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Waikato River Bioassay Study 2013-14 - Assessment of nutrient limitation

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Signed by:

Date: 10 May 2016

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Waikato River Bioassay Study 2013-14

Assessment of nutrient limitation

Prepared for Waikato Regional Council

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Cover photo: Nutrient addition and zooplankton dilution incubation system. [Photo: Max Gibbs]

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Executive summary

Waikato Regional Council monitoring data from the Waikato River for the last 20 years show that there have been trends of decreasing chlorophyll *a* but increasing total nitrogen (TN) concentrations in the water. During this period, total phosphorus (TP) concentrations have remained relatively constant suggesting a knowledge gap or a lack of understanding of the factors that affect primary production in the Waikato River system. This report presents the findings of a series of monthly bioassay studies to assess possible nutrient limitation and zooplankton grazing effects in the Waikato River at four sites — Ohakuri, Karapiro, Ngaruawahia, and Rangiriri.

Nutrient limitation

The nutrient addition bioassays used river water that had been passed through a 40 µm sieve to exclude most zooplankton. Not all zooplankton would be excluded as small rotifers and zooplankton eggs may have passed through the sieve to grow in the incubated water. Consequently, the nutrient addition bioassay results are net responses.

- **Addition of N:** The bioassay studies found little or no stimulation of phytoplankton growth by the addition of N.
- **Addition of P:** There were small stimulations of phytoplankton growth by the addition of P at the outflow from Lake Karapiro in December 2013 and January, February and April 2014. There was a strong response (7-fold increase in biomass) to the P addition at the outflow from Lake Ohakuri in March 2014. These responses may be attributable to nutrient depletion in the lakes immediately upstream during thermal stratification in summer. A small response to the addition of P was found at all sites in April 2014.
- **Addition of N+P:** There were strong positive responses at the Karapiro site on all occasions implying that some level of nutrient depletion was occurring in Lake Karapiro, immediately upstream. Strong responses also occurred at Ohakuri with a pattern of increasing response strength from December 2013 to a maximum in March 2014 (7-fold increase in biomass). The similarity of the N+P response to the P addition response at that time suggests that the N+P responses were most likely to the P, but there was also a need for a boost in N.

There were occasional positive responses to the addition of N+P at Ngaruawahia and Rangiriri – Ngaruawahia in January, February and April 2014 – Rangiriri in December 2013, and March and April 2014. This may indicate that despite elevated TN and TP concentration, dissolved inorganic nutrients may have been in short supply in the lower river.

Zooplankton grazing

Zooplankton grazing incubations indicated that zooplankton grazing could be a major factor in the amount of phytoplankton biomass in the Waikato River at all sites. These incubations also indicated that zooplankton grazing could exceed phytoplankton growth at Ohakuri and Karapiro in December 2013 and January and April 2014, at Ngaruawahia in January 2014 and at Rangiriri in January and February 2014.

Nutrient limitation – zooplankton grazing interactions

A 5-day zooplankton grazing incubation in November 2013 was uninterpretable because all phytoplankton biomass had been consumed before the end of the incubation. This raises the possibility that zooplankton grazing may have affected the phytoplankton responses in the nutrient addition bioassays. Small rotifers and zooplankton eggs that passed through the sieve and hatched would grow in the 5-day nutrient addition bioassay incubations and reduce the amount of phytoplankton biomass recorded at the end of the incubation. This could explain why some results were lower than the controls and may indicate that results showing little or no response to a nutrient addition, were actually positive responses.

Other information

- Nutrient data show a substantial increase in nitrate, dissolved phosphorus, TN and TP concentrations between the Ohakuri and Karapiro sites. Chlorophyll concentrations increased below Ohakuri from January to March 2014, with step-wise changes between Ohakuri and Karapiro and Ngaruawahia and Rangiriri.
- Particle size analysis showed that TP concentrations were most likely to be associated with fine sediment in the size range of 6-8 μm . There was no significant correlation between TP and particle size in February and March 2014 during drought-like conditions, implying that the source of fine sediment was surface runoff during rainfall events.
- Phytoplankton species composition was dominated by diatoms when the flow in the Waikato River was high, but changed to flagellates when the flow was low. This is consistent with diatoms requiring turbulent water for support in the water column and flagellates being able to adjust their position in the water column in less turbulent waters.
- Zooplankton species were dominated by Cladocerans in November 2013 at all sites, and at the lake outflow sites of Ohakuri for the whole period, and of Karapiro through to March 2014. The Ohakuri site had an abundance of Copepods and Rotifers on all occasions, whereas Karapiro had fewer Copepods, and the Rotifer biomass was highest in January, February and March 2014. Apart from the high Cladoceran biomass in November at Ngaruawahia and Rangiriri, these two sites had low abundance of zooplankton from December 2013 through to April 2014, inclusive.

1 Introduction

Waikato Regional Council's (WRC) Waikato River monitoring data from the last 20 years show that there have been trends of decreasing chlorophyll *a* but increasing total nitrogen (TN) concentrations in the water. During this period, total phosphorus (TP) concentrations have remained relatively constant, suggesting a knowledge gap or a lack of understanding of the factors that affect primary production in the Waikato River system. WRC has commissioned the National Institute of Water and Atmospheric Research Ltd (NIWA) to lead a team of scientists from NIWA and the University of Waikato (UoW) to provide information on factors influencing phytoplankton growth that will assist better understanding of the trends in nitrogen and chlorophyll *a* in the main stem of the Waikato River and will inform river models.

1.1 Background

Information presented at the Waikato Economic Impact Joint Venture, Phase 2 – Water Quality meeting on 12 September 2013 raised concerns about a knowledge gap that could affect economic modelling related to river water quality. Long-term monitoring data from various locations down the main stem of the Waikato River show variability between the phytoplankton biomass (indicated by chlorophyll *a* concentrations) and the concentrations of TN and TP in the water (Vant 2013). Data from the last 20 years show that there have been trends of decreasing chlorophyll *a* but increasing TN concentrations. During this period, TP concentrations remained relatively constant, but may now show a weak trend of decrease. The expectation would be for phytoplankton biomass to increase in response to the increase in TN, which is mainly in the form of biologically available nitrate-nitrogen (NO₃-N). That it has not, points to a knowledge gap or lack of understanding of the factors that affect primary production in the Waikato River system.

Studies on the Waikato River and elsewhere show that the main factors that influence chlorophyll *a* concentrations include nutrient limitation and phytoplankton species (e.g., Magadza, 1978, 1979, 1980; Lam, 1979; 1981), and zooplankton grazing (e.g., James 1987, Ger et al. 2014). To have reducing chlorophyll *a* concentrations but increasing TN concentrations in the river over the last 10 years would require some level of phosphorus limitation to phytoplankton growth or zooplankton grazer populations that coexist with and control phytoplankton biomass in the river.

1.2 Present study

This report presents the findings of a series of bioassay studies over the summer of 2013/14 using nutrient addition and zooplankton dilution grazing incubation techniques on freshly collected Waikato River water from four locations — the outflows from Lakes Ohakuri and Karapiro, and the Waikato River at Ngaruawahia (upstream of the confluence with the Waipa River) and at Rangiriri. These results will provide information on factors influencing phytoplankton growth in the Waikato River that will improve understanding and inform river models.

These bioassay incubations were repeated at monthly intervals for six months starting in November 2013 and ending in April 2014. To complete this study, NIWA coordinated expertise from within NIWA and the UoW to conduct laboratory-based bioassay measurements that evaluated the relationship between phytoplankton growth and biologically available nitrogen and phosphorus, total nitrogen and phosphorus, and grazing pressure by natural zooplankton populations in the water collected from the four locations. The study included characterising the water chemistry at each location on each occasion and assessing the phytoplankton and zooplankton species composition and

particle size distribution of the suspended solids. These data are compiled as tables and figures in the body of the report or included in the appendices.

2 Methods

2.1 Field work

Water samples were collected at the four sampling locations using a weighted 10-L bucket lowered from bridges at Lake Ohakuri, Ngaruawahia and Rangiriri and from a landing below the power station at Lake Karapiro (Figure 1). At the Lake Karapiro site, the bucket was thrown out into the river and quickly hauled back to shore once it had submerged.

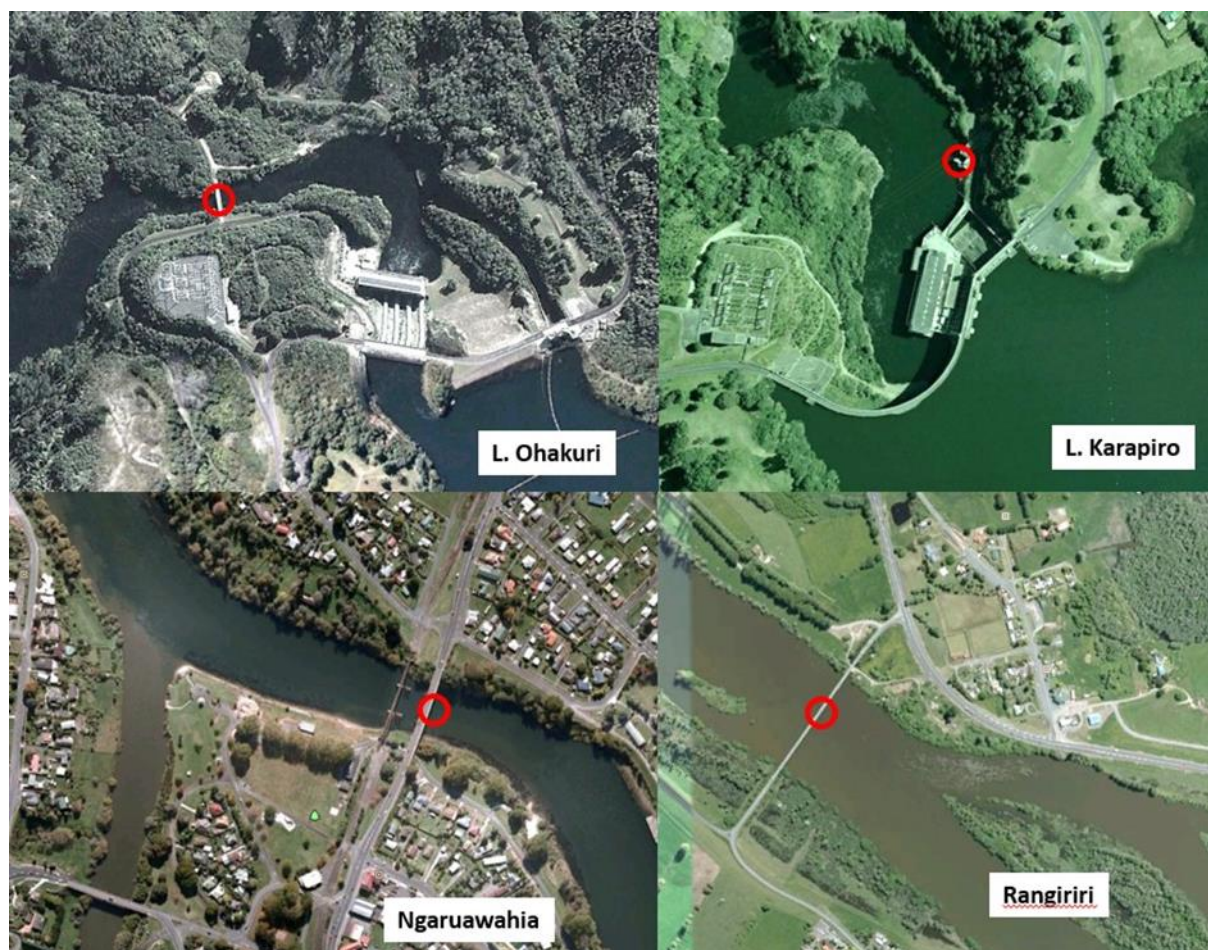


Figure 1: Sampling positions (red circles) at the four site locations on the Waikato River.

For zooplankton enumeration and biomass, 40 L of water was passed through a 40 μm mesh zooplankton net and the zooplankton collected were preserved in 50% ethyl alcohol/water mixture, on site.

Whole water was collected for water quality characterisation, phytoplankton species enumeration, particle size analysis and the zooplankton grazing incubations. Measurements on site included water temperature and pH. For the nutrient addition bioassay, water was sieved through a separate specially-cleaned funnel fitted with a 40 μm mesh to exclude the large zooplankton fraction. Additional sieved water was collected to prepare the dilution water for the zooplankton grazing experiments.

Because of the distance travelled and the potential for samples to warm up in the vehicle, the two upstream sites, Ohakuri and Karapiro, were visited in the morning and the samples were delivered to

the NIWA laboratory around midday. The two downstream sites, Ngaruawahia and Rangiriri, were visited after lunch and samples were returned to the laboratory by mid-afternoon. This schedule enabled the filtration of the pre-sieved water required for the zooplankton dilution incubations. Subsamples of whole water for water quality analysis, particle size analysis and phytoplankton enumeration were taken and refrigerated. The phytoplankton enumeration samples were preserved with Lugols iodine.

Water samples for the bioassay assessments were stored overnight at the incubation temperature in a controlled temperature room, in the dark.

2.2 Nutrient bioassay

Nutrient bioassays were done as 5-day growth assays on the 40 μm sieved water (i.e., without large zooplankton). Nutrient additions of +P, +N and +N+P were compared with control water (no addition) to estimate the nutrient status of the phytoplankton in the river at the time of sampling.

The 40 μm sieved water from each site was divided into four parts:

1. an addition of sodium nitrate to a final concentration of plus 140 mg m^{-3} as nitrate-N ($\text{NO}_3\text{-N}$)
2. an addition of potassium dihydrogen phosphate to a final concentration of plus 10 mg m^{-3} as dissolved reactive phosphorus (DRP)
3. additions of both N and P to final concentrations of plus 140 mg m^{-3} $\text{NO}_3\text{-N}$ and plus 10 mg m^{-3} DRP, and
4. no nutrient additions, to be used as the control.

The bioassay was set up using 400 mL wide-mouth, clear, PET jars on an incubation table in a controlled temperature room at 18°C. Lighting consisted of a bank of 12 daylight fluorescent lights 0.5 m above the jars. The lights cycled on and off by timer for a 16-hour light and 8-hour dark cycle. Light levels were $\sim 170 \mu\text{Mol m}^{-2} \text{s}^{-1}$, which is equivalent to 20% of the average natural daily ambient photosynthetically available radiation (PAR).

Each jar was loaded with 200 mL of water, with three replicates used for each treatment, including the control (total 48 jars). Each nutrient incubation jar was fitted with an air bubbler delivering a filtered air supply to maintain the CO_2 content in the water and to assist mixing (Figure 2).

The nutrient bioassay was run for a nominal 5-day period, allowing for setup and takedown time and delays, and used chlorophyll *a* concentrations to assess phytoplankton growth. Chlorophyll *a* concentration were measured in the initial water and then in all jars at the end of the 5-day incubation period. Phytoplankton growth was assessed as the difference between initial and final concentrations, and the growth response to nutrient additions was assessed as the proportional increase in chlorophyll *a* concentrations in the treatments relative to the control at the end of the incubation period.



Figure 2: Bioassay incubations in a controlled temperature room under artificial lighting. (Left) showing the arrangement of the 48 nutrient addition incubation jars (green aeration tubes) beside the 48 non-aerated zooplankton dilution incubation jars (no tubes). (Right) a close-up of the aeration bubblers in the nutrient addition incubation jars. Large bottles are incubations of big zooplankton.

2.3 Zooplankton grazing effects bioassay

The zooplankton grazing effects bioassay was done as a 24-hour incubation based on the sequential dilution method of Gallegos et al. (1996). The technique assumes that zooplankton filter feed on phytoplankton at a constant rate and that as the sample is diluted, a point will be reached where their filtering efficiency of the zooplankton will be so reduced that phytoplankton growth will be unaffected.

The bioassay was conducted on raw water which was progressively diluted with filtered (Whatman GF/C glass fibre filter) water from the same site. This means the dilution water has the same chemical characteristics as the raw water but without zooplankton or phytoplankton. The bioassay jars were the same as used for the nutrient bioassays (section 2.2), but without nutrient additions. Treatments consisted of diluting duplicate raw water with dilution factors of 1 (raw water control), 0.6, 0.4, 0.2, 0.1 and 0.05. Chlorophyll *a* concentration was measured in the initial water and then in all jars at the end of the 24-hour incubation period. Zooplankton grazing effects were assessed from the slope and vertical intercept of a linear regression through the chlorophyll *a* concentration data after correction for dilution at the end of the incubation period.

The grazing by big zooplankton was tested by collecting the >150 μm zooplankton from 10 L river water on a soft nylon 150 μm mesh plankton net and immediately transferring them into a 2-L glass Schott bottle of raw river water. These bottles were transferred to the laboratory in the dark at ambient temperature and subsequently incubated at 18°C under artificial lights with a 16:8 hour light:dark cycle for 24 hours next to the zooplankton dilution bioassays (Figure 2). At the end of the incubation period, duplicate water samples were taken for chlorophyll *a* measurements as an assessment of grazing pressure or growth.

2.4 Water chemistry analysis

Physical measurements made on site included temperature and pH.

In the NIWA laboratory the raw water samples were analysed for:

- dissolved reactive phosphorus (DRP)
- particulate phosphorus (PP)
- total phosphorus (TP)
- nitrate+nitrite nitrogen ($\text{NO}_3\text{-N}$)
- ammoniacal nitrogen ($\text{NH}_4\text{-N}$)
- particulate nitrogen (PN)
- total nitrogen (TN)
- particulate carbon (PC)
- total suspended solids (TSS)
- volatile suspended solids (VSS)
- turbidity
- particle size
- chlorophyll *a*.

Dissolved organic phosphorus (DOP) and nitrogen (DON) were estimated by difference.

2.5 Biological analysis

2.5.1 Phytoplankton

Raw water was assessed for phytoplankton species composition by cell counts per unit volume and biomass was expressed as biovolume.

2.5.2 Zooplankton

Zooplankton samples at each site were collected by passing 40 litres of raw river water through a 40 μm plankton net. Zooplankton species composition was determined at the UoW on the preserved sample. Zooplankton subsamples were enumerated in the laboratory at $\sim 30\times$ magnification under a stereo-dissecting microscope in aliquots until at least 300 individuals were encountered. Zooplankton biomass was estimated from the samples using length–weight relationships for crustaceans, and from tabulated median values, supplied by Lauridsen et al. (2005). Where possible, at least 10 randomly encountered individuals of each crustacean species were measured.

2.6 Physical data

2.6.1 Particle size

Unpreserved raw water samples were analysed for particle size distribution using an Eye Tech laser – based particle size analyser in an ACM-104A Liquid flow 10mm x 10mm cell. Data were presented graphically as particle volume and particle number histograms expressed as cumulative proportions (%) of the whole sample.

2.6.2 River flow data

An estimate of flow in the main stem of the Waikato River on each sampling day was obtained from the Mighty River Power web site and used the discharge from the Karapiro Hydro Dam as the reference flow.

2.7 Data handling

Where possible, analytical data has been collated into tables of related information and/or processed graphically. All data collected has been collated in separate appendices and electronic copies are held on the project drive server at NIWA in folder EVW14205.

3 Results

3.1 Nutrient addition Bioassays

The nutrient addition bioassay results are presented graphically (Figure 3) with the data expressed in proportional change over control. This interpretation assumes the value 1 represents no change while the value 2 indicated a doubling of the biomass, as indicated by chlorophyll *a* concentration, in response to the nutrient addition over the 5-day incubation period. Values less than 1 indicate that the phytoplankton biomass declined. Note differing vertical scales.

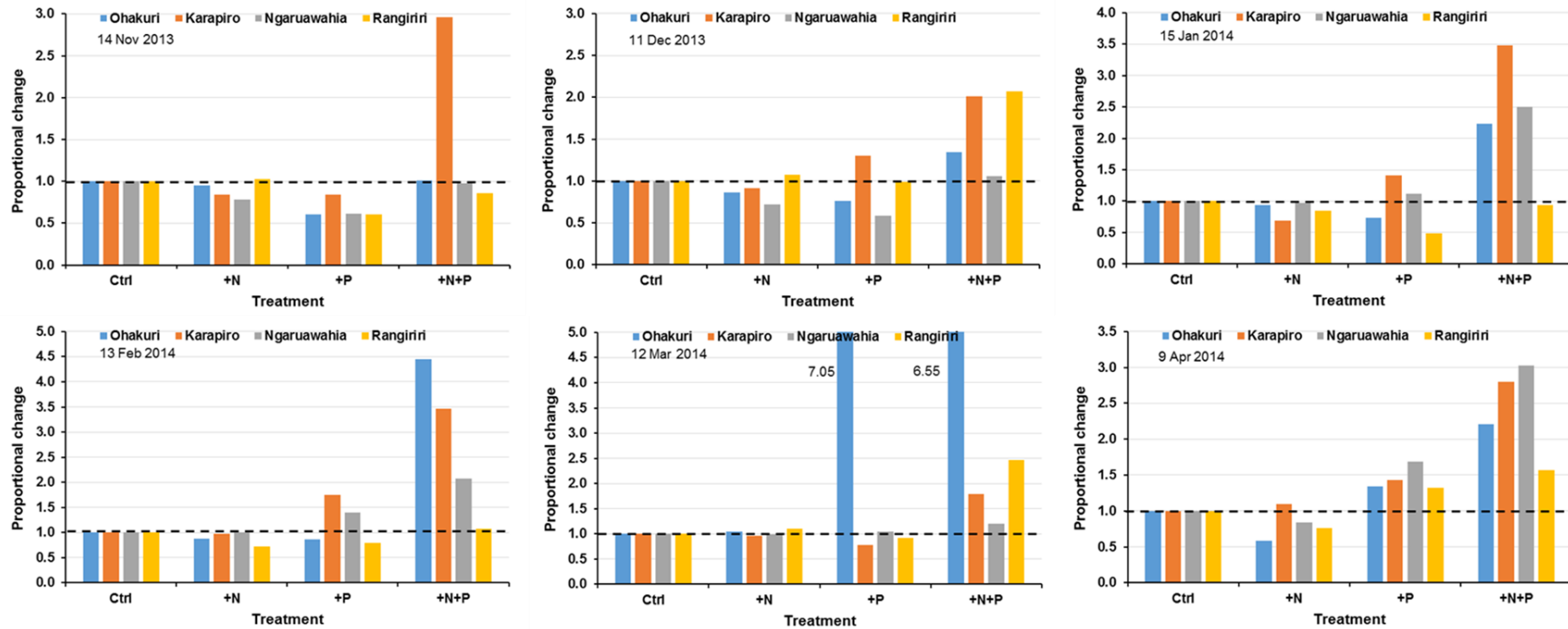


Figure 3: Nutrient addition bioassays to assess potential nutrient limitation in the Waikato River between November 2013 and April 2014. The broken line at 1 indicates no change. Above the line there has been a positive response to the nutrient added. Below the line indicates the phytoplankton biomass declined relative to the control, possibly due to grazing by small zooplankton that had passed through the plankton net.

3.2 Nutrient analyses

Nutrient concentration data (Appendix B) have been collated for each site on each sampling date for direct comparison with the nutrient bioassay data (Figure 3). The PC results from the CHN analyser have been normalised (PC-Norm) using the PartN data obtained by the wet chemistry method for PN and PP, and the PN(C/N) data from the combustion method. This allows a direct relationship between PC-Norm, PartN and PartP (i.e., C:N:P). Time series plots of selected data (Figure 4) show the concentration changes that occurred at each site over the 6-month monitoring period.

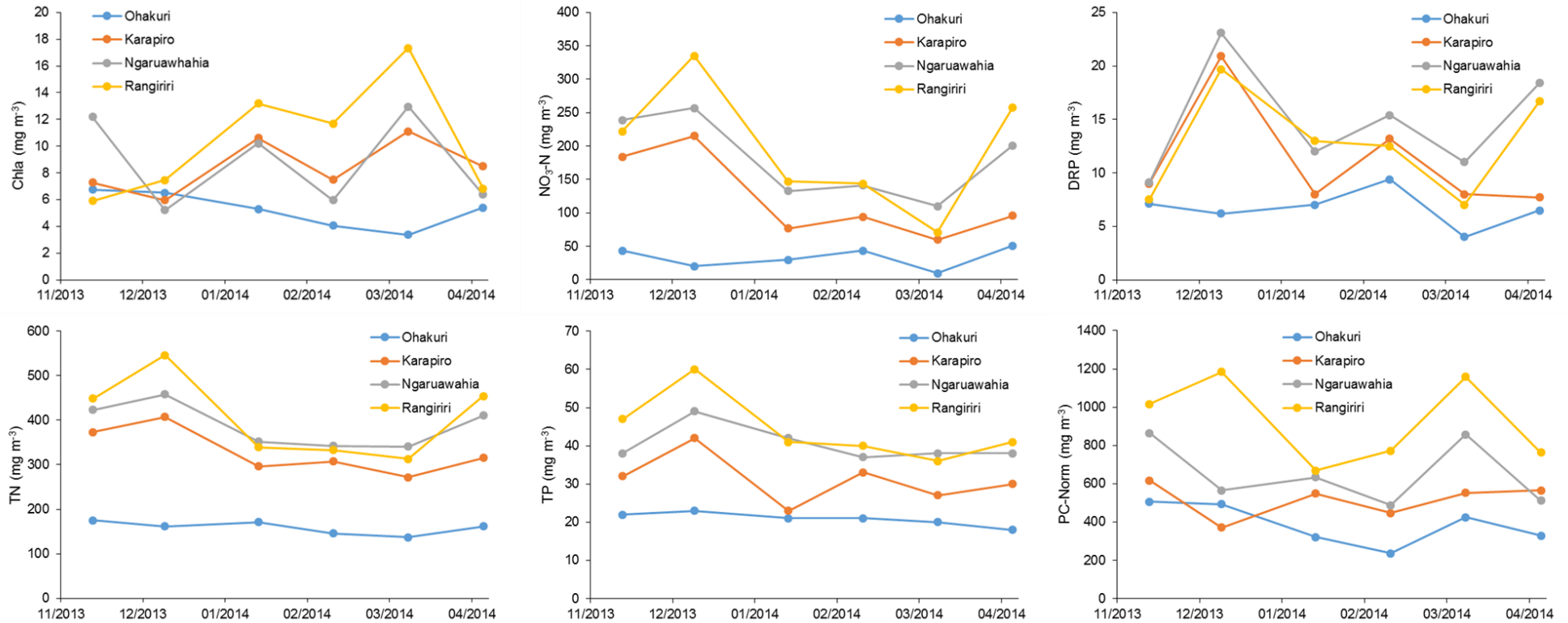


Figure 4: Time series plots of chlorophyll *a* (Chla) and selected nutrient parameters by site showing the changes in concentrations that occurred at each site over the 6-month monitoring period.

3.3 Zooplankton grazing effects

Unfortunately, the November 2013 zooplankton dilution experiment was left to run for 5 days instead of being taken down after 24 hours. Consequently, that set of results is difficult to interpret. Interpretation relies on there being phytoplankton left in the incubation jar at the end of the incubation. The chlorophyll *a* concentrations are corrected for the dilution factor and then plotted as the proportional change in chlorophyll (growth) relative to the dilution factor (Figure 5).

A simple interpretation of the example graph is as follows:

The ratio of grazing (*g*) relative to (*u*) the biomass-specific growth rate (units, per day) as a percentage (i.e., *g/u*%) gives a value of 69% for this example. This implies that zooplankton were consuming around 70% of the phytoplankton growth in the Waikato River at that site on that day. Alternatively, the chlorophyll *a* concentrations would be slowly increasing in the river.

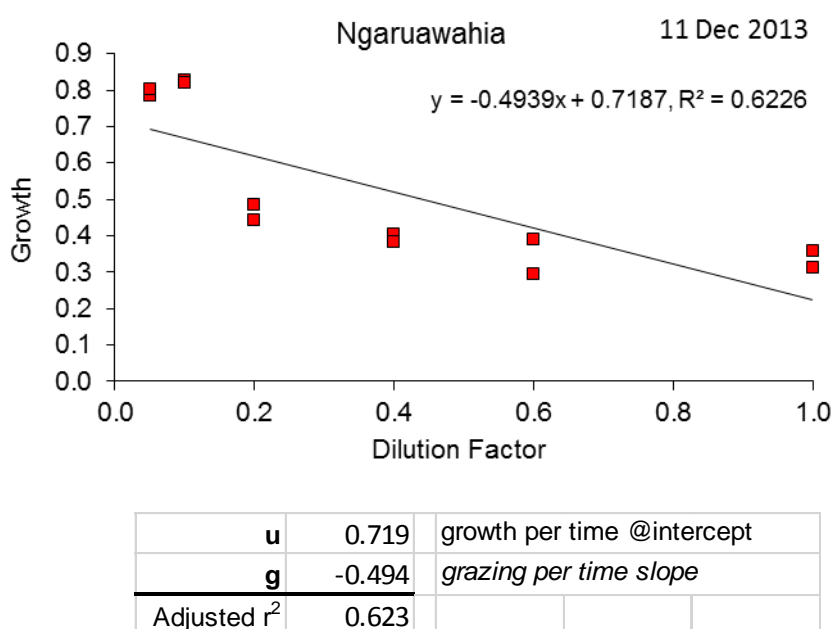


Figure 5: Example plot of growth relative to dilution in a zooplankton dilution incubation. All data are plotted. The results are interpreted below the graph as biomass-specific growth rate (*u*) = 0.719 per day and grazing pressure (*g*) removes 0.494 of that biomass per day. The results are significant with an r^2 of 0.623, ($n=12$).

Plots of all zooplankton dilution incubation results are presented in Appendix A. A summary of the results (Table 1) is collated with the results of the nutrient bioassays by month to allow cross referencing of any nutrient limitation effects with any grazing effects. In the nutrient addition table, a value of '1' equates to no change over control, a value greater than 1 is a positive response to the nutrient addition given as the number of times the biomass has increased per 5-day period. A value less than '1' indicates a reduction in phytoplankton biomass for some reason, possibly due to the grazing effect of small (<40 μ m) zooplankton over the 5-day incubation period. If correct, this implies that the numerical value of the positive response to a nutrient addition is a net value of growth minus grazing by small zooplankton in the incubation water over 5-days.

The identification of the small (<40 μ m) zooplankton in these experiments is uncertain. However, they are likely to be small rotifers that have passed through the plankton net or may be from

zooplankton eggs (probably rotifers) that have been dislodged by the sieving process and passed through the net. These latter would then hatch and have 5 days to grow and graze in the incubation jars.

The very low or negative values for growth in the November 2013 zooplankton dilution incubation, which had the full size range of zooplankton, indicated that zooplankton had removed almost all phytoplankton over the 5-day period. Consequently, those results are not interpretable.

Table 1: Summary of zooplankton grazing dilution incubation results and nutrient addition bioassays for nutrient limitation assessment. Pink shading indicated zooplankton grazing greater than phytoplankton growth without nutrient additions. Yellow shading indicates a response of more than a doubling during the 5-day incubation to that nutrient addition. Blue shading indicates grazing by small (<40µm) zooplankton may have been greater than phytoplankton growth.

Date / site	Zooplankton dilution 1-day incubations				Nutrient addition response		
	Growth	Grazing	r ²	Ratio g/u	5-day incubations		
	(u)	(g)		(%)	(+N)	(+P)	(+N+P)
14 November 2013							
Ohakuri	-0.017	-0.570	0.771	-3353	0.95	0.60	1.01
Karapiro	0.064	-0.573	0.409	895	0.84	0.84	2.96
Ngaruawahia	-0.205	-0.072	0.021	-35	0.78	0.61	0.98
Rangiriri	1.032	-0.578	0.400	56	1.03	0.61	0.86
11 December 2013							
Ohakuri	0.243	-0.296	0.617	122	0.86	0.76	1.34
Karapiro	0.301	-0.424	0.516	141	0.92	1.30	2.01
Ngaruawahia	0.719	-0.494	0.623	69	0.72	0.59	1.06
Rangiriri	0.547	-0.110	0.400	20	1.08	0.99	2.07
15 January 2014							
Ohakuri	0.319	-0.657	0.642	206	0.93	0.73	2.24
Karapiro	0.198	-0.474	0.664	239	0.69	1.41	3.49
Ngaruawahia	0.237	-0.326	0.732	138	0.97	1.12	2.50
Rangiriri	0.530	-0.631	0.712	119	0.84	0.49	0.93
13 February 2014							
Ohakuri	0.459	-0.265	0.568	58	0.87	0.87	4.45
Karapiro	0.424	-0.240	0.500	57	0.97	1.75	3.46
Ngaruawahia	0.473	-0.035	0.043	7	1.00	1.40	2.07
Rangiriri	0.932	-1.000	0.842	107	0.72	0.79	1.07
12 March 2014							
Ohakuri	0.306	-0.143	0.363	47	1.05	7.05	6.55
Karapiro	0.344	-0.129	0.167	38	0.95	0.78	1.80
Ngaruawahia	0.510	-0.337	0.643	66	0.99	1.05	1.21
Rangiriri	0.768	-0.628	0.736	82	1.10	0.91	2.47
9 April 2014							
Ohakuri	0.078	-0.488	0.578	626	0.58	1.34	2.21
Karapiro	0.184	-0.182	0.582	99	1.10	1.43	2.80
Ngaruawahia	0.374	-0.195	0.481	52	0.84	1.69	3.03
Rangiriri	0.798	-0.202	0.679	25	0.76	1.32	1.57
					Zooplankton grazing >= growth		
					>Doubling per 5 days		
					November incubation 5 days = not reliable		
					Biomass less than control		

3.4 Zooplankton

Zooplankton species composition was dominated by Cladocerans at all sites in November 2013 (Figure 6). Cladocerans were also a large component of the zooplankton species composition in the

outflows from Lakes Ohakuri and Karapiro on most occasions, although their dominance declined in favour of Rotifers in March and April 2014. In contrast, zooplankton biomass was more than 5-fold lower at the downstream sites at Ngaruawahia and Rangiriri (Figure 6). These results clearly show the effect on zooplankton biomass of having a hydrolake immediately upstream as a place for them to grow compared with the more turbulent river environment downstream which appears to inhibit growth.

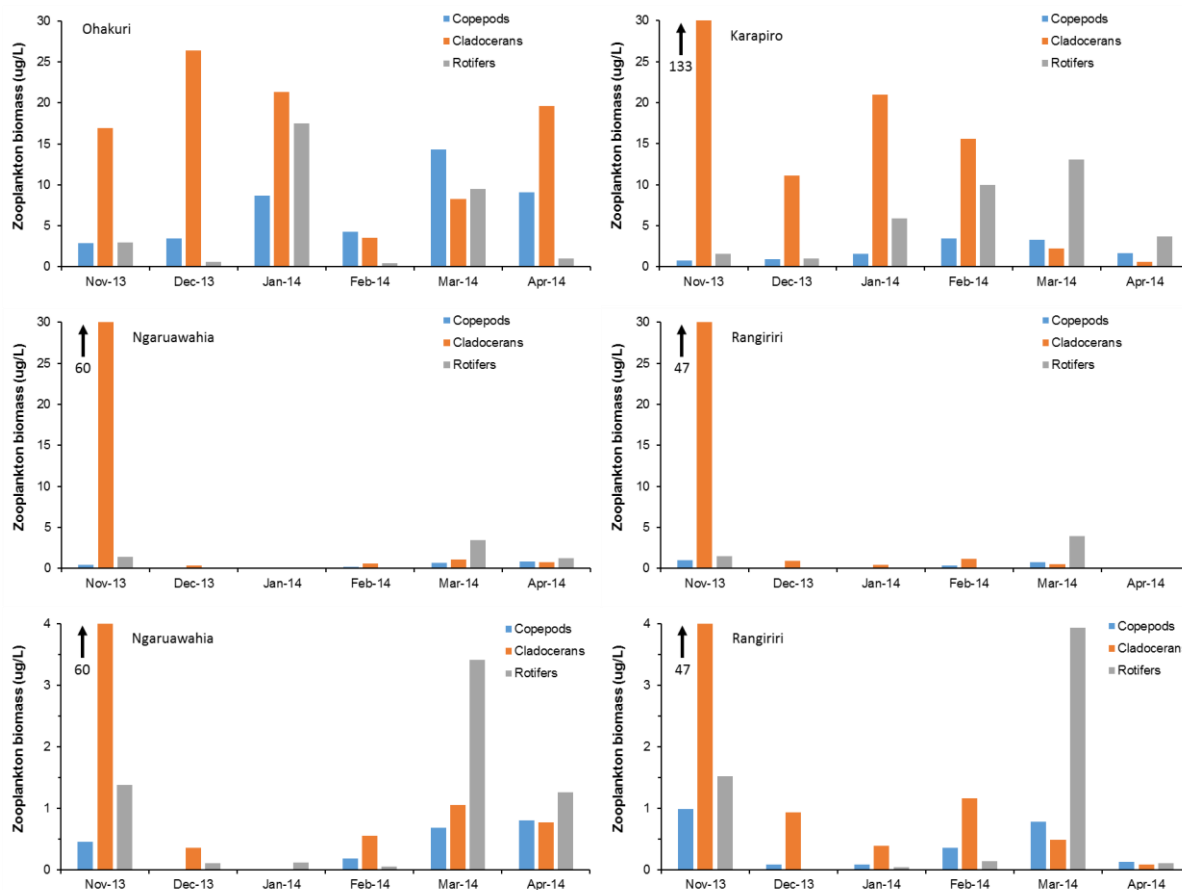


Figure 6: Summary plots of zooplankton classes at the four sampling sites over the 6-month monitoring period. Note that the bottom two graphs are the same as the middle two, but redrawn with different vertical scales for clarity.

Cladocerans comprised mostly *Daphnia galeata*, a recent North American invader, with smaller proportions of *Bosmina meridionalis*. The copepods were mostly *Calamoecia lucasi*, copepod nauplii and *Acanthocyclops robustus*. The main rotifer species were *Polyarthra dolichoptera* and *Asplanchna priodonta* with numerous (~40) minor species occurring at different times and places. A full list of zooplankton species with biomass for each site and sampling date is included in Appendix C.

Big zooplankton

The addition of large zooplankton caught on 150 μ m mesh and added to a container of raw water from the same site was expected to give an indication of large zooplankton grazing effects on phytoplankton biomass in the river. The results (Table 2) indicate that there was around 20% reduction in phytoplankton biomass in the samples from the outflows from Lakes Ohakuri and

Karapiro in December 2013 and there was <10% reduction in biomass at the Karapiro site in January and February 2014. At all other times, phytoplankton growth in the incubation container exceeded zooplankton grazing pressure.

Table 2: Big zooplankton grazing effects. Experiment was the addition of >150 µm zooplankton from 10 L river water to 2 L raw water and incubated for 24 hours at 18°C under a 16:8 hour light dark cycle.

Site	Big Zooplankton grazing per day (% change in chlorophyll)					
	14-Nov-13	11-Dec-13	15-Jan-14	13-Feb-14	12-Mar-14	9-Apr-14
Ohakuri	-	-23	54	15	23	4
Karapiro	-	-16	-4	-8	67	22
Ngaruawahia	-	-	85	29	118	74
Rangiriri	-	-	76	13	47	153

These results could be interpreted in several ways.

1. The reduction in phytoplankton biomass at the Ohakuri and Karapiro sites is consistent with an addition of large zooplankton, which probably came from the lakes immediately upstream.
2. The small increases in phytoplankton biomass could indicate that insufficient numbers of large zooplankton had been added implying that numbers of large zooplankton in the river were low at those sites consistent with observation (Figure 6).
3. The large increases in phytoplankton biomass could indicate that no large zooplankton had been caught from the 10 L of river water. This is an extreme of point 2, but is consistent with the zooplankton enumeration results (Figure 6), which indicate that copepod and cladoceran species decrease in numbers down the river and between spring and autumn.

3.5 Phytoplankton

While nutrient limitation and zooplankton grazing pressure can have obvious effects on the chlorophyll *a* concentrations in the Waikato River, a similar effect might be seen if the dominant species changed from large to small, or there was change in phytoplankton species composition over time. Examination of the phytoplankton data shows that the species composition varied down the Waikato River between sites and with time of year (Appendix E).

3.6 Particle size

Fine sediment is potentially a major source of P in the aquatic environment as the P can be iron-bound to soil particles which then get washed into the waterways during rainfall events. Linear regression of the small particle sizes with the whole TP dataset show a significant (*P*-value <0.001, *r*² = 0.44, *n*=24) positive correlation between TP and particles in the size range of 6-8 µm. There was no relationship with particle sizes <3 µm (Appendix F). When the TP data set was assessed by month, the *r*² values increased to >0.98 for some particle size ranges in November and December 2013, as well as in January and April 2014 (Appendix F). During the dry period in February and March 2014, surface runoff was minimal and fine sediment particle numbers were very low. The linear regressions

were weak or negative implying that the particles at other times were from surface runoff and that the fine sediment may have settled out of the water column. See Appendix F for more details.

3.7 Flow

Mean daily flow in the Waikato River at the outfall from Lake Karapiro on the sampling days was about $260 \text{ m}^3 \text{ s}^{-1}$ between November 2013 and February 2014, with slightly lower flow at the time of the January sampling (Figure 7). The Waikato River experienced a prolonged period of low flow (157 to $144 \text{ m}^3 \text{ s}^{-1}$) in late summer from 1 March 2014 and included the March and April samplings.

While the mean flow data (Figure 7) suggests a gradual change in flow from spring through to summer, the actual changes in flow between day and night were often large. For example, the mean daily flow on the February 2014 sampling date was around $265 \text{ m}^3 \text{ s}^{-1}$ but the discharge hydrograph from the Mighty River Power web page shows that the flow ranged from about 160 to $360 \text{ m}^3 \text{ s}^{-1}$ (Figure 8, upper). In contrast, under low flow conditions the discharge hydrograph shows that steady flow conditions at around $150 \text{ m}^3 \text{ s}^{-1}$ persisted for several days before the March (and April) samplings (Figure 8, lower).

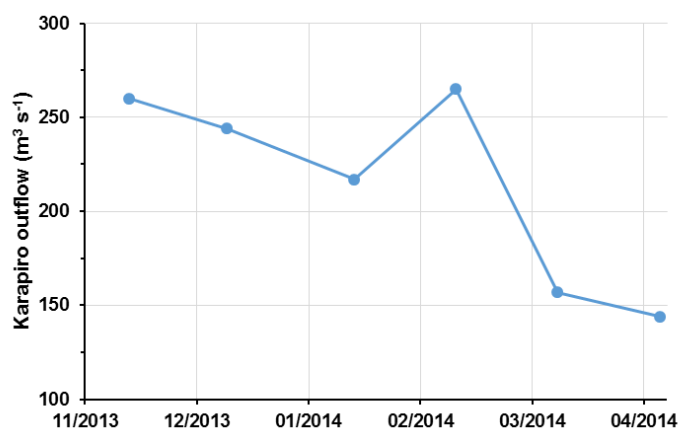


Figure 7: Mean daily discharge ($\text{m}^3 \text{ s}^{-1}$) from Lake Karapiro on the sampling days.



Figure 8: Time series discharge data from Lake Karapiro preceding the February (upper) and March (lower) sampling days (red arrows). The discharge hydrographs show rapid changes in flow under 'normal' operating conditions in February, but very steady flows under the low flow conditions in March. Images are screen grabs of the 7-day discharge charts on the Mighty River web page.

The difference between rapid changes in flow and thereby turbulence in river versus steady low flows persisting for several days to weeks during drought-like conditions is likely to be significant for phytoplankton nutrient uptake and for zooplankton growth. It would also be more conducive to sedimentation of fine sediment and phytoplankton as the dominant phytoplankton species were mostly heavy diatoms.

4 Summary

The water in the Waikato River undergoes a change from the upper reaches represented by the Ohakuri site and the middle reaches represented by the Karapiro site. On most sampling occasions, there was a step-wise increase in biochemical parameters between these two sites (Figure 4) implying a possible effect of the hydroelectric impoundments as well as changes in catchment nutrient loads on the river. Total nitrogen and NO₃-N concentrations doubled and TP concentrations increased by around 50% between Ohakuri and Karapiro. Dissolved reactive phosphorus concentrations were 4-fold higher at Karapiro in December but were subsequently <50% higher. Chlorophyll *a* concentrations were unchanged in the November and December samplings, but increased by 80-100 % at Karapiro through the January to March sampling period. As there was no time for this level of growth in the river, it is assumed that these increases reflect phytoplankton growth in Lake Karapiro immediately upstream.

The changes continued downstream from Karapiro to Ngaruawahia and Rangiriri but were generally less pronounced. Changes between Karapiro and Ngaruawahia were often small compared with changes between Ngaruawahia and Rangiriri. Exceptions were TN, TP and NO₃-N in January, February and March. The largest differences between Ngaruawahia and Rangiriri were for particulate carbon and chlorophyll *a*. Concentrations were almost always highest at Rangiriri, which may indicate the influence of the Waipa River inflow downstream of the Ngaruawahia site or effects of the Lake Waahi inflow near Huntly, and other inflows to that stretch of river.

The nutrient addition bioassays used river water that had been passed through a 40 µm sieve to exclude most zooplankton. Not all zooplankton would be excluded as small rotifers and zooplankton eggs may have passed through the sieve to grow in the incubated water. Consequently, the nutrient addition bioassay results are net responses.

The nutrient addition experiment results indicate that there were no significant positive responses to the addition of N, implying that the river water was unlikely to be N-limited to phytoplankton growth. However, there were small to large positive responses to the addition of P, either by itself or in combination with N (i.e., N+P) despite there always being measurable DRP in the water. The addition of P as DRP was able to stimulate a growth response relative to the control at Karapiro on four occasions and at Ohakuri on two occasions. The largest response (7-fold increase in biomass) to the P addition was at the Ohakuri site in March 2014. This may have been the result of stratification in Lake Ohakuri immediately upstream causing nutrient depletion in the epilimnetic waters, which are discharged via a high level intake through the power station into the downstream river. These results imply a possible tendency towards P-limitation to phytoplankton growth. P-limitation was one possible explanation for the trends of increasing N but decreasing chlorophyll *a* in the Waikato River.

The largest growth responses followed the addition of both N and P. The consistent positive response to the addition of N+P suggests that both nutrients were at low (measurable) concentrations such that the addition of both nutrients could stimulate additional growth. This implies that there was sufficient nitrogen at all sites on all dates to support the level of phytoplankton growth found in the Waikato River, but a boost in nutrient levels would support more growth.

The zooplankton dilution experiments indicated that zooplankton grazing of phytoplankton growth was important at some sites and could consume a large proportion of the primary production in the river. At the Ohakuri and Karapiro sites, on three occasions, zooplankton grazing was greater than

phytoplankton growth implying that the chlorophyll *a* concentration in the river could be reducing over time due to zooplankton grazing pressure. This could explain why there was a positive response to the addition of both N and P in the nutrient bioassays. The nutrient boost could have increased the phytoplankton growth rate sufficiently to exceed zooplankton grazing in the 5-day incubations. Zooplankton grazing could also explain why some nutrient incubation results were lower than the controls at the end of the incubation period. An implication of this logic is that net nutrient addition responses that showed little or no response may in fact represent weak positive responses.

Grazing by big zooplankton only was apparent at Ohakuri and Karapiro in December and January. If it is considered that zooplankton prefer the calm waters of a lake for growth rather than the turbulent waters of a river, these results may be explained in terms of larger numbers of zooplankton being carried out of the two upstream hydro lakes in high flow conditions compared with low flow conditions, when they would have an opportunity to avoid the out flow currents in the lakes. Downstream, at Ngaruawahia and Rangiriri, zooplankton numbers were mostly less than 10% of the numbers immediately below Lake Karapiro, which could explain the increase in chlorophyll *a* at Ngaruawahia and Rangiriri via reduced zooplankton grazing pressure.

The data in this report should be used for modelling the changes in the Waikato River along its length and over the summer season.

5 Acknowledgements

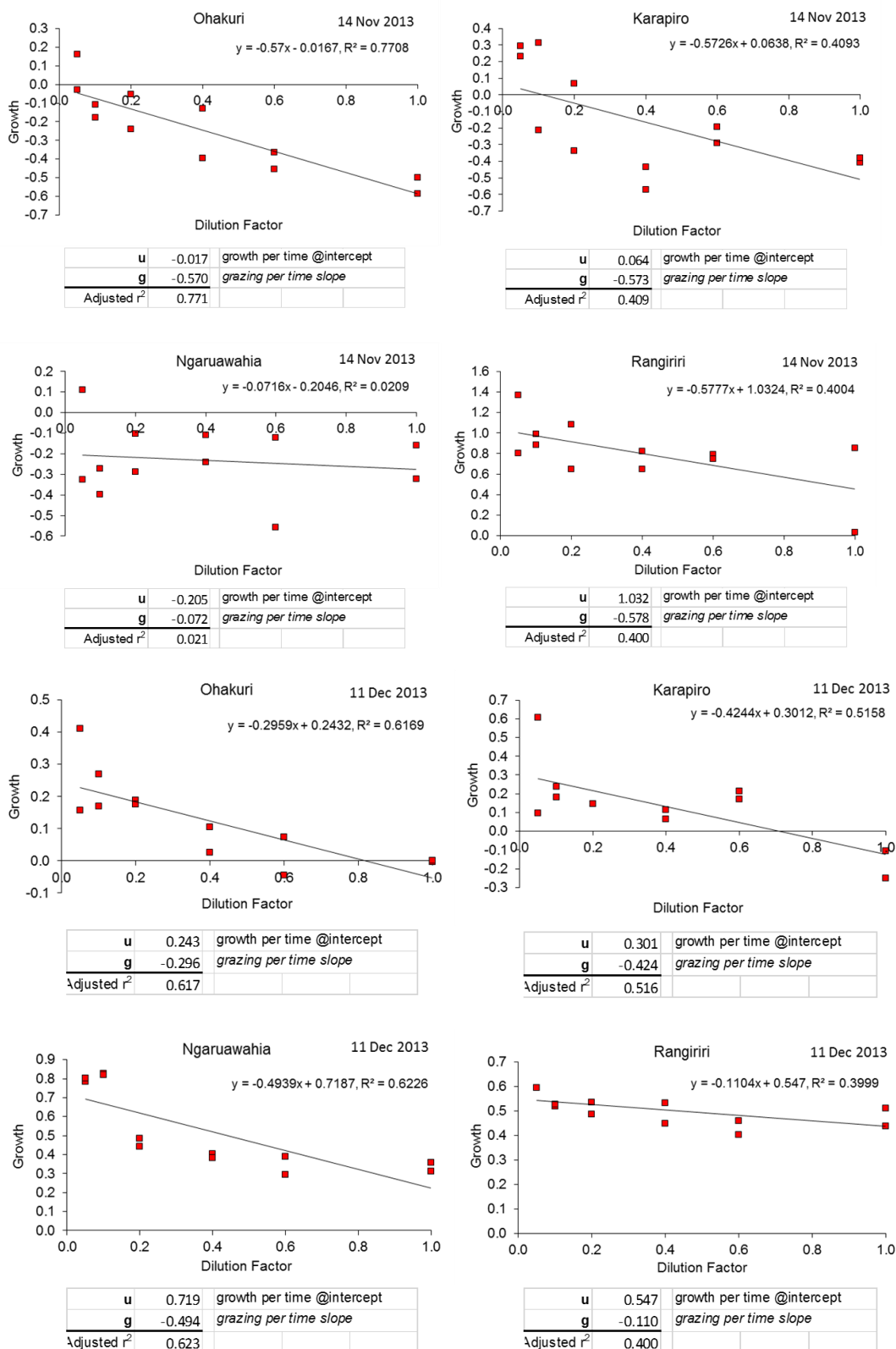
We thank Gareth Van Assema and Greg Olsen (NIWA) for field assistance collecting the water samples, and Bill Vant (WRC) for valuable discussion on the data interpretation and presentation.

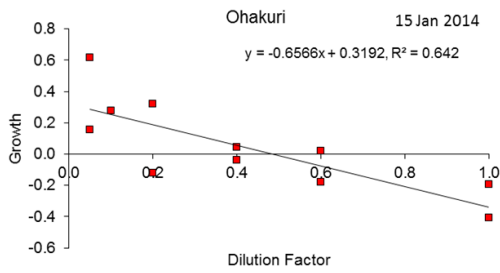
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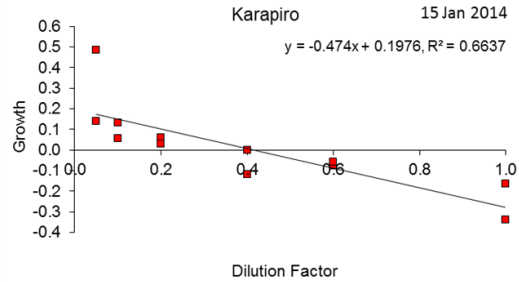
Appendix A Zooplankton dilution

The following series of graphs were used to calculate the growth (u) and grazing (g) used in Table 1. The graphs are arranged by sampling date with the four sites presented.

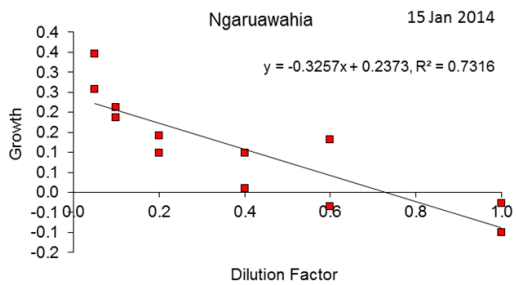




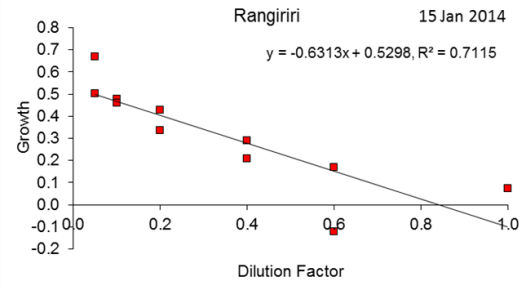
u	0.319	growth per time @intercept
g	-0.657	grazing per time slope
Adjusted r^2	0.642	



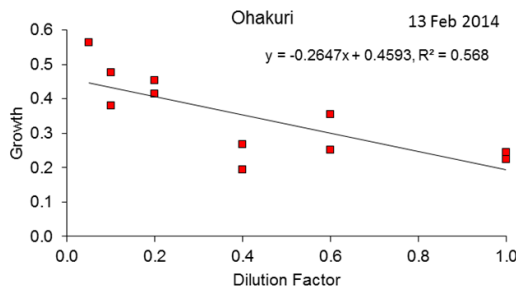
u	0.198	growth per time @intercept
g	-0.474	grazing per time slope
Adjusted r^2	0.664	



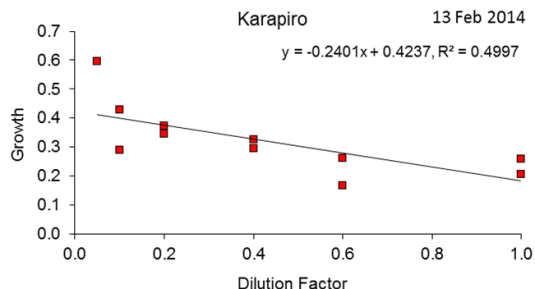
u	0.237	growth per time @intercept
g	-0.326	grazing per time slope
Adjusted r^2	0.732	



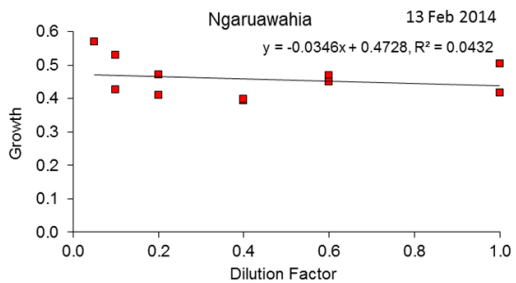
u	0.530	growth per time @intercept
g	-0.631	grazing per time slope
Adjusted r^2	0.712	



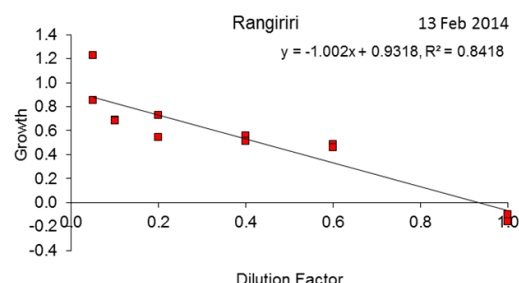
u	0.459	growth per time @intercept
g	-0.265	grazing per time slope
Adjusted r^2	0.568	



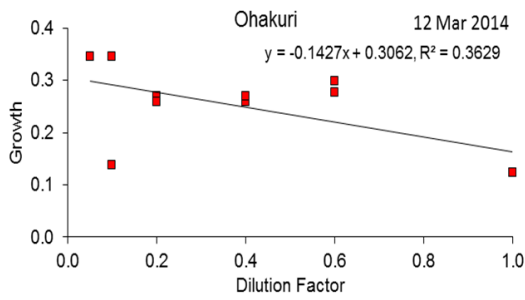
u	0.424	growth per time @intercept
g	-0.240	grazing per time slope
Adjusted r^2	0.500	



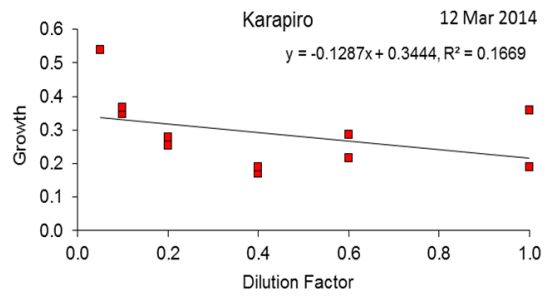
u	0.473	growth per time @intercept
g	-0.035	grazing per time slope
Adjusted r^2	0.043	



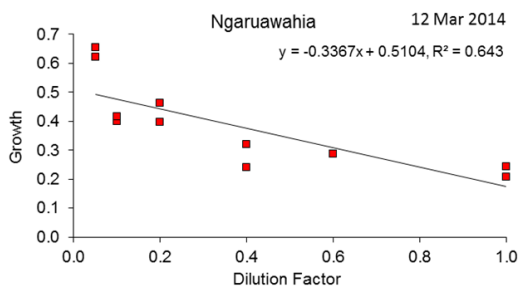
u	0.932	growth per time @intercept
g	-1.000	grazing per time slope
Adjusted r^2	0.842	



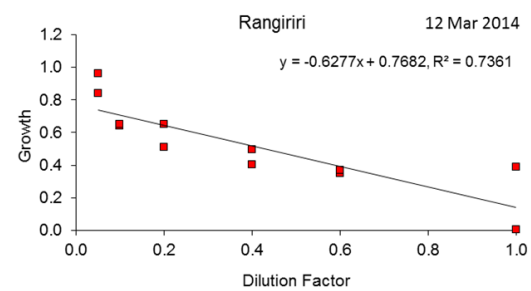
u	0.306	growth per time @intercept
g	-0.143	grazing per time slope
Adjusted r^2	0.363	



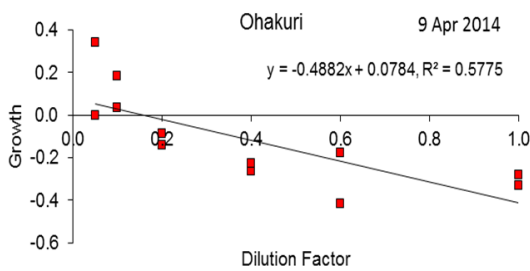
u	0.344	growth per time @intercept
g	-0.129	grazing per time slope
Adjusted r^2	0.167	



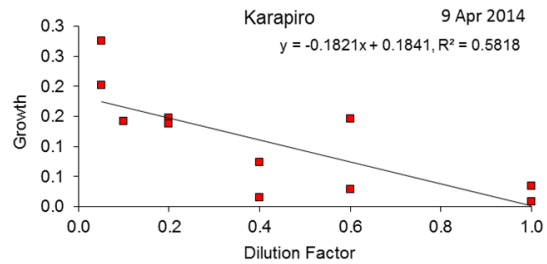
u	0.510	growth per time @intercept
g	-0.337	grazing per time slope
Adjusted r^2	0.643	



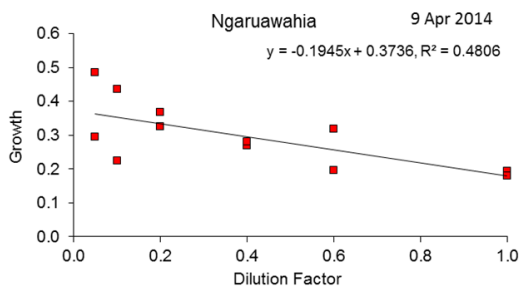
u	0.768	growth per time @intercept
g	-0.628	grazing per time slope
Adjusted r^2	0.736	



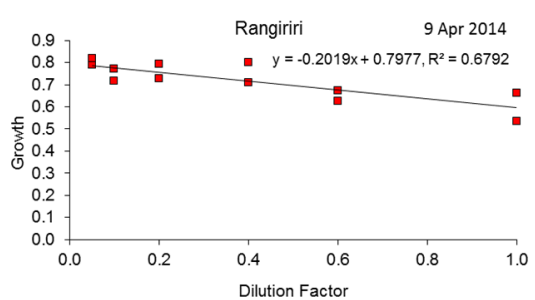
u	0.078	growth per time @intercept
g	-0.488	grazing per time slope
Adjusted r^2	0.578	



u	0.184	growth per time @intercept
g	-0.182	grazing per time slope
Adjusted r^2	0.582	



u	0.374	growth per time @intercept
g	-0.195	grazing per time slope
Adjusted r^2	0.481	



u	0.798	growth per time @intercept
g	-0.202	grazing per time slope
Adjusted r^2	0.679	

Appendix B Nutrient data

Water quality and nutrient data for each site by sampling date. PC-Norm values are the PC data normalised to the particulate N data as analysed by wet chemistry (PN/PP) and combustion (C/N).

Date/site	pH pH units	TURB NTU	SS g/m ³	VSS g/m ³	Inorg SS g/m ³	DRP mg/m ³	DOP mg/m ³	PartP mg/m ³	TP mg/m ³	NH ₄ -N mg/m ³	NO ₃ -N mg/m ³	DON mg/m ³	PartN mg/m ³	TN mg/m ³	Chla mg/m ³	PN (C/N) mg/m ³	PC mg/m ³	PC-Norm mg/m ³
14/11/2013																		
Ohakuri	-	1.3	2.3	0.8	1.6	7	1	13.5	22	2	43	47	83	175	6.8	67	407	506
Karapiro	-	1.5	3.3	1.1	2.2	9	8	15.4	32	16	184	75	98	373	7.3	84	525	617
Ngaruawahia	-	2.5	6.3	1.5	4.8	9	6	22.9	38	2	239	47	135	423	12.2	122	785	865
Rangiriri	-	5.3	13.4	2.4	11.0	8	13	26.1	47	2	222	80	145	448	5.9	162	1135	1016
11/12/2013																		
Ohakuri	9.00	1.2	1.8	0.9	0.9	6	6	11.1	23	8	20	57	76.2	162	6.5	74	478	493
Karapiro	8.38	2.2	2.5	0.8	1.7	21	10	10.7	42	39	215	92	61.0	407	6.0	62	380	372
Ngaruawahia	8.35	2.8	6.1	1.3	4.8	23	10	16.4	49	28	257	99	74.2	458	5.2	69	526	565
Rangiriri	8.93	8.0	15.2	2.4	12.8	20	3	37.2	60	3	335	63	144.7	546	7.5	120	978	1184
15/01/2014																		
Ohakuri	7.63	0.9	2.3	0.9	1.4	7	4	10.1	21	8	30	82	51	171	5.3	58	362	322
Karapiro	7.77	1.8	3.7	1.4	2.3	8	1	14.3	23	31	77	104	84	296	10.6	99	647	549
Ngaruawahia	7.68	2.6	5.3	1.6	3.7	12	11	18.9	42	12	133	105	102	352	10.2	110	678	633
Rangiriri	7.92	3.6	7.8	2.0	5.7	13	6	22.2	41	3	147	81	108	339	13.2	121	743	668
12/02/2014																		
Ohakuri	7.59	0.8	1.4	<0.5	0.9	9	4	7.7	21	6	43	56	40	146	4.1	35	206	236
Karapiro	7.80	1.9	3.1	0.9	2.1	13	7	13.1	33	44	94	90	79	307	7.5	73	415	447
Ngaruawahia	7.81	1.8	4.6	1.2	2.9	15	7	15.0	37	23	141	95	83	342	6.0	82	482	487
Rangiriri	7.88	3.1	7.3	1.7	4.5	13	5	22.4	40	2	144	66	122	333	11.7	116	734	772
12/03/2014																		
Ohakuri	7.62	0.9	2.2	0.9	1.3	4	6	9.8	20	3	10	63	61	137	3.4	55	386	425
Karapiro	7.90	1.7	3.5	1.2	2.4	8	4	14.6	27	18	60	97	97	272	11.1	103	585	552
Ngaruawahia	8.03	2.2	5.4	1.8	3.6	11	9	18.5	38	21	110	83	127	341	13.0	128	861	857
Rangiriri	8.19	3.0	6.8	2.3	4.5	7	7	22.5	36	1	71	75	166	313	17.4	158	1100	1159
9/04/2014																		
Ohakuri	7.58	0.6	1.7	0.8	0.9	7	3	8.1	18	8	51	50	53	162	5.4	45	282	329
Karapiro	7.83	0.9	2.7	1.0	1.6	8	10	12.4	30	40	96	87	92	315	8.5	96	588	566
Ngaruawahia	7.77	1.7	4.6	1.4	3.2	18	7	12.8	38	48	201	88	73	410	6.4	71	503	512
Rangiriri	7.81	2.8	7.6	1.7	5.9	17	6	18.2	41	19	258	76	102	454	6.8	94	705	763

Appendix C Zooplankton biomass

Ohakuri >40 µm zooplankton

Site	Ohakuri	Ohakuri	Ohakuri	Ohakuri	Ohakuri	Ohakuri
Date	14 November 2013	11 December 2013	15 January 2014	13 February 2014	12 March 2014	9 April 2014
Species	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)
Copepods						
Acanthocyclops robustus	0.261	1.660	3.571	1.025	1.412	0.637
Attheyella maorica	0	0	0	0	0	0
Calamoecia lucasi	0	0.868	0	0.799	9.489	3.321
Copepod nauplii	2.600	0.967	5.100	2.433	3.400	5.100
Cladocerans						
Bosmina meridionalis	4.323	1.871	2.687	1.362	7.176	19.611
Daphnia galeata	12.566	24.485	18.596	2.176	1.140	0
Ilyocryptus sordidus	0	0	0	0	0	0
Rotifers						
Ascomorphella volvocicola	0.004	0	0	0	0	0
Asplanchna brightwelli	0	0.296	16.872	0	0	0
Asplanchna priodonta	0	0	0	0.076	6.156	0
Bdelloids	0.008	0.007	0.012	0.012	0.016	0.024
Brachionus angularis	0	0	0	0	0	0
Brachionus budapestinensis	0	0	0	0	0	0
Brachionus calyciflorus	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0
Cephalodella gibba	0	0	0	0	0	0
Collotheca sp.	0.002	0.007	0.022	0.001	0.024	0.012
Conochilus dossuarius	0.002	0	0	0	0	0
Conochilus unicornis	0	0	0	0	0	0
Dicranophorus sp.	0	0	0	0	0	0
Epiphanes macrourus	0	0	0	0	0	0
Euchlanis deflexa	0	0	0	0	0	0
Euchlanis dilatata	0	0	0	0	0	0
Euchlanis meneta	0	0	0	0	0	0.016
Filinia novaezealandiae	0	0	0	0	0	0.000
Gastropus hyptopus	0	0	0	0.070	0	0.020
Hexarthra intermedia	0	0	0	0	0	0
Keratella cochlearis	0.025	0.004	0.046	0.003	0.094	0.029
Keratella procurva	0	0	0	0	0	0
Keratella slacki	0	0	0	0	0	0
Keratella tecta	0	0	0	0	0.001	0.001
Keratella tropica	0	0	0	0	0	0
Lecane bulla	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0.002	0
Lecane flexilis	0	0	0	0	0	0
Lecane luna	0	0	0	0.002	0	0.010
Lecane lunaris	0	0	0	0	0	0
Monommata sp.	0	0	0	0	0	0.006
Polyarthra dolichoptera	2.356	0.034	0.347	0.044	0.265	0.112
Pompholyx complanata	0	0	0	0	0	0
Synchaeta oblonga	0.000	0.257	0.100	0.183	2.720	0.750
Synchaeta pectinata	0.560	0	0	0	0.160	0
Trichocerca porcellus	0.018	0.002	0	0.003	0	0.005
Trichocerca pusilla	0.015	0.019	0.006	0	0.022	0
Trichocerca similis	0.017	0.013	0.040	0.029	0.023	0.017
Trichocerca stylata	0	0	0	0.001	0	0
Trichotria tetractis	0	0	0.005	0.002	0	0.009
Total zooplankton > 40 um	22.760	30.488	47.403	8.226	32.098	29.681
Copepods	2.861	3.495	8.671	4.257	14.301	9.058
Cladocerans	16.889	26.356	21.283	3.538	8.315	19.611
Rotifers	3.010	0.638	17.449	0.430	9.482	1.012

Karapiro >40 µm zooplankton

Site	Karapiro	Karapiro	Karapiro	Karapiro	Karapiro	Karapiro
Date	14 November 2013	11 December 2013	15 January 2014	13 February 2014	12 March 2014	9 April 2014
Species	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)
Copepods						
Acanthocyclops robustus	0.000	0.299	0.446	0.932	1.130	0.796
Attheyella maorica	0	0	0	0	0	0
Calamoecia lucasi	0	0	0	0	0.343	0
Copepod nauplii	0.800	0.600	1.125	2.500	1.800	0.875
Cladocerans						
Bosmina meridionalis	7.365	7.015	1.631	14.573	1.688	0.579
Daphnia galeata	125.662	4.081	19.371	0.989	0.570	0
Ilyocryptus sordidus	0	0	0	0	0	0
Rotifers						
Ascomorphella volvocicola	0	0.009	0	0.003	0.004	0
Asplanchna brightwelli	0	0	0	0	0	0
Asplanchna priodonta	0	0.513	3.135	6.840	4.332	0.285
Bdelloids	0.016	0.004	0.010	0.013	0.024	0.020
Brachionus angularis	0	0	0	0	0	0
Brachionus budapestinensis	0	0	0	0	0	0
Brachionus calyciflorus	0	0	0	0	0	0.068
Brachionus quadridentatus	0	0	0	0	0	0
Cephalodella gibba	0	0	0	0	0	0
Collotheca sp.	0.010	0.006	0.038	0.020	0.047	0.004
Conochilus dossuarius	0	0	0	0	0	0
Conochilus unicornis	0.042	0	0	0	0	0
Dicranophorus sp.	0	0	0	0	0	0
Epiphanes macrourus	0	0	0	0	0	0
Euchlanis deflexa	0	0	0	0	0	0
Euchlanis dilatata	0	0	0	0	0	0
Euchlanis meneta	0	0	0	0	0	0
Filinia novaezealandiae	0	0	0	0	0	0
Gastropus hyptopus	0	0	0	0	0	0
Hexarthra intermedia	0	0	0	0	0.046	0
Keratella cochlearis	0.022	0.012	0.011	0.022	0.015	0.045
Keratella procurva	0	0	0	0	0	0
Keratella slacki	0	0.006	0	0	0	0
Keratella tecta	0	0	0	0	0	0.006
Keratella tropica	0	0	0	0	0	0
Lecane bulla	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0
Lecane flexilis	0	0	0	0	0	0
Lecane luna	0	0	0	0	0.010	0
Lecane lunaris	0	0	0	0	0	0
Monommata sp.	0	0	0	0	0	0.008
Polyarthra dolichoptera	0.347	0.133	1.530	2.142	7.793	2.588
Pompholyx complanata	0	0	0	0	0	0
Synchaeta oblonga	0.520	0.105	0.738	0.350	0.500	0.313
Synchaeta pectinata	0	0	0	0.133	0	0.050
Trichocerca porcellus	0.046	0.009	0	0.008	0	0
Trichocerca pusilla	0.532	0.010	0	0.002	0	0.011
Trichocerca similis	0.046	0.192	0.316	0.392	0.275	0.280
Trichocerca stylata	0	0	0.133	0.017	0.008	0.005
Trichotria tetractis	0	0	0	0.008	0	0
Total zooplankton > 40 µm	135.409	12.993	28.483	28.944	18.586	5.931
Copepods	0.800	0.899	1.571	3.432	3.272	1.671
Cladocerans	133.028	11.096	21.002	15.562	2.258	0.579
Rotifers	1.581	0.998	5.909	9.950	13.055	3.681

Ngaruawahia >40 µm zooplankton

Site	Ngaruawahia	Ngaruawahia	Ngaruawahia	Ngaruawahia	Ngaruawahia	Ngaruawahia
Date	14 November 2013	11 December 2013	15 January 2014	13 February 2014	12 March 2014	9 April 2014
Species	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)
Copepods						
Acanthocyclops robustus	0.261	0	0	0.052	0.282	0.255
Attheyella maorica	0	0	0	0	0	0
Calamoecia lucasi	0	0	0	0	0	0
Copepod nauplii	0.200	0.013	0.013	0.138	0.400	0.550
Cladocerans						
Bosmina meridionalis	3.202	0.234	0.010	0.558	1.055	0.772
Daphnia galeata	56.548	0.128	0	0	0	0
Ilyocryptus sordidus	0	0	0	0	0	0
Rotifers						
Ascomorphella volvocicola	0	0	0	0	0	0
Asplanchna brightwelli	0	0.074	0	0	0	0
Asplanchna priodonta	0.228	0.014	0.014	0.014	0.798	0.057
Bdelloids	0.016	0.002	0.004	0.015	0.136	0.072
Brachionus angularis	0	0	0.002	0	0.007	0.014
Brachionus budapestinensis	0	0	0	0.001	0	0
Brachionus calyciflorus	0	0	0	0	0	0
Brachionus quadridentatus	0	0	0	0	0	0
Cephalodella gibba	0	0	0	0	0	0
Collotheca sp.	0.003	0	0	0	0.003	0
Conochilus dossuarius	0.002	0	0	0	0	0
Conochilus unicornis	0	0	0	0	0	0
Dicranophorus sp.	0	0	0	0	0	0
Epiphanes macrourus	0	0	0	0	0	0
Euchlanis deflexa	0	0	0	0	0	0
Euchlanis dilatata	0	0	0	0	0	0
Euchlanis meneta	0	0	0	0	0	0.024
Filinia novaezealandiae	0	0	0.001	0	0	0.004
Gastropus hyptopus	0	0	0	0	0	0
Hexarthra intermedia	0	0	0	0	0.012	0
Keratella cochlearis	0.041	0	0.001	0.001	0.002	0.015
Keratella procurva	0.001	0	0	0	0	0
Keratella slacki	0	0	0	0	0	0
Keratella tecta	0	0	0	0	0	0.004
Keratella tropica	0	0	0	0	0	0
Lecane bulla	0	0	0.001	0	0	0
Lecane closterocerca	0	0	0	0	0	0
Lecane flexilis	0	0	0	0	0	0.001
Lecane luna	0	0	0	0	0	0.003
Lecane lunaris	0	0	0	0	0	0
Monommata sp.	0	0	0	0	0	0
Polyarthra dolichoptera	0.296	0.009	0.060	0.006	2.234	0.908
Pompholyx complanata	0	0	0	0	0	0
Synchaeta oblonga	0.570	0.004	0.020	0.008	0.100	0.060
Synchaeta pectinata	0.040	0	0	0	0	0
Trichocerca porcellus	0.009	0	0	0	0	0
Trichocerca pusilla	0.132	0	0	0	0	0.004
Trichocerca similis	0.040	0.008	0.014	0.012	0.126	0.095
Trichocerca stylata	0	0	0.002	0	0.002	0
Trichotria tetractis	0	0	0	0	0	0
Total zooplankton > 40 µm	61.589	0.485	0.141	0.806	5.158	2.836
Copepods	0.461	0.013	0.013	0.190	0.682	0.805
Cladocerans	59.750	0.361	0.010	0.558	1.055	0.772
Rotifers	1.377	0.111	0.119	0.057	3.420	1.260

Rangiriri >40 µm zooplankton

Site	Rangiriri	Rangiriri	Rangiriri	Rangiriri	Rangiriri	Rangiriri
Date	14 November 2013	11 December 2013	15 January 2014	13 February 2014	12 March 2014	9 April 2014
Species	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)
Copepods						
Acanthocyclops robustus	0.326	0	0.067	0.070	0.282	0.016
Attheyella maorica	0	0.025	0	0	0	0
Calamoecia lucasi	0	0	0	0	0	0
Copepod nauplii	0.667	0.063	0.025	0.288	0.500	0.113
Cladocerans						
Bosmina meridionalis	3.603	0.033	0.010	1.164	0.492	0.077
Daphnia galeata	43.633	0.893	0.387	0	0	0
Ilyocryptus sordidus	0	0.007	0	0	0	0.014
Rotifers						
Ascomorphella volvocicola	0.003	0	0	0	0	0
Asplanchna brightwelli	0.740	0	0	0	0.296	0
Asplanchna priodonta	0	0	0	0.057	0.798	0
Bdelloids	0	0.003	0.016	0.027	0.052	0.010
Brachionus angularis	0	0	0	0	0.014	0
Brachionus budapestinensis	0	0	0	0.002	0	0
Brachionus calyciflorus	0	0	0.007	0	0	0
Brachionus quadridentatus	0.011	0	0.002	0	0	0
Cephalodella gibba	0	0	0	0	0	0
Collotheca sp.	0.001	0	0	0	0	0
Conochilus dossuarius	0.003	0	0	0	0	0
Conochilus unicornis	0	0	0	0	0	0
Dicranophorus sp.	0	0	0	0	0	0
Epiphanes macrourus	0	0	0	0.001	0	0
Euchlanis deflexa	0	0.001	0	0	0	0
Euchlanis dilatata	0	0	0	0	0	0
Euchlanis meneta	0	0	0.005	0.003	0.048	0.008
Filinia novaezealandiae	0	0	0.001	0	0	0.001
Gastropus hyptopus	0	0	0	0	0	0
Hexarthra intermedia	0	0	0	0	0.023	0
Keratella cochlearis	0.047	0	0	0.003	0.003	0.001
Keratella procurva	0	0	0	0	0	0
Keratella slacki	0	0	0	0	0	0
Keratella tecta	0	0	0	0	0.001	0
Keratella tropica	0	0	0	0	0.003	0
Lecane bulla	0	0	0	0	0	0
Lecane closterocerca	0	0	0	0	0	0
Lecane flexilis	0	0	0	0	0	0
Lecane luna	0	0	0	0	0	0
Lecane lunaris	0	0	0	0	0	0
Monommata sp.	0	0	0	0	0	0
Polyarthra dolichoptera	0.298	0	0.005	0.023	2.458	0.073
Pompholyx complanata	0	0	0	0	0	0
Synchaeta oblonga	0.250	0.004	0.005	0.005	0.130	0.011
Synchaeta pectinata	0.033	0	0	0	0	0
Trichocerca porcellus	0.011	0	0	0	0	0
Trichocerca pusilla	0.107	0.001	0	0	0	0.001
Trichocerca similis	0.019	0.001	0.004	0.025	0.109	0.009
Trichocerca stylata	0	0	0	0	0	0
Trichotria tetractis	0	0	0	0	0	0
Total zooplankton > 40 µm	49.753	1.031	0.533	1.666	5.210	0.332
Copepods	0.993	0.088	0.092	0.357	0.782	0.128
Cladocerans	47.235	0.933	0.397	1.164	0.492	0.091
Rotifers	1.525	0.010	0.044	0.144	3.935	0.113

Appendix D Nutrient addition bioassay

5-Day nutrient bioassay incubation results																	
14/11/2013	14/11/2013	14/11/2013	11/12/2013	11/12/2013	11/12/2013	15/01/2014	15/01/2014	15/01/2014	12/02/2014	12/02/2014	12/02/2014	12/03/2014	12/03/2014	12/03/2014	9/04/2014	9/04/2014	9/04/2014
NWA ID	Client ID	Chla mg/m3	NWA ID	Client ID	Chla mg/m3	NWA ID	Client ID	Chla mg/m3	NWA ID	Client ID	Chla mg/m3	NWA ID	Client ID	Chla mg/m3	NWA ID	Client ID	Chla mg/m3
KF1	OHA Sieved	4.1	MB105	OHA RAW	6.5	MT6	OHA Sieved	3.7	OI4	OHA Sieved	2.9	PX6	OHA Sieved	2.7	RY6	OHA Sieved	3.5
KF2	KAR Sieved	4.5	MB106	KAR RAW	6.0	MT5	KAR Sieved	6.0	OI3	KAR Sieved	5.8	PX5	KAR Sieved	9.4	RY5	KAR Sieved	7.0
KF4	NGA Sieved	6.8	MB108	NGA RAW	5.2	MT7	NGA Sieved	6.1	OI7	NGA Sieved	4.9	PX7	NGA Sieved	9.1	RY7	NGA Sieved	5.4
KF3	RAN Sieved	9.9	MB107	RAN RAW	7.5	MT8	RAN Sieved	6.9	OI8	RAN Sieved	9.3	PX8	RAN Sieved	11.8	RY8	RAN Sieved	6.1
KF5	OHA C1	2.1	MB62	OHA C1	2.9	MT80	OHA C1	2.8	OI107	OHA C1	1.4	PX77	OHA C1	4.2	RY77	OHA C1	5.4
KF6	OHA C2	2.4	MB63	OHA C2	2.8	MT81	OHA C2	2.9	OI108	OHA C2	0.4	PX78	OHA C2	1.1	RY78	OHA C2	4.3
KF7	OHA C3	2.2	MB64	OHA C3	2.3	MT82	OHA C3	2.4	OI109	OHA C3	2.1	PX79	OHA C3	3.8	RY79	OHA C3	5.7
KF8	OHA N1	2.1	MB53	OHA N1	3.0	MT77	OHA N1	2.8	OI104	OHA N1	0.1	PX80	OHA N1	4.5	RY80	OHA N1	3.5
KF9	OHA N2	2.2	MB54	OHA N2	1.5	MT78	OHA N2	2.1	OI105	OHA N2	2.1	PX81	OHA N2	2.6	RY81	OHA N2	2.7
KF10	OHA N3	2.1	MB55	OHA N3	2.4	MT79	OHA N3	2.7	OI106	OHA N3	1.3	PX82	OHA N3	2.5	RY82	OHA N3	2.7
KF11	OHA P1	1.9	MB59	OHA P1	2.3	MT83	OHA P1	2.4	OI101	OHA P1	1.3	PX86	OHA P1	18.1	RY83	OHA P1	5.2
KF12	OHA P2	0.9	MB60	OHA P2	2.0	MT84	OHA P2	2.0	OI102	OHA P2	1.3	PX87	OHA P2	16.9	RY84	OHA P2	11.5
KF13	OHA P3	1.3	MB61	OHA P3	1.8	MT85	OHA P3	1.6	OI103	OHA P3	0.8	PX88	OHA P3	29.2	RY85	OHA P3	3.9
KF14	OHA NP1	2.3	MB56	OHA NP1	3.7	MT86	OHA NP1	6.5	OI110	OHA NP1	5.9	PX83	OHA NP1	16.4	RY86	OHA NP1	12.4
KF15	OHA NP2	2.3	MB57	OHA NP2	2.9	MT87	OHA NP2	5.1	OI111	OHA NP2	4.7	PX84	OHA NP2	17.7	RY87	OHA NP2	9.0
KF16	OHA NP3	2.3	MB58	OHA NP3	4.3	MT88	OHA NP3	6.5	OI112	OHA NP3	6.9	PX85	OHA NP3	25.5	RY88	OHA NP3	12.6
KF17	KAR C1	2.9	MB74	KAR C1	4.4	MT68	KAR C1	2.8	OI83	KAR C1	1.7	PX65	KAR C1	14.8	RY65	KAR C1	9.4
KF18	KAR C2	2.7	MB75	KAR C2	3.4	MT69	KAR C2	3.1	OI84	KAR C2	3.2	PX66	KAR C2	6.1	RY66	KAR C2	3.6
KF19	KAR C3	2.7	MB76	KAR C3	5.3	MT70	KAR C3	3.7	OI85	KAR C3	4.7	PX67	KAR C3	13.6	RY67	KAR C3	5.7
KF20	KAR N1	1.7	MB65	KAR N1	3.6	MT65	KAR N1	2.4	OI86	KAR N1	4.4	PX68	KAR N1	11.2	RY68	KAR N1	6.6
KF21	KAR N2	2.4	MB66	KAR N2	4.8	MT66	KAR N2	1.5	OI87	KAR N2	1.0	PX69	KAR N2	11.7	RY69	KAR N2	8.1
KF22	KAR N3	2.9	MB67	KAR N3	3.6	MT67	KAR N3	2.7	OI88	KAR N3	3.9	PX70	KAR N3	10.0	RY70	KAR N3	5.8
KF23	KAR P1	1.9	MB71	KAR P1	5.9	MT71	KAR P1	4.6	OI77	KAR P1	5.8	PX74	KAR P1	8.6	RY71	KAR P1	4.9
KF24	KAR P2	2.5	MB72	KAR P2	5.5	MT72	KAR P2	4.8	OI78	KAR P2	5.4	PX75	KAR P2	7.4	RY72	KAR P2	9.7
KF25	KAR P3	2.6	MB73	KAR P3	5.7	MT73	KAR P3	4.1	OI79	KAR P3	5.5	PX76	KAR P3	10.9	RY73	KAR P3	12.0
KF26	KAR NP1	3.9	MB68	KAR NP1	10.7	MT74	KAR NP1	10.2	OI80	KAR NP1	11.5	PX71	KAR NP1	26.2	RY74	KAR NP1	15.5
KF27	KAR NP2	7.7	MB69	KAR NP2	9.0	MT75	KAR NP2	11.4	OI81	KAR NP2	11.9	PX72	KAR NP2	12.5	RY75	KAR NP2	19.0
KF28	KAR NP3	8.6	MB70	KAR NP3	6.7	MT76	KAR NP3	11.8	OI82	KAR NP3	9.6	PX73	KAR NP3	23.2	RY76	KAR NP3	17.7
KF29	NGA C1	8.1	MB98	NGA C1	4.7	MT92	NGA C1	5.6	OI89	NGA C1	4.0	PX89	NGA C1	16.0	RY89	NGA C1	11.8
KF30	NGA C2	6.9	MB99	NGA C2	5.2	MT93	NGA C2	4.3	OI90	NGA C2	5.5	PX90	NGA C2	17.9	RY90	NGA C2	8.6
KF31	NGA C3	7.2	MB100	NGA C3	4.9	MT94	NGA C3	4.1	OI91	NGA C3	4.2	PX91	NGA C3	17.4	RY91	NGA C3	6.6
KF32	NGA N1	4.8	MB89	NGA N1	4.8	MT89	NGA N1	4.6	OI92	NGA N1	4.4	PX92	NGA N1	16.4	RY92	NGA N1	7.8
KF33	NGA N2	6.8	MB90	NGA N2	4.3	MT90	NGA N2	3.9	OI93	NGA N2	3.6	PX93	NGA N2	17.6	RY93	NGA N2	6.7
KF34	NGA N3	5.6	MB91	NGA N3	6.9	MT91	NGA N3	5.1	OI94	NGA N3	5.7	PX94	NGA N3	16.8	RY94	NGA N3	8.2
KF35	NGA P1	4.4	MB95	NGA P1	4.0	MT95	NGA P1	3.9	OI95	NGA P1	7.1	PX98	NGA P1	17.7	RY95	NGA P1	13.9
KF36	NGA P2	4.6	MB96	NGA P2	4.8	MT96	NGA P2	5.7	OI96	NGA P2	7.0	PX99	NGA P2	15.0	RY96	NGA P2	15.4
KF37	NGA P3	4.6	MB97	NGA P3	5.9	MT97	NGA P3	6.0	OI97	NGA P3	5.0	PX100	NGA P3	21.0	RY97	NGA P3	16.2
KF38	NGA NP1	7.7	MB92	NGA NP1	9.3	MT98	NGA NP1	12.6	OI98	NGA NP1	4.7	PX95	NGA NP1	18.0	RY98	NGA NP1	26.4
KF39	NGA NP2	8.0	MB93	NGA NP2	9.6	MT99	NGA NP2	11.5	OI99	NGA NP2	11.6	PX96	NGA NP2	22.9	RY99	NGA NP2	26.3
KF40	NGA NP3	5.9	MB94	NGA NP3	11.8	MT100	NGA NP3	10.7	OI100	NGA NP3	11.9	PX97	NGA NP3	21.0	RY100	NGA NP3	29.1
KF41	RAN C1	11.4	MB86	RAN C1	11.9	MT104	RAN C1	13.1	OI68	RAN C1	8.6	PX101	RAN C1	17.0	RY101	RAN C1	12.2
KF42	RAN C2	7.5	MB87	RAN C2	14.0	MT105	RAN C2	11.6	OI69	RAN C2	9.5	PX102	RAN C2	12.3	RY102	RAN C2	12.4
KF43	RAN C3	9.5	MB88	RAN C3	12.6	MT106	RAN C3	10.9	OI70	RAN C3	9.9	PX103	RAN C3	13.7	RY103	RAN C3	11.2
KF44	RAN N1	7.3	MB77	RAN N1	8.1	MT101	RAN N1	10.4	OI65	RAN N1	7.3	PX104	RAN N1	8.0	RY104	RAN N1	8.5
KF45	RAN N2	8.9	MB78	RAN N2	7.5	MT102	RAN N2	7.6	OI66	RAN N2	4.4	PX105	RAN N2	16.7	RY105	RAN N2	9.4
KF46	RAN N3	13.0	MB79	RAN N3	12.2	MT103	RAN N3	12.0	OI67	RAN N3	8.4	PX106	RAN N3	22.8	RY106	RAN N3	9.4
KF47	RAN P1	7.6	MB83	RAN P1	9.1	MT107	RAN P1	5.1	OI74	RAN P1	8.6	PX110	RAN P1	12.2	RY107	RAN P1	21.8
KF48	RAN P2	4.6	MB84	RAN P2	7.0	MT108	RAN P2	6.3	OI75	RAN P2	6.8	PX111	RAN P2	8.4	RY108	RAN P2	10.8
KF49	RAN P3	5.1	MB85	RAN P3	6.4	MT109	RAN P3	6.0	OI76	RAN P3	6.8	PX112	RAN P3	18.6	RY109	RAN P3	14.8
KF50	RAN NP1	6.9	MB80	RAN NP1	13.5	MT110	RAN NP1	10.3	OI71	RAN NP1	11.2	PX107	RAN NP1	36.6	RY110	RAN NP1	29.6
KF51	RAN NP2	8.3	MB81	RAN NP2	13.7	MT111	RAN NP2	12.9	OI72	RAN NP2	8.5	PX108	RAN NP2	33.6	RY111	RAN NP2	13.6
KF52	RAN NP3	9.2	MB82	RAN NP3	13.5	MT112	RAN NP3	10.0	OI73	RAN NP3	10.2	PX109	RAN NP3	35.8	RY112	RAN NP3	13.0

Appendix E Phytoplankton

While nutrient limitation and zooplankton grazing pressure can have obvious effects on the chlorophyll *a* concentrations in the Waikato River, a similar effect might be seen if the dominant species changed from large to small, or there was change in phytoplankton species composition over time. Examination of the phytoplankton data shows that the species composition varied down the Waikato River between sites and with time of year (Figure E1). Diatoms were generally the most common species together with chlorophytes and flagellates.

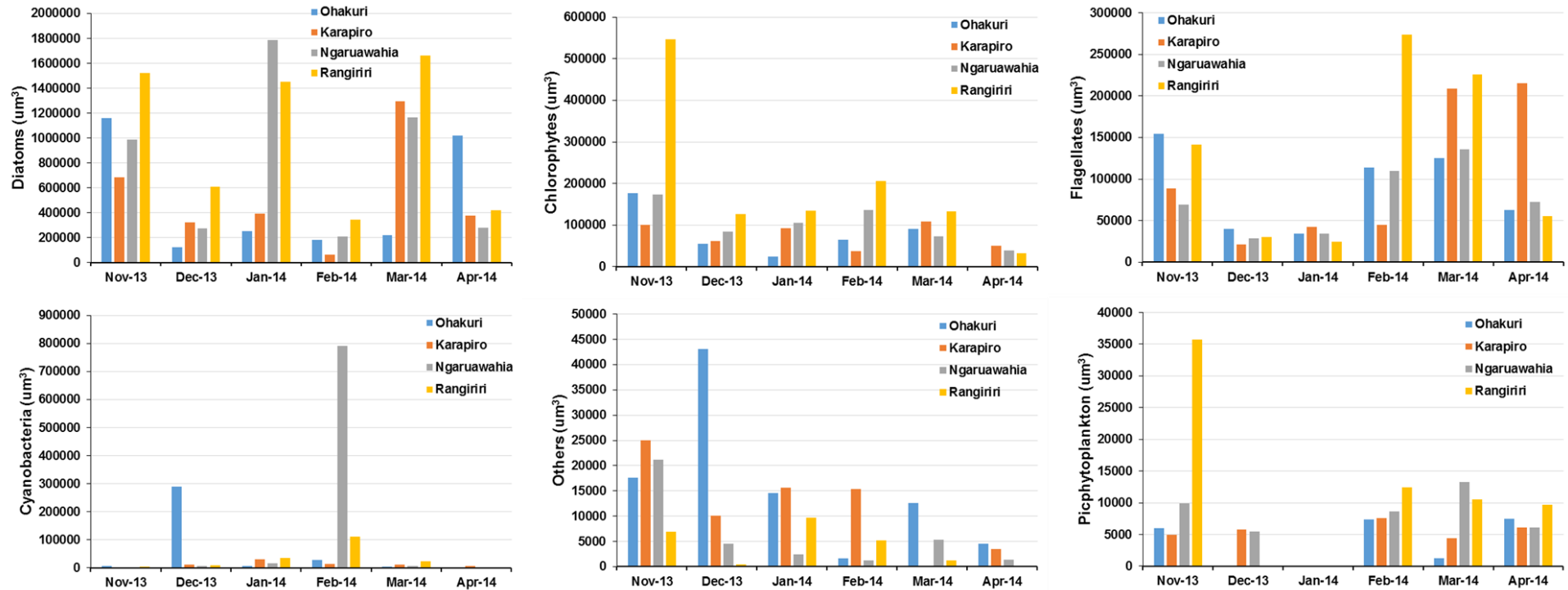


Figure E1: Time series changes in different classes of phytoplankton at the four monitoring sites in the Waikato River. Data are total biovolume (μm^3).

The most abundant diatom species were *Aulacoseira sp.* and *Fragilaria crotonensis*, which were present at most sites throughout the monitoring period (Figure E2). *Asterionella formosa* was a major component of the diatom assemblage at all sites in November 2013 but not in subsequent months. Conversely, *Attheya sp.* became a major component in the river below Lake Karapiro in March 2014, but was only a minor component before (Figure E2). Cyanobacteria became abundant at Ohakuri site in December 2013 and probably reflects conditions in the hydro dam immediately upstream (Figure E1). Cyanobacteria also became abundant at the Ngaruawahia site in February 2014, possibly in response to low flow conditions in the Waikato River at that time. At other times and sites, cyanobacteria were only present at low levels.

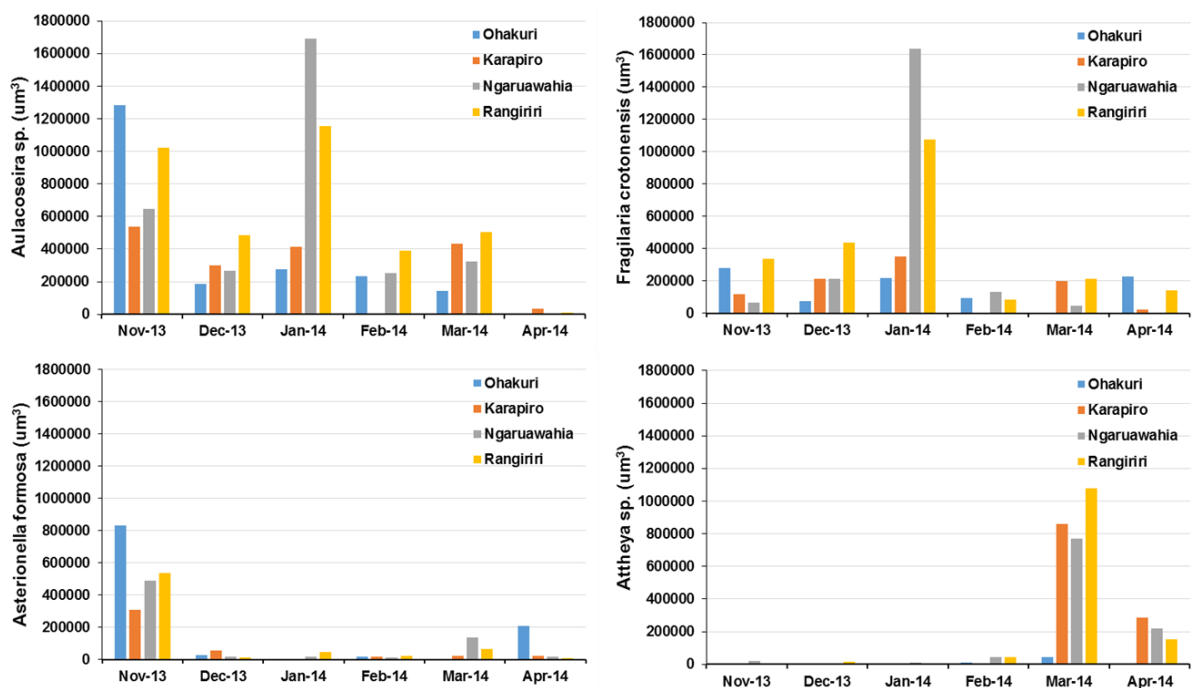


Figure E2: Breakdown of the most common diatom species at the four sites in the Waikato River.

Appendix F Particle size

Fine silt particles are typically enriched with iron-bound phosphorus and therefore may be a source of P to the Waikato River, as TP in surface runoff. The laser particle size analysis (EyeTech particle size analyser) showed differences in the volume and number of particles between sites and over time. The expectation for a numerical size distribution of fine silt would be a relatively smooth curve (Figure F1A) but there are always more of some particle sizes than others which leads to a range of high and low numbers across the particle size spectrum (Figure F1B).

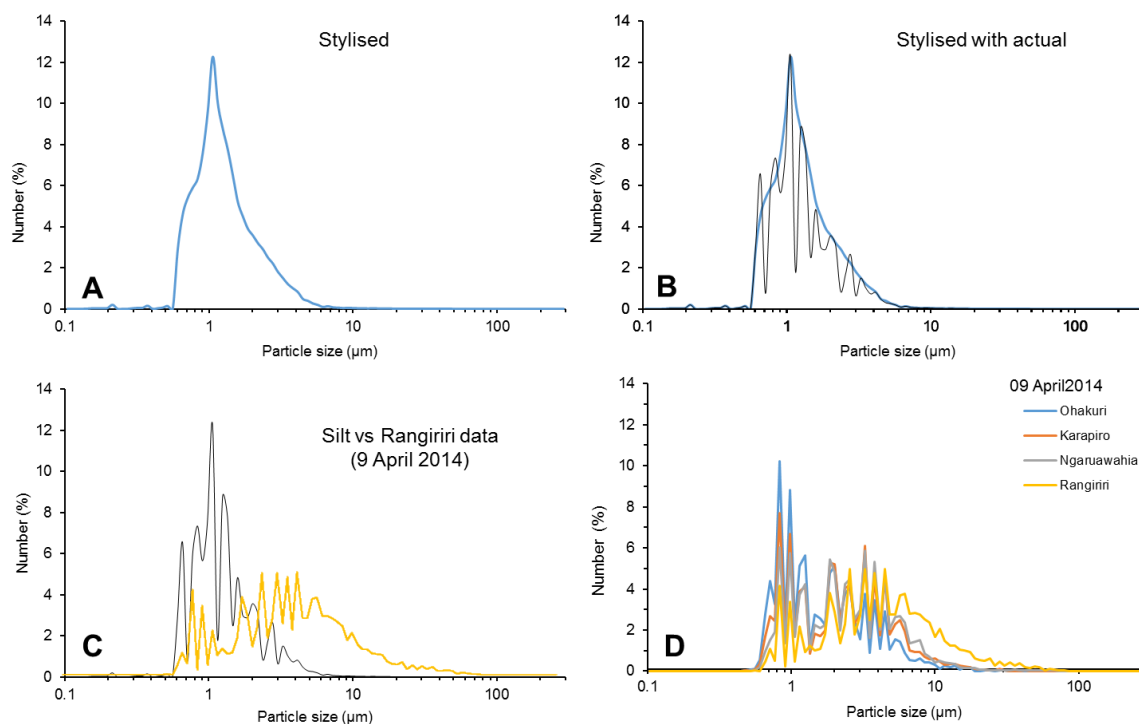


Figure F1: Illustration of how numerical particle size data is used: (A) stylised particle number distribution curve for fine silt, (B) stylised curve overlaid with actual data, (C) Actual silt data overlaid with data from Rangiriri site on 9 April 2014, (D) Complete data set for all sites on 9 April 2014 (see text for explanation).

The difference between the pure silt sample and samples from the Waikato River at Rangiriri (Figure F1C) is due to larger clay particles in catchment runoff, especially in the Waipa River. Consequently, the particle size analysis results from all four sites on 9 April 2014 (Figure F1D) show an increase in clay particles in the water between the upper catchment at Ohakuri and the lower catchment at Rangiriri. Of significance is the similarity in the data spectrum at Karapiro and Ngaruawahia, which implies very little sediment runoff in that section of river. The big increase occurs below the confluence with the Waipa River.

There were large numbers of small diameter particles (Figure F1D) mostly less than 8 µm at all sites. This size range would encompass many phytoplankton species, which could explain the consistent alignment of peaks of same particle size at all sites and sampling dates. Above 5 µm, the particle size range is more of a continuum as might be expected from a mixture of randomly sized silt and clay particles.

As a time series (Figure F2), the numerical particle size data reflect changes in fine sediment particle numbers, which were positively correlated with PP and TP (Figure F2).

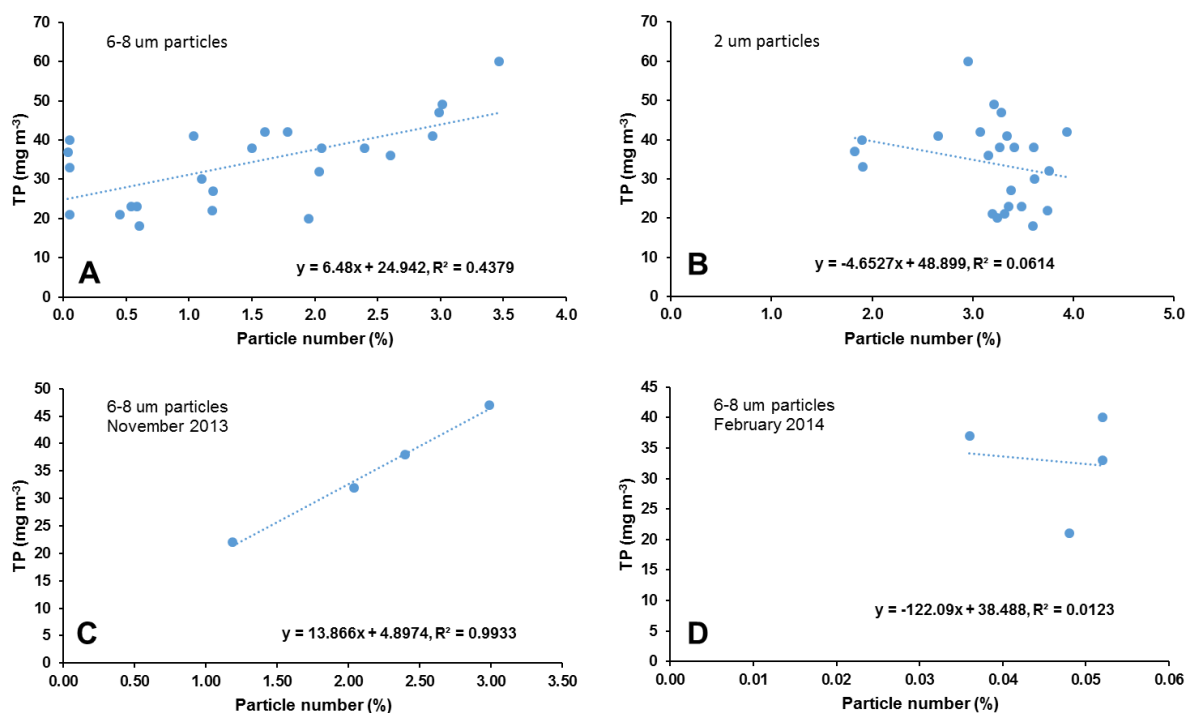


Figure F2: Relationships between fine sediment particle numbers and total phosphorus (A) in the 6-8 μm particle size range for all sampling dates, **(B)** in the 2 μm particle size range for all sampling dates, **(C)** in the 6-8 μm particle size range in November 2013, and **(D)** in the 6-8 μm particle size range in February 2014.

The data was assessed for relationships between different size fractions and TP, which was assumed to be bound to the particles. In November 2013, the strongest correlations were found with the 6-8 μm size range and the worst (negative) with particle sizes smaller than 3 μm (Table F1).

Table F1: Significance (r^2) of the linear regression between TP and different particle sizes.

Month	TP correlations (r^2) with particle size					
	Nov-13	Dec-13	Jan-14	Feb-14	Mar-14	Apr-14
Particle size (μm)						
2	-	-	-	-	0.14	-
3	-	0.63	0.99	-	0.39	0.81
4	0.82	0.91	0.93	0.35	0.02	0.96
5	0.86	0.93	0.85	0.02	0.59	0.96
6	0.96	0.98	0.89	0.07	0.13	0.79
7	0.98	0.92	0.75	-	0.34	0.72
8	0.94	0.76	0.74	0.86	0.16	0.54
4-8	0.97	0.94	0.85	0.16	0.24	0.82
5-8	0.98	0.95	0.84	0.002	0.31	0.79
6-8	0.99	0.96	0.83	-	0.23	0.72

These data show that phosphorus is correlated with different particle sizes at different times of year. In November and December 2013, there were strong correlations between TP and the 6-8 but in January 2014, the strongest correlation was with the 3 μm size range. In February and March 2014, the correlations with any size range were poor. This can be attributed to very low particle numbers

associated with the drought-like conditions and lack of surface runoff. In April 2014, after rain, the strongest correlations were with the 4-5 μm size range (Table F1).

These results are consistent with the TP load in the Waikato River being driven by surface runoff rather than by DRP.

Numerical particle size data are presented graphically in Figure F3, and volumetric particle size data are presented graphically in Figure F4.

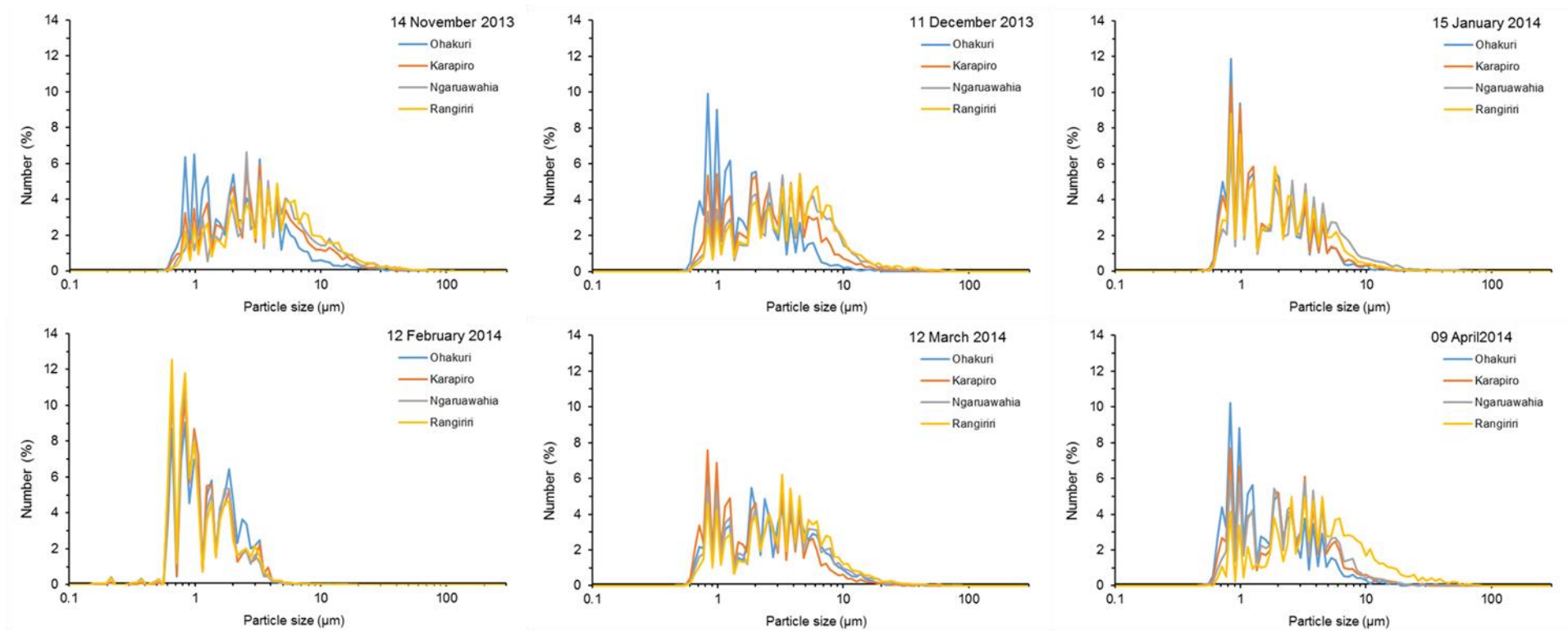


Figure F3: Time series changes in particle size distribution by number at the four sampling sites over the monitoring period.

Although few in number, the larger particles >10 μm comprise the majority of the particle volume and appear to peak in a size range between 50 to 80 μm (Figure F4) with a few of larger size. This is also consistent with the presence of large clay particles in the river water.

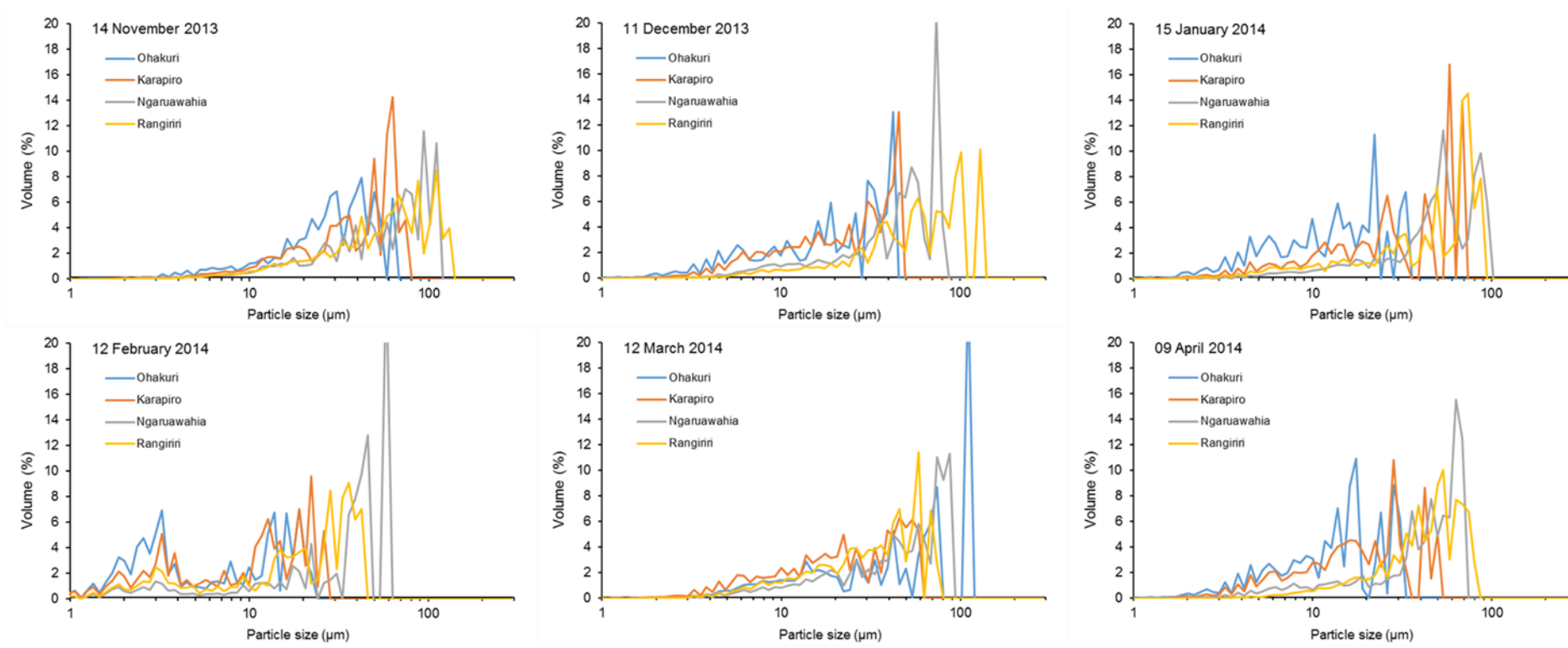


Figure F4: Time series changes in particle size distribution by volume at the four sampling sites over the monitoring period.