 2005b). The estimates – made for just 2 sites – were ~100 m^3/s (6 x median flow) at Excelsior, and 150 m^3/s (1.5 x median flow) much farther downstream at Clifden. No estimates have been made for any sites in the Mararoa River (but see Section 4 of this report). It is possible that an increase in water velocities in areas that are normally slow-moving may have

the effect of stimulating growth. In this case, a combination of flow manipulation with other control methods would be essential.

Finally note that this survey took place in mid-winter. Observations by the sampling team suggested that growth was as prolific as it had ever been since the species invaded the rivers. Highest growth in cold conditions is consistent with the taxonomic literature, which cites *D. geminata* as a cold-water species (Kilroy 2005c). Some overseas reports suggest highest growth in warmer temperatures (e.g., Reiberger 1991), but definitive data are lacking. In the case of the Mararoa – lower Waiau infestation, further observations in warmer temperatures may show up clearer, or different, responses to hydraulic parameters, and possibly different levels of biomass. For a complete picture of these within-reach patterns, a repeat survey in summer may be desirable.

4. Temporal changes in *Didymosphenia geminata*

4.1. Introduction

Although hydraulic factors are usually important for explaining the spatial distributions of periphyton in rivers at a within-reach scale, those factors fluctuate widely over time as river flows change. Thus, the periphyton biomass present in a stream at any given time, and the composition and health of the periphyton communities, depends very much on recent flow history (Biggs 1996). Other factors influence biomass and community type, particularly nutrient levels (dissolved nitrogen, phosphorus and silica), water temperature, and light availability. These factors also fluctuate over time to varying degrees.

The aim of this second component of the ecological studies on *Didymosphenia geminata* was to examine changes over time in periphyton biomass and community composition in the Mararoa and lower Waiau Rivers and to relate them to river flows, water quality and other physical factors, at a between-reach scale. The study was expected to contribute to identification of the combination of factors that are most and least favourable for *D. geminata* growth. This information, in turn, should assist in the determination of times when the community is most susceptible to losses as a result of control measures (i.e., lowest biomass or least healthy). In addition, the results may also contribute to determining the feasibility of control measures by providing quantitative biomass assessments at different times of the year and following different flow conditions.

This report summarises the results of four sampling runs undertaken on 25–27 May, 15–16 June, 7–8 July and 8 August 2005. To ensure that samples collected at different times were strictly comparable, the surveys were undertaken at approximately the same flow, viz. minimum flow (16 m^3/s) in the lower Waiau, and between the median and lower quartile of flow (~20 – 25 m^3/s, in the Mararoa. All surveys were undertaken at the six *D. geminata*-affected sites in the Mararoa and lower Waiau, the same sites as those used for the hydraulic habitat survey.

4.2. Methods

4.2.1. Field collections

On each sampling occasion, and at each site, five stones were collected from each of three water velocity categories: 0 – 0.3m/s, 0.31 – 0.7 m/s, > 0.7 m/s.

For each stone, the procedure was similar to that undertaken in the hydraulic habitat survey (see Section 3.1.1). Briefly:

- water depth at the sampling point was recorded;
- water velocity (near bed and at 0.6 depth) was measured;
- Froude (*Fr*) number was calculated for each sample (see Section 3.1.1);
- the cover of the exposed part of the stone by algae was estimated (as a percentage) and thickness category noted;
- a quantitative sample was collected by scraping algae from a 62-mm diameter circle;
- stone dimensions were recorded, and stone surface area calculated using the formula in Section 3.1.1.

At each site, two 250 ml water samples were collected from moderately flowing water for analysis of dissolved silica and nutrients. In addition, a 2 or 4-litre water sample was collected for determination of suspended *D. geminata* in the water (see Appendix D). Water temperature, pH and conductivity were recorded using a field meter.

On the first sampling occasion, a temperature logger was installed in each river (at Station Bridge and Redcliff), to record water temperature every 30 minutes. Data were downloaded on 7–8 July and the loggers returned to the river.

A supplementary objective of the fourth survey in the series was to try to improve on the visual assessment method used for the hydraulic habitat survey. As noted in Section 3.3.3, the visual biovolume index was significantly correlated with AFDM, but the proportion of variance explained was low. If a quick method that better predicted biomass was developed, then it would be feasible to track *D. geminata* biomasss in relation to flows using field-based data collection only, saving time and csots.

For a more quantitative assessment of cover, instead of assigning a mat thickness category (Section 3.1.1), the thickness was measured to the nearest mm for mats <10 mm thick, and thereafter to the nearest 5 mm (occasionally less). The maximum thickness in the August survey was 40 mm, so this system yielded at least 16 thickness categories against the six used in the hydraulic habitat survey. The new "quantitative visual index" was again the product of thickness and %coverage, with a maximum value of 4000 (though the maximum depends on the greatest thickness measured).

4.2.2. Hydrological data

The two flow recorders relevant to these sampling sites are Mararoa at Cliffs and lower Waiau at MLC (both part of Meridian Energy's monitoring system). Mean, median and maximum flows were determined for the two-week period prior to each sampling occasion. We also extracted from the flow records the number of days elapsed since flood of nominated sizes in each river, up to 100 m^3/s in the Mararoa, and 200 m^3/s in the lower Waiau. These flows represent relatively large floods in the two rivers, and over the past 2.5 years have been exceeded 5-6 times each year. The aim was to try to identify flow parameters that are correlated with *D. geminata* biomass, which would help determine the flow levels that effectively remove biomass.

4.2.3. Laboratory analyses

The algal samples were analysed for ash-free dry mass (AFDM) and chlorophyll *a* using standard methods (Biggs and Kilroy 2000, and see Section 3.1.2).

During the filtering process, pooled subsamples of the 5 replicates from each velocity band were made up for analysis for the relative abundance of algal taxa. Subsamples were examined at magnifications up to x400 under an inverted microscope and the algal taxa present were identified and listed. For each taxon we assessed by eye its relative abundance in the sample on a scale of 8 (dominant) to 1 (rare). For details of the method, see Biggs and Kilroy (2000).

Duplicate water samples were filtered in the laboratory, then analysed for soluble nitrogen and phosphorus, and soluble silica. The additional sample collected for determination of suspended cells was filtered and examined (see Appendix D).

4.2.4. Data analysis

In addition to AFDM (g/m^2 - total organic content of a sample) and chlorophyll *a* (mg/m^2 – amount of live algal material in the sample), the following biomass indices were calculated:

- AFDM:chlorophyll *a* ratio: this is the proportion of organic matter to live algal material in a sample. In the case of *D. geminata*, it indicates the relative proportions of stalks to live algal cells.

- Organic material (%): This is calculated from the ratio of AFDM to total dry weight, and indicates the proportion of silt trapped in the periphyton. High silt content (i.e., low % organic) indicates poor food quality for invertebrates.

- Phaeophytins (mg/m^2): phaeophytins are pigmented degradation products of chlorophyll *a* and are used as an indicator of algal scenescence (Petersen and Stevenson 1992). Phaeophytin concentration was calculated from the absorbance readings during chlorophyll *a* analysis, by applying the formula given in Biggs and Kilroy (2000, p. 82).

We first examined stone area, water velocity, depth and Froude number data as a check on the homogeneity of sites. Data from the hydraulic habitat study indicated that the most upstream site, Norman's Gulch, differed from the other sites in depth and Froude number (see Appendix A, Figure A1). In this study, sample selection from defined velocity bands was aimed at standardising velocity over all sites. Analysis of variance (ANOVA) was used to determine whether mean depth, Froude number or stone surface area differed among sites, using combined data from all four sampling occasions. If marked differences among sites were found, then these would need to be taken into account when comparing biomass and cover.

Differences in mean biomass values among groups of samples (by site or river) were compared using ANOVA. A correlation matrix was used too look for significant relationships between biomass and these environmental variables. Significance was taken to be a 5% chance or less that the correlation was due to chance ($P < 0.05$), although it was recognised that when running a large number of comparisons using the same biomass data there is a chance that a correlation may occur by chance. Therefore

a much lower probability than 5% is desirable to indicate a convincing correlation. Typically the value used is 5% divided by the number of relationships tested on the same data. Regression analysis was used to check individual relationships. Correlations were run using all data (six sites), followed by data for each river. Broad-scale environmental variables available were: soluble nutrients (nitrogen, phosphorus and silica, one mean value from each site on each sampling occasion), hydrological variables (values for each river on each sampling occasion), and water temperature (mean for each river). The regression analyses were run using data for the first three sampling occasions (i.e., over the period during which there were no large floods in the river), followed by data for all four sampling occasions. We expected stronger relationships between biomass and water chemistry variables in the former analysis than in the latter, and stronger relationships between biomass and hydrological variables in the latter analysis than in the former. Data were transformed as necessary to comply with the requirement of the analyses for a normal distribution.

River and site differences in periphyton community composition were illustrated using the multivariate technique of non-metric multidimensional scaling (NMDS). This technique is based on a matrix of similarity measures calculated for all combinations of samples. The ranked similarity measures are plotted in two dimensions such that the relationships between the ranks are retained as closely as possible, i.e., the most similar samples are closest together and the most dissimilar samples are farthest apart.

Regression analysis was used to investigate relationships between the new "quantitative visual index" (determined during the fourth survey only) and biomass measures.

4.3. Results

4.3.1. Hydrology

Recorded flows for the sampling period, from 5 May, are shown in Figure 6, and flow statistics for each recorder in Table 2. Small freshes preceded each survey, except for the most recent one (August) which followed a more substantial flood.

4.3.2. Temperature

Water temperatures measured at two sites over the sampling period are shown in Figure 7. Mean temperature in the Mararoa was >0.5 ºC lower than in the lower Waiau (5.91 vs. 6.45). A different pattern was evident in the two rivers, with the Mararoa having much larger diurnal variation than the lower Waiau (but see Discussion).

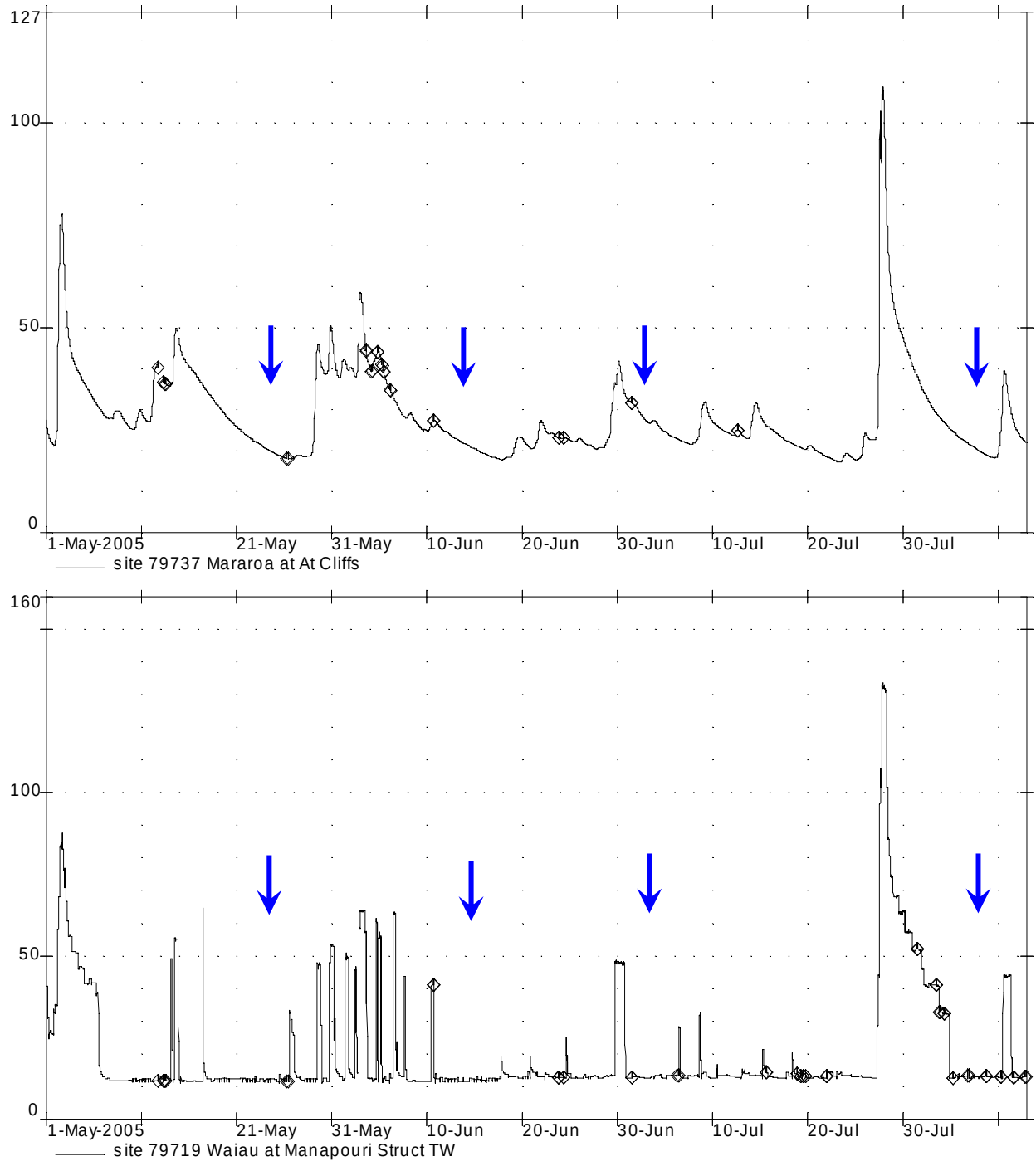

Figure 6: **Flow records for the Mararoa (top) and lower Waiau at the MLC (bottom) over the sampling period. Blue arrows indicate sampling occasions.**

Table 2: **Flow statistics for the two-week period prior to each sampling, for the two flow recorders, and times since nominated flows (mean flows for at least 12 h).**

		Flows (m^3/s) over the 2 weeks before sampling			Days since flows (m^3/s) of:			
Recorder	**Date**	**Mean**	**Median**	**Maximum**	**40**	**50**	**75**	**100**
Mararoa at Cliffs	25 May	32	31.5	50	10	23	75	76
	14 June	32.5	29	59	10	12	95	96
	3 July	26	23	42	4	31	114	115
	8 August	35	27	109	7	8	9	10
					60	**75**	**100**	**200**
lower Waiau at MLC	24 May	14.5	12.5	65	21	21	68	72
	15 June	19.5	12.5	65	11	42	89	93
	3 July	16	13	49	29	61	107	111
	8 August	38	32.5	134	9	11	11	146

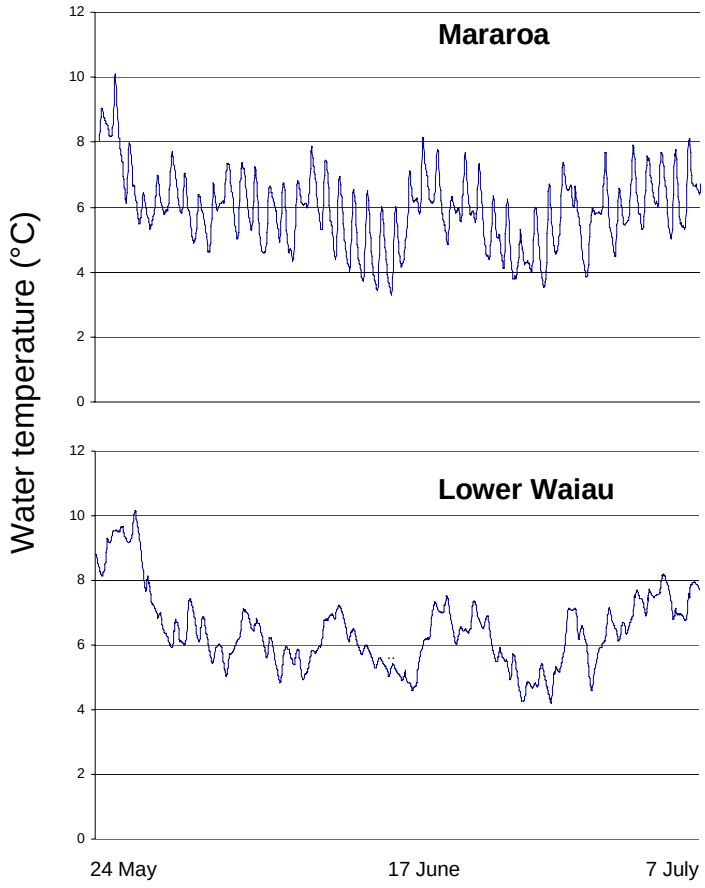

Figure 7: **Temperature records from 24/5 May to 7 July in the Mararoa River (Station Bridge) (top) and the lower Waiau (Redcliff) (bottom).**

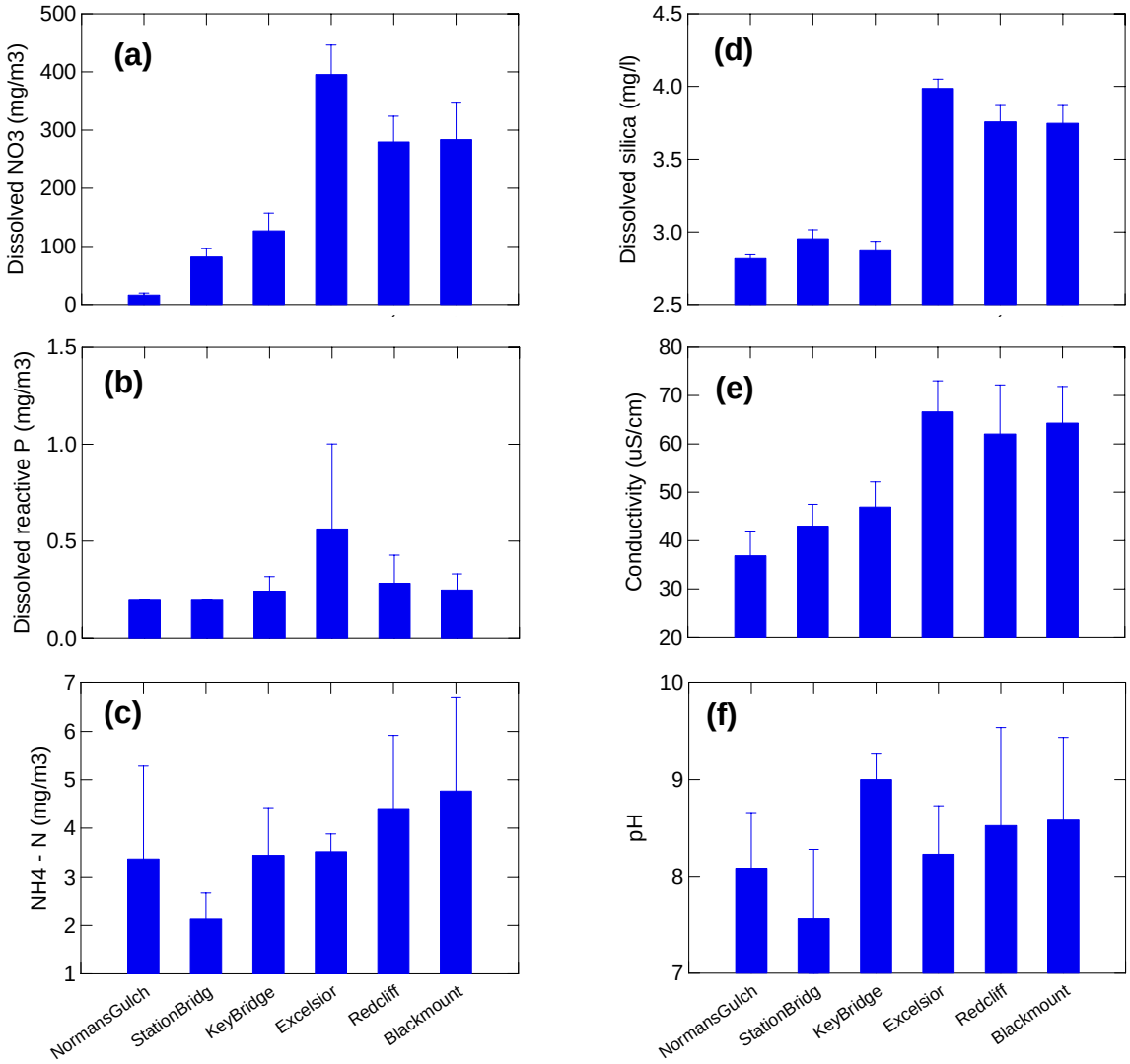

Figure 8: **Mean dissolved nitrogen (NO_3), dissolved reactive phosphorus, ammonia, dissolved silica, conductivity and pH measured at the six sites, with standard deviations, over three (pH, Si) or four sampling occasions.**

4.3.3. Water chemistry

Both dissolved nitrogen and dissolved phosphorus were mostly in inorganic form (DRP and NO_3 respectively) and varied significantly among sites (Figure 8a,b). DRP was in general low and below detection limits (0.5 mg/m^3) at the upper two sites in the Mararoa. For NO_3, there was a striking increase in a downstream direction (Figure 8a). Values measured in the Mararoa were low, contrasting with moderately high values on a national scale in the lower Waiau, e.g., with potential for eutrophic conditions given sufficiently long low flow periods over which periphyton can accumulate (Biggs 2000a). Ammoniacal nitrogen (NH_4) values were generally higher in the lower Waiau than in the Mararoa (Figure 8c), but quite variable over time (note the large error bars). In both rivers they were well within the range for which there is considered to

be a low risk of biological effects (Davies-Colley and Wilcock 2004). N:P ratios (dissolved inorganic N: dissolved reactive P) were high, ranging from 62 to 1774. N:P ratios greater than 10 have often been used to indicate limitation of periphyton growth by lack of phosphorus, although there has been debate about the reliability of this guideline (Francoeur et al. 1999). Dissolved silica was also significantly higher in the lower Waiau River than in the Mararoa (Figure 8d). Conductivity mirrored the pattern of increasing nutrient in a downstream direction, though the difference between the two rivers was less pronounced (Figure 8e).

There have been insufficient sampling occasions to determine whether there is any pattern of changes in nutrient levels over time.

Measured pH values were variable, as indicated by the large error bars (Figure 8f). This is to be expected when values are measured at different times of the day in a river containing high biomass of photosynthesising material (Davies-Colley and Wilcock 2004). Measurements taken in mid- to late afternoon were often relatively high (8.5 to >9). Note that the measurements made in August are excluded as some very low values recorded were suspected to result from a faulty meter.

4.3.4. Physical environment

The temporal data confirmed the impression from the hydraulic habitat study that the six sites were reasonably homogeneous with respect to local hydraulic variables. There were a small number of significant differences between individual sites (see Appendix B for details), but overall the sites were similar, such that any biomass differences would not be simply a result of different hydraulic conditions.

4.3.5. Periphyton biomass

Between-river comparison

For data averaged over the three sites in each river, and over four sampling occasions, the Mararoa River had significantly higher AFDM, AFDM:chla ratio and phaeophytins ($P < 0.001$ in all cases) than the lower Waiau, but the between-river difference in chlorophyll *a* was less marked. The same pattern was evident on each sampling occasion (Figure 9). Mean biomass values were extremely high: compare the mean values with the current New Zealand guideline for maximum chlorophyll *a* for maintenance of trout habitat and angling values in rivers, of 200 mg/m^3, and AFDM 35 g/m^3 (Biggs 2000; levels indicated on Figure 9a,b with green lines).

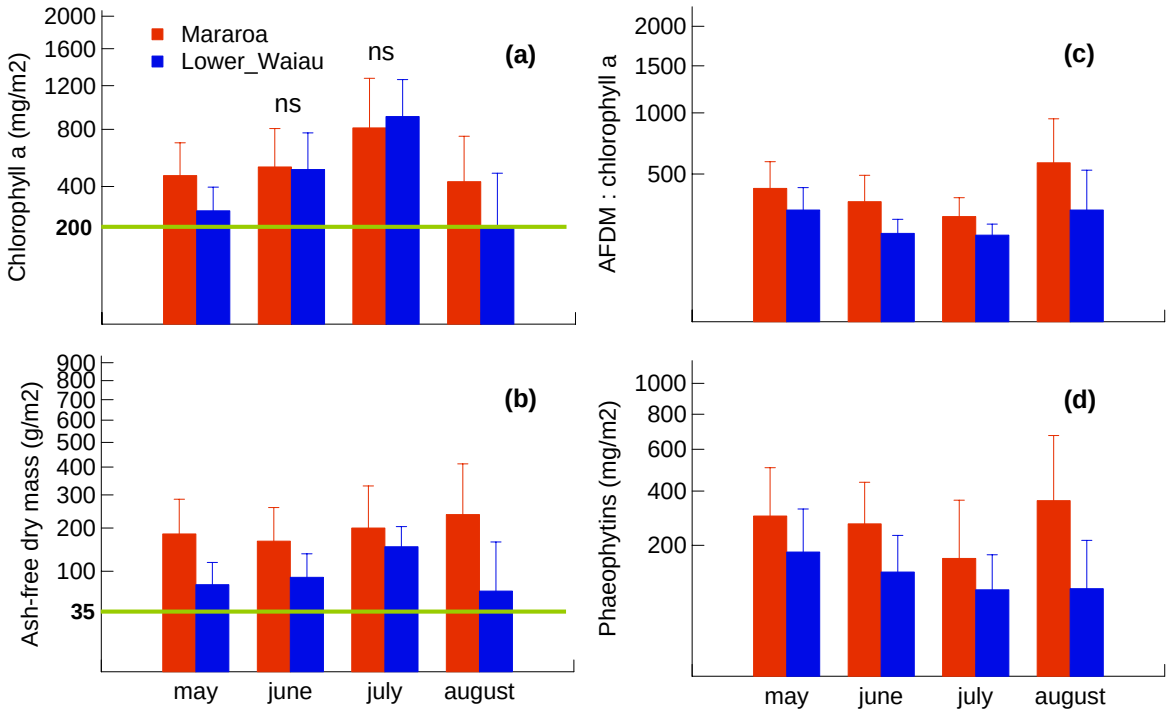

Figure 9: **Between-river comparison by sampling occasion, for chlorophyll *a* and AFDM. Bars are mean values for all three sites, with standard deviations shown. All between-river comparisons differ significantly (P < 0.001), except for those marked "ns" in (a). The green horizontal lines indicate MfE maximum recommended biomass levels in the current New Zealand guidelines for maintenance of trout habitat and fisheries values in streams. Note that the vertical scales are not linear.**

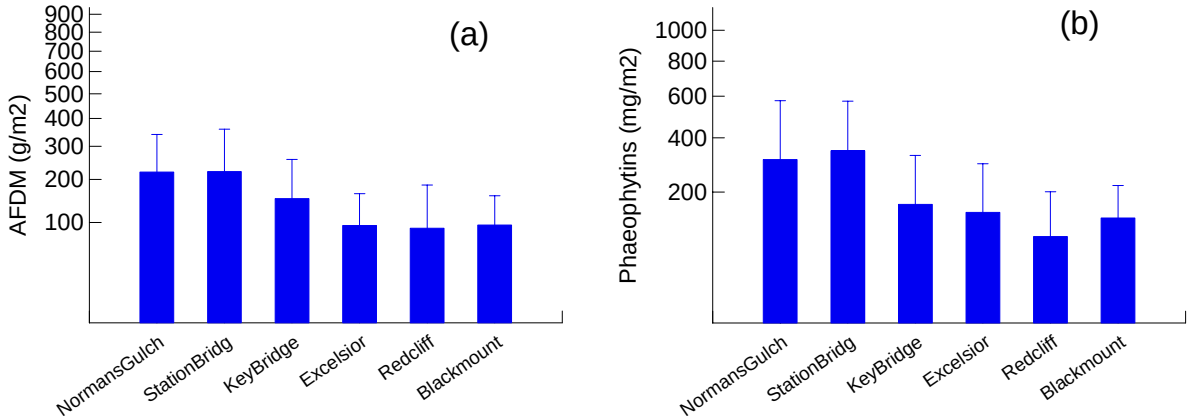

Figure 10: **AFDM (a) and phaeophytins (b) in general declined in a downstream direction. Data from all four sampling occasions, showing standard deviations.**

In both rivers, chlorophyll *a* increased between the May, June and July surveys, when there were no major floods. Chlorophyll *a* fell significantly between the July and August surveys, following the high flows on 28-29 July (Figure 6), but only to levels similar to those recorded in the first survey in late May. A similar pattern for AFDM was seen in the lower Waiau, but in the Mararoa, mean AFDM did not change significantly over all four surveys. Analysis of covariance showed that the rates of increase of both chlorophyll *a* and AFDM from May to July were significantly higher in the lower Waiau River than in the Mararoa ($P < 0.0001$ in both cases). The pattern is partly obscured because initial biomass in the Mararoa was higher (Figure 9a, b).

Between-site differences

The between river differences in biomass were reflected in a general downstream decline in AFDM and phaeophytins (Figure 10), and in AFDM: chlorophyll *a* ratio. There was no downstream trend in chlorophyll *a*. AFDM and phaeophytin concentration were closely correlated (all data, $R^2 = 0.513$, $P < 0001$).

Trends over time at individual sites

The pattern over time at each site in general reflected that seen at river-scale (averaged over the three sites), viz., a consistent trend or little change for the first three surveys, then a reversal of that trend between the July and august surveys, following the higher flows. As shown in Figures 9 and 11, the biomass reduction following the flood was much more pronounced in the lower Waiau than in the Mararoa. Indeed, while there was a 70% reduction in AFDM at Excelsior, there was a similar *increase* in AFDM at the most upstream site (Norman's Gulch) between the July and August surveys. A smaller increase was recorded at Station Bridge and at the most downstream site, Blackmount (Table 3).

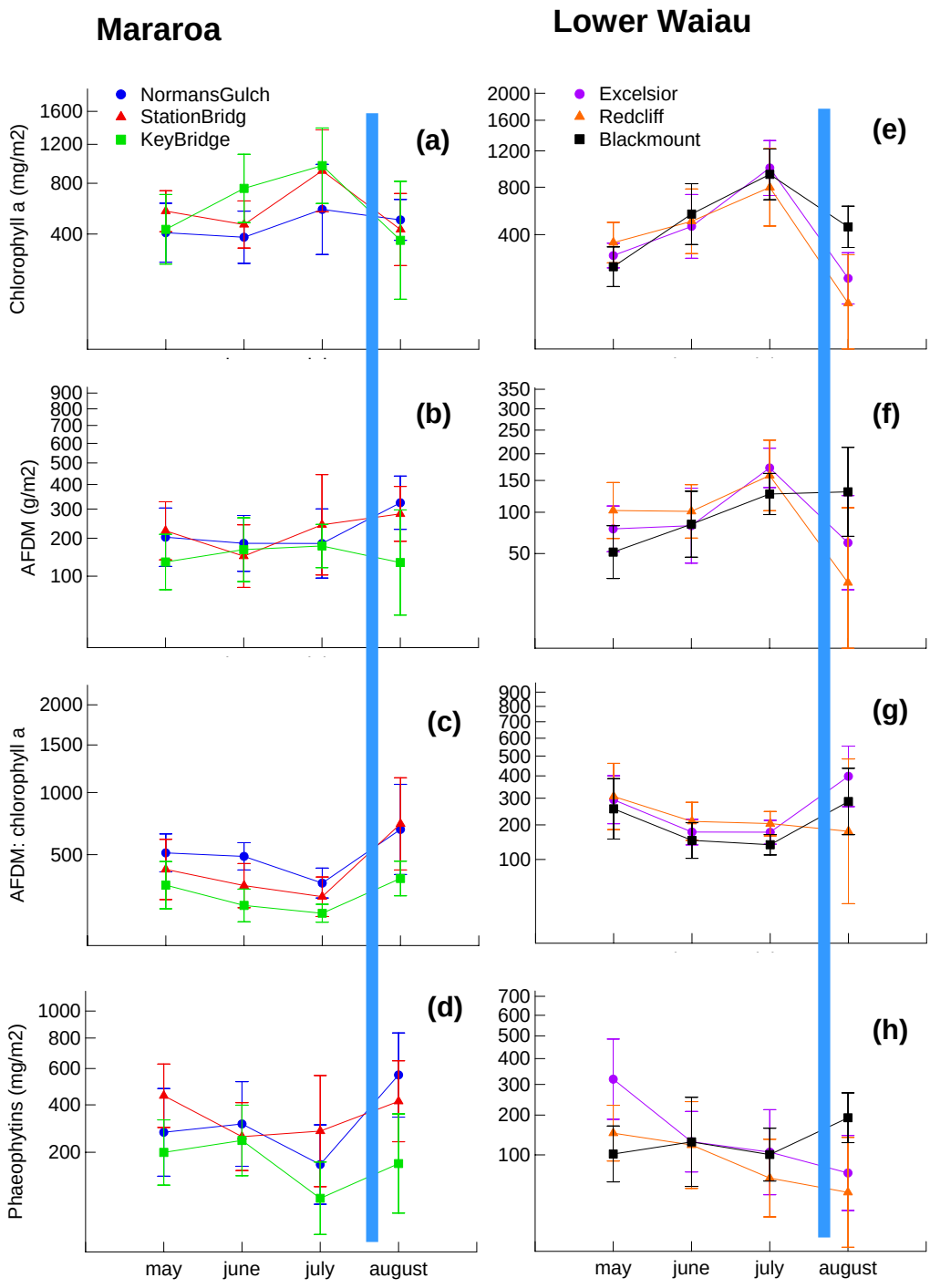

Figure 11: **Biomass parameters versus sampling occasion in each river, with the three sites plotted separately. Error bars are standard deviations. Note that the y-axis scales differ between rivers. Except for chlorophyll *a*, Mararoa values were generally higher than those in the lower Waiau. Note also the non-linear (square-root) scales on the y-axis. The vertical blue lines indicate the timing of the only substantial flood that occurred during the study (see Figure 6).**

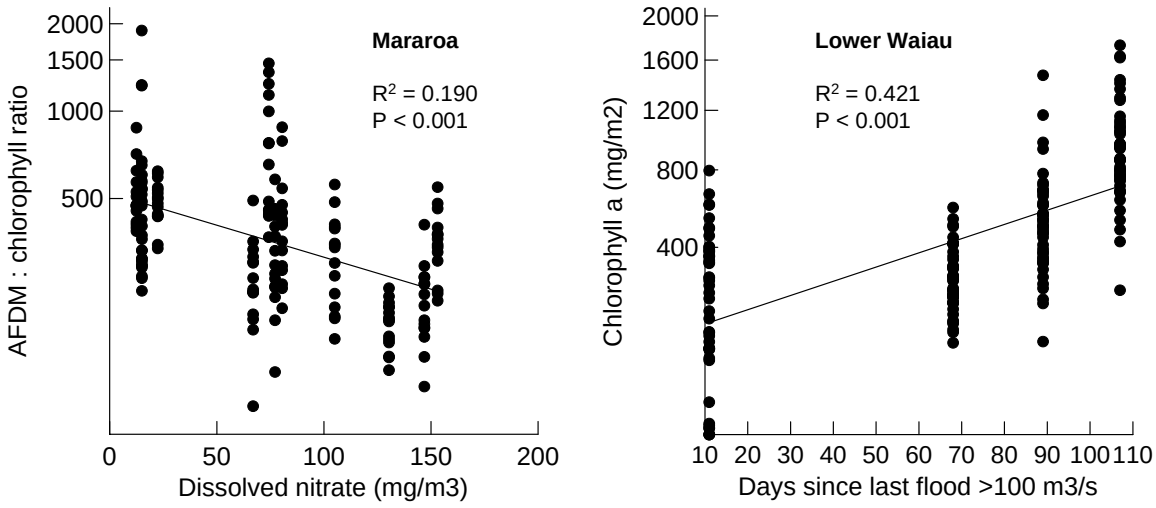

Figure 12: **Linear regression of two relatively strong relationships between *D. geminata* biomass and reach-scale environmental variables in the Mararoa and lower Waiau rivers. Note that the right-hand relationship probably better fits a non-linear function that describes logarithmic growth.**

Table 3. **Biomass responses to the flood of 28-29 July 2005 at six sites in the Mararoa and lower Waiau rivers. Surveys were undertaken on 7-8 July and 8 August. Increases following the flood are highlighted in red.**

	AFDM (g/m^2)			Chlorophyll *a* (mg/m^2)		
	July	August	% change	July	August	% change
Mararoa						
Norman's Gulch	196	332	**69**	624	507	**-19**
Station Bridge	269	290	**8**	958	460	**-52**
Key Bridge	181	160	**-12**	1003	440	**-56**
lower Waiau						
Excelsior	174	71	**-59**	1021	170	**-83**
Redcliff	164	49	**-70**	883	126	**-86**
Blackmount	129	139	**8**	949	467	**-51**

Relationships between biomass and environmental variables

The correlation matrices contained many significant relationships between reach-scale environmental variables and biomass parameters (Appendix B, Tables B1, B2). When all data were used, there were significant negative relationships associated with the general downstream increase in nutrient and conductivity (Figure 8) and the downstream decrease in ash-free dry weight and phaeophytins (Figure 10). This was most pronounced in the correlations run on data from the first three sampling occasions (the flood-free period), in which 43% of the comparisons tested were significant (Table B1, A).

When data from the two rivers were analysed separately, the correlations with biomass parameters were generally with flow variables, and tended to be stronger in the lower Waiau than in the Mararoa (Figure 12). In the Mararoa, the strongest water chemistry correlation was a negative relationship over the first three sampling occasions between the AFDM: chlorophyll *a* ratio and nitrate nitrogen (Table B1, B) . No significant relationships between biomass and water chemistry were detected in the lower Waiau.

Examination of site-specific data confirmed that the number of days since the last flood >100 m^3/s was the best predictor of AFDM and chlorophyll *a* at some sites, particularly those closer to the flow recorders. Chlorophyll *a* produced better relationships than AFDM (Figure 13).

4.3.6. Periphyton community composition

Observations during field collections suggested that the samples collected for this study comprised mostly *D. geminata* mats. The presence of other algae was noted on field sheets, but, as in the hydraulic habitat survey, this generally made up only a small proportion of the observed cover. This was confirmed by taxonomic analysis of samples from each site. *D. geminata* was assessed as "dominant" in every sample examined (Appendix B, Table B2). NMDS on the samples collected in May showed some species separation between the two rivers, but this was considered minor. Refer to Appendix B for details of the NMDS results (Appendix B, Figure B2a,b).

4.4. Suspended *D. geminata*

Water samples collected in the May and June surveys yielded a mean concentration of 238 live cells/litre river water. This concentration was largely consistent over all six sites. Refer to Appendix D for details.

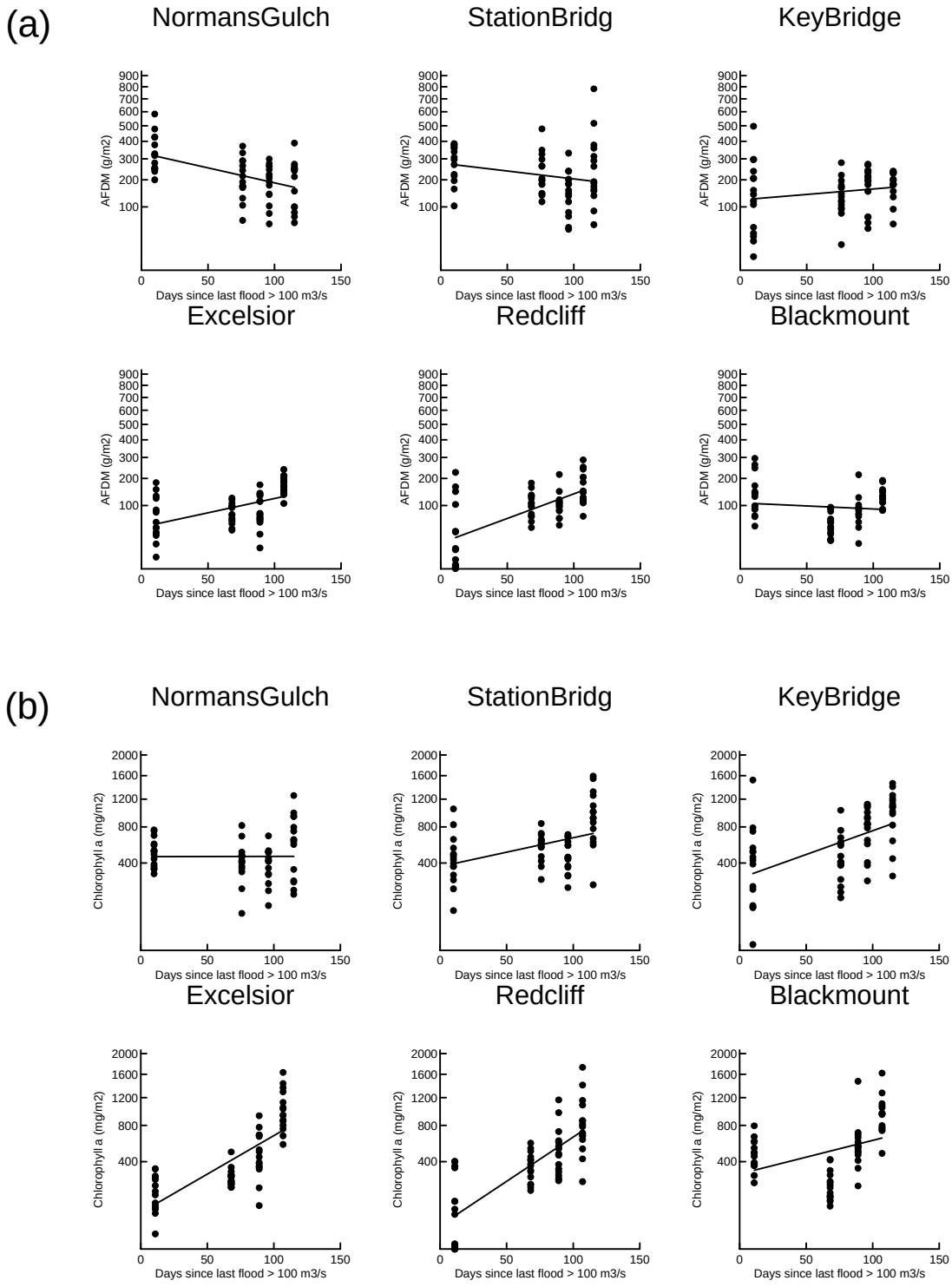

Figure 13: Relationships between days since the last flood greater than 100 m^3/s and biomass, by site. (a) AFDM; (b) chlorophyll *a*.

4.4.1. Quantitative visual index

Regression of the quantitative visual index against measured biomass parameters produced much better relationships than the original index. Compare Figure 14a below with Figure A4 (Appendix A). In particular there was an excellent relationship between the index and AFDM, with over 84% of the variance in AFDM explained by the visual index. Correlation with chlorophyll *a* was also reasonable (over 56% of the variance explained) (Figure 14b).

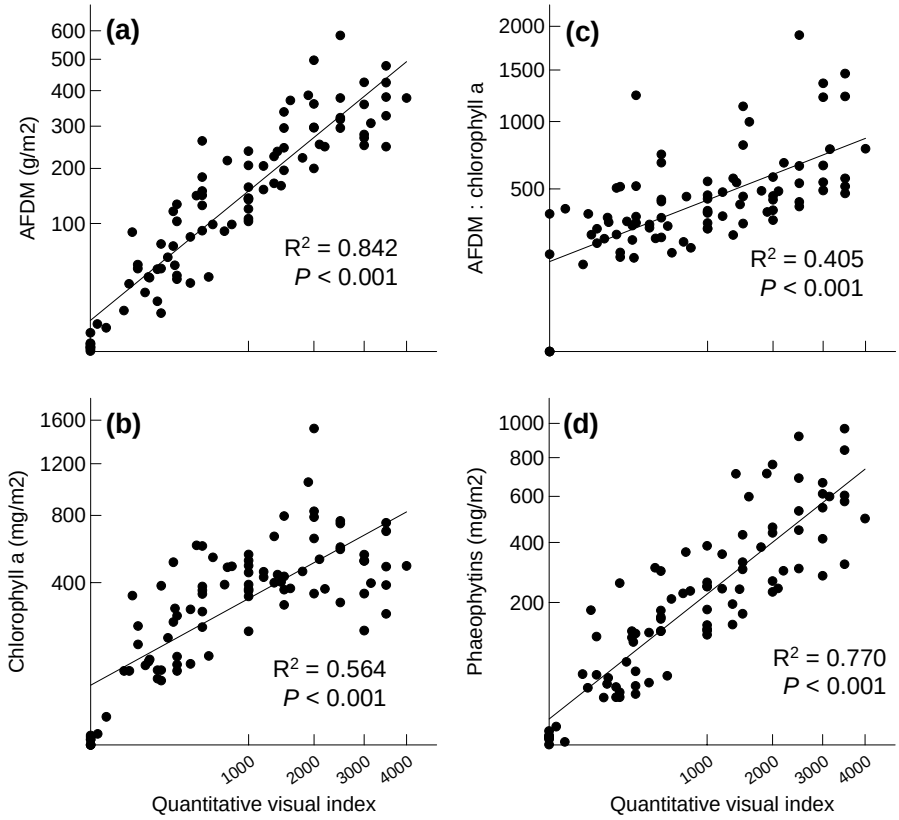

Figure 14. Relationships between the quantitative visual index trialled on the 8 August temporal survey, and measured biomass from the same sampling points.

4.5. Discussion

Several patterns of *D. geminata* biomass distributions were evident from the four surveys undertaken in this study.

1. On a river-wide scale, periphyton AFDM biomass – principally *D. geminata* – declined in a downstream direction, but chlorophyll *a* remained constant. This overall pattern was least clear in July: after 3 months with no substantial fresh, both measures of biomass reached comparable levels at all sites. The downstream decline in AFDM re-established after the flood on 28–29 July (though with higher biomass than expected at Blackmount). A downstream decline in AFDM biomass that equalized over time could be explained by lower flow variability at the upstream sites, such that biomass is less easily lost once established at peak levels. This is consistent with the increasing influence of lake inflows to the Mararoa flow moving upstream. An alternative or additional factor may be nutrient limitation in the more oligotrophic waters of the upper Mararoa (see para 5. below), although this also raises the question of why biomass was initially higher in the upper Mararoa. The lack of biomass reduction in the two upper Mararoa sites following the high flows in late July suggests that these river reaches may be more stable than the sites farther downstream (see para 4 below). Therefore the initial high biomass levels could simply have resulted from longer accrual time with no disruptive flood events. It is also possible that the slightly lower temperature in the Mararoa may be more favourable for *D. geminata* biomass accumulation. This latter hypothesis could be tested by undertaking repeat surveys under similar hydrological conditions at warmer times of the year: consistently lower biomass would support the hypothesis.

2. The downstream decline in AFDM biomass was tracked by a decline in the concentration of phaeophytins in the mat. The two indices were also closely correlated over time. This is likely due to larger, thicker growths of *D. geminata* trapping higher concentrations of scenescent and dead cells (presumably other diatom species as well as *D. geminata*). It was interesting that there was no such clear relationship between chlorophyll *a* and phaeophytins. This suggests that the areal extent, rather than the thickness, of *D. geminata* mats determines the concentration of live, photosynthesising cells, because these cells operate only at the surface of the mat.

3. Over the first three sampling occasions, with no significant flood events, chlorophyll *a* concentration increased markedly in both rivers, particularly between the June and July surveys, suggesting that the small freshes in the intervening period had no effect on growth. Flows in the period between the May and June samplings were somewhat higher, and there were fewer significant increases in biomass (only chlorophyll *a* at Key Bridge, and AFDM at Blackmount), suggesting that while the larger intervening freshes

may not have severely depleted growth, they were enough to slow down biomass accumulation.

4. An unexpected result following the flood on 28-29 July was that AFDM biomass was *higher* at three sites (Norman's Gulch, Station Bridge and Blackmount) than on the previous occasion. It may or may not be coincidence that these sites were those where biomass showed no clear relationship to hydraulic habitat factors in the hydraulic habitat study (Section 3.4). If these observations are related, then the explanation may be that the substrate at these sites is significantly more stable than that at Key Bridge, Excelsior and Redcliff. A more stable substrate over the whole reach would allow *D. geminata* to gradually colonise an area encompassing a broader range of water velocities and depths (hence no clear hydraulic habitat preferences in the range sampled). Additionally, the substrate would be less likely to mobilize under high flows and biomass loss through direct abrasion would be less likely (hence no response in AFDM to the July flood). On a river-wide scale, this means that those parts of the river with stable substrate are prone to more extensive cover by *D. geminata* over space and time.

5. The maximum biomass *loss* (AFDM) following the late July flood was 70% at Redcliff, but the amount remaining still exceeded the MfE guideline of 35 g/m^2 (Biggs 2000a). It is possible that biomass at all sites declined markedly immediately following the flood, but that the 9-10 day interval between the flood peak and the survey was sufficient time for the mats to regain some of the lost biomass. However, mats and fragments of algae were observed in the river for up to one week following the flood peak, suggesting that sloughing of material continued well after the peak (observations by BJ). The time taken for periphyton to regenerate to peak biomass following a flood depends on many factors, including flood size, nutrient supply and temperature. Reported times vary from less than 2 weeks to over 12 weeks, but are very difficult to predict (Biggs 2000a). The very low water temperatures and low supplies of dissolved nutrients in the Mararoa River would not normally be expected to favour very fast regeneration. Therefore we assume that the *increase* in biomass at Norman's Gulch occurred because the mats were not affected by the high flows, and growth was possibly stimulated through faster transfer of nutrients in higher water velcocities (Biggs & Hickey 1994). Farther down the river, especially in the lower Waiau, higher nutrient concentrations may have favoured some regeneration following biomass loss.

6. During the hydrologically benign period between the first three sampling occasions, there was a pattern of AFDM biomass continuing to increase over

time in the lower Waiau while that in the Mararoa remained stable. Chlorophyll *a* also increased in both rivers. For both biomass indices, the rate of increase was significantly higher in the lower Waiau (covariance analysis, $p<0.0001$ for both chlorophyll *a* and AFDM). The net result was that the ADFM : chlorophyll *a* ratio declined in both rivers over that period. A possible explanation for stable AFDM in the Mararoa is that at the very high biomass there, N supplies (and possibly P) were starting to become limiting. That chlorophyll *a* continued to increase could reflect diversion of nutrients to cell formation rather than stalk formation in limiting conditions. Synthesis of extra-cellular polymeric substances (EPS – of which the stalks of *D. geminata* are an example) by a related diatom *Cymbella affinis* has been shown to depend on the N:P ratio, with highest production in P-limiting conditions (N:P of 25:1 to 100:1) (see review by Hoagland et al. 1993). N:P ratios in both rivers in the present were at least 62, and generally much higher, implying P-limiting conditions. A much more focussed study would be needed to thoroughly investigate any relationship between stalk formation and nutrients. Hoagland et al. (1993) considered that much more research is need in this area, and there appears to have been little progress in the 12 years since that review.

In the present study, speculation about nutrient – biomass relationships is complicated because of the spatial (downriver) and temporal dimensions, and the interplay of many other gradients. For example, the observed weak negative relationship in the Mararoa between dissolved N and the AFDM : chlorophyll *a* ratio (Figure 12) may not be biologically significant. However, the significantly higher rates of increase of both chlorophyll *a* and AFDM in the more nutrient rich lower Waiau, strongly suggest a positive response to higher nutrient concentrations (especially nitrogen) over the flood-free period from May to July. In other words, all other things being equal, rivers with higher nutrient levels will have larger blooms. Following the flood on 28-29 July, the community was "re-set" in at least part of the river, and any temporal links to nutrient levels obscured.

7. The best hydrological predictor of biomass was the number of days elapsed since the most recent flood greater than 75 m^3/s. This worked best at Excelsior and Redcliff for chlorophyll *a*, suggesting that a flood of this size would "re-set" the community at those sites. This is in agreement with finding from an earlier study in the lower Waiau (Kilroy et al 2005b). However, it should be noted that chlorophyll *a* levels were still high even following the shortest time elapsed since a flood (10 days, the August survey). AFDM was relatively poorly predicted by all of the hydrological indices tested. None of the indices

correlated with biomass at Norman's Gulch, where biomass was consistently high (see para. 5 above). The largest flood in the study (120 m^3/s measured at Mararoa @ Cliffs) may have been a relatively minor event ~40 km upstream at Norman's Gulch. This highlights the problem of relying on a single flow recorder in a river that is affected by rainfall events in different parts of the catchment, as pointed out in Kilroy et al. (2005b).

8. Few significant relationships emerged between biomass and pH (Appendix B, Table B1), which was expected because pH measurements were made at different times of day and reflected different degrees of dissolved CO_2 uptake by photosynthesising algal mats (Davies-Colley & Wilcock 2004). In general, pH values were relatively high. For example, the long-term average for peak (mid afternoon) pH measured in the lower Waiau at Clifden is 7.7 ± 0.3, with a maximum of 8.6 (National Water Quality Monitoring Network data, NIWA). The mean value in the present study was 8.4 ± 0.8, maximum 9.3. This suggests that the presence of large *D. geminata* mats, as might be expected, has a measurable impact on pH levels, causing higher daytime peaks than normal in these rivers. Hickey & Vickers (1994) showed that a combination of pH > 9.2 and ammonia (NH_4) concentrations > 1-2 g/m^3 is toxic to some invertebrate taxa. NH_4 concentrations in the lower Waiau and Mararoa are very much lower than this (Figure 8c), therefore high pH *per se* may not significantly affect aquatic life in these river. However, the large diurnal swings in dissolved oxygen that accompany the pH changes may be a direct cause of stress to aquatic organisms.

In relation to water temperature in the two rivers, the observed difference in overall mean temperature is reasonable and seems likely to be real. However, the apparent larger diurnal variation recorded in the Mararoa may possibly be an artefact arising from the location of the two loggers. Both are in open flowing water, however the lower Waiau logger is in a lower velocity area than the Mararoa instrument. Deployment of additional loggers would be necessary to determine whether or not the records shown in Figure 7 reflect river-wide conditions.

The finding that river water flowing through sites affected by *D. geminata* contains, on average, over 240 live cells in every litre of river water is significant, confirming that the river water itself provides an important potential means for spreading *D. geminata*. Although the concentration of suspended cells may be lower along areas of the river that are not heavily affected, *any* water taken from the river could potentially hold many live cells, and not a negligible number.

Finally, the quantitative visual index trialled in the fourth temporal survey produced an excellent correlation with AFDM and a reasonable correlation with other biomass indicators. This was a marked improvement on the correlation obtained for the visual biovolume index used in the hydraulic habitat study. The method requires more time in the field than the initial method. However, if that extra time allows collection of valid information without the necessity for laboratory analyses, then it can easily be justified. Laboratory biomass analyses are objective and accurate, provide extra information on mat conditions (inorganic content, chlorophyll *a*, phaeophytins), and will always be the preferred option. However, where time and budget are limited, we suggest that use of this more detailed field technique will be an excellent alternative. Its use may be considered possible future surveys of *D. geminata* over time. In particular, since *D. geminata* appears to grow best in cold water, a repeat series of surveys over time in summer may provide valuable supplementary information. This could be achieved efficiently using this quantitative visual assessment method. If the method is used, we suggest that it should be re-validated from time to time.

5. Effects of *Didymosphenia geminata* on benthic invertebrate communities

5.1. Introduction

Anecdotal information from overseas suggests that *D. geminata* blooms may have detrimental effects on benthic stream invertebrates: some taxa typical of clean-water conditions, such as stonefly and caddisfly larvae are reduced in abundance, while densities of more tolerant taxa, such as chironomids (midge larvae) increase (e.g., Rieberger 1991 ; S. Spaulding, University of Colorado, pers. comm.). This impression has been partly corroborated by a comparison of invertebrate communities from the lower Waiau River in 2005, with communities collected from the same sites in previous years, prior to the arrival of *D. geminata* (Kilroy et al. 2005a). That comparison showed that invertebrate communities associated with *D. geminata* indeed contained much higher proportions of midge larvae and worms than in some previous years, although they were similar to those associated with high biomass of other algae. A control site in the Mararoa River, which was only mildly affected by *D. geminata*, had much higher proportions of mayfly larvae than the affected site, but this could possibly be attributed to the same conditions that prevented *D. geminata* colonizing that site (smaller substrate size, higher water velocities).

The objective of the present study was to undertake a formal comparison, using a replicated design, of invertebrate communities from affected and unaffected reaches of

the Mararoa River. The infestation of *D. geminata* starts just upstream of the confluence with Kiwi Burn (Figure 1) and has spread downstream from this point. Therefore the upstream reaches may be suitable habitat for *D. geminata*, but the alga has not yet had the opportunity to colonise there. We aimed to locate sites in this unaffected section that were similar in substrate composition and hydraulic characteristics to the downstream sampling sites, such that the main difference between upstream and downstream sites was the presence or absence of *D. geminata*.

Field collections were undertaken on 15-16 June 2005. The affected sites were at Normans Gulch, Station Bridge and Key Bridge, the three sites used for the hydraulic habitat and temporal studies. Three suitable unaffected sites were located upstream of the Kiwi Burn. The most downstream of these was approximately 150 m upstream of the start of the *D. geminata* bloom (Figure 1), and small colonies of *D. geminata* were observed on some rocks at this site. With cover of 2% or less, these colonies were considered to be too small to impact on the invertebrate communities.

The results of this study are expected to contribute to an overall assessment of the potential environmental effects of *D. geminata* on New Zealand streams and rivers.

5.2. Methods

5.2.1. Field collections

At each site, six quantitative samples of benthic macroinvertebrates were collected using a Hess sampler, from three random locations at each of 2 depths, 0.25 m and 0.35 m. The latter is the maximum depth that can be sampled without overtopping the sampler, which will lead to significant losses of invertebrates. A Hess sampler is a cylindrical metal frame (dimensions: 0.35 m diameter x 0.4 m high) covered with 50 μm nylon mesh. The sampler is driven into the streambed and oriented with the opening to a built-in mesh collecting net facing upstream (so that the water flows into it). Agitating and brushing the substrate enclosed within the sampler dislodges invertebrates and causes them to be swept into the collecting net. Invertebrates were transferred into 600 ml containers and preserved with isopropyl alcohol (final concentration approx. 50%).

Quantitative periphyton samples were collected from two replicate stones in the area immediately adjacent to the invertebrate sampling location by scraping/brushing the algae circumscribed by a 62 mm diameter lid placed over the centre of the exposed (upper) surface of each stone. Duplicate samples were pooled into a single container for analysis. At each sampling point, water depth, bed velocity and velocity at 0.6 depth were measured, as described for previous studies.

5.3. Laboratory procedures

Macroinvertebrates were separated from any algal material, gravel and sand in the samples by suspending the (lighter) invertebrates in moving water then carefully pouring off the water and invertebrates through a 300-µm mesh sieve. The sand and gravel left behind were scanned for any invertebrates remaining, which were added to the material in the sieve. Samples taken from the affected sites contained large volumes of *D. geminata*. The entire sample was first washed and pulled apart in water to suspend the invertebrates, as above. The algal mass was then examined for any remaining trapped invertebrates, which were removed. For each sample, invertebrates were sorted into taxa, identified (species level or coarser) and counted. All counts were normalised to numbers per square metre, based on the area of th Hess sampler (0.096 m^2). After identification and counting, the separated invertebrate sample was dried for 24 h at 105ºC, weighed, then ashed for 4 h at 400 ºC and re-weighed. This yielded a gross dry weight and ash-free dry weight of invertebrate biomass for each Hess sample, which was then converted to g/m^2.

Periphyton samples from each sample were analysed for AFDM and chlorophyll *a* as described for the previous studies. As before, we calculated quantities per m^2 of stone surface, based on the area of the sampling circle (0.00302 m^2). Further biomass parameters (%organic matter, AFDM:chlorophyll *a* ratio, phaeophytins) were calculated as described earlier.

5.4. Data analysis

As a check on the homogeneity of sites, we first ran a Principal Components Analysis (PCA) on stone area, water velocity, depth and Froude number data. In a PCA, combinations of variables are represented in multi-dimensional space such that the first axis explains the largest proportion of the variance, the second axis explain the next highest amount of variance, and so on. Assuming that the first two axes together explain a reasonable proportion of the total variance (e.g., 70% or more), samples plotted against these two axes are placed so that the most similar samples lie closest together, and least similar samples lie farthest apart. (For a full explanation of PCA see Clarke and Warwick (2001)).

The following were calculated from the invertebrate data for each sample:

1. Density (number of individuals per m^2).

2. Mean size of individuals (dry weight).

3. Species richness: the number of species.

4. Simpson's index of evenness:

$$D = 1 - \sum [n_i (n_i - 1)/(N (N - 1))]$$

where n_i = number of individuals in the ith species and N = total number of individuals. Increasing values of D indicate increasing diversity of a community and decreasing dominance by a single taxon, or a small number of taxa.

5. EPT species richness: the number of species belonging to the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), which comprise mainly taxa considered to be clean-water species, indicative of healthy streams.

6. %EPT: the percentage of the community made up of EPT taxa

7. MCI: the macroinvertebrate community index. This index uses a score assigned to each taxon that reflects its sensitivity to organic pollution, from 10 (most susceptible) to 1 (most tolerant) (Stark 1988, 1993). The MCI is the average score of all taxa found in a sample, scaled up by a factor of 20.

8. QMCI: the quantitative version of the MCI incorporates the abundance of individual taxa, and is the average MCI score of all individuals in a sample.

Higher values of (3) to (8) above indicate "better" communities, e.g. higher diversity, and larger proportions of taxa that are typically found in clean streams. The absolute values of the indices are not particularly informative, but relative values provide a meaningful way to compare the invertebrate communities from sites affected and unaffected by *D. geminata*. Nested analysis of variance (ANOVA) was used to compare invertebrate densities, weights and indices, and periphyton biomass measures, in the unaffected and affected parts of the river.

For a direct visual representation of invertebrate communities at each site, the identified taxa were grouped by Order, or other major taxonomic grouping (e.g., the Class Crustacea). Numbers of individuals in these groups were summed by site, then plotted as stacked histograms. To depict community differences among samples, all taxa densities were converted to percentages then NMDS (see Section 4.2.4) was used to graphically represent all samples.

Because the ANOVAs showed considerable variability among sites, therefore we also ran a correlation analysis to look for relationships between the invertebrate community indices and habitat data (periphyton biomass measures, water velocities). Variables were log-transformed where necessary, to meet the requirements of the analysis for a normal distribution of values.

6. Results

6.1. Field observations

As noted previously, *D. geminata* grew prolifically at the three affected sites. Collection of invertebrate samples was difficult and required the use of much larger collecting containers than normal. There was also significant algal growth at the unaffected upstream sites, mainly green filamentous algae.

6.2. Physical environment

The PCA run on physical habitat data showed that samples from the *D. geminata*-affected and unaffected areas of the river encompassed a similar range of hydraulic conditions (Appendix C, Figure C1). Therefore hydraulic conditions were not expected to contribute to major differences among the samples (on average).

6.3. Periphyton biomass and communities

As expected, biomass measures at the three affected sites were extremely high, and easily exceeded the current New Zealand guidelines (Biggs 2000a) (Figure 15). Both biomass measures were, overall, significantly lower at the sites unaffected by *D. geminata*, but AFDM values were surprisingly high, also exceeding the AFDM guideline. Taxonomic analysis of a subset of samples confirmed that those from the *D. geminata*-affected sites were dominated by *D. geminata*, with small diatom species also making up a portion of the community. Samples from the unaffected sites were considerably less bulky, but nevertheless contained dense populations of diatoms, green filamentous algae and cyanobacteria. Taxa here were generally typical of those found in stable lake-fed rivers.

6.4. Invertebrate communities

Seventy-four invertebrate taxa were identified, with an average of 28 taxa in each sample. For details of taxa, refer to Appendix C. The most obvious difference among

samples was that those from the *D. geminata* affected sites had much higher densities than those from the unaffected sites (Figure 16a). This meant that there were significant differences in abundances in almost all the major invertebrate groups between affected and unaffected sites (Table 4). Individual invertebrates from the unaffected sites were also larger (by dry weight) than those in the affected sites (P <0.01), though samples were very variable (Figure 16b). There was no significant difference in taxon richness between affected and unaffected sites (Figure 16c). With the exception of MCI (non- quantitative) and EPT species richness, all the calculated invertebrate indices were, on average, higher in the unaffected area than the affected area (P <0.01 in all cases). There was some variation among sites, such that not all unaffected sites differed significantly from affected sites (Figure 16c – f).

Table 4: Densities (numbers per six square metres) of invertebrates in major groups at each of the six Mararoa River sites sampled for invertebrates. Numbers are totals from 6 samples per site. *P*-values are for the comparison between affected and unaffected sites using all samples (*n* = 6 at each site). Significance level: *P*<0.05.

	Unaffected by *D. geminata*			Affected by *D. geminata*			Unaff. vs. aff.
Invertebrate group	**Upper upper**	**Upper middle**	**Upper bottom**	**Normans Gulch**	**Station Bridge**	**Key Bridge**	***P***
Cladocera	843	21	10	4110	12310	937	0.000
Coelenterata	250	10	52	0	83	0	0.001
Coleoptera	4017	4849	2768	770	4089	3392	n.s.
Crustacea	2060	510	437	770	3798	853	n.s.
Diptera	34964	11113	9240	153226	91498	33861	0.000
Isopoda	94	104	21	0	0	0	0.004
Megaloptera	42	42	10	31	52	10	n.s.
Mites	4599	499	562	978	812	166	0.049
Mollusca	2851	343	333	416	4131	499	n.s.
Nematoda	5931	1894	1894	26837	13486	5245	0.000
Platyhelminthes	531	31	42	0	0	0	0.000
Worms	10229	6514	2882	59209	149979	234683	0.000
Ephemeroptera	1800	4672	4818	4485	13996	14173	0.000
Plecoptera	3694	1800	1405	4922	5442	3382	0.001
Trichoptera (tolerant)	5099	375	614	1207	479	510	n.s.
Trichoptera (suscept.)	1051	572	354	2737	4464	5786	0.000
Totals (all inverts.)	**78 054**	**33 351**	**25 442**	**259 698**	**304 620**	**303 496**	

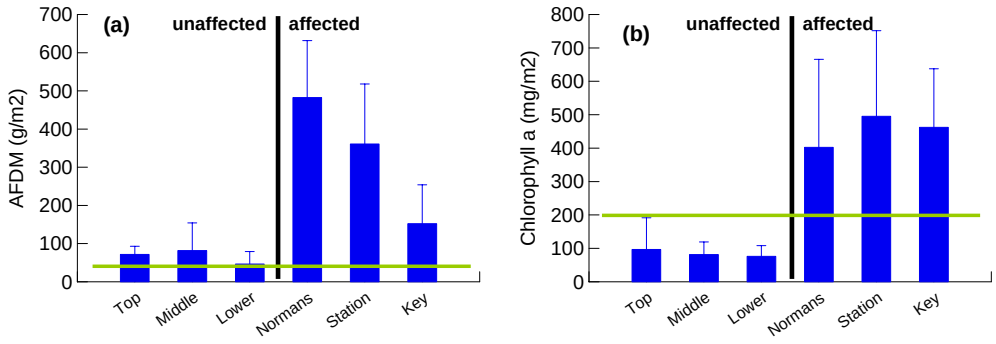

Figure 15. **Mean periphyton AFDM and chlorophyll *a* measured at three Mararoa River sites unaffected by *D. geminata* and three affected sites. Error bars are standard deviations. n = 6 at each site. Green lines show MfE guidelines for maximum periphyton biomass (35 g/m^2 AFDM, 200 mg/m^2 chlorophyll *a*).**

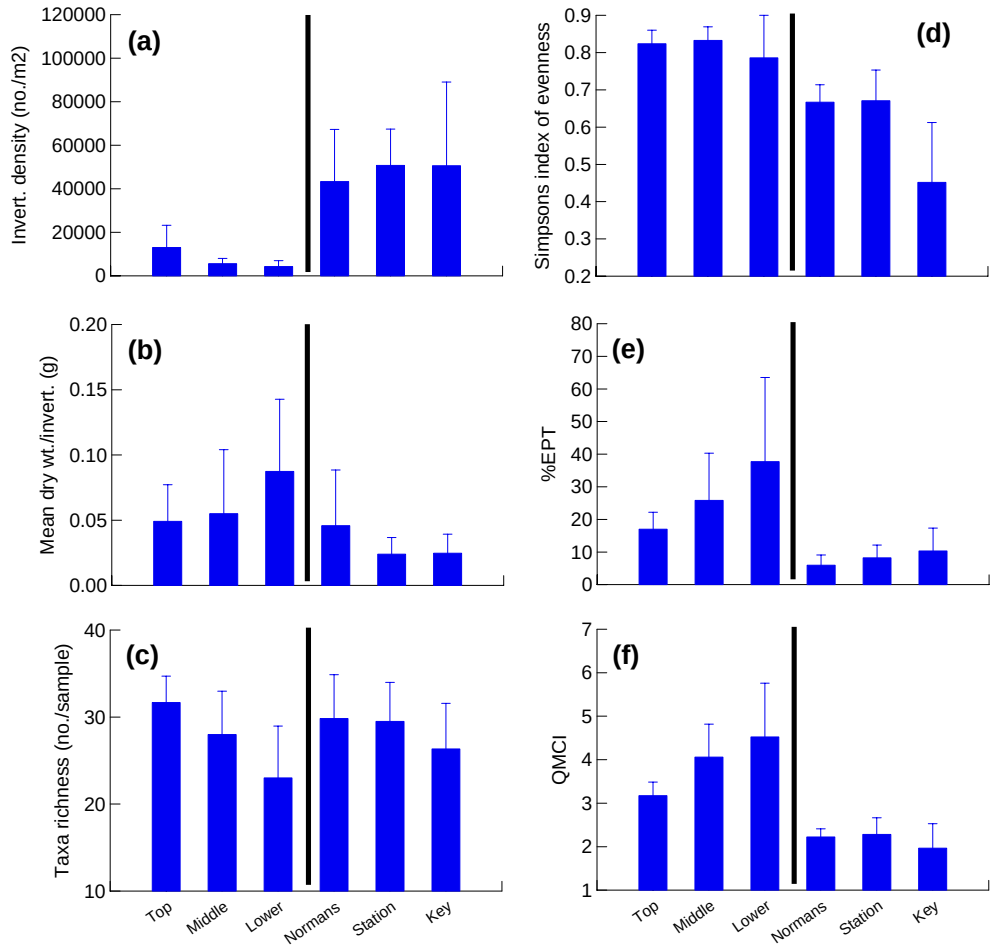

Figure 16. **Mean values of six macroinvertebrate community indices calculated from invertebrate samples from three Mararoa River sites unaffected by *D. geminata* and three affected sites. Error bars are standard deviations. n = 6 at each site. Affected and unaffected sites are as indicated in Figure 15.**

Stacked bar graphs of community composition at each site (relative abundance), with taxa placed in 12 major groups reflects the significant differences between the community indices and highlights differences among the communities in addition to abundance. In particular, there was increasing dominance going downriver by worms. Two of the unaffected sites had much higher proportions of mayflies, and all three had more beetle larvae and stoneflies (Figure 17). The NMDS analysis (Figure 18) placed samples from the three affected sites into distinct clusters, while those from the unaffected sites were much more variable within sites and not clearly separated into groups by site. (An ANOSIM analysis on the data from *D. geminata* affected vs. unaffected sites produced global R = 0.868, P = 0.001) which indicates a very significant separation between the communities.)

6.5. Relationships with environmental variables

The correlation matrix between habitat variables and the invertebrate indices revealed several significant correlations. Refer to Appendix C for details. Periphyton AFDM and chlorophyll *a* were highly correlated with some invertebrate indices, particularly invertebrate density, %EPT taxa, and QMCI. For example, AFDM and %EPT were strongly correlated (Figure 19). (Note that the indices themselves are highly intercorrelated.)

The reach-scale habitat variables conductivity and pH were significantly correlated with Simpson's index, though the relationship seems unlikely to be biologically significant.

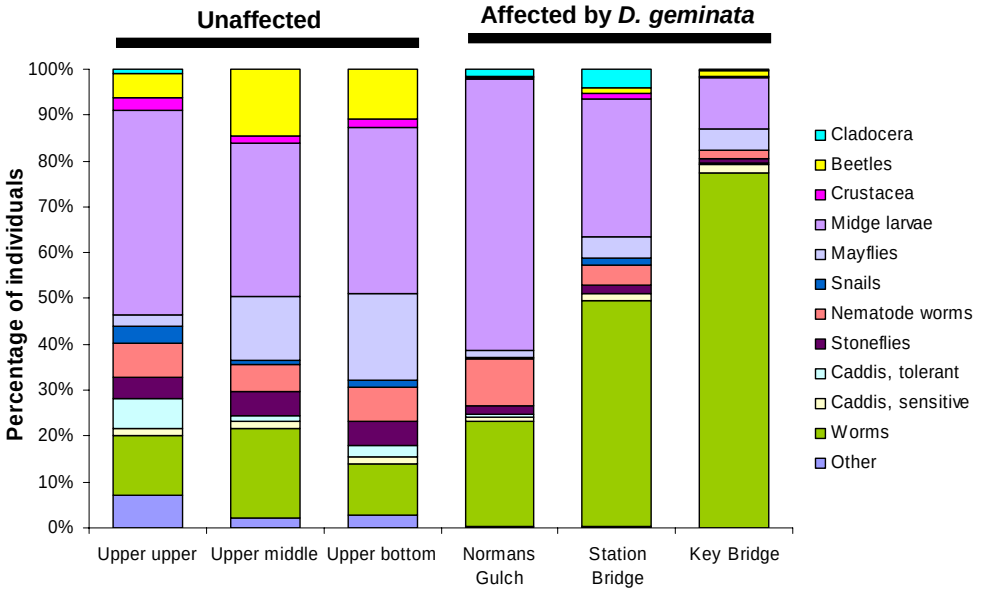

Figure 17. **Relative abundances of major invertebrate groups at three sites unaffected by *D. geminata* and three sites affected by *D. geminata*, in the Mararoa River. Averages over 6 samples at each site.**

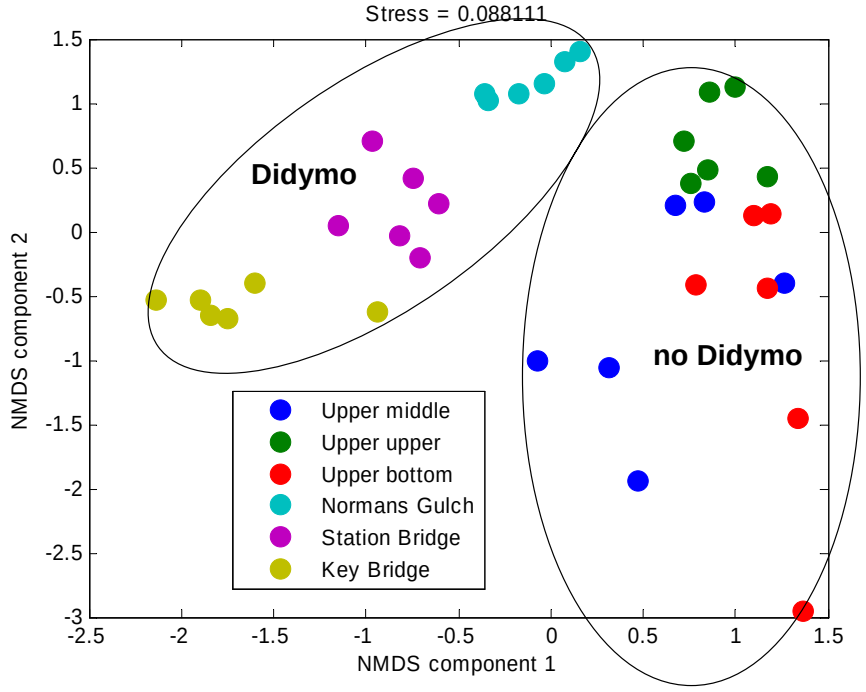

Figure 18. **NMDS plot showing similarities of invertebrate communities at three *D. geminata*-affected and three unaffected sites in the Mararoa River. Each point represents one invertebrate sample. Note that the stress for this plot is low (0.09), indicating that the relative community similarity between sites was well represented in two dimensions. The analysis was based on relative abundances.**

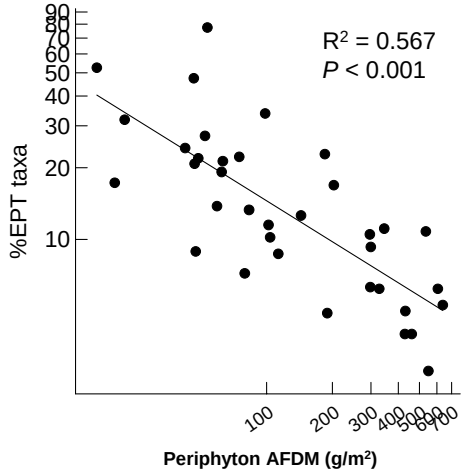

Figure 19: %EPT taxa in invertebrate communities plotted against periphyton AFDM. This was one of the best correlations between an invertebrate index and a periphyton biomass variable.

6.6. Discussion

This analysis largely corroborated previous anecdotal and more formal findings that the presence of *D. geminata* in a stream has significant effects on invertebrate communities (Mundie and Crabtree 1997; Kilroy et al. 2005a). Both the affected and unaffected sites encompassed the same range of hydraulic conditions, yet several invertebrate community indices were very much lower in the presence of *D. geminata*, viz., species evenness, %EPT, QMCI, and mean invertebrate dry weight. The first three of these indicate the presence of higher proportions of invertebrate taxa that are tolerant to degraded or polluted conditions and lower proportions of taxa typical of clean waters (Stark 1988). Smaller average invertebrate weights suggest higher proportions of typically small animals, such as midge larvae and early instar mayflies, as opposed to large individuals such as large predatory stoneflies and late-instar mayflies. The NMDS analysis also suggested more homogeneous invertebrate communities at individual sites in the *D. geminata* affected area, compared to highly variable communities in two of the three unaffected sites.

The significant correlation between %EPT and AFDM reflects a known association between periphyton biomass and invertebrate community composition (Suren et al. 2003). As suggested by Kilroy et al (2005a), it is probably not *D. geminata per se* that leads to a degradation of the invertebrate community, but the fact that the species attains such a high biomass. This is supported by the fact that when the regression analysis was repeated on the unaffected and affected samples separately, the same trend was evident in each case, although the correlations were weaker and not quite

statistically significant (Figure 20). Variability in invertebrate community composition in the sites unaffected by *D. geminata* was consistent with previous observations on specific associations between invertebrate taxa and periphyton community composition. For example, the caddisfly *Oxyethira* tends to be confined to areas with filamentous green algae (Kilroy and Suren 2002). The taxon was most common at the Upper Middle site, which had highest chlorophyll *a* of the unaffected sties, as a result of rich growth of green filamentous algae.

Although the lower values of indices mentioned indicate a decline in the quality of invertebrate communities, invertebrates densities in the *D. geminata*-affected samples were very much larger than in unaffected sites. The difference was such that although the unaffected sites had markedly higher *proportions* of mayflies and stoneflies (which are considered "desirable" invertebrates), the *D. geminata*-affected sites actually had greater *densities* of these invertebrates (Appendix C, Table C2). It was observed during sample analysis that there were many small-sized mayflies present in the *D. geminata*-affected samples. Nevertheless, it is possible that numbers of large-sized individuals (presumably living at the surface of the algal mat) were still at least as great as those in samples from unaffected sites. A complete size-class analysis of invertebrate communities would be necessary to determine whether numbers of larger-sized invertebrates are affected by the presence of thick mats of *D. geminata*.

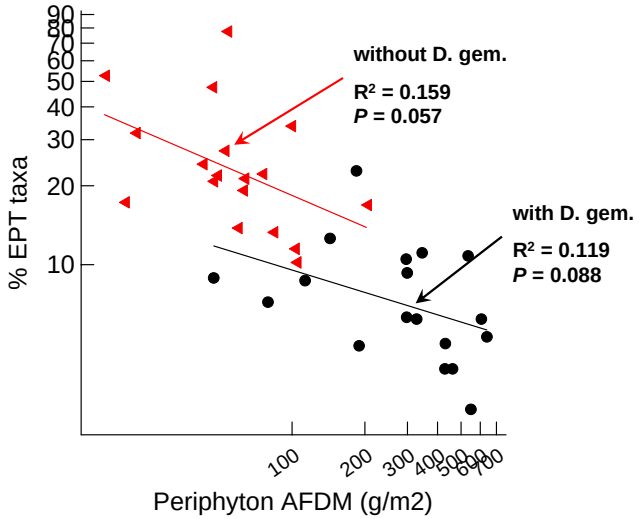

Figure 20. **%EPT taxa in invertebrate communities plotted against periphyton AFDM for *D. geminata*-affected and unaffected areas separately. (Compare with Figure 19.)**

While these dense populations of invertebrates may be grazing on *D. geminata* itself, it is also likely that the high densities of small diatoms in the mats are a more important food source, as well as detritus produced from algal growth and

scenescence. The mat itself also provides a stable habitat, protected from the water current. Midge larvae and worms in particular, thrive under these conditions (Perrin and Richardson 1997). Indeed it is possible that a thick mat of *D. geminata* provides an environment equivalent to that of the upper layers of sediment on the stream bottom (i.e., the hyporheos), Thus, in the presence of *D. geminata*, invertebrates that would normally inhabit the hyporheos move up into the algal mat. Sampling the hyorheos at unaffected and affected sites would be necessary to verify this possibility (or otherwise). Specialised methods are available for sampling the hyporheic zone in streams.

Relatively high numbers of mayflies may find suitable habitat near the surface of the mat. Unlike some other diatom accumulations, *D. geminata* is not mucilaginous or slimy. Indeed, well-developed *D. geminata* mats appear to exclude *Cymbella kappii*, which is one of the most common mucilage-producing diatom taxa in New Zealand streams (Kilroy et al. 2005a). Invertebrate taxa such as mayflies in general cannot feed easily in the presence of large amounts of mucilage (A. Suren, pers. comm..), but the less slimy *D. geminata* mats may be more favourable.

This raises the question of whether the presence of *D. geminata* in these rivers could actually be beneficial in terms of increasing the supply of food for higher trophic levels, thereby potentially enhancing the trout fishery. This phenomenon was noted in North Island lakes, when another invasive alga, water net (*Hydrodictyon reticulatum*) attained very high biomass, but was accompanied by a massive increase in invertebrate densities, leading to some excellent trout-fishing seasons (Wells and Clayton 2001). One possibility is that the dense, attached mats of *D. geminata* that harbour high densities of small invertebrates will be impenetrable to fish. In other words, the fish simply will not be able to get at this potential food source. However, without further monitoring of trout populations, including analysis of stomach contents from fish in affected and unaffected areas, it is speculation only as to whether or not *D. geminata* will have a similar effect to water net.

Like the previous two studies, this comparison took place in mid-winter. In New Zealand, seasonal patterns are rarely mentioned in relation to stream invertebrate communities (e.g., Winterbourn 2004). With the exception of a few highly seasonal taxa, an invertebrate survey in winter will yield a community similar to one in summer (A. Suren, pers. comm.). However, seasonal patterns in periphyton growth (including *D. geminata*) and fish feeding activity (Kishi et al. 2005) may lead to different patterns of invertebrate community composition under warmer conditions. It is suggested that a repeat survey in summer will help to provide a more complete understanding of the effects of *D. geminata* on invertebrate communities.

7. Overall summary and conclusions

A clear finding from all three ecological studies described in this report is that, at the time of the present surveys, *Didymosphenia geminata* biomass in both the Mararoa and lower Waiau rivers was considerably higher than that normally found for other periphyton taxa in New Zealand rivers (for examples of maximum biomass values, see Biggs 2000b). Indeed, growth was so prolific that most individual samples far exceeded the current MfE guidelines for maximum biomass of peirphyton in rivers (Biggs 2000a).

The hydraulic habitat study confirmed visual impressions that *D. geminata* thrives under a very broad range of hydraulic conditions. While an optimum water velocity was evident at some sites, growth was considerable over a wide range, from very slow up to the limit for safe sampling. Both visual assessments of biomass and quantitative biomass measures showed a great deal of scatter when plotted against hydraulic factors, with few trends evident. The broad tolerance of *D. geminata* was further emphasized by fairly specific hydraulic limits for other algae observed during the surveys.

Comparison of *D. geminata* biomass over space (6 sites spanning ~60 km of river) and time (4 sampling occasions over 10 weeks) revealed several trends. In particular we found a tendency for downstream sites (in the lower Waiau) to have lower biomass than upstream sites (in the Mararoa). Following weeks of low flows, the biomass difference almost disappeared, but re-established after a moderate-sized flood. Comparison of biomass data with measurements of dissolved nutrients strongly suggested a positive response of *D. geminata* to higher nutrient concentrations (especially nitrogen) over the flood-free period from May to July. In other words, all other things being equal, rivers with higher nutrient levels will have larger blooms. Biomass at the most upstream site in the Mararoa remained remarkably constant throughout the whole programme and it is suggested that this is related to the physical stability of that site.

At some sites, biomass was correlated with time since the most recent flood >75 – 100 m^3/s, and less so with smaller floods. This suggested that a flood of this size will "re-set" the community. Following a flood of 120 – 140 m^3/s during the sampling programme, biomass loss (AFDM) was as much as 70%. However, at all sites the amount of growth remaining was still considerable and some sites were apparently unaffected. As suggested by its broad hydraulic habitat range, *D. geminata* appears to be relatively resistant to floods and capable of growing almost everywhere in rivers where the substrate is not constantly unstable.

Effects of *D. geminata* on invertebrate communities were again associated with the ability of this alga to attain very high biomass. Sites affected by *D. geminata* had much higher invertebrate densities than unaffected sites. At the same time community composition shifted towards dominance by worms or midge larvae so that several commonly used invertebrate community indices showed significant reductions between unaffected and affected sites. Such reductions are taken to represent a fall in community health or quality. However, because of the huge increase in invertebrate densities associated with *D. geminata*, it is conceivable that higher trophic levels (fish) may actually benefit from the presence of this diatom. It is not known whether fish could utilise invertebrates living within dense algal mats. Studies on fish densities and diets would be necessary to determine this. Drift sampling and size-class analyses of benthic invertebrate communities could determine whether *D. geminata* mats are associated with reductions in densities of the large-sized mobile invertebrates favoured as food by fish.

The finding of significant concentrations of live *D. geminata* cells suspended in flowing water, even at low flows, highlights the importance of river water as a means of spread of the organism.

In terms of the initial objectives of these studies, all three have quantified the observed aesthetic effect of this alga on the Mararoa and lower Waiau river environments. Levels of biomass, breadth of hydraulic habitat, and persistence of growth through high flows appeared to be extreme at the sites sampled. There was a clear effect on invertebrate communities, although further work would be needed to determine any flow-on effects to fish.

With regard to short term responses to hydrological changes and water chemistry conditions, we reiterate that these surveys were undertaken at the coldest time of year. Since it is suspected that *D. geminata* grows best under cold conditions, different responses to hydraulic conditions, water flows and water chemistry may occur at warmer times of the year. Likewise, responses of invertebrate communities may also differ as water temperature rises. It is therefore suggested that further surveys be considered, to provide more complete information. A method for quantitative visual assessment of biomass produced excellent correlations with measured biomass values. Use of this method could be considered for any future surveys as a way of maximising the information gained with the available resources.

8. Acknowledgements

We thank Biosecurity New Zealand, particularly Christina Vieglais, for the opportunity to undertake this work. Field assistance from Lyne McFarlane, Stuart Sutherland, Ian Campbell and Gill Carr was much appreciated. Catherine Chagué-Goff, Sarah Braithwaite and Lindsay Hawke efficiently processed the many very difficult samples, and Helen Hurren produced the map (Figure 1). We thank Meridian Energy for access to hydrological data for the Mararoa and lower Waiau Rivers. Alastair Suren is thanked for his constructive review of an earlier version of this report, and Max Bothwell and Eric Edwards for their excellent contributions to the interpretation of the data.

9. References

Biggs, B. (1996). Hydraulic habitat of plants in streams. *Regulated Rivers: Research & Management 12*: 131-144.

Biggs, B. and C. Hickey (1994). Periphyton responses to a hydraulic gradient in a regulated river in New Zealand. *Freshwater Biology 32*: 49-59

Biggs, B., D. Goring, Nikora, VI. (1998). Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. *Journal of Phycology 34*: 598-607.

Biggs, B.J.F. (2000a). New Zeland periphyton guideline: detecting, monitoring and managing enrichment in streams. MfE, Wellington.

Biggs, B.J.F. (2000b). Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society 19*: 17-31.

Biggs, B.J.F.; Kilroy, C. (2000). Stream Periphyton Monitoring Manual. Published by. NIWA, for MfE.

Bourassa, N. and A. Cattaneo (1998). Control of periphyton biomass in Laurentian streams (Quebec). *Journal of the North American Benthological Society 17*: 420-429.

Clarke, K.R.; Warwick, R.M. (2001). Change in marine communities: an approach to statistical analysis and interpretation. Plymouth Marine Laboratory, UK.

Davies-Colley, R.J.; Wilcock, R.J. 2004. Water quality and chemistry in running waters. Chapter 11 in: Harding, J.S.; Mosley, M.P; Pearson, C.P.; Sorrell, B.K. (eds) Freshwaters of New Zealand. NZ Hydrological Society/NZ. Limnological Society.

Hickey, C.W.; Vickers, M.L. 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. Archives for Environmental Contamination and Toxicology 26: 292-298.

Hoagland, KD; Rosowski, JR; Gretz, MR; Roemer, SC (1993). Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. *Journal of Phycology 29*: 537-566.

Kilroy, C. 2004. A new alien diatom, *Didymosphenia geminata* (Lyngbye) Schmidt: its biology, distribution, effects and potential risks for New Zealand fresh waters. NIWA Client Report: CHC2004-128.

Kilroy, C.; Lambert, P.; Robinson, K; Blair, N. (2005a). Periphyton and invertebrate monitoring programme, lower Waiau River. Results of the 2005 survey and a commentary on the ecological effects of *Didymosphenia geminata*. NIWA Consultancy Report 2005-032.

Kilroy, C; Jarvie, B.; Sutherland, S. (2005b). Nuisance growths of periphyton in the lower Waiau River in relation to flow; a summer monitoring programme with a focus on *Didymosphenia geminata*. NIWA Consultancy report CHC05_59 51 p

Kilroy, C.; Snelder, T.; Sykes, J. (2005c). Likely environments in which the non-indigenous freshwater diatom can survive, in New Zealand. NIWA Consultancy Report 2005-043. 34 p.

Kilroy, C; Suren, A.M. (2002). Manapouri Power Scheme monitoring programme: periphyton and macroinvertebrates in the lower Waiau River. Results for 2002 and a review of five years of post-minimum flow monitoring. NIWA Client Report: CHC2002-58

Kishi, D; Murakami, M; Nakano, S; Maekawa, K (2005). Water temperature determines strength of top-down control in a stream food web. *Freshwater Biology 50*: 1315-1322.

Mundie, J.H.; Crabtree, D.G. (1997). Effects on sediments and biota of cleaning a salmonid spawning channel. Fisheries Management and Ecology 4: 111-126.

Perrin, C.J.; Richardson, J.S. (1997). N and P limitation of benthos abundance in the Nechako river, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences 54*: 2574-2583.

Peterson, C. and R. J. Stevenson (1992). Resistance and resilience of lotic algal communities: importance of disturbance timing and current. *Ecology 73*: 1445-1461.

Rieberger, K. 1991. The distribution of the diatom *Gomphonema geminata* in Vancouver Island streams. Province of British Columbia, Ministry of Environment.

Stark, J.D. (1985). A macroinvertebrate community index of water quality for stony streams. Water & Soil Miscellaneous Publication 87. Wellington, Ministry of Works and Development. 53p.

Stark, J.D. (1998). SQMCI: a biotic index for freshwater macroinvertebrate coded abundance data. *New Zealand Journal of Marine and Freshwater Research 32*: 55-66.

Stevenson, R. J. (1996). The stimulation and drag of current. Algal Ecology. R. Stevenson and R. L. Lowe, Academic Press: 321-340.

Suren, A., Biggs, B.; Duncan, M.J.; Bergey, EA, Lambert, P. (2003). Benthic community dynamics during summer low -flows in two rivers of contrasting enrichment 2. Invertebrates. *New Zealand Journal of Marine and Freshwater Research 37*: 71-83.

Wells, R. and J. Clayton (2001). Ecological impacts of water net (*Hydrodictyon reticulatum*) in Lake Aniwhenua, New Zealand. *New Zealand Journal of Ecology 25*: 55-63.

Winterbourn, MJ (2004). Stream invertebrates. Chapter 16 in Harding, J.S.; Mosley, M.P; Pearson, C.P.; Sorrell, B.K. (eds) Freshwaters of New Zealand. NZ Hydrological Society/NZ. Limnological Society.

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Appendix A: Hydraulic habitat study: details of analyses summarised in Section 3.3

Physical environment

An ANOVA run on stone sizes showed that the stones sampled at Norman's Gulch were just significantly smaller than those at Blackmount ($P = 0.044$) and Redcliff ($P = 0.048$). Norman's Gulch also differed significantly from other sites in mean water depth (shallower than Recliff ($P < 0.005$) and Excelsior ($P < 0.001$)) and Froude number (higher mean value than all other sites except Station Bridge, with P ranging from <0.001 to 0.046). Overall it was concluded that the rivers and sites were reasonably homogenous in terms of their physical characteristics, with the exception of Normans Gulch, at which shallower water and higher Froude number reflected the slightly steeper gradient that might be expected at the most upstream site.

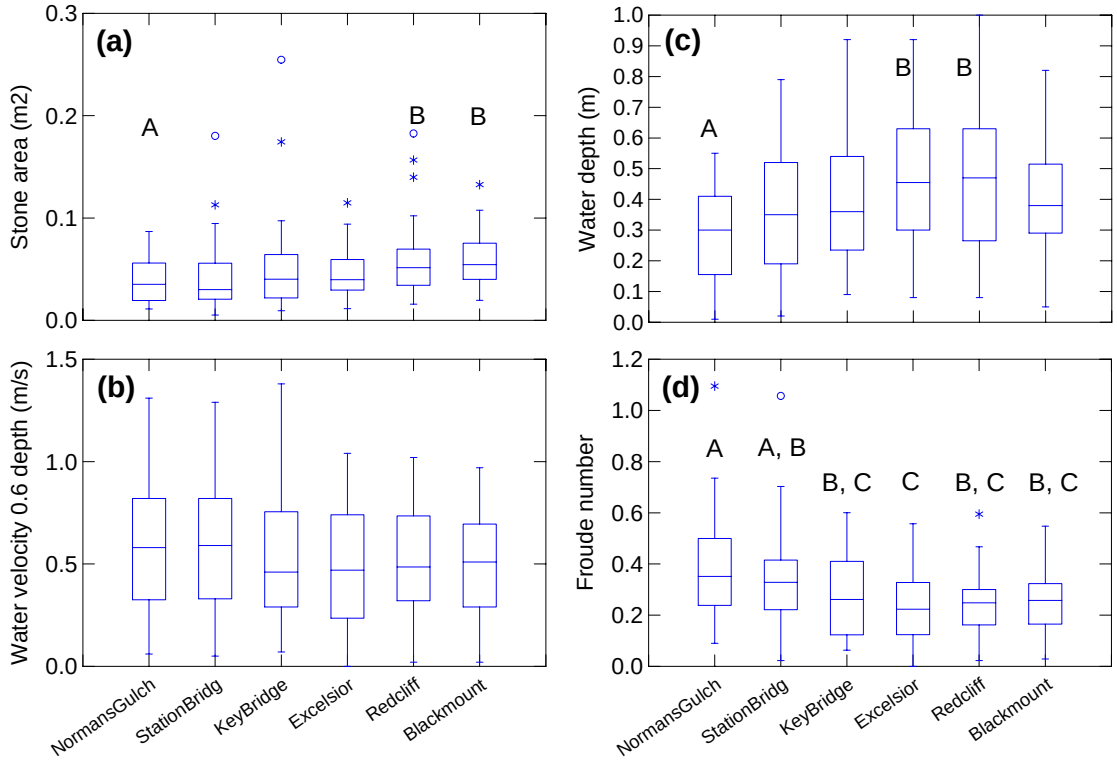

Figure A1: Box plots of physical variables vs. site, for all points sampled in the hydraulic habitat survey. The median value is shown as the central horizontal line in each box, and the upper and lower edges of the box encompass 25% of values either side of the median. The whiskers on each box show the limit of values lying within 1.5X the box height (i.e., 50% of the values) of the median. Asterisks and circles are outliers. Pairs of sites with different letters indicate significant between-site differences (stone area and Froude number only). All other comparisons were non-significant.

Biovolume and biomass relationships with physical variables

All scatter plots for AFDM and chlorophyll *a* versus the four hydraulic habitat parameters at Key Bridge and Excelsior are shown in Figures A3 and A4. Figure A5 shows details of the linear relationships between the visual biovolume index, and AFDM and chlorophyll *a* at these two sites.

Other algae

Percentage coverage by other algae in each river is shown in Figure A6, with respect to the four hydraulic variables. To enable a direct comparison with the *D. geminata* visual index, the *x*-axes are plotted using the same ranges as in Figure 4. *Cymbella* slime (red spots) clearly grew only in the lower velocity areas of both rivers. While green filaments (turquoise squares) were distributed across the whole range of velocities, at high velocities the coverage was invariably very low. In the lower Waiau River, thick mats (blue triangles) were generally confined to the mid range of water velocities and *Fr*, while thin black/brown films (green triangles) were more broadly distributed. Neither thick mats nor thin films were recorded in very shallow water (<0.2 depth). Thick mats were not recorded in water with mean water column velocity (velocity at 0.6 depth) <0.3 m/s, or $Fr < 0.1$. Overall the other algal groups identified visually showed much clearer hydraulic habitat preferences than *D. geminata*.

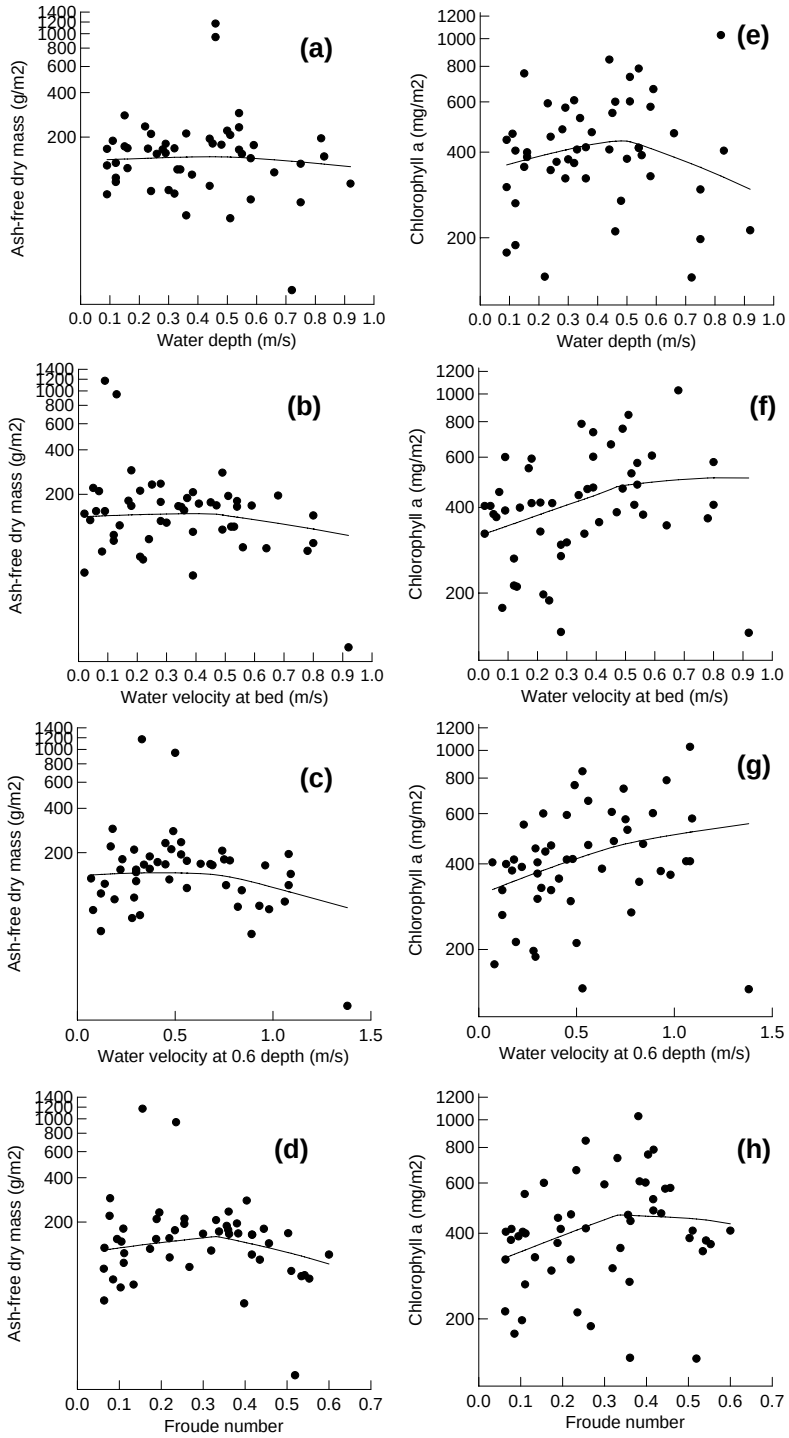

Figure A2: **Key Bridge: AFDM and chlorophyll *a* plotted against the four hydraulic parameters. Lines are locally weighed average smoothing lines (*x*-values predicted from local *y*-values).**

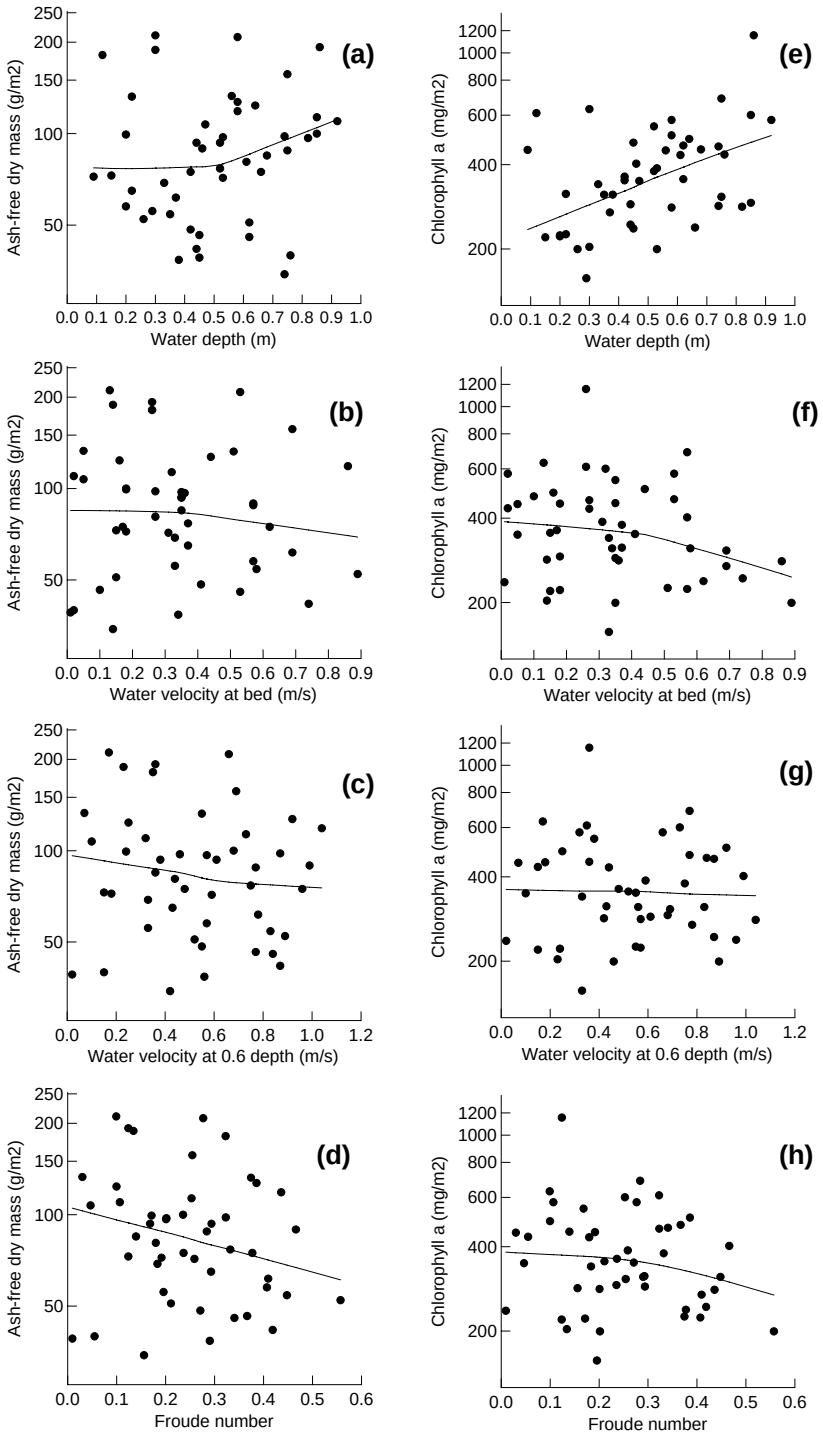

Figure A3: Excelsior: AFDM and chlorophyll *a* plotted against the four hydraulic parameters. Lines as in Figure A2.

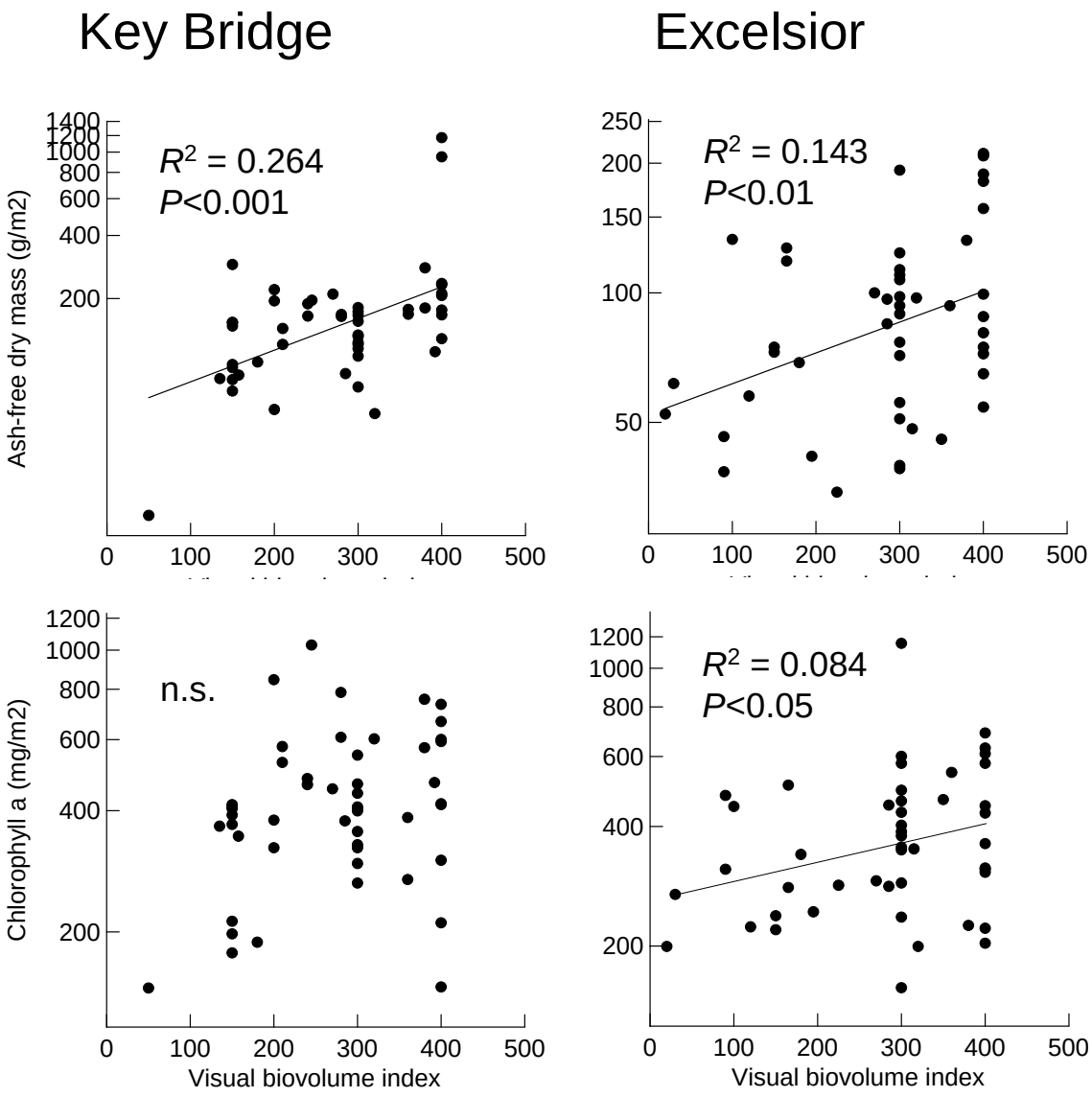

Figure A4: **Linear relationships between visual biovolume index and measured AFDM and chlorophyll *a* at Key Bridge (Mararoa River) and Excelsior (lower Waiau River). Significant relationships are indicated, but note that the broad scatter of points in all cases results in poor predictive power.**

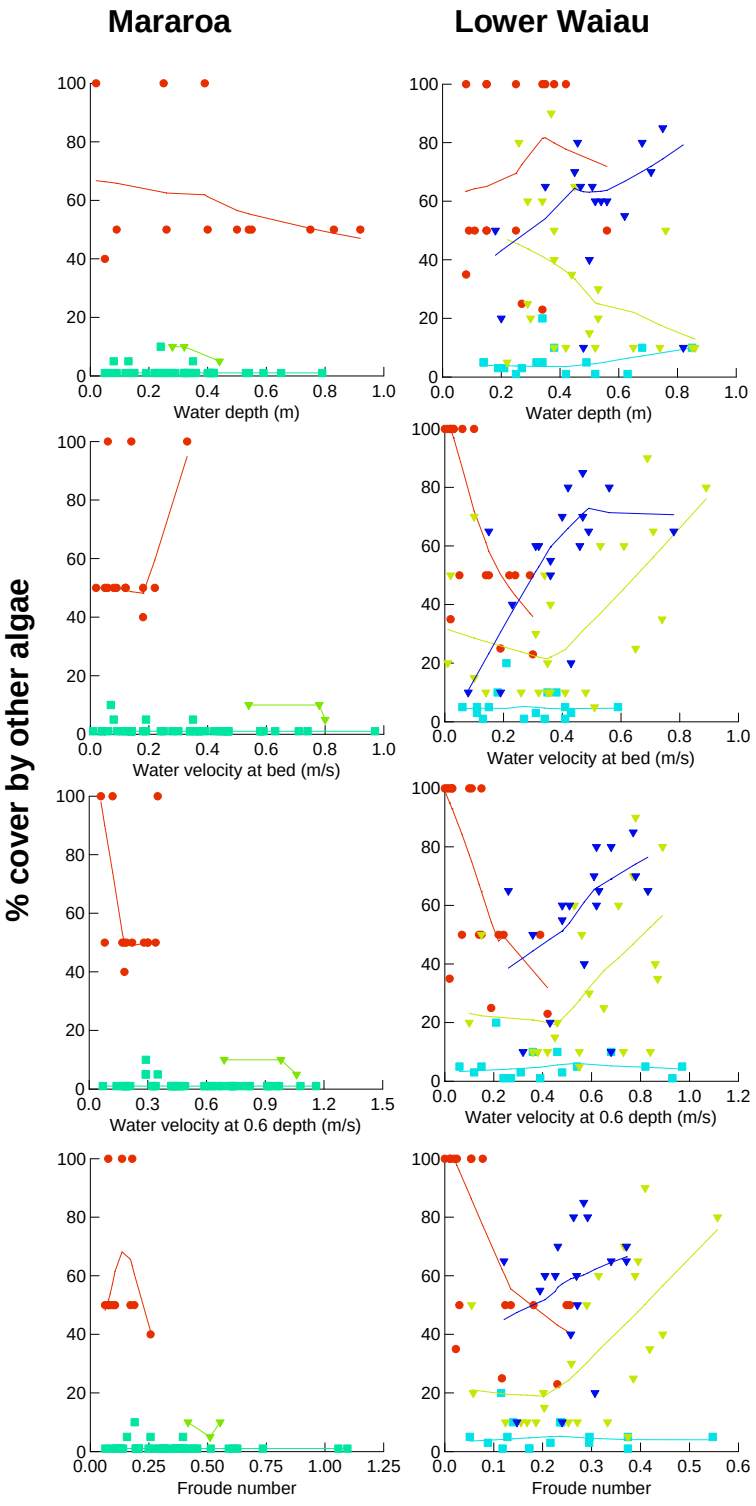

Figure A5: **Percentage cover of sampling points (stones) by algae other than *D. geminata* plotted against the four hydraulic habitat parameters in each river. ● slimy diatom mats (*Cymbella*); ■, ■ green filaments; ▼ thick black/brown mats; ▼ thin black film. Lines as in Figure A3.**

Appendix B: Temporal study: details of analyses summarised in Section 4.3

Physical environment

Figure B1 shows the distribution of values at each site (over all four sampling occasions) of sampled stone area, water velocity measured at 0.6 depth, water depth, and *Fr*. Stone areas at Redcliff were significantly larger than those at Normans Gulch (P = 0.014) and Key Bridge (P = 0.01). There were no significant differences in water velocities and water depth among any of the sites. The only significant difference in mean Froude number was between Station Bridge and Excelsior (P = 0.018). ANOVAs on a monthly basis showed no significant differences among sites in May, July and August. In June, the only significant differences were in depth: the water at sampling points was significantly deeper at Excelsior than at Key Bridge and Redcliff and Blackmount. This confirmed the overall impression of homogeneous sites.

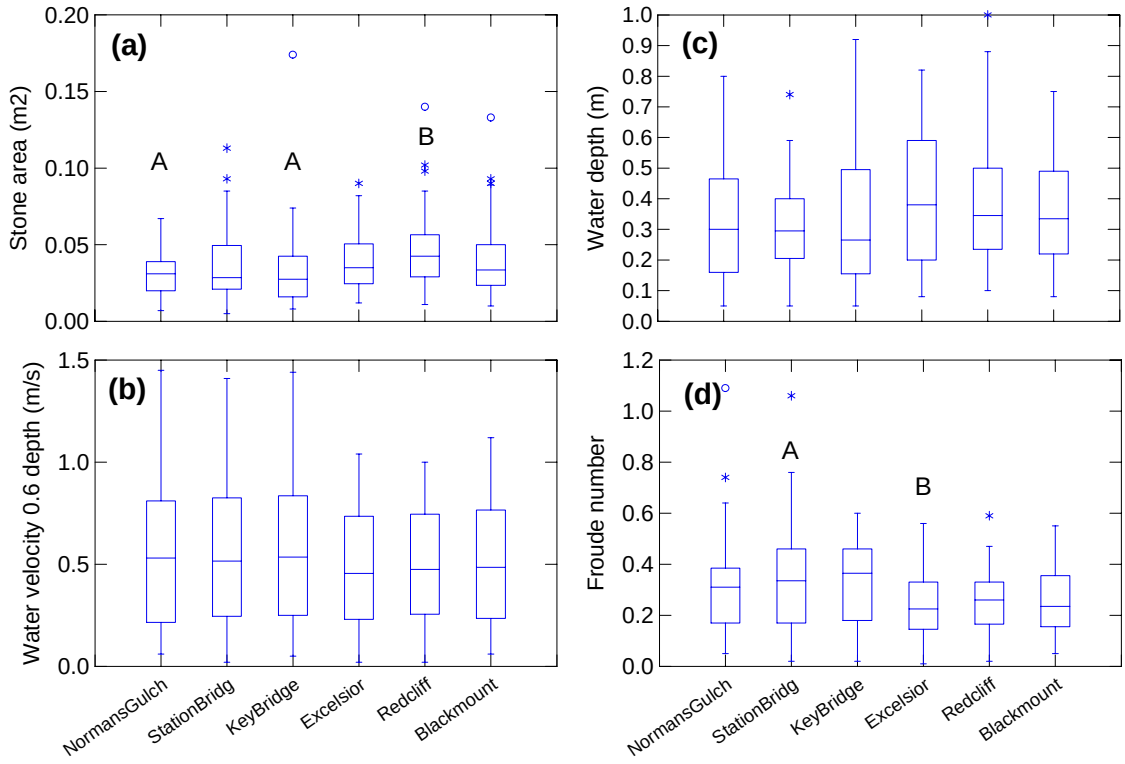

Figure B1: Box plots of physical variables vs. site (data from all four surveys). The median value is shown as the central horizontal line in each box, and the upper and lower edges of the box encompass 25% of values either side of the median. The whiskers on each box show the limit of values lying within 1.5X the box height (i.e., 50% of the values) of the median. Asterisks and circles are outliers. Pairs of sites with different letters indicate significant between-site differences (stone area and Froude number only). All other comparisons were non-significant.

Relationships between biomass and environmental variables

Correlation matrices comparing seven biomass indices with hydraulic, hydrological and water chemistry variables are presented below. Refer to Section 4.3.5.

Table B1: Pearson correlation matrices of reach-scale environmental variables vs. biomass variables, May to July (three sampling occasions over a period of stable flow). A. all data (six sites in two rivers), B. Mararoa River (three sites combined), C. lower Waiau rivers (three sites combined). Correlation coefficients in bold indicate significant relationships (Bonferroni-corrected probabilities, significance level, P < 0.05).

A. All data

	CHL	AFDM	DW	PCORG	AFDM : CH	PHAEO	CHL : PH
VEL_06	0.219	0.101	-0.158	**0.393**	-0.158	0.074	0.072
VEL_BED	-0.174	-0.105	**-0.255**	**0.315**	0.062	0.138	**-0.229**
FROUDE_06	0.198	**0.219**	-0.084	**0.387**	0.009	0.144	0.018
DEPTH	0.084	-0.121	-0.130	0.102	**-0.248**	-0.033	0.054
TEMP	-0.203	-0.069	-0.108	0.127	0.210	0.005	-0.157
COND	-0.033	**-0.467**	**-0.238**	-0.153	**-0.522**	**-0.348**	**0.239**
PH	0.168	-0.048	-0.014	-0.015	-0.237	**-0.339**	0.211
NO3_N	0.016	**-0.433**	-0.201	-0.189	**-0.556**	**-0.315**	**0.252**
NH4	-0.044	**-0.422**	-0.207	-0.102	**-0.448**	**-0.391**	0.107
TDPMGM3	-0.002	0.142	0.051	0.047	0.137	**0.327**	**-0.228**
SI	-0.127	**-0.494**	**-0.251**	-0.146	**-0.450**	**-0.326**	0.163
MEAN_FLO	0.039	**0.424**	0.208	0.153	**0.453**	**0.404**	**-0.279**
MED_FLO	0.020	0.450	0.207	0.192	**0.519**	**0.429**	**-0.316**
MAX_FLO	**-0.502**	**-0.526**	**-0.353**	-0.018	-0.067	-0.001	**-0.344**

B. Mararoa River

	CHL	AFDM	DW	PCORG	AFDM : CH	PHAEO	CHL : PH
VEL_06	0.154	-0.042	**-0.325**	*0.531*	-0.287	-0.042	0.147
VEL_BED	-0.134	-0.177	**-0.309**	*0.358*	-0.086	0.013	-0.090
FROUDE_06	0.125	0.084	-0.223	*0.481*	-0.098	0.025	0.086
DEPTH	0.041	-0.180	-0.154	0.120	-0.280	-0.116	0.115
TEMP	-0.218	0.008	-0.051	0.141	0.299	0.058	-0.187
COND	0.235	-0.105	-0.026	-0.052	**-0.395**	-0.053	0.211
PH	0.255	-0.074	-0.020	-0.038	**-0.377**	**-0.339**	0.219
NO3_N	**0.375**	-0.096	-0.008	-0.088	**-0.574**	-0.142	0.379
NH4	0.143	-0.102	-0.050	-0.033	**-0.305**	-0.079	-0.006
TDPMGM3	0.000	0.126	0.039	0.053	0.119	**0.359**	-0.283
SI	0.151	0.029	0.042	-0.039	-0.178	0.177	-0.033
MEAN_FLO	**-0.464**	-0.151	-0.127	0.119	**0.360**	**0.341**	**-0.630**
MED_FLO	**-0.475**	-0.116	-0.122	0.163	**0.421**	**0.351**	**-0.638**
MAX_FLO	**-0.358**	-0.166	-0.108	0.040	0.212	0.261	**-0.495**
DAYS_100	**0.445**	0.082	0.108	-0.181	**-0.430**	**-0.329**	**0.592**
DAYS_75	**0.445**	0.082	0.108	-0.181	**-0.430**	**-0.329**	**0.592**
DAYS_60	**0.339**	0.166	0.104	-0.029	-0.190	-0.247	**0.471**
DAYS_40	**-0.472**	-0.145	-0.127	0.131	**0.379**	**0.347**	**-0.639**

C. lower Waiau

	CHL	AFDM	DW	PCORG	AFDM : CH	PHAEO	CHL : PH
VEL_06	0.280	0.194	-0.037	0.209	-0.173	0.156	0.052
VEL_BED	-0.241	-0.145	-0.254	0.257	0.215	0.288	**-0.362**
FROUDE_06	0.272	0.195	-0.048	0.219	-0.152	0.139	0.058
DEPTH	0.152	0.095	-0.050	0.138	-0.122	0.201	-0.054
TEMP	-0.160	0.037	-0.096	0.192	**0.382**	0.109	-0.226
COND	0.088	0.021	-0.092	0.064	-0.146	0.021	0.019
PH	0.123	0.147	0.053	-0.050	0.029	-0.289	0.180
NO3_N	0.153	0.055	0.022	-0.088	-0.252	0.156	0.017
NH4	-0.022	-0.094	-0.046	0.101	-0.035	**-0.331**	0.109
TDPMGM3	-0.111	-0.166	-0.072	-0.083	-0.126	0.084	-0.096
SI	-0.208	-0.185	-0.168	0.134	0.037	0.152	-0.245
MEAN_FLO	0.176	-0.024	0.089	-0.185	**-0.402**	-0.145	0.250
MED_FLO	**0.686**	**0.597**	**0.427**	-0.140	-0.305	-0.283	**0.630**
MAX_FLO	**-0.686**	**-0.597**	**-0.427**	0.140	0.305	0.283	**-0.630**
DAYS_200	**0.759**	**0.560**	**0.461**	-0.239	**-0.521**	**-0.354**	**0.747**
DAYS_100	**0.759**	**0.560**	**0.461**	-0.239	**-0.521**	**-0.354**	**0.747**
DAYS_75	**0.705**	**0.476**	**0.423**	-0.259	**-0.565**	**-0.347**	**0.715**
DAYS_6050	**0.760**	**0.600**	**0.466**	-0.207	**-0.450**	**-0.338**	**0.728**

Table B2: **Pearson correlation matrices of reach-scale environmental variables vs. biomass variables, from May to August (four sampling occasions including before and after a moderate-sized flood). A. all data (six sites in two rivers), B. Mararoa River (three sites combined), C. lower Waiau rivers (three sites combined). Correlation coefficients in bold indicate significant relationships (Bonferroni-corrected probabilities, significance level, P < 0.05).**

A. All data

	CHL	AFDM	DM	PCORG	AFDM:CHL	PHAEO	CHL:PH
VEL_06	0.148	0.045	**-0.205**	**0.401**	-0.101	0.087	0.101
VEL_BED	-0.126	-0.126	**-0.265**	**0.303**	-0.017	0.067	-0.12
FROUDE_06	0.147	0.137	-0.15	**0.396**	0.003	0.134	0.057
DEPTH	0.064	-0.108	-0.147	0.15	-0.184	-0.005	0.091
TEMP	-0.143	-0.18	-0.154	0.094	-0.047	-0.133	-0.001
COND	**-0.272**	**-0.48**	-0.084	**-0.345**	**-0.301**	**-0.396**	0.054
NO3_N	-0.152	**-0.524**	-0.161	**-0.296**	**-0.458**	**-0.452**	0.145
NH4_N	0.074	-0.108	-0.123	0.076	-0.147	-0.103	0.095
TDPMGM3	-0.144	0.017	0.082	-0.119	0.142	0.164	-0.166
MEAN_FLO	**-0.206**	**0.22**	**0.242**	-0.125	**0.414**	**0.254**	**-0.259**
MED_FLO	-0.179	**0.232**	0.209	-0.056	**0.402**	**0.262**	**-0.248**
MAX_FLO	**-0.448**	-0.148	0.132	**-0.329**	**0.243**	-0.002	**-0.25**

B. Mararoa

	CHL	AFDM	DM	PCORG	AFDM:CHL	PHAEO	CHL:PH
VEL_06	0.1	-0.05	**-0.358**	0.544	-0.166	0	0.147
VEL_BED	-0.122	-0.22	**-0.349**	0.36	-0.137	-0.06	-0.057
FROUDE_06	0.08	0.013	-0.304	0.499	-0.089	0.01	0.107
DEPTH	0.042	-0.107	-0.153	0.169	-0.147	-0.016	0.107
TEMP	-0.11	-0.095	-0.139	0.161	0	-0.069	-0.015
COND	-0.032	-0.001	0.201	-0.253	0.058	0.035	0.038
NO3_N	0.22	-0.227	0.023	-0.219	**-0.438**	**-0.272**	0.258
NH4_N	0.025	0.184	0.085	0.034	0.162	0.237	-0.11
TDPMGM3	-0.017	0.089	0.048	0.012	0.082	**0.278**	-0.154
MEAN_FLO	**-0.45**	0.052	0.114	-0.044	**0.473**	**0.384**	**-0.436**
MED_FLO	**-0.385**	-0.119	-0.125	0.156	0.213	0.248	-0.374
MAX_FLO	**-0.28**	0.204	**0.282**	-0.187	**0.494**	**0.315**	**-0.261**
DAYS_100	**0.336**	-0.203	-0.262	0.135	**-0.542**	**-0.349**	**0.306**
DAYS_75	**0.336**	-0.203	-0.262	0.135	**-0.542**	**-0.349**	**0.306**
DAYS_6050	**0.362**	-0.063	-0.148	0.115	**-0.407**	**-0.33**	**0.362**
DAYS_40	**-0.365**	-0.155	-0.148	0.141	0.154	0.226	**-0.367**

C. Lower Waiau

	CHL	AFDM	DM	PCORG	AFDM:CHL	PHAEO	CHL:PH
VEL_06	0.173	0.05	-0.117	0.236	-0.152	0.128	0.062
VEL_BED	-0.16	-0.135	-0.233	0.242	0.097	0.22	-0.204
FROUDE_06	0.168	0.06	-0.104	0.227	-0.125	0.113	0.044
DEPTH	0.131	0.041	-0.1	0.204	-0.128	0.18	0.038
TEMP	-0.095	0.037	-0.076	0.178	0.229	0.095	-0.074
COND	**-0.351**	-0.181	0.124	**-0.33**	0.167	-0.142	-0.218
NO3_N	-0.1	-0.104	0.044	**-0.225**	-0.001	-0.028	-0.061
NH4_N	**0.27**	0.078	-0.146	**0.309**	-0.137	-0.09	0.25
TDPMGM3	**-0.416**	-0.257	0.13	**-0.409**	0.272	-0.165	-0.186
MEAN_FLO	**-0.388**	-0.204	0.182	**-0.421**	0.188	-0.185	-0.204
MED_FLO	**-0.404**	-0.188	0.185	**-0.405**	0.238	-0.173	-0.21
MAX_FLO	**-0.524**	**-0.285**	0.12	**-0.372**	0.28	-0.114	-0.327
DAYS_200	-0.062	0.026	**0.293**	**-0.438**	0.041	**-0.289**	**0.082**
DAYS_100	**0.624**	**0.343**	-0.053	**0.299**	**-0.357**	0.038	**0.425**
DAYS_75	**0.7**	**0.392**	0.088	0.101	**-0.439**	-0.117	**0.535**
DAYS_6050	**0.673**	**0.459**	0.243	-0.075	**-0.334**	-0.24	**0.593**

Periphyton community compositon

A total of 43 algal taxa was identified, most of which were diatoms. While *D. geminata* was dominant in all samples, most also contained very high numbers of small diatoms, which were assessed as abundant or common to abundant in relation to *D. geminata* (Table B2). An NMDS analysis run on community data from the May survey showed that Mararoa and lower Waiau communities were distinct (Figure B2a). However, the separation of the communities was mainly a result of differences in rare species. When species with abundances scores of 1 or 2, which also occurred at only 1 or 2 sites, were removed from the dataset, then the separation between the rivers became less clear (Figure B2b). The remaining discrimination between rivers was a result of the diatom *Synedra ulna* being more common in the Mararoa River than in the lower Waiau, and *Gomphoneis minuta* var *cassieae* being more common in the lower Waiau.

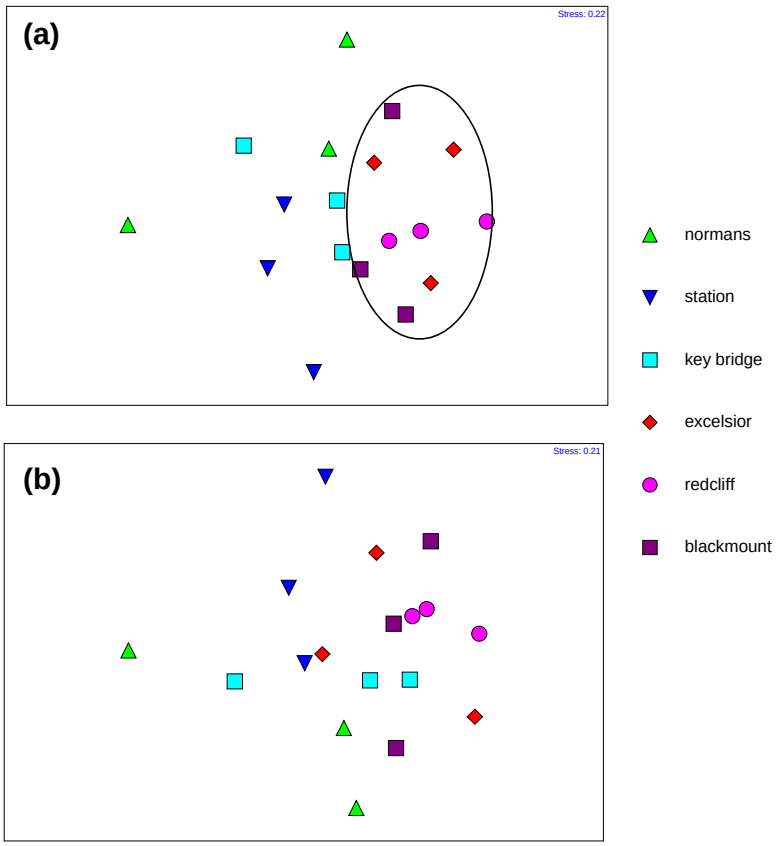

Figure B2: **Non metric multidimensional scaling (NMDS) plot of periphyton community composition data from six sites in the Mararoa – lower Waiau River system, May 2005. (a) Analysis run with complete dataset (Table B2). Note discrimination of lower Waiau sites (circled) from the Mararoa sites. (b) Analysis run omitting rare taxa.**

Table B2: **Algae taxa identified from the Mararoa and lower Waiau rivers, May 2005, with relative abundance scores. Scores range from 1 (rare) to 8 (dominant). Samples were collected from three velocity bands at each site, slow (S), medium (M), and fast (F). See main text for details.**

	River:	Mararoa River									lower Waiau River								
Algae	Site:	Normans Gulch			Station Bridge			Key Bridge			Excelsior			Redcliff			Blackmount		
type	Species	S	M	F	S	M	F	S	M	F	S	M	F	S	M	F	S	M	F
green	Ankistrodesmus spp.										1								
green	Coelastrum spp.					1											1		
green	Scenedesmus spp.																	2	
diatom	cf. Achnanthes sp.																		
diatom	Achnanthidium linearis	4		4	6	5	6	4	4	5	4		3	6	4	5	5	4	4
diatom	Achnanthidium cf. minutissimum		5	6	6	6		5	6	5	6	6	6	5	6	6	5	6	4
diatom	Achnanthidium spp. (small)	3		3						3		3			4	4	4	4	
diatom	cf. Aulacoseira spp.	3	1															1	
diatom	Cocconeis placentula (50x30µm)				3		2	1	1	2	1	1	1		1	1			1
diatom	Cymbella aspera	1				2				2									
diatom	Cymbella spp.							1	2			2		4	2		4	3	7
diatom	Cymbella cf. kappii										2		3						
diatom	Diatoma hiemale			3		3			2	2			3	3		2		4	
diatom	Diatoma tenuis	3	5	6	5	5	4	6	4	4	5	5	5	5	2	6	6	4	4
diatom	**Didymosphenia geminata**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
diatom	Diploneis spp.		1		1														
diatom	Epithemia adnata		1	1															
diatom	Epithemia sorex	2	1									1							
diatom	Encyonema spp.	6	6	6	6	6	6	6	6	6	6	6	6	6	5	6	6	6	6
diatom	Eunotia cf. incisa								1			2							
diatom	Fragilaria spp. (20 x 5µm)		3	4				3	3			2					5	5	4

Algae type	Species	River:	Mararoa River									lower Waiau River								
		Site:	Normans Gulch			Station Bridge			Key Bridge			Excelsior			Redcliff			Blackmount		
			S	M	F	S	M	F	S	M	F	S	M	F	S	M	F	S	M	F
diatom	Gomphoneis minuta var. cassieae					1		3				4	2	4	3	2	3	3	3	2
diatom	Gomphonema acuminatum					1														
diatom	Gomphonema cf. angustum		4		1						5									
diatom	Gomphonema truncatum									1										
diatom	Gomphonema spp. (small)				5		3	5	4			2		2	4	4	4	4		4
diatom	Melosira varians												1							1
diatom	Navicula cf. gregaria								1											
diatom	Navicula cf. margalithi															1				
diatom	Navicula spp.(small 30µm)		2					4												
diatom	Nitzschia spp.(small 15x5µm)		4					6	5	3		2	3		5		5	4		
diatom	Nitzschia linearis								1			1								
diatom	Nitzschia spp.(30x5µm)		4			6	4	5		3	3	2		2				5		4
diatom	Planothidium lanceolatum			1																
diatom	Reimeria sinuata																			
diatom	Rhoicosphenia spp.								1						1					
diatom	Rhopalodia novae-zealandiae			1			1													
diatom	Surirella cf. brebissonii						1													
diatom	Synedra ulna		7	5	4	3	4	3	6	2	3		3	4	3			5	5	1
diatom	Synedra ulna var ramesi		4	4				2			3	2	3	2	3	3	3	2	3	2
diatom	Tabellaria spp.			2	4	4	3	1	3	2				2	2	2		3	4	
cyan	Phormidium spp.										3			4					3	

Appendix C: Invertebrate effects study: details of analyses summarised in Section 5.3

Physical environment

The PCA run using hydraulic habitat data recorded at each invertebrate sampling point showed no real separation between sites in *D. geminata*-affected and unaffected areas Figure C1). Water velocities at one site in each area (Upper Middle and Key Bridge) were significantly higher than those at the other two sites in that area, but did not differ from each other.

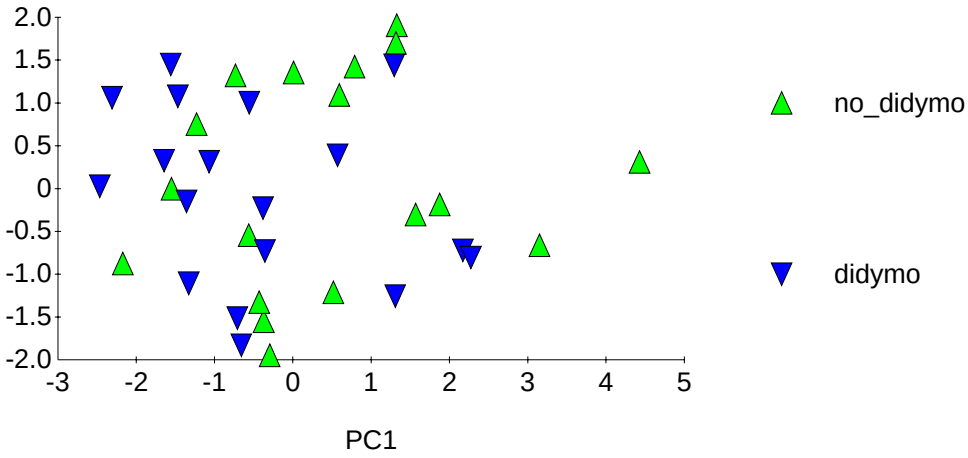

Figure C1: Principal components analysis (PCA) of four hydraulic parameters (water depth, bed velocity, velocity at 0.6 depth, Froude number) and stone surface area for all sampling points in the invertebrate survey, 15-16 June 2005. Each point represents one sampling point.

Invertebrate communities

Table C1 lists all invertebrates identified.

Invertebrate – environment relationships

The correlation matrix (Table C2) shows many significant correlations.

Table C1. **List of invertebrate taxa identified from six sites in the Mararoa River, June 2005. Under EPT, a "1" indicates EPT taxa (Ephemeroptera – mayflies; Plecoptera – stoneflies; Trichoptera – caddisflies).**

Phylum	Class		Genus, species, or common name	MCI score	EPT
COELENTERATA			Hydra	3	
PLATYHELMINTHES				3	
NEMATODA				3	
ANNELIDA	Oligochaeta		Worms	1	
ARTHROPODA	Crustacea	Cladocera	Chydoridae	5	
			Daphniidae	5	
		Copepoda	Cyclopoida	5	
			Harpacticoida	5	
		Ostracoda		3	
		Amphipoda		5	
	Insecta	Coleoptera	*Berosus*	5	
			Elmidae - larvae	6	
			Empididae	6	
		Diptera	Aphrophila neozelandica	5	
			Austrosimulium	3	
			Ceratopogonidae	3	
			Chironomiinae	4	
			Diamesiinae	5	
			Eriopterini	9	
			Muscidae	4	
			Orthocladiinae	2	
			Pupating Chironomidae	4	
			Stratiomyidae	5	
			Tanypodiinae	5	
			Tanytarsus	5	
			Stictocladius	2	
			Paucispinigera	6	
			Polypedilum	3	
		Ephemeroptera	*Austroclima/Mauiulus*	9	y
			Coloburiscus humeralis	9	y
			Deleatidium	8	y
			Nesameletus	9	y
			Rallidens mcfarlanei	9	y
		Megaloptera	*Archichaulioides*	7	
		Plecoptera	*Megaloptera diminuta*	9	
			Stenoperla sp.	10	y
			Stenoperla prasina	10	y
			Zelandobius sp.	5	y
			Zelandobius furcillatus group	5	y
			Zelandoperla sp.	5	y
			Zelandoperla decorata	5	y
			Zelandoperla fenestrata	5	y
		Trichoptera	*Aoteapsyche catherinae*	4	y
			Aoteapsyche colonica	4	y
			Oxyethira	2	y
			Costachorema sp.	7	y
			Costachorema xanthopterum	7	y
			Helicopsyche	10	y
			Hudsonema alienum	6	y
			Hudsonema amabile	6	y
			Hydrobiosis clavigera	5	y
			Hydrobiosis copis	5	y

Table C1. continued

Phylum	Class		Genus, species, or common name	MCI score	EPT
ARTHROPODA	Insecta	Trichoptera	*Hydrobiosis parumbripennis*	5	y
			Hydrobosis	5	y
			Hydroptilidae (early instars)	2	y
			Neurochorema confusum	9	y
			Neurochorema forsteri	9	y
			Neurochorema spp	9	y
			Olinga	9	y
			Polyplectropus	8	y
			Psilochorema bidens	8	y
			Psilochorema leptoharpax	8	y
			pupating *Hydrobiosis*	5	y
			Pycnocentria	7	y
			Pycnocentria evecta	7	y
			Pycnocentrodes	7	y
		Isopoda	Idoteidae	5	
		Tardiograda	Tardigrada	5	
	Acari		Mite	5	
MOLLUSCA		Bivalvia	Sphaeriidae	3	
		Gastropoda	*Gyraulus corinna*	3	
		Gastropoda	*Lymnaea* spp	3	
		Gastropoda	*Physa*	3	
		Gastropoda	*Potamopyrgus*	4	

Table C2: Pearson correlation matrices of environmental and periphyton variables vs. invertebrate community indices, run for data from six sites, three affected and three unaffected by *D. geminata*. Correlation coefficients in bold indicate significant relationships (Bonferroni-corrected probabilities, significance level, P < 0.05).

	BED_VEL	0.6_VEL	COND	PH	AFDW	CHLA	PHAEO
LOG_TOTAL	-0.441	-0.246	**0.799**	0.518	**0.724**	**0.717**	**0.789**
LOG_DWINV	0.255	0.138	-0.465	-0.351	-0.390	-0.338	-0.386
RICH	-0.247	-0.305	0.029	-0.081	0.254	0.031	0.068
SIMSON	0.130	-0.228	**-0.793**	**-0.801**	-0.396	-0.662	-0.644
EPT_RICH	-0.132	-0.118	0.326	0.206	0.433	0.326	0.336
LOG_PEPT	0.444	0.385	**-0.651**	-0.376	**-0.761**	**-0.660**	**-0.761**
MCI	-0.101	-0.071	0.408	0.304	0.443	0.416	0.429
QMCI	0.350	0.195	**-0.765**	**-0.577**	**-0.688**	**-0.699**	-0.795

Appendix D: Estimation of densities of suspended live *D. geminata* cells in flowing river water

Two-litre water samples were collected from moderately flowing water at each sampling site in the lower Waiau and Mararoa Rivers. The whole sample was strained through a double 50 μm screen, which was expected to trap most of the *D. geminata* cells. Material caught on the filter was resuspended in a small volume (up to about 80 ml). Subsamples of known volume of this concentrated sample were pipetted into the chamber of an inverted microscope and allowed to settle for 5 minuted. The entire chamber was then scanned at x100 and all live *D. geminata* cells seen (i.e., with intact chloroplasts) were counted. The procedure was repeated with up to three subsamples from each sample. The average number of cells per litre of river water was then calculated.

Counts of suspended live *D. geminata* cells from water samples collected in the May and June surveys yielded a mean concentration of 238 live cells/litre river water. This concentration was consistent over all six sites on average, although there was large variation in the counts from Norman's Gulch (Table D1).

Table D1. Concentrations of live *D. geminata* cells suspended in flowing water, Mararoa and lower Waiau Rivers.

		Mean counts (cells/litre of river water)	
River	**Site**	**May**	**June**
Mararoa	Normans	179	340
	Station	299	182
	Key	567	56
lower Waiau	Excelsior	217	205
	Redcliff	283	189
	Blackmount	194	249