The survival of *Didymosphenia geminata* in three rivers and associated spring-fed tributaries in the South Island of New Zealand

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Executive Summary

This study investigates the effect of spring-fed creeks on the survival of *Didymosphenia geminata* (didymo). The outputs of this research will provide insights on the potential control for didymo which will assist MAF Biosecurity New Zealand in assessing and developing long term management tools.

This study compared the survival of *Didymosphenia geminata* (didymo), a non-native stalked diatom, on artificial substrates placed in predominantly groundwater fed creeks to adjacent sites in three rain fed rivers in the South Island of New Zealand where *D. geminata* had become established since October 2004.

We found that *D. geminata* cells which had been established on artificial substrates died and or disappeared from the substrates placed in the spring creeks whereas they generally remained healthy in the main river sites. Water chemistry analysis showed that the spring creek sites had higher conductivity, alkalinity (measured as calcium carbonate) calcium, nitrate and to a lesser extent magnesium. Lower concentrations of organic carbon were found in the spring creeks. Macroinvertebrate abundance was variable from site to site independent of the type of stream with no clear differences between spring creeks and the main rivers.

The decline in both proportions and estimated weight of viable *D. geminata* cells to extremely low levels in all the spring creek sites in this study is a compelling result and suggests that poor survival of *D. geminata* in spring-fed creeks may be a general phenomenon.

However, no single variable common to the spring-fed creeks could be identified as being responsible for poor survival of *D. geminata*. It is possible that there is a single causal factor, which we did not measure. Alternatively, and more likely, a combination of factors, may be responsible for the observed declines.

Further field trials, such as manipulation of alkalinity and measurements of nitrite and ammonia, are recommended to determine which factor, or combination of factors, is responsible for reducing the survival of this invasive alga in New Zealand spring-fed creeks.

Introduction

Didymosphenia geminata (didymo), a stalked diatom first discovered in New Zealand in October 2004 in the Mararoa and Waiau Rivers (Kilroy 2004), forms extensive mats over the bed of rivers and streams which are characterised as having clear, relatively shallow water with large substrates that rarely move, and a stable flow. Because of its capacity to attain unprecedented biomass levels in such rivers, MAF Biosecurity New Zealand (the government department with responsibilities for responding to incursions of potentially harmful exotic organisms) declared *D. geminata* an Unwanted Organism under the Biosecurity Act 1993 within a month of its discovery. Impacts of severe blooms include aesthetic degradation, nuisance effects for recreational river users (e.g., blocking jet-boat intakes, snagging fishing equipment), effects on industry (clogging irrigation and hydro-electricity intakes), and potential effects on other parts of the river ecosystem (e.g., changes in water quality, community shifts in native algae, invertebrates and fish), Kilroy (2004).

Within a year of its discovery, *D. geminata* had spread to other rivers in Southland and other parts of the South Island. The diatom has tended to be most abundant in lake outlet rivers, but it also occurs at lower densities in rain-fed rivers, the relative abundance apparently related to the frequency of floods and freshes. However it was observed not to colonise spring-fed streams at all even though these were tributaries of the affected rivers. The lower reaches of these streams, known as "spring creeks", were frequently inundated with floodwaters from the main rivers containing *D. geminata* cells and appeared suitable for *D. geminata* since they had stable beds, with clear water and stable flows.

Fish and Game staff undertaking underwater fish censuses by drift diving noticed that where spring creeks had a large flow in relation to the mainstem *D. geminata* did not grow in the mainstem/spring creek confluence where the flow was dominated by the spring creek water. In these situations *D. geminata* was weak and brittle compared to in the mainstem where it was tough and difficult to rub off river bed stones.

As part of ecological studies on *D. geminata*, the National Institute of Water and Atmospheric Science (NIWA) undertook an experiment in two spring creeks in the Mararoa catchment. Boulders colonised by *D. geminata*, which were taken from the mainstem and placed in a similar situation in the nearby spring creek, gradually lost their *D. geminata* cover over a two-month period. No explanation for this was obvious (Larned et al. 2006).

The spring creeks in this study are associated with riparian aquifers which are recharged by rainwater and the main stream themselves by connected aquifers. Aquifers are subterranean zones of coarse gravel through which water can move, provided gradients are suitable. The rivers flow over glacial outwash plains that are composed of many meters, typically up to 20–30, of a range of fine and coarse sediments that allow for a complex pattern of underground paleochannels that ultimately discharge to the main river. Tertiary limestone deposits occur in these river catchments (Turnbull 2000). Glacial action has eroded these during the Pleistocene so that glacial outwash plains and moraines have limestone in them. During its passage

through the soils of the plains groundwaters accumulate calcium and bicarbonate ions. Other ions such as nitrate are also bound to water molecules.

Diatom abundance and distribution is known to be affected by water conductivity and ionic composition (Potatova and Charles 2003). The ultimate extent of *D. geminata* spread in New Zealand waters is not known at the moment, although NIWA has published maps showing the distribution of suitable habitat for *D. geminata* in all of New Zealand's rivers. These estimates were based on existing datasets of variables covering the entire New Zealand river network and included temperature, lake influence, flow variability and rock hardness (Kilroy et al. in press). However, it is likely that other factors such as ionic composition are also important.

Department of Conservation and Fish and Game New Zealand have introduced controls on public access to some highly valued waters such as in the Fiordland National Park. Controls on access to spring-fed streams in Canterbury that are important for salmon spawning have also been imposed. Waikoropupu (Pupu) Springs in Takaka, renowned as some of the clearest water in the world, have also been closed to direct access by the public because of concerns that they may become affected by *D. geminata* .

MAF Biosecurity New Zealand has funded studies, including this trial, into methods to control *D. geminata* and to gain a better understanding of its environmental requirements (Larned et al. 2006; Jellyman et al. 2006). This study investigates the effect of spring-fed creeks on the survival of *D. geminata*. In this report we describe studies aimed at testing the hypothesis that *D. geminata* does not survive well in spring-fed creeks. We suggest that water chemistry differences, particularly in calcium and alkalinity, may be responsible for such a response. Alternative or additional possibilities are:

(a) High densities of invertebrates (e.g. snails) in spring-fed creeks prevent substantial colonization by *D. geminata* and similar diatom taxa through their grazing activities. Snails (e.g., *Potamopyrgus antipodarum*) tend to be common in the stable conditions of spring-fed creeks.

(b) Lower water velocities and less turbulent flows in spring-fed creeks may be unfavourable for *D. geminata*.

(c) Elevated levels of nitrate in groundwater may be unfavourable for *D. geminata*.

(d) *D. geminata* may be out-competed or overgrown in spring-fed creeks by other algae already present at the trial sites.

Methods

1. Site selection

The sites selected for the trial had to meet a range of criteria. These were:

1. spring flow showed no evidence of *D. geminata* and suppressed it where it entered the infected river;
2. reasonable vehicle access required to transport heavy anchor blocks to site;
3. flows in spring creek were adequate to replicate paired site in river;
4. *D. geminata* could be cultivated on artificial substrates in the nearby river.

Site selection was more difficult than anticipated because we lacked knowledge of some spring creek summer flows and some sites were physically difficult to access. There were also time constraints, with a need to start the experiment to take advantage of a 90-day period of typically settled weather with no major floods.

The Mararoa, Oreti, Upper Clutha, Ahuriri and Waitaki Rivers were investigated because they all had blooming populations of *D. geminata*. Three sites were chosen on the Waitaki, and two each on the Mararoa and Oreti. Sites on the Waitaki were at Otiake spring near Kurow, Ferry Road South and the Hen & Chicken Spring on the true left bank three kilometres from the mouth of the river. On the Oreti they were adjacent to the Ashton Flat's road bridge and approximately 20 km downstream at the Oreti Road end access road. The Mararoa sites were at a site commonly known as Norman's Gulch (later transferred downstream to Wash Creek) and another at the confluence with Flaxy Creek spring. No spring creeks were found in the Clutha catchment or in the Ahuriri River. The final site locations are shown in Figure 1.

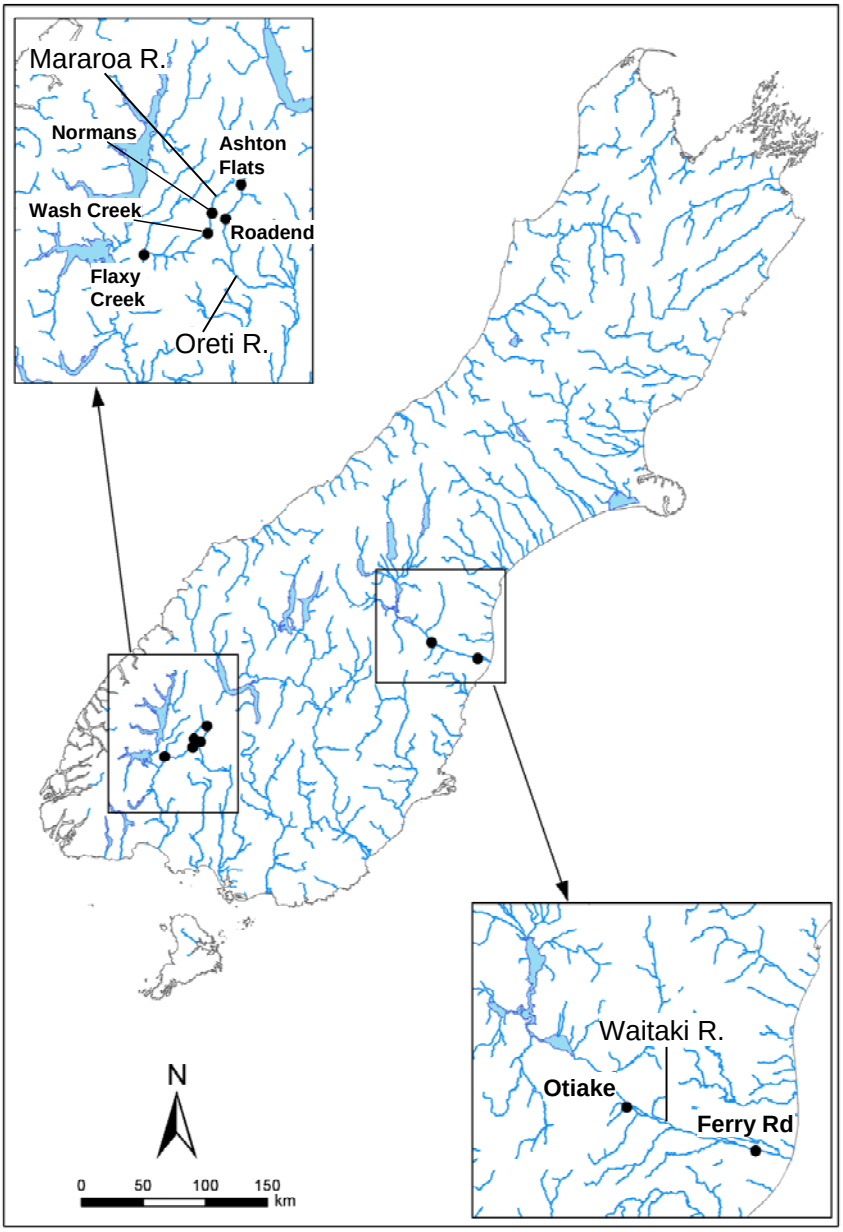

Figure 1. Location of research sites in the South Island of New Zealand.

2. Experimental design

Each spring-fed creek (unaffected by *D. geminata*) flowing into a *D. geminata* affected river was paired with a site in the river mainstem upstream from the confluence with the spring-fed creek. Plates of artificial substrates attached to concrete blocks were sunk into the river bed. They were chain anchored together, and to a metal stake which was driven into the river bed upstream (Appendix 1). Substrates comprised plastic sheets moulded into alternating rows of raised hemispheres (like the reverse side of an egg box) 40 mm in diameter (Appendix 1). The tomentose surface has been found to provide a very good surface for diatom colonisation.

Artificial substrate plates were pre-incubated in the river at each site to allow colonisation by *D. geminata*. There were six plates per site. Colonisation took 3 to 4 weeks. It was fastest in the Mararoa at Norman's and at the two Waitaki sites and slowest in the Oreti and in the Mararoa at Flaxy Creek. After colonisation three plates from each site were moved into the spring creek and three were left in the river. Ideally the latter plates should have been moved into a *D. geminata*-free section of the river but finding suitable sites for this was not possible due to the extensive colonisation of *D. geminata* in the rivers. In addition any sites in the rivers would have been far away upstream in the uppermost headwaters from the main site and transportation of the live cells over this distance would compromise their survival. It is likely that such sites would also have very different environmental conditions.

To address the problem of comparing plates transferred to the spring creeks with plates left in the river and potentially exposed to colonisation by *D. geminata*, we planned to deploy the colonised plates as soon as they were growing small/medium sized *D. geminata* colonies (3 mm to 15 mm in diameter) that were still separate from each other. To control for additional colonisation on the colonised plates, the design was to deploy new uncolonised plates (three per spring creek and three per river) at the same time the colonised plates were moved. However, it proved impossible to catch the colonised plates at a sufficiently early stage and all were well-colonised at the start of the experiment, with mean AFDM of up to 80 g/m^2 (see Appendix 2). Since colonisation was initially complete – already exceeding nuisance levels (Biggs 1990) – on the river plates we assumed that any increase in biomass was due to growth of the existing colonies and that new colonisation was insignificant. For further comments on growth on the new, uncolonised, plates placed in the rivers, refer to Appendix 2.

For the remainder of this report all results refer only to the precolonised plates that were either moved into the spring creeks or left in the river. The exception to this is in Results (7. Growth of other algae…). To determine the composition of communities developing naturally in the spring creeks, at the same time the colonised plates were moved into the creeks, we deployed clean, uncolonised plates upstream of them in the spring creeks. Biomass and community composition were analysed from these plates after 60 days (see Laboratory analyses, below).

We collected samples (see "Sample collection…" below) from each colonised plate before deploying, then moved three of the colonised plates from their location in the river to a location with similar water velocity / depth in the spring creek. We checked the water velocity / depth at all plates, at the same location relative to each plate using a "Flo-Mate" electronic velocity meter (Hach / Marsh-McBirney Inc., Maryland, USA). We aimed to get all the plates for each paired location within 20% of each other in respect of depths and velocity. Spring creek sites were also in unshaded areas as far as possible, similar to the river sites. The three plates that remained in the river were moved from their original locations and replaced in the same area to ensure that all plates received the same treatment.

Further samples were taken from all plates after approximately 30 and approximately 60 days. We also sampled at 90 days at one of the sites. All samples were analysed for viable *D. geminata* cells and ash-free dry mass (AFDM) (see "Laboratory analyses"

below). Around the time of each sampling occasion we undertook a survey of invertebrates at each site (see "Invertebrate observations" below).

Finally, on each sampling occasion we measured water velocity, depth, water temperature, conductivity and pH using a TPS field meter (WP-81, TPS Pty Ltd, Brisbane) at the location of the plates in each river and creek, and collected water samples for analysis of chemical composition. We took photographs of all plates *in situ* before lifting them out of the water to take samples. We used an underwater viewer to reduce the glare and the viewer/camera was positioned at the same location relative to each plate (e.g., just above the centre of the plate) in an attempt to estimate percentage cover visually. However, heavy colonisation from the start precluded any detection of new growth and differences on the river plates were not discernible. It was possible to detect changes in the growth of algae on the plates in the spring creeks but this was not used in the analysis. See Appendix 2.

3. Sample collection from plates

On each sampling occasion, we scraped algae from three hemispheres on each of the substrate plates in the rivers and spring creeks, and pooled them in to one sample. Pre-selection of the hemispheres sampled ensured that the samples were random, and prevented inadvertent resampling from the same hemispheres later on.

Samples for percentage of viable *D. geminata* cells (PCV) were kept separate from samples for AFDM, as follows. Each hemisphere was marked into quarters (or eighths if growth was thick: the PCV sample was kept quite small – less than ~10 x 10 x 10 mm). A small blade was used to scrape as much algal growth as possible off the hemispheres, down to the level of the flat plate. A quarter (or one eighth) of each hemisphere was scraped and the algae placed in a 120 ml screw-topped container (Elkay) with some river/creek water for the PCV sample. The remaining three-quarters (or seven-eighths) was scraped off and placed in a separate Elkay with minimal water for the AFDM sample. The proportion of the sample taken off for PCV was noted. This procedure was repeated for the other two hemispheres on that plate, pooling the material into the same PCV and AFDM containers.

The samples were kept as cool as possible after collection on ice in a chilly bin, but not frozen, and sent to the lab to arrive the following day.

4. Invertebrate / periphyton observations

On each sampling occasion a semi-quantitative assessment of invertebrate communities at each river and creek site was made on five cobbles along two transects following the method used in previous surveys (e.g., Kilroy et al. 2005). Algal cover was also noted. We calculated the number of invertebrates in a per square meter basis and compared the paired spring and river sites. We also grouped the invertebrates into algal eaters and non algal eaters and compared these.

5. Chemical analysis

Water samples were taken from both spring and river at 0, 30 and 60 days and tested for alkalinity, calcium, chloride, iron, potassium, magnesium, manganese, sodium, nitrate + nitrite nitrogen, sulphate and organic carbon. Analyses were undertaken at MLS Envirolab Ltd Invercargill and at Watercare Services Limited Auckland. Test methods were APHA 2320B (alkalinity), EPA 200.8 (calcium, magnesium and sodium), APHA 4110B (chloride), ICP OES APHA 3120 B (iron, manganese and potassium), APHA 4500-NO3 I (FIA) (nitrate+nitrite – nitrogen), APHA 4110C (sulphate) and APHA 5310B 5.20 (total organic carbon).

6. Site performance

Waitaki - Hen & Chicken Spring
Substrates for colonization were put in river in mid-December but could not be inspected due to subsequent high flows until 21/02/07. Little or no *D. geminata* had grown so the site was abandoned.

Waitaki - Ferry Road Spring
The spring creek site was vandalized severely, damaging the experiment. The damage was found prior to our 21/02/07 visit and a rescue plan was implemented where two additional plates with 30-day *D. geminata* growth were moved to the spring and placed in a less vulnerable position. On 22/03/07 it was found that the side stream of the Waitaki holding the river plates was completely dry when the normally constant 400 cumec plus flow was reduced to 240 cumecs for one week by the company operating hydro power stations upstream. The remaining plates in the spring creek were sampled out to 60 days.

Waitaki - Otiake Spring
The experiment at this site operated smoothly throughout.

Oreti - Ashton Flats Bridge
The river substrates failed to colonize with *D. geminata* sufficiently in spite of being in the river for more than three months. This result was surprising since *D. geminata* grew at this site in early 2006 and the river 1 km upstream had healthy *D. geminata* in it. It is unknown why the plates did not colonize although summer low flows were sourced mainly from groundwater as the surface flow of the Oreti stopped farther upstream.

Oreti - Oreti Road End
The 60-day cycle was completed although depleted flow in the spring reduced velocities over the substrates. The spring appeared in part to source water from the river as flow drop off was largely paralleled in the river.

Mararoa - Norman's/Wash Creek Spring
An alarming decline in the adjacent spring creek flow necessitated pairing the river site with a spring near the confluence of the Mararoa and Wash Creek 8 kilometers downstream from Norman's Gulch. This was one of the springs used in the initial

Larned et.al (2006) study. The flow in the spring creek at Norman's Gulch virtually ceased in mid summer. These paired sites were taken through to a 90-day test satisfactorily.

Mararoa - Flaxy Creek

High river flows deposited sand on the plates and delayed the start of the experiment here by 30 days but it was then completed satisfactorily. Sand was deposited on the plates at this site because the stake anchoring the concrete blocks to the river bed accumulated flood debris creating an eddy which allowed fine sediment to settle there. Otherwise this site was satisfactory.

7. Laboratory analyses

All PCV determinations were undertaken within 36 hours of sample collection. Sub-samples of each were tested for viability of *D. geminata* cells using the Neutral Red method (Kilroy 2005). Briefly, small subsamples were immersed in a 0.05% solution of Neutral Red stain for 20 – 30 mins. We ensured that pieces from each of the three hemispheres, if they were still distinguishable, were included in the tested sub-samples. After staining the sub-samples were transferred to a microscope slide, teased apart to spread the algae evenly, then covered with a coverslip and examined at 100 – 400 x under a Leica DMLB compound microscope. A count was made of at least 100 *D. geminata* cells, distinguishing stained cells (assumed alive) from unstained or empty cells (assumed dead). The counts were made in microscope fields covering the entire sub-sample, to ensure a representative count.

The composition of the entire algal community was then determined using the same sub-samples by scanning at least 20 microscope fields (at a magnification of x 400). In each field the percentage of the area occupied by *D. geminata* stalks, *D. geminata* cells (live and dead), other diatoms, green filamentous algae, green unicells, and cyanobacteria, was estimated to the nearest 5 to 10%. During the estimate the depth (i.e., biovolume) of cells was taken into account so that the final percentages were as close as practicable to estimates of the relative biovolume of the different algal groups. Again, to ensure a representative count, we included microscope fields across the whole slide. The slide was finally scanned at x100 to ensure that the 20 fields were representative. Mean percentage biovolume estimates of each algal group over all fields were then calculated.

AFDM was determined by drying the entire AFDM sample at 60 °C for approximately 72 hours until no further weight loss was noted. The dried material was weighed, ashed for 4 hours at 400 °C, then reweighed. AFDM is the difference between the ashed and dry weights. All weights were normalised to the area of the three hemispheres sampled and to g / m^2 for comparison with data from other studies.

8. Numerical analysis

Available variables were:

(a) ash-free dry mass (**AFDM**), which indicates the total amount of organic material in the sample;

(b) the percentage of viable (stained) *D. geminata* cells in each sample (**PCV**);

(c) the proportion (% by volume) of each sample made up of *D. geminata* cells, stalks and other major algal groups (other diatoms, green filamentous, green colonies/unicells, cyanobacteria).

We derived a further two variables from the above:

(d) the proportion (%) of algal volume (including extracellular stalks) in each sample made up of viable *D. geminata* cells,

PCV_all = PCV * estimated % *D.geminata* cells from (c).

(e) the total organic mass in each sample accounted for by viable *D. geminata* cells,

V_Wt = PCV_all * AFDM

(For the latter calculation we assumed a constant AFDM/biovolume for all the algal categories).

These two additional indices are important because the percentage of viable cells in a sample will not always reflect the condition of the colonies. For example, if many cells die and are quickly washed away, the remaining small number of attached cells may still show a high proportion of viable cells. Similarly, if stalk production and/or cell division cease after transfer to unfavourable conditions, the total proportion of viable cells could remain high in a sample, even though the total biomass is low.

Response of *D. geminata* in rivers and spring creeks

We used a two-way ANOVA to test for significant effects of both location (river and spring creek) and time on PCV, PCV_all and V_Wt. An ANOVA tests the hypothesis that the means of a single variable in two sets of samples are the same. A probability of less than 5% ($P < 0.05$) was taken to signify a significant difference. Again a probability of <5% that samples were the same was taken as the significance level.

Environmental variables

We compared water velocities and water chemistry variables across rivers and between the paired river and spring creek sites by visual inspection of bar graphs, then ran t-tests to check for significant differences between each spring / river pair.

To obtain an overall picture of the environmental differences among sites we carried out a non-metric multidimensional scaling (NMDS) analysis using data from all the sites and all variables. In this analysis, similarities between all pairs of sites with respect to all variables are calculated, based on normalised data (i.e. all variables converted to the same dimensionless scale). Site locations are then plotted in two dimensions to reflect their relative ranked similarities. i.e., pairs of sites that are similar are placed close to each other, and dissimilar pairs are placed far apart.

Results

1. Hydrology

As indicated in the methods section there were periods of high flows in the Oreti and Mararoa Rivers during the course of the trial. The Waitaki River is regulated and experiences less variable flows. Hydrographs from the nearest recording sites in each river clearly show the timing of these events and between-river differences (Figure 2).

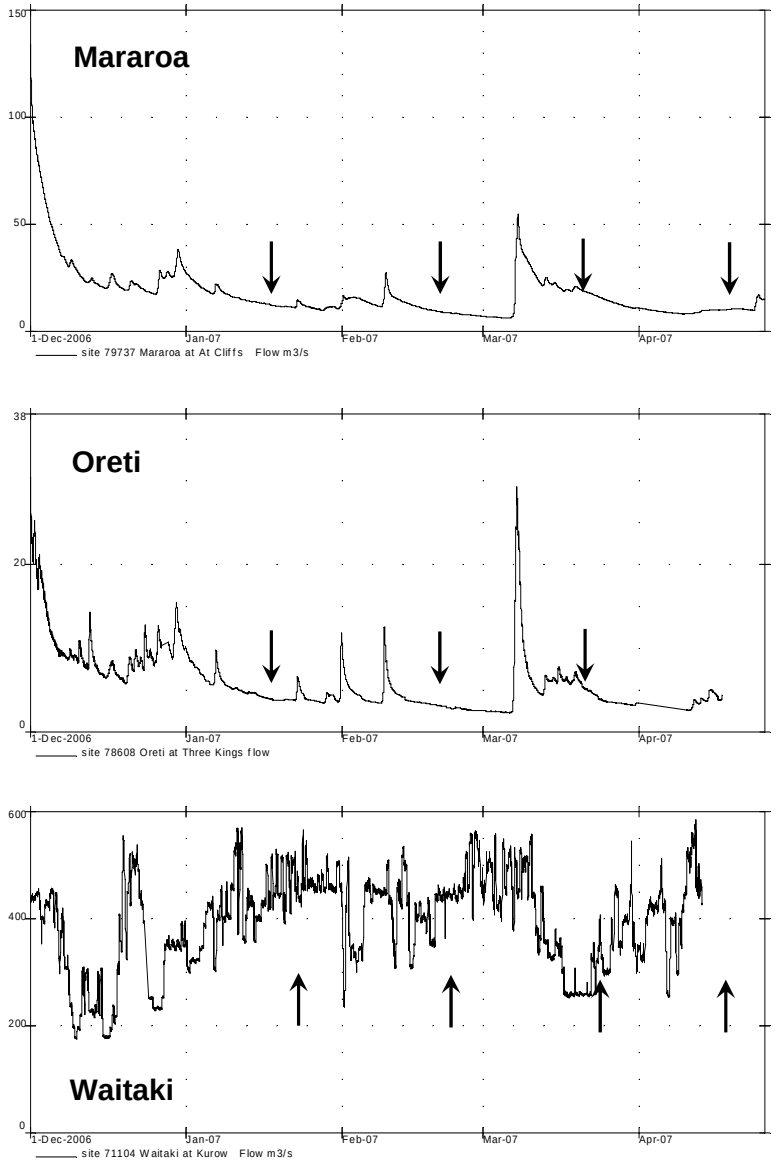

Figure 2. Hydrographs of the Mararoa, Oreti and Waitaki rivers over the time of the study (January -April 2007). Arrows indicate sampling occasions.

2. Water velocity

The substrates were placed initially so that water velocity was similar between each of the paired sites. However over the course of the summer changes in flows resulted in these similarities being lost. On average, there were significant differences between water velocity in the rivers and springs creeks at all sites, though at Flaxy and Normans, velocities in the river were very variable and were, on occasion, reduced by debris accumulated at the front of the plates. Flow velocities were restored when this was cleared. At the Oreti (Roadend) and Waitaki (Otiake) sites, water velocities were significantly lower in the spring creeks (Figure 3). This was likely caused by changes in flow over the period of the trial.

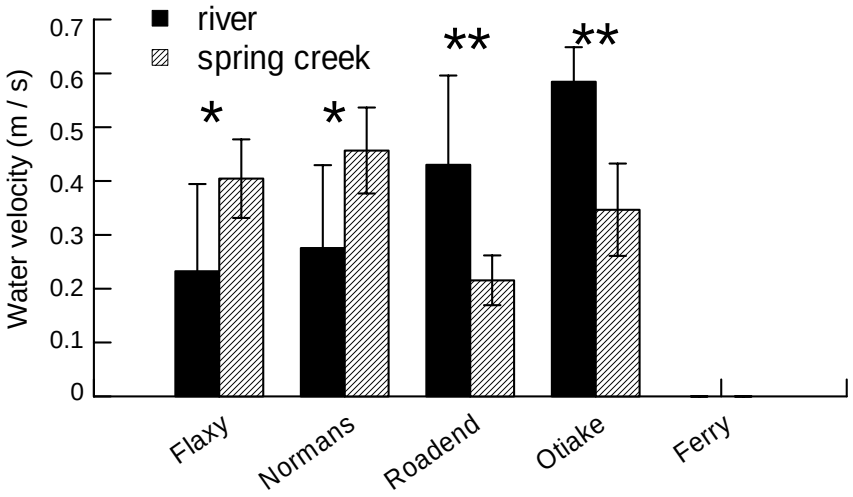

Figure 3. Mean water velocities measured in the rivers and spring creeks over three sampling occasions. Error bars are standard deviations. Asterisks above the bars indicate significant differences between river and spring creek sites (**P < 0.005, *P < 0.05). There was no water velocity data for Ferry Road.

3. Changes in proportion and abundance of live *D. geminata* cells over time.

Figures 4 a, b and d (dashed blue lines) show that survival of *D. geminata* in the spring creeks declined rapidly from the beginning of the trial so that after 30 days there were low percentages of viable *D. geminata* cells and also very low proportions of live *D. geminata* in the entire algal community. There was no recovery from this low level after 60 days. The estimated total mass of viable *D. geminata* cells (V_Wt) also declined to extremely low levels in all the springs by the end of the experiment.

Except for the apparent increase in the percentage of live cells at the Wash Creek (paired with Mararoa at Normans) and Flaxy sites after 60 days other measures of *D. geminata* survival also showed a decline after 30 days and then continued to decline up to 60 days. The plates at Normans were left in for an additional 30 days, by which time *D. geminata* had declined almost 100%, as it had at the other sites by 60 days. The result for percentage viable *D. geminata* cells after 60 days at Flaxy Creek shows how this parameter alone can be misleading. The increased percentage of viable cells in the spring creek (Figure 5a, dashed line in top graph) was caused by one replicate that contained very few *D. geminata* cells, almost all of which took up the neutral red stain.

Most of the differences between the rivers and spring creeks were statistically significant (Table 1).

Note that a fifth site (Waitaki at Ferry Road) showed the same result, viz., a steep decline of PCV and PCV_all (not illustrated). However, no data were available from the river plates at this site, for comparison (see Methods: “Site performance”).

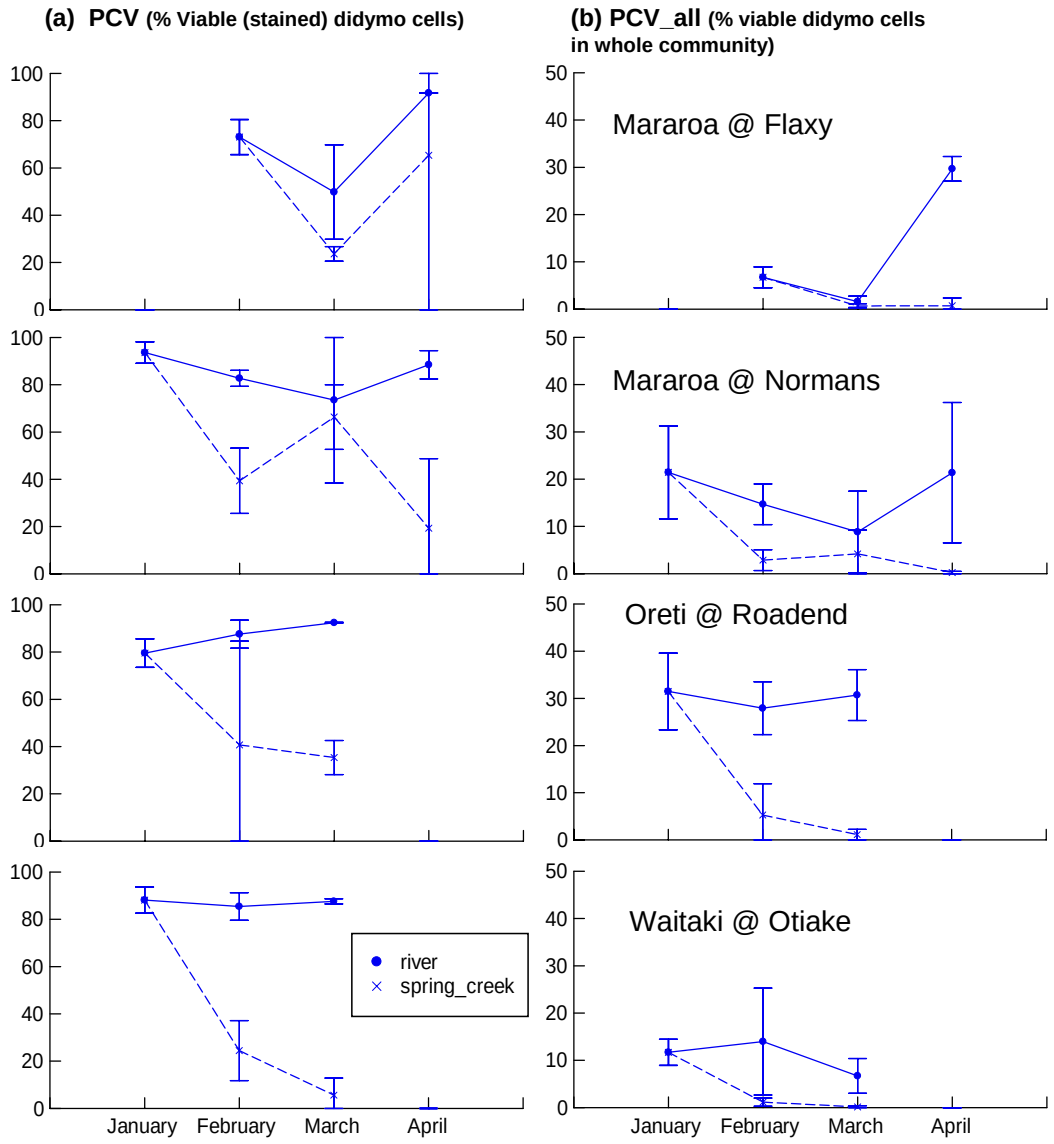

Figure 4. Summary plots showing (a) PCV, (b) PCV_all, (c) AFDM, and (d) V_Wt in the rivers (solid blue lines) and spring creeks (dashed blue lines). Sampling times are shown by month rather than days since the start of the experiment so that responses at different sites over the same time period can be compared. Error bars are standard deviations. Note that the vertical scales are the same for (a), (b) and (c), but not for (d).

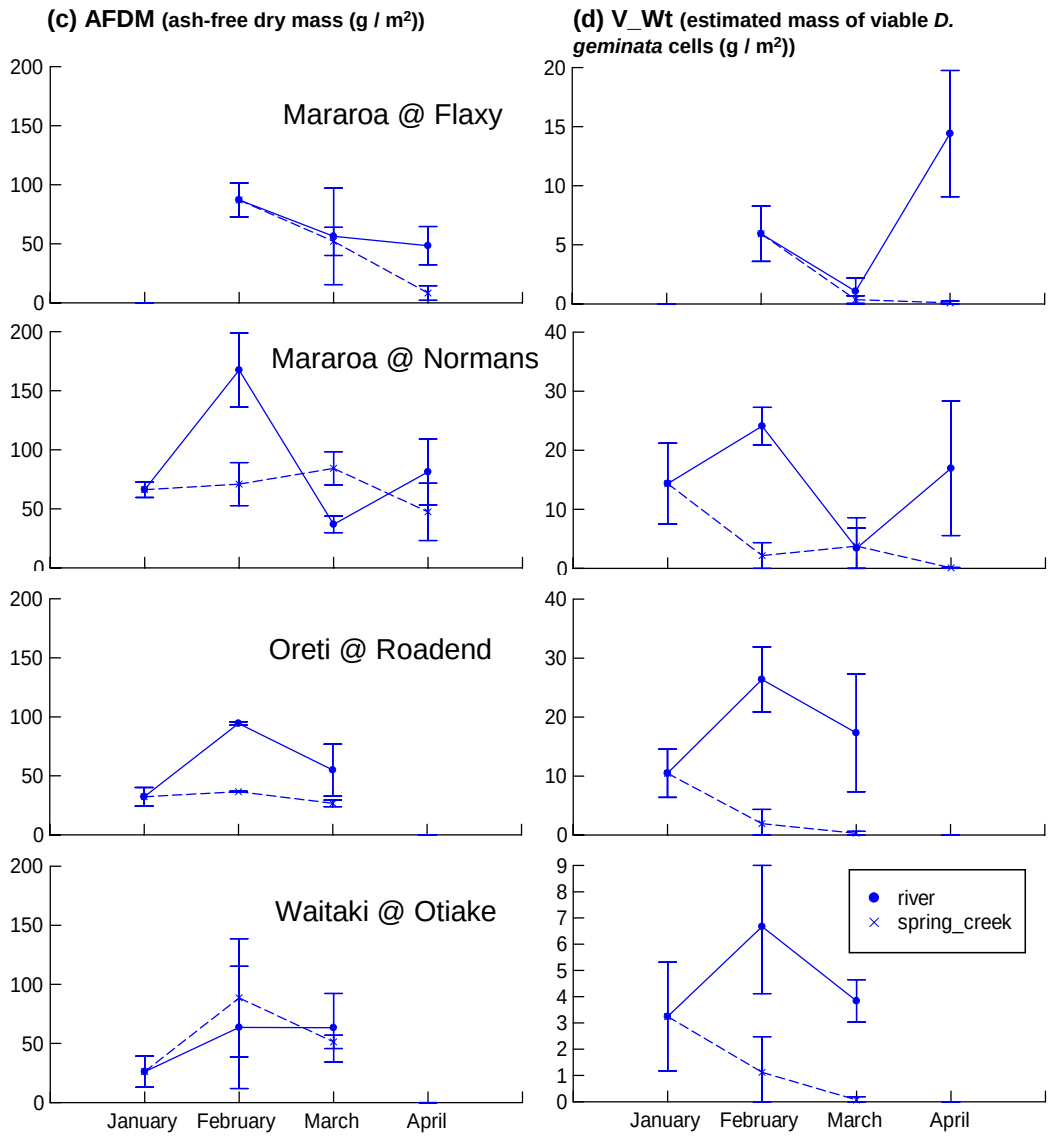

Figure 4 (continued).

Table 1. Results of two-factor (Location = river, spring creek; Time = 0, 30, 60, 90 days) ANOVA examining the effect of exposure to river and spring-creek environments at four sites on *D. geminata* cell viability (as assessed by a staining technique) and other community variables. F values are followed by probabilities, with significant values ($P < 0.05$) in bold type.

Site	Variable	Location	Time	Location x time interaction
Mararoa @ Flaxy	PCV	0.656 (0.429)	4.42 (**0.031**)	0.603 (0.558)
	PCV_all	6.793 (**0.018**)	3.648 (**0.047**)	5.642 (**0.013**)
	V_Wt	6.243 (**0.022**)	4.514 (**0.026**)	4.972 (**0.019**)
Maraoa @ Normans	PCV	44.254 (**0.000**)	19.359 (**0.000**)	13.863 (**0.000**)
	PCV_all	11.317 (**0.003**)	7.630 (**0.001**)	3.037 (0.051)
	V_Wt	23.773 (**0.000**)	6.594 (**0.002**)	8.829 (**0.000**)
Oreti @ Roadend	PCV	44.737 (**0.000**)	4.991 (**0.019**)	14.249 (**0.000**)
	PCV_all	44.756 (**0.000**)	19.013 (**0.000**)	14.479 (**0.000**)
	V_Wt	63.845 (**0.000**)	2.847 (0.084)	21.24 (**0.000**)
Waitaki @ Otiake	PCV	413.08 (**0.000**)	144.42 (**0.000**)	134.7 (**0.000**)
	PCV_all	18.014 (**0.000**)	11.251 (**0.001**)	6.803 (**0.006**)
	V_Wt	20.871 (**0.000**)	2.386 (0.120)	6.998 (**0.006**)

4. Differences in chemical composition in the springs and at the adjacent river sites.

Spring creek water had consistently higher concentrations of calcium carbonate (alkalinity) and nitrate. Calcium, magnesium, chloride, and sodium were also higher in the spring creeks but dissolved organic carbon concentrations were generally lower. (Figure 5). Potassium levels were not different. Other chemicals tested for were rarely found at detectable levels (i.e. iron, and manganese).

There were also some similarities between the springs and the rivers. The spring at Ferry Road on the Waitaki had similar chemical characteristics to the Waitaki River at that site. Organic carbon was found at very low levels in both the Waitaki River and the spring at Otiake. The Oreti River spring also had some main river chemical characteristics.

An NMDS analysis run using all water chemistry variables (Figure 6) showed that there was an overall difference in water chemistry between river and spring creek, and this was greatest at Otiake, followed by Normans, Flaxy, Oreti River at Roadend and Ferry Road. The figure confirms that water chemistry at latter two spring creeks was very similar to that of river water, except for nitrate at the Oreti Road End site.

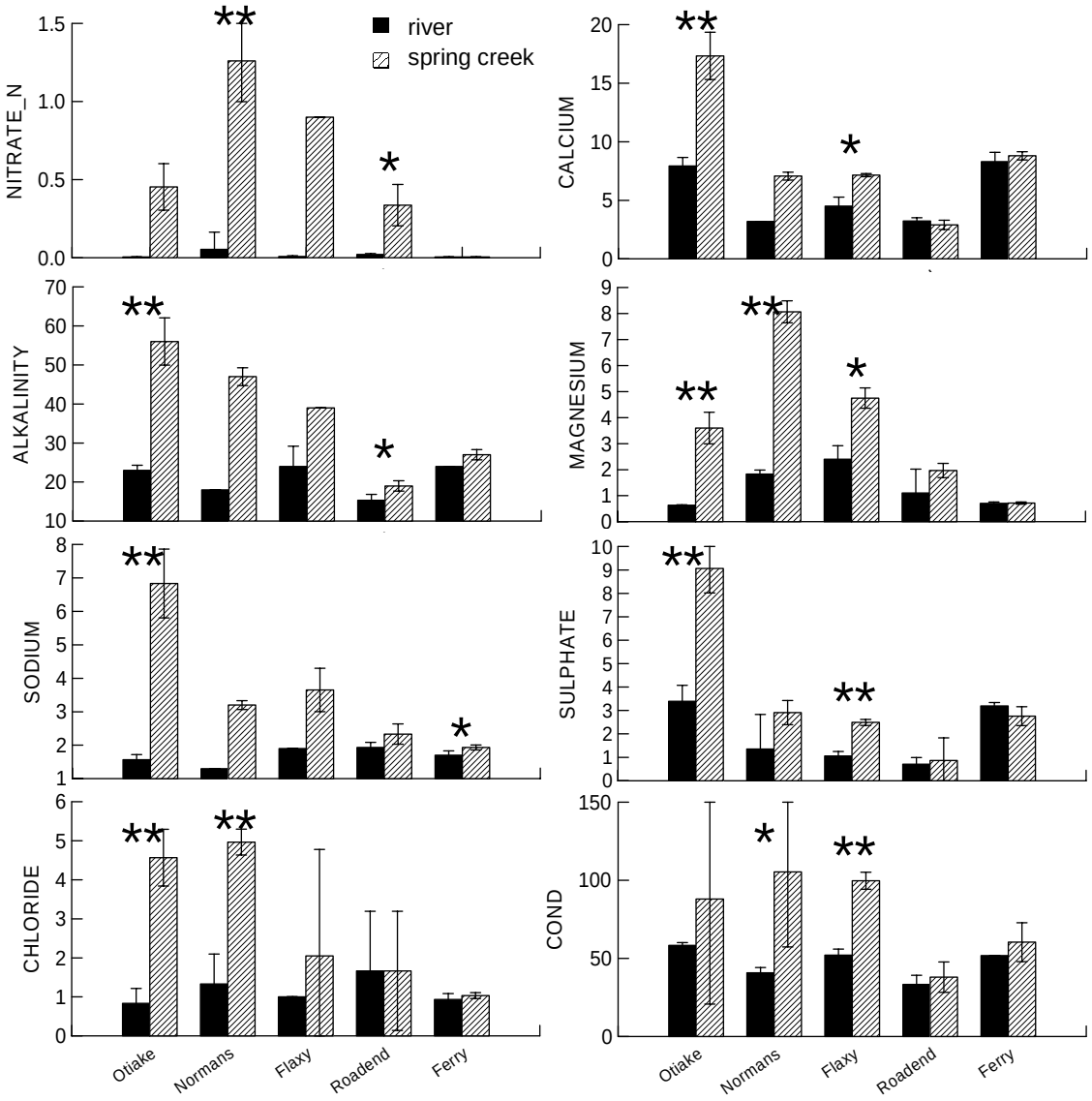

Figure 5. Mean values of water chemistry variables measured in the rivers and spring creeks over three sampling occasions (n = 3). Error bars are standard deviations. Asterisks above the bars indicate significant differences between river and spring creek sites (**P < 0.005, *P < 0.05). In cases where the graphs suggest large differences but these are not marked as significantly different, there was insufficient data to carry out a t-test. All concentrations are reported in mg/litre, with alkalinity in mg $CaCO_3$ / litre. Conductivity (COND) is in μS/cm, and temperature (TEMP) in °C. Data from the Ferry Road site are included for comparison even though the complete experiment could not be carried out (see "Site performance"). Sites are arranged in order of overall difference in water chemistry between the paired river and spring creek sites (Figure 6).

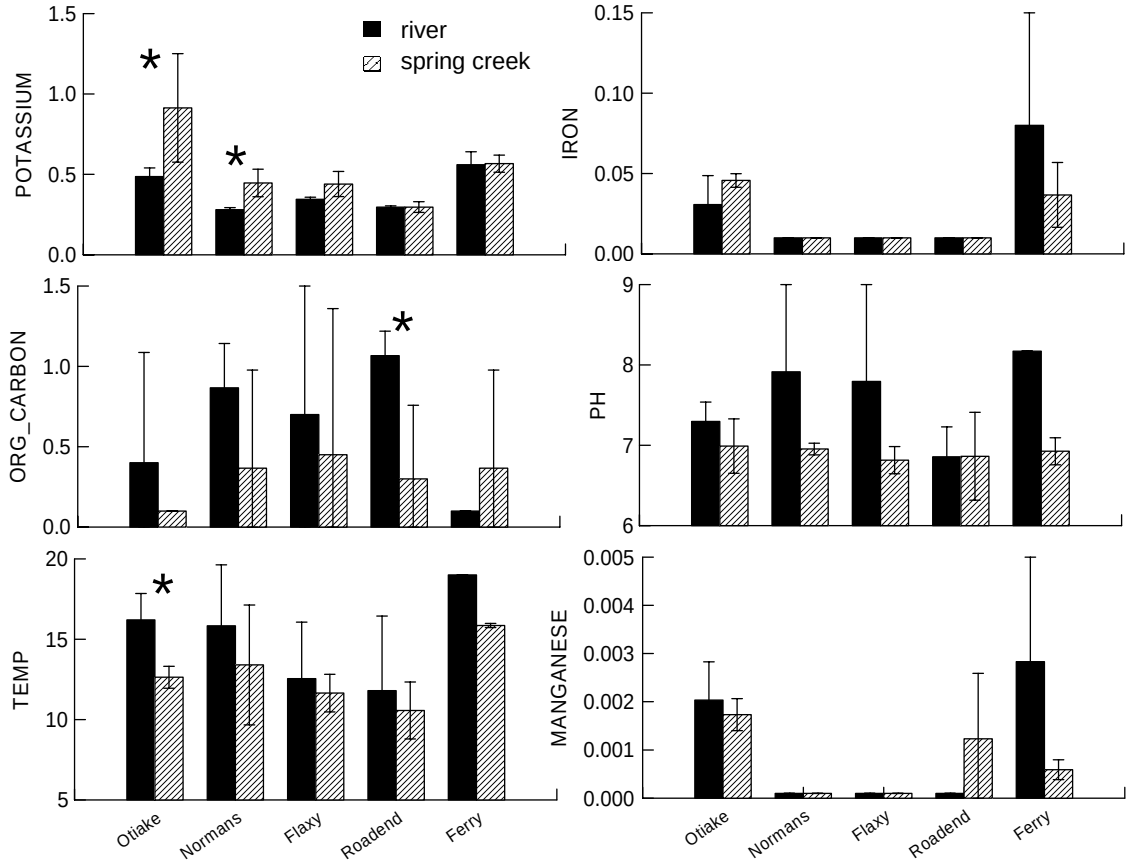

Figure 5 (continued).

5. Depth, temperature, conductivity and pH

Differences between the pairs of sites in respect to depth, temperature, conductivity and pH were also observed. Conductivity and pH measurements were generally different because of the different chemical characteristics of the spring and river water at each pair of sites. Spring creek water was also generally cooler than the river water and differed among sites (Figure 5, Table 2).

Table 2. Results of t-tests to compare the means of water depth, temperature, conductivity, and pH between spring and river sites at each location. T statistics are followed by probabilities, with significant values ($P < 0.05$) in bold type.

	Depth	Temp	Cond	pH
Waitaki Otiake	1.5 (0.078)	13.2 **(0.000)**	3.0 **(0.004)**	3.5 **(0.001)**
Oreti Road end	10.5 **(0.001)**	2.5 **(0.011)**	4.4 **(0.000)**	0.1 (0.441)
Mararoa Wash	6.0 **(0.000)**	0.03 (0.488)	9.4 **(0.000)**	7.9 **(0.001)**
Mararoa Flaxy	1.6 (0.05)	7.6 **(0.000)**	19.0 **(0.000)**	5.2 **(0.001)**

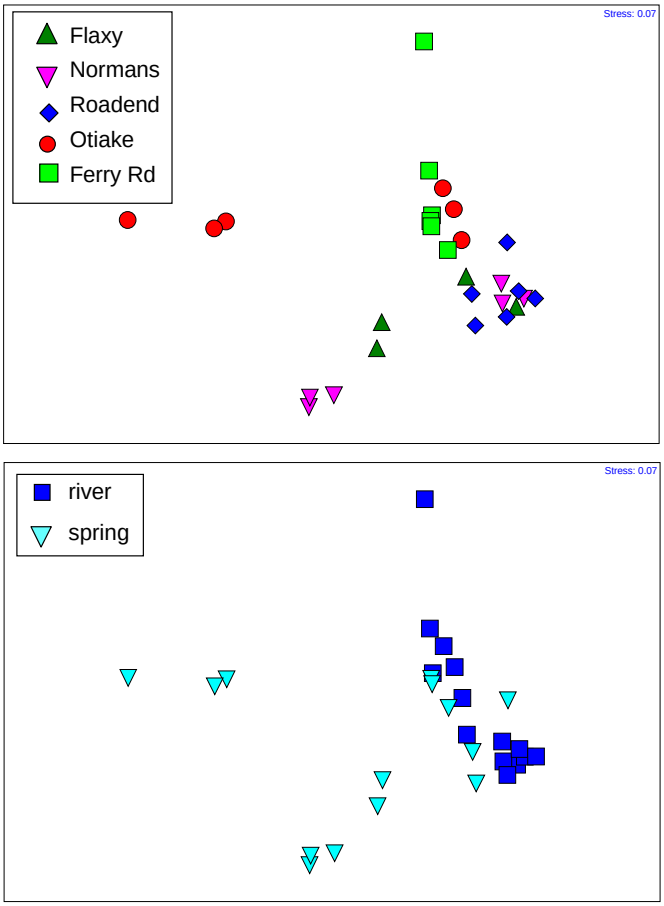

Figure 6. Non-metric multidimensional scaling (NMDS) plots summarising relative differences in water chemistry at river and spring creek sites. Each symbol represents one sampling occasion at one site. The stress value of 0.07 (in small lettering, top right-hand corner of plots) indicates a very good representation of the data in two dimensions.

6. Macroinvertebrate abundance

Free-living caddis flies (Hydrobiosidae), and stony-cased caddis flies (Hydropsychidae) were abundant at most sites. At some spring creek sites *Potamopyrgus* snails were abundant but not consistently so. These snails were also abundant in the Waitaki River at the Otiake site. Mayflies (*Deleatidium*) were also present in moderate numbers (50-100/m^2) at most sites. The Otiake spring had large numbers of crustaceans, typical of slow-flowing streams flowing from catchments with a strong limestone component (see Appendix 3).

Invertebrates were clumped into two groups, algae feeders and non-algae feeders. A plot of algae feeders in river and spring locations at each site shows markedly higher mean densities in the spring-fed creek at Normans and Ferry Road (Figure 7). However, because of high variability in the counts (Normans) and only two replicates (Ferry Road river site dried up after the first sample), the differences were not statistically significant.

Thus, there does not appear to be any consistent pattern of macroinvertebrate distribution that could be explained by differences between spring creek or main river classes.

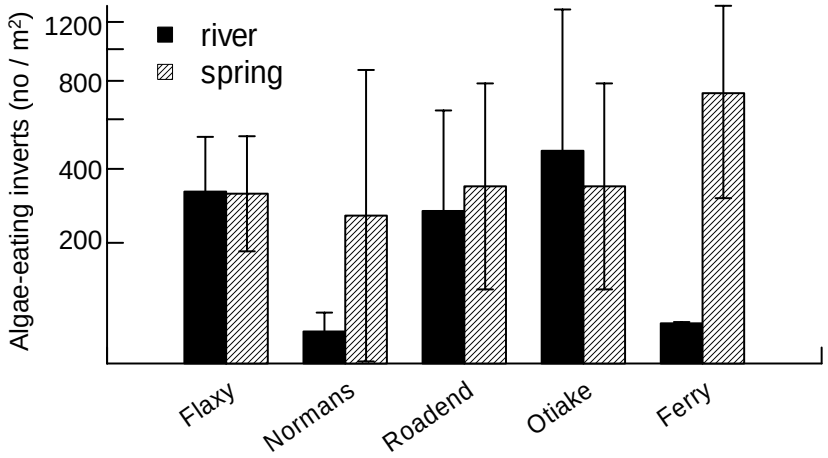

Figure 7. Mean densities of algae-eating invertebrates in the rivers and spring creeks over three sampling occasions. Error bars are standard deviations.

7. Growth of other algae in the spring creeks

Algae colonised all the uncolonised plates in the spring creeks and attained moderately high biomass in some cases (Figure 8). The pre-colonised transplanted plates were also gradually colonised by other algae to a much greater extent than those in the river (Figure 9). The algal communities colonising the plates in the spring creeks varied from site to site and did not necessarily reflect the communities overgrowing *D. geminata* on the transplanted plates (Figure 10).

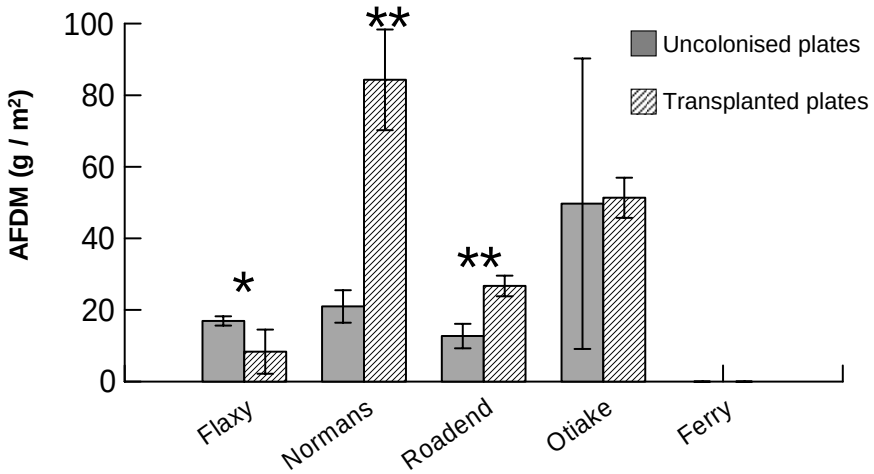

Figure 8. Mean ash-free dry mass on uncolonised and transplanted plates in the spring creeks after 60 days. Error bars are standard deviations. Asterisks above the bars indicate significant differences between river and spring creek sites (**P < 0.005, *P < 0.05). There were no data for Ferry Road.

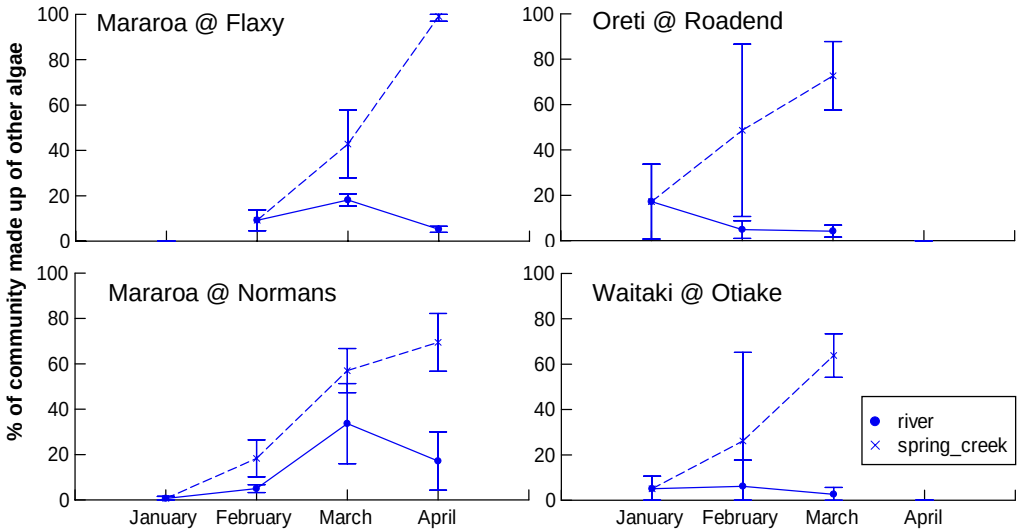

Figure 9. Percent non-*D. geminata* algae at each paired site.

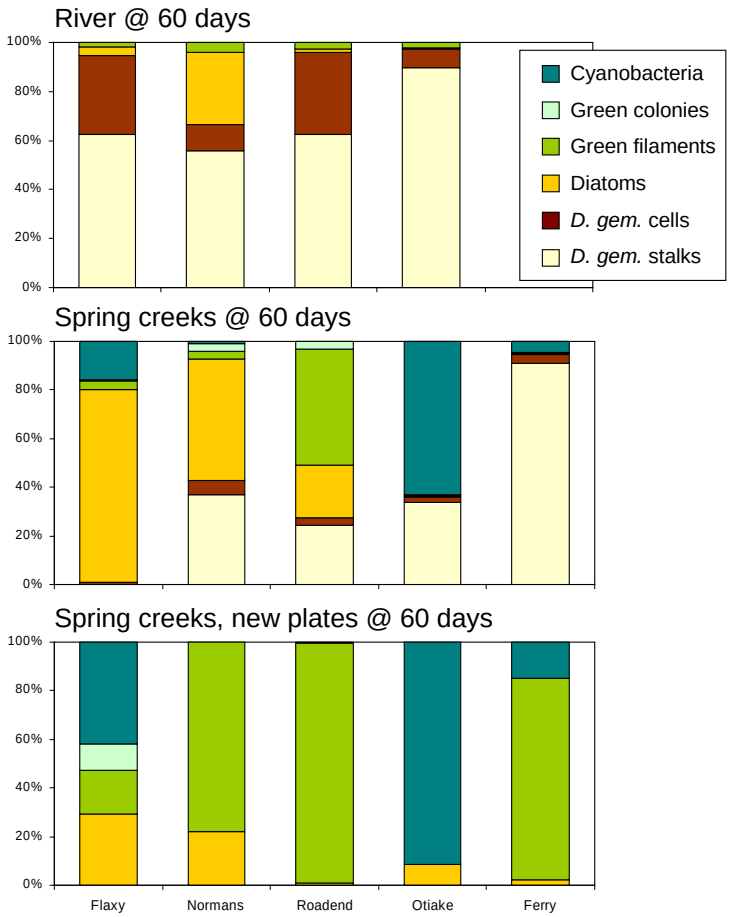

Figure 10. Mean proportions of different algal groups noted after 60 days on the pre-colonised river and spring creek plates, and on the uncolonised plates placed in the spring creeks at the time the precolonised plates were moved there from the river.

Healthy *D. geminata* cells were unexpectedly found in samples from the uncolonised plates placed in Flaxy Creek at the start of the experiment. A few cells were noted after 30 days, and slightly more after 60 days, including one visible small colony. Stalk material was minimal.

Discussion

Our main hypothesis – that *D. geminata* does not survive well in spring-fed creeks – appeared to be supported by the results of this experiment. At all sites, the colonised plates deployed in the spring creeks showed reduced cell viability and much reduced proportions and weights of live *D. geminata* cells compared to the plates left in the river. However, although the river plates were fully colonised at the start of the experiment, and therefore unlikely to have gained biomass from additional colonisation, there were also biomass declines on the river plates, which need to be explained.

AFDM biomass, cell viability and the proportions and weight of live cells declined markedly at three river sites (Flaxy, Normans and Roadend) between the February and March sampling occasions. This decline can be explained by the high flows in the Maraora and Oreti rivers on 6 – 7 March (Figure 2). In both rivers the flows peaked at least 2.5 times the median flow, which constitutes a significant flood, with potential to move portions of the bed and its attached periphyton. Simultaneous biomass loss at all three river sites would therefore be expected. Biomass on the plates on the previous sampling occasion had been relatively high (equivalent to AFDM of 80 – 150 g / m^2) suggesting that these were mature colonies, which are more likely to be scoured in a flood than the tighter adhering young colonies on the control plates. (See Appendix 1 for notes on further support for a flood-induced biomass decline.) There is no obvious explanation as to why AFDM in the river at Flaxy creek continued to decline between March and April. Nevertheless, in April the growing colonies were extremely healthy, with high PCV, PCV_all and V_Wt.

Mean AFDM at the single site in the Waitaki River (Otiake) did not decline over the course of the experiment, and there were no high flow events. This again supports the proposal that high flows were responsible for biomass loss in the Mararoa and Oreti Rivers.

No single likely reason for the decline of *D. geminata* in the spring-fed creeks was evident from the environmental data. The hypotheses are considered in turn.

Water chemistry: calcium and alkalinity

The evidence is unclear since Oreti Roadend and Ferry Road spring water was similar to river water at these sites, but the response was the same. The range of calcium in *D. geminata* affected rivers is 3.15 ± 0.2 (Monowai) to 10.9 ± 0.98 mg/l (Clutha @ Millers Flat) so only the Otiake Spring fell above this range. Alkalinity range is 11.8 ± 0.9 (Monowai) to 29.7 ± 4 (lower Waiau) [42 ± 3 (Motueka)] mg $CaCO_3$/l. The spring creeks at both Normans (Wash Creek) and Otiake had higher alkalinities (National River Water Quality Network data, NIWA]. Calcium is involved in interactions between diatom cells and the substrate they adhere to (Geesey et al. 1999) and may, in combination with other factors, affect *D. geminata* viability.

Invertebrate grazing

There is no convincing evidence for more algae eaters in the spring creeks than in the rivers.

Acidity

Low pH in the spring creeks could be limiting *D. geminata* growth. In Norway the alga was found not to occur in waters of below pH 7.0 (Eli-Ann Lindstrom pers. comm.). However at both Oreti sites the pH was less than 7.0 and growth did occur in the river.

Water velocity

The response at Oreti Roadend (where water chemistry in the river and creek were similar) could have been partly due to significantly lower water velocity in the spring creek. However *D. geminata* does have a wide water velocity tolerance with "high biomass levels occurring over a near bed velocity range of 0.02 - .065 m s^{-1}." (Larned et. al 2006).

Elevated levels of nitrate

Nitrate was elevated in all spring creeks (including Oreti @ Roadend) except Ferry Road (Figure 6), with concentrations approaching 1.5 mg/l at Normans. Of the rivers currently affected by *D. geminata* for which we have data, most have mean nitrate levels <0.3 mg/l (National Rivers Water Quality Network data, NIWA). The Oreti River at Lumsden has mean recorded nitrate of 0.4 mg/l, and *D. geminata* has occasionally grown at this site. Nevertheless, the alga is more abundant in the upper Oreti where nitrate levels are lower. Since nitrate levels spanned a wide range in the tested spring creeks, if this variable was affecting *D. geminata* viability, we might expect a negative relationship between the concentration of nitrate and *D. geminata*.

Figure 11 shows that after 30 days, *D. geminata* on all plates from the two spring creeks with highest measured nitrate (Otiake and Flaxy Creek) had declined by at least 70%. However, some plates from the other spring creek sites showed similar declines. After 60 days, plates at all spring creek sites showed >90% decline, except for the anomalous 50% survival on two plates (from Normans).

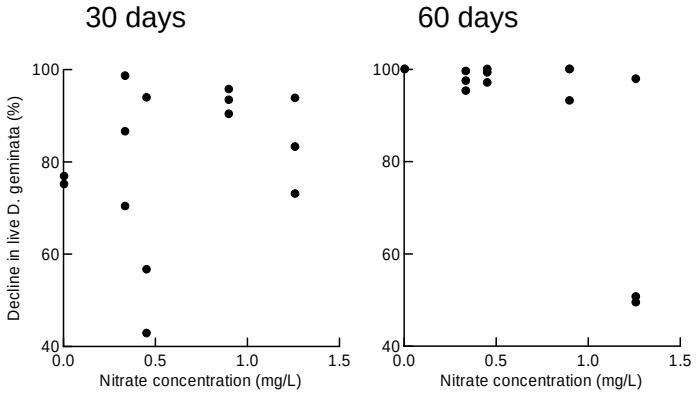

Figure 11. Percentage decline in live *D. geminata* cells plotted against nitrate concentrations in the spring creeks 30 and 60 days after the artificial substrates were transplanted into the spring creeks.

One possibility not considered previously is that high nitrite (NO_2) or ammonia (NH_4) concentrations in the groundwater that feeds spring creeks may inhibit the growth of some types of algae in these environments.

D. geminata may be outcompeted/overgrown in spring creeks by existing algae.

We observed that algae did not generally smother natural substrates in the spring creeks studied (Appendix 4). While we did not measure this, observations of the spring creeks indicated that the springs generally had low levels of periphyton even on cobbles and boulders where it would be expected to grow. In spite of this, moderate amounts of algae accumulated on the clean substrates placed in the creeks at the start of the experiment (Figure 8) and overgrew the *D. geminata*-colonised substrates (Figures 9, 10).

We considered the possibility that certain types of other algae might be more successful competitors against *D. geminata*. However Figure 12 indicates that the estimated biomass of algae in other groups over-growing the *D. geminata* mats was not clearly related to the decline in *D. geminata* for any group.

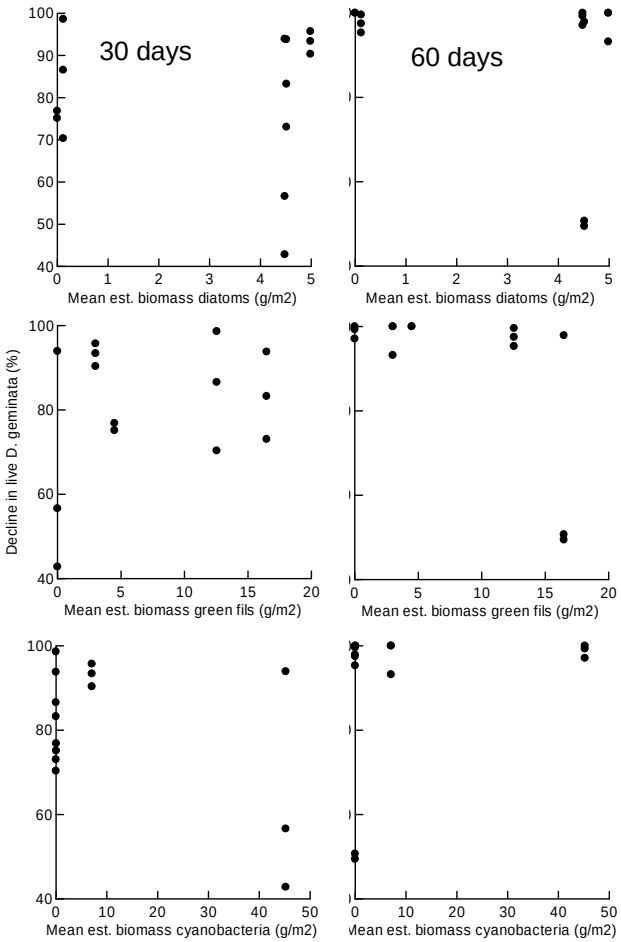

Figure 12. Percentage decline in live *D. geminata* cells plotted against mean estimated biomass of other algal groups overgrowing the *D. geminata* on colonised plates 30 and 60 days after the artificial substrates were transplanted into the spring creeks.

Observations of other spring creeks.

We have observed that other spring creeks flowing into the Mararoa and Waitaki also appear to be free of *D. geminata*. We took one water sample from three other springs in the Waitaki where we observed the absence of *D. geminata* in the lower reaches and these all had alkalinity ranging from 30 to 48 mg/l, Ca from 12-16 mg/l, nitrate from 0.33-0.91 mg/l, Mg from 1.6 – 3.2 mg/l and Na from 3.5 – 7.1mg/l. While alkalinity, nitrate and Mg were at the centre of the range measured in the other spring creeks, Ca and Na concentrations were at the high end of the range, and similar to that in Otiake Creek, in the Waitaki catchment.

Conclusion

The decline in both proportions and estimated weight of viable *D. geminata* cells to extremely low levels in *all* the spring creek sites in this study is a compelling result and suggests that poor survival of *D. geminata* in spring-fed creeks may be a general phenomenon. The observation of healthy *D. geminata* growing at low densities on the uncolonised plates placed in Flaxy Creek supports this suggestion: *D. geminata* may survive to some extent in these environments, but does not thrive.

No single variable common to the spring-fed creeks could be identified as being responsible for poor survival of *D. geminata*. It is possible that there is a single causal factor, which we did not measure. Alternatively, and more likely, a combination of factors may be responsible for the observed declines. Whether similar declines would occur if established colonies were transplanted into rivers thought to be suitable for *D. geminata* growth is also not known.

Recommendations for future investigations

1. The bulk of the findings of this project support the idea that some constituent in the water emanating from underground springs is either directly deleterious to didymo and/or indirectly adversely affects didymo's ability to compete with other species. Whatever the factor(s) is (are) they would appear to be labile and may not have been measured in our chemical survey. For these reasons we believe it is important to follow up with a more detailed examination of the water chemistry of the springs to include parameters not recorded previously, in particular nitrite (NO_2^-) and ammonia-ammonium (NH_3, NH_4^+).

2. In-stream manipulation of alkalinity – using quarried limestone rock. Liming has been used to combat the effects of acidification in northern Europe and has been largely dismissed previously as unlikely to control *D. geminata.* Indeed, raising the pH and alkalinity where these chemical variables are low may create favourable conditions for *D. geminata*. However in the present context we would be starting with streams that are already circumneutral and are affected by *D. geminata.* Limestone rock is plentiful and could be placed in streambeds already affected by the alga so that it gradually dissolved. Relatively large amounts of rock may be required; however this is unlikely to adversely affect river ecosystems. This rock is already used to control lateral erosion in rivers.

Some forms of naturally occurring limestone are more soluble than others so a range of sources of the rock would be required. Elevation of calcium and bicarbonate ions in the water may not eliminate *D. geminata* but it might reduce its vitality and adverse effects.

3. Carry out controlled experiments in an artificial stream system, varying alkalinity, Ca, Mg, nitrate and other chemical constituents in a water supply that is known to be favourable for *D. geminata*. Use a range of concentrations including high values. For example, Ca and Na concentrations in Pupu Springs are around 55 and 53 mg/l, respectively (Kim and Hunter 1997), which are, respectively, at least twice and seven times the levels measured in the springs in this study. Since a range of environmental factors may cause declines in established colonies when transplanted to new environments, a detailed experimental set up is needed to control all other factors except source water, which can be manipulated.

4. In parallel with experimental studies, collate more general information about the water chemistry of spring-fed creeks in different areas, e.g., Christchurch streams, West Coast streams. This will allow assessment of risk in different areas, in the light of experimental results.

Acknowledgements

We thank Max Bothwell (Environment Canada, University of Victoria and Malaspina University-College) for reviewing this paper and providing constructive suggestions for improvement. We are also grateful to MAF Biosecurity New Zealand for funding this project, in particular Nadene Boyle and Christina Vieglais for their support, and Frances Velvin for helpful review comments. Helen Roulston (NIWA) produced the site map. Permission to use the hydrological data was kindly granted by Environment Canterbury, Environment Southland, and Meridian Energy.

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environments at four sites on *D. geminata* cell viability (as assessed by a staining technique) and other community variables.

Table 2. Results of t-tests to compare the means of water, depth, temperature conductivity, and pH between spring and river sites at each location.

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Appendices

Appendix 1. Substrate numbering system, plate in-situ and block arrangement.


WD 4
WD 5
WD 6

Appendix 2: Notes on growth on new plates placed in the river at the time of deploying the precolonised plates.

These plates were part of the original experimental design (see Methods) but turned out to be inappropriate and are not referred to further in the Results. Here we comment on biomass on these plates compared to that on the precolonised plates in the river.

In the original design (see Experimental Design), if the river plates were still in the colonisation stage at the start of the experiment (i.e., comprising small separated colonies rather than a continuous mat) we expected that after 30 days with no scouring floods, biomass on the river plates would exceed that on the colonisation control plates (where colonisation would be at an earlier stage). Also the mass of the community comprising live *D. geminata* cells (V_Wt) would have risen and would also exceed that on the control plates. This would confirm that the original colonies on the plates when they were moved were continuing to grow, despite being disturbed by being lifted from the river at the start of the experiment.

We expected that differences between the plates would reduce as exponential growth of the colonies continued, and as other factors, especially flow variability increasingly confounded any differences. Since all the precolonised plates were fully colonised at the start of the experiment, these planned "colonisation controls" could not show the required differences. Also because the time difference between partial colonisation (small colonies) and full colonisation was very short in most cases, the controls would not have been effective except over that very short period.

We found that after 30 days, biomass (as AFDM) on the river plates was higher than that on the new plates in all cases (Figure A1.1), implying that growth was continuing on the pre-colonised plates. The exception was at the Flaxy Creek river site, when there was also barely any growth on the new plates. The fact that biomass on the control plates either declined or levelled off between February and March at all three sites in the Mararoa and Oreti supports the suggestion that declines in biomass on the precolonised plates over that period were caused by high flows (see Discussion)

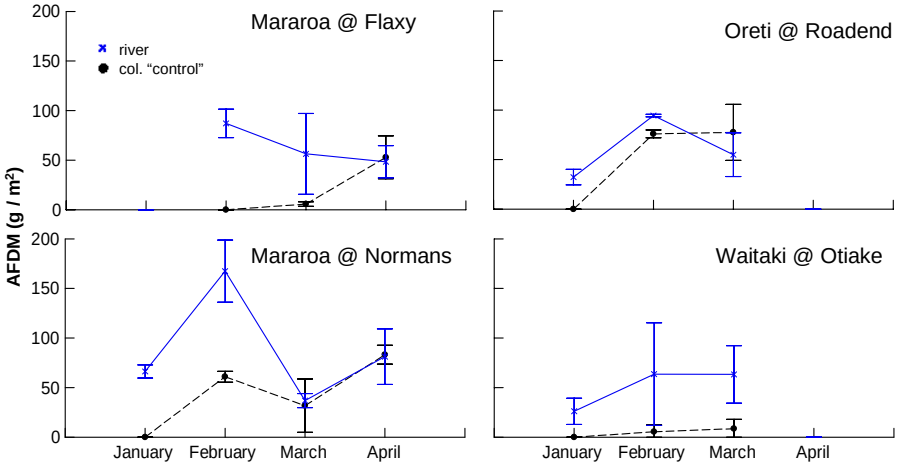
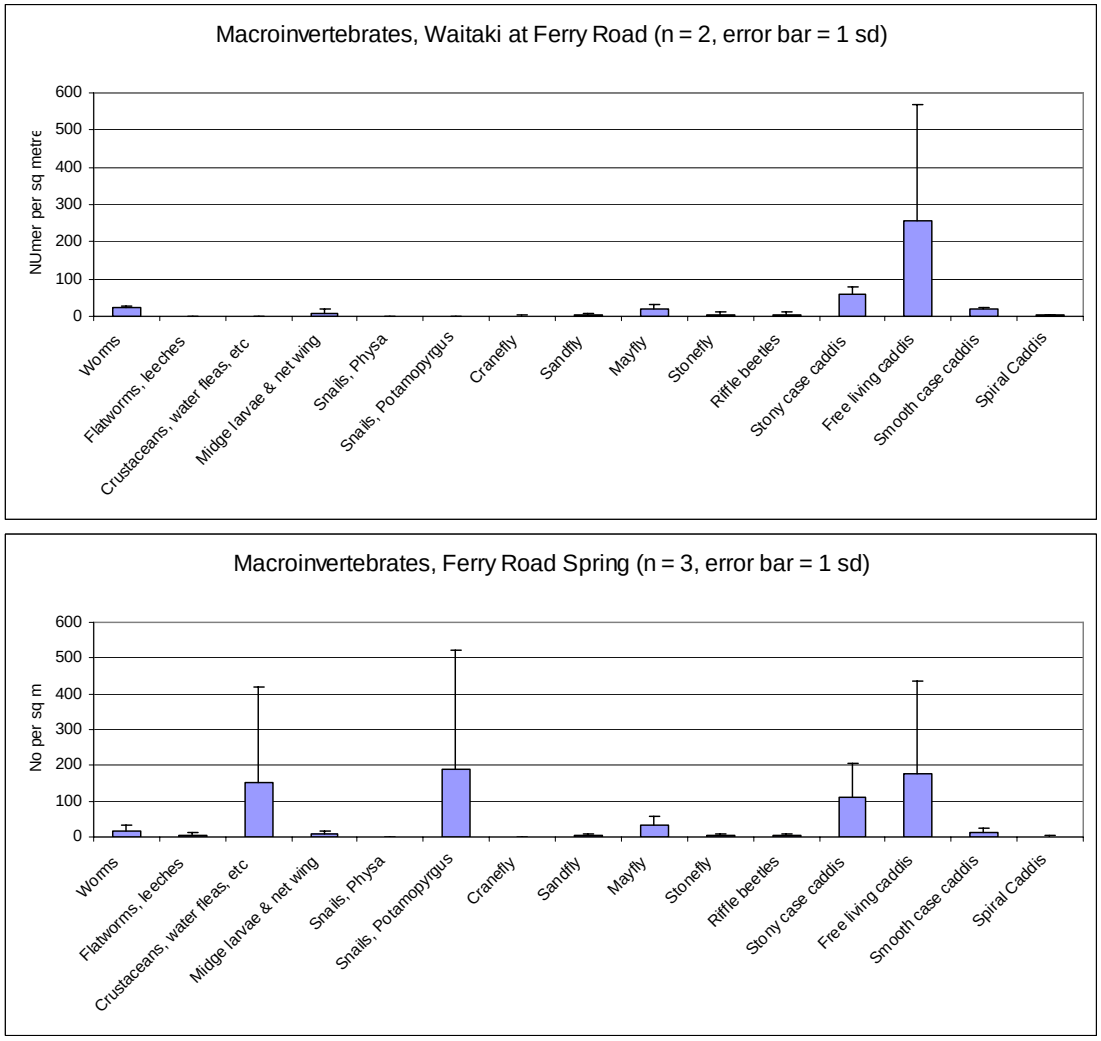
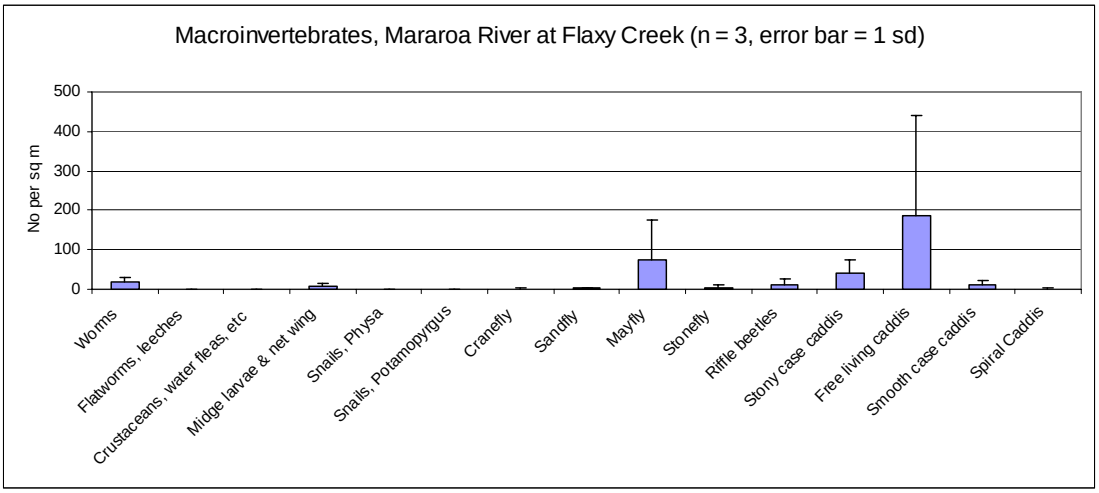


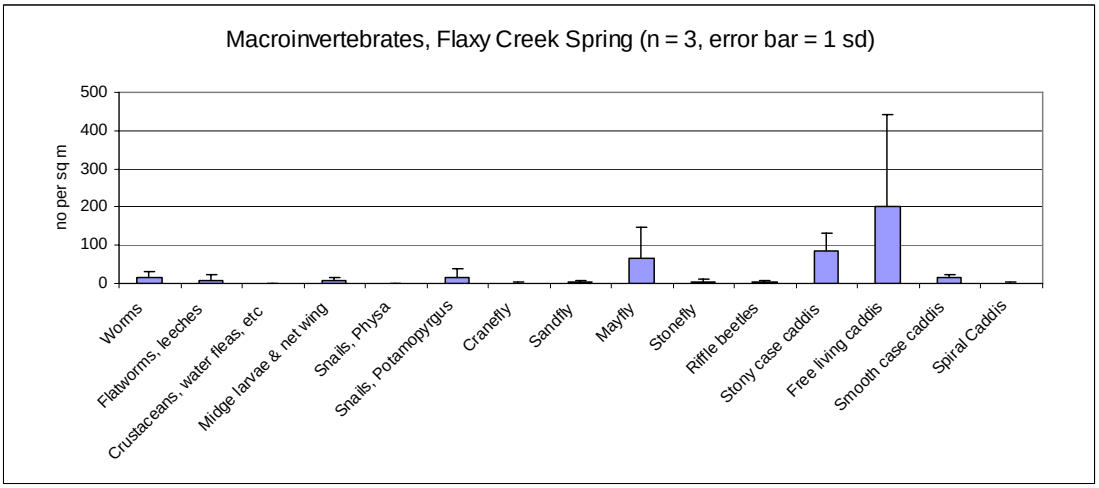
Figure A1.1. Mean ash-free dry mass (AFDM) measured on each sampling occasion on river plates and colonization controls in the rivers. To facilitate comparison with

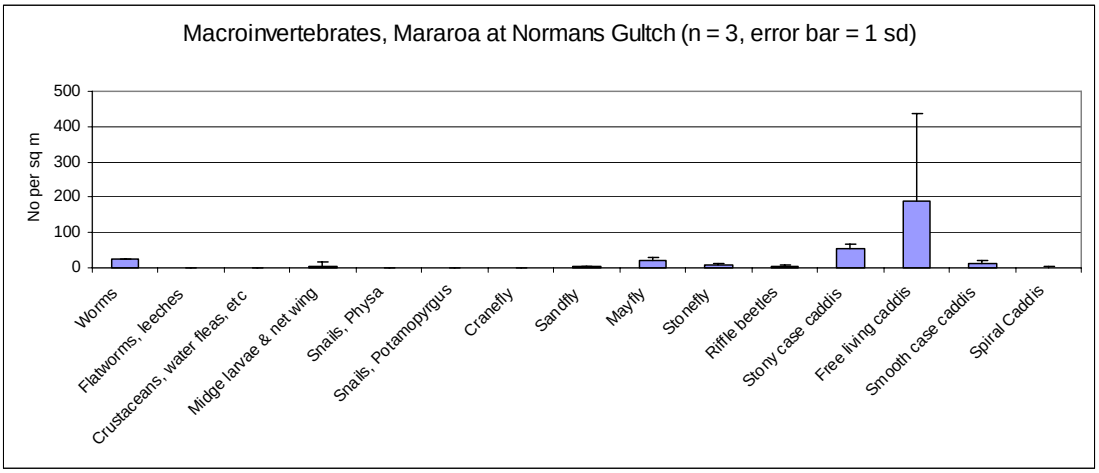
the hydrographs (Figure 2, main report) sampling occasions are shown by month. In each case, the first sampling was at time = 0, and subsequent occasions were at 30-day intervals. Error bars are standard deviations.

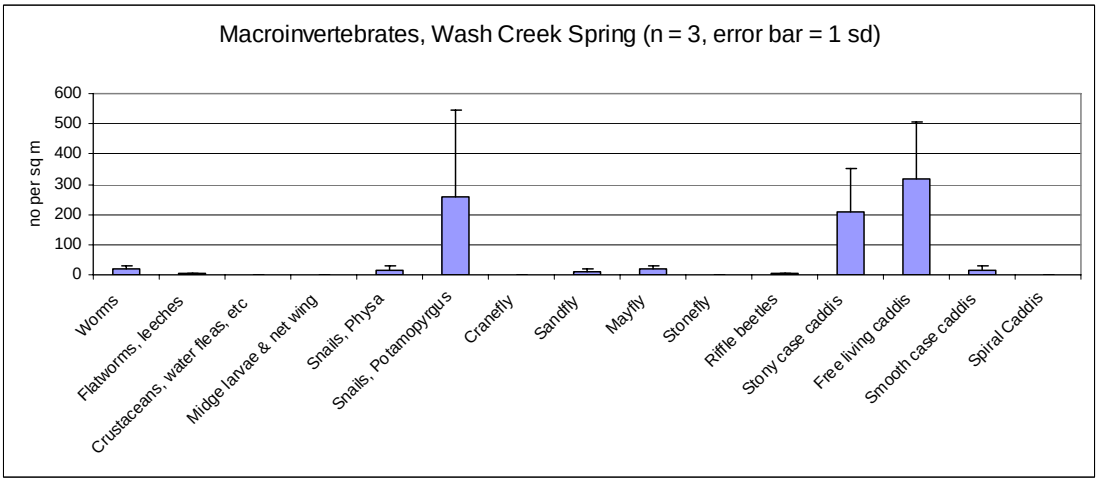
Appendix 3. Macroinvertebrate Charts.

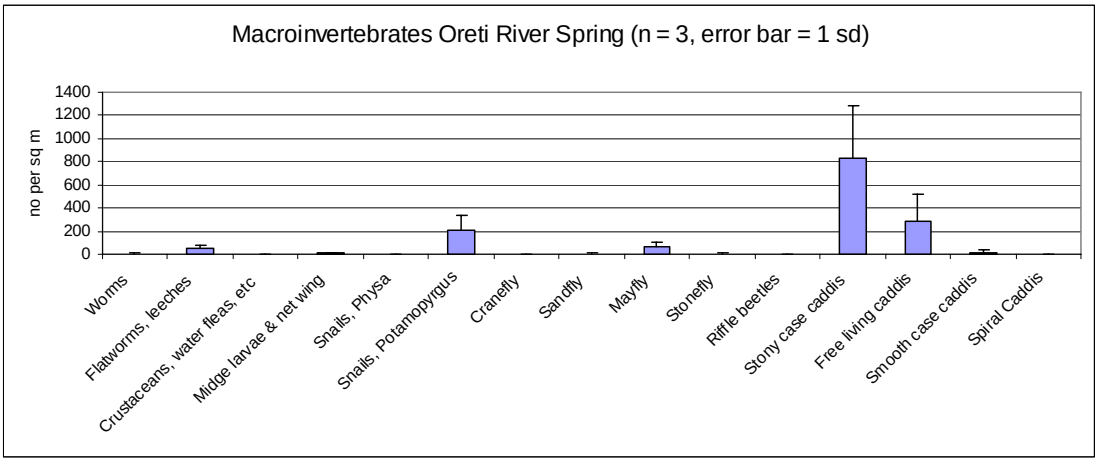


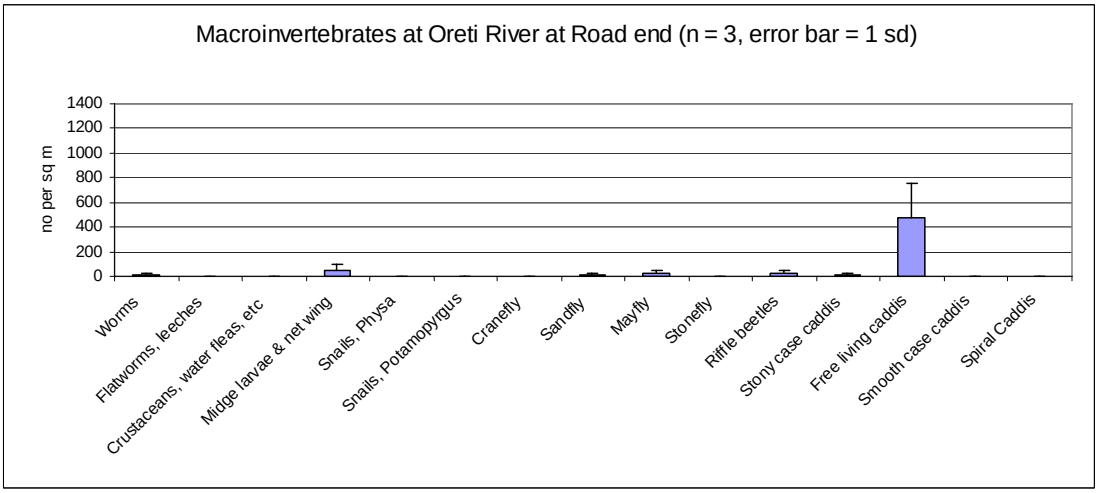

Macroinvertebrates, Mararoa River at Flaxy Creek (n = 3, error bar = 1 sd)
No per sq m
500
400
300
200
100
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis

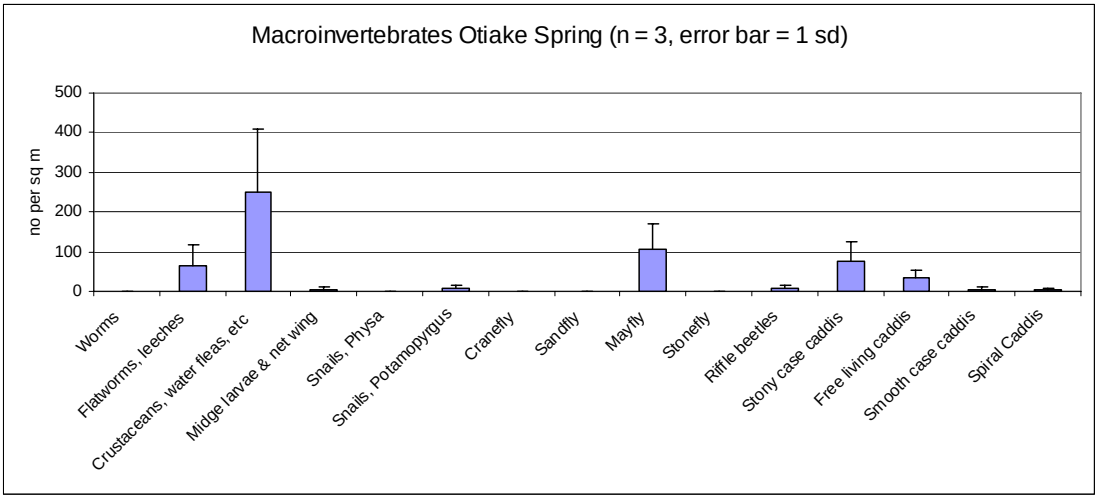

Macroinvertebrates, Flaxy Creek Spring (n = 3, error bar = 1 sd)
no per sq m
500
400
300
200
100
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis

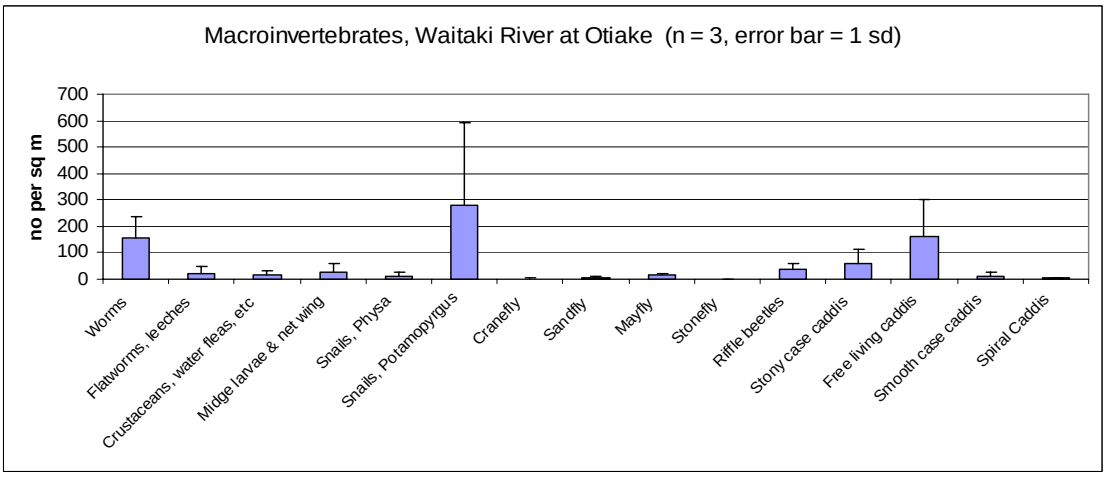

Macroinvertebrates, Mararoa at Normans Gultch (n = 3, error bar = 1 sd)
No per sq m
500
400
300
200
100
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis


Macroinvertebrates, Wash Creek Spring (n = 3, error bar = 1 sd)
no per sq m
600
500
400
300
200
100
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis


Macroinvertebrates Oreti River Spring (n = 3, error bar = 1 sd)
no per sq m
1400
1200
1000
800
600
400
200
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis


Macroinvertebrates at Oreti River at Road end (n = 3, error bar = 1 sd)
no per sq m
1400
1200
1000
800
600
400
200
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis


Macroinvertebrates Otiake Spring (n = 3, error bar = 1 sd)
no per sq m
500
400
300
200
100
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis


Macroinvertebrates, Waitaki River at Otiake (n = 3, error bar = 1 sd)
no per sq m
700
600
500
400
300
200
100
0
Worms
Flatworms, leeches
Crustaceans, water fleas, etc
Midge larvae & net wing
Snails, Physa
Snails, Potamopyrgus
Cranefly
Sandfly
Mayfly
Stonefly
Riffle beetles
Stony case caddis
Free living caddis
Smooth case caddis
Spiral Caddis

Appendix 4. Photographs of Spring Creeks.

Wash Creek Spring Creek.


Flaxy Creek confluence with the Mararoa.

Otiake Spring Site.