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**Ecological studies of *Didymosphenia  
geminata* in New Zealand, 2006-2007**

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**NIWA Client Report: CHC2007-070  
December 2007**

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## **Ecological studies of *Didymosphenia geminata* in New Zealand, 2006-2007**

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## Executive Summary

The non-native benthic alga *Didymosphenia geminata* has been detected in approximately 53 rivers and streams (including tributaries in the same catchment) in southern New Zealand between October 2004 and October 2007. The presence of *D. geminata* over large areas of channel at affected rivers poses a risk of substantial changes in ecological properties (e.g., species diversity, population sizes, nutrient pools), and ecological processes (e.g., ecosystem metabolism, nutrient cycling). Few of the affected rivers were sites of environmental monitoring prior to the *D. geminata* incursion, so baseline ecological conditions are not well-documented. However, monitoring data for some currently affected reaches (e.g., the Waiau River, monitored for Meridian Energy) suggests that benthic algal biomass was low, and that benthic macroinvertebrate assemblages were diverse and stable.

Early assessments of the ecological impacts of *D. geminata* to New Zealand were estimated to be high. These predictions were based primarily on anecdotal information because documented statistical or observational information was lacking. Quantitative information concerning the effects of *D. geminata* on river ecosystems and the environmental factors that affect *D. geminata* growth and biomass is needed to better define the national risks associated with the invasion. MAF Biosecurity New Zealand contracted NIWA to produce this information through survey and experimental studies. Based on an expert evaluation of how *D. geminata* might interact within a conceptualized model of New Zealand's freshwater ecosystem, and building upon the preliminary ecological studies conducted in 2005, three general objectives were set for the NIWA 2006 – 2007 ecological studies:

1. Identify and quantify impacts of *D. geminata* mats on native fish, benthic invertebrates and river chemistry.
2. Determine the extent to which nutrient availability limits growth and biomass accumulation in *D. geminata*.
3. Quantify spatial and temporal variation in *D. geminata* abundance (i.e., biomass and areal cover) and identify correlations between abundance and the physical and chemical factors that are likely to control or limit abundance or growth.

For clarity, the work has been grouped into five studies:

1. Effects of *D. geminata* on native fish
2. Effects of *D. geminata* on native macroinvertebrates
3. Effects of *D. geminata* on water quality

4. Nutrient limitation in *D. geminata*
5. *D. geminata* biomass dynamics

### **Effects of *D. geminata* on native fish**

Twenty four of New Zealand's native fish fauna (total of 49 species) are non-diadromous (i.e., non-migratory and restricted to freshwater), and most of these species forage, spawn and rest in benthic habitats. Thick algal mats that may cover a substantial portion of a river reach, such as those formed by *D. geminata* in New Zealand, pose a risk for co-occurring native fish. To help assess this risk, a geographic information system (GIS) study was carried out to compare the distribution of individual native fish species (excluding lake-dwelling and estuarine species, as these live in habitats that *D. geminata* has not colonised), with distributions of rivers representing a range of *D. geminata*-colonisation risk. All 10 diadromous fish species were ranked as slightly vulnerable to *D. geminata* colonisation. Of the 15 non-diadromous galaxiids, five were ranked as highly vulnerable, nine as moderately vulnerable, and one as slightly vulnerable; both non-diadromous bully species had low vulnerability. This classification highlighted the vulnerability of the small non-diadromous galaxiids species with restricted distributions, to adverse effects of *D. geminata*.

A survey was conducted to assess native fish populations in *D. geminata*-affected and *D. geminata*-free river reaches of the Oreti and Aparima Rivers, Hamilton Burn and Irthing Stream. At the time of sampling, there was substantial *D. geminata* cover at only two of the survey sites, both in the upper Oreti River. Contrary to expectations, the highest density of galaxiids among the sites was measured at one of the Oreti River sites with moderate *D. geminata* cover. Few other differences between *D. geminata*-affected and non-affected sites were detected. Long-term monitoring over a broader gradient in *D. geminata* biomass will be needed to develop quantitative fish- *D. geminata* relationships. Many of the sites in which no *D. geminata* was visible during this preliminary survey eventually were colonised from affected reaches upstream, but funding resources were not available to continue this work to determine the effects of this increased algal biomass.

### **Effects of *D. geminata* on macroinvertebrates**

Two types of direct interactions with *D. geminata* are likely to affect the abundance and diversity of benthic macroinvertebrates, habitat interactions and trophic interactions. In the case of habitat interactions, invertebrate species that require exposed sediment surfaces to forage, respire, and reproduce are likely to be negatively affected when channel surfaces are covered by *D. geminata* mats. In the case of trophic interactions, invertebrate species that can utilize *D. geminata* as a food source are likely to be favoured over those that do not consume *D. geminata* (and whose food availability may be inversely related to *D. geminata* abundance). Studies of both types of interactions were carried out; habitat interactions were assessed by monitoring invertebrate assemblages and periphyton



biomass at 12 river reaches over a 13 month period, and trophic interactions were assessed for four common invertebrate taxa in laboratory experiments.

Results of laboratory feeding trials with herbivorous *Deleatidium* spp. (mayfly larvae), *Pycnocentroides* spp. (caddisfly larvae), *Potamopyrgus antipodarum* (gastropod), and Chironomidae (midge larvae), indicated that the first three taxa consume both *D. geminata* cells and stalks, and the chironomid larvae consume neither. The laboratory experiments provided the first evidence for invertebrate consumption of *D. geminata*. However, the contribution that *D. geminata* makes to the nutrition of invertebrates in *D. geminata*-affected rivers is not known, and the conditions under which invertebrate grazing can substantially reduce *D. geminata* abundance in rivers are not known.

A monitoring programme at 12 reaches in three *D. geminata*-affected rivers was used to quantify relationships between invertebrate assemblage variables and *D. geminata* biomass over a wide biomass range (0-600 g AFDM m<sup>-2</sup>). The invertebrate variables were total density, total biomass, taxon richness, sizes of individuals, and the proportion of invertebrate density and biomass composed of Ephemeroptera, Plecoptera, and Trichoptera (EPT) which are considered pollution-sensitive insect orders in degraded streams. Quantitative relationships between invertebrate variables and *D. geminata* biomass were generally weak due to high within-reach and between-reach variability. However, in all cases where statistically significant relationships were detected, invertebrate densities, biomass, and taxon richness increased with increasing periphyton biomass, whereas the proportional density and biomass composed of EPT decreased with increasing periphyton biomass. In general, *D. geminata* proliferations led to increased invertebrate abundance and increased diversity, but the assemblages shifted from a predominance of EPT taxa to a predominance of crustaceans, non-EPT insects, and worms. The preceding results are consistent with results of a June 2005 study, in which invertebrate assemblages in *D. geminata*-affected and *D. geminata*-free reaches of the Mararoa River were compared (Kilroy et al. 2006a). In that study, sites with *D. geminata* contained much higher densities of invertebrates than non-colonised sites, including more than twice the density of combined pollution intolerant insect orders EPT, and more than 20 times the density of pollution tolerant worms, and these relative densities resulted in concomitant reductions in the proportion of EPT (by taxa). No effect of *D. geminata* on the sizes of common invertebrates was detected in the current study. The lengths of *Deleatidium* sp (mayflies), *Zelandobius* sp. (stoneflies), Orthocladinae (midges), and Oligochaeta (segmented worms) were determined in each sample and regressed on *D. geminata* biomass; in each case, the regression did not explain a significant proportion of the variation in length data.

Threshold levels of periphyton biomass above which % EPT biomass fell below a guideline level of 50% were 12.5, 18.3 and 166.7 g AFDM m<sup>-2</sup> in the Mararoa, Waiau, and Oreti Rivers, respectively. The large threshold biomass value for the Oreti River is due to the greater proportion of EPT biomass in the Oreti at very low periphyton biomass levels compared with the Mararoa and Waiau Rivers.

One of the potential effects of *D. geminata* on native fish is alterations in food availability. Most of the native fish are generalist predators of benthic invertebrates, so information on changes in the density,

biomass and size structure of invertebrate assemblages caused by *D. geminata* is needed to assess possible effects on fish. As noted above, invertebrate densities and biomass generally increase with increasing *D. geminata* biomass; these are potential benefits for native fish. The absence of detectable effects of *D. geminata* on size of the most abundant invertebrate taxa suggests that selection for large prey items by fish may be unaffected by *D. geminata*. The reductions in % EPT abundance and % EPT biomass suggest that proportions of preferred prey items may decline at sites of high *D. geminata* biomass; the effect of these changes on fish behaviour needs to be tested by comparing predation at *D. geminata*-affected, and *D. geminata*-free reaches. Results of a preliminary study on trout drift foraging and bioenergetics found no significant negative effect attributable to *D. geminata* biomass on invertebrate drift density and biomass (Shearer et al. 2007). However, the drift data should be interpreted with caution as they were limited to two sampling dates, and predation was estimated from a bioenergetics model, not direct measurements of predation.

### Effects of *D. geminata* on water quality

Dissolved oxygen (DO) is required by all aerobic organisms, and DO depletion can have adverse biological effects. Oxygen is produced in rivers during daylight by algal photosynthesis and is removed by respiration. Rivers with high photosynthetic production and respiration also show marked diurnal changes in pH, which in turn affects chemical equilibrium, solubility and speciation. Thus rivers with large diurnal swings in DO often have similarly wide swings in pH, although this is also affected by the geochemical buffering capacity. Native freshwater fish and shrimp in New Zealand have been shown to be sensitive to pH levels above and below a circum-neutral range.

*D. geminata*-affected reaches in the Mararoa and Oreti Rivers were monitored continuously over several days for DO, pH and temperature, and these data were used in a diurnal model to compute values for  $P_{\max}$  (maximum photosynthetic rate value in a 24-h period) and  $R_{20}$  (the 24-h average respiration rate at a reference temperature of 20° C). The Mararoa reach was characterised by high *D. geminata* abundance, and the Oreti reach by low *D. geminata* abundance. The Mararoa sites had much higher rates of photosynthetic production and respiration, larger daily pH ranges and higher maximum pH levels, compared with the Oreti sites. These results indicated that DO in the Mararoa River is strongly influenced by *D. geminata*, and the Oreti River much less so. The maximum pH levels (> pH 9) associated with high *D. geminata* abundance are potentially deleterious for aquatic organisms.

*D. geminata* frequently covers river channel surfaces from bank to bank. Under these conditions, it is reasonable to predict that *D. geminata* will alter the chemical environment inhabited by benthic and epiphytic organisms. It is also likely that *D. geminata* modifies the benthic environment in ways that contribute to its own ecological success (e.g., by trapping nutrient-rich organic material and reducing diffusive nutrient losses). To determine how *D. geminata* affects the environment immediately above, within and below mats, electrochemical microsensors were used to construct profiles of dissolved oxygen and pH above and within mats in the Mararoa River. These in situ measurements are the first

of their kind from turbulent river environments, and are likely to be more realistic than measurements made in laboratory systems. The microsensor measurements were made in conjunction with fluorometric measurements of photosynthetic performance by *D. geminata* mats. Microelectrode measurements revealed DO peaks at the mat–water interface. These peaks are due to photosynthetic oxygen production by the pigmented *D. geminata* cells at mat surfaces, and this result was expected. However, we observed larger DO peaks below the surface of most mats, and gradients of increasing oxygen concentration from the pigmented surface down to the subsurface peak. These results were not expected, and lead us to speculate that a metabolically-active assemblage of photoautotrophic organisms inhabits *D. geminata* mats. This assemblage may take advantage of dissolved nutrients that are generated by remineralisation of organic matter at the bases of mats, and diffuse upwards.

### **Nutrient limitation in *D. geminata***

A nutrient enrichment experiment was carried out in seven *D. geminata*-affected rivers to assess nutrient limited biomass accrual, and to identify the predominant limiting nutrient(s). Nitrogen and phosphorus are the most common limiting nutrients in the temperate oligotrophic rivers that *D. geminata* has affected in New Zealand. The bioassay units were deployed for short (15–23 days) and long (26–37 days) periods to increase the likelihood of detecting treatment effects.

Algal biomass levels in the bioassays varied widely among sites, and between deployment times. After the initial deployment (15 – 23 days), biomass levels varied among sites from  $< 1$  to  $> 100$  mg chl *a* m<sup>-2</sup>. This range roughly corresponds to nutrient levels in the rivers; lowest biomass levels were measured in the most oligotrophic reaches (e.g., Ahuriri, Aparima, and Oreti Rivers) and the highest in the most enriched rivers (e.g., Waiau River). Results of two-way analyses of variance indicated that there were significant enhancement effects of nitrate enrichment at 2 rivers, the Ahuriri and Mararoa, and significant enhancement effects of phosphate enrichment at 3 rivers, the Aparima, Waiau and Waitaki. There were significant positive nitrate  $\times$  phosphate interactions at the Waiau and Waitaki. The three reaches in which significant enhancement effects could not be detected (Monowai River and the Oreti at Ashton Flat and Gravel Pit) were also reaches in which NDS units were lost to floods, and complete analyses could not be made. The data that were available after floods at those sites suggests that nutrient limitation was in effect after 27–28 days.

Results of the bioassays indicated that periphyton were nutrient-limited in five of the eight reaches tested. As *D. geminata* was present on all bioassay units and dominant on many, it appears that *D. geminata* is nutrient-limited across most of its current range in New Zealand. Comparisons of algal biomass from nutrient-enriched and control treatments indicate that on average, benthic algae accrual under ambient conditions is  $\leq 60\%$  of the potential, nutrient-enriched rate. Therefore, increased nutrient loading to affected rivers is likely to be followed by increased growth of *D. geminata*.

### ***D. geminata* biomass dynamics**

A 13-month monitoring programme was carried out to assess spatial and temporal variation in *D. geminata* abundance, and to identify and quantify relationships between *D. geminata* abundance and major environmental variables. Twelve reaches in the Oreti, Mararoa and Waiau Rivers were monitored at fortnightly-monthly intervals. To reduce habitat-based variability while ensuring that samples are representative of each reach, the monitoring programme focused on run habitats. A total of 3212 estimates of periphyton (predominately *D. geminata*) cover and Kilroy Biomass Index (KBI; product of % cover and average mat thickness), and 1830 periphyton biomass measurements were made between April 2006 and May 2007. Regressions were used to evaluate relationships between *D. geminata* abundance and solar radiation, water temperature, near-bed velocity, water depth, and elapsed time following bed-mobilising floods.

Periphyton abundance in most of the *D. geminata*-affected reaches peaked in August 2006, declined in September-October 2006, remained low through December 2007, and began increasing in January 2007. The September-October 2006 decline in periphyton abundance coincided with a series of floods that were moderate in magnitude, but sufficient to mobilize bed material. The sustained period of low abundance in summer 2006 coincided with a series of large-magnitude floods on each river from mid-November to mid-December.

In most of the study reaches, periphyton abundance decreased as a function of daily solar radiation on the sampling dates. In each of the Oreti River reaches, periphyton abundance decreased as a function of mean water temperature on the sampling dates. These negative relationships between periphyton and solar radiation and temperature were unexpected, because the ranges of solar radiation and temperature in the study area were not close to levels expected to inhibit algal productivity. The negative relationships are probably artefacts of the temporal effects of flood flows. Specifically, initial reduction of periphyton during the September-October 2006 floods coincided with the spring increase in solar radiation and water temperature, and the low levels of periphyton during the period of large floods in November and December coincided with the annual maxima in solar radiation and water temperature. The lack of a detectable statistical relationship between periphyton abundance and cumulative degree-days provides circumstantial support for this interpretation.

Relationships between periphyton abundance and instantaneous hydraulic variables were generally weak; regressions using near-bed velocity and Froude number explained 2 to 14% of the variability in periphyton biomass within reaches or rivers. No regressions of periphyton biomass on water depth were significant. In contrast, elapsed time following bed-mobilising floods was closely related to periphyton biomass in the Mararoa (two reaches), Oreti (five reaches) and Waiau (one reach) Rivers. Coefficients of determination for these regressions were higher than for regressions on near-bed velocity or Froude numbers, suggesting that time since bed-mobilising floods is a more important determinant of periphyton abundance than instantaneous hydraulic conditions. Support for this prediction comes from a hydraulic habitat study of the Mararoa and lower Waiau Rivers (Kilroy et al.

2006a). Results of that study indicated that *D. geminata* occupied a wide depth and velocity range, *D. geminata* biomass did not vary systematically with depth or velocity, and there were substantial reductions in biomass immediately after large floods.

Periodic measurements of dissolved nutrients in the Mararoa, Oreti and Waiau Rivers revealed no longitudinal gradients in ambient nutrient concentrations. There were no detectable relationships between average *D. geminata* biomass and ambient nutrient concentrations. These results suggest that instantaneous nutrient concentrations, like instantaneous hydraulic conditions, are poor predictors of long-term *D. geminata* growth and standing stock.

The preceding observations suggest that artificial floods are a potentially important means of controlling *D. geminata* proliferations in dammed rivers. However, several cautions are warranted concerning the effectiveness of artificial floods: 1) they are unlikely to be highly effective in rivers with armoured beds or with little fine sediment in storage; 2) they are unlikely to be effective far downstream of dams due to flood attenuation; 3) a minimum artificial flood magnitude (several times larger than baseflow) is required to generate sufficient bed shear to remove algae, particularly in cases of limited abrasion; 4) a possible unintended consequence of artificial floods for *D. geminata* is the dispersal of viable material downstream, leading to rapid range expansion within rivers.

Observations by Fish and Game field officers suggest that *D. geminata* is excluded from spring-fed streams, and from mixing zones downstream of spring-fed stream confluences with run off-fed rivers. These observations suggest that a physical or chemical property of groundwater prevents *D. geminata* from establishing or sustaining net growth. To assess the viability of *D. geminata* in spring-fed streams, a transplant study was carried out in May and June 2006. Cobbles with attached *D. geminata* mats (~ 100% cover) were collected from Mararoa River donor sites and shifted to two spring-fed streams, Flaxy Creek and Spring Creek. At intervals from 0 to 50 days after transplanting, samples of the mats on each cobble were taken for determination of percentage live *D. geminata* cells. Water samples were collected from the spring-fed streams and the Mararoa River donor sites to assess differences in chemistry. The percent live cells at each experiment site decreased from a starting value of ~ 80% to 15-20% over 50 days. This trend may have been due to a chemical constituent, but it may also have been due to grazing invertebrates. There was no evidence that flood flows caused the trend. The largest differences in chemistry between the spring-fed streams and Mararoa River sites were in nitrate, magnesium and sulphate concentrations; differences between other nutrients, major ions and heavy metals were generally small, and heavy metal concentrations were usually below the detection limit. Although these preliminary results indicate the viability of *D. geminata* declined when transported from areas of *D. geminata* proliferation in run-off-fed rivers to adjacent spring-fed streams, this pilot study provided no direct evidence supporting the prediction that groundwater chemistry excludes *D. geminata* from spring-fed streams.

## 1. Introduction

The invasive mat-forming diatom *Didymosphenia geminata* was first detected in the Waiau River, Southland, in October 2004. At the time of this report (October 2007), *D. geminata* has been recorded in approximately 53 rivers and streams (including tributaries in the same catchment) in Southland, Otago, Canterbury, Tasman, and Westland. In most of the affected rivers, extensive (10's-100's of metres) and thick (1-20 cm) mats have been observed. Mats in the mid to lower range of these dimensions have been observed in rivers of other temperate regions (e.g., British Columbia and North-central Europe; Rieberger 1991, Kaweka & Saneki 2003).

The current study is an assessment of the ecological effects that *D. geminata* has on river ecosystems, and of the environmental factors that control or influence *D. geminata* growth and abundance. Early assessments of the potential impacts of *D. geminata* to New Zealand (Kilroy 2004, Campbell 2005) indicated that perceived ecological impacts were high to extreme, but these assessments were, by necessity, based on anecdotal information because documented statistical or observational information was lacking. The current study is expected to add experimental evidence, observational surveys and inductive reasoning to the ongoing assessment of the potential and measured impacts of this relatively new introduction to New Zealand. The ecological endpoints measured for the current study were selected from amongst a comprehensive list developed by a panel of scientific experts and prioritized against the following list of operational criteria:

- current state of knowledge about the endpoint
- urgency to know more information about the endpoint
- feasibility of study completion within the timeframe and resources available
- economic and social impacts of the information
- relevance of the information to controlling *D. geminata*
- relevance of the information to resource management decisions.

### 1.1. Ecological effects

High levels of *D. geminata* biomass and cover in some river reaches suggests that the alga has strong effects on components of river ecosystems, including fish, macro- and microinvertebrates, other algae, flow dynamics, and biogeochemical processes. Direct effects of *D. geminata* on any of these components are likely to have indirect effects on others. For example, direct alteration of dissolved oxygen dynamics may affect fish and invertebrates, and direct effects on invertebrates may affect fish feeding



behaviour. We narrowed the focus of this study to examine the ecological effects of *D. geminata* on native fish, macroinvertebrates, and water quality.

No previous studies have been carried out to assess the effects of *D. geminata* on native fish distributions. However, it is logical to predict that *D. geminata* may have deleterious effects on native fish. Most of the native fish species in New Zealand rivers inhabit benthic habitats, consume benthic prey, and nest beneath or between cobbles. High benthic *D. geminata* cover may alter the quantity or quality of habitat or food available to native fish, and/or elicit behavioural changes in some fish species.

Preliminary studies in the Mararoa and Waiau Rivers indicated that sites with extensive *D. geminata* mats contained higher densities of invertebrates than non-colonised sites (Kilroy et al. 2006a), including more than twice the density of combined pollution intolerant insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), and more than 20 times the density of pollution tolerant worms. Hence, community composition also differed, with higher proportions of pollution- and fine sediment-tolerant invertebrates in *D. geminata*-affected areas. Also, mean invertebrate dry weights were lower in the *D. geminata*-affected areas, but without size class analyses, the mechanism for the weight reduction and what role the higher densities played were not discernable. In sum, these observations were informative, but they came from a very limited dataset (two rivers and one season of sampling). The sampling regime detailed in the present study is intended to generate more conclusive evidence about the effects of *D. geminata* on invertebrates.

Two types of direct interactions with *D. geminata* are likely to affect the abundance and diversity of benthic macroinvertebrates, habitat interactions and trophic interactions. In the case of habitat interactions, invertebrate species that require exposed sediment surfaces to forage, respire, and reproduce are likely to be affected when channel surfaces are covered by *D. geminata* mats. In the case of trophic interactions, invertebrate species that can utilize *D. geminata* as a food source are likely to be favoured over those that do not consume *D. geminata* (and whose food availability may be inversely related to *D. geminata* abundance). Studies of both types of interactions were carried out; habitat interactions were assessed by monitoring invertebrate assemblages and periphyton biomass at 12 river reaches over a 13 month period, and trophic interactions were assessed for four common invertebrate taxa in laboratory experiments.

Among the potential deleterious effects of *D. geminata* on river ecosystems are alterations in dissolved oxygen (DO), pH, and dissolved nutrients. Large increases in photosynthetic biomass, as occur during *D. geminata* blooms, are likely to increase the

amplitude of diurnal DO and pH cycles. Increased photosynthesis is predicted to cause higher daytime DO and pH levels, and increased respiration is predicted to cause lower DO and pH levels at night. If dead algal tissue or other organic material accumulates on river beds over long periods (weeks to months), bacterial decomposition may cause hypoxic or anoxic conditions to persist throughout the day. DO concentrations below ~ 5 mg/l can exclude fish and benthic invertebrates, reduce physiological performance, or cause shifts in species composition. Acidification or alkalisation can cause direct animal tissue damage and disrupt osmoregulation. Smaller reductions in pH can have sublethal effects, including avoidance behaviour and reduced growth. In the current study, effects of *D. geminata* on DO and pH levels were measured at two spatial scales, river reaches and individual *D. geminata* mats. The river reach-scale measurements were used to identify potential effects of altered diurnal DO and pH cycles caused by *D. geminata* on river fauna. The mat-scale measurements were used to explore the possibility that nutrient regeneration and restricted water flow within mats contribute to the rapid growth of *D. geminata* in oligotrophic water.

In large, oligotrophic, grassland rivers such as the Oreti, Ahuriri and Waitaki, low nutrient concentrations, high light levels and high *D. geminata* biomass suggest that the alga is relatively efficient at utilising scarce levels of nutrients, but nevertheless may be limited by the availability of dissolved nutrients, and that *D. geminata* is a major sink for dissolved nutrients. Testing for nutrient limitation may provide information about how *D. geminata* can be controlled or reduced in abundance (e.g., by reducing the transfer of growth-limiting nutrients from land to rivers). Examining effects of *D. geminata* on water column nutrient levels can provide information on changes in nutrient availability for other primary producers (e.g., periphyton, bacteria and aquatic macrophytes). Finally, anecdotal observations suggest that *D. geminata* cannot establish or does not sustain net growth in spring-fed streams. If true, this restriction may be due to high concentrations of dissolved minerals, metals or organic contaminants. An initial assessment of these observations was made by transplanting healthy *D. geminata* mats into spring-fed streams and monitoring growth responses, and by comparing water chemistry in samples from spring-fed streams lacking *D. geminata* with samples from adjacent run-off fed sites with abundant *D. geminata*.

## 1.2. Controlling factors

Controlling factors can be assessed in terms of temporal variation (i.e., controls on proliferation within sites over time), and in terms of spatial variation (i.e., controls on the locations of proliferations within rivers, within catchments, and across New Zealand). Despite the fact that observations of *D. geminata* proliferation date back over 100 years (Vallin 1951, Whitton 1984), the environmental conditions that



promote or limit proliferations are very poorly understood. Literature searches used to summarize environmental conditions favourable for *D. geminata* growth yielded broad ranges for most environmental factors (Kilroy 2004; Kilroy et al. 2005b). For example, the temperature and nitrate concentration ranges reported for reaches containing *D. geminata* mats were 6-20° C, and 0.007-0.32 mg/L, respectively. The environmental conditions reported in the literature were used in a geographic information system (GIS) to develop maps of suitable habitat for *D. geminata* in New Zealand (Kilroy 2005b), but these maps could not be validated until a new set of predictive maps was developed, after *D. geminata* had expanded its range. A new set of prediction maps have been completed this year, based on updated *D. geminata* distribution, a large number of potential explanatory variables, and boosted regression tree modeling (Kilroy et al. 2007). Results of the new mapping exercise predict that the proportion of run-off from lake-fed sub-catchments, the proportion of hard-rock lithology in a catchment, and elapsed time since significant flooding are the primary determinants of *D. geminata* abundance in a river (Kilroy et al. 2007). The first two variables are presumed to correspond to flow stability and substrate stability; all three variables are related to the time available for *D. geminata* biomass accrual between bed-mobilising floods. The accuracy of the predictive maps are limited by the range of environmental conditions at rivers currently occupied by *D. geminata*, and more generally, by the fact that *D. geminata* is still expanding its range in New Zealand. More accurate relationships between environmental factors and *D. geminata* abundance will require considerably more field data. For this reason, all field measurements of *D. geminata* abundance in the current research programme are accompanied by measurements of environmental factors. Environmental data from *D. geminata*-affected New Zealand rivers have been compiled for only 2 years. These data consist of measurements made by Kilroy et al. (2006a) in May-August 2005, and in the present study, from April 2006 to May 2007. The resulting datasets are small and highly variable; a substantial amount of additional sampling will be required to develop more reliable relationships.

Within affected rivers, environmental controls on *D. geminata* abundance have not yet been conclusively identified. It is possible that light, nutrient and/or temperature levels exert physiological control, and may produce a seasonal growth cycle. However, it is also possible that some factor or factors never exceed a threshold that limits *D. geminata*. For example, under conditions of severe nutrient limitation, natural variation in water temperature may have no detectable effect on growth. It is also likely that flood flows of sufficient magnitude to remove *D. geminata* mats by drag or bed-movement will influence standing stock. Preliminary data from Kilroy et al. (2006a) suggest that flood flows do not eliminate or substantially reduce *D. geminata* in a river reach unless the bed is mobilized. Flood flows are superimposed on seasonal cycles in light, nutrients and temperature, so effects of bed-mobilizing floods may

mask regular seasonal growth cycles. The interactions among the major controlling factors can only be teased apart by amassing sufficient data over time.

In addition to the field measurements of *D. geminata* and environmental factors, a separate study was devoted to measuring nutrient limitation in *D. geminata*. Long-term information about nutrient effects is needed for four reasons: 1) to determine whether nutrient controls on rivers could affect *D. geminata* proliferation; 2) to assess relationships between nutrient loads and the presence and absence of *D. geminata* within and among river systems; 3) to assess *D. geminata* survival and biomass development in highly eutrophic systems, and 4) to determine whether the current guidelines for nutrients and flows to prevent periphyton proliferations used for RMA-based decisions/consenting are adequate for *D. geminata*. At a multi-year time scale, differences between rivers and river reaches in *D. geminata* proliferation may be due to multiple factors, including differences in light, temperature, flow regime, nutrient availability and grazing intensity.

In the following report, the three studies on ecological effects of *D. geminata* (i.e., native fish, macroinvertebrates and water quality) are presented first, followed by the two studies on controlling factors affecting *D. geminata* growth and biomass (i.e., nutrient limitation and biomass dynamics). Each of the five studies is presented in sequence, and each has a separate methods, results and discussion section. A brief description of the sites used for all studies is described in the following section.

### 1.3. Study sites

River reaches used in the study are located in Otago and Southland, South Island (Figure 1, Table 1). Study sites were selected following reconnaissance trips in April and May 2006. The Mararoa, Oreti and Waiau Rivers of Southland were used as focal rivers for periphyton biomass and invertebrate monitoring and field experimentation. The upper Mararoa and Oreti Rivers each comprise a gradient of periphyton abundance; these gradients were used to develop relationships between periphyton abundance and other environmental variables (e.g., invertebrate assemblages, hydrological conditions). A single reach on the Waiau River, Excelsior Creek, has been the site of periphyton monitoring for several years, and was included in the present study to maintain continuity with the previous ecology project (Kilroy et al. 2006a). On the Mararoa and Oreti Rivers, 50-m long permanent reaches were selected for monitoring. At the time of the reconnaissance trips, the 5 Mararoa reaches and 6 Oreti reaches represented a 0 to > 90% range in periphyton cover. A single reach on the Oreti River, Mt Nicholas, was free of visible *D. geminata* during the first 10 months of the study, and was dry for the remaining four months of the study.

## 2. Effects of *D. geminata* on native fish

### 2.1. Vulnerability of native fish to *D. geminata* colonisation: a GIS analysis

Many of New Zealand's 49 native fish species forage, spawn and rest in benthic habitats. Unusually high algal biomass in benthic habitats such as that formed by *D. geminata* in New Zealand poses a risk for co-occurring benthic fish through reductions in habitat and food quality and quantity. Additional risks posed by are deleterious changes in water chemistry (e.g., reductions in night dissolved oxygen concentrations due to algal respiration). Twenty-four of the native fish species are non-diadromous and therefore non-migratory. Many of these species have restricted ranges. If the presence of *D. geminata* causes reductions to or losses of populations of non-diadromous species, population restoration may be slow, or may not occur. To help assess the risk of *D. geminata* to native fish species, a GIS study was carried out to compare the distribution of individual native fish species with distributions of rivers representing a range of *D. geminata*-colonisation risk. The risk to fish was assessed based on the percent of their habitat likely to be colonised by *D. geminata*, their threatened status according to the Department of Conservation, their potential for recruitment from other populations and their benthic substrate association.

#### 2.1.1. Methods

##### Model of potential colonisation by *D. geminata*

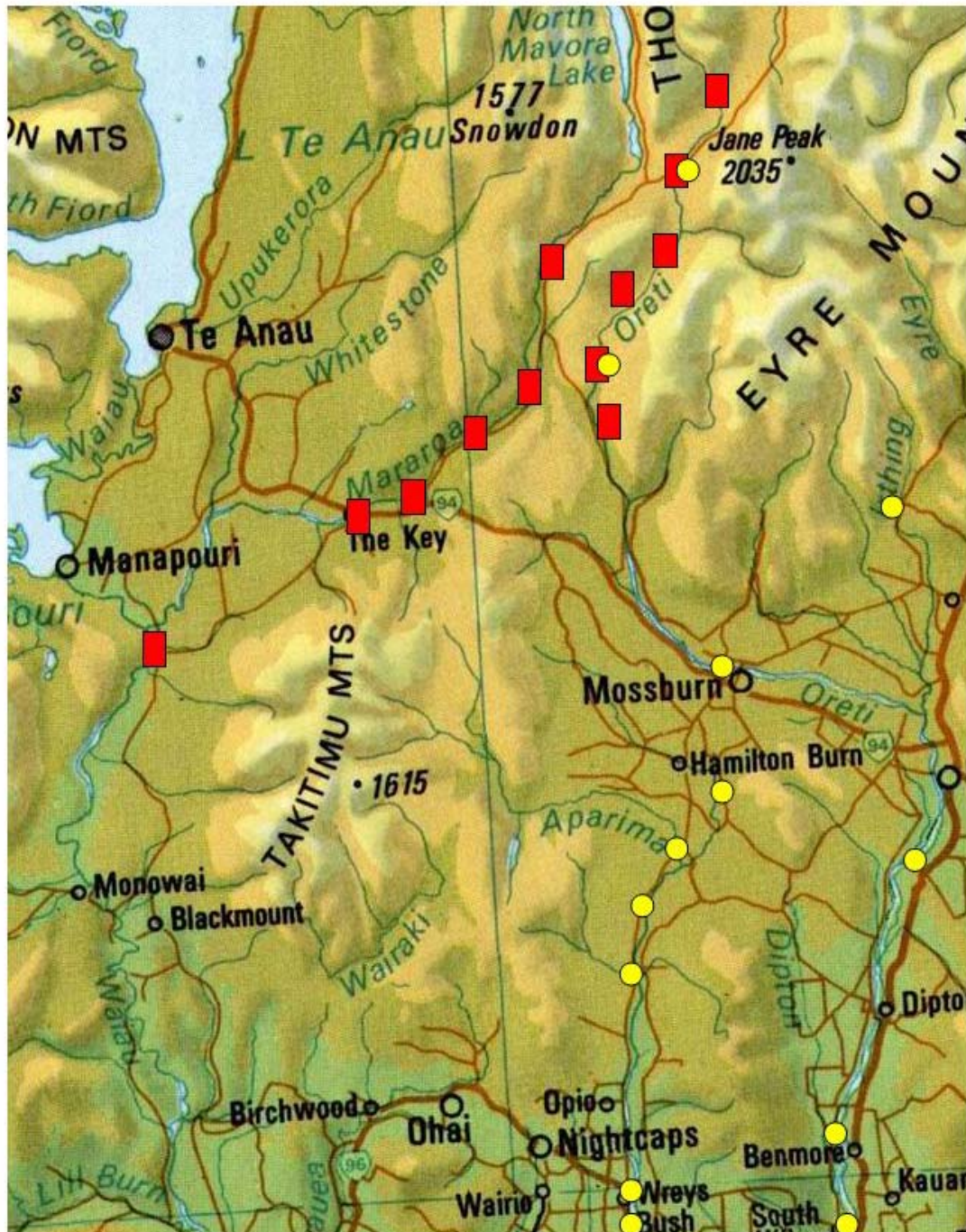
We used an existing stratification of New Zealand's rivers in terms of their suitability for *D. geminata* colonisation, based on the River Environment Classification (REC; Snelder et al. 2004). Environment factors that are assumed to strongly determine the distribution of the alga such as water temperature and flow stability contributed to the stratification; dispersal vectors were not included. A detailed description of the stratification process is in Kilroy et al. (2005b). Maps of potential colonisation by *D. geminata* in New Zealand are given in Appendix 1.

##### Model of native fish distributions

Data on fish distributions were compiled from records in the New Zealand Freshwater Fish Database (NZFFDB) (<http://niwascience.co.nz/services/nzffd>). Native species that are restricted to estuarine reaches of rivers (giant bullies, common and Stokell's smelt, yelloweye and gray mullet, flounders, kahawai) were excluded from the analysis because they will not co-occur with *D. geminata*. Lamprey were excluded because there are insufficient data for this cryptic species. Although inanga is a coastal species, it was included as it is a species of high recreational value. Inanga were also used to



assess model performance for a widespread coastal species. A list of the species evaluated, together with an indication of their distribution (North and/or South Island) and relative abundance is given in Table 2.



**Figure 1:** Map of Southland study area. Red boxes: invertebrate and periphyton monitoring reaches. Yellow circles: native fish survey reaches.

**Table 1:** Summary table of rivers and reaches used in ecology studies.

River	Reach	Native fish	Study			
			Macroinvertebrates	Water quality	Biomass dynamics	Nutrient effects
Ahuriri	Killermont Station					X
Aparima	Mossburn Road Bridge	X				X
Aparima	Dunrobin	X				
Aparima	Avondale	X				
Aparima	Wrey's Bush	X				
Aparima	Otautau	X				
Aparima	Thornbury	X				
Hamilton Burn		X				
Irthing Stream		X				
Mararoa	Key Bridge		X		X	
Mararoa	Station Bridge		X	X	X	X
Mararoa	Princhester Creek		X		X	
Mararoa	Haycock Hills		X		X	
Mararoa	Normans Gulch		X		X	
Monowai	Power Station					X
Oreti	Mt. Nicholas Station		X		X	
Oreti	Ashton Flats	X	X	X	X	X
Oreti	Haybarn		X		X	
Oreti	Three Kings	X	X	X	X	
Oreti	Centre Hill (Farm Manager's House)		X		X	
Oreti	Gravel Pits		X		X	X
Oreti	Mossburn	X				
Oreti	Josephville Road	X				
Oreti	Benmore Road Bridge	X				
Oreti	Wrey's Bush Bridge	X				
Waiau	Excelsior Creek		X		X	
Waiau	Monowai confluence					X
Waitaki	Otiati confluence					X

**Table 2:** List of New Zealand native species used in the present study. Distribution: N = North Island, S = South Island, W = widespread, R = restricted.

Common Name	Scientific Name	Distribution	Number of records in NZFFDB
<b>NATIVE SPECIES</b>			
<b>Diadromous species</b>			
Longfin eel	<i>Anguilla dieffenbachii</i>	N,S. W	9492
Shortfin eel	<i>Anguilla australis</i>	N,S. W	4581
Banded kokopu	<i>Galaxias fasciatus</i>	N,S. W	3047
Shortjaw kokopu	<i>Galaxias postvectis</i>	N,S. R	590
Koaro	<i>Galaxias brevipinnis</i>	N,S. W	2241
Inanga	<i>Galaxias maculatus</i>	N,S. W	2975
Torrentfish	<i>Cheimarrichthys fosteri</i>	N,S. W	1877
Redfin bully	<i>Gobiomorphus huttoni</i>	N,S. W	3602
Common bully	<i>Gobiomorphus cotidianus</i>	N,S. W	4349
Bluegill bully	<i>Gobiomorphus hubbsi</i>	N,S. W	934
<b>Non-diadromous</b>			
Alpine galaxias	<i>Galaxias paucispondylus</i>	S,R	234
Bignose galaxias	<i>Galaxias macronasus</i>	S,R	44
Canterbury galaxias	<i>Galaxias vulgaris</i>	S,W	855
Clutha flathead galaxias	<i>Galaxias</i> species D	S,R	140
Dusky galaxias	<i>Galaxias pullus</i>	S,R	58
Dwarf galaxias	<i>Galaxias divergens</i>	S,R	504
Eldon's galaxias	<i>Galaxias eldoni</i>	S,R	78
Lowland longjaw galaxias	<i>Galaxias cobitinis</i>	S,R	49
Northern flathead galaxias	<i>Galaxias</i> "northern"	S,R	105
Otago roundhead galaxias	<i>Galaxias anomalus</i>	S,R	93
Southern flathead galaxias	<i>Galaxias</i> "southern"	S,R	36
Southern roundhead galaxias	<i>Galaxias gollumoides</i>	S,R	101
Taieri flathead galaxias	<i>Galaxias depressiceps</i>	S,R	212
Teviot galaxias	<i>Galaxias</i> "Teviot"	S,R	10
Upland longjaw galaxias	<i>Galaxias prognathus</i>	S,R	77
Cran's bully	<i>Gobiomorphus basalis</i>	N,W	1000
Upland bully	<i>Gobiomorphus breviceps</i>	N,S,W	2763

Three methods of assessing distributions of individual species were used; all these methods were based on the REC geographic framework, and on the NZFFDB distributions.

- known distributions directly from the NZFFDB
- models of distribution using boosted regression trees, for migratory (diadromous) species. An advantage of this method was that it could “populate” areas where a species has not been recorded, but environmental parameters indicated there was a strong likelihood of that species being present.
- for non-diadromous fish distributions, boosted regression trees were used with addition of a catchment restriction factor (to constrain distribution to known catchments) to obtain better resolution for less widespread fish species like the central Otago galaxiid complex.

A boosted regression tree (BRT) is a form of stochastic gradient boosting (Friedman et al. 2000, Hastie et al. 2001). Boosted methods such as BRT differ from conventional regression-based methods, which are intended to identify a single model describing relationships between the response and predictor variables. Boosted models progressively build a “committee” of relatively simple models, each of which is adapted to explain features of data that are not well-explained by the preceding models (Hastie et al. 2001). Results of the ensemble of models are then averaged to form a final prediction. In recent comparative analyses, BRT has been shown to outperform conventional regression-based techniques (Leathwick et al. 2007).

#### **Estimating areas of overlap between distributions of individual fish species and likely colonisation by *D. geminata***

Data were imported into a GIS format using ArcMap, and maps of likely colonisation by *D. geminata* were determined at 3 levels (high, medium, and low likelihood). Maps of the distribution of native fish were produced, either showing known distribution (from NZFFDB) or potential distribution (based on a  $> 0.5$  probability of occurrence) for both diadromous and non - diadromous species. Distributions of some of the more widespread species are given in Appendix 1, Fig. 3 – 22.

REC “reach length” segments where *D. geminata* and fish distributions overlapped were then summed to provide the total length of waterway per species that could be colonised by *D. geminata*; these calculations were made for each of the three levels of

likely colonisation. Finally, data for each level were expressed as percentages of the total fish habitat per fish species likely to be colonised on the North and South Islands.

### **Estimation of overall vulnerability of individual fish species to habitat colonisation by *D. geminata***

An overall assessment of the vulnerability of individual species was made using the potential for colonisation data in Table 2, and the following criteria:

- whether a species is migratory or non-migratory (non-migratory species are more vulnerable to local extinction, as migratory species recruit from other catchments)
- threatened status, as defined by Department of Conservations (DoC; Hitchmough 2002)
- perceived association with substrates (e.g. whether a species spends much of its life concealed and feeding within substrates, whether it spawns within substrates)

For the threatened species scores, DoC rankings (Hitchmough 2002) in order of decreasing concern are as follows:

1. Nationally critical
2. Nationally endangered
3. Nationally vulnerable
4. Serious decline
5. Gradual decline
6. Sparse
7. Range restricted
8. Data deficient

If a species did not have one of these scores, it was classified as “not threatened”.

The present analysis used the first five categories. For the overall assessment of impacts, the categories were assigned the following values:



Nationally critical	5
Nationally endangered	4
Nationally vulnerable	3
Serious decline	2
Gradual decline	1
Sparse	0
Range restricted	0
Data deficient	0

As the DoC classification was made several years ago, it is incomplete for some species. Thus bignose galaxias was not listed, while the southern flathead and Teviot galaxias were listed as “data deficient”, meaning insufficient information was available at that time for a realistic assessment to be made. For the sake of completeness and between-species comparisons, assessments were made for these species based on the number of records on the NZFFDB, using a similar designation as that given below for diadromy. Therefore the following scores were given:

bignose galaxias	5
southern flathead	5
Teviot galaxias	5

For the overall assessments of the likelihood of *D. geminata* colonisation of habitat occupied by individual fish species, the total percentages for both North and South Islands (Table 1) were added. Scores were then apportioned according to the following ranges:

1	0 – 20 % habitat potentially colonised
2	21 – 40 % habitat potentially colonised
3	41 – 60 % habitat potentially colonised
4	61 – 80 % habitat potentially colonised
5	81 – 100 % habitat potentially colonised

Scores were assigned to non-migratory fish species corresponding to the risk posed by geographic restriction. The most isolated or restricted species were expected to have the lowest probability of population augmentation from neighbouring populations in resident populations and to be negatively affected by *D. geminata*. Scores were based

on the number of records for each non-migratory fish species in the NZFFDB. Migratory (diadromous) fish species were assigned scores of 0 to reflect the low risk posed to species that move between catchments via the coastal zone. The geographic restriction scores were assigned as follows:

0. diadromous species
1. 500 records
2. 250 – 499 records
3. 100 – 249
4. 50 – 99
5. < 50

Individual fish species show varying degrees of association with the substrates within their habitat. For example, it would be anticipated that more pelagic species like inanga would have a lower level of habitat association than a benthic-dwelling species like lowland longjawed galaxias. *D. geminata* is estimated to have significant impacts on river habitats (Campbell 2005), by affecting invertebrate assemblages, and infilling interstitial spaces used by some native fish for spawning and rearing.

To assess substrate associations, a qualitative approach was used, using the consensus of four experienced fishery ecologists. Scores were based on the preceding ordinal scale, with the weakest substrate associations assigned a score of 1, and the strongest assigned a score of 5. Two species were used to delineate the ends of the substrate association gradient, inanga (weak association) and “pencil” galaxiids (strong association); other species were scored relative to these extremes.

### 2.1.2. Results

The figures (Appendix 1, Figs. 3 – 22) show a number of contrasts, with some species having extensive areas of habitat potentially colonised (e.g., longfin eel), while other species have relatively small areas. The proportion of river kilometres occupied by individual fish species with a medium to high probability of colonisation by *D. geminata* is presented in Table 3.

For the overall assessment, the scores awarded to status, likelihood of colonisation, importance of diadromy, and substrate association were added, and the overall vulnerability of each species was assessed by:

Score of < 10 = L (low)

Score of 10 – 14 = M (medium)

Score of > 15 = H (high)

Scores and overall risk levels are shown in Table 4.

**Table 3: Percent of habitat (as river length) occupied by native fish species with a medium to high probability of colonisation by *D. geminata*.**

Species	North Island			South Island		
	Potential for colonisation			Potential for colonisation		
	Medium	High	Total	Medium	High	Total
<b>Diadromous species</b>						
Longfin eel	3.0	0.1	<b>3.10</b>	18.7	4.8	<b>23.5</b>
Shortfin eel	0.3	0.0	<b>0.3</b>	3.0	0.1	<b>3.2</b>
Banded kokopu	0.3	0.0	<b>0.3</b>	14.7	21.4	<b>36.1</b>
Shortjaw kokopu	2.0	0.0	<b>2.0</b>	2.2	0.0	<b>2.2</b>
Koaro	13.5	4.9	<b>18.4</b>	35.6	15.9	<b>51.6</b>
Inanga	0.2	0.0	<b>0.2</b>	8.1	0.7	<b>8.8</b>
Torrentfish	3.2	0.1	<b>3.2</b>	32.0	8.1	<b>40.1</b>
Redfin bully	1.3	0.0	<b>1.3</b>	13.9	14.1	<b>28.1</b>
Common bully	1.5	0.0	<b>1.6</b>	12.9	4.9	<b>17.9</b>
Bluegill bully	1.0	0.0	<b>1.0</b>	23.1	6.1	<b>29.2</b>
<b>Non-diadromous</b>						
Alpine galaxias				24.9	26.4	<b>51.2</b>
Bignose galaxias				25.7	15.7	<b>41.4</b>
Canterbury galaxias				43.2	36.5	<b>79.7</b>
Clutha flathead galaxias				76.9	11.1	<b>88.0</b>
Dusky galaxias				45.9	54.1	<b>100.0</b>
Dwarf galaxias	25.1	10.7	<b>35.8</b>	5.4	2.1	<b>7.5</b>
Eldon's galaxias				52.2	33.7	<b>86.0</b>
Lowland longjaw galaxias				87.0	2.1	<b>89.1</b>
Northern flathead galaxias				35.9	53.2	<b>89.1</b>
Otago roundhead galaxias				57.4	1.6	<b>58.9</b>
Southern flathead galaxias				55.7	40.9	<b>96.6</b>
Southern roundhead galaxias				48.6	26.8	<b>75.5</b>
Taieri flathead galaxias				38.8	59.9	<b>98.7</b>
Teviot galaxias				70.6	29.4	<b>100.0</b>
Upland longjaw galaxias				39.7	12.2	<b>51.9</b>
Cran's bully	0.1	0.0	<b>0.1</b>			
Upland bully	6.2	0.2	<b>6.4</b>	21.1	5.9	<b>27.0</b>

**Table 4:** Overall risk assessment of native fish species to colonisation by *D. geminata*. † = not recorded on DoC database, but relative assessment made.

	Threat status	Likelihood of colonisation by <i>D. geminata</i>	Geographic restriction	Substrate association	Overall risk
<b>Diadromous species</b>					
Longfin eel	2	1	0	2	L
Shortfin eel	0	1	0	1	L
Banded kokopu	0	1	0	1	L
Shortjaw kokopu	2	1	0	2	L
Koaro	0	2	0	3	L
Inanga	0	1	0	1	L
Torrentfish	0	2	0	5	L
Redfin bully	0	1	0	4	L
Common bully	0	1	0	2	L
Bluegill bully	0	1	0	5	L
<b>Non-diadromous species</b>					
Alpine galaxias	0	3	3	5	M
Bignose galaxias	5 <sup>†</sup>	2	5	4	H
Canterbury galaxias	0	5	1	5	M
Clutha flathead galaxias	3	5	3	5	M
Dusky galaxias	1	5	4	5	M
Dwarf galaxias	1	2	1	5	L
Eldon's galaxias	1	5	4	5	H
Lowland longjaw galaxias	5	5	5	5	H
Northern flathead galaxias	0	5	3	5	M
Otago roundhead galaxias	1	3	4	4	M
Southern flathead galaxias	5 <sup>†</sup>	5	5*	4	H
Southern roundhead galaxias	0	4	3	5	M
Taieri flathead galaxias	1	5	3	5	M
Teviot galaxias	5 <sup>†</sup>	5	5	5	H
Upland longjaw galaxias	1 <sup>†</sup>	3	4	5	M
Cran's bully	0	1	1	4	L
Upland bully	0	2	1	3	L

## 2.2. Impacts of *D. geminata* on the distribution and abundance of native fish

A preliminary electric fishing survey was conducted in May 2006 to compare the diversity, abundance and well-being (growth and condition) of native fish between comparable *D. geminata*-affected and *D. geminata*-free river reaches.

### 2.2.1. Methods

#### Study sites

The locations of river reaches used for quarterly sampling of fish distributions are listed in Table 5. The plan in the original proposal was to compare the diversity, abundance and well-being (growth and condition) of native fish at *D. geminata*-affected reaches in the Oreti River with similar data from (then) non-affected sites in the Aparima River. In the Oreti River, we had planned to sample *D. geminata*-free (control), and *D. geminata*-affected reaches. To incorporate at least one representative small non-migratory galaxiid (the species group of most concern to DoC), one site was to be within the area where the alpine galaxias has been recorded. In the Aparima River, we planned to sample control reaches. While it had been recognised that there was a high likelihood that some portion of the Aparima River would be colonised by *D. geminata* during the following year, the pre-colonisation electric fishing data would have provided an opportunity to carry out a robust before-after-control-impact analysis.

**Table 5:** Location of sampling sites (East and North = GPS references).

Sites	Site number	East	North
Aparima above Dunrobin	1	2124523	5484108
Hamilton Burn at Goodalls Rd	2	2132652	5488748
Aparima at Mossburn Rd bridge	3	2134993	5480443
Aparima at Avondale (near Etal stream)	4	2131831	5473346
Aparima at Wreys Bush	5	2131798	5452646
Aparima at Otautau	6	2123863	5441004
Aparima at Thornbury	7	2131619	5424628
Upper Oreti (near Mavora Lakes)	8	2134570	5531048
Oreti at Oreti road	9	2129212	5518647
Oreti at Mossburn	10	2140236	5494644
Irthing Stream by Five Rivers	11	2129213	5518649
Oreti at Josephville Rd	12	2152677	5480404
Oreti at Benmore Rd bridge	13	2147603	5462562
Oreti at Wreys Bush/Winton Rd bridge	14	2146651	5443277

Shortly after the project proposal was submitted (February 2006), *D. geminata* was recorded from the Aparima River, and this river could no longer function as a true control. *D. geminata* cover at colonised sites was generally low during the February

2006 survey, presumably because colonisation was at an early stage. Rather than a before-after study, we used the Aparima sites to assess fish assemblages over a narrow gradient of *D. geminata* abundance..

### **Reconnaissance survey and site selection**

A reconnaissance of the Oreti River was made on 4 May 2006 to identify suitable sampling reaches and access (mainly provided by Fish and Game angler access points). The Aparima River was not included in this initial survey as we were familiar with it from a previous study (e.g., McCleave and Jellyman 2004). Sampling was carried out the week of 21 May 2006, at six sites each in the Aparima and Oreti, and two uncolonised control sites (Table 2).

At the Oreti River, the uppermost site was just beyond the Mavora Lakes turn off on the Mt. Nicholas Station road, and sites downstream of here were selected every 15-20 km, with the lowest site near Wreys Bush-Winton Road bridge. Below Winton, no suitable sites could be found as the Oreti is channelised and the water is too deep for electric fishing. A site was also chosen on Irthing Stream to serve as a control.

The Aparima River sites were selected from the NZMS 260 series maps. The upper site was immediately above Dunrobin Station, and the downstream sites were spaced approximately 15-20 km apart until the last site above the Thornbury bridge. A control site was chosen in the Hamilton Burn near Mossburn.

### **Electric fishing**

#### **Native fish surveys of sites in the Aparima and Oreti Catchments**

Five lanes were electric-fished at each site; each lane was 20 m<sup>2</sup>, and water types were riffles or runs, or a mix of each (riffles usually have greatest species diversity and highest densities of fish). Sites were referenced by GPS. Measurements included channel width, fish cover, water temperature and conductivity. Substrate provided most of the cover for fish, and ranged from gravel to boulders.

Within each lane, the following parameters were recorded:

- Habitat type (% run, riffle)
- Three measurements of water velocity, at the ends and middle of each lane.
- The range and percentages of substrate types present
- The lengths of individual fish and weight of a sample of up to 20 specimens (for calculation of condition factors)

- Percent cover by *D. geminata*, and mat thickness on 5 rocks.

All sites were sampled with a Kainga EFM300 battery-operated backpack electric fishing machine, set on 300-400 volts and at a pulse frequency of 100 hz. Sampling was conducted by fishing downstream into a fine-mesh stop net that covered ~1 m width of stream. All captured fish were returned to their original habitat following completion of sampling at that site. One lane at each site was repeatedly fished (up to three times), so that the resulting depletion in successive catches could be used to estimate total fish populations.

### 2.2.2. Results

The May 2006 electric fishing survey generated a fish dataset corresponding to low levels of *D. geminata* abundance at newly-colonised sites in the Aparima and Oreti Rivers. *D. geminata* was present at 2 of the 14 survey sites, both in the Oreti River (Table 6). The percent cover ranged from 5 - 85% of lane areas; there were no thick mats blanketing the entire channel. The May 2006 dataset was intended for comparison with data from subsequent surveys corresponding to higher levels of *D. geminata* abundance. Rapid growth of *D. geminata* was forecast to occur at newly-colonised sites during spring and summer. However, funding was not available to carry out the subsequent surveys. Results of the May 2006 survey are reported here.

Fish densities in the Aparima and Oreti Rivers were similar, but composition and relative abundance differed. The Aparima River contained eight species (seven natives, one introduced) compared with seven (six natives, one introduced) for the Oreti River. Upland bullies were the dominant species (44%) in the Aparima, followed by galaxiids (30%), and fish were distributed relatively evenly throughout the catchment. In contrast, galaxiids dominated samples in the Oreti River (88 %) followed by upland bullies (6 %), and fish densities declined markedly with distance downstream (Table 7). The galaxiids species group included several of the “Otago-galaxiids” whose taxonomy is in revision. Contrary to expectations, the highest density of galaxiids among the sites was measured at one of the Oreti River sites with moderate *D. geminata* cover (124 fish m<sup>-2</sup>, 14% *D. geminata* cover).

Diadromous species (longfin eel, torrentfish, common bully) were only collected from the lower sites in each river, reflecting arrival of juveniles from the sea and gradual upstream penetration. Size and condition data (Table 8) were intended for comparison with future samples. There were no obvious or consistent trends between fish sizes and distance upstream (as indicated by “Site” in Table 8).

**Table 6:** Summary of physical data at native fish survey sites. Width, water temperature and electrical conductivity (EC) were measured once at each site; other variable values are averages of 5 lanes per site. ElCond: Electrical conductivity

Site	Width	Water temp.	EC	% run	% riffle	Lane depth	Lane velocity	<i>D. geminata</i>		Substrate %					
	Mean (m)	Mean (°C)	Mean (µS/cm)	Mean	Mean	Mean (m)	Mean (m/s)	Mean % cover	Mean mat depth	Mud	Sand	Fine gravel	Coarse gravel	Cobble	Boulder
Aparima 1	15	6.8	40	50	50	0.26	0.53	0		0	2	0	4	18	76
Aparima 2	12	8.1	80	62.5	37.5	0.26	0.64	0		0	0	9	23	45	24
Aparima 3	20	6.4	40	40	60	0.24	0.58	0		0	0	10	39	49	1
Aparima 4	30	7.6	70	50	50	0.22	0.67	0		0	0	0	29	69	2
Aparima 5	50	5.6	80	40	60	0.15	0.60	0		0	0	3	29	62	6
Aparima 6	90	7.1	110	40	42	0.13	0.47	0		0	0	12	53	33	0
Aparima 7	60	6.9	120	60	40	0.15	0.50	0		0	1	38	41	20	0
Oreti 1	15	8.3	20	58	42	0.28	0.73	18	3	0	6	24	45	25	0
Oreti 2	20	8.1	20	0	100	0.23	0.65	44	4	0	2	14	28	42	14
Oreti 3	25	7.6	40	20	80	0.22	0.74	0		0	0	13	39	44	4
Oreti 4	11	6.9	10	75	25	0.20	0.74	0		0	3	38	36	23	0
Oreti 5	35	7	50	80	20	0.18	0.84	0		0	0	22	41	37	0
Oreti 6	50	8.2	60	50	50	0.19	0.70	0		0	0	21	59	20	0
Oreti 7	55	6.5	70	24	76	0.15	0.70	0		0	0	40	48	12	0



**Table 7:** Summary of area fished, total fish numbers and densities by site, and densities of all galaxiids and upland bullies (sites listed from upstream to downstream). Note that densities are for first pass electric fishing only and have not been adjusted for sampling efficiency.

Site	Area fished (m <sup>2</sup> )	All fish		All galaxiids	Upland bullies
		Total No	No/m <sup>2</sup>	No/m <sup>2</sup>	No/m <sup>2</sup>
Aparima River					
1	100	21	0.21	0.03	
2	100	5	0.05	0.03	0.01
3	100	74	0.74	0.77	0.31
4	100	28	0.28	0.28	0.15
5	100	12	0.12	0.10	0.08
6	100	12	0.12	0.05	0.02
7	120	72	0.60	0.34	0.34
Totals/mean	720	224	0.31	0.23	0.14
Oreti River					
1	100	124	1.24	1.24	0.00
2	100	35	0.35	0.27	0.03
3	100	56	0.56	0.48	0.08
4	100	30	0.30	0.23	0.04
5	80	2	0.03	0.03	
6	100	3	0.03	0.01	
7	100	11	0.11	0.03	
Totals/mean	680	261	0.38	0.34	0.02

**Table 8:** Summary of electric fishing catches, Aparima and Oreti Rivers.

River	Species	Site	No.	Length (mm)		No.	Weight (g)		Condition	
				Mean	SD		Mean	SD	Mean	SD
Aparima	Longfin eel	6	4	120.3	4.5	4	2.81	0.28	0.161	0.003
	Torrentfish	5	2	79.0	9.9	2	6.18	1.77	1.244	0.108
		6	2	79.5	6.4	2	5.63	0.82	1.122	0.106
		7	16	58.6	20.3	16	3.59	3.52	1.299	0.093
	Galaxiids	1	3	61.3	9.0	3	1.86	0.92	0.751	0.096
		2	2	59.5	7.8	2	1.11	0.01	0.553	0.206
		5	2	61.5	7.8	2	1.62	0.59	0.679	0.006
		6	3	71.0	9.5	3	2.88	1.39	0.763	0.068
	Flathead galaxias	3	43	71.2	15.5	10	3.78	2.62	0.786	0.079
		4	13	70.9	17.0	12	3.24	2.61	0.741	0.070
	Upland bully	2	1	58.0		1	2.22		1.138	
		3	31	54.0	12.8	12	2.82	0.61	1.189	0.112
		4	15	54.5	9.8	8	2.22	0.96	1.116	0.090
		5	8	39.6	5.0	8	0.82	0.45	1.170	0.366
		6	2	50.5	13.4	2	1.52	1.11	1.062	0.004
		7	41	37.2	6.3	2	0.89	0.54	1.169	0.029
	Common bully	7	15	40.0	6.9	3	1.14	0.70	1.083	0.103
	Brown trout	1	18	88.6	9.8	17	8.36	2.85	1.166	0.112
		2	2	96.5	23.3	1	5.80		1.133	
		6	1	125.0		1	20.03		1.026	
	Total	Total	224							
Oreti River	Longfin eel	14	2	119.5	17.7	2	3.33	1.27	0.191	0.010
	Torrentfish	14	6	89.0	19.4	6	9.81	7.75	1.218	0.063
	Galaxiids	8	123	52.5	8.7	23	1.41	1.02	0.706	0.171
		9	27	80.7	19.6					
		10	43	68.3	14.0	12	3.49	2.01	0.732	0.109
		11	23	68.3	15.5	17	3.38	2.54	0.763	0.079
		12	2	90.5	12.0	2	5.58	1.31	0.757	0.123
		13	1	65.0		1	2.13		0.776	
	Flathead galaxiid	13	2	68.5	7.8	2	1.98	0.27	0.626	0.128
		14	3	83.0	11.5	3	4.46	1.71	0.750	0.065
	Alpine galaxias	8	1	60.0						
		10	5	61.2	6.5					
	Upland bully	9	3	58.7	3.5					
		10	8	40.3	14.8	8	1.18	1.49	1.194	0.181
		11	4	52.8	14.6	3	2.62	0.89	1.202	0.054
	Brown trout	9	5	101.8	18.3					
		11	3	109.3	22.8	3	16.39	11.33	1.114	0.094
	Total	Total	261							

### 2.3. Discussion

All ten diadromous fish species were ranked as low risk to colonisation by *D. geminata*. This group included the commercially and recreationally important longfin and shortfin eels, and the whitebait species. Of the 15 non-diadromous galaxiid species, 5 were ranked as highly vulnerable, 9 as moderately vulnerable, and one, the dwarf galaxias, as having low vulnerability. Both non-diadromous bully species, Cran's bully and upland bully, were assessed as having low vulnerability.

This outcome seems reasonable for two reasons. First, diadromous species are generally widespread, and hence the potential impacts of colonisation by *D. geminata* are "diluted" across wide areas. Second, most non-diadromous species have restricted distributions, usually within areas with a high likelihood of colonisation by *D. geminata*.

Exceptions to these generalisations are the non-diadromous species Canterbury galaxias, dwarf galaxias, Otago roundhead galaxias, possibly southern roundhead galaxias, upland longjaw galaxias, Cran's bully and upland bully. These species have relatively broad distributions, and populations that are negatively affected by *D. geminata* could be replaced or augmented by recruitment from adjacent catchments.

The greatest uncertainty in the GIS analysis is the model of colonisation by *D. geminata* (Kilroy et al. (2005b)). The model was based on outflows from lake-fed catchments, hard geology, cool water temperatures, and hydrological stability. Additional information on factors affecting *D. geminata* distribution would probably increase model accuracy. The model predicts a much greater likelihood of *D. geminata* colonisation in South Island rivers compared with North Island rivers, due to lower temperatures in South Island rivers. Sparse colonisation predicted for the South Island West Coast is due to hydrological stability terms in the model, i.e., duration of stable flows and flood frequency; the West Coast has higher flood frequencies and a lower stable flow duration than elsewhere in New Zealand.

After the GIS analysis was completed for the current study, a new modelling approach was developed to analyse *D. geminata* colonisation patterns and predict future expansion of the *D. geminata* range in New Zealand river (Kilroy et al. 2007). We recommend that subsequent GIS-based assessments of the risk posed by *D. geminata* employ the Kilroy et al. (2007) model.

Criticisms can be levelled about the techniques used in the current risk assessment. For example:

- Four factors were used to assess species vulnerability (threat status, geographic restriction, substrate association, and *D. geminata* colonisation potential). Other factors could have been added or substituted. However, much of the existing geographic information was subsumed in the *D. geminata* colonisation potential factor. Overall it is considered that the factors used adequately covered the important features of the biology of the fish species.
- The four factors were not differentially weighted. For consistency and to avoid making somewhat arbitrary decisions on relative weightings, all factors were assumed to be of equal importance.
- The number of records in the NZFFDB was used to assess the conservation status of three species whose status was not listed on the DoC database, and to rank the geographic restriction of non-diadromous species. Consequently, conservation status and geographic restriction were not independent factors. However, only three species were affected, and all three have restricted ranges. A reduction in their status from “nationally critical” to “nationally endangered” would not have affected their overall risk.
- Substrate association ranks were assigned by a group of experienced fishery biologists, and it is possible that additional information for the central Otago galaxiids could have made slight differences to the evaluations of these species. Ideally, a less subjective technique like the use of substrate suitability preferences would be used for such assessments, but suitability preference data are only available for common species (Jowett and Richardson 1995).

Many native fish species have strong associations with substrates, spending most of their lives in the spaces between cobbles, and feeding and spawning in the same habitat. Many of the non-diadromous fish species burrow in gravels (Cadwallader 1976, McDowall and Eldon 1997, Dunn and O’Brien 2006). At least one fish species (lowland longjaw galaxias) deposits eggs at gravel depths > 20 cm (P. Ravenscroft, DoC, pers. comm.). Spawning success in these species depends on rapid exchange of water between the surface and the spawning gravel to supply oxygen to eggs. Foraging and spawning in substrate-dependent fish species taxa could be compromised by extensive *D. geminata* mats.

A more conservative approach to the final assessment, based solely on the likelihood of colonisation by *D. geminata*, resulted in an overall assessment similar to the assessment with four factors (Table 3). There were no changes in risk for the diadromous species when the conservative approach was applied, and only three changes for the non-diadromous species. Canterbury galaxias and Taieri flathead

galaxias would shift from moderate to high risk, and bignose galaxias from high to low risk. Bignose galaxias is confined to the McKenzie basin, and although it is quite widespread within that area, a conclusion of low risk would be inappropriate.

Whether the final risk analysis was based on multiple factors, or solely on the likelihood of colonisation by *D. geminata*, the process highlighted the vulnerability of the small non-diadromous galaxiid species to *D. geminata*. This in turn emphasises the need to contain the spread of *D. geminata*, and prevent introductions in rivers like the Kauru (Kakanui River tributary) and Teviot, which contain some of New Zealand's rarest native fish species.

### **3. Effects of *D. geminata* on macroinvertebrates**

#### **3.1. Field study**

The primary objective of the field study was to develop empirical relationships between invertebrate assemblages and periphyton abundance across a broad gradient of *D. geminata* abundance. These relationships are needed to predict the effects of subsequent *D. geminata* colonisation, and to provide information about potential effects of *D. geminata* on aquatic biodiversity and on food supplies for fish.

##### **3.1.1. Methods**

A quarterly sampling campaign was carried out at 12 reaches in the three focal rivers (Fig. 2). The reaches were selected to provide a wide range of *D. geminata* abundance levels, and subsequent monitoring indicated that this range was maintained through the 13-month study (see Section 6). For example, time-averaged % cover of *D. geminata* in the Oreti River ranged from 0-89%. All monitoring sites were runs (rapidly-flowing, relatively deep water), which were the predominant habitats in each reach. The first quarterly sample collection was disrupted by a large flood in late April 2006 (discussed in detail in Section 6), so samples were collected during two periods, 24-25 April 2006 and 17 May 2006. Subsequent sampling dates were 1-2 August 2006, 13-15 December 2006, and 27-28 March 2007. At each site, six samples were collected using a Surber sampler (area 0.06 m<sup>2</sup>, mesh size 250 µm). Invertebrate samples were transferred to 600 ml containers and preserved with isopropyl alcohol (final concentration ~ 50%). Two rocks immediately adjacent to the Surber sampler were selected at random for algal biomass determination. Algal samples were collected and processed following the procedures in Section 6.1.





**Figure 2:** River reaches used for invertebrate and *D. geminata* monitoring on the Mararoa and Oreti Rivers.

Invertebrate samples were processed at Cawthron Institute, Ltd. Briefly, samples with large amounts of *D. geminata* were rinsed and the algae pulled apart in water to suspend invertebrates. The bulk of the invertebrates were separated from algal material, gravel and sand by elutriation through a 300- $\mu$ m mesh sieve. The remaining sand, gravel, and algae were scanned for invertebrates, which were removed by hand. Samples with little or no *D. geminata* were processed entirely. Invertebrates from the samples were identified (species level or coarser), counted, and measured (standard lengths for insect larvae, crustaceans, and mollusks, total lengths for worms). Counts

were converted to areal densities. Excel macros with taxon-specific length-biomass relationships were used to estimate invertebrate biomass in each sample.

Comparisons of invertebrate assemblage parameters were made by one-way analysis of variance and post hoc pair-wise Tukey tests. Comparisons focused on among-site, within-river differences, based on the rationale that each river comprises a pool of invertebrates that is hydrologically-connected across sites. If invertebrate assemblages at different sites within each river were broadly similar (i.e., differences in assemblage properties are small in magnitude), it was reasonable to pool data from multiple sites to produce river-scale invertebrate-periphyton relationships.

Univariate relationships between periphyton biomass and invertebrate parameters were identified and quantified by linear regression. The independent variable in these relationships is periphyton biomass, rather than *D. geminata* biomass, because the periphyton samples associated with the invertebrate samples were entirely *D. geminata* (see Section 6.1.2, below). Each invertebrate sample was paired with the average periphyton biomass on the two rocks collected next to the sampler. Data from all four sampling dates were included in each regression. The primary invertebrate assemblage parameters were: taxon richness, total invertebrate density, total biomass, lengths of the most common taxa, % EPT abundance, and % EPT biomass. EPT refers to the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). EPT taxa are frequently used as indicators of general stream health, as some species from the EPT orders are sensitive to contaminants and suspended sediment concentrations, low dissolved oxygen, and high temperatures (Quinn and Hickey 1990, Wallace et al. 1996). EPT-indices were originally developed for biological assessments of chemical water quality (Lenat 1988), but have since been used to assess a wide variety of environmental stressors, including periphyton proliferations. It is not always clear that EPT-indices are the most accurate or precise variable for these assessments. In the current study, EPT-indices were used because reductions in the relative abundance of EPT taxa with increasing periphyton biomass have been recorded in many New Zealand streams (Biggs & Kilroy 2000). The parameters % EPT abundance, and % EPT biomass refer to proportions of EPT individuals per sample and proportions of EPT taxa by biomass, respectively. Absolute EPT density and EPT biomass were not included in the analyses because these parameters were highly correlated with total invertebrate density and total invertebrate biomass, respectively (linear correlation for total and EPT density  $r = 0.59$  for all sites; linear correlation for total and EPT biomass  $r = 0.84$  for all sites).

Negative relationships between periphyton biomass and % EPT biomass were used to estimate threshold periphyton abundance levels, above which invertebrate assemblages appear to be negatively affected. In a large-scale assessment of

macroinvertebrates in New Zealand rivers, Quinn and Hickey (1990) reported the EPT comprised > 60% of invertebrates in gravel/cobble-bed rivers with low nutrient concentrations, within upland catchments dominated by tussock and unimproved pasture. The monitoring sites used in the present study are in similar settings, and the 50% level is a conservative threshold between rivers dominated by “clean water” invertebrates and rivers dominated by molluscs, crustaceans, Chironomidae, Diptera and other taxa associated with poor water quality. Note that Quinn and Hickey (1990) calculated % EPT as the proportion of EPT taxa in the total taxon richness; biomass data were not provided in their study.

The effect of periphyton on invertebrate community structure was analysed by principal components analysis (PCA) using PCORD software. The co-variance matrix of the invertebrate community was reduced to 2 principal components and sites were plotted in two-dimensional ordination space. The rarest taxa (present in < 4 samples) were removed from the dataset before analysis.

Effects of *D. geminata* biomass on body sizes of common benthic invertebrates were assessed by regression. The four invertebrate taxa with the highest abundance across all sites in the Mararoa and Oreti rivers were used in the analysis: *Deleatidium* sp (Ephemeroptera, mayflies), *Zelandobius* sp. (Plecoptera, stoneflies), Orthocladiinae (Diptera, midges), and Oligochaeta (Annelida, segmented worms). Standard lengths of all individuals were recorded during quarterly sample processing. The abundance of each taxon at each site and date was calculated by summing over six replicate samples. The proportions of individuals in each of 14 3-mm length classes were determined and these proportions were multiplied by the mid-range of each length class; each product is an abundance-weighted length. The abundance-weighted mean lengths for the four taxa were regressed on the corresponding mean *D. geminata* biomass levels.

### 3.1.2. Results

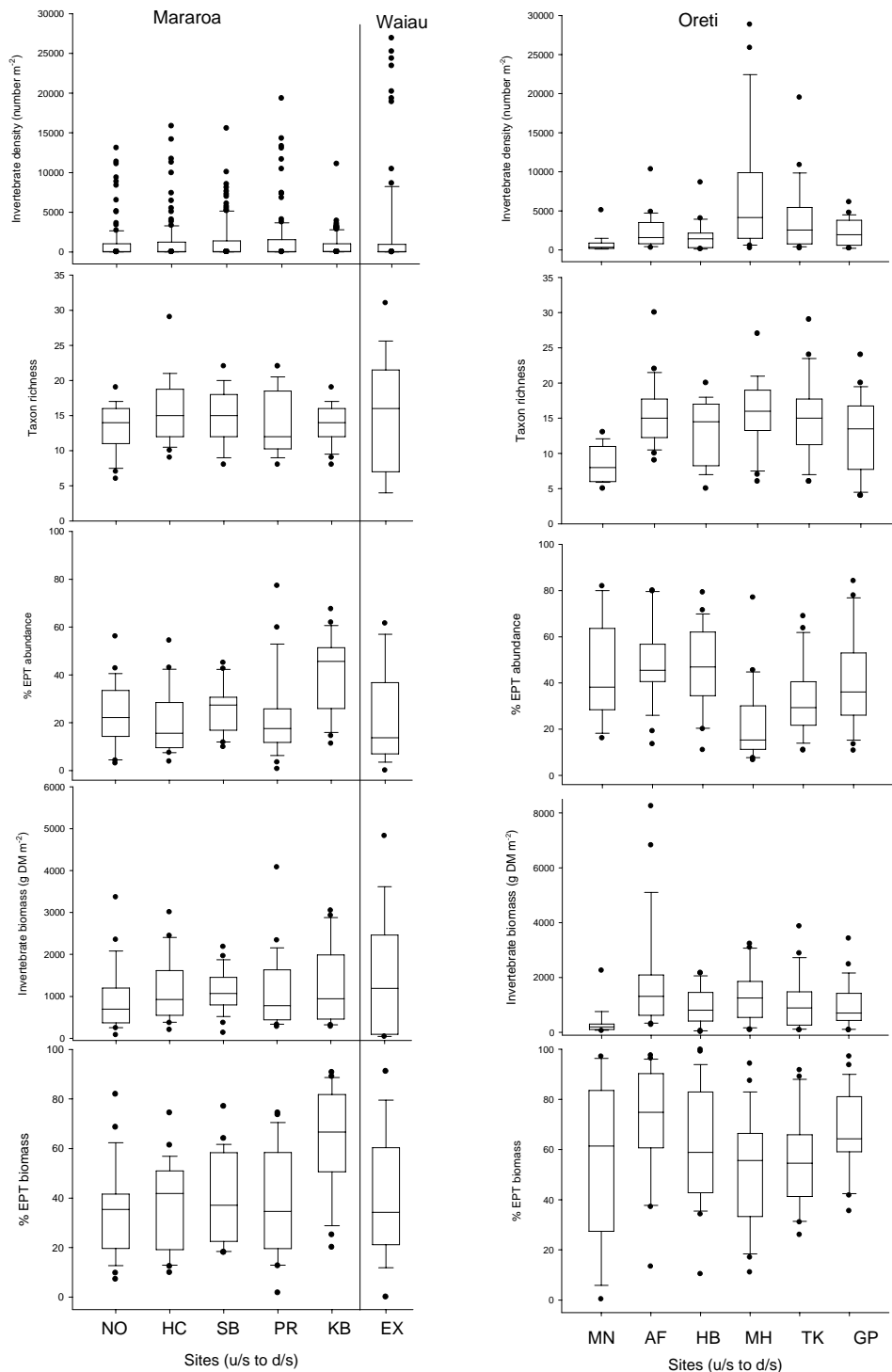
As noted in the methods section, absolute EPT density and absolute EPT biomass were highly correlated with total invertebrate density and total invertebrate biomass, respectively. Proportional EPT abundance and proportional EPT biomass were not significantly correlated with total density or biomass. The relationships reported here are limited to the non-correlated invertebrate parameters. The distributions of invertebrate assemblage parameters are shown as box plots in Figure 3. Pair-wise comparisons between reaches (Tukey tests following significant one-way ANOVAs) within the Mararoa and Oreti Rivers indicated that both rivers have a single reach that is dissimilar to others in the same river. In the Mararoa, the furthest down-stream reach at Key Bridge, had significantly ( $P \leq 0.05$ ) lower invertebrate densities, and



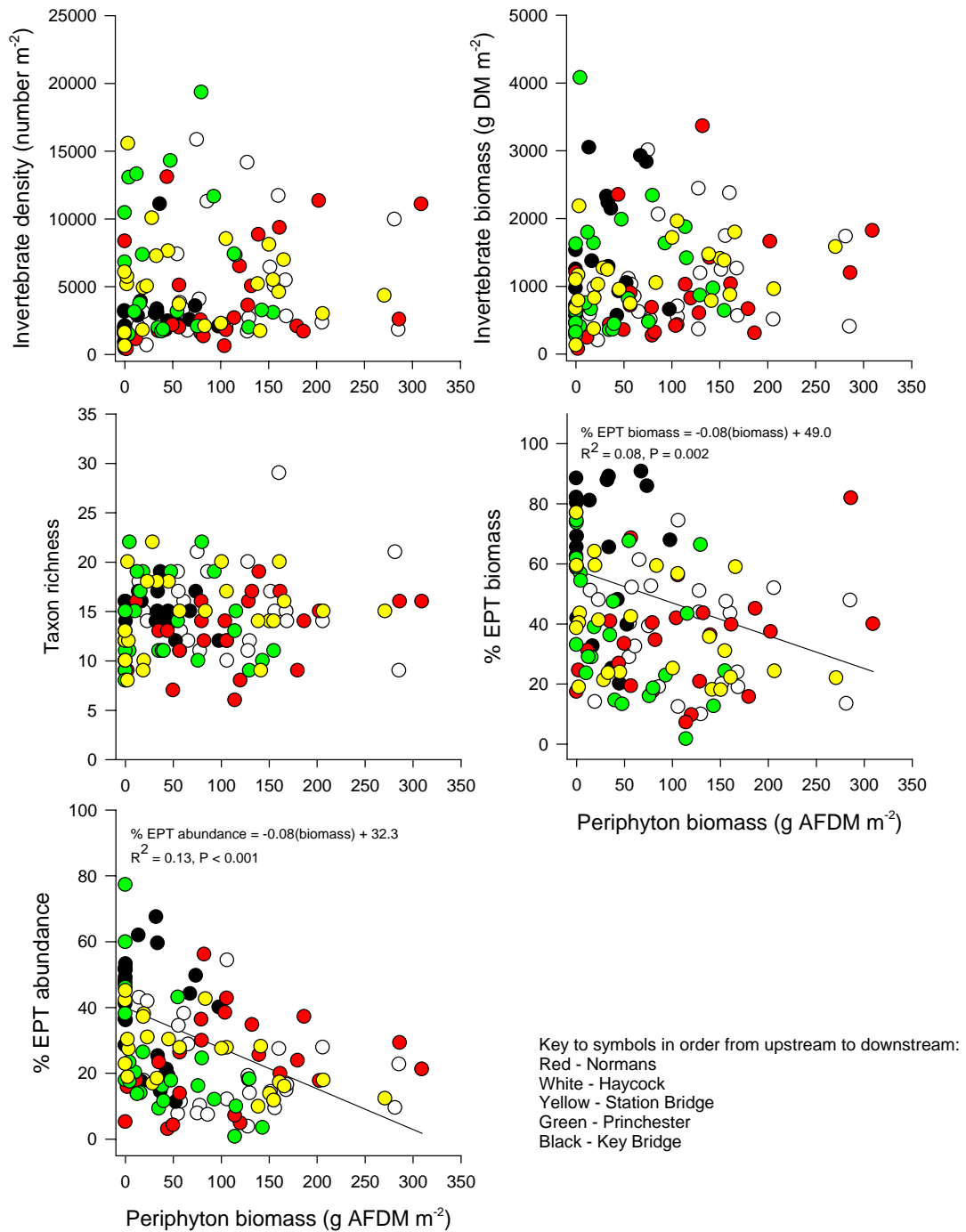
higher % EPT abundance and % EPT biomass than upstream sites. The Key Bridge reach is characterised by relatively fine gravel compared to the cobble and boulder-dominated reaches upstream. No other between-reach differences were detected in the Mararoa. In the Oreti, the upstream-most reach at Mt. Nicholas had significantly lower invertebrate densities than all downstream reaches, lower taxon richness than the Ashton Flat, Manager's House, and Three Kings reaches, and lower total biomass than the Ashton Flat reach. The Manager's House reach had significantly lower EPT abundance than all other sites. The Mt. Nicholas reach dried in January 2007, and flow did not resume during the remainder of the study. A total of 17 out of 130 pair-wise comparisons were statistically significant.

Relationships between invertebrate assemblage parameters and periphyton biomass at the whole-river scale are shown in Figures 4-6. Results for individual reaches, and for all reaches combined, are compiled in Table 9. The % EPT abundance and % EPT biomass were the only significant invertebrate-periphyton linear regressions in the Mararoa River data. In contrast, each regression was significant for the Oreti and Waiau Rivers. Scatterplots for the Mararoa suggest that among-reach variability is higher than in the Oreti River. A comparison of regression results for each reach substantiates that pattern; fewer within-reach regressions were significant in the Mararoa compared with the Oreti (Table 9). Pooling all rivers and reaches resulted in significant relationships for each invertebrate parameter. In the latter case, large sample sizes ( $N = 276$ ) and high statistical power resulted in significant regressions, but the regressions accounted for  $< 20\%$  of the variability in each parameter. In all cases of significant linear regressions, invertebrate densities, invertebrate biomass, and taxon richness increased with increasing periphyton biomass, and % EPT abundance and % EPT biomass decreased with increasing periphyton biomass.

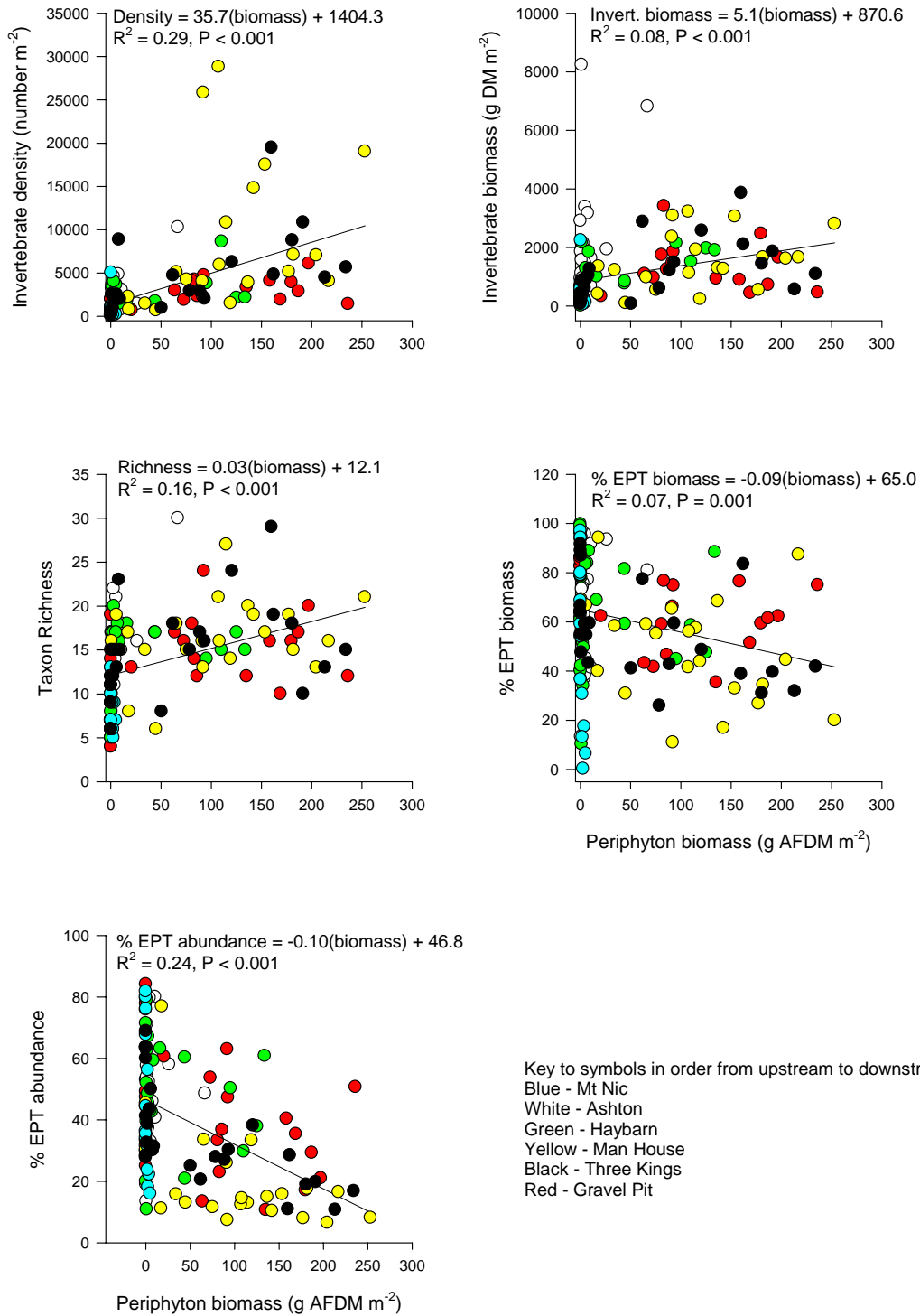
The generally weak relationships between invertebrate assemblage parameters and periphyton biomass indicate that factors in addition to periphyton biomass affect invertebrate assemblages. Hydraulic habitat conditions are likely to be prominent among those factors. To determine whether the variability explained by periphyton biomass was comparable to that explained by hydraulic habitat conditions, regressions were calculated for each invertebrate assemblage parameter as a function of depth, near-bed velocity and Froude number (Table 10). The Froude number ( $Fr = v/(gd)^{0.5}$ , where  $v$  is velocity,  $g$  is gravity acceleration, and  $d$  is depth) is an index of flow force in the downstream direction. Near-bed velocity and Froude number explained a small (1 to 5%) but significant amount of the variation in invertebrate assemblage parameters in all three rivers. Invertebrate parameter-depth regressions were not significant. A comparison of regression coefficients of determination indicates that periphyton biomass generally explains more variation in invertebrate assemblages than hydraulic habitat variables.



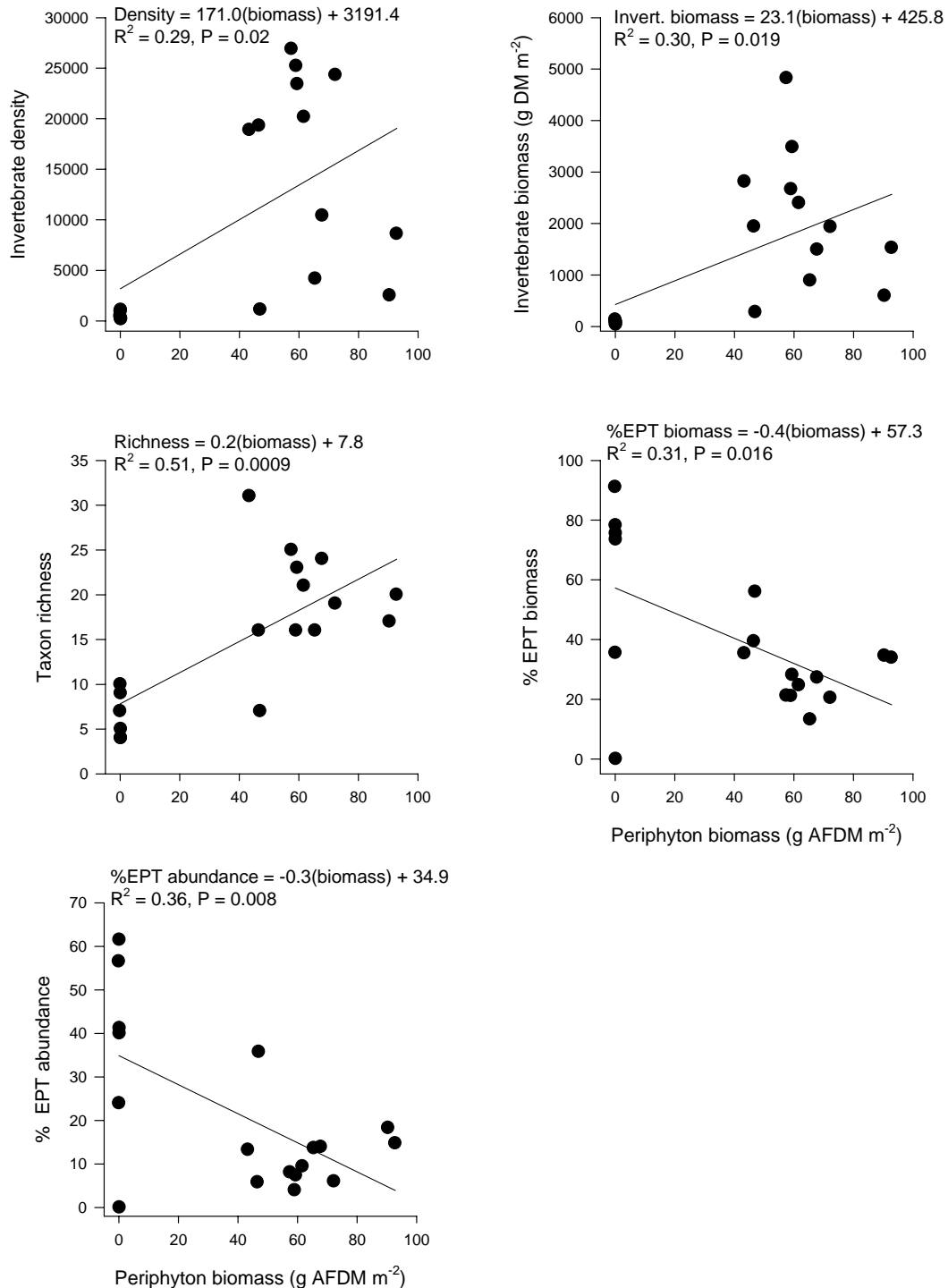
**Figure 3:** Invertebrate assemblage parameters at Mararoa, Waiau and Oreti River reaches. Boxes: 25<sup>th</sup> and 75<sup>th</sup> percentiles, central lines: medians, whiskers: 5<sup>th</sup> and 95<sup>th</sup> percentiles, points: outliers. Site codes: NO: Normans, HC: Haycock, SB: Station Bridge, PR: Princhester, KB: Key Bridge, EX: Excelsior, MN: Mt Nicholas, AF: Ashton Flat, HB: Haybarn, MH: Manager's House, TK: Three Kings, GP: Gravel Pits.



**Figure 4:** Relationships between invertebrate assemblage parameters and periphyton biomass in the Mararoa River. Lines and equations indicate significant regressions ( $P < 0.05$ ).



**Figure 5:** Relationships between invertebrate assemblage parameters and periphyton biomass in the Oreti River. Lines and equations indicate significant regressions ( $P < 0.05$ ).



**Figure 6:** Relationships between invertebrate assemblage parameters and periphyton biomass in the Waiau River at Excelsior Creek. Lines and equations indicate significant regressions ( $P < 0.05$ ).

**Table 9:** Invertebrate-periphyton regression results. River reaches are listed from upstream to downstream within each river. Regression equations are included for statistically significant regressions (in bold).  $N = 24$  for individual reaches, except Mt Nicholas ( $N = 18$ ). Biomass in regression equations refers to periphyton.

River	Reach	Density	Taxon richness	% EPT abundance	Total biomass	% EPT biomass
Mararoa	Normans	$R^2 = 0.09, P = 0.16$	$R^2 = 0.09, P = 0.15$	$R^2 = 0.04, P = 0.30$	$R^2 = 0.11, P = 0.12$	$R^2 = 0.12, P = 0.09$
Mararoa	Haycock	$R^2 = 0.02, P > 0.5$	$R^2 < 0.01, P > 0.5$	$R^2 = 0.06, P = 0.20$	$R^2 = 0.01, P > 0.5$	$R^2 = 0.02, P = 0.5$
Mararoa	Station Br	$R^2 = 0.01, P > 0.5$	$R^2 = 0.03, P = 0.44$	<b><math>Y = -0.08(\text{biomass}) + 31.9</math> <math>R^2 = 0.37, P = 0.002</math></b>	$R^2 = 0.12, P = 0.09$	<b><math>Y = -0.09(\text{biomass}) + 45.6</math> <math>R^2 = 0.15, P = 0.05</math></b>
Mararoa	Princhester	$R^2 < 0.01, P > 0.5$	$R^2 < 0.01, P > 0.5$	<b><math>Y = -0.18(\text{biomass}) + 31.9</math> <math>R^2 = 0.27, P = 0.009</math></b>	$R^2 < 0.01, P > 0.5$	<b><math>Y = -0.18(\text{biomass}) + 47.3</math> <math>R^2 = 0.19, P = 0.04</math></b>
Mararoa	Key Br	$R^2 = 0.08, P = 0.19$	$R^2 = 0.12, P = 0.11$	$R^2 = 0.06, P = 0.25$	<b><math>Y = 14.1(\text{biomass}) + 888.4</math> <math>R^2 = 0.20, P = 0.02</math></b>	$R^2 < 0.01, P > 0.5$
Mararoa	All reaches $N = 120$	$R^2 = 0.02, P = 0.11$	$R^2 = 0.02, P = 0.14$	<b><math>Y = -0.08(\text{biomass}) + 32.3</math> <math>R^2 = 0.13, P &lt; 0.0001</math></b>	$R^2 = 0.02, P = 0.12$	<b><math>Y = -0.08(\text{biomass}) + 49.0</math> <math>R^2 = 0.08, P = 0.002</math></b>
Oreti	Mt. Nic	$R^2 = 0.04, P > 0.5$	$R^2 = 0.11, P = 0.17$	<b><math>Y = -8.9(\text{biomass}) + 56.8</math> <math>R^2 = 0.39, P = 0.006</math></b>	$R^2 = 0.05, P = 0.37$	<b><math>Y = -16.5(\text{biomass}) + 75.4</math> <math>R^2 = 0.63, P &lt; 0.0001</math></b>
Oreti	Ashton	<b><math>Y = 116.3(\text{biomass}) + 1512.2</math> <math>R^2 = 0.55, P &lt; 0.0001</math></b>	<b><math>Y = 0.22(\text{biomass}) + 14.3</math> <math>R^2 = 0.48, P &lt; 0.0001</math></b>	$R^2 < 0.01, P > 0.5$	<b><math>Y = 70.7(\text{biomass}) + 1410.1</math> <math>R^2 = 0.25, P = 0.012</math></b>	$R^2 = 0.02, P > 0.5$
Oreti	Haybarn	<b><math>Y = 23.1(\text{biomass}) + 1239.9</math> <math>R^2 = 0.28, P = 0.007</math></b>	$R^2 = 0.13, P = 0.09$	$R^2 < 0.01, P > 0.5$	<b><math>Y = 10.3(\text{biomass}) + 649.5</math> <math>R^2 = 0.42, P = 0.0006</math></b>	$R^2 < 0.01, P > 0.5$
Oreti	Manager House	<b><math>Y = 48.2(\text{biomass}) + 2510.6</math> <math>R^2 = 0.20, P = 0.03</math></b>	<b><math>Y = 0.03(\text{biomass}) + 13.1</math> <math>R^2 = 0.18, P = 0.04</math></b>	<b><math>Y = -0.1(\text{biomass}) + 33.6</math> <math>R^2 = 0.32, P = 0.004</math></b>	<b><math>Y = 6.6(\text{biomass}) + 698.5</math> <math>R^2 = 0.26, P = 0.01</math></b>	<b><math>Y = -0.1(\text{biomass}) + 62.9</math> <math>R^2 = 0.17, P = 0.04</math></b>
Oreti	Three Kings	<b><math>Y = 34.2(\text{biomass}) + 1580.7</math> <math>R^2 = 0.38, P = 0.001</math></b>	$R^2 = 0.15, P = 0.07$	<b><math>Y = -0.1(\text{biomass}) + 41.9</math> <math>R^2 = 0.46, P = 0.0003</math></b>	<b><math>Y = 6.4(\text{biomass}) + 645.2</math> <math>R^2 = 0.28, P = 0.008</math></b>	<b><math>Y = -0.1(\text{biomass}) + 63.6</math> <math>R^2 = 0.24, P = 0.02</math></b>
Oreti	Gravel Pits	<b><math>Y = 14.9(\text{biomass}) + 1019.8</math> <math>R^2 = 0.45, P = 0.0003</math></b>	<b><math>Y = 0.3(\text{biomass}) + 10.1</math> <math>R^2 = 0.24, P = 0.015</math></b>	<b><math>Y = -0.1(\text{biomass}) + 49.4</math> <math>R^2 = 0.16, P = 0.05</math></b>	<b><math>Y = 4.2(\text{biomass}) + 631.0</math> <math>R^2 = 0.16, P = 0.05</math></b>	<b><math>Y = -0.08(\text{biomass}) + 73.8</math> <math>R^2 = 0.16, P = 0.05</math></b>
Oreti	All reaches $N = 144$	<b><math>Y = 35.7(\text{biomass}) + 1404.4</math> <math>R^2 = 0.29, P &lt; 0.0001</math></b>	<b><math>Y = 0.03(\text{biomass}) + 12.1</math> <math>R^2 = 0.16, P &lt; 0.0001</math></b>	<b><math>Y = -0.1(\text{biomass}) + 46.5</math> <math>R^2 = 0.24, P &lt; 0.0001</math></b>	<b><math>Y = 5.1(\text{biomass}) + 870.6</math> <math>R^2 = 0.09, P = 0.0004</math></b>	<b><math>Y = -0.09(\text{biomass}) + 65.0</math> <math>R^2 = 0.07, P = 0.001</math></b>
Waiau	Excelsior	<b><math>Y = 171.0(\text{biomass}) + 3191.4</math> <math>R^2 = 0.29, P = 0.02</math></b>	<b><math>Y = 0.2(\text{biomass}) + 7.8</math> <math>R^2 = 0.51, P = 0.0009</math></b>	<b><math>Y = -0.3(\text{biomass}) + 34.9</math> <math>R^2 = 0.36, P = 0.008</math></b>	<b><math>Y = 23.1(\text{biomass}) + 425.8</math> <math>R^2 = 0.30, P = 0.019</math></b>	<b><math>Y = -0.4(\text{biomass}) + 57.3</math> <math>R^2 = 0.31, P = 0.016</math></b>
All rivers	All reaches $N = 276$	<b><math>Y = 23.6(\text{biomass}) + 2868.2</math> <math>R^2 = 0.10, P &lt; 0.0001</math></b>	<b><math>Y = 0.02(\text{biomass}) + 12.7</math> <math>R^2 = 0.09, P &lt; 0.0001</math></b>	<b><math>Y = -0.1(\text{biomass}) + 40.0</math> <math>R^2 = 0.20, P &lt; 0.0001</math></b>	<b><math>Y = 3.3(\text{biomass}) + 928.7</math> <math>R^2 = 0.05, P = 0.0001</math></b>	<b><math>Y = -0.1(\text{biomass}) + 57.9</math> <math>R^2 = 0.10, P &lt; 0.0001</math></b>

**Table 10:** Invertebrate-hydraulic habitat regression results. V: velocity, Fr: Froude number. Regression equations are included for statistically significant linear regressions (in bold).  $N = 276$ .

Habitat variable	Density	Taxon richness	% EPT abundance	Total biomass	% EPT biomass
Depth (m)	$R^2 < 0.01, P = 0.28$	$R^2 < 0.01, P = 0.28$	$R^2 = 0.01, P = 0.07$	$R^2 = 0.15, P = 0.07$	$R^2 < 0.01, P > 0.5$
Near bed velocity ( $\text{m s}^{-1}$ )	<b><math>Y = -5939.4(V) + 6183.8</math> <math>R^2 = 0.10, P &lt; 0.0001</math></b>	<b><math>Y = -6.7(V) + 16.1</math> <math>R^2 = 0.04, P = 0.0005</math></b>	<b><math>Y = 27.4(V) + 23.8</math> <math>R^2 = 0.05, P = 0.0003</math></b>	<b><math>Y = -959.3(V) + 1436.7</math> <math>R^2 = 0.02, P = 0.018</math></b>	<b><math>Y = 36.2(V) + 39.8</math> <math>R^2 = 0.05, P = 0.0001</math></b>
Froude number	<b><math>Y = -8971.5(\text{Fr}) + 5932</math> <math>R^2 = 0.02, P = 0.010</math></b>	<b><math>Y = -9.3(\text{Fr}) + 15.7</math> <math>R^2 = 0.03, P = 0.004</math></b>	<b><math>Y = 44.5(\text{Fr}) + 24.4</math> <math>R^2 = 0.04, P = 0.0006</math></b>	<b><math>Y = -1342.9(\text{Fr}) + 1376.5</math> <math>R^2 = 0.01, P = 0.05</math></b>	<b><math>Y = 60.8(\text{Fr}) + 40.2</math> <math>R^2 = 0.05, P = 0.0002</math></b>

For each river, the threshold periphyton biomass corresponding to 50% EPT biomass was calculated from the regressions, as discussed above (Section 4.1.1). The threshold biomass values are 166.7 g AFDM m<sup>-2</sup> for the Oreti, 12.5 g AFDM m<sup>-2</sup> for the Mararoa, and 18.3 g AFDM m<sup>-2</sup> for the Waiau at Excelsior Creek. The large threshold biomass value for the Oreti is due to the greater average % EPT biomass in the Oreti (60.5%) compared with the Mararoa (42.6%) and Waiau (39.4%).

Responses of individual invertebrate taxa to periphyton biomass in the Oreti, Mararoa and Waiau Rivers were indicated by the sign and magnitude of correlations between invertebrate density and periphyton biomass (Table 11). Out of 51 invertebrates used in the analysis, the densities of 27 were significantly related to periphyton biomass in either the Oreti or Mararoa Rivers. Sample sizes from the Waiau at Excelsior were generally too small to analyse, so the Waiau data were pooled with the Oreti or Mararoa to provide correlations for all rivers combined. In general, densities of EPT taxa and acarinids were negatively correlated with periphyton biomass, and other insect groups (e.g., Chironomidae), crustaceans, and annelids were positively related to periphyton biomass. Several taxa were highly sensitive to changes in periphyton biomass, as indicated by large correlation coefficients. The largest negative correlation was for the mayfly *Deleatidium* sp. ( $r = -0.34$ ). The largest positive correlations were for annelid worms ( $r = 0.43$ ), the free-living caddisfly *Hydrobiosis* sp. ( $r = 0.43$ ), and the stonefly *Zealandobius* sp. ( $r = 0.59$ ).

Results of the principle components analysis indicated that the first two PCA axes accounted for about 30% of the total variance in the invertebrate data (Axis 1: 19.9%, Axis 2: 10.5). The ordination diagram from the PCA is shown in Figure 7. Site scores on the first axis were negatively correlated with periphyton biomass ( $r = -0.43$ ). These results indicate that approximately 9% of the variability associated with invertebrate assemblages from different reaches and seasons was due to differences in periphyton biomass.

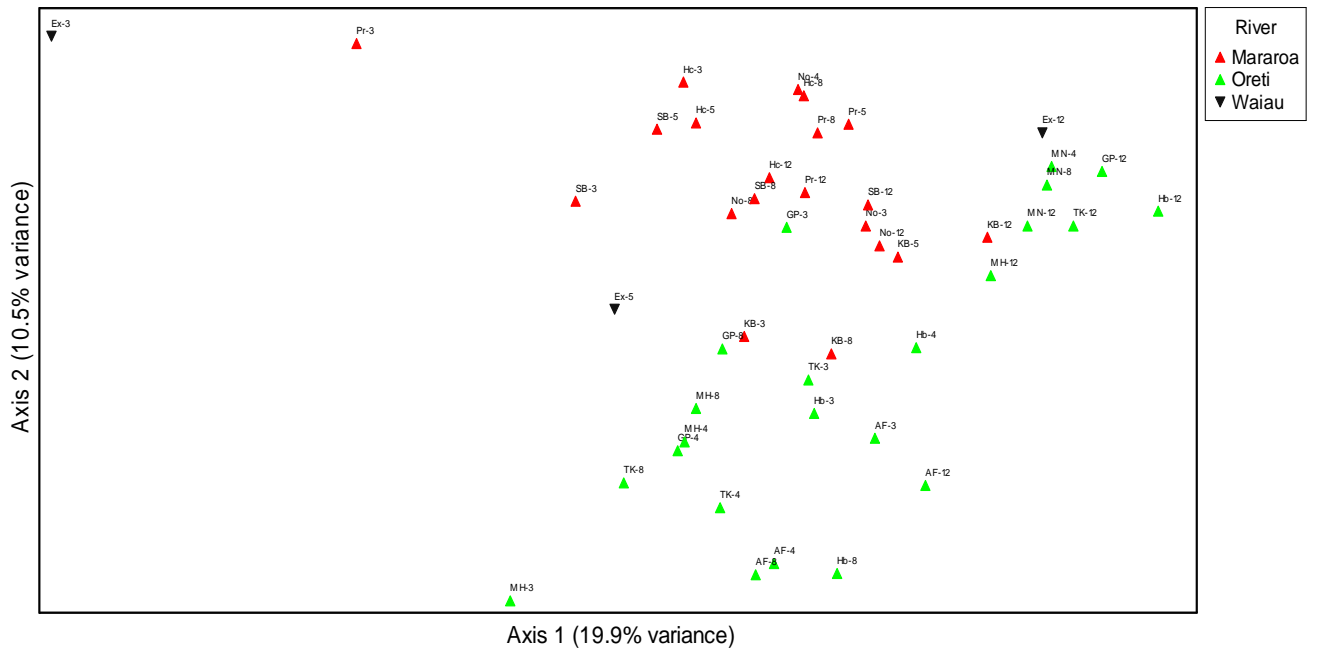
Linear regressions of *D. geminata* biomass on the lengths of *Deleatidium* sp., *Zealandobius* sp., Orthocladinae, and Oligochaeta did not explain a significant proportion of the variation in length data. Mean lengths for each taxon are shown by river reach and date in Table 12. In all cases, the regression statistic  $R^2$  was less than 0.04, and the probability of type 1 error was greater than 20% ( $P > 0.2$ ).



**Table 11.** Abundance-periphyton biomass correlations for the 51 most common invertebrates. Values are linear correlation coefficients for the average density of each taxon and average periphyton biomass. Significant correlations are in bold. Sample sizes are the product of reach number and sampling dates. Average invertebrate densities are from 6 samples on each quarterly sampling date. Blanks: taxon did not occur in samples from one river. Sample size for the single Waiau River site was too small for analysis.

Taxon	All rivers (N = 46)	Mararoa (N = 20)	Oreti (N=23)
<i>Deleatidium</i>	<b>-0.34</b>	<b>-0.59</b>	-0.18
Elmidae	-0.12	<b>-0.32</b>	-0.07
<i>Austrosimulium</i>	-0.15	-0.26	-0.21
<i>Psilochorema</i>	0.06	<b>-0.44</b>	<b>0.37</b>
<i>Aoteapsyche</i>	-0.12	<b>-0.51</b>	0.11
<i>Stenoperla</i>	-0.11	0.07	-0.05
Eriopterini	-0.04	-0.20	0.03
<i>Aphrophila</i>	-0.04	-0.08	-0.01
<i>Coloburiscus</i>	-0.14	-0.27	-0.06
<i>Olinga</i>	0.14	-0.13	<b>0.47</b>
<i>Zelandoperla</i>	-0.02	0.18	-0.15
<i>Plectrocnemia</i>	-0.15	0.03	-0.23
<i>Neocurupira</i>	-0.06		0.02
<i>Chironomus</i>	-0.07	-0.13	-0.13
<i>Oniscigaster</i>	0.11		0.25
ACARINA	-0.08	<b>-0.37</b>	-0.11
<i>Oxyethira</i>	0.03	0.03	-0.04
<i>Costachorema</i>	-0.02	<b>-0.40</b>	0.09
Staphylinidae	-0.05	-0.15	0.03
<i>Tanytarsus</i>	-0.01	-0.25	0.10
<i>Pycnocentroides</i>	0.30	<b>0.40</b>	<b>0.49</b>
<i>Maoridiamesa</i>	<b>0.31</b>	<b>0.55</b>	-0.07
Tanypodinae	0.18	0.07	<b>0.38</b>
Hydraenidae	0.06	-0.29	0.21

Taxon	All rivers (N = 46)	Mararoa (N = 20)	Oreti (N=23)
<i>Nesameletus</i>	0.07	-0.22	0.23
<i>Pycnocentria</i>	0.14	<b>0.31</b>	0.19
<i>Pycnocentrella</i>	0.05	0.07	-0.14
COLLEMBOLA	<b>0.39</b>	<b>0.34</b>	<b>0.48</b>
Ceratopogonidae	0.06	-0.07	0.20
Empididae	<b>0.30</b>	0.06	<b>0.57</b>
NEMATOMORPHA	0.13	0.22	0.00
<i>Physa</i>	-0.01	0.04	
<i>Archichauliodes</i>	0.16	0.11	0.12
Anthomyiidae	0.09	-0.11	<b>0.36</b>
<i>Polyplectropus</i>	0.06	-0.01	
<i>Hydrobiosis</i>	<b>0.43</b>	0.25	<b>0.80</b>
NEMATODA	0.14	0.12	0.19
Cladocera	0.14	0.19	
<i>Polypedilum</i>	<b>0.34</b>	0.24	<b>0.46</b>
Stratiomyidae	0.20	0.19	0.22
<i>Stictocladius</i>	0.29	0.18	<b>0.85</b>
<i>Berosus</i>	0.14	0.06	0.29
Ostracoda	0.05	0.09	<b>0.65</b>
<i>Neurochorema</i>	<b>0.33</b>	<b>0.35</b>	<b>0.49</b>
<i>Potamopyrgus</i>	0.19	0.09	0.27
Orthoclaadiinae	<b>0.40</b>	0.14	<b>0.64</b>
<i>Hudsonema</i>	0.19	0.08	<b>0.50</b>
<i>Gyraulus</i>	0.14	<b>0.38</b>	<b>0.44</b>
Sphaeriidae	<b>0.42</b>	<b>0.53</b>	
<i>Zelandobius</i>	<b>0.59</b>	<b>0.48</b>	<b>0.70</b>
ANNELIDA	0.43	0.31	0.72



**Figure 7:** Ordination diagram from principle components analysis of benthic macroinvertebrate data (log X+1-transformed densities). Each symbol represents the mean of six samples in a reach on one sampling date.

**Table 12:** Standard lengths (mm) of common invertebrate taxa in the Oreti and Mararoa Rivers. Values are mean abundance-weighted lengths for each site and date. Abundance weights were based on total numbers in six samples at each site and date. Taxa shown are most abundant taxa across all sites and dates. NP: not present in samples.

Date	River	Site	<i>Deleatidium</i> sp.	<i>Zelandobius</i> sp.	Orthocladinae	Oligochaeta
April 2006	Oreti	Ashton Flat	1.78	1.64	1.89	1.50
		Haybarn	0.82	1.50	1.11	1.60
		Manager House	1.35	1.02	1.03	1.15
		Three Kings	1.16	1.22	1.09	1.31
		Gravel Pit	1.11	1.37	0.85	0.58
May 2006	Mararoa	Normans	1.00	1.30	0.85	0.56
		Haycock	1.10	0.95	1.20	0.43
		Station Br.	1.24	1.26	1.35	1.29
		Princhester	NP	1.43	1.49	0.88
		Key Br.	1.02	1.11	0.97	0.65
August 2006	Oreti	Ashton Flat	1.01	1.42	1.18	0.46
		Haybarn	0.77	1.07	1.11	1.08
		Manager House	0.94	1.05	1.19	1.17
		Three Kings	1.28	0.82	0.79	1.65
		Gravel Pit	1.06	1.87	1.11	0.64
August 2006	Mararoa	Normans	0.89	1.06	0.95	0.56
		Haycock	0.84	0.89	1.50	0.79
		Station Br.	1.66	1.32	0.99	0.73
		Princhester	1.53	1.78	1.21	0.99
		Key Br.	0.95	1.43	0.91	0.42
December 2006	Oreti	Ashton Flat	1.01	1.42	1.34	0.44
		Haybarn	0.77	1.07	1.18	1.07
		Manager House	0.94	1.05	1.26	1.17
		Three Kings	1.32	0.82	1.50	1.65
		Gravel Pit	1.08	1.94	1.35	0.64
December 2006	Mararoa	Normans	0.89	1.06	1.44	0.75
		Haycock	1.97	0.89	1.25	0.85
		Station Br.	1.66	1.32	1.41	0.73
		Princhester	1.53	2.49	1.26	0.99
		Key Br.	0.95	1.43	1.06	0.44
March 2007	Oreti	Ashton Flat	0.67	1.11	0.84	4.50
		Haybarn	0.63	1.00	0.93	1.16
		Manager House	1.01	0.90	0.78	1.19
		Three Kings	0.83	1.16	1.02	1.06
		Gravel Pit	1.17	0.94	1.66	0.94
March 2007	Mararoa	Normans	1.13	0.89	0.96	1.05
		Haycock	1.30	1.07	1.03	0.70
		Station Br.	1.14	0.82	0.81	1.05
		Princhester	1.10	0.80	0.59	0.89
		Key Br.	1.09	1.50	1.18	0.77

### 3.2. Laboratory feeding trials

The primary objectives of the laboratory experiment were 1) to determine whether any of the dominant invertebrate taxa that consume algae in the absence of *D. geminata* consume *D. geminata* after it has colonised; and 2) whether *D. geminata* is a highly preferred diet item for some. The rationale for the study is that invertebrate taxa that utilize or prefer *D. geminata* as a food source are likely to be favoured over those that do not consume *D. geminata* in colonised reaches. This information will improve predictions about the effects of *D. geminata* colonisation on the composition of biotic communities.

#### 3.2.1. Methods

Laboratory feeding trials were conducted using invertebrate taxa that were common at *D. geminata*-affected sites. Chironomids (midge larvae), *Deleatidium* spp. (mayfly larvae) and *Pycnocentroides* spp. (caddisfly larvae) were collected from the Clutha River near Albertown with a Surber sampler. The freshwater snail, *Potamopyrgus antipodarum*, was collected using a dip net from Lake Waihola. Invertebrate samples were transported on ice to the University of Otago, where they were identified, sorted, and held in Clutha River water for 48 h to clear digestive tracts. Individuals of each taxon were randomly sorted into aquaria (2-litre containers, half-filled with Clutha River water), and maintained at 17°C in a 15:9 hour day:night cycle. Three *D. geminata*-covered rocks (100 – 150 mm diameter) from the Clutha River were added to each aquarium. To determine whether invertebrates consumed periphyton under experimental conditions, "control" treatments were set up using rocks covered with non-*D. geminata* periphyton from an unaffected site at the Clutha River. The aquaria were aerated to prevent hypoxia. Individual animals from each treatment were sacrificed daily to determine whether they had ingested *D. geminata* cells, stalks, both cells and stalks, or other periphyton. The invertebrates were rinsed three times in distilled water, and then the intact digestive tracts were removed. Gut contents were stained with Brilliant Cresyl Blue and mounted on microscope slides for viewing. Algal cells within the invertebrate digestive tract were identified using Cox (1996).

No statistical analysis was necessary because all invertebrates exposed to *D. geminata* consumed it, except chironomids, which never consumed it. All invertebrates exposed to other periphyton had algal cells in gut contents, though it was not possible to identify algal cells ingested by chironomids because of the large amount of amorphous material.

### 3.2.2. Results

*D. geminata* was found in the digestive tracts of *D. geminata*-naive *Deleatidium* spp., *Pycnocentrodes* spp., and *Potamopyrgus*, but not in chironomid larvae (Table 13; Fig. 8). When non-*D. geminata* periphyton was presented to invertebrates, all animals consumed it, although algae ingested by chironomids were not identifiable. In experiments where *D. geminata*-covered rocks were presented to *D. geminata*-naive invertebrates, all *Deleatidium* spp., *Pycnocentrodes* spp., and *Potamopyrgus* consumed *D. geminata* cells. *D. geminata* stalks were found in all *Pycnocentrodes* spp, but only 30% of *Potamopyrgus*, and 15% of *Deleatidium* spp. (Table 13). Analysis of the digestive tracts of the three taxa that consumed *D. geminata* indicated that 43% of the individuals fed on other algae while feeding on *D. geminata*.

**Table 13:** Analyses of the digestive tracts of invertebrates fed either *D. geminata* or other periphyton. Percentages are for individuals containing algal materials in their digestive tracts. *D. geminata*-covered rocks also contained other algae; the "alternative algae" treatment was *D. geminata*-free.

Taxonomic group	Treatment	No. of organisms (n)	Didymo present (%)	Only didymo cells present (%)	Only didymo stalk present (%)	Didymo cells and stalk present (%)	Other algae present (%)
<i>Deleatidium</i> spp.	<i>D. geminata</i>	10	100	0	85	15	50
<i>Pycnocentrodes</i> spp.		9	100	0	0	100	56
<i>Potamopyrgus antipodarum</i>		13	100	70	0	30	23
Chironomidae		10	0	0	0	0	0*
<i>Deleatidium</i> spp.	Alternative algae	10	0	0	0	0	100
<i>Pycnocentrodes</i> spp.		10	0	0	0	0	100
<i>Potamopyrgus antipodarum</i>		5	0	0	0	0	100
Chironomidae		4	0	0	0	0	0*

\* algal cells were not identifiable

### 3.3. Discussion

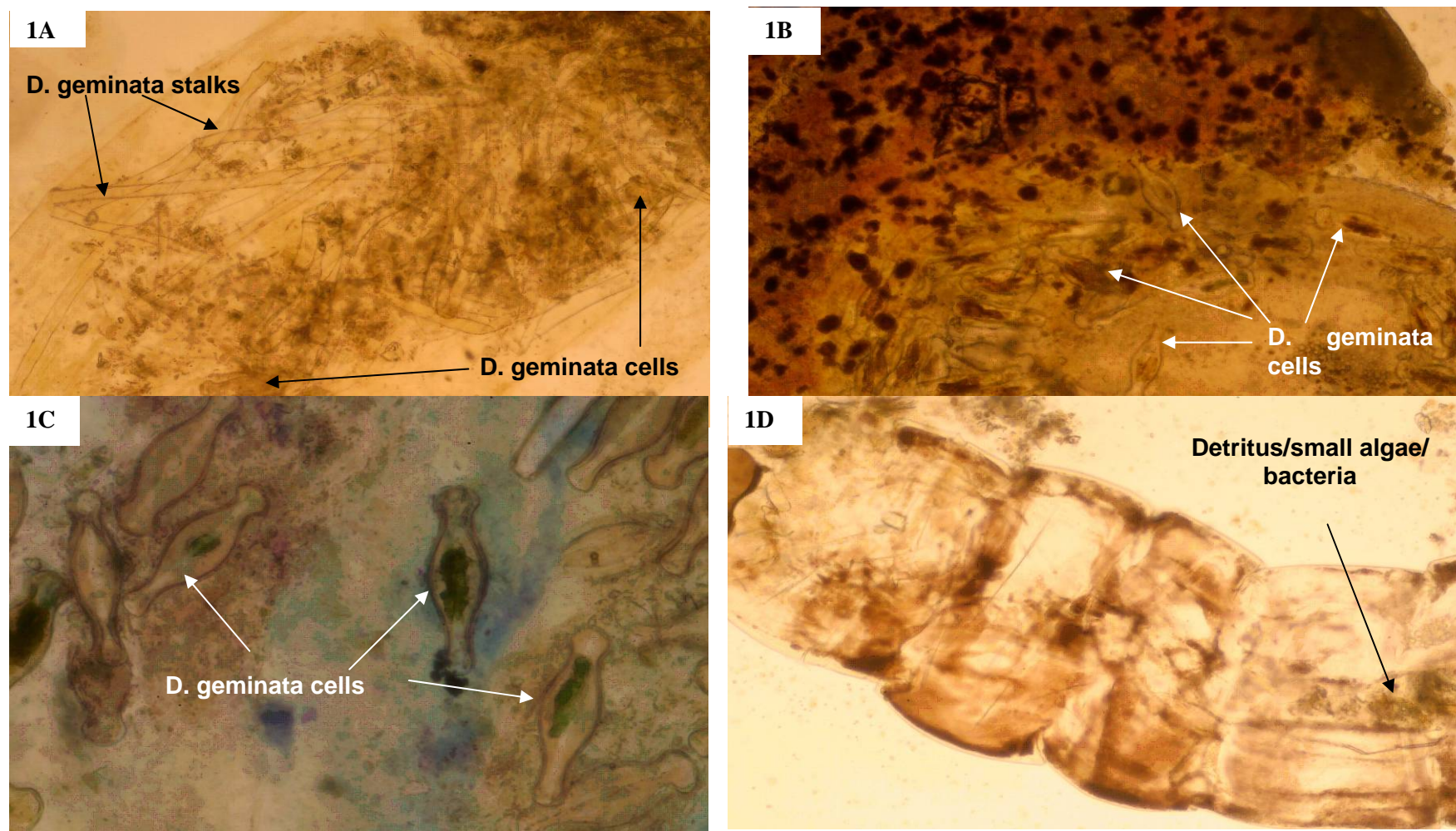
When all sampling sites were combined to assess large-scale patterns in invertebrate assemblages, five invertebrate-*D. geminata* relationships emerged: positive relationships between *D. geminata* biomass and invertebrate density, invertebrate biomass and taxonomic richness, and negative relationships between *D. geminata* biomass and % EPT abundance and %EPT biomass. A previous study of the Waiau and Mararoa Rivers reported a positive relationship between *D. geminata* biomass and invertebrate density, and a negative relationships between *D. geminata* biomass and % EPT abundance (Kilroy et al. 2006a). It is premature to infer from these results to other *D. geminata*-affected rivers, but the relationships listed above suggest that *D. geminata* may have a generally positive effect on invertebrate biomass and a generally negative effect on the predominance of EPT invertebrates in rivers.

It is not yet clear whether the negative EPT-*D. geminata* relationships are caused by an intrinsic property of *D. geminata* (e.g., a chemical defence), or by the generalized effects of high algal biomass. Positive relationships between total invertebrate abundance and benthic algal biomass, and negative relationships between the dominance of EPT taxa and benthic algal biomass have been observed for a broad range of algal taxa (e.g., Dudley et al. 1986, Biggs 2000). These observations suggest that negative EPT-*D. geminata* relationships were primarily due to generic effects of algal biomass

Invertebrate grazers can have a major impact on attached algae in streams (Power 1990). No published information is available on invertebrate feeding on *D. geminata*. The analysis of invertebrate communities from the sampled rivers showed that the presence or abundance of *D. geminata* was positively related to the abundance of some taxa, including *Pycnocentroides* spp. and *Potamopyrgus*, both of which were found to feed on *D. geminata* in feeding trials. Other taxa with densities positively correlated with *D. geminata* biomass (e.g., *Hudsonema*, *Pycnocentria*, *Olinga*, beetle larvae, and possibly *Aoteapsyche*) may graze on *D. geminata*. These observations suggest that the potential for invertebrate grazing as a form of biological control of *D. geminata* proliferations. Biological control of nuisance planktonic algae in lakes has been well-studied, and effective invertebrate, protozoan and fungal control agents have been identified (Sigee et al. 1999, Schindler 2006). In contrast, the topic of biological control of benthic algal proliferations is unexplored. A wider range of feeding trials, and quantification of *D. geminata* consumed are needed to assess the potential for native herbivorous invertebrates to contribute to the control of *D. geminata*. Trials with native invertebrates should logically occur before non-native biological control agents are considered, as the risk of unintended impacts following the intentional release of non-native herbivores is very high.

A caveat is in order regarding invertebrate assemblage responses to *D. geminata*. “*D. geminata* mats” are actually complex mixtures of *D. geminata*, other unicellular and filamentous algae, particulate organic matter, fungi, invertebrates and microbes (see Section 6.1.2 below). It is likely that invertebrate assemblages respond to the presence and abundance of aggregate periphyton, rather than to *D. geminata* per se. Kilroy et al. (2006a) provided circumstantial evidence supporting this proposition: % EPT in Mararoa River invertebrate assemblages was inversely related to periphyton biomass at sites dominated by *D. geminata*, and at sites with little or no *D. geminata*. Similar observations were made in the current study. At the Ashton Flat reach on the Oreti River, *D. geminata* is relatively uncommon (< 10% of live algal cells; discussed below in Section 4.3), and the invertebrate-periphyton relationships listed in Table 9 are primarily due to non-*D. geminata* periphyton





**Figure 8:** Gut contents of *D. geminata*-naïve invertebrates presented with *D. geminata* as a food source. A = *Deleatidium* (X 200 magnification), B = *Pycnocentroides* (X 100), C = *Potamopygus* (X 400), D = Chironomid (X 100).



## 4. Effects of *D. geminata* on river chemistry

### 4.1. Whole-reach effects of *D. geminata* on dissolved oxygen and pH

The primary objective of this study was to determine whether *D. geminata* proliferations cause substantial alterations in diurnal dissolved oxygen (DO) and pH cycles. If respiration or photosynthesis in mats causes large reductions in DO or large increases in pH, fish and invertebrates could be negatively affected.

#### 4.1.1. Methods

Sondes (DataSonde, Hydrolab Corp., Austin, Texas) were used to monitor DO, temperature and pH with accuracies of  $\pm 0.2 \text{ mg L}^{-1}$ ,  $\pm 0.1^\circ\text{C}$  and  $\pm 0.2$  units, respectively. Sondes were calibrated following the manufacturer recommendations and were deployed from 11 to 13 April 2006, at four sites: Mararoa River at Haycock Hills (the upstream Mararoa site), Mararoa River at Station Bridge (downstream site); Oreti River at Ashton Flats Bridge (upstream Oreti site) and Oreti River at Three Kings (downstream Oreti site).

The Mararoa reach (distance from upstream to downstream site) was 7 km-long, and had an average *D. geminata* biomass of  $153 \text{ g AFDW m}^{-2}$ . The Oreti reach was 25 km-long, and had an average *D. geminata* biomass of  $47 \text{ g AFDW m}^{-2}$ . River flow was  $11.8 \text{ m}^3 \text{ s}^{-1}$  at the downstream Mararoa site, and  $6.4 \text{ m}^3 \text{ s}^{-1}$  at the downstream Oreti site on 11 April 2006. The Mararoa was consistently warmer than the Oreti, by  $\sim 2.5^\circ\text{C}$ .

Data from the Sondes were used to analyse variation in DO ( $\text{mg L}^{-1}$  and % saturation), pH and temperature with time. DOFLOW model simulations were carried out following the procedure described in Wilcock et al. (1995). DOFLOW computes  $P_{\max}$  (maximum photosynthetic rate value in a 24-h period),  $R_{20}$  (24-h average respiration rate for a mean temperature of  $20^\circ\text{C}$ ),  $K_2$  (reaeration coefficient) and  $P/R$  (24-h average ratio of photosynthetic production-to-respiration). The average daily deficit,  $\bar{D}$  (the difference between saturation DO at ambient temperature and the actual DO), is related to  $K_2$  and to 24-h average values of  $R$  and  $P$  by:

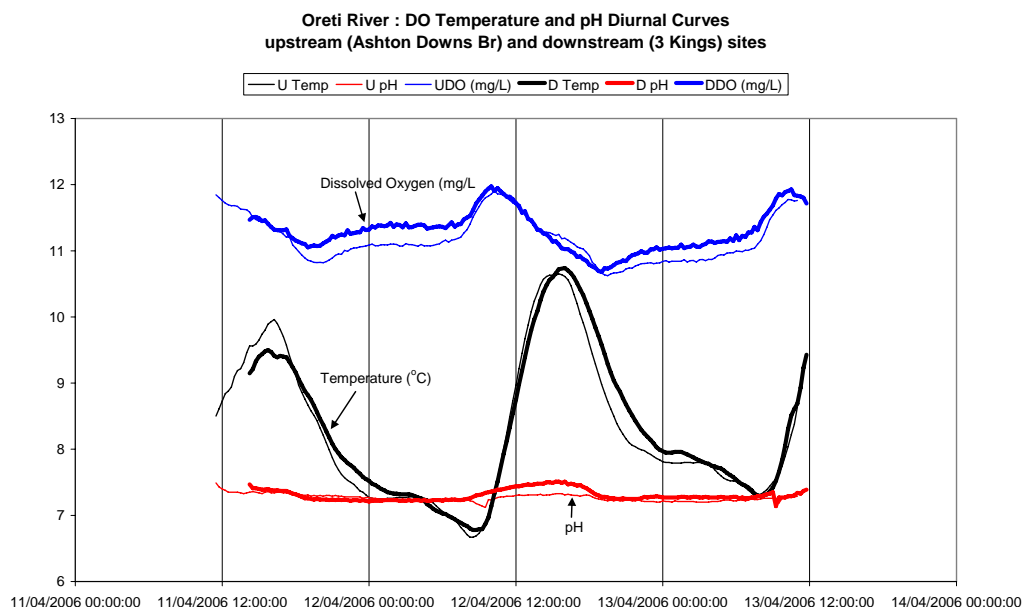
$$\bar{D} = \left( \frac{R - P}{K_2} \right)$$

Chapra & Di Toro (1991). Thus, when  $P/R = 1$ , the daily average deficit is zero. When  $P/R > 1$  average DO is supersaturated, whereas when  $P/R < 1$  average DO is below saturation.

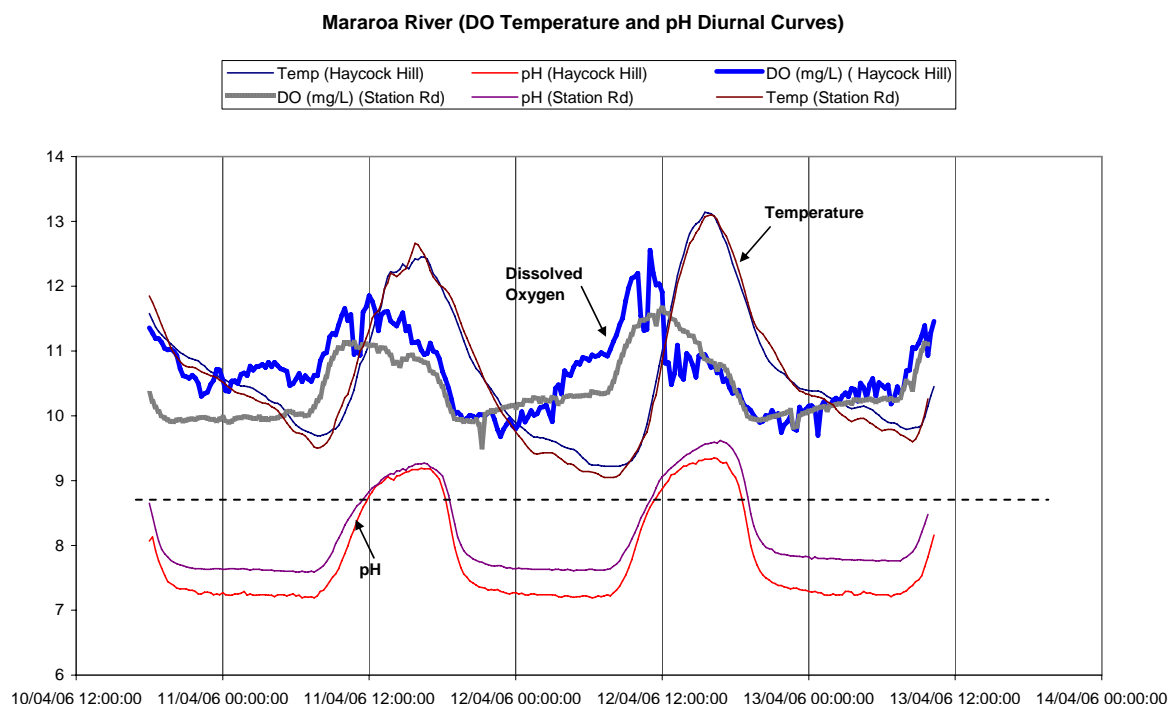
Flow gauging data were used to calculate  $K_2$  values as a check on the validity of the DOFLOW calculations (Wilcock 1984). Water samples were analysed for alkalinity (as  $\text{CaCO}_3$ ) using a standard titration method, and had an uncertainty of  $\pm 0.5 \text{ mg L}^{-1}$  (APHA 1998).

#### 4.1.2. Results

Diurnal curves for DO, temperature and pH for the Oreti (Fig. 9) and Mararoa River (Fig. 10) have several differences in structure. The Oreti DO curve was predominantly influenced by water temperature, as indicated by maximum DO levels at the lowest daily temperature (at  $\sim 0800$  hours). In contrast, maximum DO levels in the Mararoa occurred at mid-day, indicating the influence of algal photosynthesis. Diurnal maximum and minimum values of DO and pH for the Mararoa and Oreti Rivers are summarised in Table 14. There were large changes in DO and pH between sites on the Mararoa (i.e., large gaps between lines representing the upstream and downstream sites on Figure 10), and no changes between sites on the Oreti (i.e., no gaps between lines of Figure 9). This suggests that there were substantial cumulative effects of *D. geminata* on parcels of water as they moved down the Mararoa Reach, but no such effects in the Oreti. This supposition is strengthened by the fact that the Oreti reach was 3.6 times longer than the Mararoa reach.



**Figure 9:** Diurnal curves for DO ( $\text{mg L}^{-1}$ ), temperature ( $^{\circ}\text{C}$ ) and pH at two Oreti River sites, Ashton Flats Bridge and Three Kings.



**Figure 10:** Diurnal curves for DO ( $\text{mg L}^{-1}$ ), temperature ( $^{\circ}\text{C}$ ) and pH at two Mararoa River sites, Haycock Hills and Station Rd Bridge. Dotted line indicates pH guideline value for protection of river ecosystems (ANZECC 2000).

**Table 14:** DOFLOW results for the Mararoa and Oreti Rivers, 10-13 April 2006, compared with field data and calculated  $K_2$  values. Units are  $\text{g O m}^{-3} \text{ d}^{-1}$  for  $P_{\text{max}}$  and  $R_{20}$ ,  $\text{d}^{-1}$  for  $K_2$  and  $\text{g m}^{-3}$  for DO.

River/site	$P_{\text{max}}$	DOFLOW simulation results and sonde data				$\text{DO}_{\text{max}}$	$\text{DO}_{\text{min}}$
		$R_{20}$	$P/R$	$K_2$			
<i>Mararoa</i>							
Haycock Hills	25	18	0.51	12		12.6	9.68
<i>Oreti</i>							
Ashton Flats Bridge	4	1.5	0.6	7		11.9	10.6

### Dissolved oxygen

Parameter values from the DOFLOW simulations and the Sonde measurements were in close agreement for both the upstream and downstream sites on both rivers. Comparisons of the upstream sites indicate the influence of *D. geminata* on dissolved oxygen dynamics (Table 14). The Mararoa River, with greater *D. geminata* biomass, has higher rates of photosynthetic production ( $25$  versus  $4 \text{ g m}^{-3} \text{ d}^{-1}$ ) and respiration ( $18$  versus  $1.5 \text{ g m}^{-3} \text{ d}^{-1}$ ) than the Oreti River. The effect on DO is masked somewhat by the high reaeration rate coefficients caused by turbulent river flows. DOFLOW

values of  $K_2$  were broadly in agreement with experimental values determined from velocity and depth data measured at each site during the Sonde deployments. At lower river flows and velocities the amplitude of DO diurnal changes may be more pronounced (i.e., lower minimum and higher maximum values), but this would be offset somewhat by reduced water depths at low flows.

## pH

Alkalinity in the Mararoa ( $17.5 \text{ g m}^{-3}$  as  $\text{CaCO}_3$ ) and Oreti Rivers ( $13.9 \text{ g m}^{-3}$ ) was low in comparison with other New Zealand rivers, for which the average is  $\sim 35 \text{ g m}^{-3}$  (Close & Davies-Colley 1990). This indicates that the Mararoa and Oreti rivers have low buffering capacity and are therefore susceptible to large diurnal and seasonal pH changes, which may be caused by *D. geminata*. The presence of large amounts of *D. geminata* in the Mararoa at the Haycock Hills and Station Bridge sites resulted in an average diurnal change in pH of about 2 (Fig. 9, Table 15). In contrast, the Oreti River sites with much lower *D. geminata* biomass levels had a diurnal pH change of only 0.4, which is within experimental uncertainty (Fig. 9 Table 15). The Mararoa River pH values in April were close to the upper limit tolerated by native fish (West et al. 1997). This value may be exceeded, and the diurnal range greater, in summer, when flows are reduced and photosynthetic production higher. The upper guideline pH level for upland New Zealand rivers in daylight is 8.0 (ANZECC 2000). This value is clearly exceeded in the Mararoa River.

**Table 15: Maximum and minimum diurnal values of DO (% saturation) and pH for the Mararoa and Oreti Rivers, 10-13 April 2006.**

	DO (% saturation)		pH	
	minimum	maximum	minimum	maximum
<i>Mararoa</i>				
Haycock Hills	86.1	111.5	7.19	9.35
Station Rd Bridge	85.6	106.0	7.59	9.62
<i>Oreti</i>				
Ashton Flats Bridge	90.7	101.7	7.12	7.43
Three Kings	93.0	102.7	7.14	7.51

## 4.2. Fine-scaled effects of *D. geminata* mats on water chemistry and metabolism

In this study, microscale patterns in chemistry and metabolism associated with *D. geminata* mats were measured with electrochemical microsensors and a pulse-amplitude-modulated fluorometer. The objective of the study was used to contribute to understanding the means by which *D. geminata* achieves rapid growth and sustains high biomass levels in oligotrophic river water. One potential strategy is to restrict

water flow and trap organic matter within mats, which may enhance nutrient regeneration. The measurements made in this study address that strategy.

The microsensor measurements were the first to be made in a natural river environment. This is noteworthy because in situ measurements ensure that hydraulic conditions (e.g., turbulence and boundary layer dynamics) are realistic, a goal that is difficult to achieve in laboratory systems.

#### 4.2.1. Methods

The fieldwork was carried out in April 2006 in the Mararoa River at Station Bridge, in a shallow (10-30 cm depth) reach with ~ 90 % *D. geminata* cover. To measure dissolved oxygen concentration [O<sub>2</sub>] microprofiles across the mat–water interface, we used a Unisense O<sub>2</sub> microelectrode (Revsbech 1989) and a micromanipulator (Märzhäuser Wetzlar) mounted on an aluminium frame on the river bed (Fig. 11). An underwater picoammeter PA 3000U (Unisense) provided the polarization voltage for the microelectrode, which had an outside tip diameter of 50 µm, a 90% response time of ~1 s, and a stirring sensitivity of <2%. The micromanipulator moved the electrode in 0.2-mm steps from a position above the mat to a depth of 6–8 mm into the mat.



**Figure 11:** Microprofiling system in the Mararoa River at Stations Bridge. The aluminium frame holds the micromanipulator and O<sub>2</sub> microelectrode. The pulse-amplitude-modulated fluorometer and picoammeter are on the left side of the frame.

During the profiling measurements, a time-series of the downwelling irradiance of photosynthetically active radiation (PAR; 400–700 nm) incident to the mat surface was measured with a submersible quantum sensor (LI-COR Environmental LI-192SA) and a data logger (LI-COR-1000). The quantum sensor was mounted with the cosine sensor face parallel to the river bed. Water temperature, conductivity, and pH were recorded with two PortaMess 913 meters, a SE 204 conductivity sensor, and a SE 102 pH/Pt1000 electrode.

A Walz pulse-amplitude-modulated fluorometer (PAM) was used to produce rapid light curves (RLC), which can provide insight to the photoadapted status of *D. geminata*. RLCs were recorded by exposing dark-adapted mats of *D. geminata* to a sequence of actinic irradiances in 7 discrete PAR steps from 0 to 480  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ . The actinic irradiance supplied by the PAM was measured using the quantum sensor and data logger. Each period of actinic light lasted 8 s, resulting in quasi steady-state fluorescence,  $F$ . At the end of each actinic period, a saturation pulse of white light was used to determine the maximal fluorescence yield,  $F'_m$ , and the quantum yield of charge separation in PSII,  $\phi_p = (F'_m - F) \times F'_m{}^{-1}$ . The photosynthetic electron transport rate (ETR) between PSII and PSI was estimated as  $\text{ETR} = \phi_p \times \sigma_a \times E_d$  where  $\sigma_a$  is the absorption cross section of PSII, and  $E_d$  is the incident downwelling irradiance of PAR. The relative ETR was estimated as  $\text{rETR} = \phi_p \times E_d$  (Hofstraat et al. 1994). Figure 10 shows one of the experimental mats used for profiling and photosynthesis measurements. The mat is  $\sim 100 \text{ cm}^2$  in surface area, and 6 cm thick.

### Methodological considerations

Modeling metabolic activity of algal mats using solute concentration profiles across mat–water interfaces requires precise determination of the sensor tip position relative to the mat surface. This requirement can be met visually in calm water, but not in the turbulent flow at the study site. The position of the mat surface relative to the measured profile can also be estimated from the profile itself by an abrupt change in the slope of the solute concentration profile. The inflection occurs because mass must be conserved in the flux across the interfaces despite the difference in apparent diffusivity between the water and the mat. This approach requires that profiles are measured at a resolution of  $<0.2 \text{ mm}$  to produce sufficient data points in the diffusive boundary layer, which is usually  $<0.5 \text{ mm}$  thick in high flows. Our profiles were measured at a resolution of  $0.2 \text{ mm}$ . Consequently, there is some uncertainty in the positions of profiles relative to mat surfaces.

Modelling metabolic activity in mats of *D. geminata* using solute concentration microprofiles also requires the measurements to be made under steady state conditions



(i.e., solute production, consumption, and transport do not change over time, and the concentration gradient is stable). Such conditions were not met during our field study because variation in PAR (due to clouds), temperature, and flow speed affected the processes that shape concentration profiles.

The vertical range of the micromanipulator used for manual profiling is 10 mm, and the thicknesses of natural mats at Station Bridge were > 1 cm, so we were not able to profile entire mats. Therefore, solute concentrations at the detritus-rich base of mats (rock–mat interface, Figure 12C, D) could not be assessed; at this depth, we expected steep gradients due to bacterial decomposition of organic matter and associated processes of nutrient regeneration.

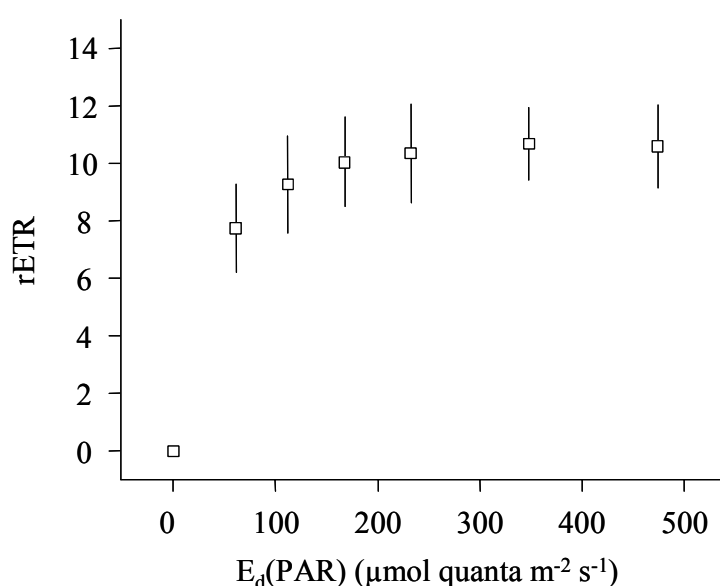


**Figure 12:** A: Top view of a *D. geminata* mat from Station Bridge. B: Cross section showing the surface layer of pigmented *D. geminata* cells, and the mat composed of unpigmented stalks. C and D: Two images showing detritus at the underside of the mat.

## 4.2.2. Results

### Fluorescence yield

Our first attempt to measure chlorophyll fluorescence of light- and dark adapted samples of *D. geminata* revealed that the fluorescence of the surface layer of pigmented cells is sufficiently strong to be investigated with the existing fluorometer. An example rapid light curve (RLC) is shown in Figure 13. For future RLC measurements, the sequence of actinic irradiances will have to be modified to include more data in the lower PAR range ( $0 - 80 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ).



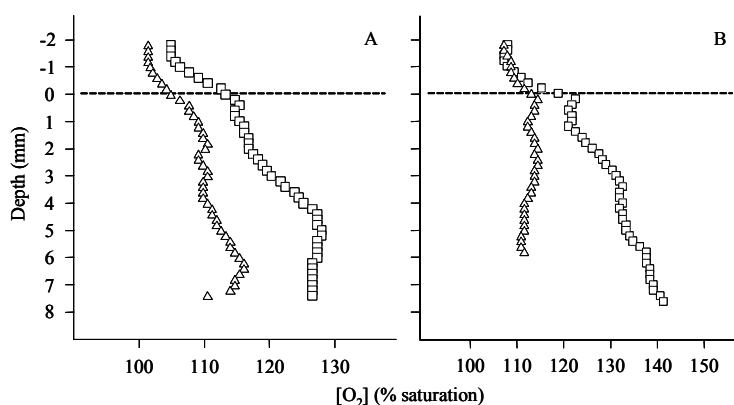
**Figure 13:** Relative rate of electron transport (mean,  $n = 4$ ) in dark-adapted *D. geminata* cells during exposure to a sequence of actinic irradiances of 7 discrete PAR steps from 0 to  $480 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ . Vertical lines are standard deviation.

### *In situ* dissolved oxygen concentration profiles

We measured a total of 15 *in situ*  $[\text{O}_2]$  microprofiles across the mat–water interface. The shapes of the profiles can not be explained solely on the basis of current understanding of photosynthetic activity in algal mats. As expected, we found peaks in  $[\text{O}_2]$  at the mat–water interface due to activity of photosynthetically active *D. geminata* cells (Fig. 14). As shown in Figure 12 A and B, pigmented *D. geminata* cells are concentrated in a surficial layer 1 – 2 mm deep. However, we observed larger  $[\text{O}_2]$  peaks well below the surface of most mats, and gradients of increasing  $[\text{O}_2]$  from the pigmented surface down to the subsurface peak. Most subsurface peaks occurred at depths of 5 mm or deeper (Fig. 14), but limitations of the micromanipulator prevented us from extending profiles deeper and confirming the depths of maximum  $[\text{O}_2]$ . Assuming that solute transport inside the mat is diffusive, increasing  $[\text{O}_2]$  in the



deeper mat and subsurface  $[O_2]$  peaks can only be explained by the activity of other autotrophic microbes within the mat. If the pigmented *D. geminata* cells at mat surfaces were the only source of dissolved oxygen, maximum values in the profiles would occur within the surface layer. At this point, we have not identified the source(s) of dissolved oxygen within mats. If the sources are phototrophic organisms, they require PAR to generate oxygen. The mechanism by which light is transmitted to the inner portions of dense *D. geminata* mats is entirely unknown. As a working hypothesis, we suggest the following:



**Figure 14:** *Didymosphenia geminata*. Examples of  $[O_2]$  microprofiles measured normal to the mat surface from a position above the mat to a depth of 8 mm. The mat surface is located at 0 mm (dashed line). The profiles in panel A were measured on 12 April 2006 at 1140 h (triangles) and at 1230 h (squares). The profiles in panel B were measured on 11 April 2006 at 1015 h (triangles) and 1200 h (squares).

The mucopolysaccharide stalks of *D. geminata* direct PAR into deeper layers of the mat where other phototrophic organisms occur and generate oxygen. If the organisms producing oxygen in the mats are photoautotrophs, they may take advantage of dissolved nutrient efflux from the organic matter that accumulates at the bases of *D. geminata* mats (Fig. 12 C and D).

### 4.3. Discussion

The whole-reach metabolism study revealed some major differences between river reaches that were heavily affected by *D. geminata* (e.g., the Upper Mararoa), and reaches that are moderately affected (e.g., the Upper Oreti). There were large cumulative effects of *D. geminata* on water chemistry over the short Mararoa reach, and no detectable effect over the longer Oreti reach. The two reaches differed in discharge (by 80%), average temperature (by  $\sim 2.5^\circ C$ ), and *D. geminata* biomass ( $>$  three times greater in the Mararoa reach). Differences in diurnal DO and pH variation, and in the timing of DO and pH peaks, indicated that differences in *D. geminata*

biomass had a greater influence than differences in temperature or discharge. *D. geminata* biomass in the long Oreti reach (47 g AFDW m<sup>-2</sup>) appeared to be below a threshold at which *D. geminata* is the primary control on DO and pH. The biomass in the shorter Mararoa reach (153 g AFDW m<sup>-2</sup>) was above the threshold, as indicated by mid-day DO maximum, large diurnal DO cycles, and the diurnal change in pH, which is in phase with the DO change. However, it is possible that other differences between rivers (e.g., depth, channel geometry) could modify the direct effect of *D. geminata*. Future assessments of *D. geminata*-effects should include enough reach measurements over space and time to quantify the relationship between biomass and reach metabolism.

On both measurement days, pH in the Mararoa reach temporarily exceeded the level considered potentially deleterious for aquatic ecosystems in New Zealand rivers, 8.0 (ANZECC 2000). The peak pH level observed in the late autumn study, 9.6, is likely to be exceeded in subsequent spring and summer seasons when photosynthetic carbon uptake by *D. geminata* is higher. These pH levels should be cause for concern. If pH exceeds 9, physiological effects on fish may be lethal (Alabaster and Lloyd 1982). In laboratory studies of New Zealand native fish, most species exhibit avoidance behaviour near water patches with pH > 9.5 (West et al. 1997).

While the effects of algae on river ecosystems are generally studied at moderate to large scales, i.e., 10<sup>0</sup> to 10<sup>3</sup> m, we consider the need to undertake fine-scaled studies, such as the microelectrode measurements in this report, critical. The rate-limiting steps for *D. geminata* growth (e.g., nutrient mass-transfer, nutrient uptake, light supply), and the processes by which *D. geminata* affects water chemistry all operate at scales of 10<sup>-6</sup> to 10<sup>-2</sup> m.

We hypothesize that *D. geminata* attains its remarkable biomass by creating microhabitats that generate inorganic nutrients and control nutrient flux. To test this hypothesis, we must first demonstrate nutrient regeneration from organic matter within mats, or nutrient trapping between the channel bed and the overlying water. Then we need to identify the source(s) of organic matter. Potential sources are decomposing *D. geminata* cells and stalks, bacteria, protists and metazoans living in the mats, or benthic algae and biofilms that are overgrown during initial *D. geminata* colonisation. If decomposition of overgrown biofilms is the main nutrient source for *D. geminata* mats, then the mats are likely to have finite lifespans, as the underlying material will eventually be depleted. The generation of oxygen within mats (Fig. 11) provides circumstantial evidence for an assemblage of photoautotrophic bacteria living in the *D. geminata* microhabitat. It is likely that a consortium of microorganisms live in the mats, and function jointly to modify the physical and chemical environment; such consortiums are common in algal mats (e.g., Glud et al. 1999).

Gradients of PAR, pH, oxygen and dissolved nutrients measured through *D. geminata* mats will provide information about the structure and functions of the consortium.

## 5. Nutrient limitation in *D. geminata*

An in situ nutrient enrichment experiment was carried out in seven *D. geminata*-affected rivers to assess nutrient limitation of biomass accrual, and to identify the predominant limiting nutrient(s). The experiment was designed to identify sites at which *D. geminata* accrual was nutrient-limited, and to identify the limiting nutrient.

### 5.1. Methods

The nutrient-enrichment experiment was conducted in winter (May and June 2006). Either inorganic nitrogen or phosphorus is nearly always the growth-limiting nutrient in temperate oligotrophic rivers (Francoeur 2001). A bioassay technique using nutrient diffusing substrata (NDS) was used to provide consistent levels of nitrogen and phosphorus enrichment to benthic algae in a wide range of river conditions (Francouer et al. 1999). NDS bioassays consist of large steel trays that hold twenty 400-ml containers of 2% agar. In each tray, five containers are assigned to each of four nutrient treatments: plus nitrate, plus phosphate, plus nitrate and phosphate, control (no nutrient enrichment). The agar corresponding to each treatment is amended with sodium nitrate, sodium phosphate monobasic, both, or none, as necessary. Hardened ashless filters are secured to the top of each container, and when the containers are placed in the steel tray, the filters are flush with the top of the tray and exposed to the overlying stream water. Ridges in the plastic lid of the steel trays run parallel to the flow direction, and minimize cross-contamination among the nutrient treatments. In the water, amended nutrients diffuse from the agar into the filters, and enrich the microzone of water from which benthic algae receive nutrients. The trays remain in the study rivers for periods sufficient for periphyton, including *D. geminata*, to establish and to exhibit preferential growth on containers from at least one nutrient treatment. If exposure to one nutrient results in significantly higher periphyton biomass than the others, that nutrient is considered to limit biomass accrual in the periphyton assemblage.

NDS trays were installed in eight river reaches in May 2006: Waiau near Monowai Power Station, Monowai near Waiau confluence, Aparima at Wrays Bush Bridge, Mararoa at Station Bridge, Ahuriri at Killermont Station, Oreti at Ashton Flat, Oreti at Gravel Pit, Waitaki at Otiata confluence. Four or six trays of 20 containers were installed at each reach in run habitats with no adjacent tree canopies, 2-3 m from either bank, at depths of 40-60 cm  $s^{-1}$ . Trays were retrieved at two subsequent intervals, 10 to

21 days after installation. Upon retrieval, the filters were transferred from each agar container to a plastic vial, then transported on ice to the NIWA Christchurch laboratory for determination of chlorophyll *a* concentrations. Procedures for extracting chlorophyll and determining chlorophyll *a* concentrations on the filters are given in Biggs and Kilroy (2000). Chlorophyll data from each tray were analysed by factorial analyses of variance with two levels (present or absent) of two factors (nitrate and phosphate). Factorial ANOVAs quantify the interaction of the two factors; in this case the interaction refers to the nitrate-plus-phosphate treatment, which may be indicative of co-limitation. Ratios of the mean biomass from nutrient enriched treatments to the mean biomass from control treatments were used to estimate the severity of nutrient limitation at each reach. The nutrient enriched treatment with the highest mean biomass was used for each ratio.

A sample of periphyton from a filter from each treatment at each site was retained after chlorophyll extraction for examination by light microscopy. The dominant algal taxa in each sample were visually ranked on an 8-point scale, with 1 the least common taxon and 8 the most common. These visual assessments yield repeatable results when carried out by experienced analysts (Biggs and Kilroy 2000). The primary intent of the visual estimates was to determine whether *D. geminata* was a dominant taxon in the experiment at each site.

To assess dissolved nutrient availability during the April to June 2006 study, and to begin compiling data on temporal variability in dissolved nutrient concentrations at *D. geminata*-affected river reaches, water samples were collected for nutrient analysis on 2 to 5 dates at the 12 study sites on the Mararoa, Oreti and Waiau Rivers. Water samples were collected with acid-washed syringes, filtered in the field through glass-fibre filters into acid-washed, deionized water-leached, and sample-rinsed plastic bottles, then transported on ice to the NIWA Hamilton analytic laboratory. Analytes were nitrate+nitrite, ammonium, dissolved reactive phosphate, total dissolved nitrogen and phosphorus, dissolved organic carbon, and dissolved reactive silicate.

## 5.2. Results

Nutrient-enhanced algal accrual was detected at seven out of eight river reaches. In some cases, the identity of the limiting nutrient could be determined by examining the trays before chlorophyll analysis. Figure 15 illustrates a clear case of phosphate limitation in a *D. geminata*-dominated algal assemblage in the Waitaki River. The exception was the Oreti River at Ashton Flat. Initial rates of accrual were very low at this site, and all but one of the phosphate-enriched replicates was lost during high

flows before the final retrieval. Therefore, no conclusions can be drawn concerning nutrient limitation at this site.

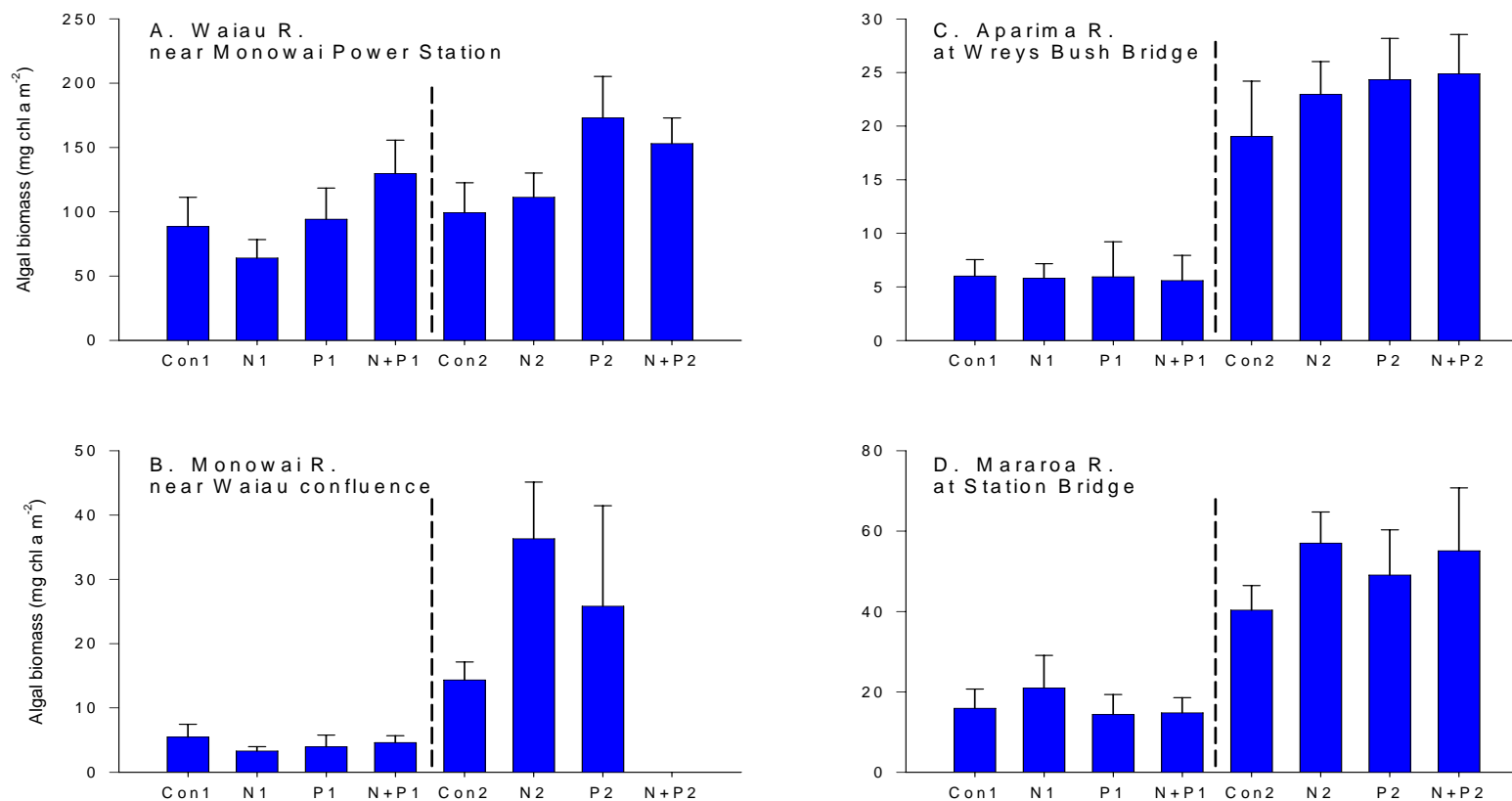
Microscopic examination of filters indicated that *D. geminata* was present in each nutrient treatment at each site (data not presented). *D. geminata* cells were the most common taxa encountered on filters from all sites except the Waitaki and Aparima Rivers; at these sites, *D. geminata* was the second or third most abundant taxon. The visual estimates are conservative, because cells were counted in the extracts, not diatom stalks. *D. geminata* cells generally account for less material in samples than *D. geminata* stalks.



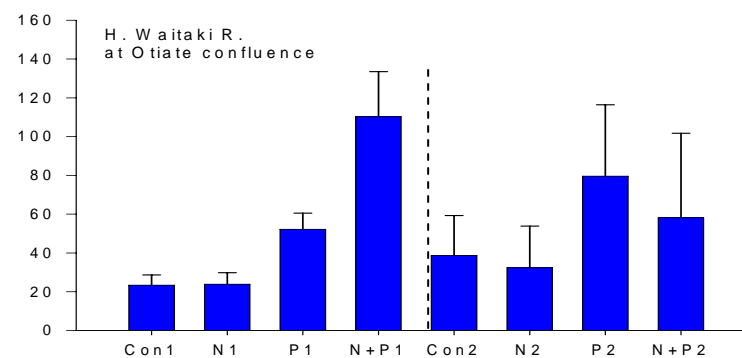
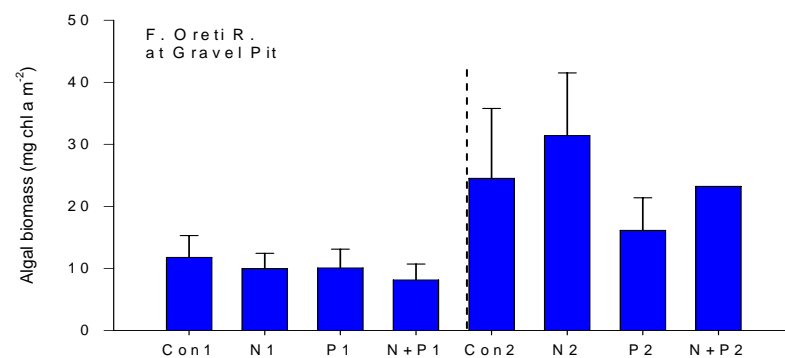
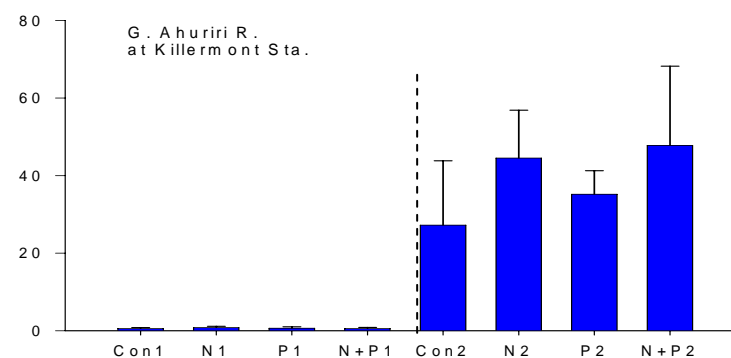
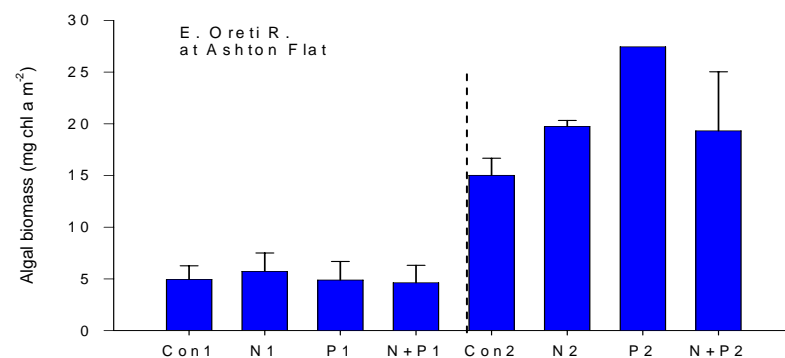
**Figure 15:** Example of a tray of nutrient-diffusing substrata from the Waitaki River, 22 days after deployment. Nutrient treatments are aligned in horizontal rows. Row 1: control, Row 2: phosphate, Row 3: nitrate + phosphate, Row 4: nitrate

Algal biomass levels varied widely among sites, and between deployment times. After the initial deployment (15 – 23 days), biomass levels varied among sites from  $< 1$  to  $> 100 \text{ mg chl } a \text{ m}^{-2}$  (Fig. 16). This range roughly corresponds to nutrient levels in the rivers; lowest biomass levels were measured in the most oligotrophic reaches (e.g., Ahuriri, Aparima, Oreti) and the highest in the most enriched rivers (e.g., Waiau).

In most reaches, differences in biomass levels among treatments increased with exposure time (Fig. 16). For this reason, the second set of NDS units to be retrieved was used for statistical comparisons of treatments whenever possible. The first set of NDS units retrieved were analysed in cases where numerous units were lost during high flow periods midway through the study. In those cases, the first sets to be retrieved were used. Exposure times are indicated in Table 16. Results of two-way analyses of



**Figure 16:** Results of NDS surveys. Values are means,  $\pm 1$ SD. Dashed line separates two retrieval dates. Note that all replicates of the N+P treatment were lost before the second retrieval at Monowai River.



**Figure 16 continued** Results of NDS surveys. Dashed line separates two retrieval dates. Columns with no error bars indicate that only one replicate was recovered.



**Table 16:** Results of comparisons of nitrate-enrichment and phosphate-enrichment on benthic algal growth in NDS assays. Results are significance levels for two-way analyses of variance using ln-transformed concentrations of chlorophyll *a* (ln mg Chl *a* m<sup>-2</sup>). Statistically significant values (< 0.1) are in bold. Headings: +N and +P: main effects of nitrate and phosphate. +N × +P: interaction term. DIN:DRP ratios are for water samples collected at or near the experiment site during the current study, during 2005 (Kilroy et al. 2006a), or from Jan 2005 – Jan 2006 for the NIWA National River Water Quality Network database.

River	Exposure time (d)	+N	+P	+N × +P	DIN:DRP
Ahuriri	27	<b>0.07</b>	0.48	0.76	10.9
Aparima	28	0.23	<b>0.06</b>	0.36	7:1
Mararoa	26	<b>0.03</b>	0.49	0.29	47:1
Monowai	14	0.29	0.88	0.20	30:1
Oreti at Ashton Flat	14	0.62	0.28	0.33	15:1
Oreti at Gravel Pit	14	0.59	0.71	0.94	23:1
Waiau	23	0.61	<b>&lt; 0.001</b>	<b>0.06</b>	~ 1000:1
Waitaki	37	0.64	<b>0.005</b>	0.52	22:1

variance indicated that there were significant enhancement effects of nitrate enrichment at two reaches, Ahuriri, and Mararoa (Table 16). There were significant enhancement effects of phosphate enrichment at 3 reaches, Aparima, Waiau, and Waitaki. There were significant nitrate × phosphate interactions at the Waiau, and Waitaki. In both cases of significant interactions, the effects were positive (i.e., N+P enrichment resulted in significantly higher biomass levels than either nitrate or phosphate enrichment alone). The three reaches in which significant enhancement effects could not be detected (Monowai, Oreti at Ashton Flat and Gravel Pit) were also the reaches in which flood flows led to the loss of experimental units or trays, and complete analyses could not be made. Data that were available after floods at those sites suggests that nutrient limitation was in effect after 27-28 days (Fig. 16). Therefore the results in Table 16 should be viewed as conservative.

Ratios of algal biomass from nutrient enriched treatments to biomass on control treatments were used as an index to the severity of nutrient limitation. At the time of the first retrieval (after 15-23 days of exposure), enriched:control biomass ratios ranged from 0.8 at the Monowai and Oreti at Gravel Pit reaches to 4.7 at the Waitaki reach (average across sites: 1.6). At the time of the second retrieval (after 26-37 days

of exposure), the ratios were always greater than 1, and ranged from 1.3 at the Aparima and Oreti reaches to 2.5 at the Monowai reach (average across sites: 1.6).

Ratios of dissolved inorganic nitrogen and phosphorus (DIN:DRP ratios) in the water at each NDS reach are shown in Table 17. These ratios are expected to correspond to the relative availability of nitrogen and phosphorus to benthic algae. When one nutrient is scarce in relation to demand, that nutrient is expected to limit biomass accrual. As a broad generalization, DIN:DRP ratios less than 20:1 are expected to cause nitrogen limitation in periphyton, and ratios greater than 20:1 to cause phosphorus limitation (Hillebrand & Sommer 1999). Two sites with unusually high DIN concentrations, Haycock Hills and Key Bridge, are located immediately downstream of tributaries that drain intensively-farmed land, Wash and Princhester Creeks. These tributaries are presumably net sources of DIN.

**Table 17:** Nutrient concentrations, ratios and conductivity at Oreti and Mararoa River sites. Values are averages of 2-5 samples. EC: electrical conductivity. Nutrient concentrations in  $\mu\text{g L}^{-1}$ . Conductivity in  $\text{uS cm}^{-1}$ .

River	Site	EC	DRP	DIN	DON	DOP	DIN:DRP	DIN:DON
Oreti	Mt Nic Sta	24.3	2.70	18.50	164.50	7.88	12.7	0.2
	Ashton Flats	27.2	1.50	20.38	64.63	7.00	15.4	0.4
	Haybarn	30.8	1.67	8.17	76.50	4.33	5.2	0.2
	Managers House	32.9	1.50	11.25	63.75	4.00	8.0	0.2
	Three Kings	31.1	1.75	16.00	76.33	3.75	9.1	0.2
	Gravel Pits	32.7	0.50	11.25	95.75	2.50	22.5	0.2
Mararoa	Kiwi Burn	36.8	1.00	6.50	86.17	2.00	6.5	0.1
	Normans Gulch	38.6	1.25	25.63	101.13	4.00	13.2	0.7
	Haycock Hills	42.6	2.50	394.13	109.00	4.25	136.8	0.5
	Station Bridge	44.8	1.00	47.25	142.25	10.00	47.3	0.4
	Princhester	49.9	2.00	18.00	104.00	5.00	9.0	0.2
	Key Bridge	46.2	3.33	414.50	78.00	3.00	70.2	0.5

Dissolved nutrient concentrations and ratios for the April to June 2006 period at the study sites along the Mararoa and Oreti Rivers are shown in Table 17. On average, the Mararoa and Oreti Rivers had comparable DRP and DON concentrations, but DIN concentrations were higher in the Mararoa. Consequently, DIN:DRP ratios and

DIN:DON ratios in the Mararoa were also higher. The DIN:DRP ratios suggest that nitrogen limited algal growth in the Oreti (average ratio 12:1), and phosphorus limited algal growth in the Mararoa (average ratio 47:1) during Winter 2006. These predictions were not supported by NDS experiment results (Table 16). There were no detectable relationships between *D. geminata* biomass and ambient nutrient concentrations within or across rivers.

### 5.3. Discussion

Results of the NDS assays indicate algal assemblages were nutrient-limited in five of the eight reaches tested. As *D. geminata* was present on all NDS units and dominant on many, these comparisons of treatments suggests that *D. geminata* is nutrient-limited across most of its current range in New Zealand. If dissolved nutrient levels increase in these rivers, it is likely that *D. geminata* biomass levels will also increase. Comparisons of algal biomass from nutrient-enriched and control treatments indicate that on average, benthic algae accrual rates in the reaches under ambient conditions are ~ 60% of the potential rate under nutrient enriched conditions (assuming an average enriched:control ratio of 1.6). The nutrient availability from the NDS units may have been below saturating levels for algal growth, so the 60% figure above may underestimate the actual severity of nutrient limitation.

The NDS assays and the dissolved nutrient data from each NDS site indicate that nitrogen, phosphorus and nitrogen+phosphorus limitation occurred over a wide range of DIN:DRP ratios. Similar results have been reported from streams in Canterbury and Otago (Francoeur et al. 1999). These results suggest that biomass accrual can be decoupled from nutrient availability by growth supported by stored nutrients, and by uptake into algal nutrient pools that are not used for immediate growth. It is also important to note that the algal assemblages in field experiments such as the NDS assays are composed of multiple species, some of which have dissimilar nutrient requirements. This situation causes variability in responses to nutrient enrichment. Finally, comparisons between dissolved nutrient ratios and the results of the NDS assays illustrate the fact that nutrient ratios are not always accurate indicators of nutrient limitation. High DIN:DRP ratios are not always indicative of phosphorus limitation, and low DIN:DRP ratios are not always indicative of nitrogen limitation. Dissolved nutrient ratios convey no information about the specific nutrient requirements of algae for growth, or about nutrient storage capacities that may uncouple nutrient availability from growth. In general, growth assays are more accurate indicators of nutrient limitation than dissolved nutrient ratios.

## 6. *D. geminata* biomass dynamics

### 6.1. Monthly and fortnightly sampling

A monitoring programme was carried out to assess spatial and temporal variation in *D. geminata* abundance, and to identify and quantify relationships between *D. geminata* abundance and major environmental variables. The variables considered as potential determinants of *D. geminata* abundance were solar radiation, water temperature, near-bed velocity, water depth, and elapsed time following bed-mobilising floods.

#### 6.1.1. Methods

The *D. geminata* biomass monitoring programme ran from April 2006 to May 2007. Monthly samples for determination of *D. geminata* biomass were collected at the same 12 reaches in the Oreti, Mararoa and Waiau Rivers used for invertebrate monitoring (Table 1, Fig. 2). As noted in Section 3.1, the reaches were selected in April 2006 to provide a wide gradient in *D. geminata* abundance. A single reach in the Oreti River at Mt. Nicholas Station appeared to be upstream of all *D. geminata*-affected reaches, and was selected as a control site. However, this reach dried in January 2007. All samples were collected in runs (rapidly-flowing, no emergent rocks), which were the predominate habitats in each reach. Sediment grain size measurements were made at each reach in April and May 2006. Randomly-selected particles ( $N = 84\text{--}153$ ) from runs were measured using a Wolman frame (Wolman 1954), and mean and median particle sizes calculated.

*D. geminata* samples for biomass determination consisted of  $32.1\text{ cm}^2$  portions of mat removed from the surfaces of 12 randomly selected stones at each reach. For the first three months of the programme, additional samples from each stone were collected for determination of the proportions of *D. geminata* and other algal taxa in total algal cell counts. This analysis was used to estimate the proportion of *D. geminata* in biomass samples. Water depth and near-bed velocity was measured next to each sampled rock, to facilitate regression analyses relating *D. geminata* biomass and hydraulic habitat parameters. Biomass samples were frozen within 6 hours of collection, and shipped by refrigerated courier to the NIWA Christchurch analytical laboratory, where they were stored frozen until analysis. On the day prior to biomass analysis, the samples were freeze-dried and ground. Chlorophyll *a* concentrations and ash-free dry mass (AFDM) biomass levels were determined from each sample for the first three months of the sampling programme. Samples from subsequent dates were analysed for AFDM biomass only. Chlorophyll *a* ( $\text{mg m}^{-2}$ ) is a measure of live algal material in the sample, and AFDW ( $\text{g m}^{-2}$ ) is a measure of the total organic content of a sample, including cell contents and *D. geminata* stalk material. Chlorophyll *a* and AFDM in ground samples were determined using the procedures of Biggs and Kilroy (2000).

The subsamples for assessing proportions of *D. geminata* were thawed, homogenized, and 1 ml subsamples were examined by light microscopy at 100 $\times$ . Three samples from each of the 12 biomass monitoring reaches were assessed. A stage micrometer was used as a transect line in random fields of view, and *D. geminata* cells, stalks and other algal cells that intersected the line were recorded at 200  $\mu$ m intervals. At least 100 counts were made for each sample. Freezing and thawing the samples appeared to cause degradation of stalk material, so the proportions of *D. geminata* material in samples that included other algal species are greatly underestimated. Every periphyton sample examined included live cells of non-*D. geminata* benthic algae. Consequently, the results of the study are hereafter expressed in terms of quantities of total periphyton, not *D. geminata*.

Fortnightly visual estimates of periphyton abundance were made at the same sites used for biomass monitoring. These estimates combined high-frequency monitoring with low-intensity sample processing to gain information about short-term changes in abundance. As with the monthly samples, fortnightly samples consisted of 12 randomly-selected cobbles at each site. The % periphyton cover on the top surface of each cobble was estimated, and the thickness of the periphyton mat, if present, was measured with a ruler. Two workers made all visual estimates during the monitoring programme. The product of the % cover and thickness estimate for each cobble was used as an index of biomass, the “Kilroy Biomass Index” (KBI). This is a modified version of the “quantitative visual index used” in Kilroy et al. (2006a). KBI values are more accurate indicators of temporal changes in *D. geminata* abundance than areal cover because they account for three dimensions (area and thickness), rather than two. KBI values are closely correlated with AFDM biomass (linear correlation coefficient  $r = 0.92$ ,  $P < 0.0001$ , reported in Kilroy et al. 2006a).

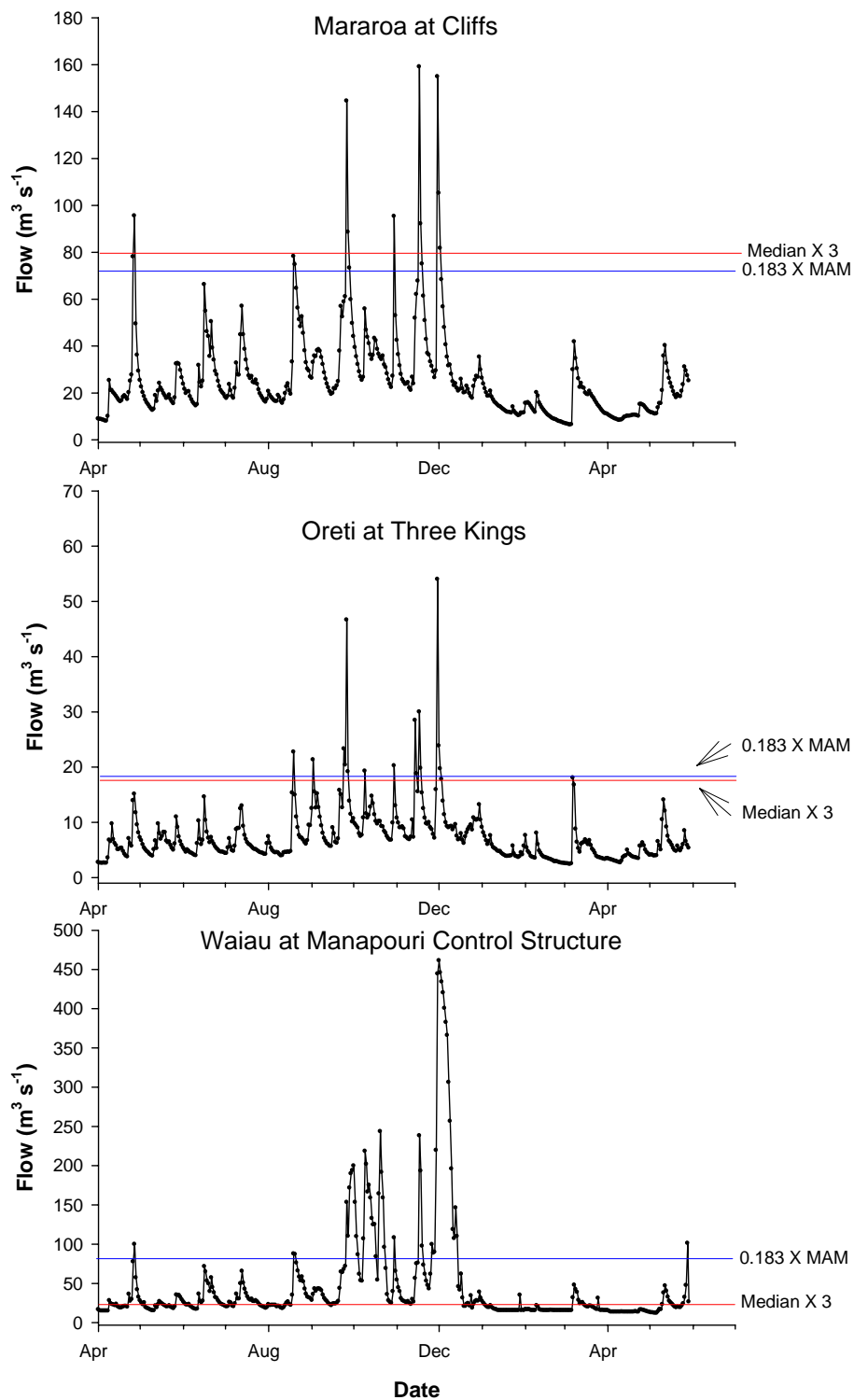
Relationships between periphyton abundance and light and temperature data were carried out using the periphyton monitoring data and data acquired from field sensors. Irradiance sensors (Odyssey Corp.) with integrated loggers were installed at sites on the Mararoa River (Station Bridge) and the Oreti River (Ashton Flat) on 6 May 2006. Water temperature data for the Oreti River was acquired from Environment Southland from their sensor at the Three Kings flow recorder, within the study area. No long-term water temperature data were available for the Mararoa or Waiau Rivers. After downloading the irradiance sensors, it was apparent that the batteries had failed several months before retrieval; the Mararoa sensor stopped recording on 4 December 2006, and the Oreti sensor stopped recording on 25 September 2006. To augment this data, total solar radiation data (direct + diffuse) was acquired from the NIWA climate station at Manapouri Airport, 20 km west of the western-most study reach. The Manapouri data spanned the entire study period. Daily total solar radiation ( $\text{MJ m}^{-2} \text{d}^{-1}$ ) at Manapouri Airport was closely correlated with the Odyssey sensor data

at the Mararoa and Oreti sites (linear correlation coefficients  $r = 0.97$ ,  $N = 213$  for the Mararoa site,  $r = 0.89$ ,  $N = 143$  for the Oreti site). Periphyton abundance-solar radiation relationships were then assessed using the Manapouri dataset.

Relationships between periphyton abundance and hydrologic variables were carried out using the periphyton cover, KBI and biomass data, depth and velocity measurements made during monthly biomass sampling, and river flow data acquired from Environment Southland (Oreti River) and NIWA (Mararoa and Waiau Rivers) flow recorders. The flow recorders are located at Three Kings (Oreti), Cliffs (Mararoa), and Manapouri Control Structure (Waiau). The river flow data were used to estimate the number and dates of flood flows capable of mobilizing river bed substrata. The rationale for this assessment was based on observations that bed-mobilizing flows control algal accrual in many gravel-bed rivers in New Zealand (e.g., Biggs 1995). Bed-mobilising flood flows were defined as flow events  $\geq 0.183 \times$  mean annual maximum daily flow, referred to hereafter as 0.18(MAM). The 0.18(MAM) estimator was developed by Clausen and Plew (2004), who calculated flows required to mobilise median grain sizes ( $d_{50}$ ) and 84<sup>th</sup>-percentile grain sizes ( $d_{84}$ ) in 40 New Zealand rivers. On average, flows that mobilised  $d_{50}$  material in that study were 0.18(MAM). An alternative flow statistic used to estimate bed-mobilising capacity is  $3 \times$  median flow. The frequency of  $3 \times$  median flows was negatively correlated with periphyton biomass in a range of New Zealand rivers (Clausen and Biggs 1997). This correlation is presumably due to shear stress related to elevated water velocity, and/or bed mobilisation. In the Oreti, Mararoa and Waiau Rivers, 0.18(MAM) and  $3 \times$  median flows were similar in magnitude (Fig. 17). The 0.18(MAM) estimator was used in this study due to the direct relationship with bed mobilization. To test the hypothesis that periphyton biomass is a function of accrual time between bed-mobilising flows, periphyton biomass was regressed on days since 0.18(MAM) events. The application of the 0.18(MAM) estimator to the 12 study reaches assumes that flows at each reach within a river are similar to the flows at the single flow recorder on each river. This assumption is reasonable for cases where there are no major tributaries joining the river between the study reaches and the recorder. This is the case for the Waiau at Excelsior, for the Oreti at the three downstream sites, and for the Mararoa at Key Bridge (the downstream-most site). Spot gauging at reaches upstream of Key Bridge indicate that flows at these reach are within 75% of the flows at the recorder.

### 6.1.2. Results

The AFDM of periphyton samples was significantly correlated with chlorophyll *a* biomass (linear correlation coefficient  $r = 0.40$ ,  $N = 198$ ). On average, AFDW



**Figure 17:** Hydrographs for the Mararoa, Upper Oreti and Lower Waiau Rivers during the current study. The Waiau River is dam-controlled and the difference between 0.183(MAM) and  $3 \times$  median flow values is primarily due to the low operating flow.



biomass was 277 times greater than chlorophyll *a* biomass. This comparison indicates that *D. geminata* mats are essentially large accumulations of stalk material with a thin layer of pigmented cells (Fig. 12, Section 4.2.2). AFDW biomass is an appropriate response variable for estimating the effects of environmental conditions on periphyton, as reported below. Furthermore, correlations between percent areal cover of periphyton and AFDW biomass were stronger than for chlorophyll *a* biomass. Finally, AFDW biomass represents the quantity of periphyton that elicits responses by invertebrates, fish, and other river biota, not chlorophyll *a* biomass. For these reasons, the results and discussions below focus on AFDW biomass. The percentage live *D. geminata* cells in total algal cell counts ranged from 1% in the Oreti River at Mt Nicholas, to 78% in the Oreti River at Three Kings (Table 18).

**Table 18:** Percentage of *D. geminata* cells in total algal cells from biomass samples collected at the focal rivers. Values are means of three samples, collected in April and May 2006.

River	Site	% <i>D. geminata</i>
Oreti	Mt Nic Sta	1.0
	Ashton Flat	7.3
	Haybarn	43.8
	Managers House	67.6
	Three Kings	78.2
	Gravel Pit	67.5
Mararoa	Normans Gulch	35.2
	Haycock Hills	43.8
	Station Bridge	36.5
	Princhester Creek	52.0
	Key Bridge	35.0
Waiau	Excelsior	45.1

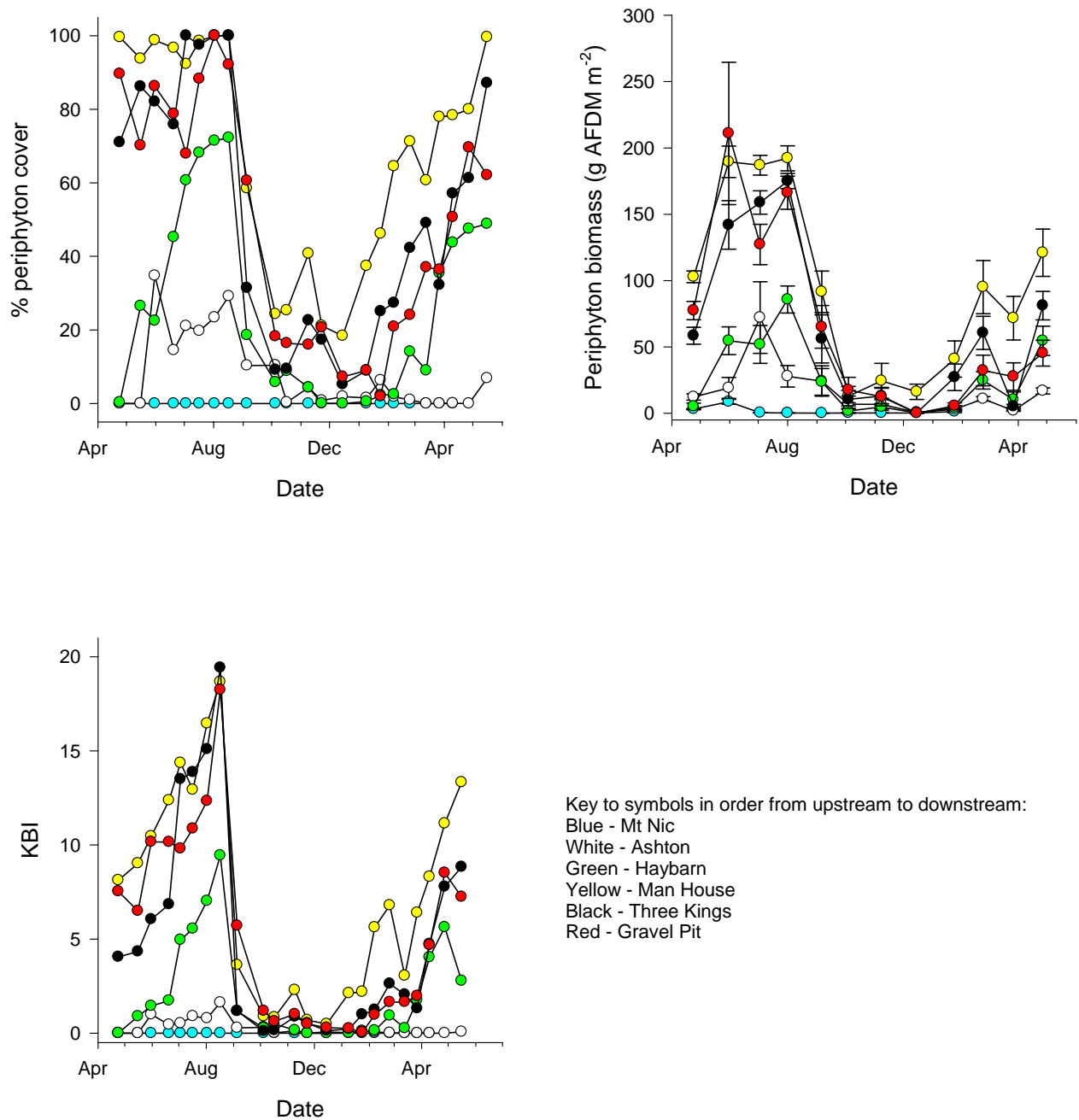
Frequency distributions of periphyton cover, KBI and biomass indicated that the data cover and KBI datasets for each river were left-skewed (due to large proportions of low and zero values); arcsine transformation did not appreciably improve normality. Biomass data were normally distributed; for all linear correlations between data and normal scores,  $r > 0.70$ . Therefore, standard deviations were calculated for biomass data, but not for cover and KBI data. Regressions were computed using untransformed values. With datasets as large as those in the present study, regressions are robust to deviations from normal distributions.

Time series of periphyton abundance are shown in Figures 18 (Oreti River), 19 (Mararoa River) and 20 (Waiau River at Excelsior). A total of 3212 periphyton cover and KBI estimates, and 1830 periphyton biomass measurements were made between April 2006 and May 2007. There was no visible *D. geminata* in the Mt Nicholas reach of the Oreti River during the study. Microscopic examination of samples from this reach indicated that *D. geminata* was present at extremely low levels (Table 18). All other reaches were visibly colonised throughout the study. Periphyton abundance in most of the *D. geminata*-affected reaches peaked in August 2006, declined in September-October 2006, remained low through December 2007, and began increasing in January 2007. This pattern was highly coherent at reaches in the Oreti, but was obscured due to high among-reach variability in the Mararoa (Figs. 18 and 19). The September-October 2006 decline in periphyton abundance coincided with a series of moderate spring floods in late September and late October, and the sustained period of low abundance in summer 2006 coincided with a series of large-magnitude floods on each river from mid-November to mid-December (Fig. 17). The role of flood flows in controlling periphyton abundance is examined in detail below.

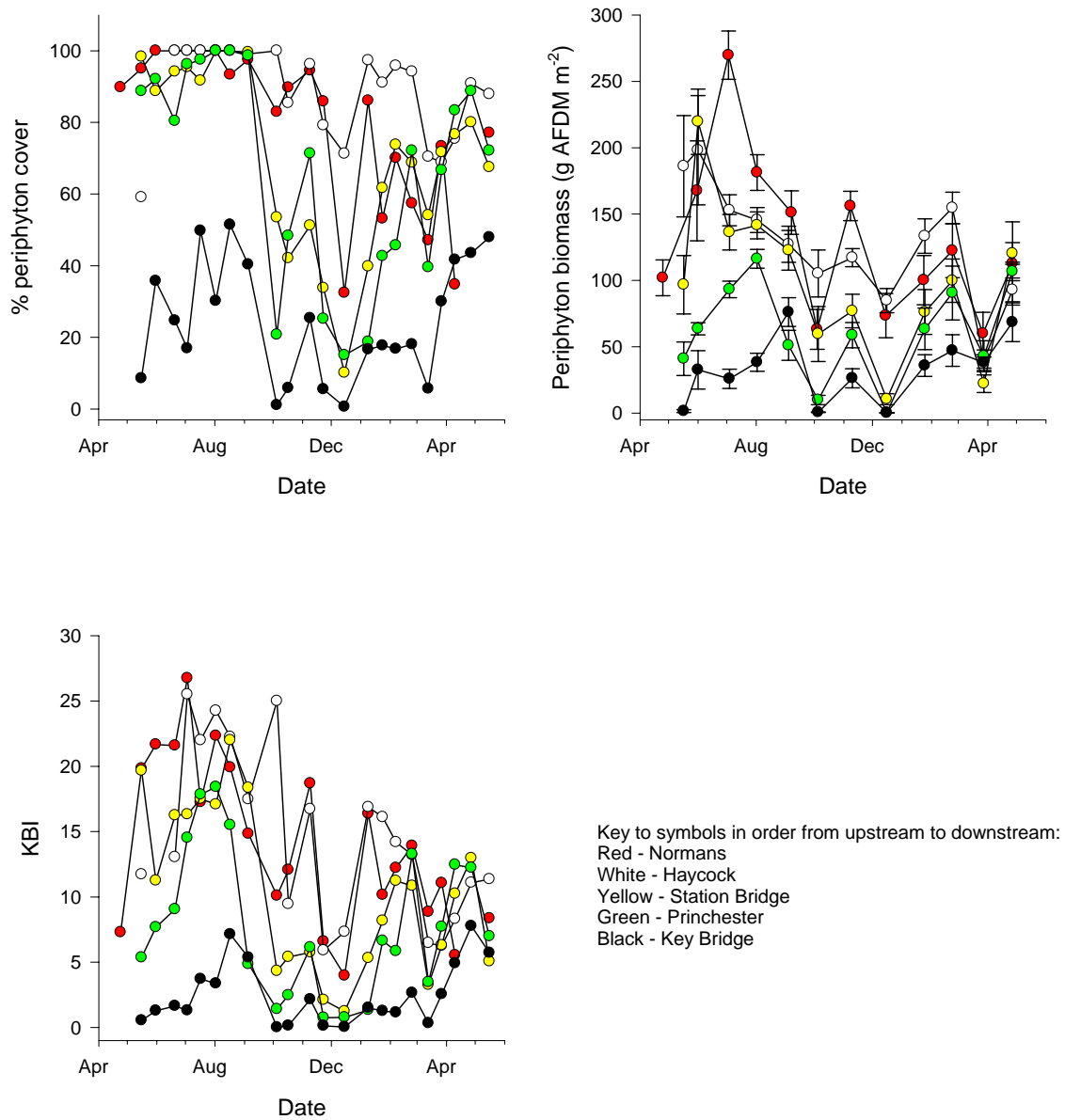
Long-term changes in periphyton biomass were assessed at the Waiau River at Excelsior, by combining data from the current study with annual monitoring data from the same site provided by Meridian Energy Ltd (Fig. 21). The average periphyton biomass at this site prior to the discovery of *D. geminata* in the Lower Waiau (October 2004) was 11.3 g AFDM m<sup>-2</sup>. The average biomass following colonisation (May 2005-May 2007) was 54.2 g AFDM m<sup>-2</sup>, a 5-fold increase.

### **Solar radiation and water temperature.**

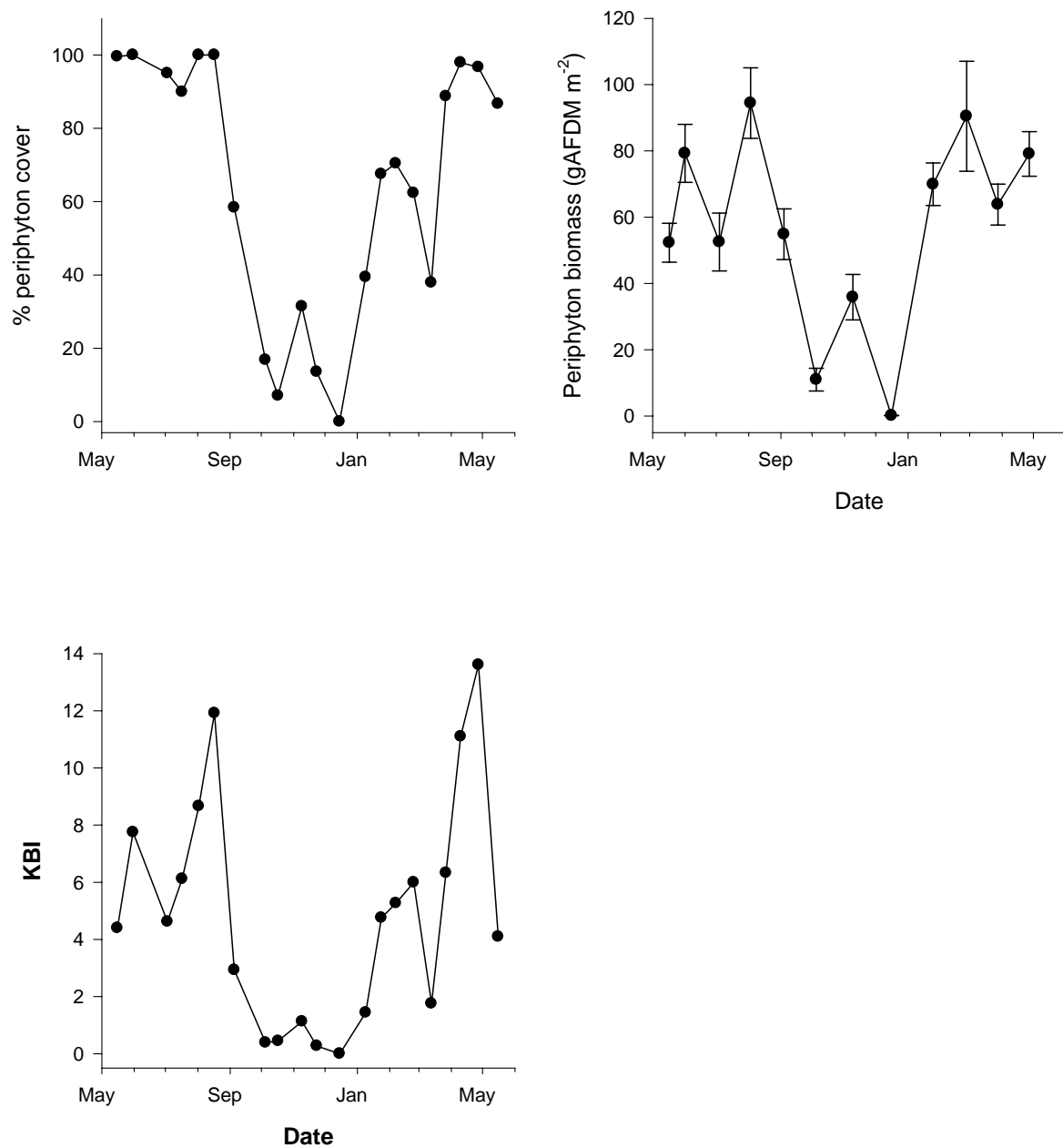
If seasonally-varying factors such as solar radiation and water temperature strongly influence *D. geminata* abundance, the periphyton time-series should have a sinusoidal pattern with a 1-year period. Graphs of the data from Mararoa River reaches are not sinusoidal (Fig. 19). Graphs of the data from the Oreti and Waiau Rivers appear sinusoidal, with maximum abundance in late autumn-early winter, and minimum abundance in late spring-early summer (Figs. 18 and 20). However, it is not clear whether these temporal patterns are primarily due to the timing of a flood-related reduction in abundance, and subsequent recover, or to regular seasonal variation in a limiting resource such as light. The lack of replication (i.e., no replicate seasons) precluded an assessment of seasonal periodicity in periphyton abundance by spectral analysis. Instead, relationships between periphyton abundance and solar radiation and temperature were tested.



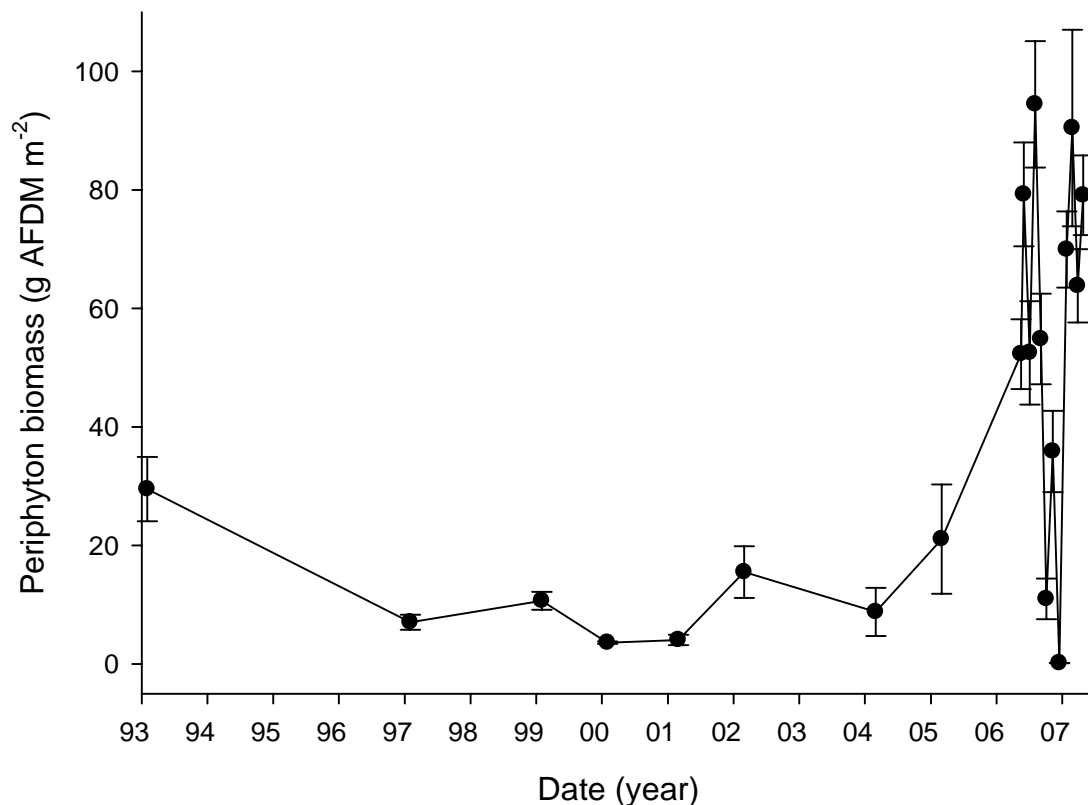
**Figure 18:** Time series of periphyton abundance in the Oreti River. Values are means of 12 cover and KBI estimates, 6 biomass samples. Error bars:  $\pm 1$  standard error. KBI: Kilroy Biomass Index; see text for explanation.



**Figure 19:** Time series of periphyton abundance in the Mararoa River. Values are means of 12 cover and KBI estimates, 6 biomass samples. Error bars:  $\pm 1$  standard error.

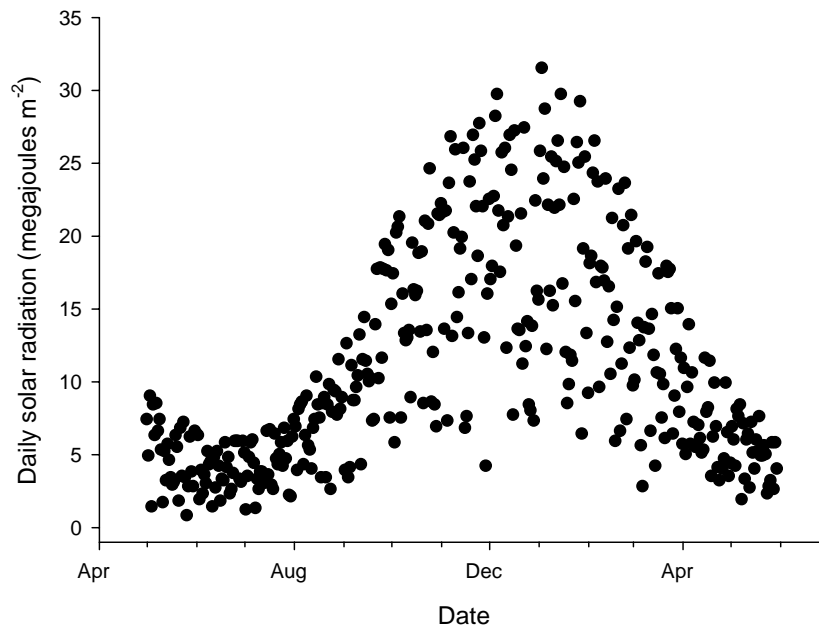


**Figure 20:** Time series of periphyton abundance in the Waiau River at Excelsior Creek. Values are means of 12 cover and KBI estimates, 6 biomass samples. Error bars:  $\pm 1$  standard error.

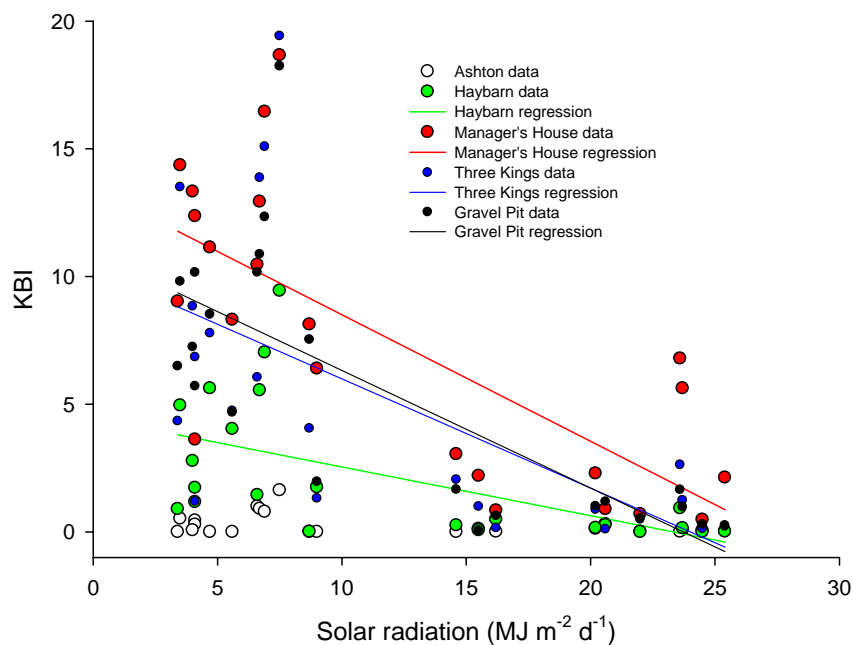


**Figure 21:** Long-term periphyton biomass time series from the Waiau River at Excelsior Creek. Values are means 9 (1993-2005) and 6 (2006-2007) biomass samples. Error bars:  $\pm 1$  standard error. Pre-2005 samples were collected in February or March of each year.

During the study period, daily solar radiation at the Manapouri Airport ranged from 0.8 to 31.5 MJ m<sup>-2</sup> d<sup>-1</sup> (mean  $\pm$  1SD: 11.1  $\pm$  7.4). Maximum daily solar radiation levels occurred in December 2006 and January 2007, and minima in May 2006 and May 2007 (Fig. 22). The annual solar radiation pattern is sinusoidal, but frequent cloud cover results in days with low total radiation throughout the year. Periphyton KBI values were used to assess periphyton-solar radiation relationships because there were more KBI data per site than biomass data, and because KBI and biomass values were shown to be highly correlated (Kilroy et al. 2006a). In the Oreti River, KBI values in four reaches downstream of Ashton Flat decreased with increasing daily solar radiation (Fig. 23, Table 19). The periphyton-solar radiation regression for the Ashton Flat reach was not significant, and KBI values at the remaining Oreti River reach at Mt. Nicholas were generally zero. In the Mararoa River, KBI values in four reaches decreased with increasing daily solar radiation (Fig. 24, Table 19). The regression for the Haycock reach was not significant. In the Waiau River, KBI values decreased with increasing daily solar radiation (Fig. 25, Table 19).

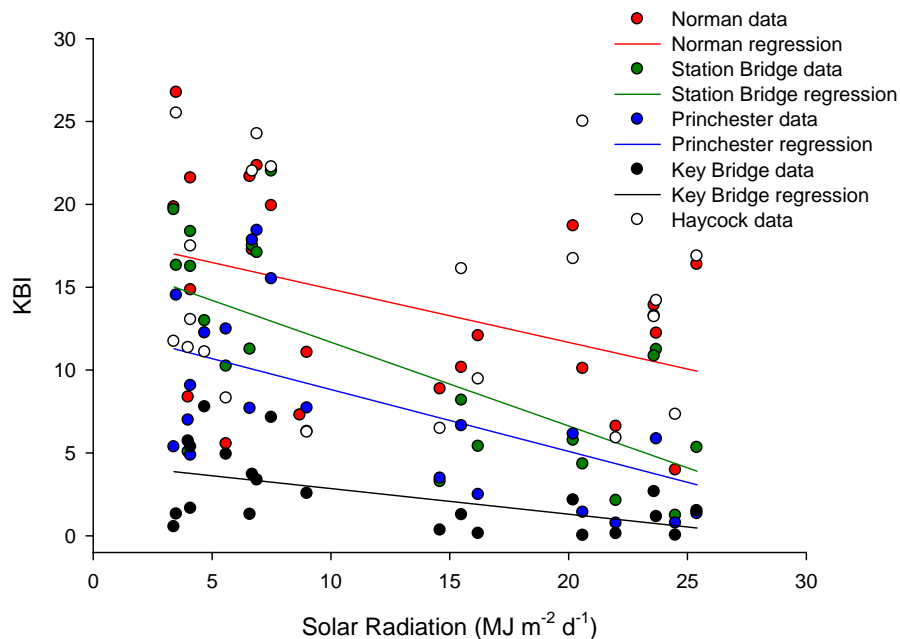


**Figure 22:** Daily total solar radiation during monitoring period (26 April 2006 to 30 May 2007). Solar radiation was measured at the Manapouri Airport climate station.



**Figure 23:** Periphyton abundance (as Kilroy Biomass Index, KBI) in the Oreti River as a function of daily solar radiation at Manapouri Airport on the date of periphyton sampling. Lines are for significant linear regressions ( $P < 0.05$ ).

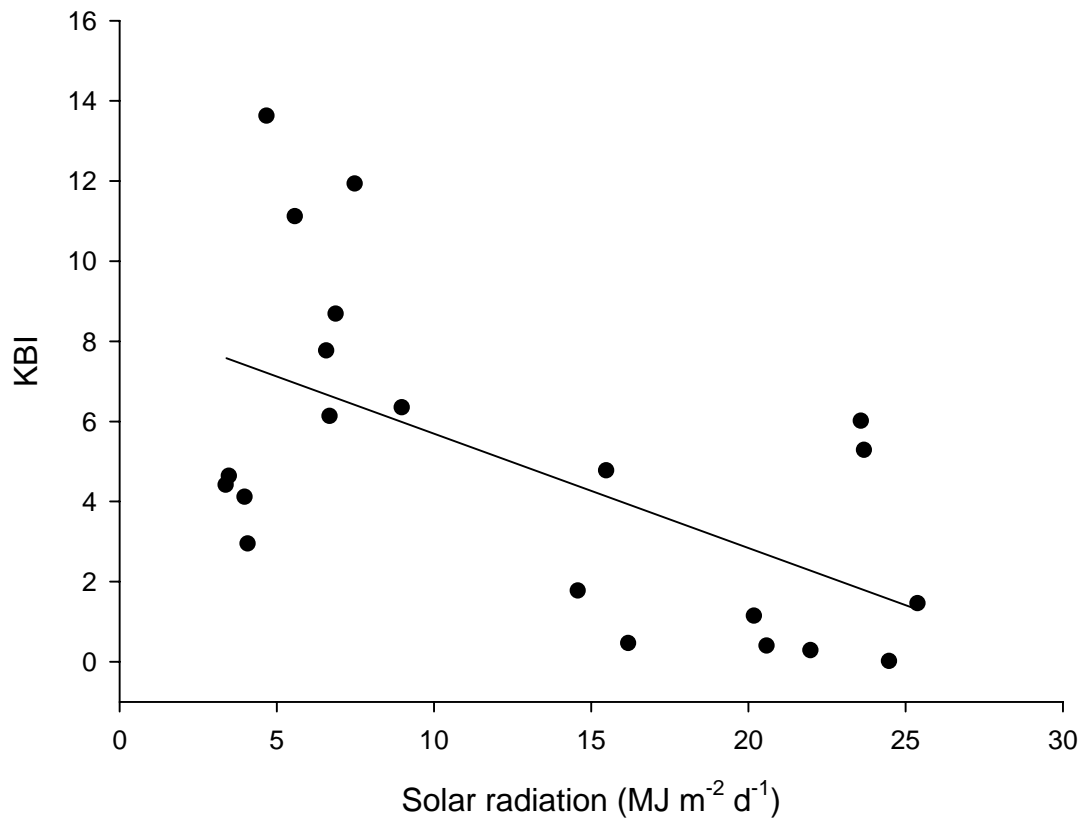




**Figure 24:** Periphyton abundance (KBI) in the Mararoa River as a function of daily solar radiation at Manapouri Airport on the date of periphyton sampling. Lines are for significant linear regressions ( $P < 0.05$ ).

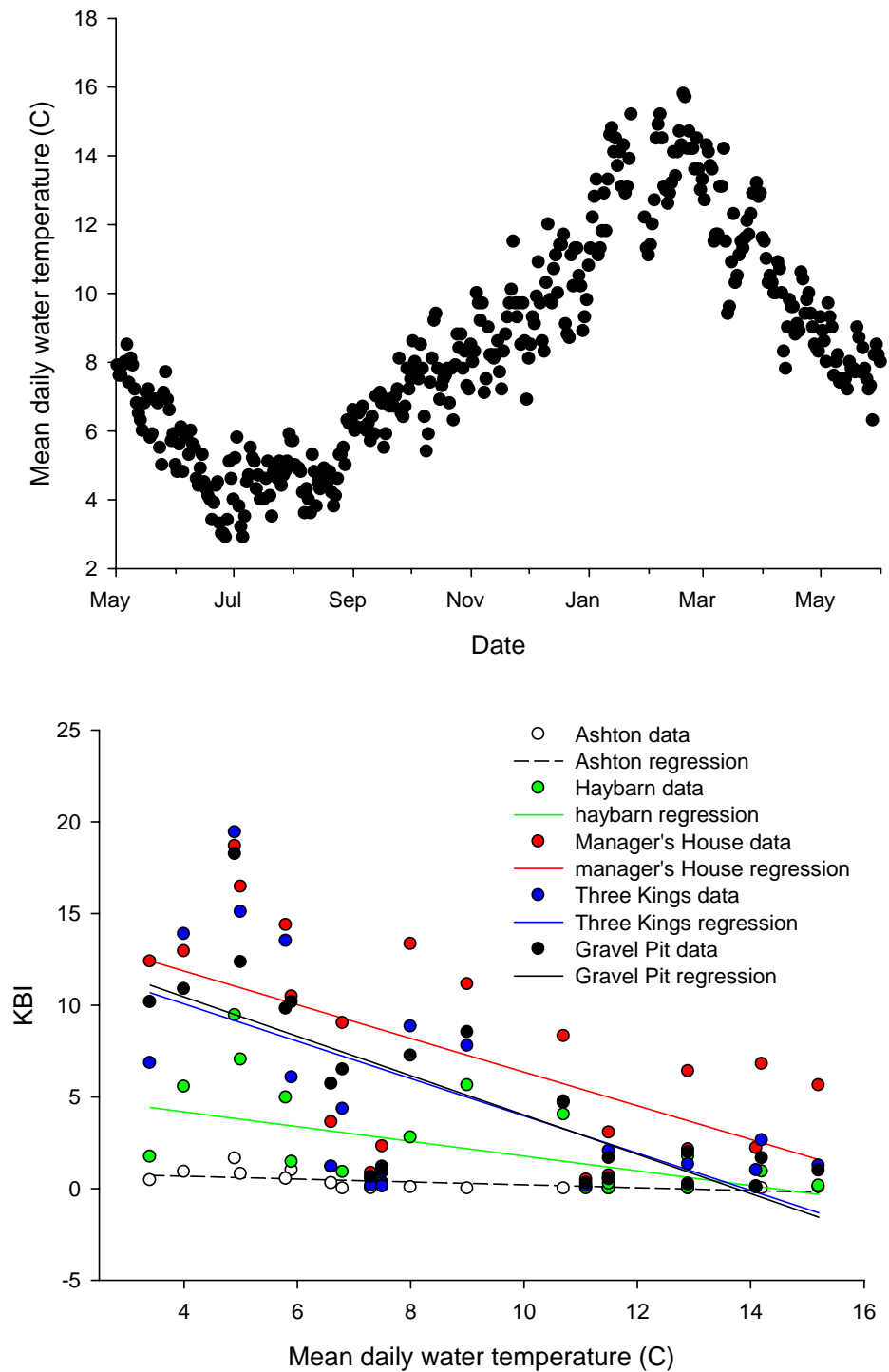
**Table 19:** Regression equations for periphyton-solar radiation relationships shown in Figures 22 and 23. NS: regression not statistically significant.

River	Reach	Regression	$R^2$	$P$
Oreti	Ashton	NS		0.09
	Haybarn	$KBI = -0.2(\text{solar radiation}) + 4.5$	0.33	0.004
	Manager's House	$KBI = -0.5(\text{solar radiation}) + 13.5$	0.53	<0.0001
	Three Kings	$KBI = -0.4(\text{solar radiation}) + 10.3$	0.38	0.002
	Gravel Pit	$KBI = -0.5(\text{solar radiation}) + 10.9$	0.55	< 0.0001
Mararoa	Normans	$KBI = -0.2(\text{solar radiation}) + 18.1$	0.17	0.023
	Haycock	NS		0.45
	Station Br	$KBI = -0.5(\text{solar radiation}) + 16.7$	0.44	0.0007
	Princhester	$KBI = -0.4(\text{solar radiation}) + 12.6$	0.31	0.007
	Key Br	$KBI = -0.2(\text{solar radiation}) + 4.4$	0.29	0.009
Waiau	Excelsior	$KBI = -0.3(\text{solar radiation}) + 8.5$	0.35	0.012



**Figure 25:** Periphyton abundance (KBI) in the Waiau River at Excelsior as a function of daily solar radiation at Manapouri Airport on the date of periphyton sampling. Lines are for significant linear regressions ( $P < 0.05$ ).

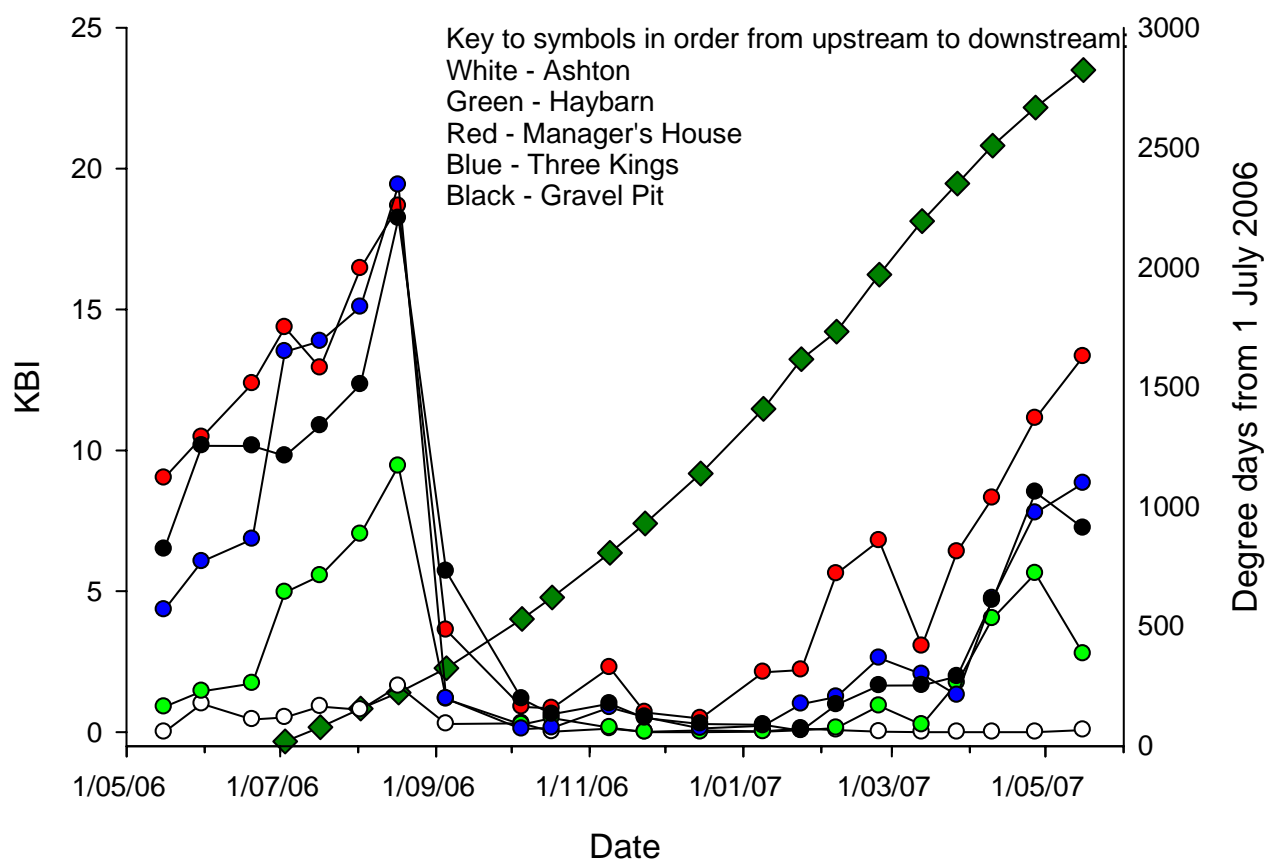
Daily mean temperature in the Oreti River at Three Kings ranged from 2.9 to 15.8° C during the study period (Fig. 26 top panel). The annual temperature pattern was sinusoidal; July 2006 was the coldest month and February 2007 the warmest. Plots of KBI values from the Oreti reaches versus daily mean water temperature indicates that periphyton abundance decreased with increasing temperature (Fig. 26 bottom panel). Regression equations for these relationships are listed in Table 20. As was the case for solar radiation, it was not clear whether the periphyton abundance-temperature relationships are a direct effect of temperature, or an artifact of flood flows that removed periphyton during spring and summer. Further, it is likely that periphyton abundance responds primarily to long-term thermal conditions, not daily average conditions. Therefore, periphyton abundance was compared to cumulative degree-days, calculated from the coldest period (July 2006). From 1 July 2006 to 16 May 2007 (the last sampling date), the Oreti River at Three Kings experienced 2823 degree-days (Fig. 27). There was no detectable relationship between periphyton abundance and cumulative degree-days (regression  $P > 0.5$ ).



**Figure 26:** Top: daily mean water temperature in the Oreti River at Three Kings. Bottom: periphyton abundance (as Kilroy Biomass Index, KBI) in the Oreti River as a function of daily mean water temperature at Three Kings on the date of sampling. Lines are for significant linear regressions ( $P < 0.05$ ).

**Table 20:** Regression equations for periphyton-water temperature relationships in the Oreti River. Regressions correspond to graphs in Figure 24, bottom panel.

Reach	Regression	$R^2$	$P$
Ashton	$KBI = -0.08(\text{temperature}) + 1.0$	0.42	0.006
Haybarn	$KBI = -0.4(\text{temperature}) + 5.8$	0.28	0.01
Manager's House	$KBI = -0.9(\text{temperature}) + 15.5$	0.33	0.009
Three Kings	$KBI = -1.0(\text{temperature}) + 14.1$	0.40	0.007
Gravel Pit	$KBI = -1.1(\text{temperature}) + 14.7$	0.57	< 0.0001



**Figure 27:** Cumulative degree days in the Oreti River at Three Kings (green diamonds), and periphyton KBI values at Oreti River reaches. Degree-days begin accumulating on the date of lowest recorded water temperature (1 July 2006).

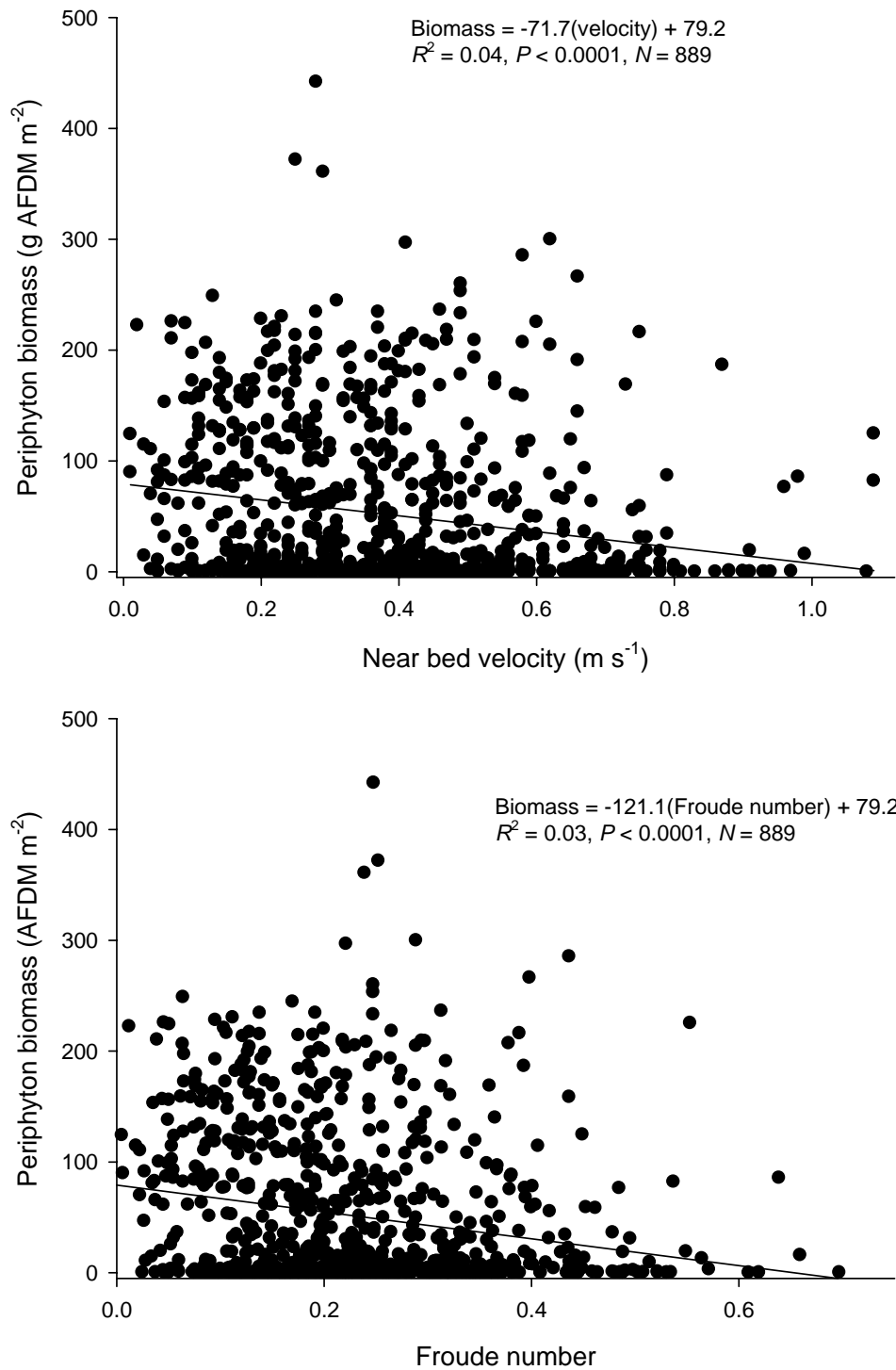
## Hydraulic variables

Periphyton biomass was weakly, negatively related to near-bed velocity and to Froude number in most of the study reaches (Table 21). No regressions relating periphyton biomass to water depth were significant ( $P > 0.05$ ). Coefficients of determination for significant regressions using near-bed velocity and Froude number ranged from 0.02 to 0.14, indicating that these instantaneous hydraulic properties only explained 2 to 14% of the variability in periphyton biomass within reaches or rivers. In general, biomass-flow relationships were stronger for reaches in the Oreti River than the Mararoa River. The regressions for the Waiau River at Excelsior were not significant. Plots of periphyton biomass versus near-bed velocity and Froude number in the Oreti River (Fig. 28) and Mararoa River (Fig. 29) indicate that the detection of significant linear relationships is primarily due to large sample sizes and consequently, high statistical power, rather than obvious trends in the data.

Relationships between periphyton abundance and bed-mobilising flows were assessed by regressing abundance (% periphyton cover, KBI, and biomass) on elapsed time since the last flood in each river that was predicted to mobilise the median-size bed sediment. This flood magnitude corresponds to  $\geq 0.18 \times$  the mean annual maximum daily flow, 0.18(MAM), as discussed in Section 6.1.1. Elapsed time after 0.18(MAM) flows explained a significant amount of the variation in periphyton abundance in two reaches on the Mararoa River (Fig. 30), in 5 reaches on the Oreti River (Fig. 31), and in the Waiau River at Excelsior Creek (Fig. 32). Periphyton biomass at the heavily *D. geminata*-affected Haycocks reach on the Mararoa River decreased with time after 0.18(MAM) flows, and a power function fit the data from that reach (Fig. 30). In all other cases, periphyton abundance increased with time after 0.18(MAM) flows. The regression equations quantifying these relationships for the Oreti River are listed in Table 22. Coefficients of determination for the regressions in all three rivers (range: 0.25–0.91) were higher than for regressions on near-bed velocity or Froude numbers, suggesting that time since bed-mobilising floods is a more important determinant of periphyton abundance than the instantaneous hydraulic variables. Most of the run habitats sampled in the Oreti River were dominated by medium-to-coarse gravels (median grain sizes 16–45 mm; Table 23), and most of the run habitats sampled in the Mararoa and Waiau Rivers were dominated by small-to-medium cobbles (median grain size range 45.3–91 mm; Table 23). Differences between the rivers in the relationships between periphyton abundance and time after floods may be related to the fact that the relatively fine sediments in the Oreti River will be mobilized more frequently than the coarser sediments in the Mararoa and Waiau Rivers.

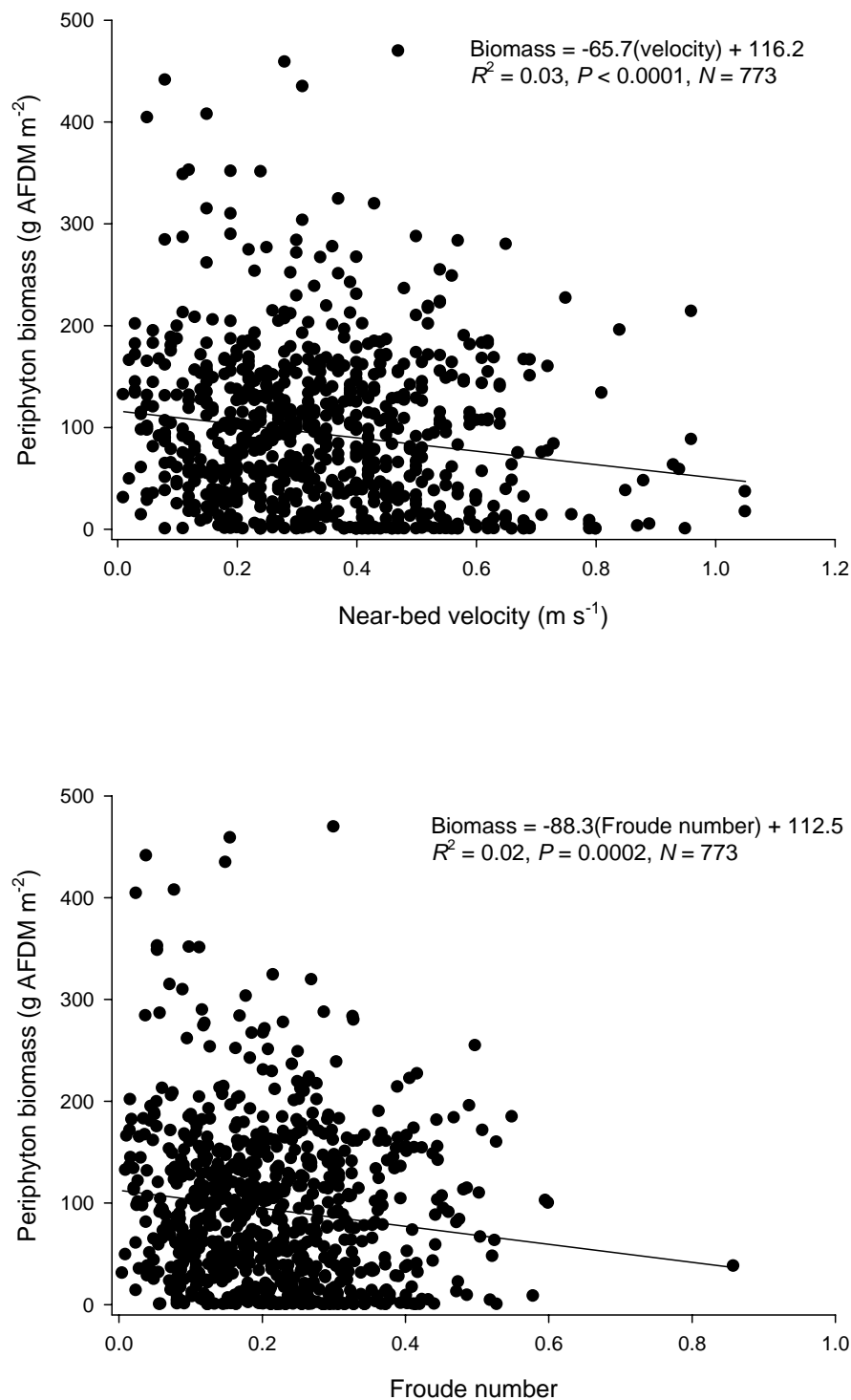
**Table 21:** Regression equations for periphyton biomass and near-bed velocity, and periphyton biomass and Froude number. V (velocity,  $\text{m s}^{-1}$ ), Fr: Froude number (dimensionless), NS: regression not statistically significant.

River	Reach	Regression equation	R <sup>2</sup>	P
Mararoa	Normans	Biomass = $-94.1(V) + 167.7$	0.04	0.01
		Biomass = $-164.0(\text{Fr}) + 165.5$	0.03	0.03
	Haycock	V: NS		0.59
		Fr: NS		0.95
	Station Br	V: NS		0.21
		Fr: NS		0.17
	Princhester	Biomass = $-57.2(V) + 82.9$	0.03	0.03
		Fr: NS		0.40
	Key Br	Biomass = $-43.4(V) + 52.3$	0.06	0.003
		Biomass = $-55.2(\text{Fr}) + 47.6$	0.03	0.049
	All reaches	Biomass = $-65.7(V) + 116.2$	0.03	<0.0001
		Biomass = $-88.3(\text{Fr}) + 112.5$	0.02	0.0002
Oreti	Mt. Nic	Biomass = $-7.4(V) + 3.6$	0.09	0.0008
		Biomass = $-13.8(\text{Fr}) + 3.8$	0.09	0.0007
	Ashton	Biomass = $-39.6(V) + 35.9$	0.05	0.007
		Biomass = $-56.7(\text{Fr}) + 33.6$	0.03	0.002
	Haybarn	Biomass = $-78.2(V) + 62.8$	0.13	<0.0001
		Biomass = $-127.7(\text{Fr}) + 61.8$	0.14	<0.0001
	Manager House	Biomass = $-94.5(V) + 136.5$	0.05	0.006
		Biomass = $-214.8(\text{Fr}) + 146.2$	0.06	0.002
	Three Kings	Biomass = $-121.3(V) + 119.1$	0.07	0.0008
		Biomass = $-272.2(\text{Fr}) + 133.0$	0.11	<0.0001
	Gravel Pits	Biomass = $-84.7(V) + 106.7$	0.03	0.027
		Fr: NS		0.52
	All reaches N	Biomass = $-71.7(V) + 121.1$	0.04	<0.0001
		Biomass = $-121.1(\text{Fr}) + 79.2$	0.03	<0.0001
Waiau	Excelsior	V: NS		0.24
		Fr: NS		0.55



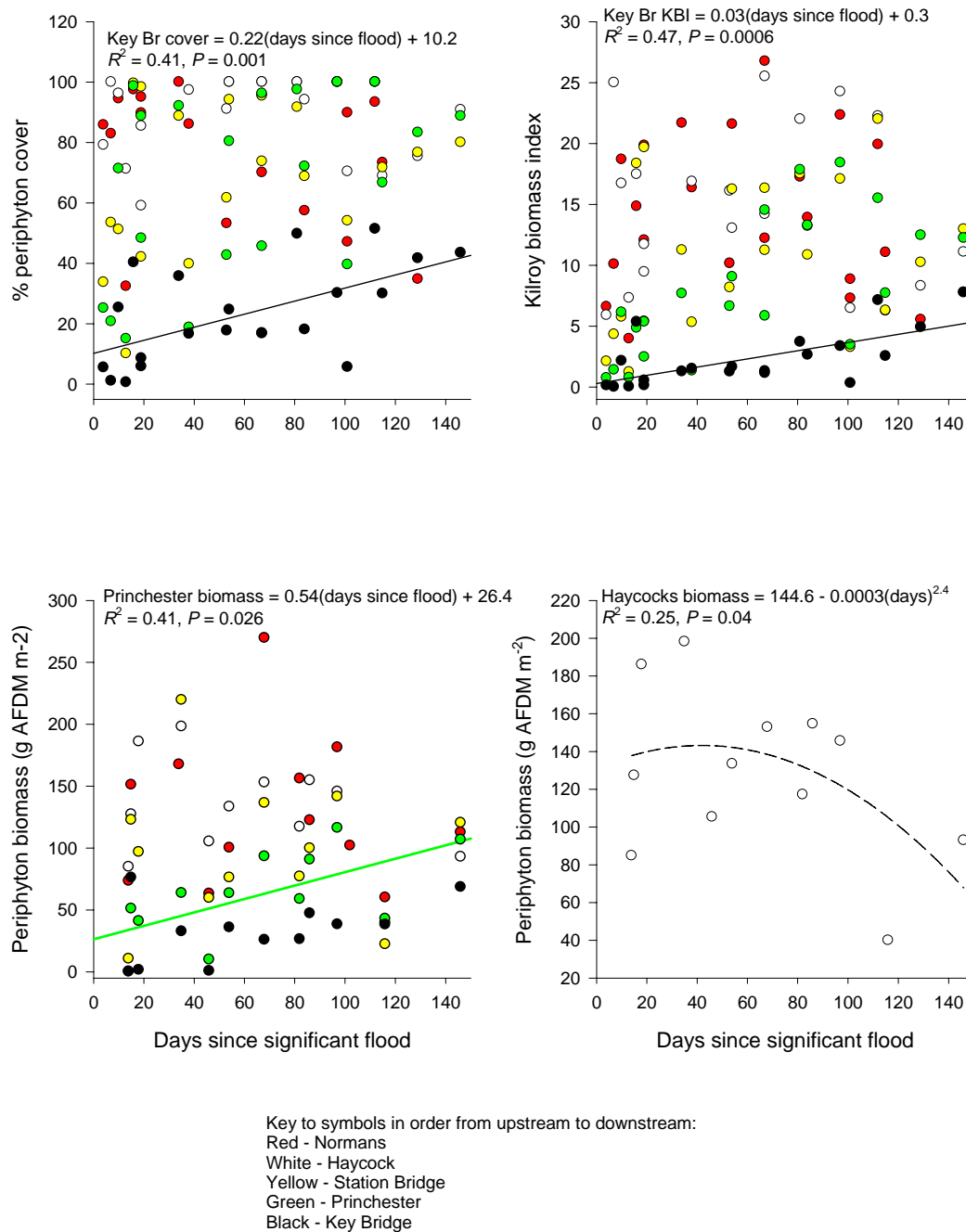
**Figure 28:** Periphyton biomass in the Oreti River (all reaches) as a function of near-bed velocity (top) and Froude number (bottom). Lines indicate significant linear regressions.



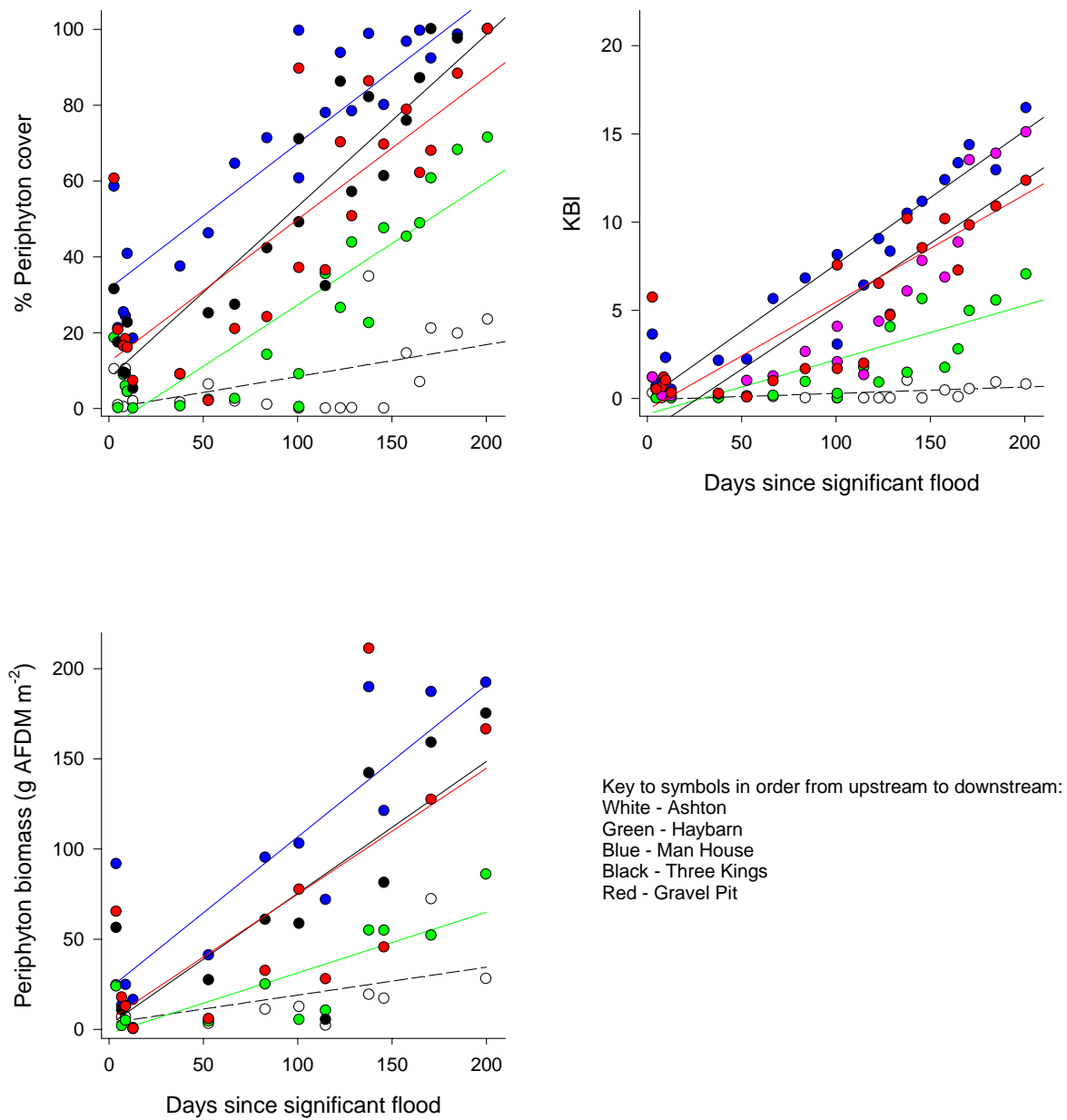


**Figure 29:** Periphyton biomass in the Mararoa River (all reaches) as a function of near-bed velocity (top) and Froude number (bottom). Lines indicate significant linear regressions.

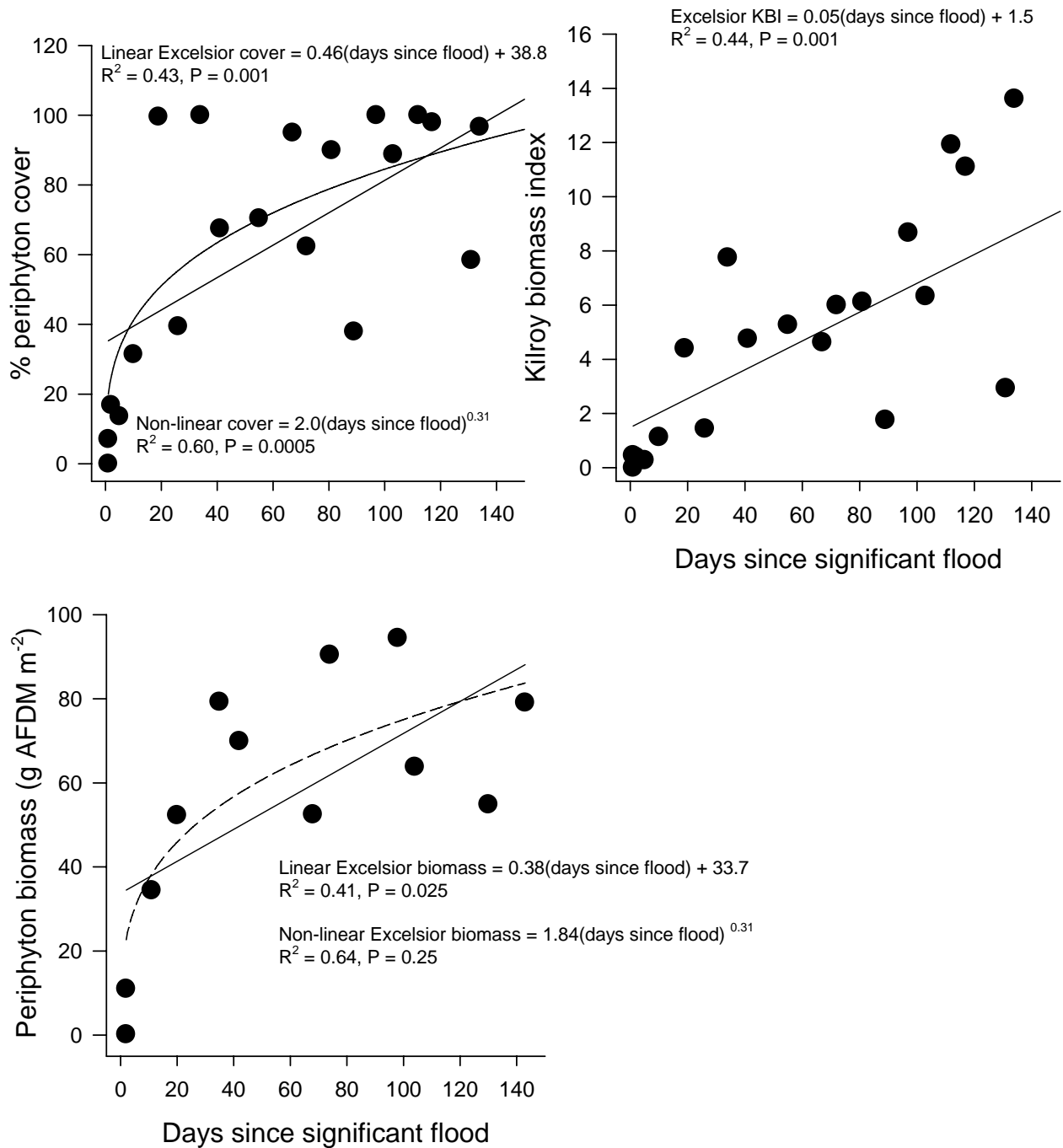
Mararoa River



**Figure 30:** Periphyton abundance as a function of days since significant flood flows in the Mararoa River. Lines indicate significant linear regressions.



**Figure 31:** Periphyton abundance as a function of days since significant flood flows in the Oreti River. Lines indicate significant linear regressions.



**Figure 32:** Periphyton abundance as a function of days since significant flood flows in the Waiau River at Excelsior Creek. Lines indicate significant linear regressions.

**Table 22:** Regression equations for relationships between periphyton abundance and days since 0.18(MAM) flood in the Oreti River.

Reach	Regression equation	R <sup>2</sup>	P
Ashton	% cover = 0.8(days since flood) + 0.4	0.31	0.006
	KBI = 0.004(days since flood) -0.07	0.34	0.003
	Biomass = 0.2(days since flood) + 3.4	0.30	0.05
Haybarn	% cover = 0.3(days since flood) -5.1	0.78	<0.0001
	KBI = 0.03(days since flood) -0.9	0.64	<0.0001
	Biomass = 0.3(days since flood) -2.3	0.68	0.0009
Manager House	% cover = 0.4(days since flood) + 31.6	0.82	<0.0001
	KBI = 0.1(days since flood) + 0.02	0.91	<0.0001
	Biomass = 0.8(days since flood) + 22.5	0.76	0.0002
Three Kings	% cover = 0.5(days since flood) + 8.0	0.86	<0.0001
	KBI = 0.1(days since flood) -1.9	0.79	<0.0001
	Biomass = 0.7(days since flood) + 2.3	0.67	0.001
Gravel Pits	% cover = 0.4(days since flood) + 12.3	0.66	<0.0001
	KBI = 0.1(days since flood) -0.6	0.72	<0.0001
	Biomass = 0.7(days since flood) + 5.2	0.50	0.01

**Table 23:** Sediment grain sizes from run habitats in the study reaches in the Mararoa, Oreti and Waiau Rivers. Units are mm.

River	Reach	Median	Mean
Mararoa	Normans	45.3	88.7
	Haycock	64.0	96.5
	Station Br	90.5	79.3
	Princhester	64.0	82.1
	Key Br	32.0	42.2
Oreti	Mt. Nic	22.6	32.5
	Ashton	16.0	29.8
	Haybarn	22.6	34.5
	Manager House	32.0	49.3
	Three Kings	16.0	34.5
	Gravel Pits	45.3	78.7
Waiau	Excelsior	64.0	85.2

## 6.2. Effects of groundwater in spring-fed streams on *D. geminata*

Fish and Game field officers have reported that the mixing zones where spring-fed tributaries enter *D. geminata*-affected reaches of the Mararoa and Oreti Rivers generally have no visible *D. geminata*. These observations suggest that a physical or chemical property of groundwater prevents *D. geminata* from establishing or sustaining net growth. To assess the viability of *D. geminata* in spring-fed streams, a transplant study was carried out in May and June 2006.

### 6.2.1. Methods

On 8 May 2006, large cobbles (15-20 cm diameter) with 100% *D. geminata* cover were transplanted from the Mararoa River into two spring-fed tributaries of the Mararoa River, Flaxy Creek near the Whitestone River confluence, and Spring Creek near the Wash Creek confluence. The intent of this pilot study was to assess *D. geminata* viability while immersed in groundwater. Donor sites in the Mararoa River were within 200 m of the two recipient sites. Each cobble was photographed with an identification number, sampled, then placed in the recipient stream in numerical order. Samples consisted of small fragments of pigmented mat; three fragments were composited from each cobble. The fragments were transported on ice in water-filled containers to the laboratory for *D. geminata* cell counts using light microscopy and neutral red stain. The neutral-red staining procedure for distinguishing live and dead *D. geminata* cells is described in detail in Kilroy 2005a). On three subsequent sampling dates (26 May, 7 June, 27 June 2006), new sets of fragments were collected from each cobble for cell counts. The proportion of live cells to total cell counts was plotted against elapsed days after transplanting to quantify changes in viability.

On three sampling dates, water samples were collected from Flaxy Creek and Spring Creek, and from the two donor sites in the Mararoa River, for chemical analysis. These samples were used to screen a broad range of chemical parameters, to gain some understanding of differences in chemistry between run off-fed rivers that support *D. geminata*, and spring-fed streams that do not. The water samples were filtered in the field through ashed glass-fibre filters (Whatman GF/F) into acid-washed polyethelene bottles, and transported on ice to Hill Laboratories Ltd. for determination of 24 chemical parameters, including concentrations of seven metals. In addition, pH, DO and conductivity were measured at each sampling site.

### 6.2.2. Results

After 30 days of immersion in the spring-fed streams, the percentage of living *D. geminata* cells on the surfaces of transplanted rocks declined by ~ 25%; from an initial

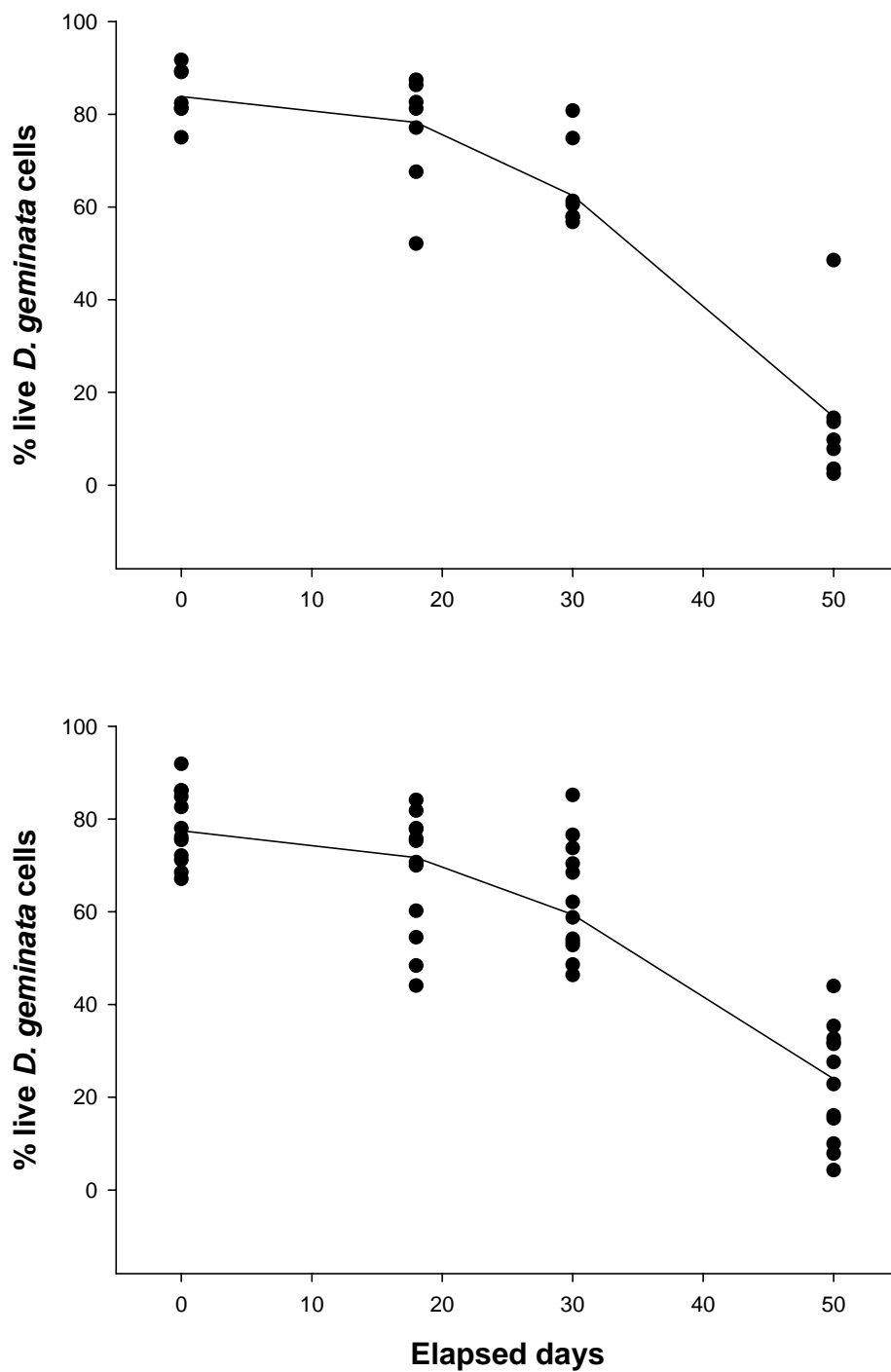
level of ~ 80% to ~ 60% in both streams (Fig. 33). In the next 20 days, percent live cells declined more steeply in both streams, to an average of 15 to 20%. Due to the non-linear change in percent live cells, polynomial regressions fit these data from both streams much better than linear regressions. The reason(s) for non-linear changes in percent live cells is not clear; no substantial changes in chemistry were apparent between sampling dates, and no flood flows were observed during the study. It is assumed that flood flows would have scoured the channels as well as removing *D. geminata* cells from the experimental cobbles, but no scouring was evident (e.g., by the removal of periphyton or macrophytes, or by deposition on banks). However, abundant snails (*Potamopyrgus antipodarum*) were observed on the experimental cobbles on the last sampling date, and the preceding invertebrate grazing trials indicated that *P. antipodarum* can consume *D. geminata*.

Results from the chemistry analyses are shown in Table 24. The largest differences in chemistry between the spring-fed streams and Mararoa River sites were in nitrate concentrations (9-79 times higher in spring-fed streams), and magnesium and sulphate concentrations (2-4 times higher in spring-fed streams). The highest nitrate, magnesium and sulphate concentrations measured in the spring fed streams were 1.5, 8.1, and 2.8 mg L<sup>-1</sup>, respectively. These concentrations are unlikely to inhibit periphyton growth. Differences between other nutrients, major ions and heavy metals were generally small, and heavy metal concentrations were usually below the detection limit (0.1 to 1 ppb). On the basis of this pilot study, although viability of *D. geminata* declined when mats were transplanted from areas of proliferation to adjacent spring-fed streams, there was little evidence to support the prediction that groundwater chemistry excludes *D. geminata* from spring-fed streams.

### 6.3. Discussion

The relationships showing periphyton biomass as a function of daily solar radiation and water temperature are unlikely to be direct causative relationships. Under the ambient conditions in the Southland, New Zealand study area, light and water temperature are very unlikely to exceed tolerance levels for benthic diatoms. Periphyton mats have been tested for light-saturation or photoinhibition in several New Zealand rivers at the same latitude range (45-46° S) as the reaches used in the present study (Young and Huryn 1996, Dodds et al. 1999). In these studies, periphyton photosynthesis increased continuously with light level, and no saturation was indicated. The maximum water temperature measured in the study area in 2006-2007, 16° C, is very unlikely to be near a maximum threshold for benthic diatoms. While cryophilic algae are known from alpine and polar areas, most of these are functionally eurythermal (DeNicola 1996). It is unlikely that *D. geminata* is a





**Figure 33:** Temporal pattern in percentage of live cells in *D. geminata* mats transplanted from the Mararoa River to two spring-fed tributaries. Top panel: Flaxy Creek. Bottom panel: Spring Creek. Lines, equations and coefficients are for polynomial regressions.

**Table 24:** Chemistry in spring-fed streams and in adjacent Mararoa River sites. All analytes in dissolved phase. Concentrations and alkalinity units are mg L<sup>-1</sup>. Conductivity units are  $\mu\text{S cm}^{-1}$ . BDL: below detection limit. Values are means of 3 to 5 samples.

Analyte	Flaxy Creek	Mararoa River (Flaxy Creek control)	Spring Creek	Mararoa River (Spring Creek control)
Electrical conductivity ( $\mu\text{S/cm}$ )	102.2	53.2	114.6	42.1
pH	7.58	7.52	7.22	9.38
Total alkalinity	40.0	24.5	44.5	19.3
Nitrate	1.20	0.13	1.41	0.02
Ammonium	0.001	0.001	0.001	0.001
Organic nitrogen	0.39	0.145	0.162	0.073
Reactive phosphorus	0.004	0.00001	0.003	0.004
Organic phosphorus	0.005	0.003	0.003	0.005
Organic carbon	0.20	0.80	0.30	1.40
Silicate	6.85	2.51	5.79	2.48
Bicarbonate	49.0	30.0	54.3	23.0
Calcium	8.20	5.16	7.72	3.55
Magnesium	5.35	2.60	7.96	2.04
Sodium	4.32	2.29	3.67	1.56
Potassium	0.51	0.33	0.55	0.25
Chloride	3.08	1.70	3.80	1.20
Sulphate	2.40	1.05	2.33	0.55
Arsenic	BDL	BDL	BDL	BDL
Cadmium	BDL	BDL	BDL	BDL
Chromium	BDL	BDL	0.001	BDL
Copper	BDL	BDL	BDL	BDL
Nickel	BDL	0.001	0.003	0.001
Lead	BDL	BDL	BDL	BDL
Zinc	BDL	0.005	0.007	0.002

cryophilic species, as the native range includes northern temperate continental Europe. Positive growth responses for temperate diatoms in culture are often reported up to maxima of 20-30° C (DeNicola 1996).

The negative relationships observed between periphyton abundance and solar radiation and water temperature are likely to be artifacts of flood effects on periphyton. A series of floods occurred through the spring-summer period, which appeared to progressively reduce periphyton abundance from September to November 2006, then maintain periphyton at low levels until January 2007. The onset of these floods coincided with increasing solar radiation and water temperature levels, and the very large floods of December 2006 and January 2007 coincided with annual maxima in solar radiation and water temperature. Based on these observations, we conclude that large floods limit periphyton abundance in the study rivers (discussed in detail below), and while light and temperature at sub-saturating levels may limit algal growth rates, they do not reach growth-inhibiting levels.

*D. geminata* is broadly distributed across hydraulic habitats in the Mararoa, Oreti and Waiau Rivers. High biomass levels ( $> 100 \text{ g AFDW m}^{-2}$ ) were recorded in both very shallow ( $< 10 \text{ cm}$ ) and the deepest water sampled (70 cm), and over a near-bed velocity range of  $0.01$  to  $1.1 \text{ m s}^{-1}$ . Quantitative relationships between biomass (both AFDW and chlorophyll *a*) and depth, near-bed velocity and Froude number were generally weak, indicating that instantaneous hydraulic conditions explain little of the variability that exists in *D. geminata* biomass at reach scales. This is a reasonable conclusion, because periphyton accrual is a gradual process, and occurs over a time scale longer than changes in depth and velocity. In dammed and spring-fed rivers with few or no floods, fine-scaled differences in near-bed hydraulics have been shown to influence periphyton biomass (e.g., Biggs and Hickey 1994). However, bed-mobilising flood flows swamp the finer scaled effects of hydraulic habitats, and periphyton in flood-prone rivers may be in a near-constant state of recovery from preceding floods. This proposition can be tested if *D. geminata* colonises rivers with highly stable flows in the future. The Waikoropupu River downstream of Pupu Springs, Tasman, is a possible example, if *D. geminata* spreads to this tributary from the affected Takaka River. Flow in the Waikoropupu River ranges from  $7\text{--}20 \text{ m}^3 \text{ s}^{-1}$ , but is typically stable, as catchment runoff is attenuated by the lentic springs.

The patterns in *D. geminata* biomass reported here corroborate the results of the previous ecology study, where no upper limits were detected in velocity, Froude number or depth beyond which *D. geminata* did not attain high biomass (Kilroy et al. 2006a). During periods of lower flow, these hydraulic variables will generally decline, and it is therefore unlikely that depth, velocity or shear stress prevents the development of *D. geminata* mats at any time of year in most rivers. Exceptions may

include river beds  $> 1$  m deep, and very steep channels with velocities  $> 1$  m s<sup>-1</sup> or Froude numbers close to 1.0. The physical variable that is likely to limit biomass development in the Mararoa, Oreti and Waiau Rivers are large floods that mobilize bed material, exert high shear stress on algal mats, and/or carry a sufficient quantity of abrasive sediment in suspension to remove periphyton. Numerous studies have demonstrated that bed-mobilisation and abrasion by suspended sediment and bedload are primary controls on periphyton biomass in gravel rivers (Biggs & Kilroy 2000). While water velocity, depth and shear at base flow may set the upper and lower limits on periphyton survival, the annual and shorter-term variability in periphyton abundance often reflects the flood regime of a river (i.e., flood frequency, magnitude and duration). The patterns in *D. geminata* biomass reported here also lend support to the recent GIS model developed recently to predict the suitability of New Zealand rivers to support *D. geminata* (Kilroy et al. 2007). In that model, the environmental variables with the greatest predictive power were related to flood frequency, bed mobility, and hydrological stability (also related to flood frequency).

Taken together, the preceding conclusions about nutrient limitation (Section 5.3), potential light and temperature limitation, and flood effects suggest the following conceptual model: Physiological growth limitation is a likely condition in mid-latitude rivers with cool water temperatures and relatively low nutrient concentrations (Francoeur et al. 1999). In the absence of physical disturbances such as bed-mobilisation, light or temperature limitation would result in a seasonal cycle of periphyton abundance. In the absence of disturbances, severe nutrient limitation would result in increased periphyton abundance following floods due to pulsed nutrient input from terrestrial sources. However, physical disturbances occur frequently in flood-prone gravel-bed rivers, and the effects of floods on periphyton abundance masks effects of resource limitation. Resource-limited growth may only be apparent as the rate of recovery in the intervals between disturbances. Given several years of data, we predict that the time course of *D. geminata* abundance in affected rivers would be composed of rapid declines following bed-mobilising flows, slow recovery during winter periods, and more rapid recovery during summer periods.

The observation that flood flows are primary controls on periphyton biomass suggests that *D. geminata* proliferation could be partially controlled in dammed rivers by releasing flood-magnitude flows (“artificial floods”). The effects of artificial floods on periphyton abundance have been assessed on several rivers, and results generally indicate a detectable, but temporary reduction in periphyton (Patten et al. 2001, Uehlinger et al. 2003). In New Zealand, artificial floods are being monitored in the Moawhango River, North Island and Opuha River, South Island (D. Arscott, S. Larned, unpublished data). Results of three years of monitoring have lead to the following observations. First, the magnitude of the flood relative to preceding base-

flow discharge partly determines effectiveness; floods  $\leq 2\times$  baseflow over the preceding weeks have had minor or undetectable effects on periphyton abundance. Second, effectiveness of periphyton removal may be rapidly attenuated downstream of dams. On the Opuha, changes in periphyton abundance have been undetectable 15 km downstream of the dam. Third, lack of bedload movement and scour by fine sediments can reduce effectiveness. Many dams act as sediment traps, and the river channels downstream receive highly erosive water with low sediment concentrations. Consequently, downstream channels have little mobile sediment in storage, and beds become armoured. Under these conditions, artificial floods of moderate magnitude may be incapable of mobilising bed material, and carry little suspended load; the potential effects of these floods on periphyton is limited to drag forces associated with bed shear. Effects of moderate sized floods (e.g.,  $5\times$  baseflow) in the sediment-starved Opuha River have been limited to partial removal of long filamentous green algae, but not algal mats, crusts or biofilms (D. Arscott, S. Larned, unpublished data). It is likely that fine sediment augmentation and/or mechanical disruption of armour layers will be needed if artificial floods are used to remove *D. geminata*. Fourth, a frequent observation following natural floods in *D. geminata*-affected river reaches is that mats are transported downstream but remain viable and within the river channel. Upon flood recession, this material is likely to colonise new substrate. Therefore, a cautious approach to using artificial floods for *D. geminata* control is warranted; floods are also vectors for *D. geminata* dispersal.

The near-absence of *D. geminata* in mixing zones where spring-fed tributaries enter *D. geminata*-affected rivers, and from the tributaries themselves, remains unexplained. Concentrations of nutrients and some metals appear too low in the two spring-fed streams used in the current study to kill *D. geminata* cells. A second transplant study concerning groundwater effects on *D. geminata* has recently been completed (Sutherland et al. 2007). In that study, paired spring-fed stream and river sites were used on the Waitaki, Mararoa and Oreti Rivers. As in the current study, *D. geminata* viability declined in all spring-fed streams in the second study. In both studies, alkalinity, conductivity, and nitrate, magnesium, calcium, and chloride concentrations were higher and dissolved organic carbon concentrations were lower in the spring-fed streams than in the adjacent rivers. Four general mechanisms have been proposed to explain the low viability of the *D. geminata* in spring-fed streams: chemical stress, grazing pressure, unsuitable hydraulic conditions, and competition with other algae (Sutherland et al. 2007). The chemistry data from the spring-fed streams do not indicate a solute or toxicant present at lethal concentrations. No significant differences in grazer densities were detected by Sutherland et al. (2007) in comparisons of spring-fed streams and adjacent rivers. Near-bed water velocities in the spring-fed streams were generally lower than in the adjacent river reaches, but were within the range in which *D. geminata* achieves high biomass levels ( $0.02 - 0.65 \text{ m s}^{-1}$ ). There was some

indication in the Sutherland et al. (2007) study that native algae overgrew *D. geminata* in spring-fed streams, but there were no quantitative relationships between *D. geminata* viability and non-*D. geminata* algal biomass. Three additional mechanisms should be assessed in future research. First, a solute or chemical property that was not measured may be responsible for poor survival in spring-fed streams. No non-metal contaminants have been measured, and pesticide or herbicide residues, or other contaminants may be present at elevated levels in local groundwater. This explanation seems unlikely, in view of the number of spring-fed streams in which the absence of *D. geminata* has been noted. Second, invertebrate herbivore communities may differ in spring-fed streams and adjacent rivers, but more intensive sampling is required to detect differences. Third, cumulative effects of differences in chemistry, grazing and competition may explain poor *D. geminata* survival in spring-fed streams. These factors may have minor individual effects, but their combined effects may be substantial.

## 7. Conclusions

### 7.1. Effects of *D. geminata* on river ecosystems

In this study, surveys of native fish were limited to a single date on each of 14 river reaches; only two of which were substantially colonised by *D. geminata*. There was no detectable effect of *D. geminata* at the two affected sites. This limited dataset precludes the development of relationships linking native fish abundance to *D. geminata* abundance, despite that the highest density of galaxiids among the sites was measured at one of the two sites with moderate *D. geminata* cover (124 fish m<sup>-2</sup>, 14% *D. geminata* cover). While some work has been completed that addresses possible mechanisms by which *D. geminata* may affect fish populations (e.g., by altering invertebrate drift, or producing drifting algae that reduces foraging efficiency; Hayes et al., 2006, Shearer et al., 2007), more datasets collected over time and space to capture seasonal and site differences are required to develop quantitative relationships which are still critically needed for assessing risks to fish populations. No statistically significant relationships have been detected from recent salmonid surveys in *D. geminata*-affected rivers in North America and Europe (MDDEP 2007), and to our knowledge, no relationships have been identified that link *D. geminata* to benthic riverine fish taxa such as galaxiids, suckers, catfish, sturgeons and anguillid eels. The native anadromous fish fauna of New Zealand are almost entirely benthic, and *D. geminata*–fish interactions are likely to be very different for benthic and mid-water fishes such as trout.

The diets of native fish in alluvial rivers in New Zealand are dominated by benthic invertebrates (Sagar and Elton 1983, McIntosh 2000). Three properties of benthic invertebrate assemblages potentially affect native fish: composition (e.g., proportions of EPT taxa), biomass, and size structure. Most of the native fishes in New Zealand alluvial rivers are diet generalists that take prey in proportion to availability. Generalist predators are not as reliant on individual invertebrate taxa or groups of taxa (e.g., EPT) as drift-feeding salmonids (Allibone & Townsend 1998, McIntosh 2000). To our knowledge, no relationships have been identified that link proportions of “clean water” taxa or other indicator invertebrates to native New Zealand fish abundance, growth, reproductive success or other dependent variables. Broad diets reduce the likelihood that elimination of single sensitive invertebrate taxa, or groups of taxa, would strongly affect native fish abundance. The positive relationships between *D. geminata* biomass and benthic invertebrate density and biomass reported here and in a previous ecology study (Kilroy et al. 2006a) suggest that *D. geminata*-colonisation can have benefits for fish that forage in benthic zones. However, there is no empirical evidence to support this proposition, and the energy required to prey on invertebrates within dense *D. geminata* mats may equal or exceed the energetic benefits of higher prey abundance. The last general property of invertebrate assemblages that may affect predatory native fish abundance is size structure. Prey-size selectivity has been demonstrated for at least one native galaxiid from New Zealand alluvial rivers (Glova & Sagar 1989), and this may be a general pattern. Presumably, large prey items are energetically preferable to small prey items. However, the size-analysis of the most abundant invertebrates in the Oreti and Mararoa study reaches did not indicate any effect of *D. geminata* on size. This finding further reduces the likelihood that *D. geminata* negatively affects native fish by altering invertebrate assemblage.

As noted above, the energetic costs of benthic foraging within *D. geminata* mats and drift feeding in the presence of drifting *D. geminata* may represent a negative effect of *D. geminata* on fish. Alternatively, primary effects of *D. geminata* colonisation may be related to habitat or river chemistry, rather than feeding. It is likely that proliferations of *D. geminata* alter the availability of spawning and resting habitat, and the demonstrated changes in dissolved oxygen and pH associated with *D. geminata* (discussed below) may affect physiological function in eggs and post-hatch stages.

Results of the whole-reach metabolism study indicated that high levels of *D. geminata* biomass can strongly alter the chemistry of river water. Maximum pH levels recorded in the Mararoa River in late autumn were 9.6. Sustained pH levels > 9 pose a high risk of deleterious physiological and behavioural effects on fish (Alabaster and Lloyd 1982, West et al. 1997). Night-time dissolved oxygen declined to lower levels in the Mararoa than in the Oreti River, and calculated respiration rates in the Mararoa were



an order of magnitude higher than in the Oreti; these differences are due in large part to higher *D. geminata* biomass in the Mararoa. However, dissolved oxygen was  $> 9.5 \text{ mg l}^{-1}$  in both rivers throughout the study, and hypoxia is unlikely to have developed. Elevated pH appears to pose a greater risk to river biota than reduced dissolved oxygen concentrations. The whole-reach metabolism study was carried out in April 2006, when water temperatures ranged from  $7\text{--}10^\circ \text{C}$ , and *D. geminata* biomass levels were approximately half of the maximum measured during the study. This observation suggests that the effects of *D. geminata* on river chemistry could be substantially greater during periods of maximum water temperature and/or maximum *D. geminata* biomass. A second run of the whole-reach metabolism study is planned for summer 2008, and the prediction of greater impact on river chemistry will be tested at that time.

Positive relationships between *D. geminata* abundance and both invertebrate density and taxon richness, and negative relationships between *D. geminata* abundance and % EPT taxa have been previously reported from New Zealand (Kilroy et al. 2006a). While we cannot be certain that these relationships hold for all *D. geminata*-affected rivers, they are sufficiently well-established that some consideration of mechanistic explanations is warranted. Increased invertebrate abundance with increasing algal biomass or algal productivity has been demonstrated for herbivorous invertebrates in New Zealand rivers (Biggs & Lowe 1994). Presumably, the mechanism underlying these relationships is the provision of algal food. Herbivorous taxa (e.g., *Pycnocentroides* sp., some Orthocladinae) dominated many invertebrate samples from *D. geminata*-affected sites, and laboratory feeding trials indicated that some common invertebrate herbivores consume *D. geminata*. Therefore, food resources may partially explain the invertebrate density-periphyton biomass relationship. Alternatively, invertebrate densities may increase with increasing periphyton biomass because the effectiveness of periphyton as a refuge from predators and/or turbulent flow increases with mat thickness (Power 1990), or simply because periphyton biomass and the invertebrate populations that occupy periphyton mats both increase with time following mat establishment. Invertebrate taxonomic diversity has been shown to increase with both *D. geminata* biomass and non-*D. geminata* periphyton biomass (Suren et al. 2003, Death & Zimmerman 2005, this study). At low periphyton biomass levels, these positive relationships may reflect food limitation or the availability of flow refuge provided by periphyton. However, the high *D. geminata* biomass levels observed in this study make it unlikely that there are direct causative periphyton biomass-diversity relationships over the entire biomass range. Instead, increased invertebrate diversity over time may reflect successional changes, while increased periphyton over time reflects accrual (Death & Zimmerman 2005).

## 7.2 Environmental controls on *D. geminata*

Quantitative relationships between *D. geminata* biomass and depth, near-bed velocity and Froude number were generally weak, indicating that instantaneous hydraulic conditions explain a small proportion of variability in biomass at reach scales. This is a reasonable finding, because periphyton accrual is a gradual process, and occurs over time scales longer than those associated with changes in depth and velocity. In dammed rivers and spring-fed rivers with few or no floods, fine-scaled differences in near-bed hydraulics have been shown to influence periphyton biomass (e.g., Biggs and Hickey 1994). However, bed-mobilising flood flows obscure the finer scaled effects of time-averaged hydraulic habitats, and periphyton in flood-prone rivers may be in a near-constant state of recovery from preceding floods. This proposition can be tested in *D. geminata*-affected rivers with relatively stable flows like the Lower Waitaki and Clutha. *D. geminata* biomass in these rivers is predicted to have a spatial distribution that corresponds to optimal and suboptimal hydraulic habitat conditions.

In general, hydraulic habitat variables such as depth, near-bed velocity and Froude number may be more valuable as indicators of habitat tolerance limits than as indicators of biomass accrual. Presumably, there are habitats in which *D. geminata* cannot persist because the hydraulic conditions exceed tolerance limits. For example, in extremely high-velocity conditions, shear stress may exceed the capacity for *D. geminata* cells or mats to adhere to bed forms (Biggs and Thomsen 1995). In extremely low-velocity conditions, the resistance imposed on nutrient transport by near-bed layers of viscous flow may reduce nutrient supplies to levels that do not meet physiological requirements (Larned et al. 2004). If the extreme habitat conditions above and below which *D. geminata* cannot persist are identified, these conditions can be used to refine risk assessments and risk maps. The data compiled in this study and in the previous ecology study (Kilroy et al. 2006a) do not contribute to the identification of tolerance limits, because *D. geminata* was present across the entire gradient sampled in both studies. The recent prediction maps for *D. geminata* in New Zealand (Kilroy et al. 2007) have contributed to the identification of tolerance limits, but additional research is needed before the susceptibility of uncolonised rivers can be predicted with high accuracy and precision. The 2007 prediction maps are preliminary, because *D. geminata* is still expanding its range in New Zealand. As more rivers are colonized, and the presence or absence of *D. geminata* is determined with greater sensitivity (e.g., Cary et al. 2007), relationships between environmental variables and *D. geminata* presence and abundance will improve. In addition, we suggest that a targeted, subjective sampling programme will be more effective for identifying physical habitat tolerance limits than the stratified random (i.e., random sampling within fixed reaches) approach used in the previous studies. Targeted sampling refers to sampling focused in extreme hydraulic habitats identified by field

teams. These habitats should include bedrock chutes, cascades, and stagnant eddies and backwaters.

The physical variable that is most likely to limit biomass development in the Mararoa, Oreti and Waiau Rivers is the frequency of large floods, which mobilize bed material, exert high shear stress on algal mats, and/or carry a sufficient quantity of abrasive sediment in suspension to remove periphyton. This prediction is supported by relationships linking *D. geminata* biomass and time since major floods (Kilroy et al. 2006a, 2007, this study). Similar relationships have been developed for non-*D. geminata* periphyton in temperate and desert gravel bed rivers in New Zealand (Grimm and Fisher 1989, Uehlinger et al. 1996, Biggs et al. 1999).

Flood flows appear to be a primary control on periphyton development in all flood-prone rivers with mobile beds. As flood flows are one of the few mechanisms that have been unequivocally shown to reduce *D. geminata* biomass, they represent a tool for managing *D. geminata* in affected rivers. The only sites at which flood flows could be used deliberately are dammed rivers, on which the dams are rated for releases of sufficient magnitude to remove large proportions of *D. geminata* from channel substrate. Unpublished observations of dam releases suggests that some dams can provide effective releases (e.g., Lower Waiau River), but others cannot release floods of sufficient magnitude, frequency, and/or duration to remove more than a fraction of the periphyton biomass present prior to the release (e.g., Opuha River). Before dam releases can be used for managing *D. geminata*, a substantial amount of information is needed. The attenuation of flows downstream from dam faces must be considered; if attenuation is rapid, *D. geminata* removed from the bed will simply be transported downstream rather than to the floodplain or sea. The shear force required to remove algae from stable substrate, and to mobilize channel beds needs to be considered. Many dammed rivers in New Zealand have armoured beds due to sediment trapping behind the dam. In these rivers, large sediment particles can be embedded and require very high flows for mobilization. The same rivers have little fine surficial sediment, and this material may be required to abrade *D. geminata* from immobile rocks. The most cost-effective approach for using flood flows to reduce *D. geminata* biomass may be to combine dam releases of moderate magnitude with additions of fine sediment at points downstream of the dam. This approach is currently being trialed at the Opuha Dam.

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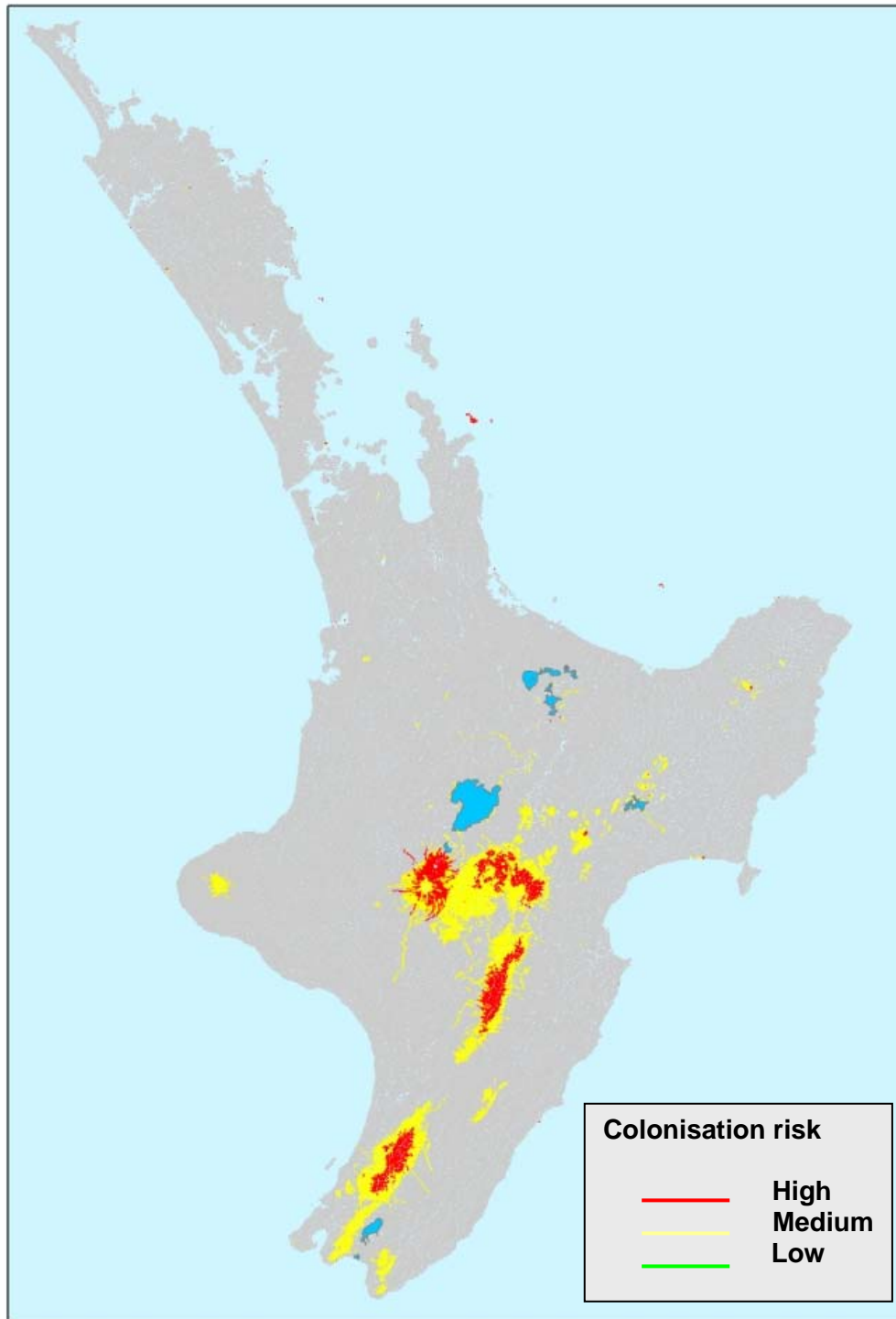
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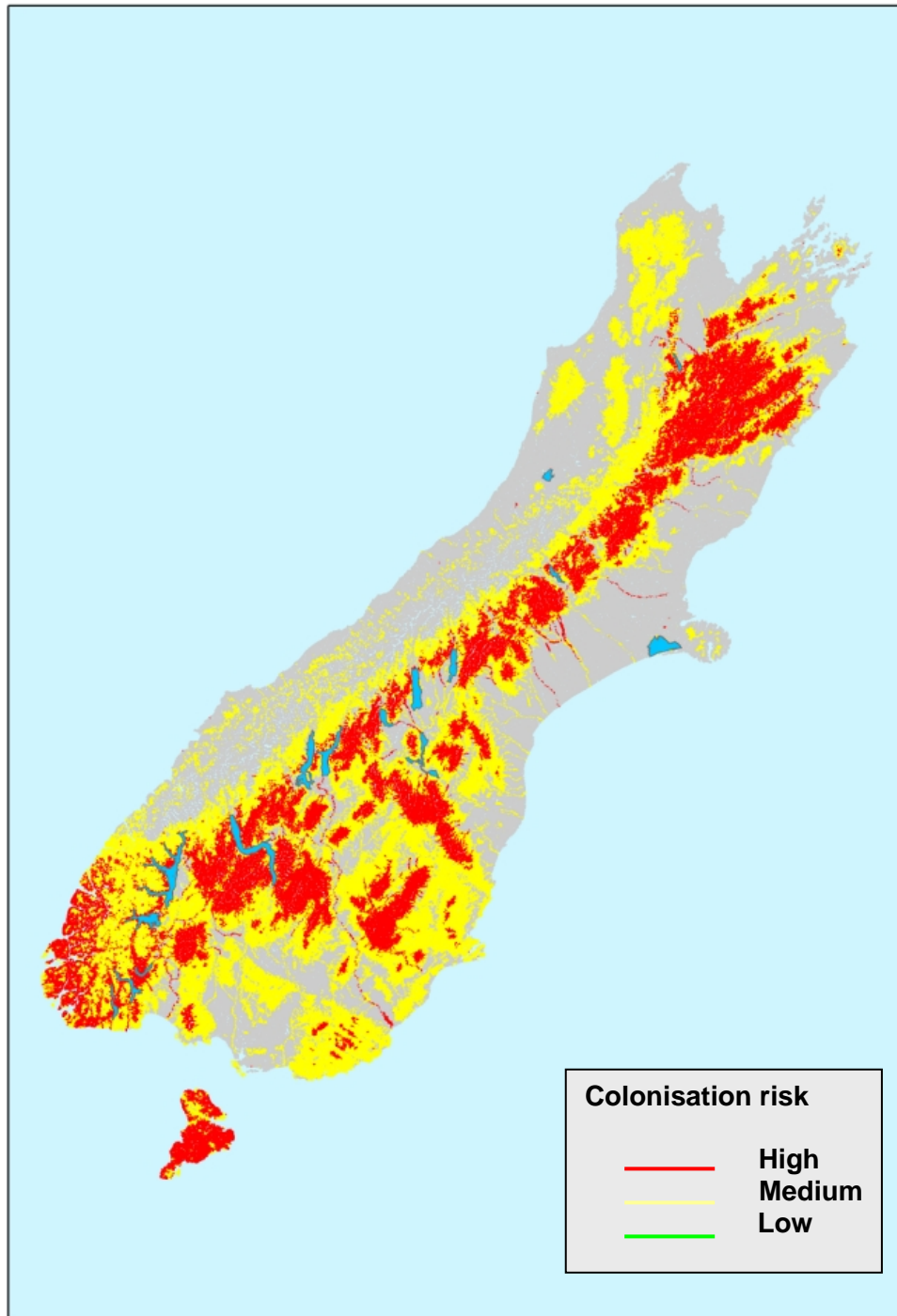
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## Appendix

Maps of the potential area of North and South Islands that could become colonised by *D. geminata*, and the potential area of the habitats of a range of native fish species that could become colonised.

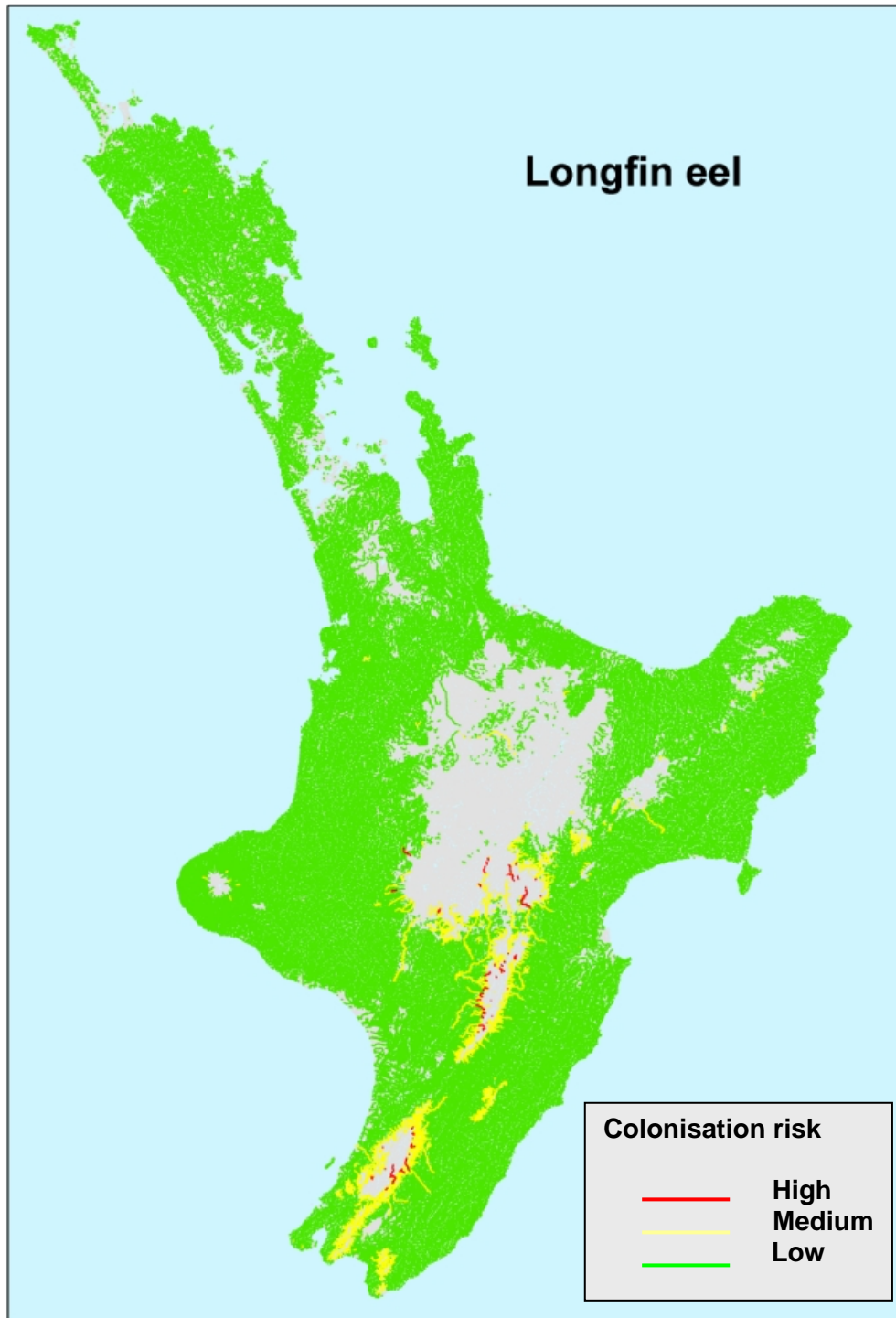


**Figure A1:** Likelihood of colonisation of freshwater habitats in the North Island by *D. geminata*.

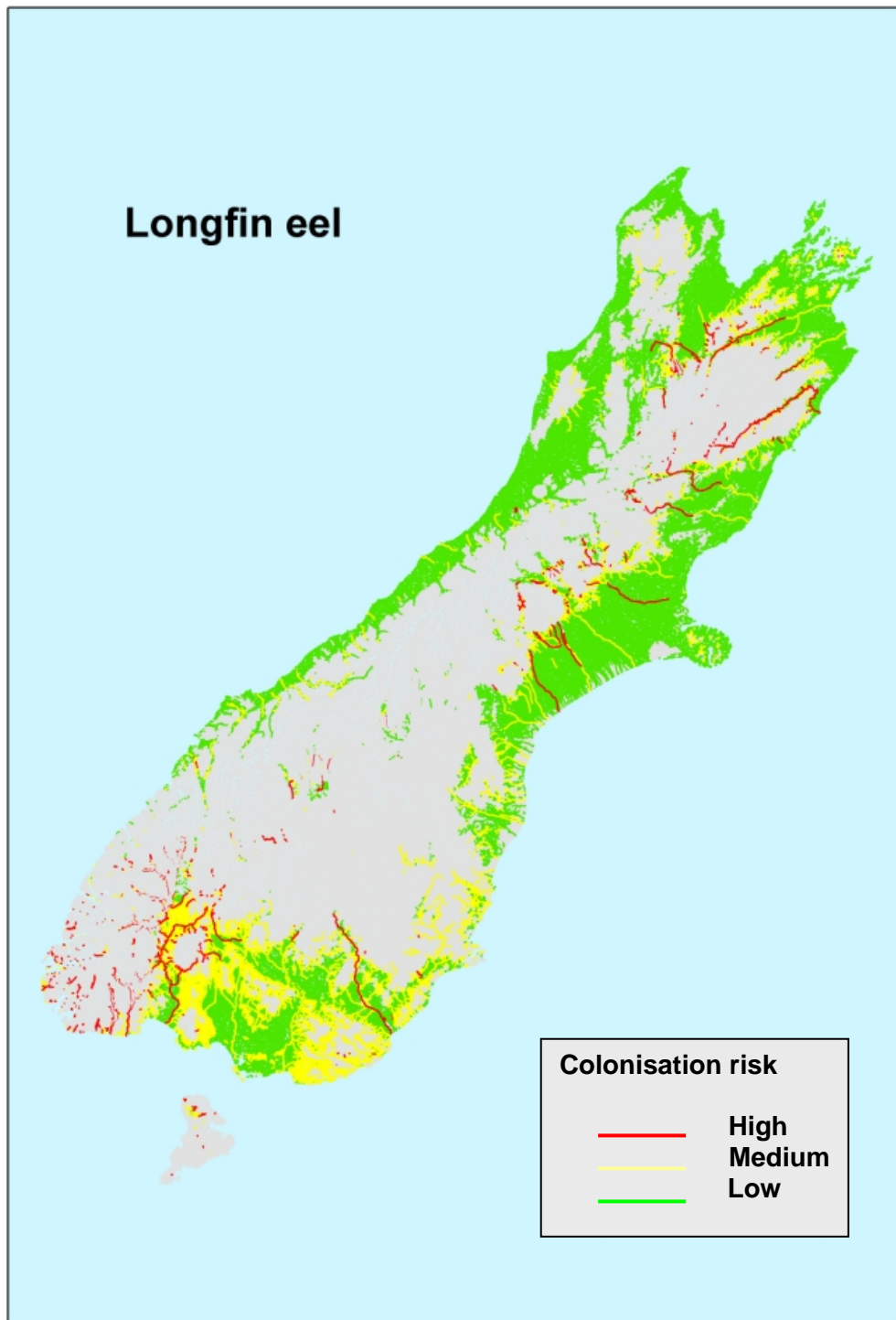


**Figure A2:** Likelihood of colonisation of freshwater habitats in the South Island by *D. geminata*.

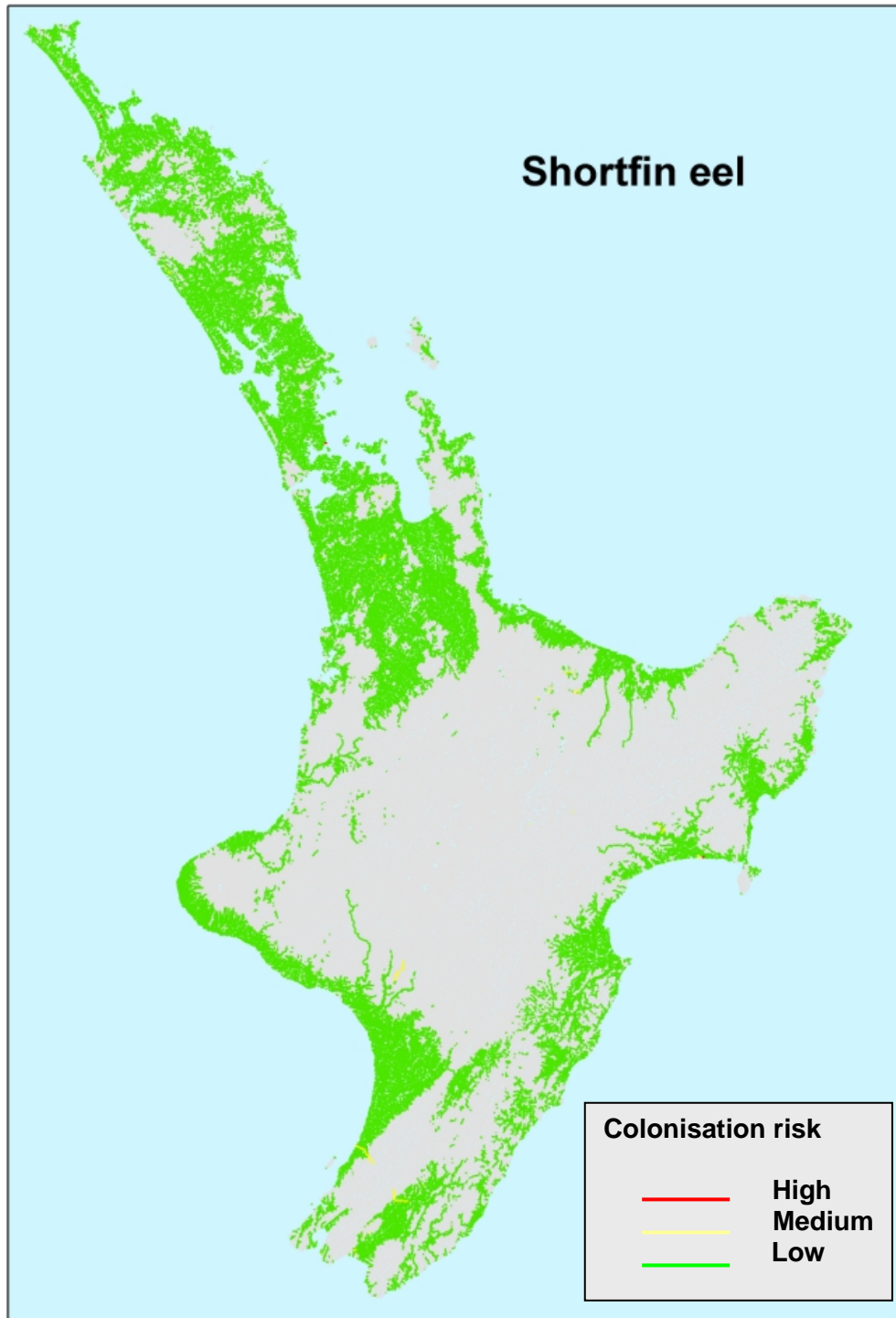




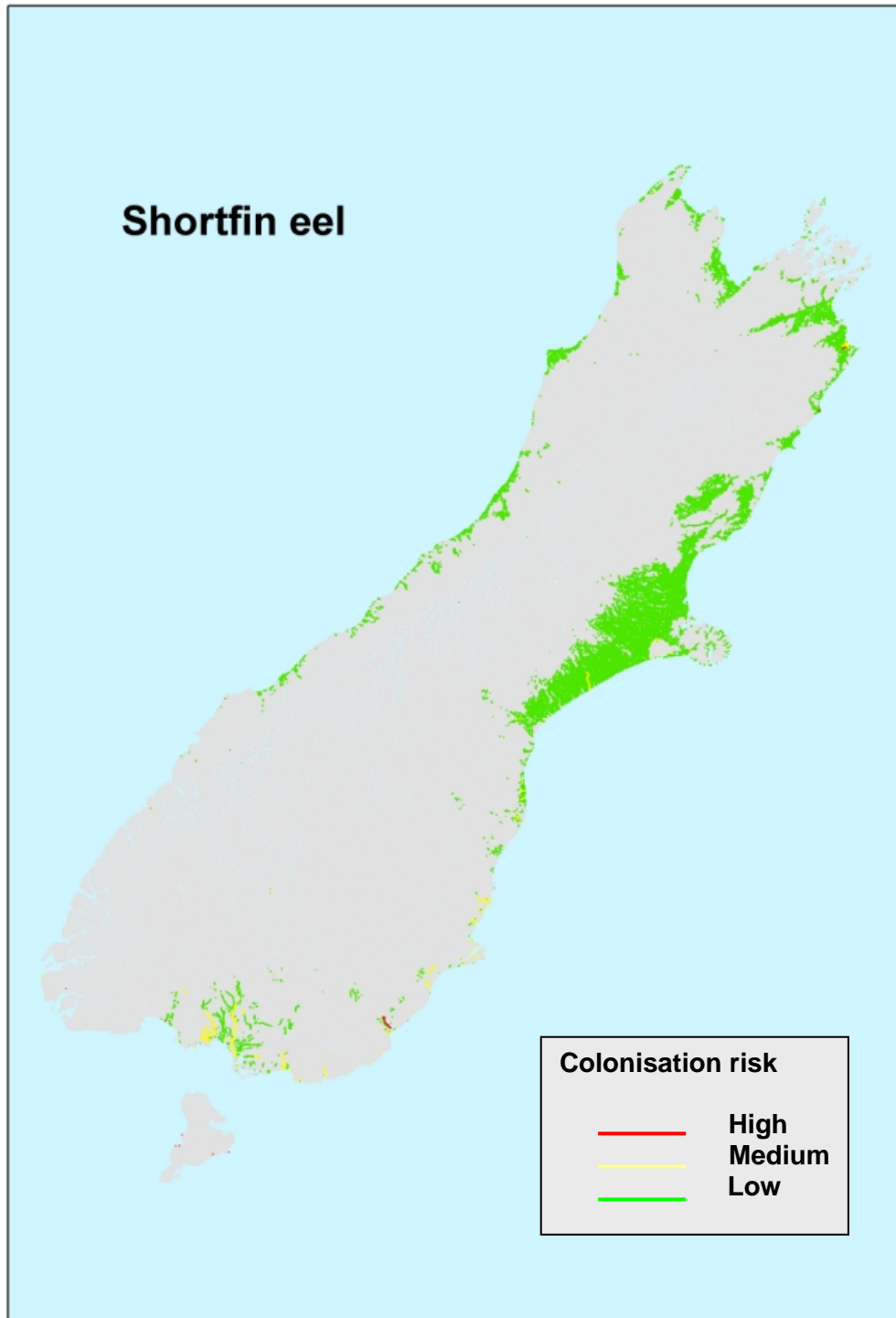
**Figure A3:** Likelihood of colonisation of longfin eel habitat in the North Island by *D. geminata*.



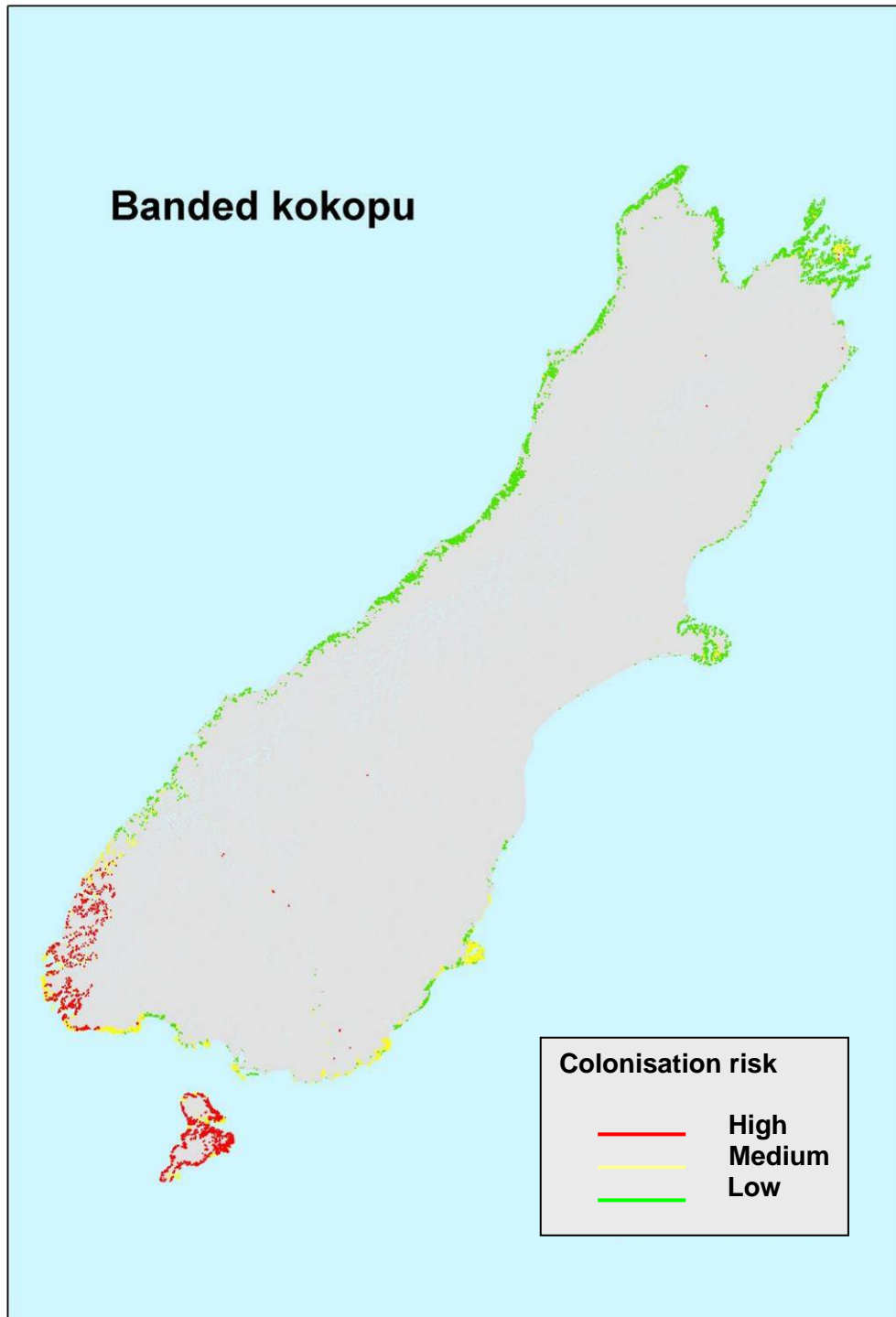
**Figure A4:** Likelihood of colonisation of longfin eel habitat in the South Island by *D. geminata*.



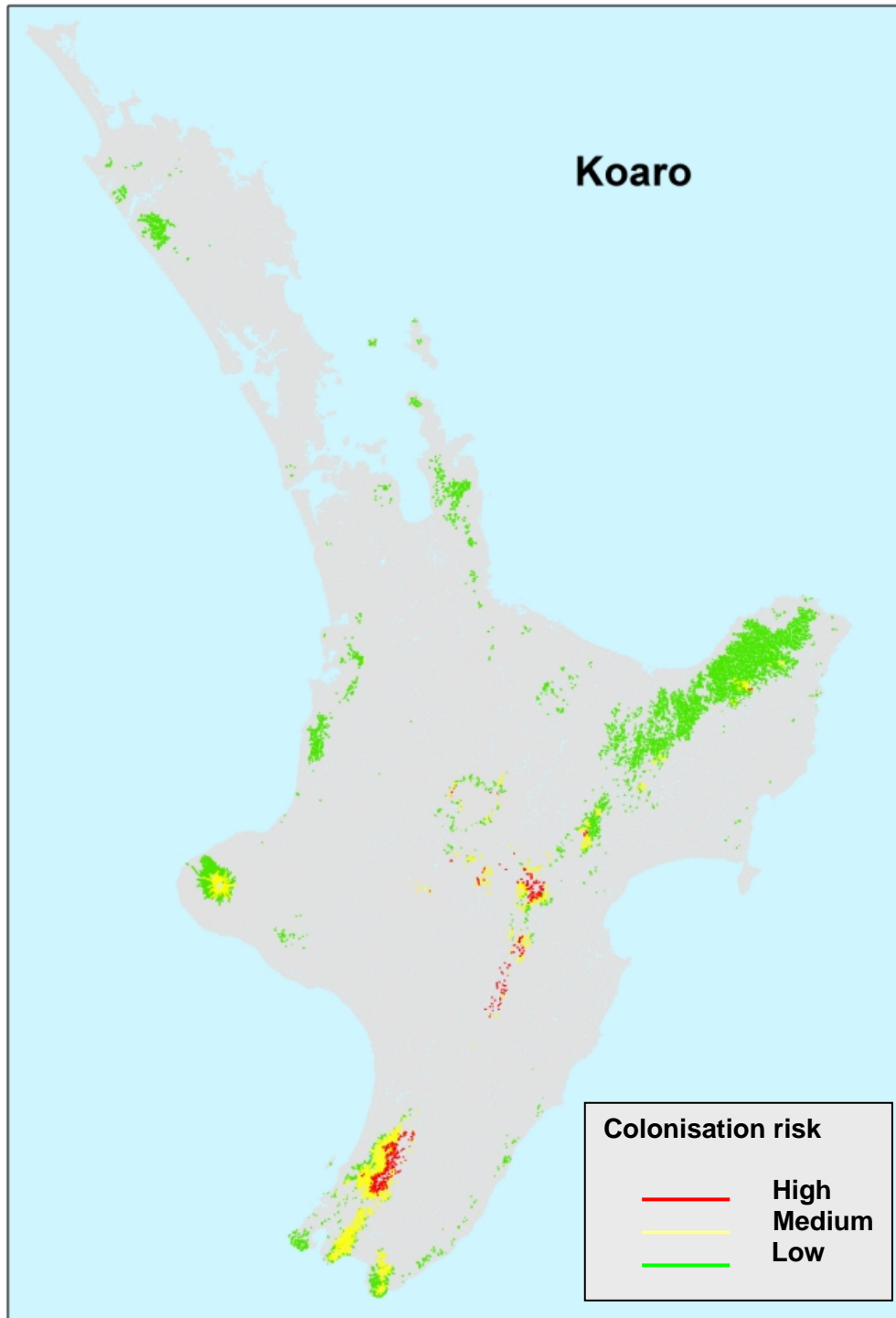
**FigureA5:** Likelihood of colonisation of shortfin eel habitat in the North Island by *D. geminata*.



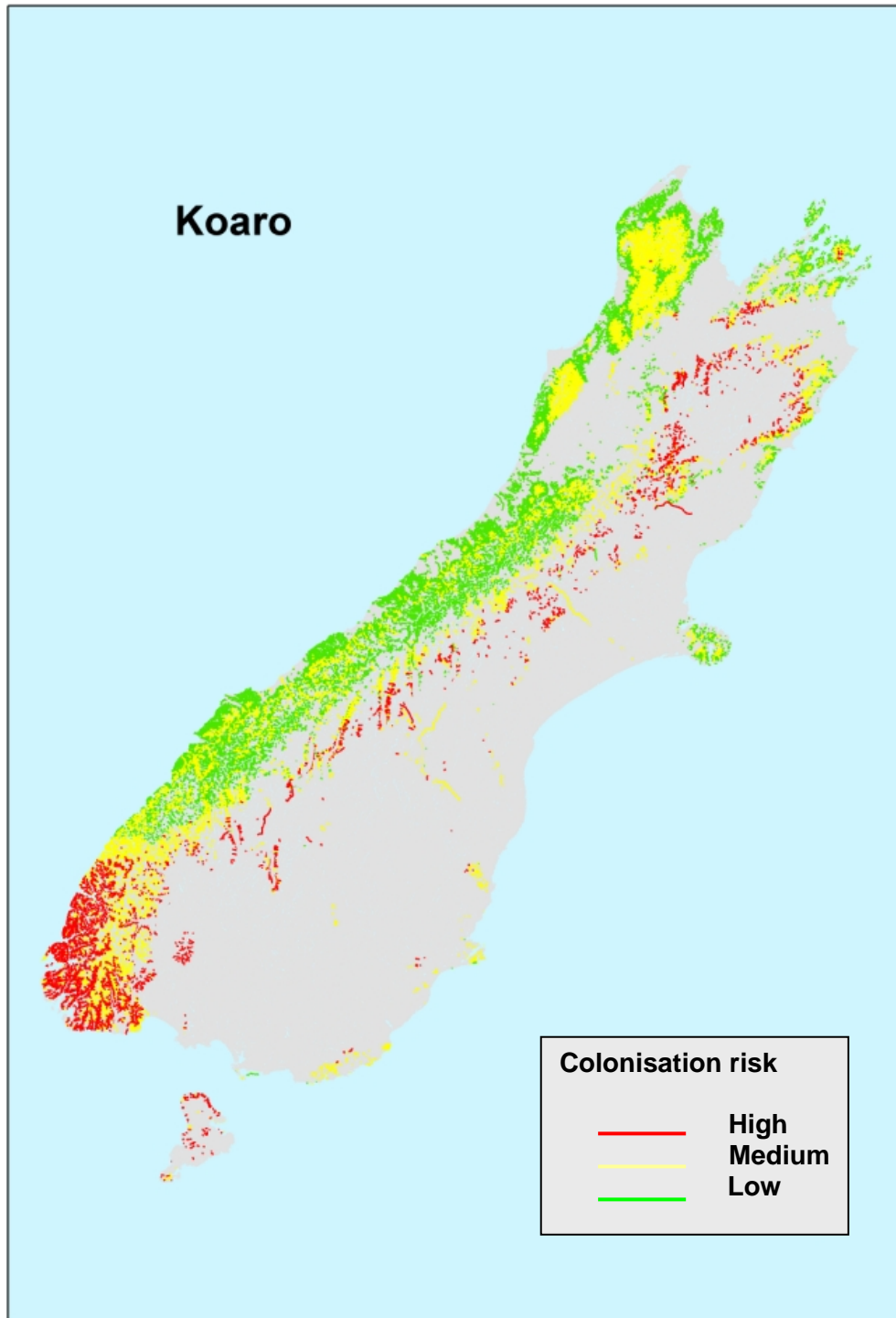
**Figure A6:** Likelihood of colonisation of shortfin eel habitat in the South Island by *D. geminata*.



**Figure A7:** Likelihood of colonisation of banded kokopu habitat in the South Island by *D. geminata*.

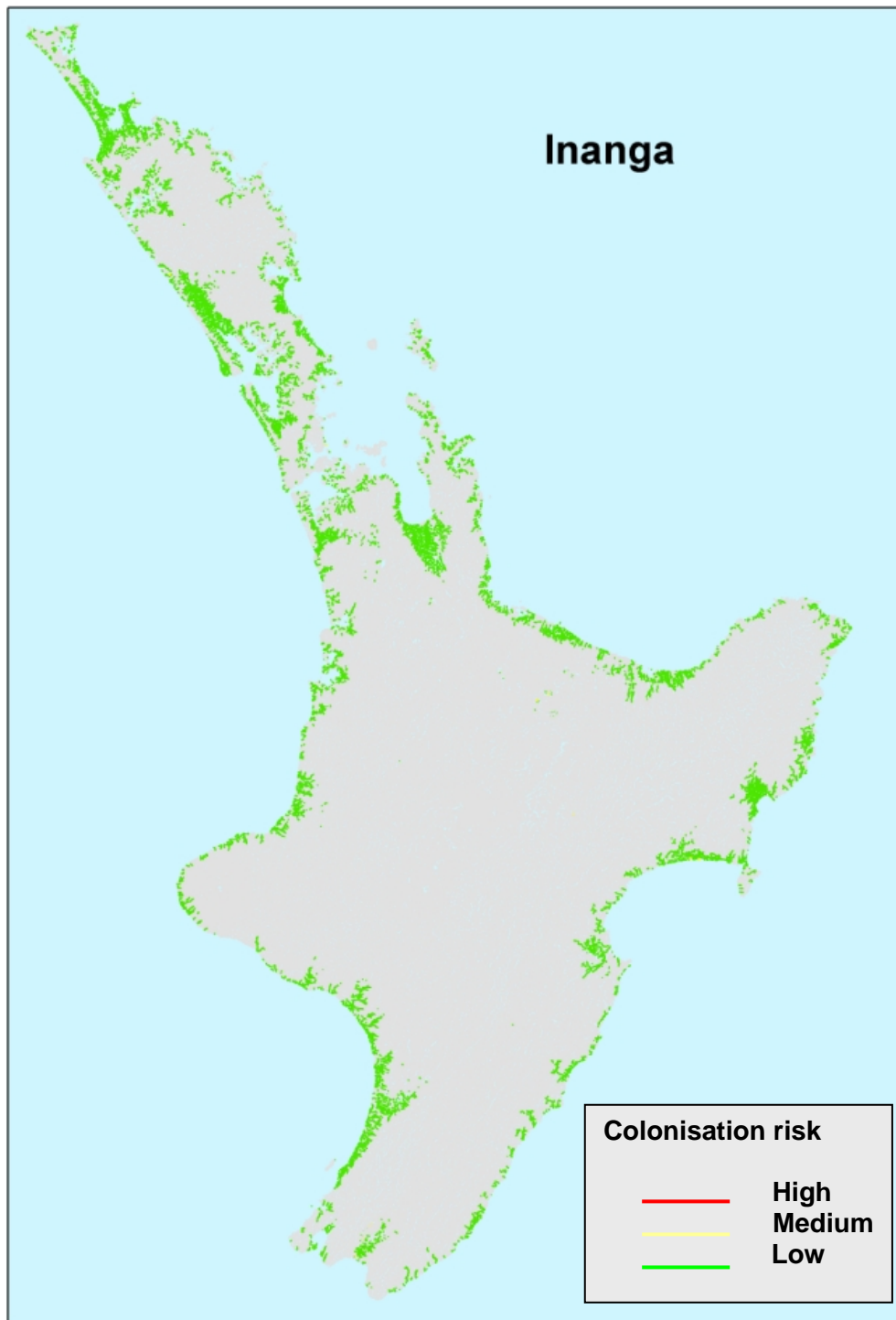


**Figure A8:** Likelihood of colonisation of koaro habitat in the North Island by *D. geminata*.

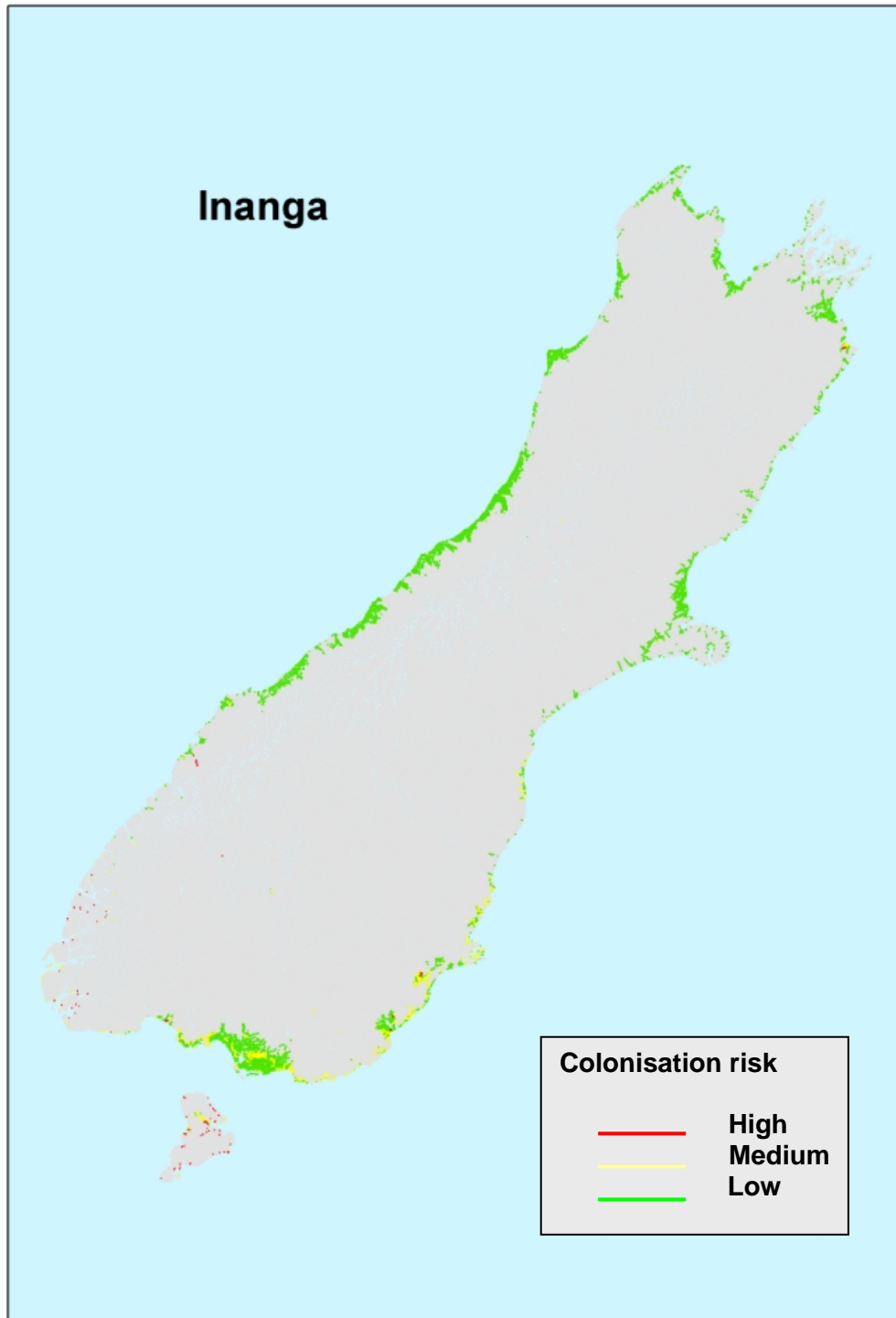


**Figure A9:** Likelihood of colonisation of koaro habitat in the South Island by *D. geminata*.

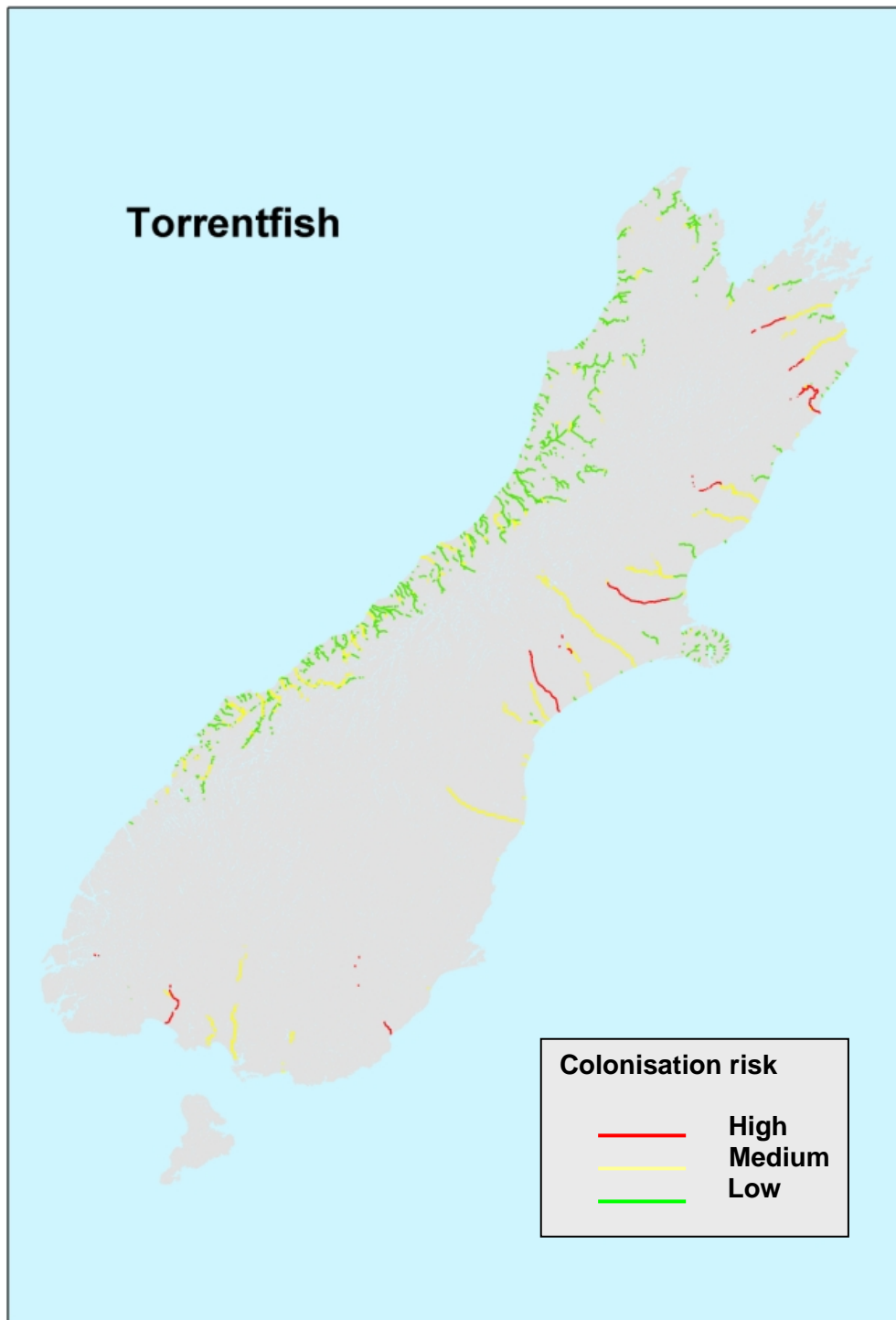




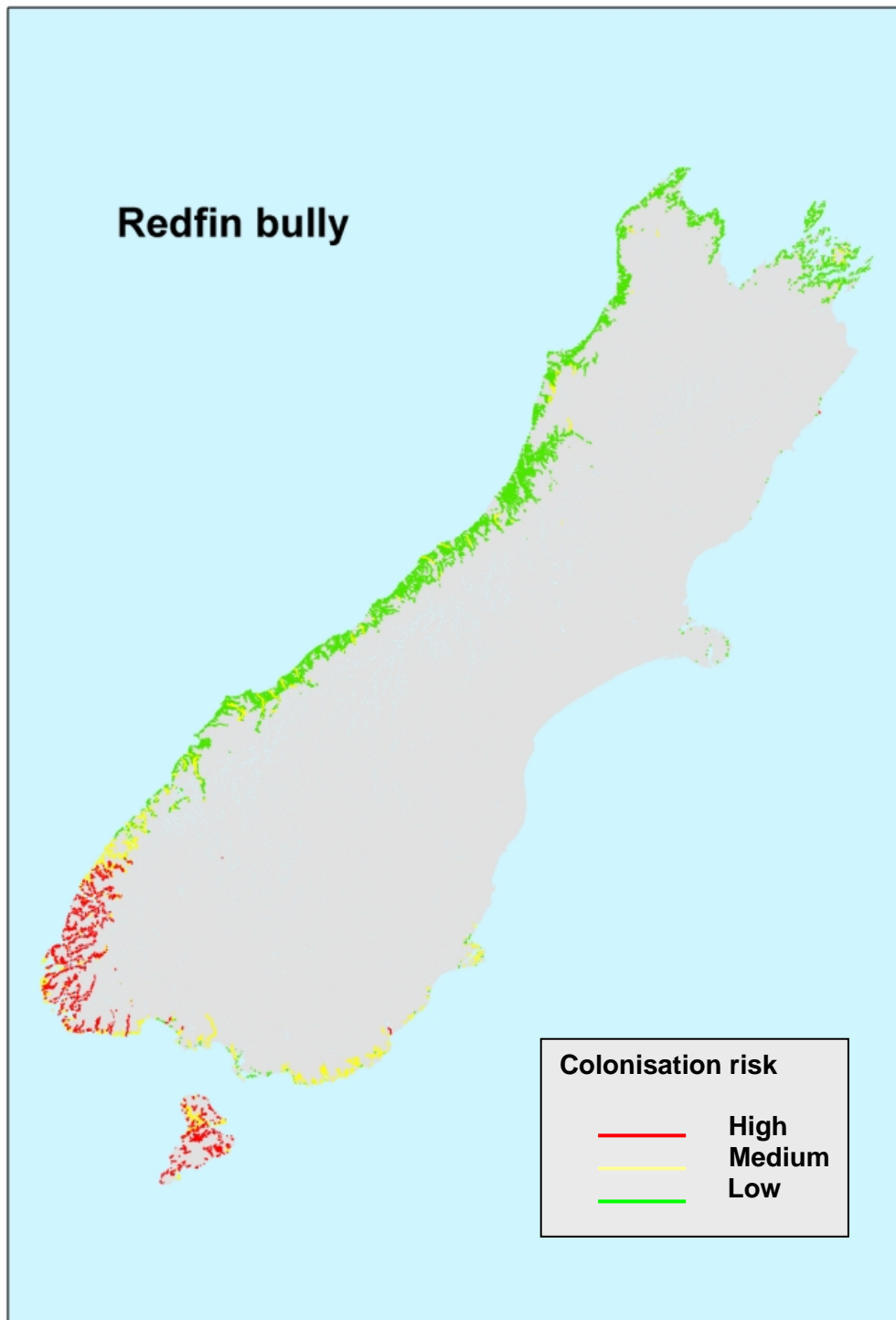
**Figure A10:** Likelihood of colonisation of inanga habitat in the North Island by *D. geminata*.



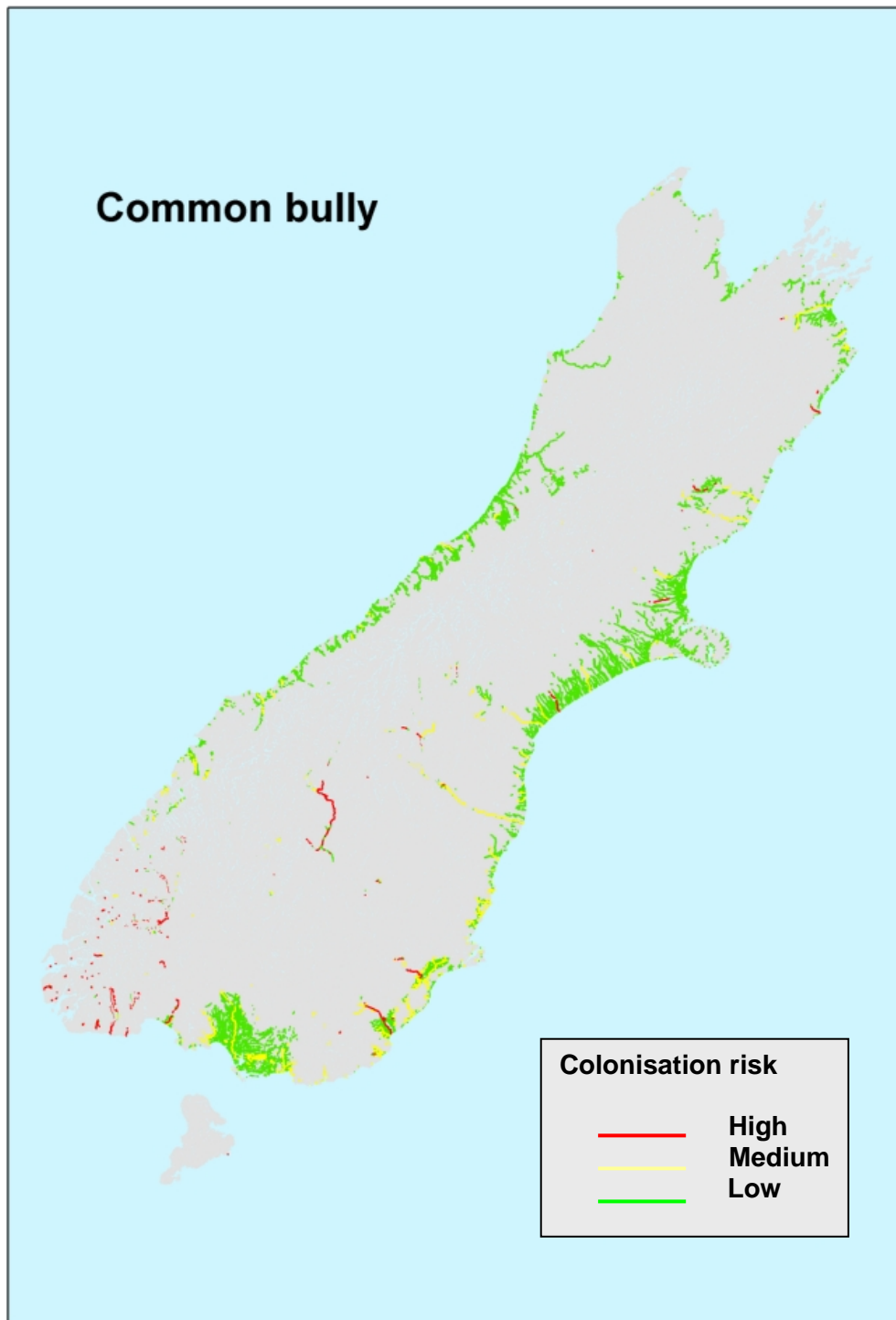
**Figure A11:** Likelihood of colonisation of inanga habitat in the South Island by *D. geminata*.



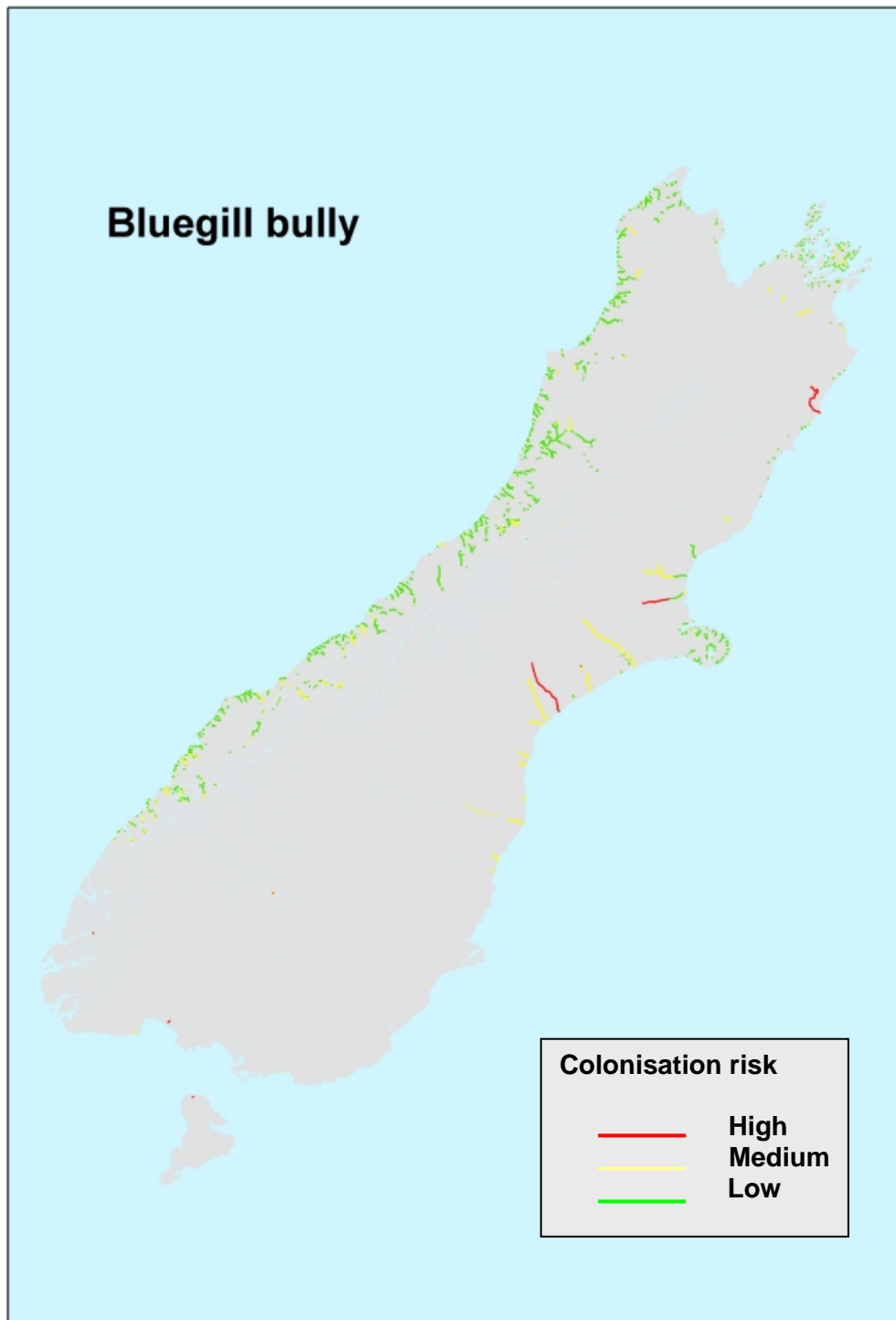
**Figure A12:** Likelihood of colonisation of torrentfish habitat in the South Island by *D. geminata*.



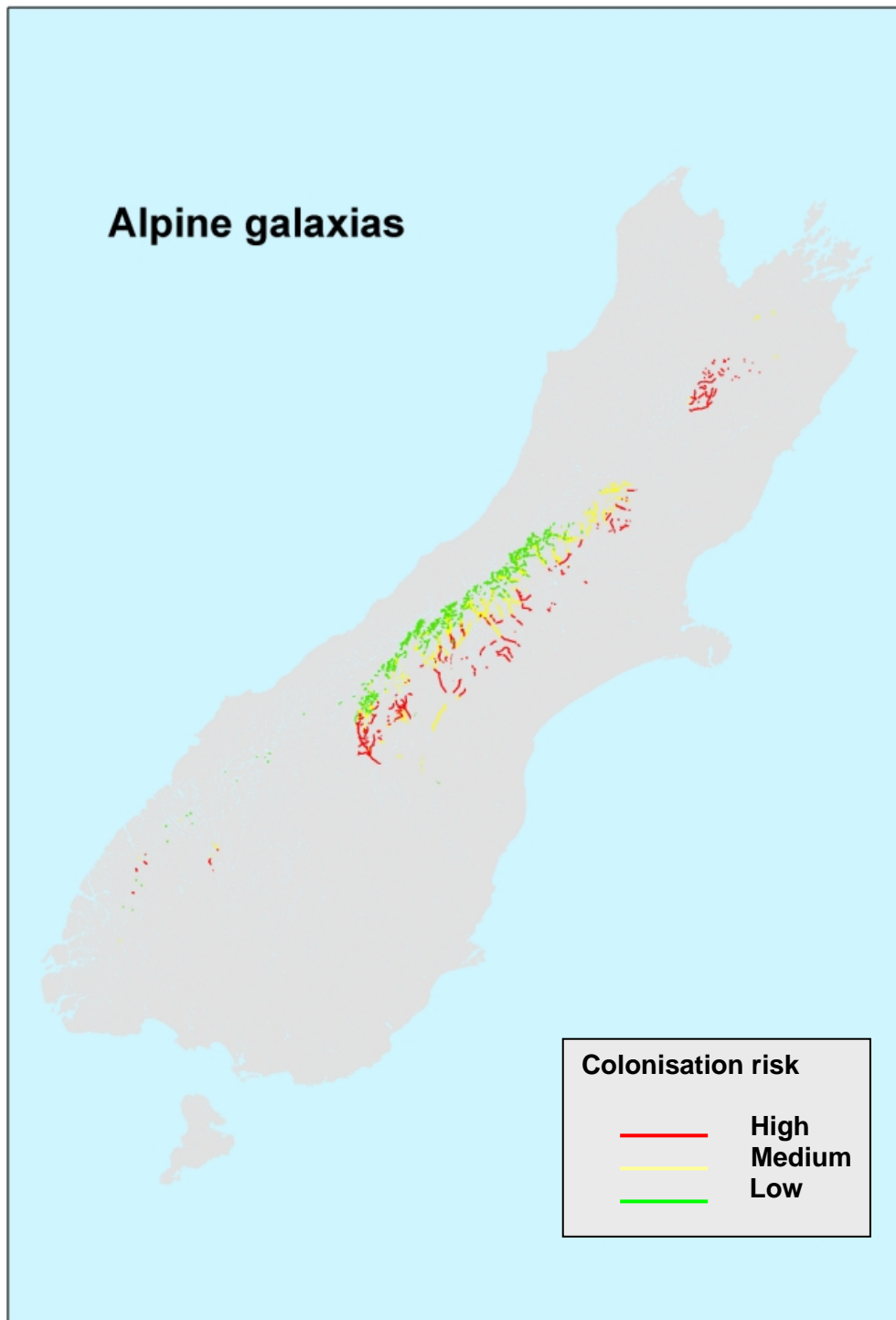
**Figure A13:** Likelihood of colonisation of redfin bully habitat in the South Island by *D. geminata*.



**Figure A14:** Likelihood of colonisation of common bully habitat in the South Island by *D. geminata*.

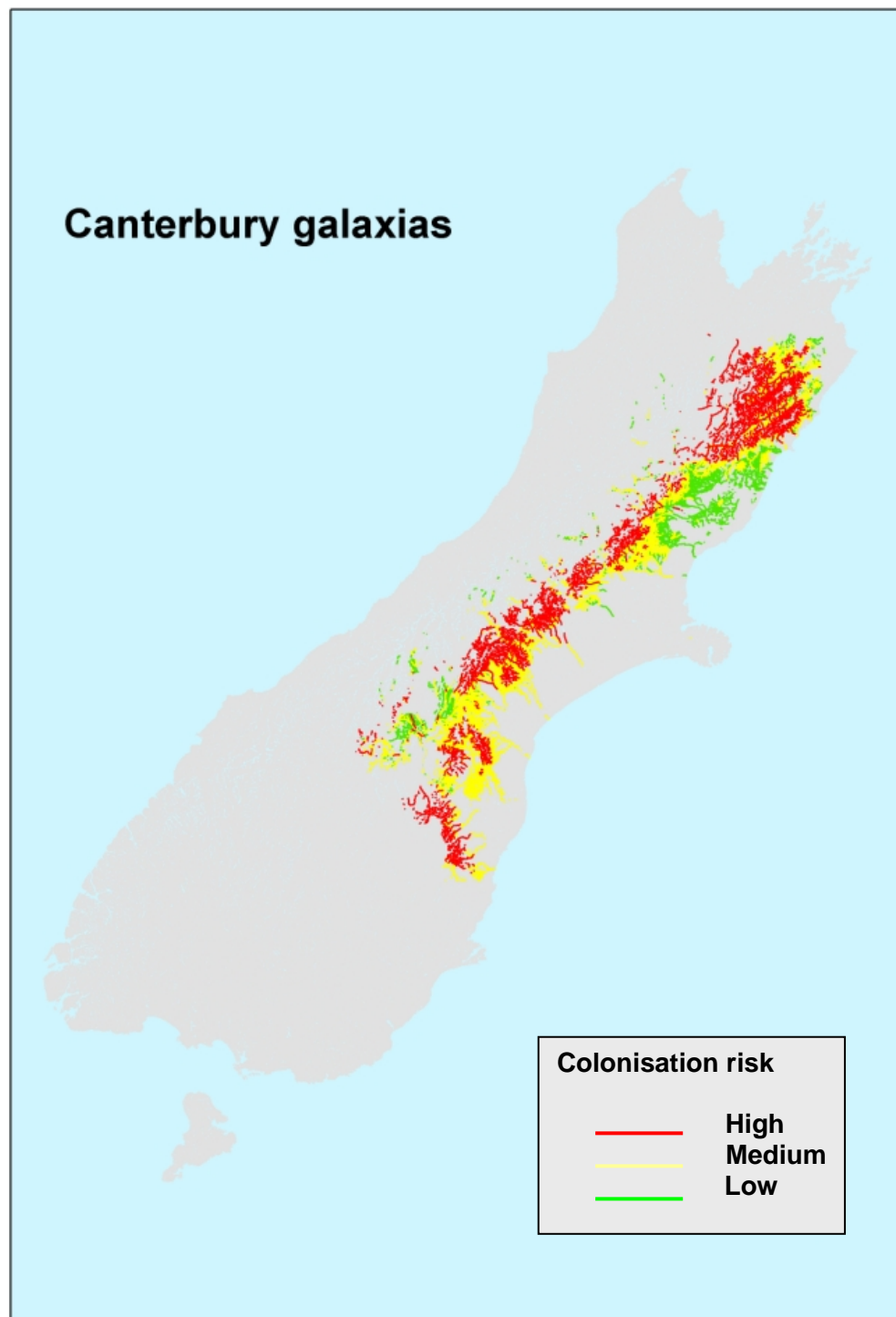


**Figure A15:** Likelihood of colonisation of bluegill bully habitat in the South Island by *D. geminata*.

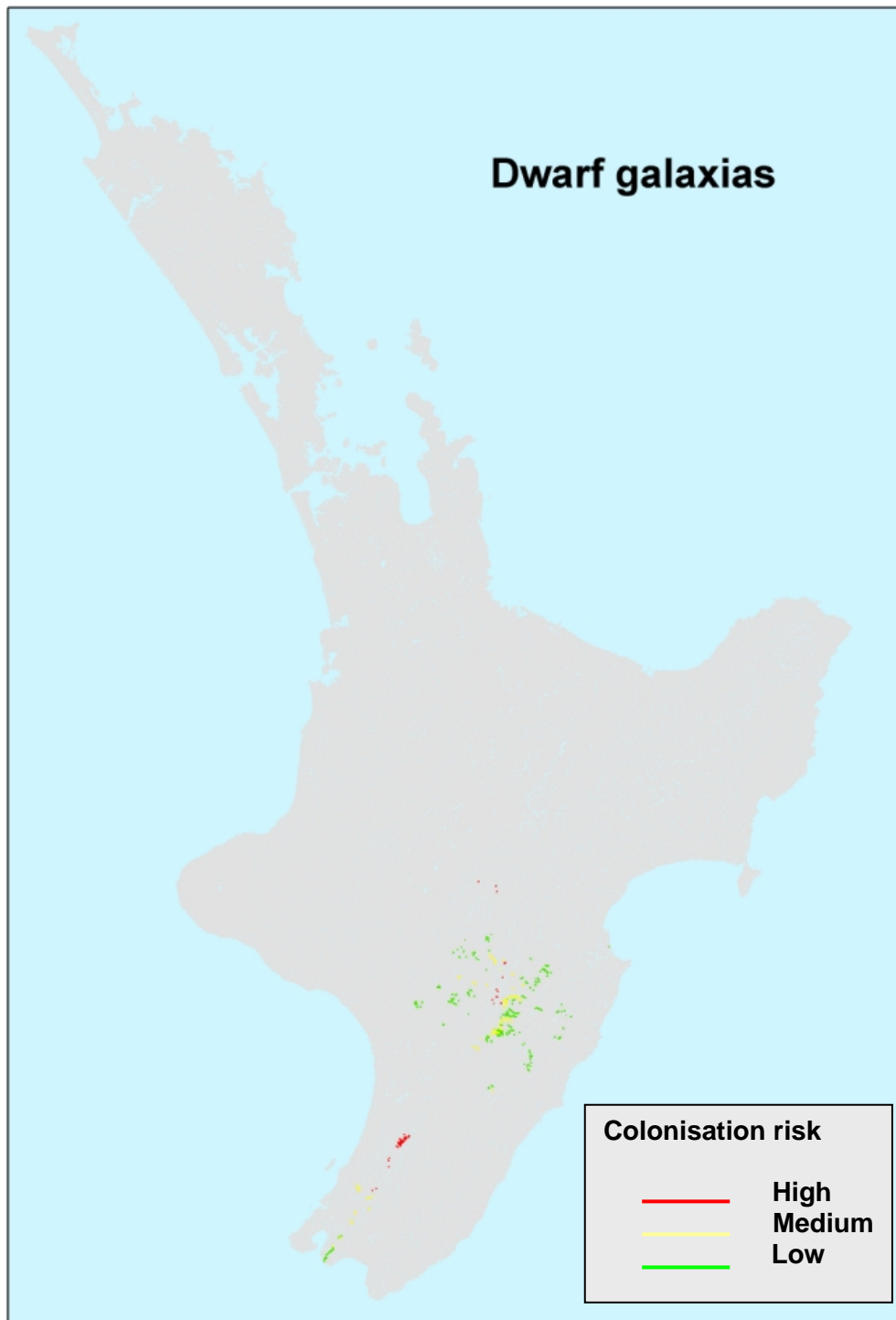


**Figure A16:** Likelihood of colonisation of alpine galaxias habitat in the South Island by *D. geminata*.

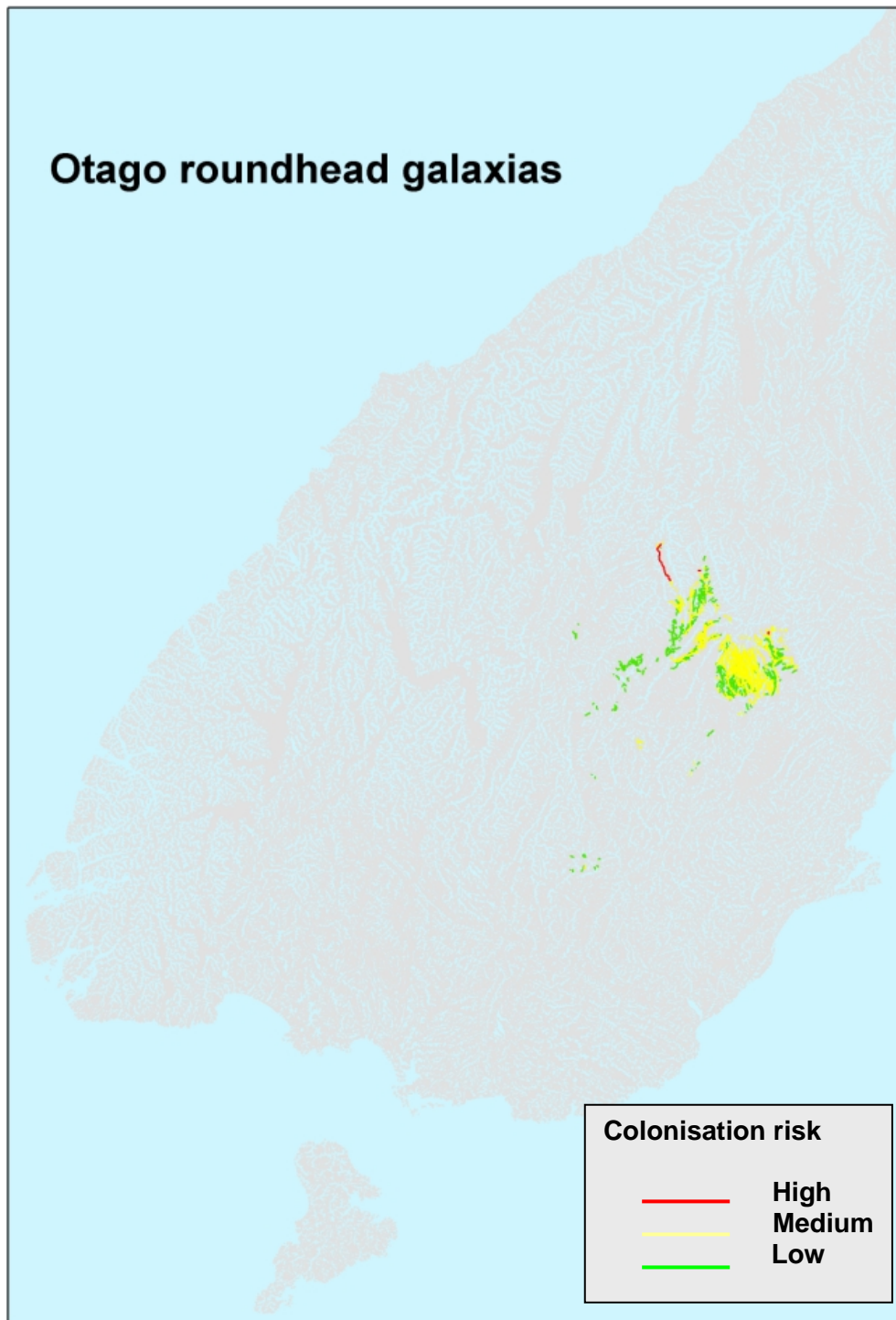




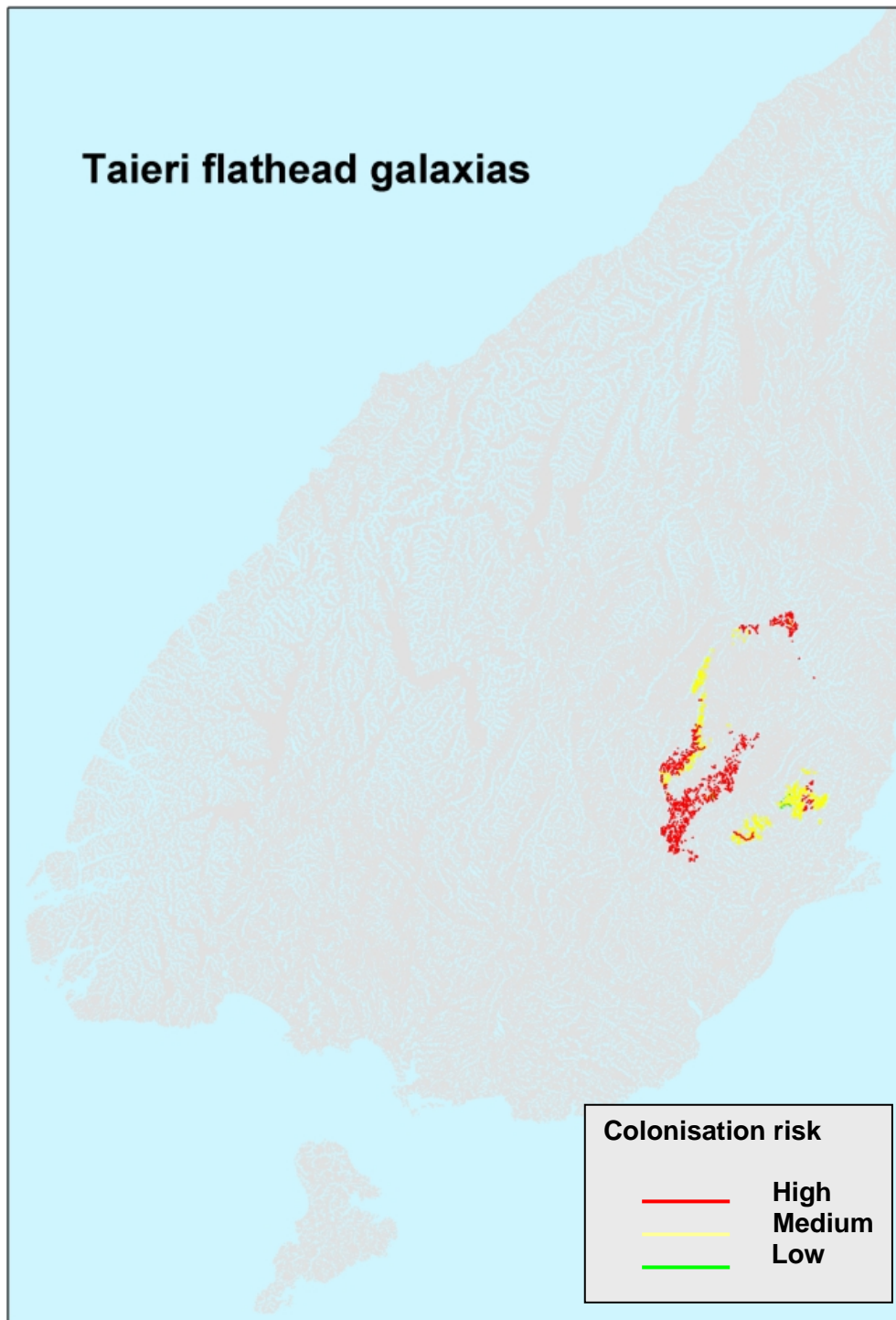
**Figure A17:** Likelihood of colonisation of Canterbury galaxias habitat in the South Island by *D. geminata*.



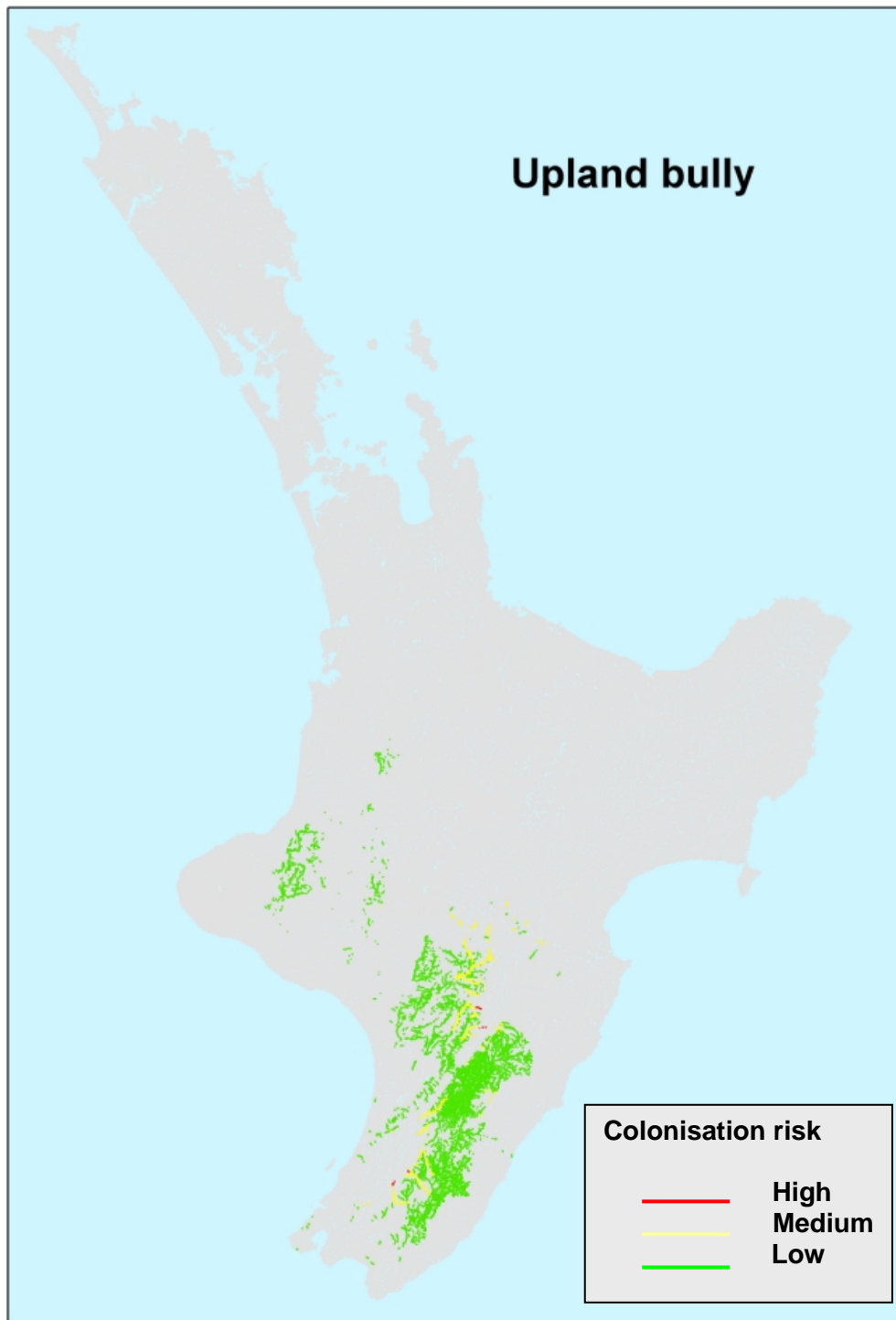
**Figure A18:** Likelihood of colonisation of dwarf galaxias habitat in the North Island by *D. geminata*.



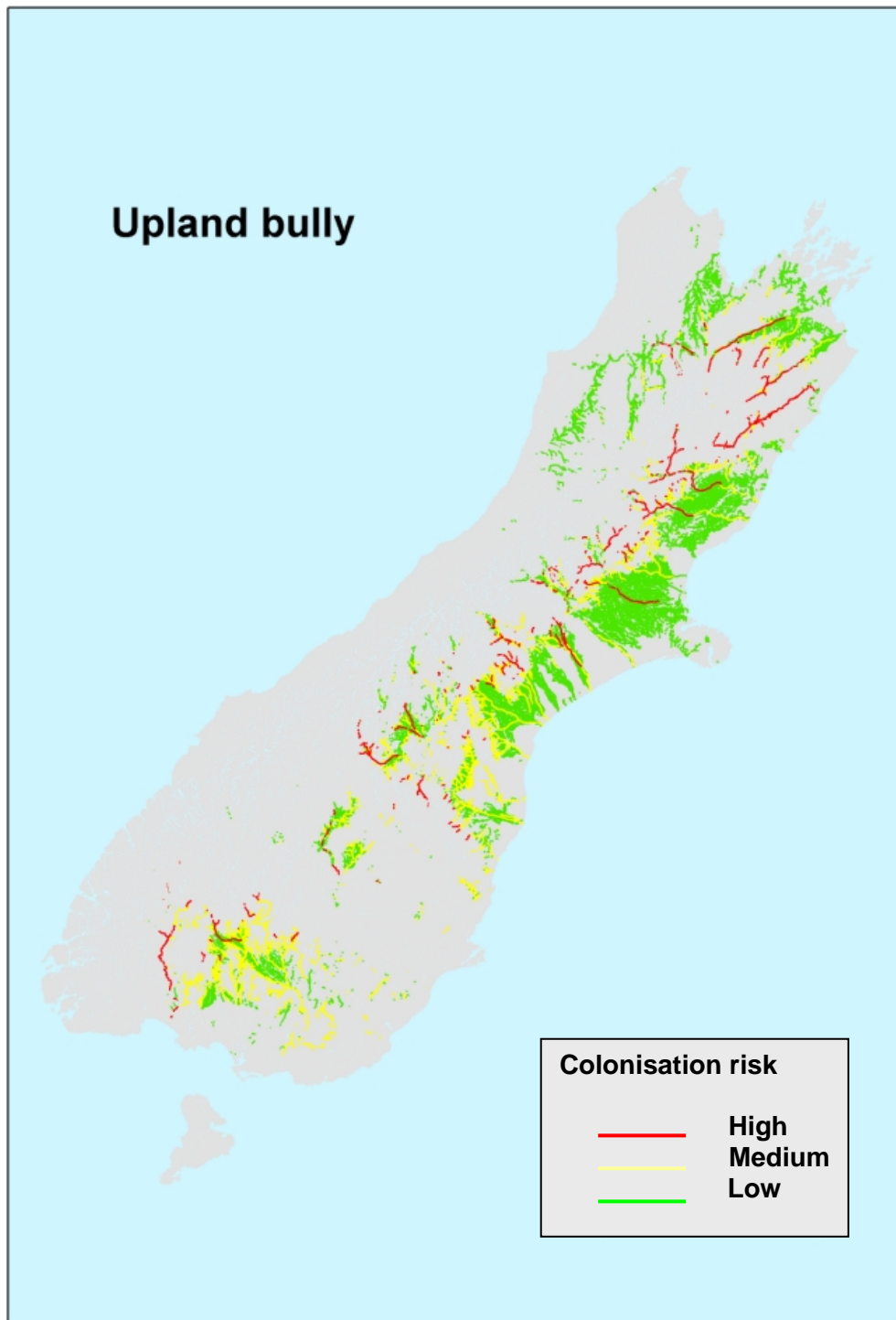
**Figure A19:** Likelihood of colonisation of Otago roundhead galaxias habitat in the South Island by *D. geminata*



**Figure A20:** Likelihood of colonisation of Taieri flathead galaxias habitat in the South Island by *D. geminata*



**Figure A21:** Likelihood of colonisation of upland bully habitat in the North Island by *D. geminata*.



**Figure A22:** Likelihood of colonisation of upland bully habitat in the South Island by *D. geminata*.