Effect of groundwater inputs on benthic algal biomass and species composition in the Waikanae River

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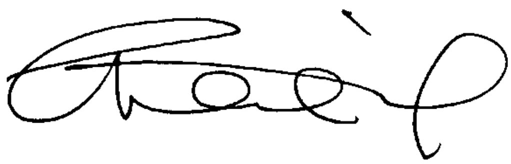

Cathy Kilroy

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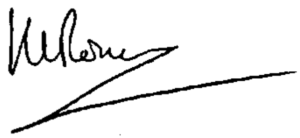

Helen Rouse

Executive summary

- The Kāpiti Coast District Council (KCDC) has investigated a range of options to secure a reliable, sustainable water supply to meet expected population growth. The preferred option over the next 50 years is River Recharge with Groundwater (RRwG). The RRwG option involves pumping groundwater into the river below Waikanae Water Treatment Plant (WTP) to replace river water taken upstream for town supply, thus maintaining minimum flows below the WTP as required by the resource consent for water abstraction. Augmenting flows below the WTP with groundwater would therefore allow abstraction of more river water than is currently permitted.

- Changes in water chemistry to the Waikanae River as a result of the groundwater discharge will depend on the relative volume of groundwater discharged into the river, its dilution, and the duration of discharge. Under a worst-case scenario (i.e. a 50 year low flow in the Waikanae River and peak day demand by 2060), water flow below the WTP would comprise *ca.* 70% groundwater to 30% river water. In practice, at Day 1 the ratio of groundwater to river water would be much less than this, with gradual increases to meet demand over the period the recharge would be in use.

- The ecological effects of groundwater discharge into the Waikanae River were investigated during January to April 2011 using within-river experimental channels (Suren et al. 2011). While algal biomass was higher in the experimental zone (with groundwater addition) than in the control zone (river water only), dissimilarities were attributed to differences in substrate size between the two zones, rather than differences in water chemistry.

- Some uncertainty still exists as to the effects of groundwater addition to the Waikanae River. As such, a further study using out-of-river channels was designed to determine what effect (if any) the addition of groundwater at 70% concentration would have on periphyton community composition and biomass from the Waikanae River over a 60-day period.

- Two treatments were tested: river water only and a mix of approximately 70% groundwater / 30% river water (called bore-mix hereafter), with five replicate channels each. Natural (cobbles) and a range of artificial substrates were selected to colonise and support algal growth within each channel. Only one artificial substrate (i.e. felted substrate) was selected for subsequent analyses.

- Dissolved reactive phosphorus (DRP) was variable in both river and groundwater, with concentrations consistently higher in groundwater than river water, while concentration of soluble inorganic nitrogen (SIN) was higher in river water than groundwater. The temperature of groundwater ranged between 14.2 and 15.4℃, while the temperature of river water within the supply tank was more variable, ranging from 5.5℃ to 16.5℃.

- Bore-mix water had a relatively warm, stable temperature (mean 14.5℃), low water velocity (~0.1 m/s) and high DRP and SIN concentrations. Under these conditions, only the filamentous diatom *Melosira varians* dominated periphyton biomass throughout the study. The Cyanobacterium *Phormidium* was present but dominated the community only on the first sampling occasion (Day 19). By Day 44 its dominance had reduced dramatically, and continued to decline over the course of the study.

- In comparison, periphyton exposed to river water at similar velocities and SIN concentration, but cooler temperatures and lower DRP concentration, did not achieve biomass levels as high as that measured in bore-mix channels. Unlike the channels containing bore-mix, both *Melosira varians* and *Phormidium* dominated the cobble substrate exposed to river water throughout the experiment.
- The observed temperature differences between the channels were not ideal but reflected the inherent background conditions during the experiment. Whether this would have affected the results cannot fully be ascertained, but the reduction in *Phormidium* cover in the bore-mix channels was surprising given its preference for warmer water, which should have favoured its growth.
- Invertebrate communities found within both river and bore-mix channels at the end of the experiment were similar, comprising of taxa indicative of healthy river ecosystems.
- Overall, this study found that:
    - Bore-mix water (with higher concentration of DRP and warmer temperatures) significantly increased periphyton biomass, but did not stimulate *Phormidium* growth;
    - There was no significant difference in community composition of periphyton exposed to bore-mix water compared to periphyton exposed to river water only.
- RRwG will have no effect on flushing flows or sustained low flow events (factors that influence cyanobacterial mat proliferations), but will have a low and temporary effect on water temperature and nutrient concentrations. Mitigation and adaptive management responses include using a hierarchy of bore preference (using groundwater with lower phosphorus concentrations first) and implementing a comprehensive monitoring regime above and below the groundwater discharge.
- It is likely that RRwG would be implemented in summer during extended low flow periods typically caused by drought. Repeating this out-of-stream experiment under a summer scenario of warmer temperatures, which are likely to favour *Phormidium* growth, would further test the hypothesis that RRwG is unlikely to increase the biomass and cover of *Phormidium* over and above what is found presently during summer low flows.
- Lines-of-evidence thus far from Suren et al. (2011) and this study show that RRwG is unlikely to increase biomass and cover of *Phormidium* over and above what is found presently during summer low flows in the Waikanae River.

1 Introduction

The Waikanae River supplies water to the Waikanae-Paraparaumu-Raumati district, via a run-of-river abstraction at the Waikanae Water Treatment Plant (WTP). During periods of low flow, groundwater from the Waimea aquifer is augmented with river water for public water supply. The taste and water hardness of groundwater makes this source of water unacceptable to the community (CH2M Beca 2010). The Kāpiti Coast District Council (KCDC) has investigated a range of options to secure a reliable, sustainable water supply to meet expected population growth. The preferred option over the next 50 years is River Recharge with Groundwater (RRwG), with a further 50 years supply secured by the Lower Maungakotukutuku Dam (CH2M Beca 2010). The RRwG option involves pumping groundwater into the river below the WTP to replace river water taken upstream for town supply, thus maintaining minimum flows below the WTP as required by the resource consent for water abstraction. Augmenting flows below the WTP with groundwater would therefore allow abstraction of more river water than is currently permitted.

Changes in water chemistry to the Waikanae River as a result of RRwG will depend on the relative volume of groundwater discharged into the river, its dilution, and the duration of discharge. Modelling has shown that the longest continuous period of bore use would be *ca.* 60 days (CH2M Beca 2010). Under a worst-case scenario (i.e. a 50 year low flow in the Waikanae River and peak day demand by 2060), water flow below the WTP would comprise *ca.* 70% groundwater to 30% river water (CH2M Beca 2010). In practice, at Day 1 the ratio of groundwater to river water would be much less than this, with gradual increases to meet demand over the period the recharge would be in use (CH2M Beca 2010).

The ecological effects of groundwater discharge into the Waikanae River were investigated during January to April 2011 using within-river experimental channels (Suren et al. 2011). In this study, benthic algae (periphyton) samples were collected from a partitioned experimental zone of the river receiving approximately 70% groundwater / 30% river water and a control zone receiving river water only. While algal biomass was higher in the experimental zone (with groundwater addition) than in the control zone (river water only), dissimilarities were attributed to differences in substrate size between the two zones, rather than differences in water chemistry. This conclusion was supported by substantial cyanobacterial mat cover occurring on large stable substrates within the river outside but immediately adjacent to the experimental zone, with significantly higher algal biomass here compared to that found within the experimental zone (Suren et al. 2011). Furthermore, algal biomass measured at two additional sites downstream (1.2 km and 4.4 km, respectively) where the groundwater influence would have been negligible was comparable with that found within the experimental zone.

Periphyton is an important, natural component of river ecosystems, converting energy from sunlight into organic matter, which is then utilised by invertebrates (and ultimately fish) for food. While periphyton also creates habitat for invertebrates and fish, under certain conditions it can proliferate and degrade the habitat. Increased algal growth can cause a shift in invertebrate community composition and change the appearance of streams and rivers, affecting the recreational values of rivers (Biggs 1996).

Some uncertainty still exists as to the effects of groundwater addition to the Waikanae River. As such, a further study using out-of-river channels was designed to determine what effect the addition of groundwater at 70% concentration would have on periphyton communities in the Waikanae River (Kilroy 2012). Out-of stream channels would allow direct comparison of the colonisation and growth of algae under the two different water types over a 60-day period, while all other environmental conditions remain the same. While algal development in channels is not expected to exactly mimic that growing naturally in the river, comparing river periphyton at the start and end of the study would help demonstrate how the proposed groundwater inputs might affect algal communities within the Waikanae River (Kilroy 2012).

Specifically, this study sought to address the following questions:

1. Is there a difference in biomass between periphyton exposed to groundwater / river water and periphyton exposed to river water only?
2. Is there a difference in community composition of periphyton exposed to groundwater / river water compared to periphyton exposed to river water only?

2 Methods

2.1 Channel construction

Channels were designed and constructed at Waikanae WTP by KCDC staff following specifications by Kilroy (2012) and are shown in Figure 2-1. Briefly, two 2 m^3 tanks were set up approximately 1 m off the ground to provide a continuous supply of river water and aerated[1] groundwater water to experimental channels. Algal experimental channels (2230 mm long x 120 mm wide x 100 mm deep) were constructed of 20 mm plywood and lined with black polythene sheets. A total of 10 channels were made allowing five replicate river channels and five replicate groundwater / river water channels (hereafter called bore-mix channels). A timber frame supported the channels off the ground at working height, with the head of the channels raised 50 mm to enhance the flow of water. Stainless steel gate valves (50 mm) and flexible hosing (Black Magic®; Blackwoods) connected tank water from one or both tanks into the appropriate channel, allowing water flows of 40-50 L/minute in each channel and correct mixing of groundwater and river water in bore-mix channels.

Figure 2-1: Experimental set-up showing layout of water supply tanks and channels (Day 1). Substrate layout and water flow are similar for all channels. Water drains into single large channel and returned to the Waikanae River.

Five channels were set up to receive river water only, and five channels were set up to receive both river and groundwater (i.e. bore-mix). Plywood baffles were placed at the head of each channel to allow for water mixing and comparable flow dynamics for all channels. Placement of channels alternated along the timber frame, so that river channels were

[1] Lack of exposure to atmospheric oxygen means that groundwater often has a low concentration of dissolved oxygen. Groundwater is therefore routinely pumped through an aeration tower at Waikanae WTP before use, so that the oxygen concentration in groundwater is comparable to that found in river water.

interspersed with bore-mix channels. A removable plywood outflow plate was constructed for each channel, which could be attached when the Waikanae River was in flood. These plates allowed river water supply pumps to be switched off, preventing sediments from flood waters entering the channels and allowing substrates to remain submerged within the remaining ponded channel water. Channels were placed in an un-shaded area facing west at the Waikanae WTP, and all channels and tank overflows drained into a single channel that returned water to the Waikanae River.

2.2 Periphyton substrates

Natural and a range of artificial substrates were selected to colonise and support algal growth within each channel as accrual rates can be highly variable (Biggs 1988). Therefore, artificial substrates with a range of different surface textures known to colonise algae were used. Pre-colonised cobbles retrieved from the Waikanae River and four different types of artificial substrates were placed in each channel, with each channel laid out in exactly the same way (Figure 2-2). The artificial substrates were:

- Moulded plastic artificial cobbles, with a tomentose (felt-like) surface (hereafter called felted substrate);
- Ceramic tiles (unglazed 22 mm x 72 mm);
- Open-celled Styrofoam® slab (Floracraft Corp., USA); and
- Brick pavers.

Only felted substrate was subsequently be chosen for analysis (see Section 3.2).

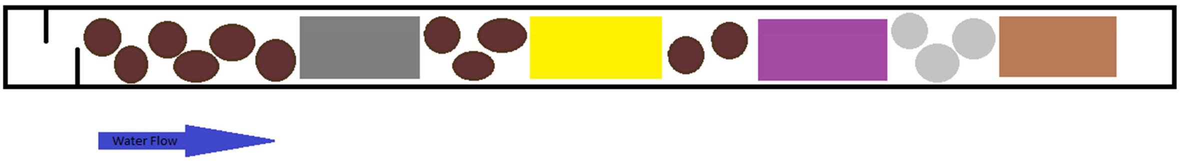

Figure 2-2: Schematic drawing of channel showing baffles at the head of the channel and the number and layout of substrates. Pre-colonised river cobbles are dark brown and bare cobbles (no periphyton) are light grey. Rectangles show type and location of artificial substrates: tiles (grey rectangle), Styrofoam (yellow), felted substrate (pink) and brick paver (light brown). Blue arrow indicates direction of water flow.

Felted substrate, tiles and Styrofoam were attached to brick pavers using either water-resistant glue (FixAll®; HOLDFAST) or Velcro (Dual Lock®; 3M). Sheets of felted artificial cobbles were pre-cut into individual 'cobbles' and glued to the paver to aid sample collection (Figure 2-3). Pre-colonised river cobbles were placed at the top end of each channel to help 'seed' artificial substrates with algal cells, and both pre-colonised and bare cobbles were interspersed between artificial substrates throughout the channels to enhance water depth and flow dynamics, ensuring water covered all substrates at a similar rate of flow (Figure 2-2).

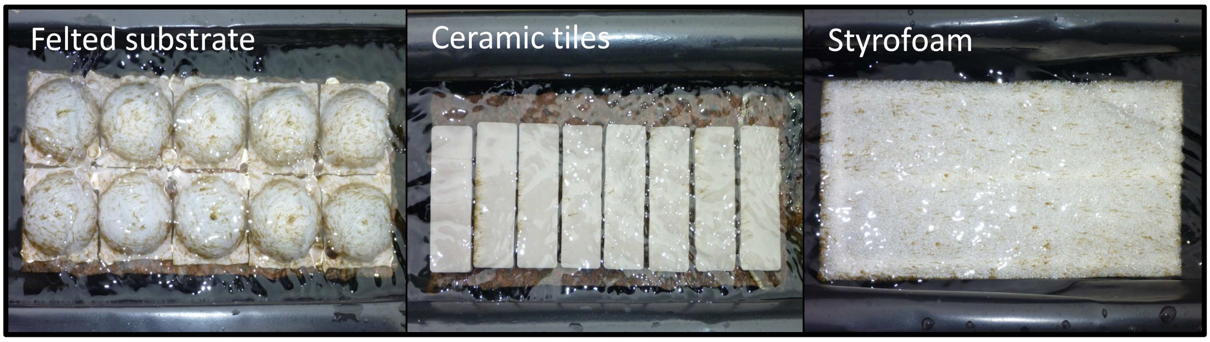

Figure 2-3: Artificial substrates in-situ on Day 1 of the experiment. Total depth of felted substrate was 70mm; ceramic tiles 60 mm; and Styrofoam 55 mm.

Pre-colonised cobbles were selected from the river and placed into the channels, ensuring mats of cyanobacteria and diatoms were included in each channel. Specifically, the first and third cobble in each channel had at least 60% cyanobacteria mat cover (~0.3 to 0.5 mm thick), while diatom mats of varying thickness dominated the periphyton cover on the second, fourth and fifth cobbles in each channel. All other pre-colonised cobbles contained a mix of the above periphyton communities as well as green filamentous algae. The experiment commenced on 29 April 2012 (Day 1) and concluded on 27 June 2012 (Day 60).

2.3 Environmental monitoring

Groundwater for this experiment was sourced from K10 bore. Spot readings of conductivity, pH and temperature from river and groundwater supply tanks, as well as from experimental channels, were measured regularly using a handheld meter (WP-81; TPS Australia). Conductivity measurements were used to determine the mix of groundwater and river water within the bore-mix channels ensuring approximately 70% dilution of groundwater in river water. Temperature was monitored continuously using temperature loggers (iButton®; Maxim) installed in each channel and in each supply tank. River water and groundwater samples were collected from the corresponding supply tank at the start of the experiment and then at Days 19, 44 and 60. These were filtered (0.45 µm cellulose syringe filter) and frozen within 2 hours of collection for subsequent nutrient analyses using Lachat methods (NIWA Laboratories, Hamilton).

Water flowing out of each channel was monitored regularly using a measuring jug and stopwatch to ensure flows between 40 to 50 L/minute were maintained. During periphyton sampling, when Styrofoam pavers were removed, water velocity was measured (using a Flo-Mate®; Marsh-McBirney) mid-way along each the channel where approximately 30 cm of unrestricted water flow within the channel allowed realistic, reproducible measurements. Water depth within channels and water depth of artificial substrates were also measured, and water flow across substrate surfaces were checked visually to ensure water coverage and flows across surfaces were similar for all channels.

2.4 Periphyton sampling

Similarities between periphyton growing in channels and periphyton naturally occurring in the river can be assessed by collecting samples from the river at the start and the conclusion of the study, and comparing biomass and communities with that in the channels. This comparison is only possible if there are no significant floods in the river during the course of the experiments, which would affect communities in the river but not in the channels (Kilroy

2012). Periphyton samples were collected from five river cobbles randomly selected on Day 1 from the same area where cobbles for the channels were retrieved. Periphyton was collected by first defining a circle on the cobble (diameter 40mm), then by scraping and scrubbing algae using brushes and scalpel blade from this area into a sample container. Unfortunately, several floods occurred in the Waikanae River during the experimental period, scouring much of the periphyton from the river bed so that further sampling was not practical. Therefore, comparisons between channel and river periphyton communities cannot be made.

Samples from natural cobbles and artificial channel substrates were collected on three occasions on Days 19, 44 and 60 of the experiment. Pre-colonised cobbles placed in the channel on Day 1 and selected for sampling on Day 19 and Day 60 had at least 60% *Phormidium* mat cover recorded on Day 1. Cobbles selected on Day 44 had mixed periphyton cover (diatom/*Phormidium* mats) recorded on Day 1.

One sample from each artificial substrate type was collected on Day 19, to determine which substrate colonised the most successfully, and was therefore appropriate to analyse. Periphyton from tiles and felted substrate were removed by scraping as described above, but with all periphyton collected. Periphyton samples from Styrofoam substrate were collected using a cookie-cutter (30 mm diameter) to cut two algal-covered discs from the Styrofoam slab for each sample. All samples were frozen within 4 hours and stored frozen until laboratory analysis. Analysis of periphyton from artificial substrates subsequently focused only on felted substrates (see Section 3.2).

2.5 Periphyton laboratory analysis

Periphyton samples (where n = 5 for each substrate / treatment type) were analysed for chlorophyll *a*, which is widely-used to estimate algal biomass. Chlorophyll *a* is a measure of the amount of live algae in a sample and therefore reflects the amount of organic matter that has accumulated from algal growth on the substrate. Laboratory analysis of chlorophyll *a* followed the methods described in Biggs and Kilroy (2000), with values normalised subsequently to give weight per m^2 of river bed.

Subsamples (where n = 3 for each substrate / treatment type) were also examined under a microscope at magnifications of up to 400*x*, for algal taxa identification. Relative abundance of each taxon was assessed qualitatively on a scale of 1 (rare in sample) to 8 (dominant in sample) (Biggs and Kilroy 2000). Note that the scores are on a non-linear scale: thus a score of 8 represents much more than 8 times a score of 1. Each taxon was assigned to one of the following groups:

- Cyanobacteria, such as *Phormidium*;
- Green algae, including filamentous taxa, such as *Ulothrix*; and non-filamentous taxa, such as *Scenedesmus;*
- Red algae, such as *Audouinella;*
- Filamentous diatoms, such as *Melosira varians* and *Fragilaria;*
- Diatoms that grow on long stalks, such as *Gomphoneis* and *Gomphonema*; or
- Single cell diatoms, such as *Synedra* and *Navicula.*

2.6 Invertebrates

The presence of invertebrates was noted when cleaning and dismantling substrates from river and bore-mix channels at the conclusion of the experiment. Each taxon encountered was identified and Macroinvertebrate Community Index (MCI) scores calculated for river and bore-mix treatments. The MCI is based on presence/absence of invertebrate taxa and was developed to assess nutrient enrichment/sedimentation in stony streams (Stark and Maxted 2007). The metric is commonly used to describe overall invertebrate community "health", where a score above 120 is interpreted as excellent, above 100 is good, and a score of less than 80 is interpreted as poor (Stark and Maxted 2007).

2.7 Data analysis

Periphyton relative abundance scores were scaled up in the following way to better reflect what was actually seen in a sample. Scores of 1, 2, 3, 4, 5, 6, 7, and 8 were therefore converted to 1, 2, 4, 8, 16, 32, 64, and 128 (i.e., a factor of two between each converted score), before standardising to percentage relative abundance for the analyses described below.

Bar graphs were used to explore differences in periphyton group composition (defined in Section 2.5) within bore-mix and river channels, with respect to substrate type. Ordination of the community data was then used to examine how periphyton communities, within river and bore-mix channels, differed from each other. Ordination using non-metric multi-dimensional scaling (NMDS; PRIMER) graphically represents community relationships, showing samples that are very similar in community composition closer together, and samples that are less similar plotted further apart (Clarke and Warwick 2001). Simply, if sample A is more similar to sample B than it is to sample C, then sample A will be placed closer on the ordination plot to sample B than to sample C. In preparation for ordination, data were converted into a Bray-Curtis dissimilarity matrix (McCune & Grace 2002).

Interpretation of how well NMDS represents the data is given by a measurement of 'stress', where a stress value of less than 0.1 indicates a good ordination with no real risk of drawing false inferences, and a stress value of greater than 0.2 indicates a plot that is difficult to interpret (McCune & Grace 2002). Species bubble plots of two dominant taxa (i.e. abundance scores of 8; see Section 2.5) were overlain on the NMDS ordination to better visualise of how these taxa were most likely responsible for the arrangement of the ordination.

Analysis of similarity (Two-way crossed ANOSIM; PRIMER) was used to test whether there were any differences in community composition between treatments (river water versus bore-mix) and time. Two-way analysis of variance (ANOVA; SYSTAT v. 12) was used to determine if any differences in algal biomass (as Chlorophyll *a*) existed between treatments (river water versus bore-mix) and time. The two-way ANOVA also tests the interaction between treatment and time, with a significant interaction effect indicating that biomass differs between water types over time. Chlorophyll *a* data was log-transformed to meet assumptions of normality and equality for parametric testing.

3 Results

3.1 Water chemistry / environmental parameters

The temperature of groundwater ranged between 14.2 and 15.4℃, while the temperature of river water within the supply tank was more variable, ranging from 5.5℃ to 16.5℃ with an average of 8.9℃ (Table 3-1). Mean temperature of w ater within the bore-mix channels ranged between 11.5 to 15.9℃ after mixing with riv er water (Figure 3-1). Mean temperature within the river channels ranged between 6.2 to 16.9℃ (Figure 3-1). The difference in water temperature between river and bore-mix channels was as high as 7℃ with an average difference of 3.2℃. Two temperature spikes were re corded in the river channels and the river water supply tank (on 25 May for ~ 1 hour, and 20 June for ~1.5 hours), indicating times when the river water pumps were temporarily inactive, reducing water flow and exposing loggers and substrates (Figure 3-1).

Table 3-1: Water chemistry and nutrient concentrations of river water and aerated groundwater. SIN (soluble inorganic nitrogen) concentration is the sum of nitrate-N, nitrite-N and ammoniacal-N. Measurements and samples were taken from water storage tanks. *n* = 4.

Determinant	River water	Ground water
Physico-chemistry parameters: mean (range)		
pH (units)	7.58 (7.29 - 7.66)	8.05 (7.86 - 8.12)
Temperature (℃)	8.9 (5.5 -16.5) †	14.5 (14.2 - 15.4)
Conductivity (µS/cm)	98 (92.8 - 101.8)	763 (677 - 803)
Nutrients: median (range)		
Ammoniacal-N (mg/m^3)	3.3 (2 - 5)	252 (249 - 256)
Dissolved Reactive Phosphorus (DRP) (mg/m^3)	9 (4 - 10)	24 (17 - 38)
Nitrate-N (mg/m^3)	318 (65 - 386)	1.8 (1 - 2)
Nitrite-N (mg/m^3)	< 1	< 1
SIN (mg/m^3)	323	254

† Maximum temperature spikes of 22.5℃ and 20℃ on 25 May 2012 and 20 June 2012 not included here. (Water level in river water tank was below temperature logger, and therefore ambient air temperature was being recorded).

Mean conductivity of groundwater and river water was 763 µS/cm and 98 µS/cm, respectively (Table 3-1). Mean conductivity within bore-mix channels was 567 µS/cm, equating to *ca.* 74% dilution of groundwater (Table 3-2). Water depth, water velocity and discharge were similar between river and bore-mix channels (Table 3-2). Depth of water flowing over substrates was similar across all channels for each substrate type (Figure 3-2).

Ammoniacal-N concentration in groundwater was higher (median 252 mg/m^3), compared to river water (3.3 mg/m^3) (Table 3-1). Nitrate-N concentration was elevated and variable in river water (ranging 65 to 386 mg/m^3) compared to groundwater (median 1.8 mg/m^3). Nitrite-N concentration in both water types was less than the detection limit of 1 (Table 3-1). Dissolved reactive phosphorus (DRP) was variable in both river and groundwater, with concentrations consistently higher in groundwater (median 24 mg/m^3) than river water (median 9 mg/m^3). Concentration of soluble inorganic nitrogen (SIN), which is a measure of all bioavailable soluble inorganic nitrogen compounds available for algal growth (i.e. ammoniacal-N + nitrate-N + nitrite-N) was higher in river water than groundwater (323 mg/m^3

and 254 mg/m^3, respectively) (Table 3-1). Nutrient concentrations in bore-mix channels were calculated using the ratio of groundwater to river water, determined from water conductivity (i.e. 74%). Concentration of DRP within bore-mix ranged from 14 to 30 mg/m^3 (median 20 mg/m^3), and concentration of SIN ranged from 206 to 288 mg/m^3 (median 272 mg/m^3).

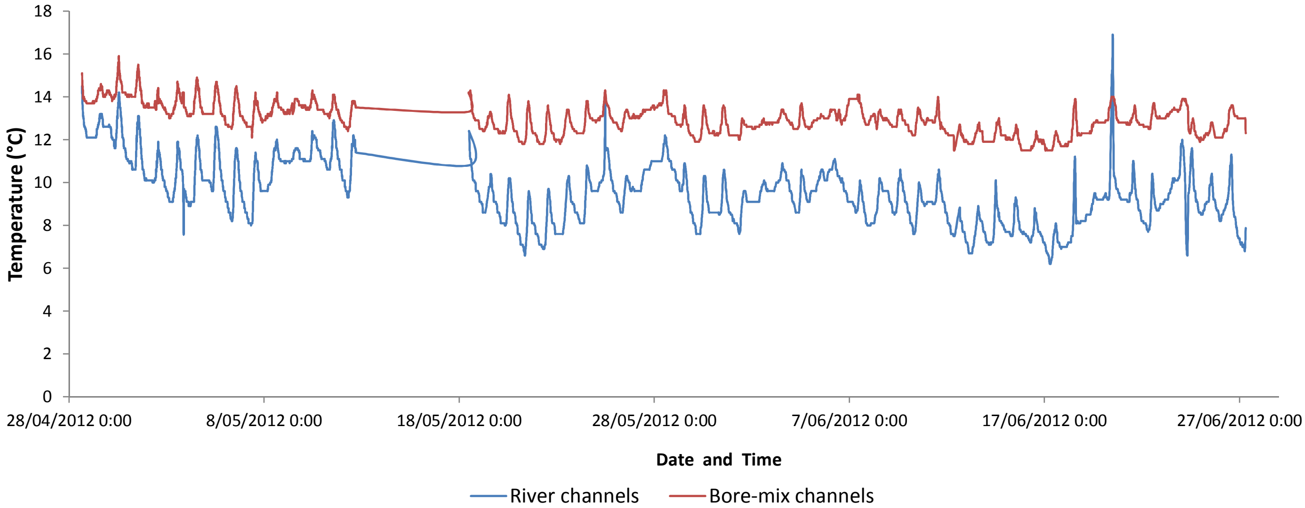

Figure 3-1: Temperature in river and bore-mix channels. Temperature spikes in river channels on 25 May and 20 June correspond to low water levels in the river water supply tank. Straight lines for both river and bore-mix temperature data between 12 and 18 May indicate temperature logger failure.

Table 3-2: Conductivity, pH and hydrological parameters of experimental channels.

Parameter	River channels	Bore-mix channels
Physico-chemistry parameters: mean (range)		
Conductivity (µS/cm)	100 (77.6 – 109)	567 (347 - 715)
pH (units)	7.51 (± 0.33)	8.07 (± 0.10)
Hydrological parameters: mean		
Water depth (mm)	66.2	67.4
Water velocity (m/s)	0.1	0.09
Discharge (m^3/s)	0.00079	0.00075

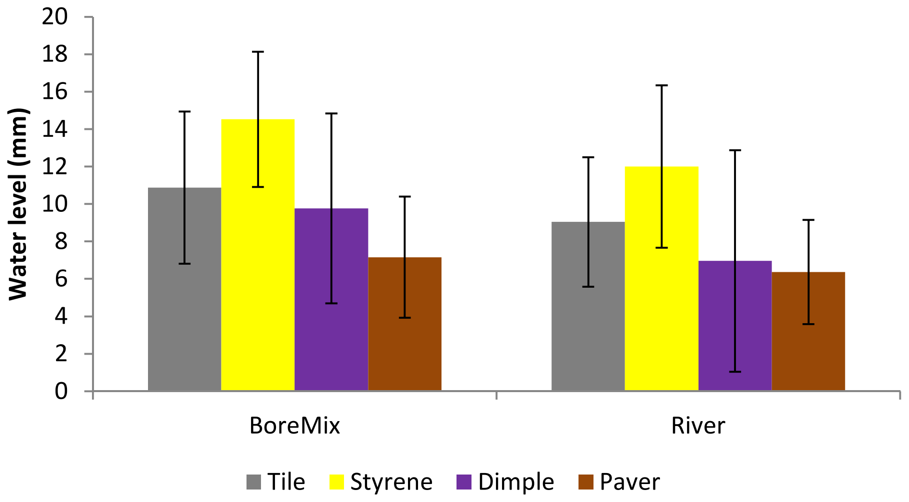

Figure 3-2: Mean water level flowing over artificial substrates in river and bore mix channels. Error bars show standard deviation.

3.2 Periphyton community composition

Abundant to dominant taxa (i.e. abundance scores of 7 and 8; see Section 2.5) from cobbles collected from baseline river samples at the start of the experiment were the cyanobacterium *Phormidium*, and the stalked diatoms *Gomphoneis minuta* var. *cassieae* and *Gomphonema parvulum*. Stalked diatoms made up approximately 60% of the algal community, with single cell diatoms and Cyanobacteria making up approximately 20% and 10%, respectively. Filamentous diatoms and green and red algae made up the remainder of the community. Several large flood events in the Waikanae River during the experiment precluded baseline samples being collected at the conclusion of the study, therefore comparisons between river and channel communities cannot be made.

Periphyton cover was observed on all artificial substrates by Day 19 (i.e. the first sampling event) (Figure 3-3). Analyses focused on natural cobble substrates and artificial felted substrates, with the latter appearing to successfully colonise and support growth of periphyton mats more than the other artificial substrate types. Prior to sampling on Day 19, a layer of grey coloured fine silt was deposited into (mainly) river water channels over periphyton on substrates. This occurred as a result of a ~43 m^3/s flood in the Waikanae River on 15 May 2012 when silt-laden river water flowed into channels before the water supply pumps could be shut down. One sample collected from a cobble at this time showed large amounts of silt present. By Day 44 (the second sampling event), the amount of silt covering the periphyton had reduced and none was noted in samples.

A total of 69 taxa made up the periphyton community across all samples. Of the periphyton collected from cobbles, 45 taxa were found in the river channels and 48 taxa in the bore-mix channels. Of taxa found on the felted substrate, 52 were in the river channels and 56 in the bore-mix channels. Abundant to dominant taxa found on natural and artificial substrates within the channels varied between treatment and time, with up to four taxa dominating the community throughout the experiment (Table 3-3). All four taxa were found on cobbles in the river channels. In these river channels, by Day 19 the filament-forming diatom *Melosira varians,* the stalked diatom *Gomphoneis minuta* var. *cassieae*, and cyanobacterium *Phormidium* were the dominant taxa, while by Day 44 *Melosira varians* and *Phormidium* dominated, and by Day 60 *Gomphoneis minuta* var*. cassieae*, *Melosira varians*, *Phormidium* and the green filamentous alga *Ulothrix* dominated. In comparison, only two taxa dominated cobble substrates in bore-mix channels by Day 19 (*Phormidium* and *Melosira varians*), while by Day 44 and Day 60, the only dominant taxon was *Melosira varians*.

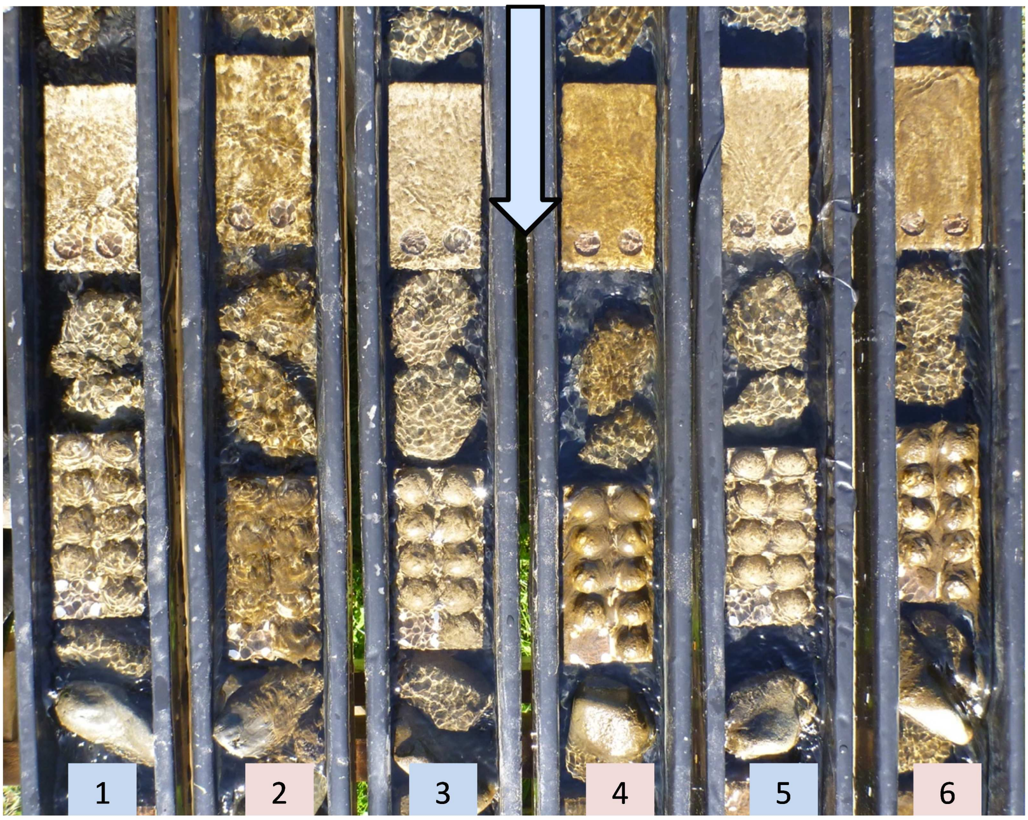

Figure 3-3: Section of Channels 1 to 6 after sampling event at Day 19. Styrofoam substrates are at top of photo and felted substrates are near bottom of photo. River cobbles are interspersed throughout channels. River channels are 1, 3 and 5; Bore-mix channels are 2, 4 and 6. Arrow shows direction of water flow. Khaki-green filamentous periphyton is clearly seen in bore-mix channels.

The number of dominant taxa on artificial substrates in river channels was also greater than in bore-mix channels. On day 19, both *Melosira varians* and *Gomphoneis minuta* var. *cassieae* dominated the periphyton. At Day 44, *Melosira varians* was the only dominant taxa, and on Day 60 both *Melosira varians* and *Ulothrix* were dominant. In comparison, there was one dominant taxon (*Melosira varians*) on artificial substrates from bore-mix channels at Days 19, 44 and 60 (Table 3-3).

Differences in community composition of periphyton groups between river and bore-mix channels are shown in Figure 3-4. Periphyton communities on pre-colonised cobble substrates within the river channels were generally similar at each sample event, apart from the final sample event when green algae increased to 10% by Day 60. While the proportions of stalked and single-celled diatoms were similar in cobble periphyton from bore-mix channels, the proportion of filament-forming diatoms increased from 20% (Day 19) to 60% (Day 60), and the proportion of cyanobacteria reduced from 40% (Day 19) to 3% (Day 60). In comparison, the proportion of cyanobacteria averaged 20% in the river channels over the 60-day experiment.

Table 3-3: Presence/absence of dominant algal taxa in river and bore-mix treatments. Taxa shown are where abundance scores are 7 and 8, and are listed with respect to substrate type and day of sample collection. Highlighted cells show presence of taxa.

Dominant Taxa	Cobbles						Felted Substrate					
	River			Bore mix			River			Bore mix		
Day	19	44	60	19	44	60	19	44	60	19	44	60
Melosira varians	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Phormidium	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No
Gomphoneis minuta	Yes	No	Yes	No	No	No	Yes	No	No	No	No	No
Ulothrix	No	No	Yes	No	No	No	No	No	Yes	No	No	No

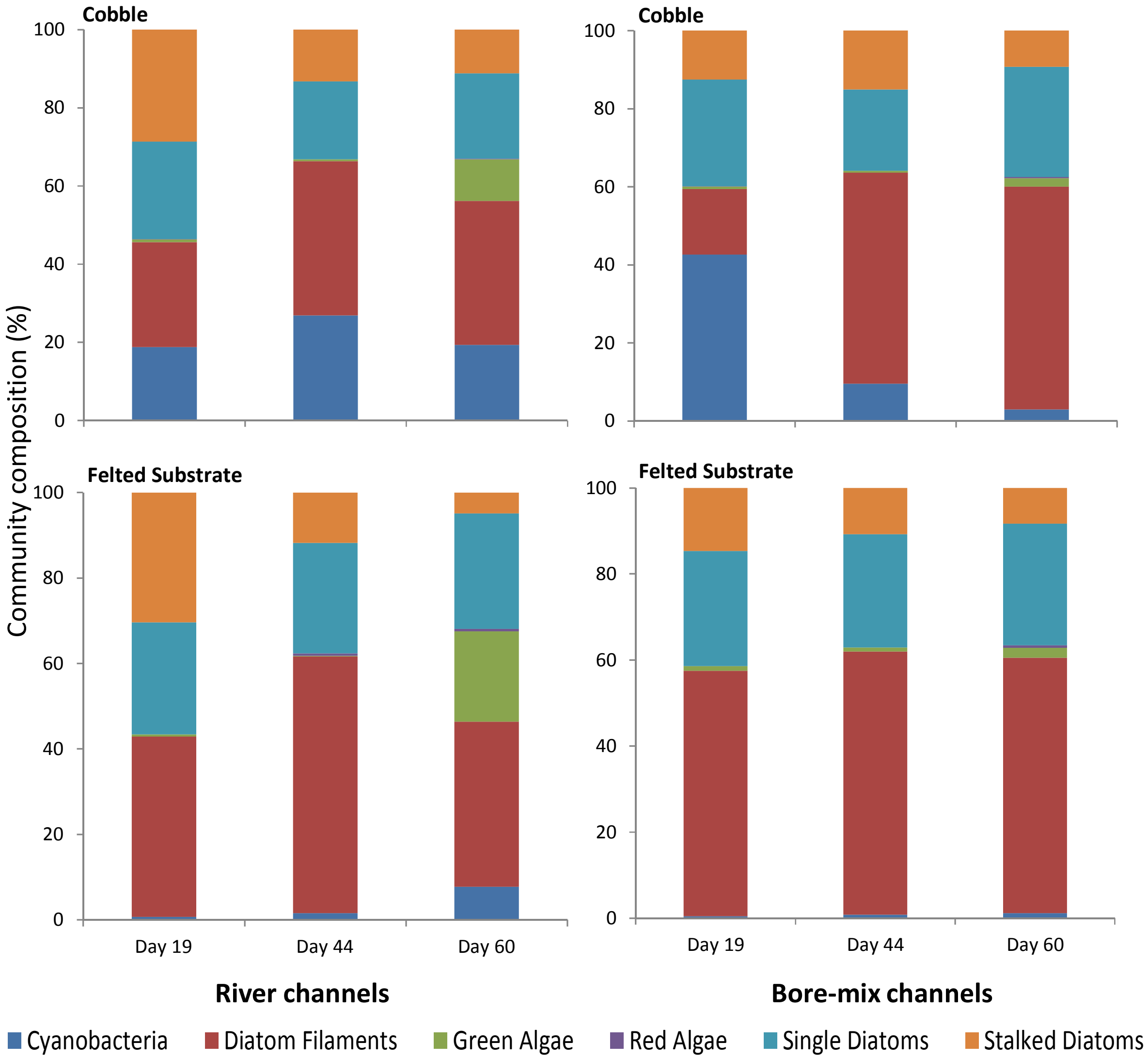

Figure 3-4: Comparison of community composition (%) of periphyton groups between river and bore-mix treatments on cobble and felted substrate over time. Periphyton groups are defined in Section 2.5.

Composition of the periphyton community colonising the artificial felted substrates was more variable in river treatments than in bore-mix treatments, with the latter supporting similar communities throughout the experiment. Cyanobacteria increased from 0.7% at Day 19 to 8% in the river treatments by Day 60, but made up just 1% of the community in the bore-mix treatments over the same period. The proportion of green algae was also higher in the river treatments, making up 20% of the community at Day 60 compared to 2% of the community in the bore-mix treatments.

The NMDS ordination shows that community composition within river channels was more variable than in bore-mix channels, with greater spread of samples (collected from both substrates) from river channels (blue triangles; Figure 3-5), compared to the grouping of samples from bore-mix channels (green squares; Figure 3-5). The ordination clearly illustrates the change in community on substrates exposed to bore-mix between Day 19 and Days 44 and 60. At Day 19, three samples (collected from cobbles) from bore-mix channels were more similar to river samples collected on three separate occasions (also collected from cobbles) (dark circle; Figure 3-5). The separate and tight grouping of all other samples from bore-mix channels demonstrates that communities exposed to bore-mix were relatively homogenous (red circle; Figure 3-5).

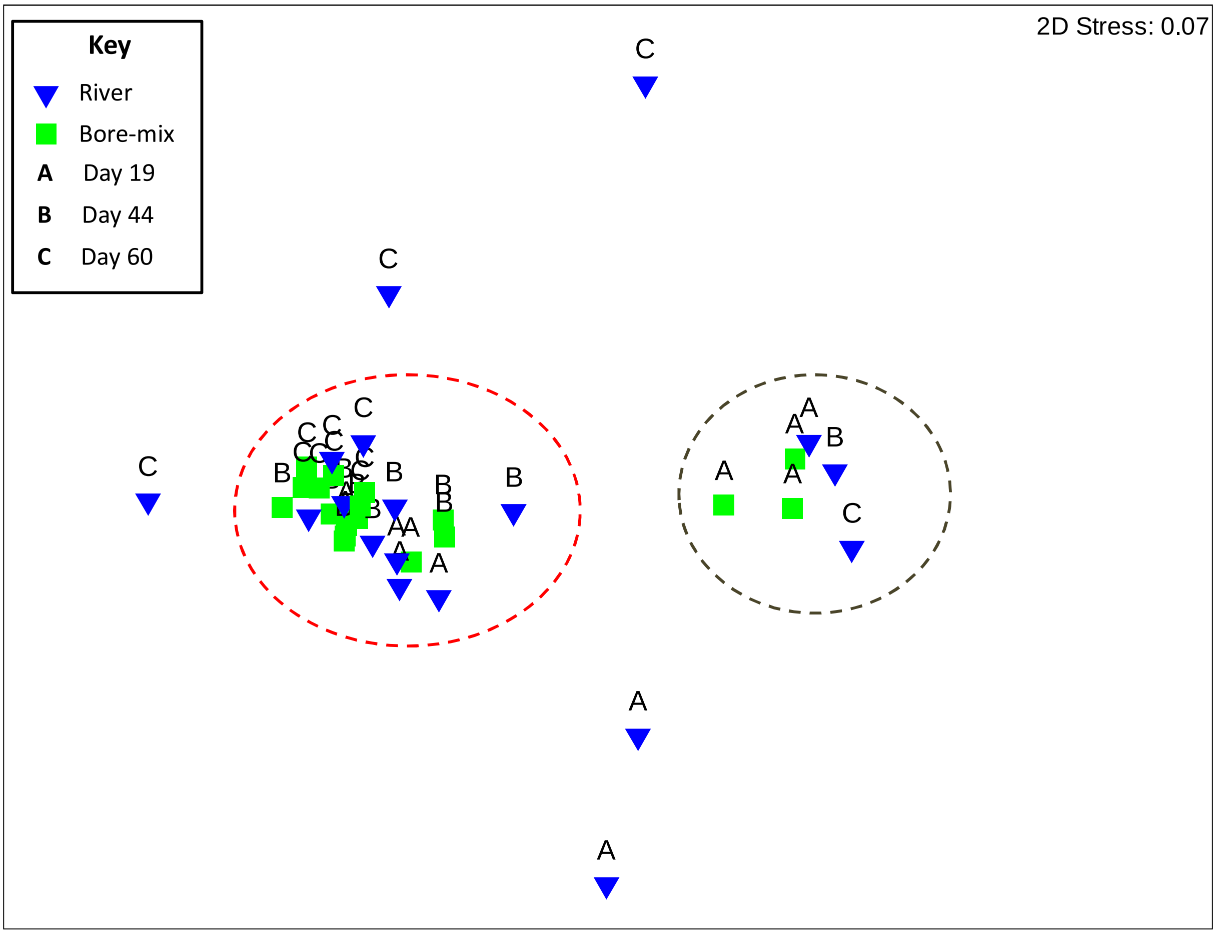

Figure 3-5: NMDS ordination of all periphyton data collected from river (blue triangles) and bore-mix (green squares) channels. Letters refer to date of periphyton collection (see Key for definition). Circles illustrate two discrete clusters of bore-mix samples: the dark circle contains three samples from bore-mix channels on Day 19, the red circle groups all other samples from bore-mix channels. A stress value of 0.07 indicates the ordination provides good representation of the samples.

Superimposing relative abundance of dominant taxa *Melosira varians* and *Phormidium* onto the ordination, using bubble plots, illustrates how these taxa are responsible for the two clusters observed within the ordination (Figure 3-6). Dominance of the cyanobacterium, *Phormidium,* in communities within bore-mix channels at Day 19 is responsible for the clustering of samples defined by the dark circle in Figures 3-5 and 3-6. By Day 44, periphyton communities within the bore-mix channels are dominated by the filamentous diatom *Melosira varians*, the alga responsible for clustering of samples defined by the red circle (Figures 3-5 and 3-6).

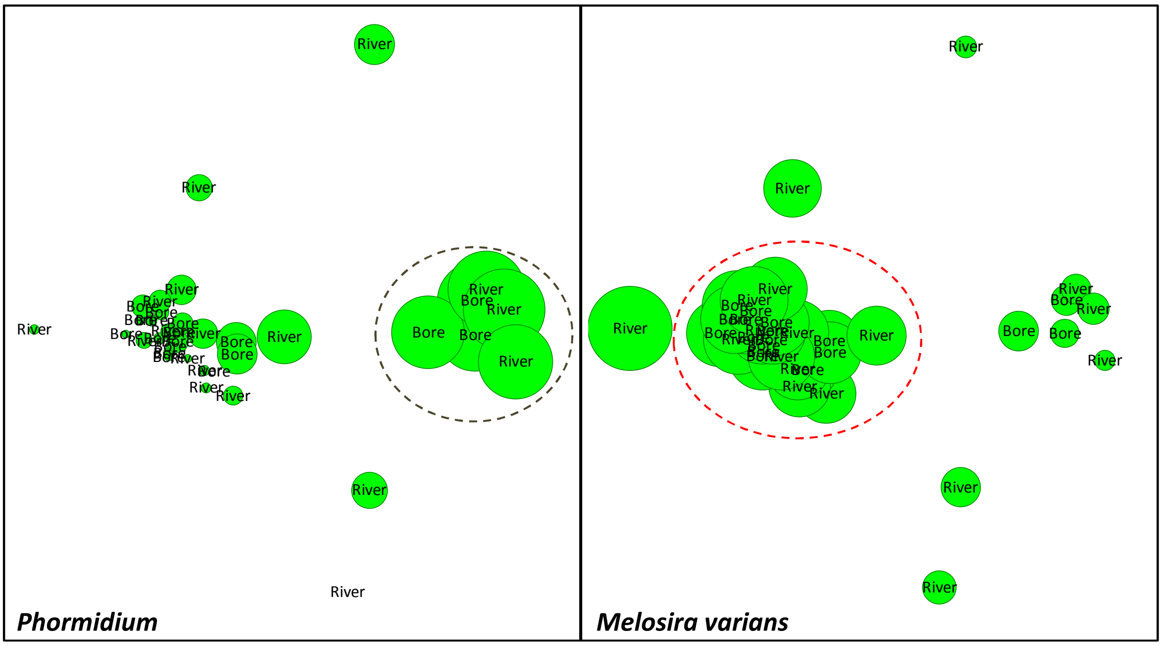

Figure 3-6: Bubble plots superimposed on NMDS ordination showing relative abundance of dominant taxa *Phormidium* and *Melosira varians* within samples. 'River' and 'Bore' refers to samples collected from river and bore-mix channels, respectively. Green circles increase in size as relative abundance increases. No green circle indicates the taxon was absent in that sample. The dark circle contains three samples from bore-mix channels on Day 19, the red circle groups all other samples from bore-mix channels (see Figure 3-5).

ANOSIM showed that there was no significant difference in community composition of periphyton between river or bore-mix treatments, on either cobbles or felted substrate. However communities did change significantly over the course of the experiment, on both cobbles (Global R statistic: 0.342, p-value = 0.023) and felted substrates (Global R statistic: 0.358, p-value = 0.001).

3.3 Periphyton biomass

Chlorophyll *a* from baseline river samples (Day 1) ranged from 94 to 614 mg/m^2, with a mean chlorophyll *a* of 242 mg/m^2. Several large flood events occurred after the experiment commenced, scouring substrate within the Waikanae River and removing around 90% of the periphyton. Therefore, further samples were not collected from the river on Day 60 as planned.

Chlorophyll *a* from pre-colonised cobbles was significantly higher in bore-mix channels compared to river channels (ANOVA; p-value = 0.020), and significantly different over time (ANOVA; p-value = 0.000). On Day 19, chlorophyll *a* on cobbles from river channels ranged from 636 to 1390 mg/m^2, with a mean chlorophyll *a* of 883 mg/m^2, while chlorophyll a in bore-mix channels ranged from 1049 to 1582 mg/m^2, with mean 1301 mg/m^2 (Figure 3-7). By Day 60, mean chlorophyll *a* was only 89 mg/m^2 in river channels and 199 mg/m^2 in bore-mix channels. The interaction effect of treatment and time was not significant indicating that biomass growth within the two treatments was similar at each sampling event.

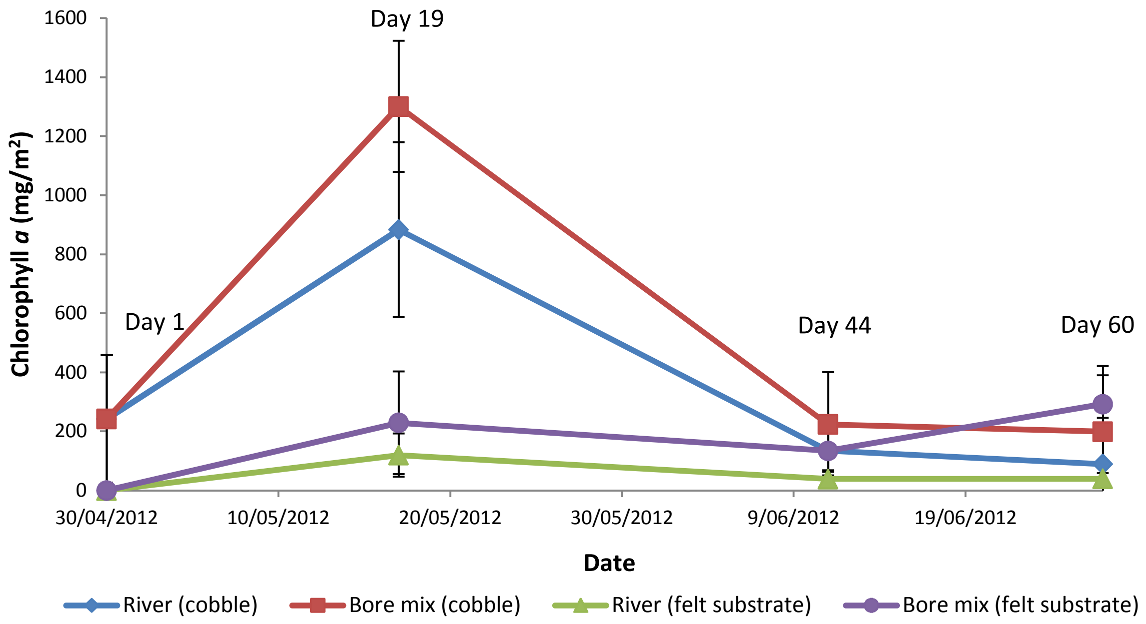

Figure 3-7: Mean chlorophyll *a* (as biomass) measured from cobble and felted substrate exposed to either river water or bore-mix. Error bars give standard deviation.

Similarly, chlorophyll *a* was significantly higher on felted substrates exposed to bore-mix channels than those exposed to river water (ANOVA; p-value = 0.000). Chlorophyll *a* on felted substrates within river channels ranged from 27 to 206 mg/m^2 (mean 120 mg/m^2), while in bore-mix channels chlorophyll *a* ranged from 97 to 531 mg/m^2 (mean 229 mg/m^2) (Figure 3-7). There was also a significant difference in chlorophyll *a* across time (ANOVA; *p*-value = 0.034), however the interaction effect of treatment and time for felted substrates was not significant.

3.4 Invertebrates

A total of 18 freshwater invertebrate taxa were identified at the end of the experiment (Table 3-4). Thirteen taxa found were “clean-water” EPT invertebrates (i.e., Ephemeroptera, Plecoptera, Trichoptera taxa), with 9 of these recovered from river channels and 11 recovered from bore-mix channels. The MCI score was 104 in the river channels and 127 in the bore-mix channel.

Table 3-4: Invertebrates found in river and bore-mix channels. Presence / absence of all invertebrates recovered from channels at the end of the experiment.

Invertebrate taxa		River channels	Bore mix channels
Trichoptera (caddisflies)			
	Hydrobiosis	Yes	Yes
	Olinga	Yes	Yes
	Baeroptera	No	Yes
	Costachorema	Yes	No
	Pycnocentria	No	Yes
	Pycnocentrodes†	Yes	Yes
	Neurochorema	Yes	Yes
	Oxyethira†	Yes	No
	Aoteapsyche	Yes	Yes
	Psilochorema	No	Yes
Ephemeroptera (mayflies)			
	Deleatidium	Yes	Yes
Plecoptera (stoneflies)			
	Zelandobius	No	Yes
	Zelandoperla	Yes	Yes
Diptera (two-winged flies)			
	Orthocladiinae	Yes	Yes
	Maoridiamesa	Yes	No
	Austrosimulium	Yes	No
	Aphrophila	Yes	Yes
Non-insects			
	Hirudinea	Yes	No

† denotes pupating invertebrates

4 Discussion

This study experimentally assessed the effects of simulated RRwG on algal communities during a late autumn / winter scenario. It addressed whether periphyton exposed to either bore-mix or river water differed in biomass or community composition. This work was done because of uncertainties in the findings of Suren et al. (2011) who attributed greater periphyton biomass in the experimental zone to differences in substrate, where the experimental zone contained larger, more stable substrate compared to the control zone. This experiment sought to establish whether the high algal biomass observed in channels exposed to groundwater / river water was due to increased nutrients from the groundwater, with substrates similar between both channel types. Specifically, this study sought to answer the following questions:

1. Is there a difference in biomass between periphyton exposed to groundwater / river water and periphyton exposed to river water only?
2. Is there a difference in community composition of periphyton exposed to groundwater / river water compared to periphyton exposed to river water only?

Artificial channels containing bore-mix and river waters were used to clarify substrate size / nutrient concentration ambiguities. Both substrate stability and nutrients influence periphyton accrual (Biggs 2000). Periphyton communities within artificial channels are not expected to mimic river communities where the water is sourced because the environment within the channels will be different. This is especially the case if algae are grown on artificial substrates (Biggs 1988; Nielsen et al. 1984). However, using channels and artificial substrates is a direct and reliable way of determining whether differences in water quality will affect algal growth (Kilroy and Bothwell 2011).

Groundwater conductivity, pH, temperature and SIN concentration from the K10 bore used in this study were similar to groundwater from bores used in 2011 field experiment (K4 and K6), while DRP concentration was much lower than in 2011 (72 mg/m^3) (Suren et al. 2011). During this experiment, DRP concentration was consistently higher in groundwater than in river water, whereas SIN was generally much higher in river water. Similar results were found by Suren et al. (2011) reinforcing the fact that the RRwG will increase phosphorus concentrations but likely reduce nitrogen concentrations.

Over the 60-day experiment using out-of-river channels, periphyton biomass was consistently and significantly higher on both natural and artificial substrate types exposed to bore-mix water. However, differences in community composition between bore-mix and river water were not significant over the course of the experiment, with the filamentous diatom *Melosira varians* being dominant or co-dominant within the periphyton community on both substrate types. The cyanobacterium *Phormidium* remained well established within algal mats on cobbles exposed to river water, but reduced over time on cobbles exposed to bore-mix waters. Expansive blooms of *Melosira varians* have been associated with elevated SIN concentration, while the effects of phosphorus enrichment on periphyton communities, particularly cyanobacteria, are variable (Biggs 2000; Borchardt 1996; Heath et al. 2011). In the Wellington region, Health et al. (2011) found that nitrogen may be more important than phosphorus in determining the presence of *Phormidium*, with cyanobacterial cover more prevalent at sites with proportionally more nitrogen than phosphorus.

Temperature also influences species composition and periphyton biomass (DeNicola 1996). Diatoms generally tend to dominate at cooler temperatures, cyanobacteria at warmer temperatures and green algae in between (DeNicola 1996). Season also affects *Phormidium* mat development, with cyanobacteria mats more likely to occur during summer (December to March) when river flows are below average and when water temperatures are above 14℃ (Heath et al. 2011; Nielson et al. 1984). In this study, bore-mix water had a relatively warm, stable temperature (mean 14.5℃), low velocity (~0. 1 m/s) and high DRP and SIN concentrations. Under these conditions, *Melosira varians* dominated periphyton biomass throughout the study. *Phormidium* was present but dominated the community only on the first sampling occasion (Day 19). By Day 44 its dominance had reduced dramatically, and continued to decline over the course of the study.

In comparison, periphyton exposed to river water at similar velocities and SIN concentration, but cooler temperatures and lower DRP concentration, did not achieve biomass levels as high as that measured in bore-mix channels. Unlike the channels containing bore-mix, both *Melosira varians* and *Phormidium* dominated the cobble substrate exposed to river water throughout the experiment. The observed temperature differences between the channels were not ideal but reflected the inherent background conditions during the experiment. Whether this would have affected the results cannot be fully ascertained, but the reduction in *Phormidium* cover in the bore-mix channels was surprising given its preference for warmer water, which should have favoured its growth. These results further imply that *Phormidium* mats are unlikely to increase as a result of discharging groundwater into the Waikanae River.

During summer, Suren et al. (2011) also found periphyton proliferated after exposure to bore-mix waters with much greater biomass within the experimental zone compared with periphyton exposed to river water within a control zone. Although periphyton samples were not analysed for community composition, visual assessments showed that biomass in the experimental zone was dominated by cyanobacterial mats and filamentous green algae. However, they also found that differences in substrate sizes between these two zones were likely to have confounded the experiment; quantities of periphyton biomass similar to that measured in the experimental zone occurred elsewhere within the river where substrates were stable and the groundwater influence was negligible.

Invertebrate communities found within both river and bore-mix channels at the end of the experiment were similar, comprising mostly of taxa usually found in stony streams with good water quality. The high MCI score from bore-mix channels reflects that these conditions supported an invertebrate community usually found in streams in good ecological condition. Algal scraper/grazers, such as the mayfly *Deleatidium* and the caddisflies *Olinga* and *Aoteapsyche* were common to both channel types, so grazing pressure on algae was likely to be similar across channels. These findings are in agreement with earlier studies that show invertebrate communities within the Waikanae River would not be adversely affected under the RRwG scenario (Suren et al. 2010; Suren et al. 2011).

5 Conclusion and recommendations

This study assessed the effects of simulated RRwG under an extreme worst-case scenario, using out-of-river experimental channels, on algal communities during late autumn / winter. The key findings are that:

- There was a difference in biomass between periphyton exposed to groundwater / river water and periphyton exposed to river water only. Bore-mix water (with higher concentration of DRP and warmer temperatures) significantly increased periphyton biomass, but did not stimulate *Phormidium* growth; and
- There was no significant difference in community composition of periphyton exposed to groundwater / river water compared to periphyton exposed to river water only.

Invertebrate communities present within both bore-mix and river channels at the end of the experiment were similar, comprising of taxa indicative of healthy river ecosystems. This is in agreement with earlier studies that show invertebrate communities within the Waikanae River would not be adversely affected by RRwG under a worst-case scenario (Suren et al. 2010; Suren et al. 2011).

This study adds to investigations conducted by Suren et al. (2011) where within-river experimental channels were used to assess the effects of groundwater discharge on algal, invertebrate and fish communities of the Waikanae River. Both studies were undertaken to determine the potential environmental effects of RRwG on the Waikanae River to help support a resource consent application made under the Resource Management Act.

Cyanobacteria are widespread in rivers and streams throughout New Zealand, occurring in a wide range of water-quality conditions, including low-nutrient waters (Biggs and Kilroy 2000). Cyanobacterial mats occur naturally in the Waikanae River, and as with other rivers in the Wellington region, can dominate the periphyton community during warm, dry summer months when river flow is low and stable (Heath et al. 2011; Milne and Watts 2007). While nutrients (along with temperature and light) are important for periphyton growth generally, factors controlling cyanobacteria mat cover instead appear to be the length of time between flushing flows[2] (where periphyton mats can be sloughed off substrate with increasing water turbulence) and sustained low river flows (Biggs 2000; Heath et al. 2011; Milne and Watts 2007).

RRwG will have no effect on flushing flows or sustained low flow events, but it will have a low and temporary effect on water temperature and nutrient concentrations. Other factors that influence periphyton biomass would not be affected (e.g. sunlight, grazing by invertebrates, substrate stability). Table 5-1 summarises the effect of RRwG on these main causal factors (reduced flushing flow events, elevated water temperatures, sustained low flow conditions, and nutrient inputs), and suggests mitigation or further action that may be required. Monitoring and adaptive management responses may include:

1. A hierarchy of bore preference, using bores supplying groundwater with the lowest phosphorus concentrations in the first instance;

[2] A flushing flow is one that can scour algal mats from the river bed, and is defined as a flow three times the median flow (Clausen and Biggs 1997; Wood et al. 2009).

2. Implement a monitoring regime in the river above and below the groundwater discharge point to record periphyton cover and water quality (including nutrients); and

3. Continue with monitoring protocols and action plans currently in place by Greater Wellington Regional Council and KCDC to assess cyanobacteria cover that naturally occurs during summer months to manage the risk to public health (e.g. Wood et al. 2009).

Table 5-1: Summary of main causal factors controlling cyanobacteria proliferations and the degree of effect these factors have on the Waikanae River under RRwG. Suggested mitigation or actions are also listed.

Causal Factor	Degree of effect of RRwG	Suggested mitigation / Action
Reduced or no flushing flow events	No effect. RRwG will have no effect on the magnitude or frequency of flushing flows in the Waikanae River.	No specific mitigation required.
Elevated water temperatures.	Low effect if RRwG is implemented during Autumn. Groundwater temperature was warmer than river water during the late autumn / winter out-of-river channel experiment, which may have stimulated periphyton growth in the experimental channels and slowed algal growth in the river channels. Unlikely to have an effect if RRwG is implemented during Summer. Groundwater is likely to be cooler than river temperatures during summer months.	Repeat out-of-channel periphyton experiment under a summer scenario to further test the hypothesis that biomass and cover of *Phormidium* is unlikely to increase over and above what is found presently during summer low flows in the Waikanae River.
Sustained low flow conditions	No effect. RRwG will have no effect on the low flows in the Waikanae River. The minimum flow of 750 m/s to protect in-stream river values will be maintained and not be affected.	No specific mitigation required.
Nutrient inputs	Low effect. RRwG will increase dissolved reactive phosphorus concentration. RRwG will likely lower soluble inorganic nitrogen concentration.	Consider developing a hierarchy of bore preference to use when implementing RRwG; discharge water first from bores where phosphorus concentration in groundwater is lowest. Implement a periphyton monitoring regime in conjunction with RRwG following protocols by Wood et al. (2009). Monitor nutrient concentration of groundwater discharge, and river water upstream and downstream of discharge point
Season	No effect if RRwG is implemented during Autumn. Unlikely to have an effect if RRwG is implemented during Summer.	Repeat out-of-channel periphyton experiment under a summer scenario to further test the hypothesis that biomass and cover of *Phormidium* is unlikely to increase over and above what is found presently during summer low flows in the Waikanae River.

This study was based on a scenario of a 1 on 50 year low flow and projected water demand of 32,000 m^3/day in the year 2060 (CH2M Beca 2010). Under this scenario, approximately 70% of the river flow below the WTP would be groundwater, discharging over a 60-day period. This is an extreme and worst-case scenario. In reality, there would be some years where there will be no need to implement RRwG. Further, when RRwG is implemented, 70% of groundwater is unlikely to be discharged from Day 1. The discharge is more likely to gradually increase to meet demand over the period the RRwG would be in use to a maximum discharge ratio of *ca.* 70% to 30% river water.

Overall, although the water chemistry of the Waikanae River below the WTP will change as a result of RRwG, for the duration of the groundwater discharge, lines-of-evidence thus far from this study and Suren et al. (2011) show that the addition of groundwater to the river is unlikely to increase the biomass and cover of *Phormidium* in the river over and above what is found presently during summer low flows.

6 Acknowledgements

I would like to thank Dave Bassett at the Waikanae Water Treatment Plant, along with Bruce, Brian, Les, and Dave, for their expertise and help, including building the experimental channels, measuring physico-chemical parameters and keeping a watchful eye on flood events throughout the experiment. Without their help, this experiment would not have been so successful. I would also like to thank Helena Campbell and Chris Cunningham (NIWA) for processing samples (for periphyton identification and chlorophyll analysis) so efficiently. Thanks also to Cathy Kilroy (NIWA) who provided valuable advice and comments throughout the study, and to Cathy and the team from CH2M Beca for their comments on the draft report.

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Appendix A List of periphyton taxa and abundance scores, which ranges from 1 (rare) to 8 (dominant); 0 is absent.

Taxa	Bore-mix	River	River-bed	Taxa	Bore-mix	River	River-bed
Cyanobacteria				Single-celled diatoms (continued)			
Chamaesiphon	1	1	0	*Cymbella aspera*	1	2	1
Heteroleibleinia	0	0	1	*Cymbella cuspidata*	0	1	0
Lyngbya	2	0	0	*Cymbella kappii*	4	4	4
Merismopedia	0	1	0	*Cymbella tumida*	6	6	4
Oscillatoria	1	1	1	*Encyonema gracile*	1	1	0
Phormidium	8	8	8	*Encyonema minutum*	5	5	4
Filamentous diatoms				*Epithemia adnata*	1	0	0
Aulacoseira	0	1	0	*Frustulia vulgaris*	1	1	0
Aulacoseira granulata	0	1	0	*Hantzschia*	1	0	0
Diatoma hiemale	1	1	1	*Meridion circulare*	1	0	0
Diatoma tenuis	2	2	1	*Navicula capitatoradiata*	3	2	1
Eunotia	1	1	0	*Navicula cryptocephala*	1	1	0
Fragilaria capucina	0	1	0	*Navicula gregaria*	1	1	0
Fragilaria crotonensis	0	1	0	*Navicula lanceolata*	5	4	3
Fragilaria vaucheriae	4	5	3	*Navicula margilithi*	2	1	0
Melosira varians	8	8	6	*Navicula rhynchocephala*	2	1	0
Tabellaria flocculosa	0	1	0	*Nitzschia*	4	4	5
Green algae				*Nitzschia acicularis*	2	0	2
Ankistrodesmus	2	1	1	*Nitzschia dissipata*	3	1	0
Cladophora	3	3	0	*Nitzschia linearis*	2	1	1
Closterium	1	0	0	*Pinnularia gibba*	1	1	0
Cosmarium	1	1	0	*Pinnularia viridis*	1	1	0
Microspora	2	2	0	*Planothidium lanceolata*	3	3	1
Oedogonium	2	3	3	*Reimeria*	3	3	1
Scenedesmus	1	1	1	*Rhoicosphenia curvata*	4	4	4
Spirogyra	1	4	0	*Rossithidium linearis*	6	6	5
Staurastrum	1	0	0	*Sellaphora*	1	0	0
Stigeoclonium	2	2	4	*Stauroneis*	1	0	0
Ulothrix	2	8	2	*Surirella ovalis*	1	1	1
Red algae				*Synedra acus*	4	4	3
Audouinella/Batrachospermum	1	2	1	*Synedra ulna* var. *biceps*	2	2	2
Single-celled diatoms				*Synedra ulna* var. *contra*	1	2	1
Achnanthes	2	3	2	Stalked diatoms			
Achnanthes oblongella	0	1	0	*Gomphoneis minuta* var. *cassieae*	6	8	8
Achnanthidium minutissimum	3	5	2	*Gomphonema angustum*	2	2	1
Cocconeis placentula	4	4	3	*Gomphonema minutum*	5	5	4
Cyclotella	1	0	0	*Cymbella aspera*	5	5	7