Effects of Palmerston North City's Wastewater Treatment Plant discharge on water quality and aquatic life in the Manawatu River

Joint monitoring programme between Palmerston North City Council and Horizons Regional Council

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Contents

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Executive Summary

The Palmerston North wastewater treatment plant (WWTP) was upgraded in 2008 to reduce phosphorus (P) in the discharge during periods of low flow in the Manawatu River. The aim of the upgrade was to reduce periphyton biomass and the impact on the aquatic macroinvertebrate community. It was predicted that this would result in a partial improvement but that periphyton biomass would still exceed guideline values after about 18 days of accrual.

Concern was raised about the effect of the WWTP discharge on aquatic life after sampling in early 2011 found more periphyton and a decline in the quality of the macroinvertebrate community (as measured by QMCI) downstream of the discharge. This resulted in Palmerston North City Council and Horizons Regional Council undertaking a joint programme to investigate the impacts of the Totara Road WWTP discharge on water quality, periphyton and macroinvertebrates in the Manawatu River.

Monitoring and investigations were undertaken in the Manawatu River from November 2011 to May 2012. It included weekly monitoring of water quality and periphyton cover, a synoptic survey to identify if the WWTP discharge was the main source of nutrients to the river, aquatic macroinvertebrate sampling and experiments to determine nutrient limitation. It was a wet summer so some of the investigations were delayed until a period of low flow in April 2012.

The purposes of the joint work programme were to identify:

- The extent and magnitude of effects occurring in the periphyton and aquatic macroinvertebrate communities downstream of the WWTP.
- The causes of these effects.
- What future management actions may be required for the treatment processes to reduce adverse effects on the river environment.

The magnitude and extent of effects on aquatic life

Periphyton grew substantially faster at sites downstream of the WWTP discharge, so that during periods of low flow periphyton biomass exceeded the targets in the Proposed One Plan (POP) after 17 and 19 days of accrual (for sites 800m downstream and 1400m downstream respectively) compared to 37 days of accrual required upstream of the discharge.

The quality of the aquatic macroinvertebrate community (as measured by QMCI) declined as periphyton biomass increased, and during a period of low flow was significantly lower downstream of the discharge. The second week of macroinvertebrate sampling (after 29 days of periphyton accrual and 15 days of less than half median river flows) found a substantial decline in the abundance of mayfly and other EPT taxa at the downstream sites, which represented a significant adverse effect on aquatic life. This degree of impact was predicted to occur, on average, 1.2 times per summer at sites about 800m downstream of the discharge.

The cause of these effects

The investigations found that the effects on periphyton and macroinvertebrates were not simply due to phosphorus in the discharge during periods of low flow. When the discharge was being treated for P, there was more bioavailable P measured in the river than could be explained by the discharge. At the site 800m downstream a 160% increase in DRP was measured compared to upstream, but only 7% to 23% could be explained by the effluent discharge occurring at the time. The unexplained loads estimated during the synoptic surveys were 7 to 11 kg/day.

Furthermore, while the site 1400m downstream had similar periphyton growth than what was predicted based on measured dissolved reactive phosphorus (DRP) concentrations, the site upstream had substantially less periphyton than predicted, and the site 800m downstream had substantially more.

Two mechanisms might explain the additional DRP measured in the river. Firstly, the synoptic survey found evidence of groundwater seepage entering the river. This might be contributing to the load of DRP although it is doubtful that this could explain the magnitude of load observed. Secondly, river sediments might be desorbing P to the overlying water during periods of low flow when the WWTP is treating for P.

Two mechanisms might explain the faster than predicted periphyton growth at the site 800m downstream. Firstly, periphyton might be experiencing higher DRP concentrations at the sediment surface than that measured in the water as P enters from groundwater seepage and/or desorption from sediments. Secondly, heterotrophic biofilms, stimulated by carbon in the discharge, might be conditioning the substrate and stimulating periphyton growth.

The weight of evidence from bioassay experiments indicated that periphyton biomass at the upstream site was primarily limited by something other than nitrate or phosphorus, but phosphorus provided secondary limitation. We speculated that the primary limiting factor(s) at the upstream site might be micronutrients (e.g. cobalt, molybdenum, silica), and/or grazing by macroinvertebrates. Limitation by ammoniacal nitrogen could not be ruled out.

Future management actions to reduce the effects on aquatic life

Potential management actions to reduce the effect of the discharge on aquatic life include:

- Keep treating the discharge for P during periods of low river flow because it is helping limit the extent of periphyton growth.
- Once started continue treating the discharge for P until periphyton cover is reduced to avoid pulses of P while there is still periphyton cover.
- If river sediment is found to be desorbing DRP then start treating for P before the current trigger of half median flow so there is less sediment derived P by the time the river reaches low flow.

Some further investigations would help direct and refine the most appropriate management actions to reduce the effect of the discharge on aquatic life. The key questions to address are:

- To what extent (if any) are river sediments desorbing P during periods of low flow when the discharge is removing P? This has implications for the period of time that P should be removed from the discharge.

- Do heterotrophic biofilms stimulate periphyton growth and P availability at the site 800m downstream? This may have implications for the treatment of BOD in the discharge.

- Are low concentrations of micronutrients (e.g. Si, Co, Mo) or ammoniacal nitrogen limiting periphyton growth upstream? If so, there may be implications of specific treatment processes.

- How much dissolved P does groundwater contribute to this section of the river and what is elevating dissolved P in the groundwater? If nutrient loads from groundwater loads are found to be significant than more attention would be justified for reducing nutrient sources to groundwater, these are most likely to be leakage from ponds at the WWTP or, to a lesser extent, the landfill.

- To what extent are macroinvertebrate grazers controlling periphyton growth? This has more general implications for understanding periphyton dynamics in the Manawatu River.

1 Introduction

1.1 Background

In 2008 the Totara Road Wastewater Treatment Plant (WWTP) was upgraded to remove phosphorus by alum dosing. This upgrade reduced the phosphorus load entering the Manawatu River with the discharge and was anticipated to reduce the amount of periphyton downstream and reduce the consequent impact on the aquatic macroinvertebrate community.

At the time of granting the consent it was predicted that the upgrade would result in a partial improvement and that in-stream periphyton guidelines would still be exceeded downstream of the discharge after 18 to 19 days of accrual[1] (respective values from Cameron 2002, Biggs and Kelly 2002).

In January 2011 a survey was undertaken by Palmerston North City Council (PNCC) of benthic ecology in the Manawatu River upstream and downstream of the Totara Road Wastewater Treatment Plant (WWTP) (Cameron 2011). This survey found that despite a reduction and phosphorus accrual since the 2008 upgrade, periphyton cover at sites between 800m to 1400m downstream of the WWTP could still reach high levels. During a period of low flow the site downstream of the WWTP had a statistically significant elevation of periphyton cover and a corresponding decline in the quality of the aquatic macroinvertebrate community (as indicated by the SQMCI) compared to upstream sites.

Horizons Regional Council expressed concern about the reported decline in the SQMCI beyond the 20% target in the Proposed One Plan, and concern that this might have constituted a breach of Condition 3 f of the discharge permit (number 101829)[2] which states that:

> *"3. The discharge shall not:*
>
> *f. cause significant adverse effects on aquatic life".*

After a series of discussions between PNCC and Horizons it was agreed to develop of a joint programme of work to further investigate the issue. The joint river work programme was endorsed in a Memorandum of Understanding (MoU) between PNCC and Horizons RC dated 7th November 2012.

1.2 Purpose of joint work programme

This report describes the result of the joint river work programme. The purposes of the joint work programme as stated in the MoU were to address:

- The effects that are occurring in the periphyton and aquatic macroinvertebrate communities downstream of the WWTP.

[1] Assuming average in-stream DRP concentration of 15 mg/m^3.

[2] This was issued under the Manawatu Catchment Water Quality Regional Plan.

- The causes of these effects (including effects of flows and other sources of contaminants besides the WWTP).

- What future management actions may be required for the treatment processes to lessen adverse effects on the river environment.

The technical aims of the monitoring programme were to:

- Clarify the extent to which the PNCC WWTP discharge is affecting periphyton growth and the aquatic macroinvertebrate community in the Manawatu River.

- Identify the extent to which other sources of contaminants may influence the Manawatu River between the upstream and downstream sample points.

- Identify which if any nutrient is limiting periphyton growth upstream and downstream of the discharge – particularly at flows less than 40 m^3/s when the P treatment is occurring.

- Provide guidance on modifications to the treatment process to reduce any adverse effect on the river environment, which may include refining the timing and extent of wastewater phosphorus removal in order to limit downstream periphyton growth.

1.3 One Plan Water Quality Targets

Schedule D of the Proposed One Plan (POP) sets water quality targets for rivers in the Manawatu-Whanganui Region. The Manawatu River near the PNCC WWTP discharge is in zone "Lower Manawatu Mana_11a", water quality targets particularly relevant to this zone are:

- *The algal biomass on the river bed must not exceed 120 mg of chlorophyll a per square metre.*

- *The maximum cover of visible river bed by periphyton as filamentous algae more than 2 centimetres long must not exceed 30%.*

- *The maximum cover of visible river bed by periphyton as diatoms or cyanobacteria more than 0.3 centimetres thick must not exceed 60 %.*

- *The annual average concentration of dissolved reactive phosphorus (DRP) when the river^ flow is at or below the 20th flow exceedance percentile must not exceed 0.001 grams per cubic metre, unless natural levels already exceed this target.*

- *The annual average concentration of soluble inorganic nitrogen (SIN)3 when the river^ flow is at or below the 20th flow exceedance percentile must not exceed 0.444 grams per cubic metre, unless natural levels already exceed this target.*

- *The Macroinvertebrate Community Index (MCI) must exceed 100, unless natural physical conditions are beyond the scope of application of the MCI.*

- *There must be no more than a 20 % reduction in Quantitative Macroinvertebrate Community Index (QMCI) score between appropriately matched habitats upstream and downstream of discharges to water."*

1.4 Overview of the joint work programme

The joint work programme consisted of five investigations in the Manawatu River upstream and downstream of the Totara Road WWTP. These were:

- A synoptic survey river water quality to identify whether there are other potential nutrient sources (other than the WWTP) between the upstream and downstream monitoring sites (undertaken on 19 April and 26 April).

- Weekly water quality monitoring of the Totara Road WWTP discharge and in the Manawatu River at one site upstream and two sites downstream of the discharge. An analysis of the effects of the treatment wetland on the discharge quality was also undertaken (see Appendix 6).

- Weekly monitoring of periphyton cover and biomass to better understand periphyton dynamics.

- Nutrient limitation bioassay (in the river for 13 days from 14th to 27th April) and analysis of cellular nutrient concentrations (25th January, 21st April and 28th April) to better understand which nutrient, if any, is limiting periphyton growth upstream and downstream of the discharge during a period of low flow when the WWTP is treating for phosphorus. A bioassay array was also placed in the river for five days from 29th March to 3rd April but had to be removed due to a flood.

- Aquatic macroinvertebrate and periphyton sampling to clarify the extent to which the Totara Rd WWTP is affecting the aquatic macroinvertebrate community. This involved sampling four sites (two upstream and two downstream) on two sequential occasions during a period of low flow when the WWTP was treating for phosphorus (P) (undertaken on 20th and 27th April).

The data collection and field surveys of the river were primarily undertaken by Horizons RC field staff and was integrated with other monitoring of the Manawatu River occurring during the summer.

Many of the planned investigations required sampling during a period of low flow and when the WWTP was treating for phosphorus. The summer of 2011/12 was very wet, and an extended period of low flow conditions did not occur until mid-April. A summary of when the different monitoring occurred with respect to flow is shown in Figure 1.1.

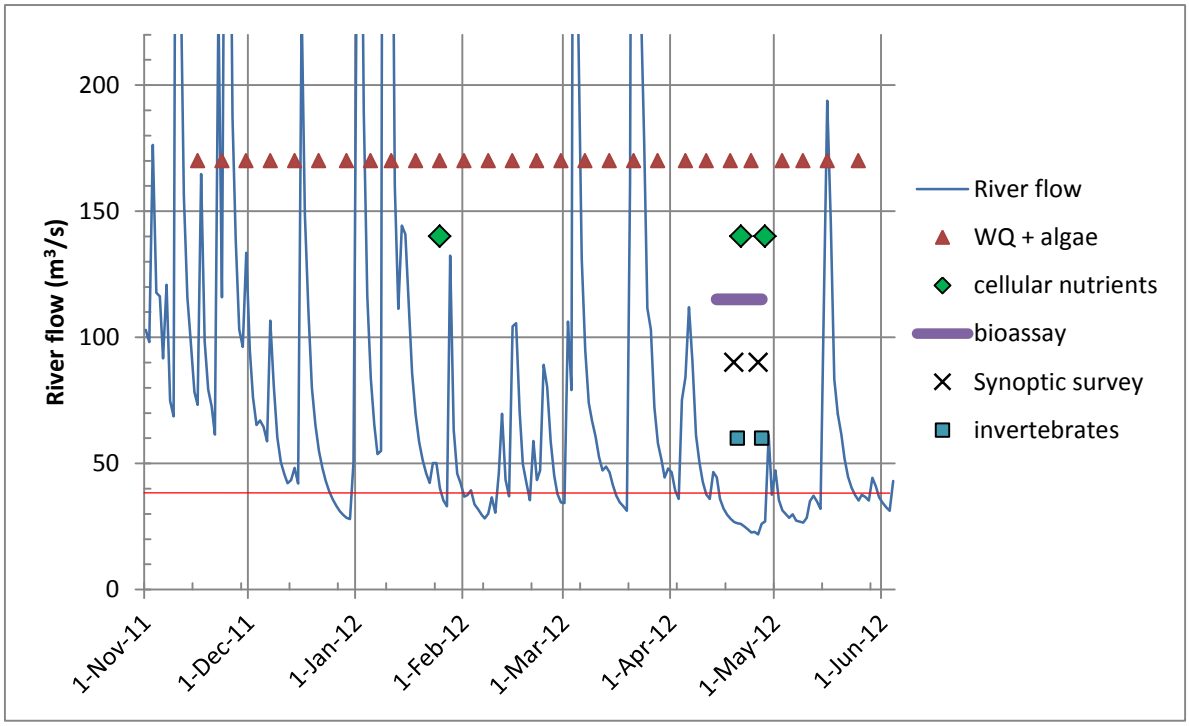


Figure 1.1: Timing of surveys and investigations overlaid on flow in the Manawatu River (at Teachers College). The red line indicates flows of 37 m^3/s (half median flow) below which the WWTP must be treating for phosphorus. The top of the scale (220 m^3/s) represents a flow of three times median flow.

1.5 Statistical analysis

The statistical significance of water quality results, periphyton cover and macroinvertebrate indices was compared using a non-parametric Mann-Whitney test and an equivalence test in the software 'TimeTrends'. The data from upstream sites and downstream sites were pooled, which incorporates both within site diversity and between site diversity. A difference was considered statistically significant if the *p*-value was < 0.05.

Equivalence tests incorporate both testing of means (using a student t-test) and testing of a meaningful change (interval testing). One advantage of equivalence tests is that it is less sensitive to sample size. Increasing the sampling effort may make it either more or less likely that an equivalence hypothesis will be rejected, unlike a statistic test comparing mean values, where more data makes it more likely that the hypothesis will be rejected.

2 Synoptic surveys

2.1 Aim

There has been speculation that in addition to the PNCC WWTP discharge there might be some other potential sources of nutrients in the 1.8 km between the upstream and downstream sampling points. We were asked to consider the possibility of alternative nutrient sources to the Manawatu River which could include:

- Turitea Stream entering on the true left opposite the upstream sample point;
- Palmerston North City Council closed landfill on the true right;
- Possible leaching via groundwater from the sludge storage ponds;
- Kahuterawa Stream on the true left just upstream of the first downstream sampling site.

2.2 Method

Surveys of a range of water quality variables were conducted in the river reach from 1 km upstream of the WWTP discharge to 2.5 km downstream of the discharge (150m downstream of Mangaone Stream confluence). The surveys took place on 19 and 26 April during a period of low flow when the WWTP was removing phosphorus, with the aim of identifying changes in water quality associated with the potential additional nutrient sources.

Electrical conductivity, temperature and dissolved oxygen measurements were made in the field at approximately 100m intervals between the discharge point and the 800m downstream site in the main stem of the Manawatu River. Water quality samples were collected at nine sites on the Manawatu River, as well as from the discharge, from the Turitea Stream (entering about 1100m upstream on the true left), the Kahuterawa Stream (entering about 820m downstream on the true left) and the Mangaone Stream (entering about 2250m downstream on the true right). All Manawatu River samples from 800m downstream upwards were collected from the true right bank, while from 1400m downstream they were collected on the true left due to access. The sample locations are shown in Figure 2.1. Photo 1 shows the Manawatu River at 400m downstream at this point there is evidence of fly tipping or possibly old landfill material (e.g. plastic wrap and concrete) amongst the eroded gravel bank of the river (see Photo 2).

Water quality samples were stored in a cool chilli-bin and sent to Watercare Laboratories overnight to test for the following variables: electrical conductivity (EC), pH, $cBOD_5$, total nitrogen (TN), nitrate-nitrogen, nitrite-N, total ammoniacal-N (NH_4-N), total phosphorus (TP), total dissolved phosphorus (TDP), dissolved reactive phosphorus (DRP), *E. coli* bacteria, total hardness, a suite of organic compounds, and a suite of trace elements and metals (dissolved and total fractions) including: aluminium (Al), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), sodium (Na), zinc (Zn), arsenic, boron (B), chloride (Cl) and sulphur (S). Soluble inorganic nitrogen (SIN) was calculated from nitrate, nitrite and total ammonia.

Variables that are typically elevated as a result of any landfill influence include: iron, boron, chloride, potassium, zinc and total ammonia.

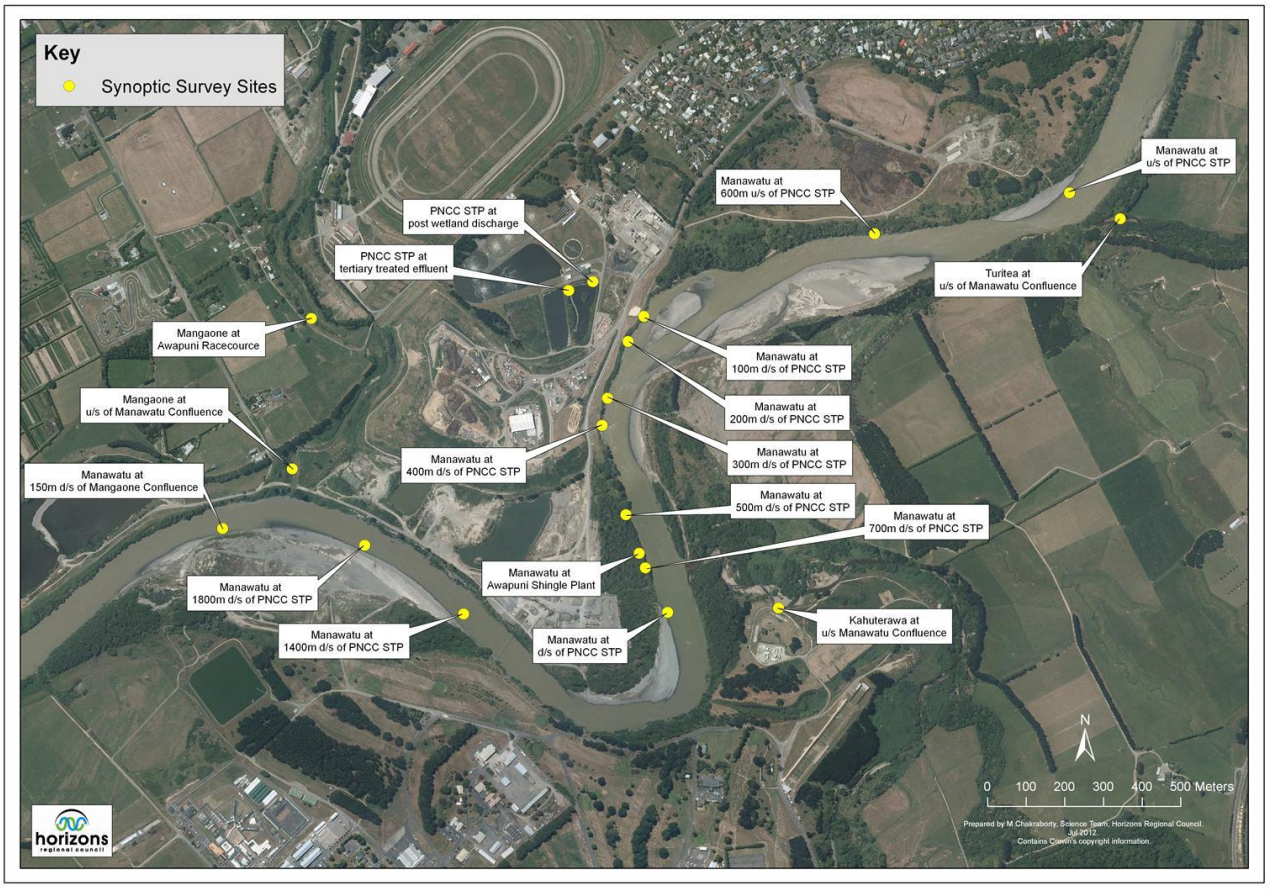


Figure 2.1: Water quality sample sites for the synoptic survey on 19th and 27th April. White circles indicate the discharge and tributaries. Additional electrical conductivity measurements were taken every 100m between the discharge point and the 800m downstream monitoring site.

Photo 1: Manawatu River at 400m downstream of the discharge, facing upstream during about median flow.

Photo 2: Evidence of fly tipping or possibly old landfill material exposed by the river at about 400m downstream of the discharge.

2.2.1 Adjusting for dilution

Salinity or electrical conductivity (EC) is often used as a conservative tracer in mixing studies. Because the WWTP discharge has considerably higher EC compared to the Manawatu River upstream (i.e. about 808 µS/cm compared to 216 µS/cm), the EC in the discharge itself can be used to estimate dilution at sampling points downstream.

Dilution was calculated using a mass balance approach over a 'control volume' of the river after mixing. The formula for the dilution factor (D) for downstream sampling points is:

$$D = (EC_{eff} – EC_{u/s})/(EC_{d/s} – EC_{u/s})$$

Where: D = dilution factor; EC_{eff} = electrical conductivity of the effluent; $EC_{u/s}$ = EC of the river upstream; $EC_{d/s}$ = EC of the river downstream.

The dilution factor obtained from this method for each sample location was then used to calculate a theoretical concentration (assuming no in-river attenuation) for variables of interest using the following formula:

$$C_{d/s} = ((C_{eff} – C_{u/s}) / D) + C_{u/s})$$

Where: D = dilution factor, C_{eff} = concentration in the effluent; $C_{u/s}$ = concentration in the river upstream; $C_{d/s}$ = concentration in the river downstream.

An estimate was also made of the theoretical dilution available if the effluent was fully mixed with the river. This was done using measurements of electrical conductivity and discharge volume from the effluent and the Manawatu River upstream. The following formula was used:

$EC_{\text{fully mixed}}$ = ((upstream EC x river flow) + (discharge EC x discharge flow)) / (river flow + discharge flow)

These methods assume a relatively constant EC in the discharge and in the river over the period of sampling. EC in rivers can vary diurnally as a result of photosynthesis and respiration. Photosynthesis uses carbon dioxide from the water which shifts the balance of bicarbonate ions in the water, increasing pH and, to a much lesser extent, increasing the EC during the day. Samples were collected over a four hour period from downstream to upstream so any diurnal change would result in a slight under-estimate of dilution, however fifteen minute measurements of EC in the Manawatu River at the Teaches College shows that there was very little change in electrical conductivity during the day.

2.3 Results and discussion

2.3.1 General results

The results of water quality sampling on 19th April and 26th April are shown in Appendix 3. The discharge had elevated concentrations of a number of variables including: $cBOD_5$, NH_4-N, nitrate-nitrite-N, TN, DRP, *E.coli* bacteria, zinc, aluminium and dissolved iron. For a number of variables the discharge had lower concentrations than the tributaries. Concentrations of all organic compounds were below detection and the results are not shown in the table.

The Soluble Inorganic Nitrogen (SIN)[3] concentration in the Manawatu River upstream of the discharge was 0.232 g/m^3 and 0.051 g/m^3 on 19th and 26th April respectively. These values had respectively increased to 0.88 g/m^3 and 0.586 g/m^3 at 800m downstream of the discharge. The increased nitrogen was primarily due to total ammoniacal nitrogen from the discharge.

The DRP concentration in the Manawatu River upstream of the discharge was 0.007 g/m^3 and <0.005 g/m^3 on 19th and 26th April respectively[4]. These values had respectively increased to 0.018 g/m^3 and 0.011 g/m^3 at 800m downstream of the discharge. As will be discussed, not all of the increase in DRP can be explained simply by the discharge itself.

2.3.2 Electrical conductivity and dilution

The electrical conductivity[5] in the Manawatu River downstream of the WWTP is shown in Figure 2.1. The calculated dilution factors with distance downstream are shown in Table 2.1. The dilution factors assume dilution due to mixing of the effluent with the river water and do not account for the influence of tributaries or any groundwater influence. By a distance of 800m downstream dilution factors based on EC were 60% to 90% of estimates of full mixing based on mass loads. This is consistent with a mixing study by Rutherford *et al*. (1997) who found that the effluent was not fully mixed across the river until after the bend (probably in the vicinity of 1200-1400m downstream of the discharge). At a location 850m downstream of the discharge on the true right bank the total ammonia concentrations were found to be 1.5 times fully mixed.

EC appeared to be influenced by factors other than dilution of the discharge. Figure 2.2 shows a change in EC on a more detailed scale. As would be expected, EC declined after the Kahuterawa Stream confluence (entering about 820m downstream) and slightly rised after the Mangaone Stream confluence (about 2250m downstream). However there was also a slight increase in EC between sites 600m downstream (Awapuni Shingle Plant) and 800m downstream – suggesting a possible groundwater influence at this point raising the EC by 1 to 3 µS/cm on the 19th and 26th April respectively. Small EC changes of this magnitude are sufficient to explain the lower dilution factor calculated from EC compared to the estimated dilution factor at full mixing based on mass loads.

[3] SIN is nitrate-nitrite nitrogen + total ammoniacal nitrogen and generally considered the forms most available for periphyton and plant growth.
[4] Calculations based on the 'less than' value on 27 April will have wide error margins.
[5] Using EC measured with a meter in the field.

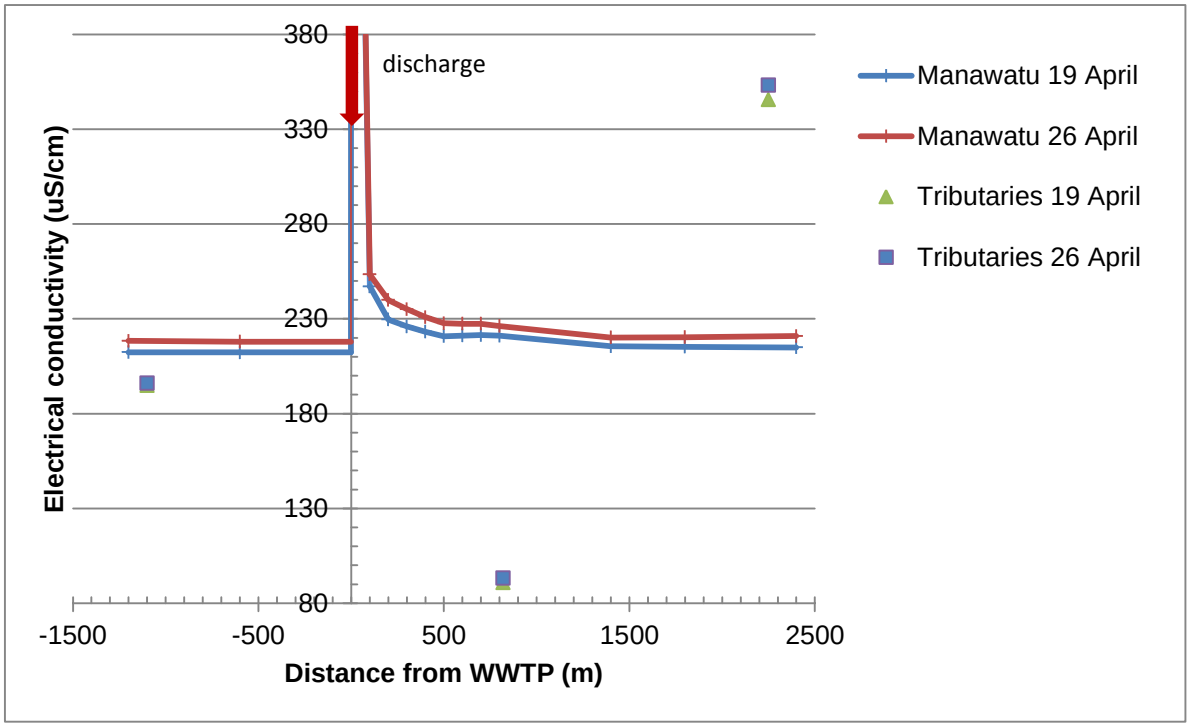


Figure 2.1: Change in electrical conductivity with distance upstream and downstream from the WWTP discharge (sampling from true right bank).

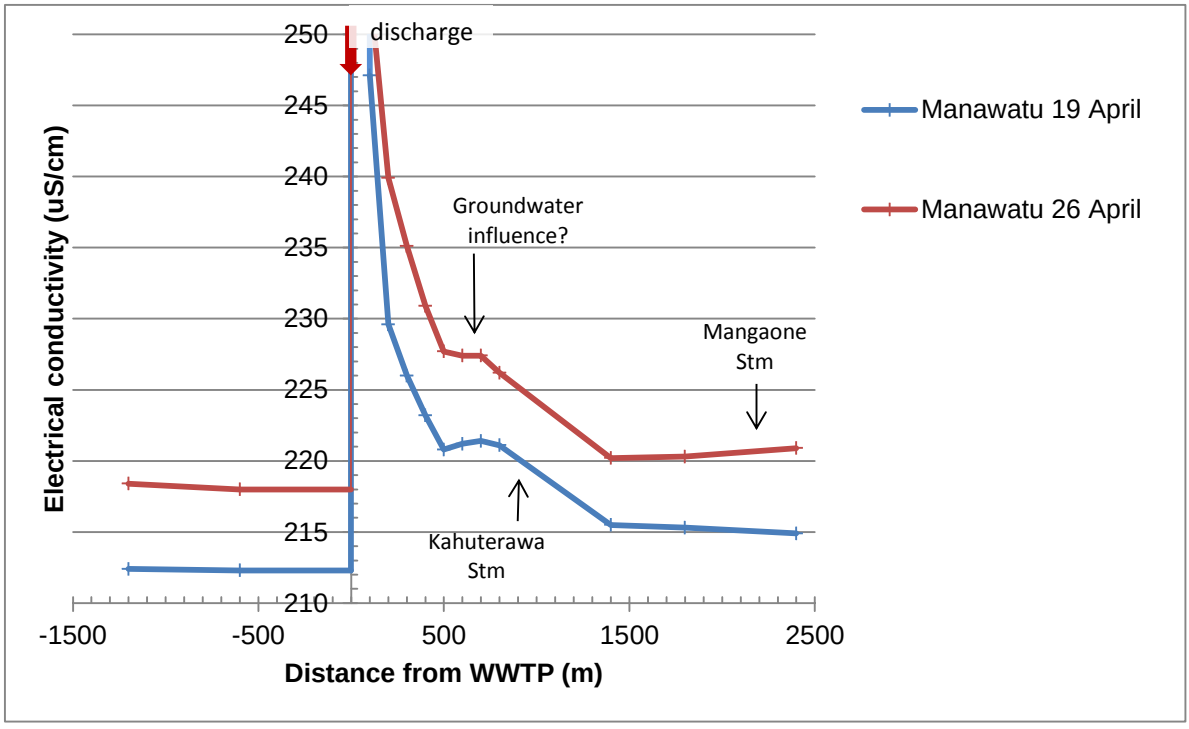


Figure 2.2: Fine scale change in electrical conductivity with distance upstream and downstream from the WWTP discharge (sampling from true right bank).

Table 2.1: Dilution factor on true right bank of Manawatu River with distance downstream from WWTP discharge (calculated from electrical conductivity).

Distance d/s (m)	Dilution factor 19th April	Dilution factor 26th April	Comment
100	17.1	16.9	
200	34.4	27.4	
300	43.5	35.0	
400	54.7	46.4	
500	70.1	61.8	
600	66.9	63.7	
700	65.5	63.7	
800	67.7	73.0	
Full mixing	**114.6** [6]	**80.3** [7]	Calculated from daily load
820			Kahuterawa Stream influence
1400	186.2	272.3	
1800	198.6	260.4	
2250			Mangone Stream influence
2400	229.1	206.6	

2.3.3 Change in nutrient concentration

Changes in the concentration of total nitrogen (TN) and soluble inorganic nitrogen (SIN) along the study reach are shown in Figure 2.3 and Figure 2.4 respectively. The graph compares measured concentrations with hypothetical concentrations that would occur solely due to dilution. The observed change in total nitrogen was strongly related to dilution due to mixing of the WWTP effluent. SIN concentrations were also strongly related to dilution due to mixing of the effluent, although on 19th April there was about 18% [8] more SIN measured than expected due to dilution at sites 400m to 800m downstream of the discharge; this suggests that a small amount of TN may be converting into SIN in this reach of the river. TN and SIN showed a similar pattern by the time the river was full mixed at 1400m downstream.

Upstream of the discharge about two thirds of the total nitrogen concentration was in the form of nitrate, one third in the form of organic nitrogen and only about 4% in the form of total ammonia (NH_4-N). The discharge contributes a considerable load of nitrogen to the river – predominantly (88% to 90%) in the form of NH_4-N and organic N (10% to 12%). Thus 800m downstream of the discharge total nitrogen was predominantly in the form of NH_4-N (72% to 75%), with a smaller proportion of nitrate (11% to 27%) and organic nitrogen (1% to 14%). On 19th April there was evidence of about 0.1 mg/l of organic nitrogen being converted to NH_4-N between the sites upstream and 800m downstream of the discharge.

The change in concentration of total phosphorus (TP) and dissolved reactive phosphorus (DRP) is shown in Figure 2.5 and Figure 2.6 respectively. As with TN, the results for TP

[6] Based on effluent discharge of 0.2361 m^3/s, effluent EC 808 µS/cm, river discharge of 26.822 m^3/s, river EC 212.3 µS/cm.
[7] Based on effluent discharge of 0.2755 m^3/s, effluent EC 817 µS/cm, river discharge of 21.844 m^3/s, river EC 218 µS/cm.
[8] 18% more SIN at site 800m downstream, 0.88 g/m^3 measured compared to 0.747 g/m^3 predicted.

show that the observed change in TP was strongly related to dilution due to mixing of the WWTP effluent. However DRP measured at the site 800m downstream on 19 April was 160% above background concentrations and about 2.4 higher[9] than concentrations expected by simple dilution of the effluent (as estimated by EC). The discharge was predicted to cause a 7% increase in DRP concentrations[10], with the additional measured increase probably due to other sources.

Effluent sampling (post-wetland) by PNCC had higher values than effluent samples collected during the synoptic survey (i.e. 0.114 mg/l compared to 0.04 mg/l on 19th April, and 0.13 mg/l compared to 0.019 mg/l on 26th April). The change in DRP due to dilution was calculated using both values and graphed in Figure 2.6. Even based on these higher values measured concentrations was 2.1 times higher than expected due to dilution. On 19 April the discharge was predicted to cause a 23% increase in DRP concentrations[10], with the additional measured increase probably due to other sources.

Some studies have total dissolved phosphorus (TDP) to be a better indicator of bioavailable phosphorus because it included dissolved organic forms that can also be bioavailable (e.g. Turner et al. 2003). In this survey TDP followed a similar pattern to DRP (see Figure 2.7). Results from weekly monitor (see next section) found TDP to be, on average, 36% and 14% higher than DRP at sites 800m downstream and 1400m downstream respectively when flows were less than 40 m^3/s.[11] At the site 800m downstream, for 19 and 26 April respectively, the measured TDP was 0.019 g/m^3 and 0.015 g/m^3 i.e. 171% and 150% higher than upstream. In contrast the predicted TDP for this site was 0.0083 g/m^3 and 0.007 g/m^3, i.e. 19% and 17% increase from upstream.

One feature of Figure 2.6 and 2.7 is that DRP and TDP concentrations predicted from dilution calculations appear to be relatively constant with distance downstream compared to the other variables graphed. This is because the discharge had a relatively low DRP concentration compared to the river (about 5.7 times the background river concentration), whereas other variables were proportionally much higher in the discharge e.g. total phosphorus in the discharge was about 109 times above background river concentrations.

2.3.4 Change in phosphorus load

Another way to examine the potential effects of the WWTP discharge is to compare the nutrient load from the river and the discharge. Table 2.2 compares the load to DRP and TP in the Manawatu River upstream and downstream (after full mixing) and from the discharge and Managone Stream. The results are consistent with the dilution analysis – i.e. there was an unexplained source of DRP (about 6.9 to 11 kg/day) but a relatively consistent TP.

[9] At site 800m downstream on 19th April measured DRP was 0.018 g/m^3 compared to a predicted value of 0.0075 g/m^3. Values from 26th April were unreliable for this comparison because upstream values were <0.005 g/m^3

[10] On 19th April, upstream DRP was 0.007 g/m^3, 800m downstream was 0.018 g/m^3 and predicted was 0.0075 g/m^3 using synoptic survey data and 0.0086 g/m^3 using PNCC effluent data. On 26 April upstream DRP was <0.005 g/m^3, making comparisons uncertain.

[11] Mean TDP was 0.0116 and 0.0098 g/m^3 at site 1400m downstream, mean DRP was 0.00855 and 0.00856 g/m^3 at site 1400m downstream. Mean concentrations of TDP and DRP in the discharge were 0.0879 and 0.033 g/m^3 respectively

Table 2.2: Load of TP and DRP in the Manawatu River on 19th April 2012 and during low flows between 11 April – 25th May (n=6).

Site	19th April		11th April – 25th May	
	DRP load (kg/day)	TP load (kg/day)	DRP load (kg/day)	TP load (kg/day)
Manawatu Rv u/s	16.22	25.49	21.23	44.8
Discharge	*0.82*	*24.48*	*0.577*	*17.11*
Manawatu Rv 1400m d/s	-	-	28.69	60.68
Manawatu Rv 1800m d/s	28.10	44.5	-	-
Mangaone Stm	*0.067*	*0.34*	-	-
Manawatu Rv 2400m d/s	25.79	37.52	-	-

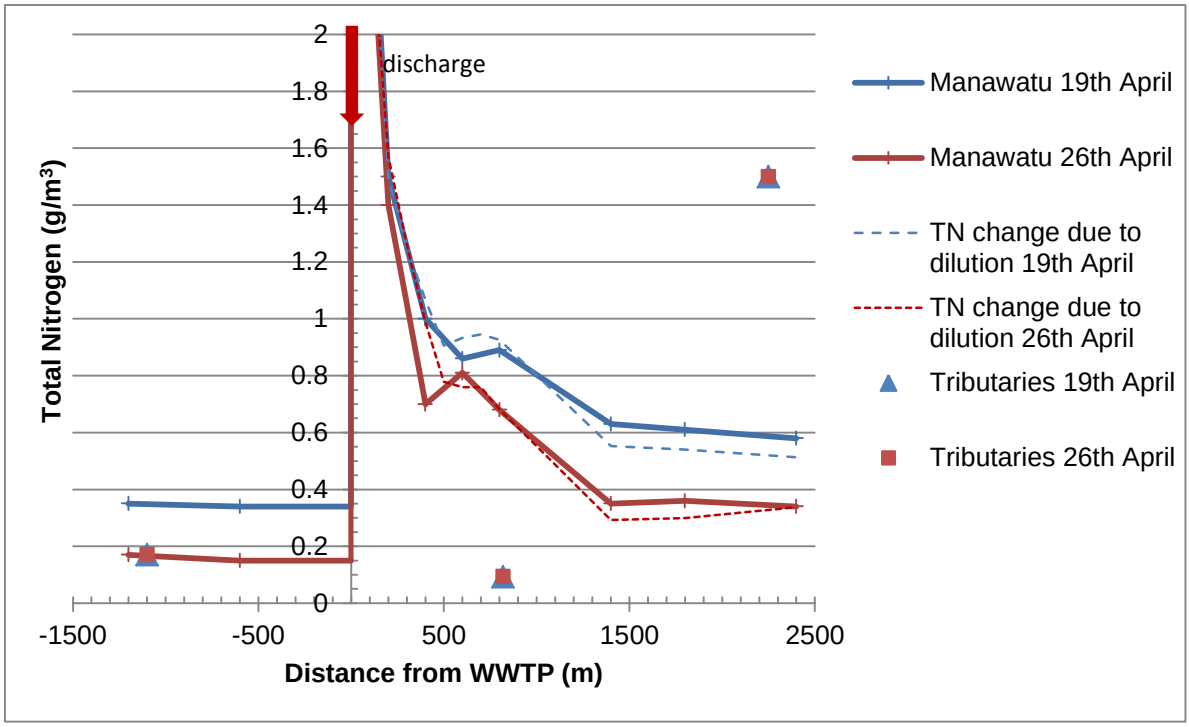


Figure 2.3: Change in total nitrogen with distance upstream and downstream from the WWTP discharge (sampling from true right bank).

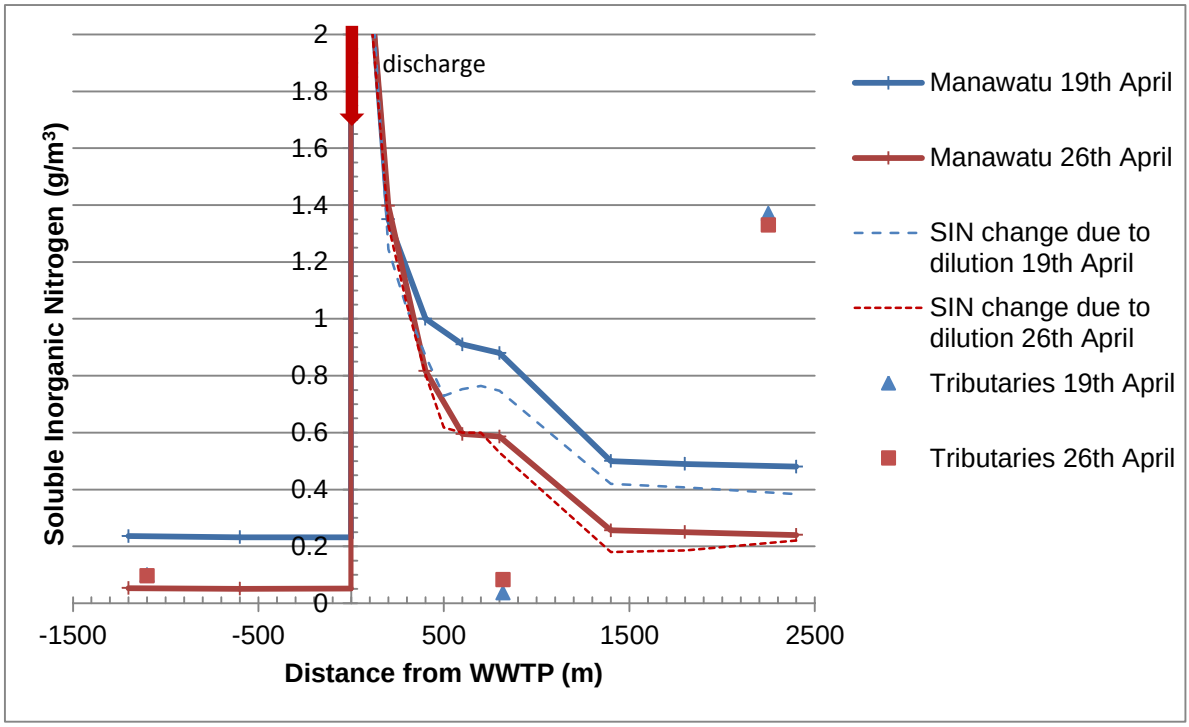


Figure 2.4: Change in soluble inorganic nitrogen with distance upstream and downstream from the WWTP discharge (sampling from true right bank).

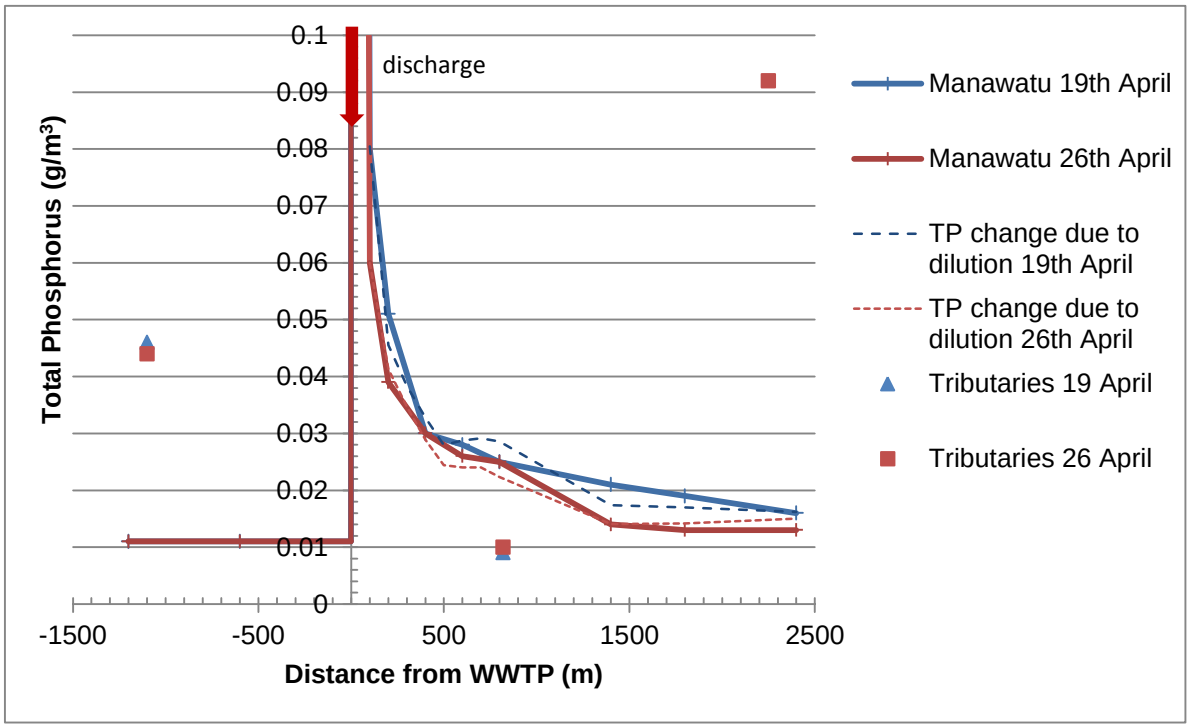


Figure 2.5: Change in total phosphorus with distance upstream and downstream from the WWTP discharge (sampling from true right bank).

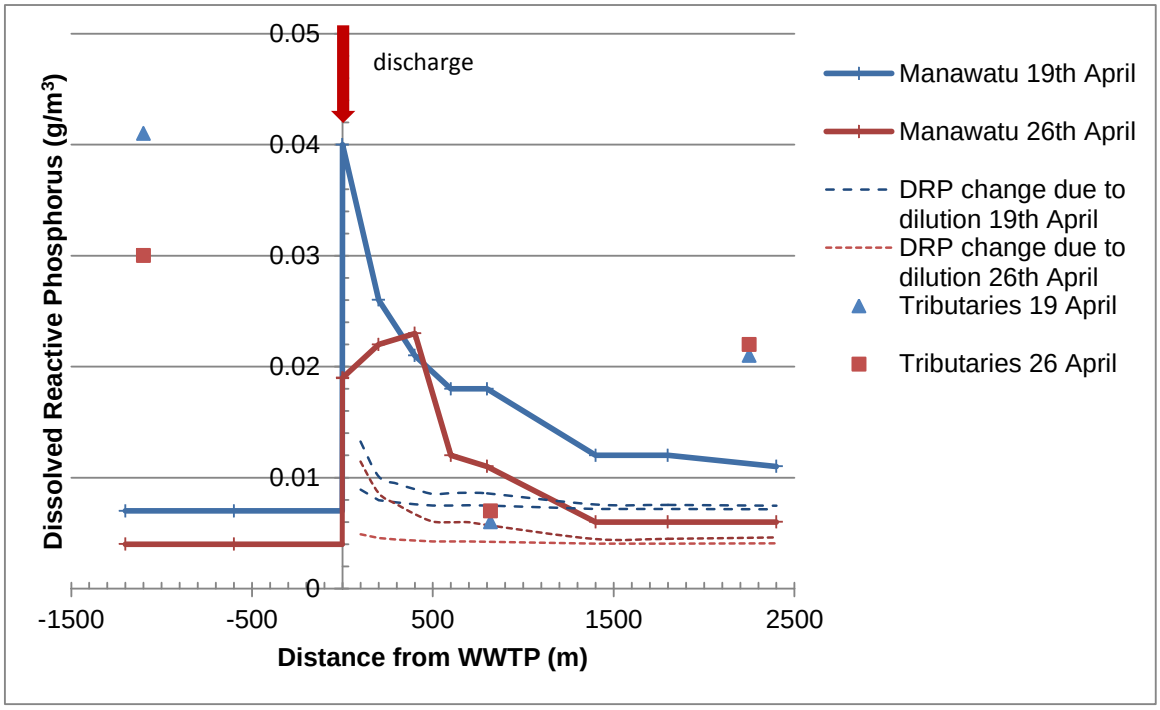


Figure 2.6: Change in dissolved reactive phosphorus with distance upstream and downstream from the WWTP discharge (sampling from true right bank). The dashed lines show expected change in DRP due to dilution; two estimates are graphed for each date based on: effluent sample from synoptic survey (lower line), effluent sample from PNCC compliance sampling (higher line).

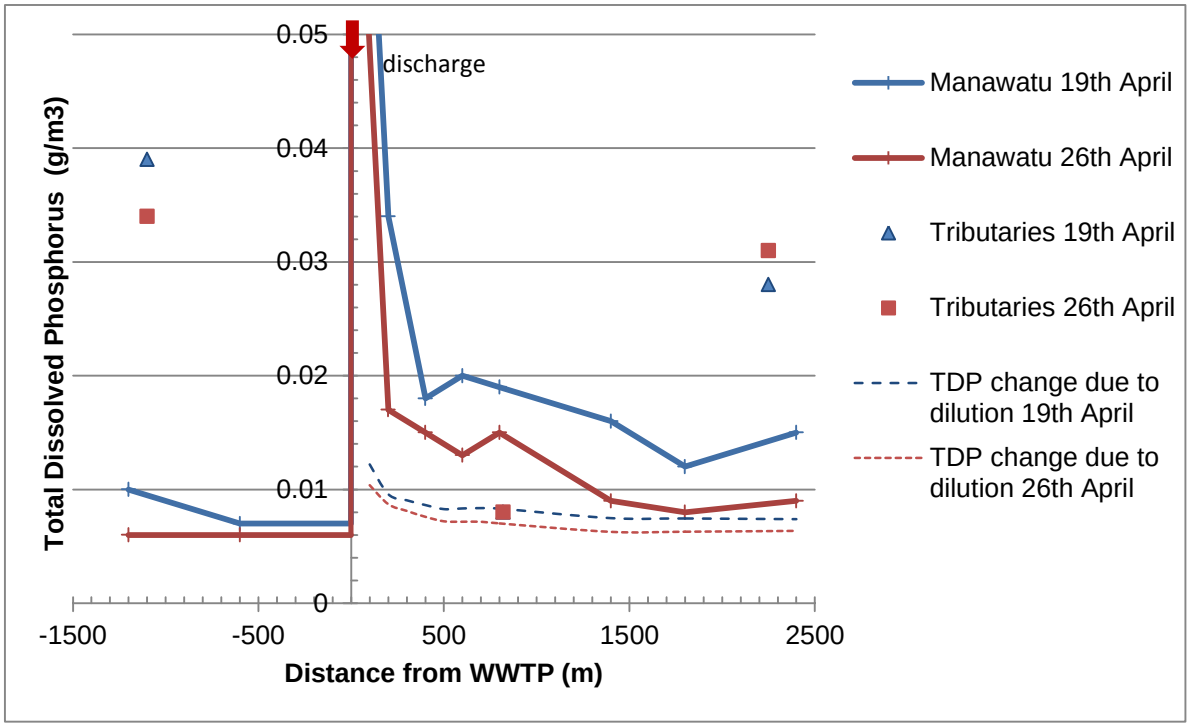


Figure 2.7: Change in total dissolved phosphorus (TDP) with distance upstream and downstream from the WWTP discharge (sampling from true right bank). The dashed lines

show expected change in DRP due to dilution; two estimates are graphed for each date based on: effluent sample from synoptic survey (lower line), effluent sample from PNCC compliance sampling (higher line).

2.3.5 Potential sources of DRP in addition to the discharge

The doubling in DRP concentration compared to the predicted concentration cannot be simply explained by desorbing of phosphorus attached to particles to a dissolved form because both pH and dissolved oxygen were high in the river compared to the effluent – the opposite conditions than what would be expected for desorbing P. A more plausible explanation is that two separate processes are occurring, whereby some particulate phosphorus is settling in the river prior to full mixing while at the same time there is another source of DRP influencing the river downstream of the WWTP discharge.

Potential sources of DRP to the river downstream of the discharge could be:

a) Seepage of groundwater rich in P.

b) Desorption of dissolved phosphorus from the river sediments as the sediments come to equilibrium with lower P concentrations in the overlying water; or

c) Late mixing of the Turitea Stream (but we this),

Seepage of groundwater carrying nutrients derived from the ponds near the WWTP or the landfill could be a source of DRP. If this is occurring then the predicted nutrient concentrations (based on EC) would be under-estimates and thus the change in DRP concentration compared to what would be expected from the WWTP discharge would be more than 2.4 times.

It is possible that when the discharge is not treating for P the sediment acts as a P sink (i.e. increasing the equilibrium P concentration). When the WWTP is alum dosing for P the discharge and river concentrations are lower, and some of the P stored in the sediments could be released back into the water column (see Lucci *et al* 2010, Haggard and Stoner 2009).

The Turitea Stream had DRP concentrations slightly higher than the treated effluent. The Turitea Stream confluence with the Manawatu River is on the true left about 1100m upstream of the discharge, but during the study period the flow of the Manawatu River was concentrated on the true left so it was considered possible that the Turitea Stream did not fully mix across the river until some point after the site 600m upstream. However during the period the Turitea Stream had low flows (about 0.06 m^3/s), about a quarter of the volume from the WWTP and a slight decline (0.1 to 0.4 µS/cm) in EC was observed between the sites 1200m upstream and 600m upstream, that that was consistent with full mixing of the Turitea Stream with the Manawatu River. Furthermore, previous monitoring of the Turitea Stream has shown much lower DRP concentration which counts against this being a long term contributor of nutrients.

2.3.6 Potential influence of groundwater on the Manawatu River

The results of the synoptic surveys provides some evidence that groundwater may be entering the Manawatu River between 600m and 800m downstream of the discharge. This was evidenced by an increase in the concentration of electrical conductivity (see Figure 2.2), and nitrate-nitrite-nitrogen compared to sites upstream of the discharge and immediately downstream of the discharge. The concentrations of some other variables do not decline as quickly as would be predicted by simply dilution of this discharge, these include: DRP, SIN, and potassium.

Variables are typically elevated as a result of landfill leachate include: total ammonia, iron, boron, potassium, sodium, manganese and chloride. Groundwater monitoring in the vicinity of the WWTP and the landfill has shown concentrations of a number of these variables elevated compared to the river including EC (320 to 1080 µS/cm), total ammonia (0.05 to 76 g/m^3), nitrate-nitrite-N (0.1 to 32.8 g/m^3), DRP (up to 0.76 g/m^3). Groundwater DRP concentrations were highest downstream of the WWTP ponds (and upstream of the landfill). River measurements were taken near the true right bank of the river and will be detecting these concentration increases before full mixing of any groundwater with the river.

It is plausible that nutrients derived from the WWTP ponds or landfill is moving with groundwater to the river. To confirm the degree of influence and the sources of contamination would require some further work including collecting groundwater samples from shallow groundwater adjacent to the river.

Horizons RC surveyed groundwater levels in bores around the landfill and WWTP site in 15th June 2012 when the river flow was about 49 m^3/s. This showed groundwater gradients shifting towards the southwest as the river flows around the bend. It is likely that groundwater gradients under the Awapuni shingle plant, landfill and WWTP shift westwards (towards Mangaone Stream confluence) at higher river levels.

Note that during periods of low flow the Mangaone Stream has very little flow, i.e. about 37 l/s at half median flow which is about 15% of the volume of effluent discharged by the WWTP. Thus the potential influence of the Managaone Stream on water quality in the Manawatu River is small.

Groundwater investigations under the Awapuni landfill in 1993 (Earthtec Consulting 1995) estimated the groundwater flow of the unconfined aquifer to be 1670 m^3/day and upward groundwater leakage in the base of the unconfined aquifer to be 20 m^3/day. Assuming a DRP concentration of 0.4 g/m^3 (average of bore MW4) this translates to a DRP load of 0.716 kg P/day. This is about the same DRP load to the river as the discharge during low flows in April when treating for P (0.6 kg/day) (see Table 2.2), but does not fully explain the additional 7 kg/day measured downstream. If these estimates are correct then groundwater seepage would be only a partial explanation for the additional dissolved P concentrations.

2.4 Summary of the synoptic surveys

- Electrical conductivity was used a tracer to calculate dilution factors as the treated effluent from the WWTP progressively mixed with the Manawatu River.
- Changes in EC values indicated that a groundwater source may have been entering the river on the true right bank above sites 600m to 800m downstream of the discharge.
- Soluble inorganic nitrogen (SIN) was about 18% higher than what would be predicted simply by dilution of the effluent. This may be due to organic nitrogen changing to a dissolved inorganic form, but a contribution of nitrate from a groundwater influence may also be a contributing factor.
- At a distance of 800m downstream, the discharge increased the SIN concentration in the river from 0.232 g/m^3 to 0.88 g/m^3 on 19th April, and 0.051 g/m^3 to 0.586 g/m^3 on 26th April. The increased SIN was primarily in the form of total ammoniacal nitrogen and exceeded guideline concentrations for control of periphyton growth.
- At a distance of 800m downstream, the discharge and other sources substantially increased the DRP concentration in the river from 0.007 g/m^3 to 0.018 g/m^3 on 19th April (a 157% increase), and <0.005 g/m^3 to 0.011 g/m^3 on 26th April. The direct discharge was predicted to cause a 7% to 23% increase in DRP concentrations (i.e. 0.0005 to 0.0016 g/m^3) with the rest due to other sources.
- At a distance of 800m downstream, total dissolved phosphorus was measured to be 150% to 170% higher than upstream, while the WWTP was predicted to cause an increase of only 17% to 19% above upstream concentrations.
- Changes in TP concentration was consistent with dilution based on EC. However DRP measured 800m downstream was 2.4 times higher than concentrations predicted simply by dilution of the effluent.
- Total phosphorus (TP) concentrations were similar to (based on dilution calculations) or less than (based on load calculations) what was expected due to the WWTP discharge. This suggests that some particulate P from the alum treated effluent was precipitating in the river downstream of the discharge.
- The disproportionate increase in DRP compared to EC observed during the April low flow may be explained by:
    - Dissolved P carried with groundwater seepage to the river (although load estimates suggest this might be only a partial explination),
    - Desorption of dissolved phosphorus from the river sediments as the sediments come to equilibrium with lower P concentrations in the overlying water.

- The difference between measured and predicted DRP was less on 26th April compared to 19th April. This would be consistent with both a reduction in groundwater head (and thus discharge volume) in the weeks following rain, or DRP sorbed to sediments coming into equilibrium with overlying river water.

- Potential sources of contaminants to groundwater have not been confirmed but could include the closed landfill site or seepage from WWTP storage ponds. The location where groundwater enters the river is likely to shift westward towards Mangaone Stream at higher river flows.

- To confirm the degree to which groundwater influences the river water quality and the source of any contaminants would require some further work including collecting groundwater samples from shallow groundwater adjacent to the river and surveying water levels during periods of low flow (i.e. less than half median flow).

- To confirm potential desorption of dissolved phosphorus from the river sediments this would require further work to test the sorption capacity of river sediments.

3 Water quality survey

3.1 Aim

Weekly river water quality sampling was undertaken to provide information to interpret observed changes in periphyton cover and composition and to allow more accurate calculation of mass loads during periods of stable river flow. This in turn was used to assess the influence of other nutrient sources to the Manawatu River during periods of stable river flow.

The results presented in this section are further discussed in section 4 in the context of periphyton dynamics.

3.2 Method

Water quality sampling was undertaken weekly from 16th November 2011 to 25th May 2012 at the same time as assessments of periphyton cover. The sampling was usually done on a Wednesday or Thursday. The location of these sites are shown in Figure 3.1, the sites sampled were:

- Manawatu River about 1000m above the discharge point;
- Manawatu River about 800m below the discharge point,
- Manawatu River about 1400m below the discharge point,
- PNCC WWTP discharge prior to the wetland and
- PNCC WWTP discharge after the wetland.

Water quality samples were stored in a cool chilli-bin and sent to Watercare Laboratories overnight to test for the following variables: total nitrogen, nitrate-nitrite nitrogen (NNN), total ammoniacal nitrogen (NH_4-N), total phosphorus (TP), dissolved reactive phosphorus (DRP), dissolved iron, dissolved magnesium and dissolved aluminium. Instream field measurements were made for the following parameters in the Manawatu River: electrical conductivity (EC), dissolved oxygen (DO), pH, and temperature.

In addition, DRP was measured daily from the Totara Road WWTP during periods when the Manawatu River is half median flow (37 m^3/s) and when the WWTP was treating for phosphorus. Daily flow weighted composite samples were collected prior to the effluent entering the wetland pond and a daily grab samples was collected after the wetland pond.

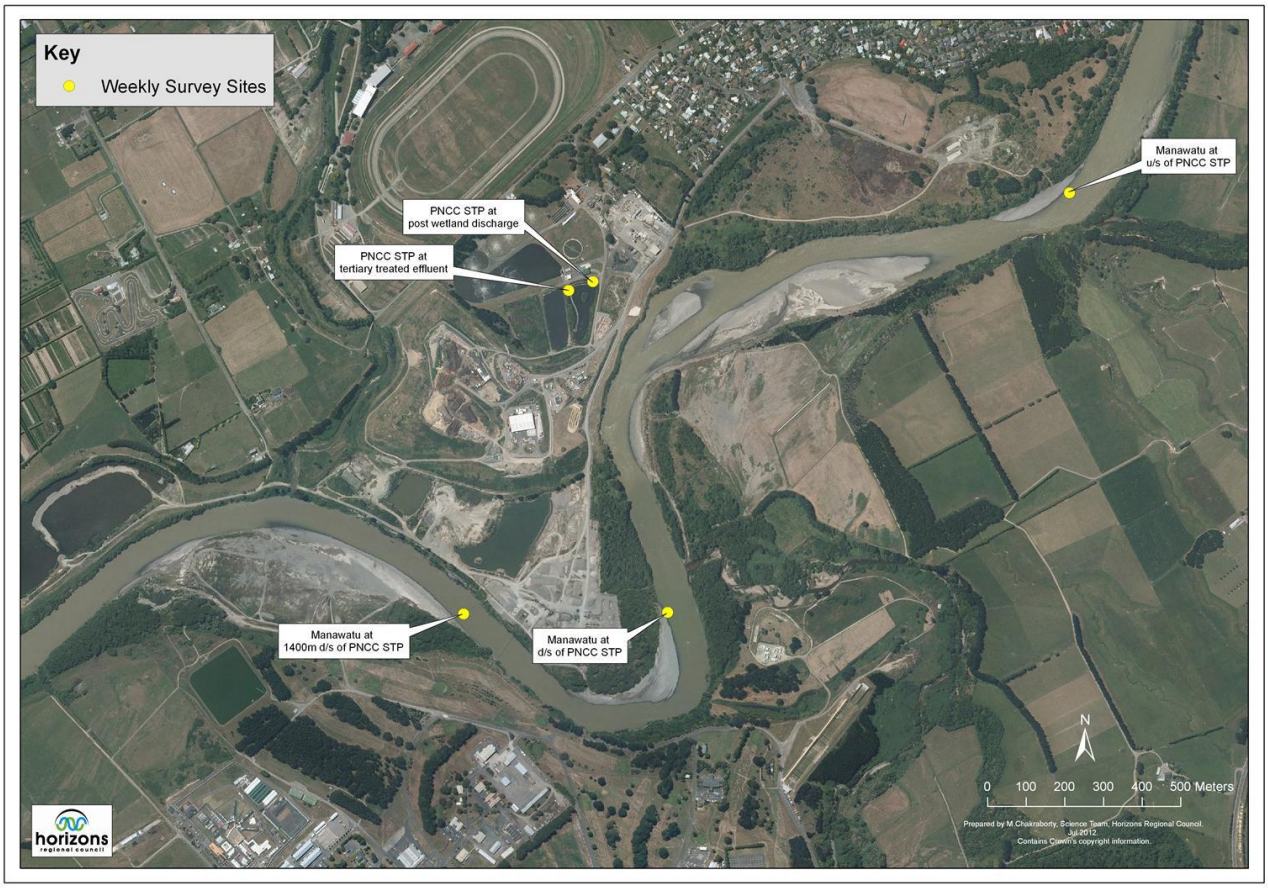


Figure 3.1: Location of water quality monitoring sites on the Manawatu River and the WWTP discharge. The location of the discharge is indicated with a yellow arrow.

3.3 Results

3.3.1 Totara Road WWTP discharge monitoring

The concentration of DRP leaving the treatment wetland during low flow periods since November 2012 is shown in Figure 3.1. The mean DRP concentration during this period when the river was at half median flow was 0.096 g/m^3. In contrast, when the WWTP was not treating for P the mean concentration discharged was 2.62 g/m^3 (mean of monthly samples between April 2009 and April 2012). During the period when the nutrient bioassay was in the river (15th April to 28th April 2012) the mean DRP concentration of the discharge was 0.078 g/m^3 and the mean load of DRP was 1.81 kg/day.

The long term mean DRP discharged during phosphorus treatment (April 2009 to May 2012) was 0.109 g/m^3 and the corresponding long term DRP load was 2.63 kg/day.

A comparison of sampling before and after the wetland found that the concentration of DRP was on average about 24% lower after the wetland than before it, but there is considerable variability in the data (see Appendix 6).

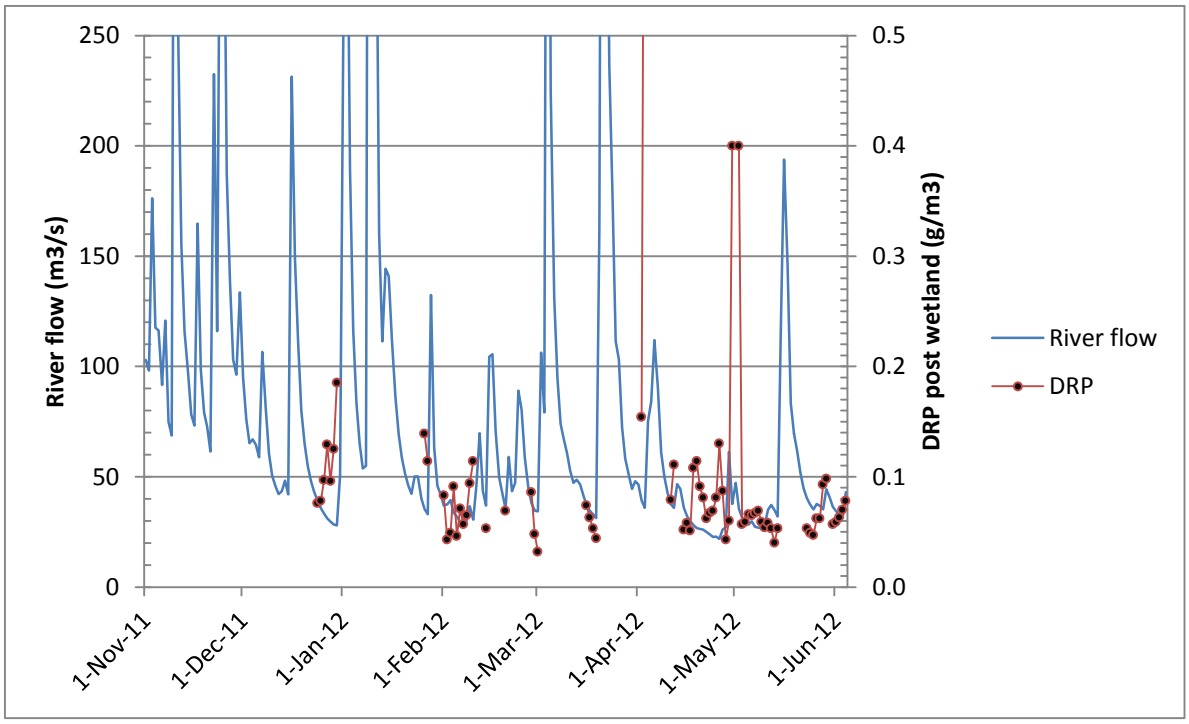


Figure 3.1: Dissolved Reactive Phosphorus concentration in the discharge from the treatment wetland and corresponding river flow.

3.3.2 River and effluent monitoring

The results of water quality monitoring during November 2011 to May 2012 are shown in Appendix 4. Table 3.1 shows the median of selected variables for the full period and Table 3.2 shows the median when the WWTP was treating for phosphorus i.e. the flow in the Manawatu River was about half median flow. The results of a Mann-Whitney statistical test are shown in Table 3.3.

All variables had median values within the targets set in the One Plan and guideline values (i.e. ANZECC 2000, periphyton guidelines (Biggs 2000) with the exception of: nitrate, soluble inorganic nitrogen (SIN), dissolved reactive phosphorus (DRP), periphyton biomass (as measured by chlorophyll *a*) and periphyton cover (as measured by the Periphyton Proliferation Index (PPI)).

SIN was within the One Plan target value (i.e. 0.44 g/m^3) at the upstream site but only dropped to within concentrations that the Biggs (2000) model predicts are needed to control excessive periphyton growth during low flow periods (i.e. 0.1 g/m^3 to 0.295 g/m^3 for accrual periods of 27 to 30 days respectively). There was a statistically significant increase in SIN between the upstream and both downstream sites, which were primarily due to additional ammonium from the discharge (see Figures 3.2 and 3.3).

For the full data set, median DRP in the river upstream and downstream of the discharge exceeded the One Plan target value[12] (i.e. 0.01 g/m^3) but were within periphyton guideline values to avoid excessive periphyton growth assuming a 20 day accrual period (i.e. <0.026 g/m^3) (Biggs 2000). The DRP concentrations were significantly higher at the site 800m downstream but during low flow periods (when the WWTP was treating for P) the difference between upstream and downstream sites was less and not statistically significant (see Figures 3.2 and 3.3).

DRP concentrations during low flow periods were within One Plan target values and could potentially limit periphyton growth at the upstream site and downstream sites. Removal of phosphorus by the WWTP during periods of low flow reduced median DRP concentrations in the effluent to 0.018 g/m^3 (or 0.098 g/m^3 as a mean) which is similar to the values based on PNCC monitoring data for the same period.

Total phosphorus was also lower during periods when the WWTP was treating for P and a statistically significant difference could be detected at 800m downstream but not after full mixing at 1400m downstream.

Alum used for removing P from the effluent caused an increase in dissolved aluminium downstream of the discharge during low flow periods. However the absolute concentrations were low and less than the ANZECC (2000) guideline value even in the effluent prior to discharge.

Table 3.1: Median water quality results for sites in the Manawatu River during period November 2011 to May 2012.

Site	EC (uS/cm)	Field pH	NH4-N (g/m^3)	TN (g/m^3)	Nitrate (g/m^3)	SIN (g/m^3)	DRP (g/m^3)	TP (g/m^3)	Chl *a* (mg/m^2)	PPI (%)	Al (g/m^3)	B (g/m^3)	Cu (g/m^3)	Fe (g/m^3)	Ni (g/m^3)	Zn (g/m^3)
Instream guidelines *			**0.900**			**0.440**	**0.010**		**120 / 200**		**0.0550**	**0.3700**	**0.0014**		**0.0110**	**0.0080**
1000m u/s	169.4	7.7	0.01	0.555	0.310	0.320	0.013	0.026	3.9	0.7	0.023	0.021	0.0005	0.061	0.0003	0.0005
800m d/s	180.6	7.6	0.17	0.77	0.330	0.525	0.019	0.049	33.5	20.2	0.026	0.022	0.0005	0.06	0.0003	0.0006
1400m d/s	174.5	7.7	0.099	0.695	0.310	0.464	0.016	0.040	34.6	10.1	0.022	0.021	0.0005	0.064	0.0003	0.0007
WWTP post wetland	710		29	34	0.007	29.007	2.100	3.100			0.045	0.048	0.002	0.096	0.002	0.017
WWTP pre-wetland	730		30	35	0.007	30.002	2.100	3.000			0.047	0.048	0.002	0.092	0.002	0.017

Metals are dissloved fractions

NZ Periphyton guidelines (Biggs 2000) set limits for SIN of <0.29 g/m^3 and DRP of <0.026 g/m^3 assuming 20 days accrual period.

[12] This is equivalent to the values in the periphyton guidelines assuming a 27 day accrual period.

Table 3.2: Median water quality results when Manawatu River flow was less than 40 m^3/s (period November 2011 to May 2012).

Site	EC (uS/cm)	Field pH	NH4-N (g/m^3)	TN (g/m^3)	Nitrate (g/m^3)	SIN (g/m^3)	DRP (g/m^3)	TP (g/m^3)	Chl *a* (mg/m^2)	PPI (%)	Al (g/m^3)	B (g/m^3)	Cu (g/m^3)	Fe (g/m^3)	Ni (g/m^3)	Zn (g/m^3)
Instream guidelines *			**0.900**			**0.440**	**0.010**		**120 / 200**		**0.0550**	**0.3700**	**0.0014**		**0.0110**	**0.0080**
1000m u/s	193.7	7.8	0.008	0.41	0.215	0.228	0.006	0.014	41.3	1.6	0.005	0.022	0.0003	0.0435	0.0002	0.0002
800m d/s	202	7.7	0.23	0.7	0.225	0.495	0.008	0.018	225.9	41.2	0.0195	0.0225	0.0003	0.0395	0.0002	0.0005
1400m d/s	199.1	8.0	0.16	0.61	0.220	0.427	0.007	0.017	93.9	22.1	0.017	0.022	0.0003	0.04	0.0002	0.0003
WWTP post wetland	750		33	37	0.008	33.01	0.018	0.960			0.049	0.0425	0.0003	0.0435	0.0016	0.0135
WWTP pre-wetland	770		33	37	0.022	33.02	0.030	0.910			0.0645	0.0455	0.0003	0.05	0.0017	0.0135

Metals are dissloved fractions

NZ Periphyton guidelines (Biggs 2000) set limits for SIN of <0.29 g/m^3 and DRP of <0.026 g/m^3 assuming 20 days accrual period.

Table 3.3: Results of Mann-Whitney non-parametric statistical test comparing upstream and downstream sites. Bold values <0.005 indicate a statistically significant difference.

Variable	upstream vs. 800m downstream		upstream vs. 1400m downstream	
	All data	**Flow <40 m^3/s**	**All data**	**Flow <40 m^3/s**
Conductivity	0.069	0.151	ns	ns
Field pH	0.155	ns	ns	0.086
NH4-N	**<0.0001**	**0.0002**	**<0.0001**	**0.0002**
TN	**0.001**	**0.002**	0.076	**0.013**
Nitrate	ns	ns	ns	ns
SIN	**0.0003**	**0.003**	**0.020**	**0.002**
DRP	**0.008**	ns	0.089	ns
TP	**0.037**	**0.041**	ns	0.176
Periphyton biomass (Chl a)	**0.069**	**0.017**	0.109	**0.045**
Periphyton cover (PPI)	**0.0002**	**0.001**	**0.013**	**0.008**
Cyanobacteria cover %	**<0.001**	**<0.001**	**0.030**	**0.010**
Dissolved Al	0.181	**0.0002**	ns	**0.0002**
Dissolved B	ns	ns	ns	ns
Dissolved Cu	ns	ns	ns	ns
Dissolved Fe	ns	ns	ns	ns
Dissolved Ni	ns	ns	ns	ns
Dissolved Zn	ns	0.167	ns	ns

ns = not statistically significant

p-values are shown where they are less than 0.02.

p-values <0.05 were considered statistically significant.

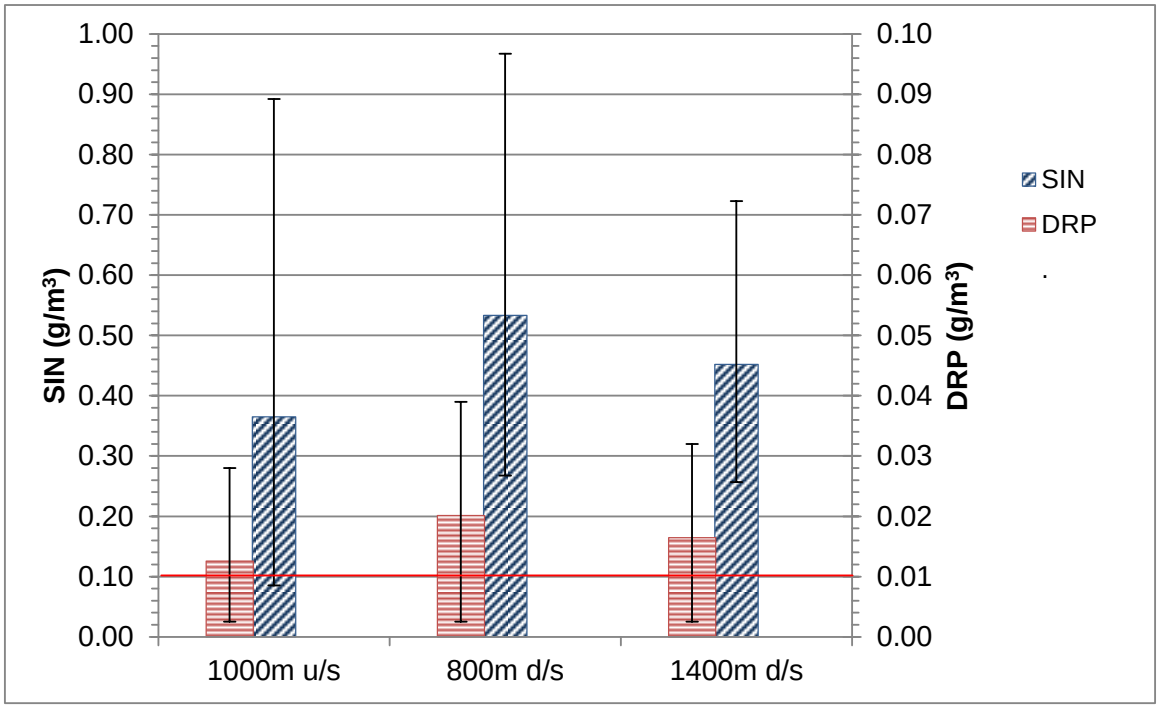


Figure 3.2: Mean DRP and SIN in the Manawatu River during period Nov 2011 to May 2012. Error bars show maximum and minimum values. The red line indicates mean monthly values to control periphyton growth assuming 27 day accrual period (Biggs 2000).

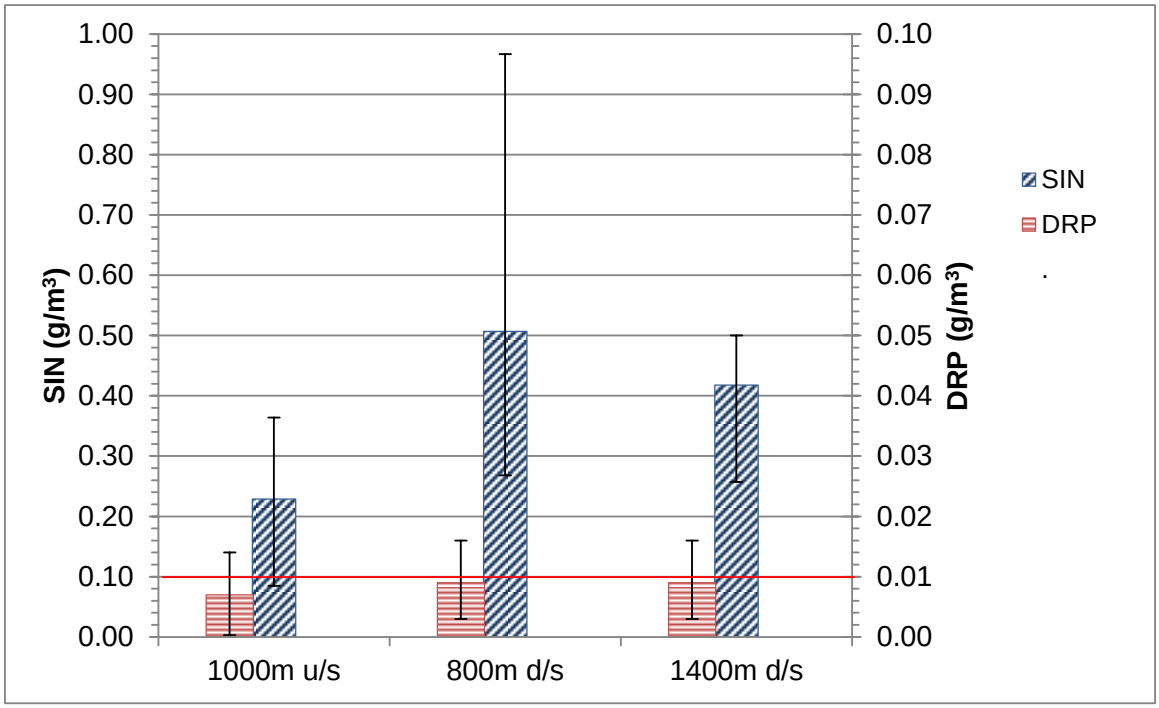


Figure 3.3: Mean DRP and SIN for sites in the Manawatu River during period when river flow was < 40 m^3/s (i.e. WWTP was treating for phosphorus). Error bars show maximum and minimum values. The red line indicates mean monthly values to control periphyton growth assuming a 27 day accrual period (Biggs 2000).

3.3.3 Potential for nutrient limitation

The potential for nutrient limitation of periphyton growth can be estimated by comparing both the concentration of SIN to DRP with guidelines of when each nutrient starts to become limiting as is done in Figure 3.4 and 3.5 for measurements at < half median river flow and between half median flow and two times median flow. Note that the guidelines for assessing nutrient limitation given in these graphs are applicable to mean monthly values and there will be a graduation in the degree of nutrient limitation. Nevertheless the graphs help illustrate that, based on nutrient concentrations at high flows there was little possible P limitation for a small percent of time at the upstream site but no limitation at the downstream sites. During periods of low flow, when the WWTP is treating for P, there was potential P limitation or co-limitation occurring most of the time at the upstream site, and potential P limitation occurring 60% to 70% of the time at the downstream sites. The degree to which nutrients were actually limiting periphyton growth is discussed in the following sections of this report.

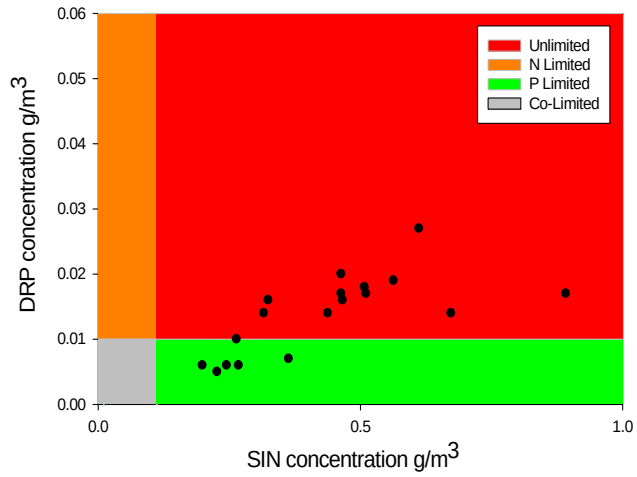
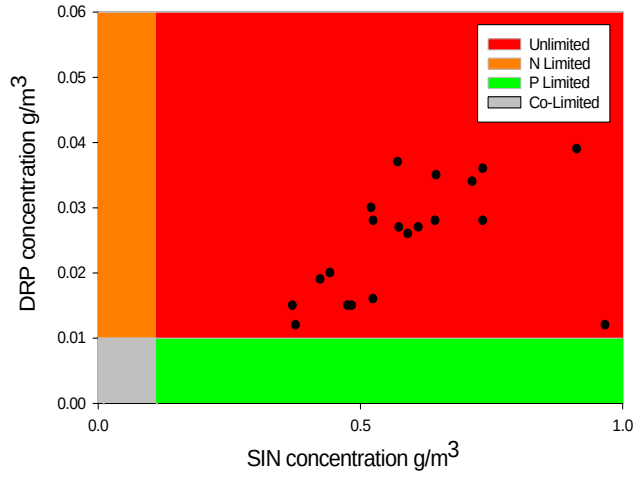
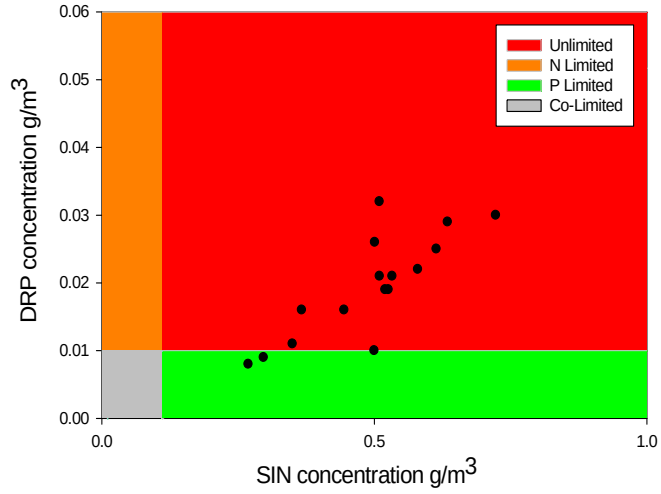
	**Manawatu River upstream** Potential limitation status percentage of results Unlimited = 67% N limited = 0% P limited = 33% Co-limited = %0
	**Manawatu River 800m downstream** Potential limitation status percentage of results Unlimited = 100% N limited = 0% P limited = 0% Co-limited = 0%
	**Manawatu River 1400m downstream** Potential limitation status percentage of results Unlimited = 81.25% N limited = 0% P limited = 18.75% Co-limited = 0%

Figure 3.5: Potential nutrient limitation when Manawatu River flow is between half median flow and two times median flow.

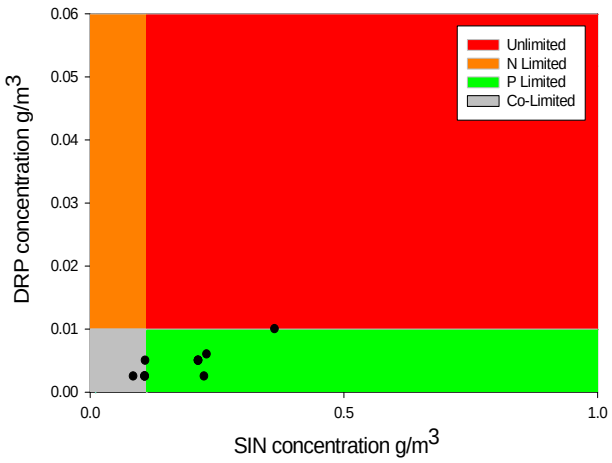
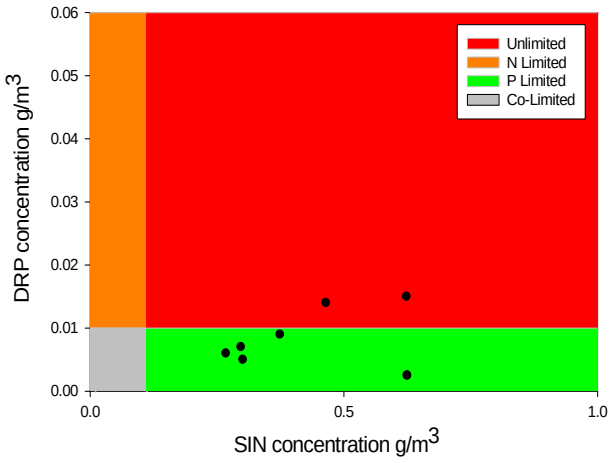
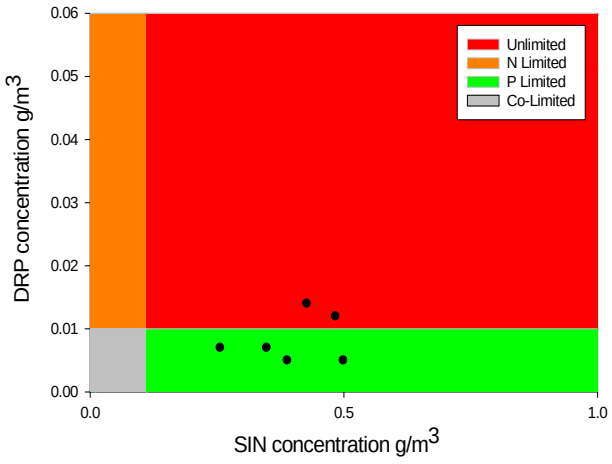
	**Manawatu River upstream** Potential limitation status percentage of results Unlimited = 0% N limited = 0% P limited = 57% Co-limited = 43%
	**Manawatu River 800m downstream** Potential limitation status percentage of results Unlimited = 29% N limited = 0% P limited = 71% Co-limited = 0%
	**Manawatu River 1400m downstream** Potential limitation status percentage of results Unlimited = 33.3% N limited = 0% P limited = 66.6% Co-limited = 0%

Figure 3.4: Potential nutrient limitation when Manawatu River flow is less than half median flow.

3.3.4 DRP predicted compared to measured

The synoptic surveys identified that groundwater seepage may be contributing to the nutrient load (and particularly DRP) downstream of the discharge during periods of low flow. To further investigate this possibility we estimated dilution and DRP concentrations at site 800m downstream of the discharge during low flow periods (Manawatu River flow < 40 m^3/s) using changes in electrical conductivity and same method as described in Section 2. For the site 800m downstream predicted median DRP during low flow periods (n=10) was 0.0057 g/m^3 compared to a measured concentration over the same period of 0.008 g/m^3. This predicted DRP was only 4% higher than measured upstream concentrations of 0.0055 g/m^3 while the measured median concentration was 50% higher. This adds weight to the conclusions of the synoptic survey. The differences between predicted and measured values were not statistically significant using the Mann-Whitney test. However testing the data using a cumulative binomial distribution returned a binomial distribution probability of 0.945, i.e. about 5% probability that the predicted DRP was lower than the measured DRP due to chance[13].

3.4 Summary

- During low flow periods DRP and SIN reduced to concentrations that could potentially control periphyton growth at the upstream site. However, at the downstream sites only DRP was sufficiently low to potentially control periphyton growth during low flow periods.

- Total ammonia and SIN concentrations were significantly higher at downstream sites under all flow conditions.

- DRP and TP concentrations were significantly higher at the downstream sites when the WWTP was not removing P from the effluent. Removal of phosphorus by the WWTP during periods of low flow reduced the median DRP concentration in the effluent to 0.018 g/m^3 (or 0.098 g/m^3 as a mean). DRP concentration at the downstream site was still higher than upstream but the difference was not statistically significant.

- Treating for P also elevated dissolved aluminium at the downstream site but the absolute concentrations remained low relative to the ANZECC guideline value.

- Predicted DRP based on dilution of the effluent during all low flow periods (n=10) was less than measured values and only 4% higher than upstream values, while measured values were 50% higher; adding weight to conclusion of the synoptic survey that there was another source of DRP downstream of the discharge.

[13] Based on predicted values being less than measured values 7 times out of 10.

4 Periphyton dynamics

4.1 Aim

Weekly monitoring of periphyton cover and biomass was undertaken to improve our understanding of periphyton temporal dynamics, variability in space and time, how quickly periphyton cover/biomass increases after floods, and how much periphyton growth occurs before and after the discharge is treated for P. The key aims of this monitoring were to:

- To clarify the extent to which the Totara Road WWTP discharge was affecting periphyton growth in the Manawatu River.
- To inform any decision to refine the timing of wastewater phosphorus removal in order to limit downstream periphyton growth.

4.2 Method

Periphyton monitoring was undertaken weekly from 16th November 2011 to 25th May 2012 at the same time as water quality sampling. The sampling was usually done on a Wednesday or Thursday.

Samples were collected from the same river monitoring sites as water quality samples:

- Manawatu River about 1000m above the discharge point;
- Manawatu River about 800m below the discharge point; and
- Manawatu River about 1400m below the discharge point (commenced in week 5 of the sampling programme).

All sites were at the upstream side of a gravel beach and had similar conditions in terms of lighting, clarity, water depth, water velocity and substrate size.

The monitoring involved visual estimates of periphyton cover in runs and collecting a representative sample for analysis of chlorophyll *a* and species composition. Where cyanobacteria mats were present, an additional sample was collected and analysed for toxicity[14].

The method for periphyton assessment was:

1. A visual assessment of the percentage cover of filamentous algae, algal mats, 'sludge', 'films' and 'clean' at 5 points across each of 10 transects (4 transects at 1400m downstream) encompassing run habitat and extending across the wadable width of the river (i.e. to a maximum depth of about 0.6m). The first transect location was marked to allow sampling at a similar location each week. The transects were at least 5 metres apart.

[14] Toxicity analysis contributed to additional investigations being undertaken by Horizons Regional Council.

2. The visual monitoring methods followed the protocols outlined in Appendix 2 of "A periphyton monitoring plan for the Manawatu-Wanganui Region" (Kilroy *et al*. 2008). Reported estimates included the following categories:

    - percentage cover of visible river bed by bacterial and/or fungal growths (sewage fungus) visible to the naked eye;
    - percentage cover of visible river bed by slimy filamentous algae more than 2 cm long;
    - percentage cover of visible river bed by coarse filamentous algae greater than 2 cm long;
    - percentage cover of visible river bed by diatoms mats more than 0.3 cm thick ;
    - percentage cover of visible river bed by cyanobacteria mats more than 0.3 cm thick;
    - percentage cover of visible river bed by film (typically diatoms) less than 0.3 cm thick; and
    - percentage cover of visible river bed that is clean.

3. The collection of a periphyton biomass sample at the same established monitoring sites and transects as above, using method QM-1b from the Stream Periphyton Monitoring Manual (Biggs & Kilroy 2000). This involved removing all periphyton from a 5.0 cm diameter area on the surface of ten (10) rocks collected across a single transect and the samples bulked to produce a single sample (i.e. a total area sampled of 0.02 m^2). Samples were frozen and sent to NIWA for analysis of chlorophyll *a* and species composition (using quantitative 200 fixed counts). Analysis of periphyton samples followed the Biggs & Kilroy (2000) guidelines for chlorophyll *a* analysis.

4. The substrate type, water depth and water velocity were recorded using the ruler method (see Harding *et al* 2009) at each point where samples are collected along the transect.

5. Additional information was collected including the distance from the marker stake to the water line and an estimate of the percent cover of any periphyton exposed above the water line. River substrate were photographed on each occasion.

6. A sample of cyanobacteria (if present) was collected at each site and sampling occasion by scaping mat material from 10 rocks into a plastic container. The samples was frozen as soon as possible and then sent for toxin extraction and analysis.

To help assess the biological health of the river, the following ecological indices were calculated based on Collier *et al*. (2007):

- Periphyton Proliferation Index (PPI). This is an indicator of biomass. It is the percent cover of long filaments algae (>2cm) and diatom + cyanobacteria mats (>0.3cm).

- Periphyton Slimyness Index (PSI). This is an indicator of biomass and is the weighted percent cover of each thickness category. We used a modified calculation because our method grouped 'median' and 'thick' mats; it was calculated as: PSI = {(%Thin mat <0.3cm) + (%mats 0.3-3cm * 2) + (% Slimy filamentous >2cm * 4) + (Coarse filamentous >2cm and %cyanobacteria mat*5)} / 5.

4.3 Results and discussion

The results of weekly periphyton monitoring during November 2011 to May 2012 are shown in Appendix 4. The median values of periphyton biomass and cover are shown in Tables 3.1 and 3.2, and the results of the statistical test shown in Table 3.3.

Periphyton biomass (as measured by chlorophyll *a*) and periphyton cover (as measured by the Periphyton Proliferation Index (PPI) were within POP trigger values (and guideline values) at the upstream site but exceeded these values at the downstream sites (see Figure 4.1). Both periphyton cover and biomass were significantly higher at the two downstream sites compared to the upstream site during low flow periods.

As expected, there was a strong correlation between periphyton cover and periphyton biomass (see Figure 4.2). For the site 800m downstream the relationship became non-linear at high biomass – possibly due to co-correlation with more filamentous green algae (with more chlorophyll a) at higher biomasses . Another interesting feature was that the relationship between the two measures appeared to be different at different sites. At a biomass of 120 mg chlorophyll *a* /m^2, periphyton cover was estimated to be about 14% at the upstream site and about 28% at the site 800m downstream. The relationship at the downstream site corresponds better to the corresponding values in the NZ periphyton guidelines (Biggs 2000). It is not clear why relative estimates of cover and biomass differed between the sites – it could be because the method for estimating cover did not distinguish long strands of diatoms from thicker filamentous green algae. The rest of this section focuses on relationships based on periphyton biomass because biomass correlations between accrual period and biomass were stronger than correlations based on cover (PPI) (e.g. for the upstream site the r^2 = 0.70 for PPI verse days since last flood, compared to a r^2 of 0.93 for chlorophyll *a* verse days since last flood).

4.3.1 Periphyton species

The periphyton species that numerically dominated samples were the diatoms *Gomphonema* sp., *Rossithidium* sp., *Melosira varians*, *Diatom* sp., *Rhoicosphenia* sp., and the filamentous green algae *Stigeoclonium* sp. Diatoms were consistently most dominant after periods of flood and the filamentous green algae *Stigeoclonium* sp. become more dominant during periods of low flow – especially at the site 800m downstream. A comprehensive analysis of changes in species composition has not been undertaken.

All sites had only a small proportion of cyanobacteria cover during the monitoring period. When cyanobacteria species were present they were mostly *Phormidium* sp. or *Leptolyngbya* sp. with *Oscillaroria* sp. less common. The maximum cyanobacteria cover

recorded at the upstream site and 1400m downstream site was less than 1% (0.7% and 0.3% respectively). The site 800m downstream had a mean of 3% cover of cyanobacteria with a maximum cover recorded of 8.6%. Cyanobacteria cover between sites was statistically significant (see Table 3.2), however the overall cover was much less than the 20-50% trigger levels in the cyanobacteria guidelines (MfE and MoH 2009).

More cyanobacteria cover (albeit small) is consistent with what would be expected due to a higher N:P ratio found downstream of the discharge and higher absolute nutrient concentrations. Horner *et al.* (1990) found that diatoms were favoured by relatively high velocities and low phosphorus concentrations, whereas the blue-green algae *Phormidium* tend to be dominate at higher DRP concentrations. Green algae preferred higher DRP and lower velocities.

No cyanotoxins were found in the cyanobacteria samples but in two out of six samples where cyanobacteria were present the sampling analysis found trace amounts of dihydro-homoanatoxin-a which is a degradation product of anatoxin-a. The concentrations present on these occasions were very low compared to concentrations found by Wood *et al*. (2010) in a survey of 7 rivers throughout NZ or by Wood and Young (2011) in a survey of benthic of cyanobacteria in Manawatu Rivers.

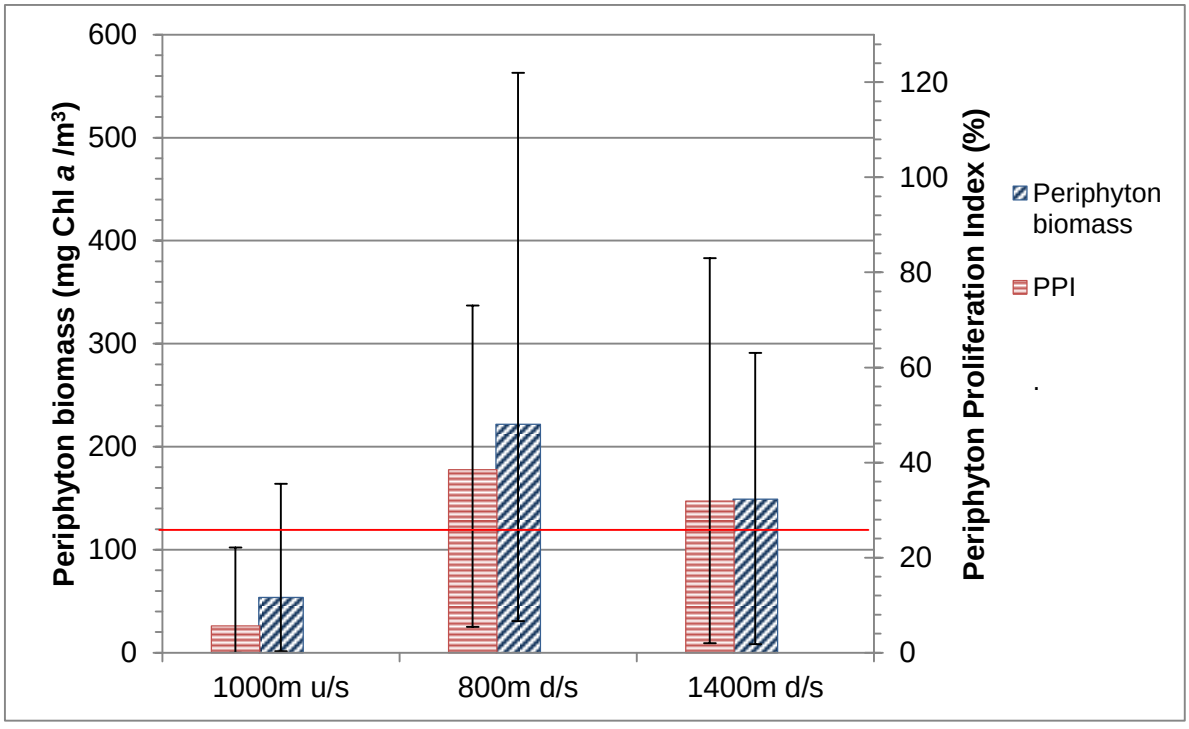


Figure 4.1: Mean periphyton biomass and cover measured during periods of low flow (<40 m^3/s). Error bars indicate maximum and minimum values. The red line indicates guideline values for biomass and cover for filamentous green algae (Biggs 2000).

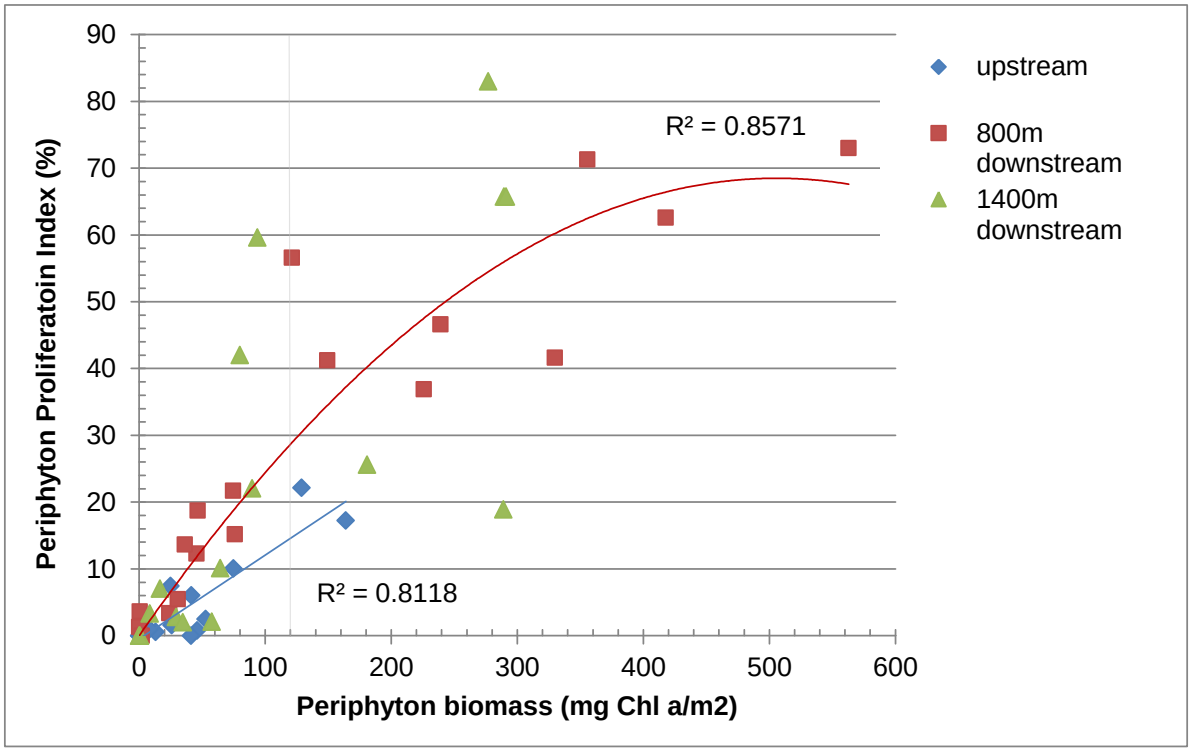


Figure 4.2: Relationship between periphyton biomass and periphyton cover as measured by the Periphyton Proliferation Index.

4.3.2 River flow as a controlling factor in periphyton biomass

Figure 4.3 shows that change in periphyton biomass at each site during the summer monitoring. It shows more rapid growth and higher periphyton biomass at the downstream sites with declines in periphyton biomass driven by flow events.

The relationship between periphyton biomass and flow is illustrated in Figures 4.4 and 4.5. Flood flows greater than three times the median flow[15] often approximates the level of flow to move bed sediment and remove periphyton biomass (e.g. Clausen and Biggs 1997). During this study even a 2x median flow (about 146 m^3/s) reduced biomass to less than 1 mg chl *a* $/m^2$ (see Figure 4.4). The correlation between periphyton biomass and days since a 3x median flow flood had an r^2 of 0.93 and 0.83 for the upstream and 800m downstream site respectively. There was a slightly better correlation with a 2x median flow and generally there was only one or two days difference between accrual periods calculated with a 2x median flow event and accrual due to a 3x median flow event.

High velocities caused by high flow reduced periphyton biomass through scour. Loss rates increase with increasing suspended sediment due to addition scour effects but also with additional biomass. An elevation of velocity above which periphyton is accustomed can lead to increased loss rates and temporarily reduce biomass (Horner *et al*. 1990); this

[15] About 219 m^3/s in the Manawatu River at Teaches College.

probably explains occasional dips in biomass (e.g. on 3rd May at 800m downstream) associated with only small flow increases.

Not only did periphyton biomass increase with the days of accrual since the last flood but so did the rate of periphyton accrual (see Figure 4.6). The rate of periphyton growth was much faster at the 800m downstream site compared to the upstream site but at both sites growth rate increased over time. This was partially because the rate of increase (measured as mg chl *a* /m^2/day) was strongly associated with the initial periphyton biomass (see Figure 4.7). The higher the initial biomass the faster the periphyton growth rate up to the point where periphyton biomass was sufficiently high (i.e. above about 200 mg/m^2) (or thick) so that growth rates slow. Periphyton at the site 1400m downstream appeared to slough off and scour at lower biomass levels compared to the site 800m downstream.

If periphyton biomass accrual is expressed as a percentage change of the initial biomass then the highest percentage increase in periphyton biomass was observed when the initial biomass was low, however the r^2 values for this correlation were very poor (e.g. about 0.14).

There was no positive relationship between nutrients and periphyton biomass or growth. In fact there was a weak negative log-log relationship between DRP concentration and periphyton biomass (r^2 =0.66 at upstream site and r^2 =0.48 at 800m downstream site). Similarly there was a weak negative log-log relationship between DRP concentration and periphyton accrual. This reflects background nutrient concentrations in the river declining at all sites as the river recedes after a flood (possibly due in part to uptake by periphyton).

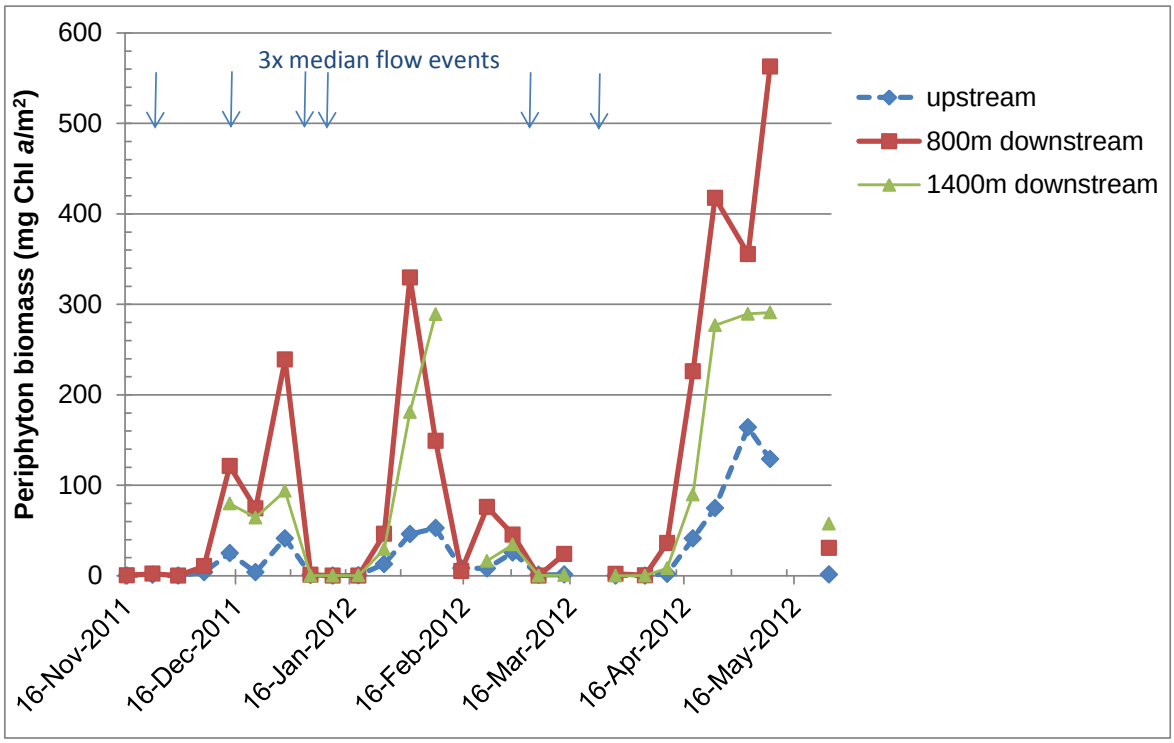


Figure 4.3: Change in periphyton biomass over time during the summer 2011-2012

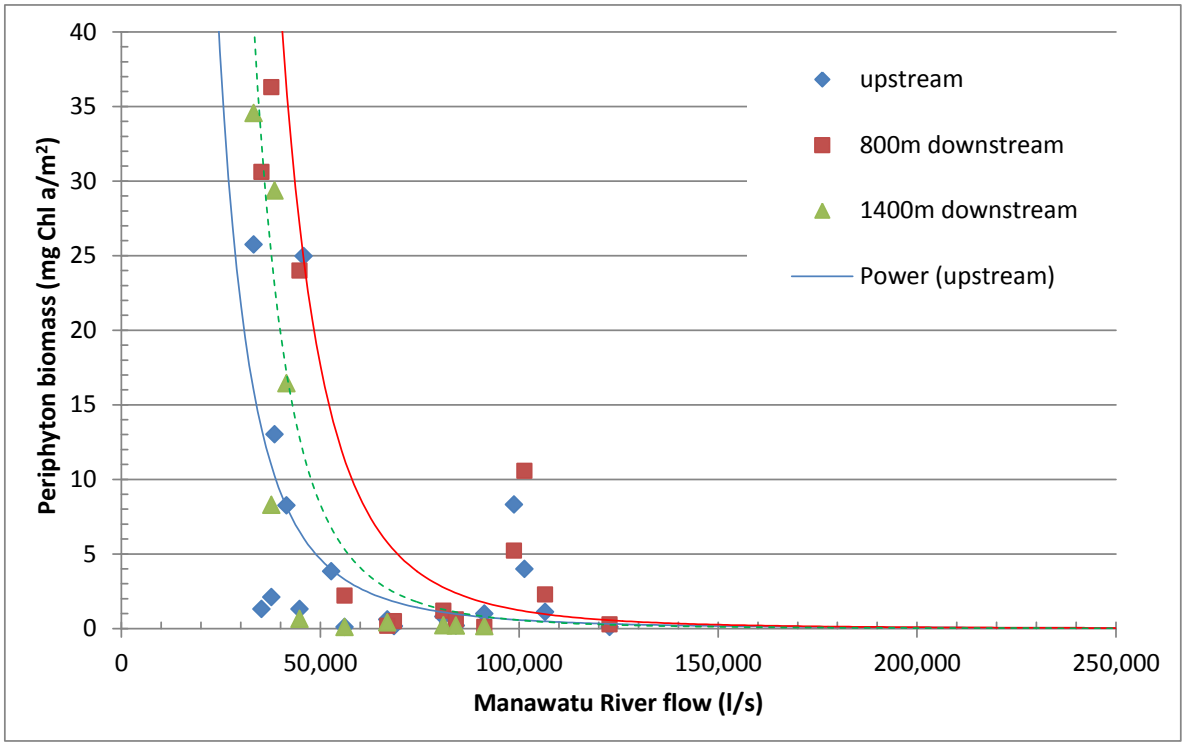


Figure 4.4: Periphyton biomass in relation to flow in the Manawatu River (Nov 2011 – May 2012) (scales are truncated).

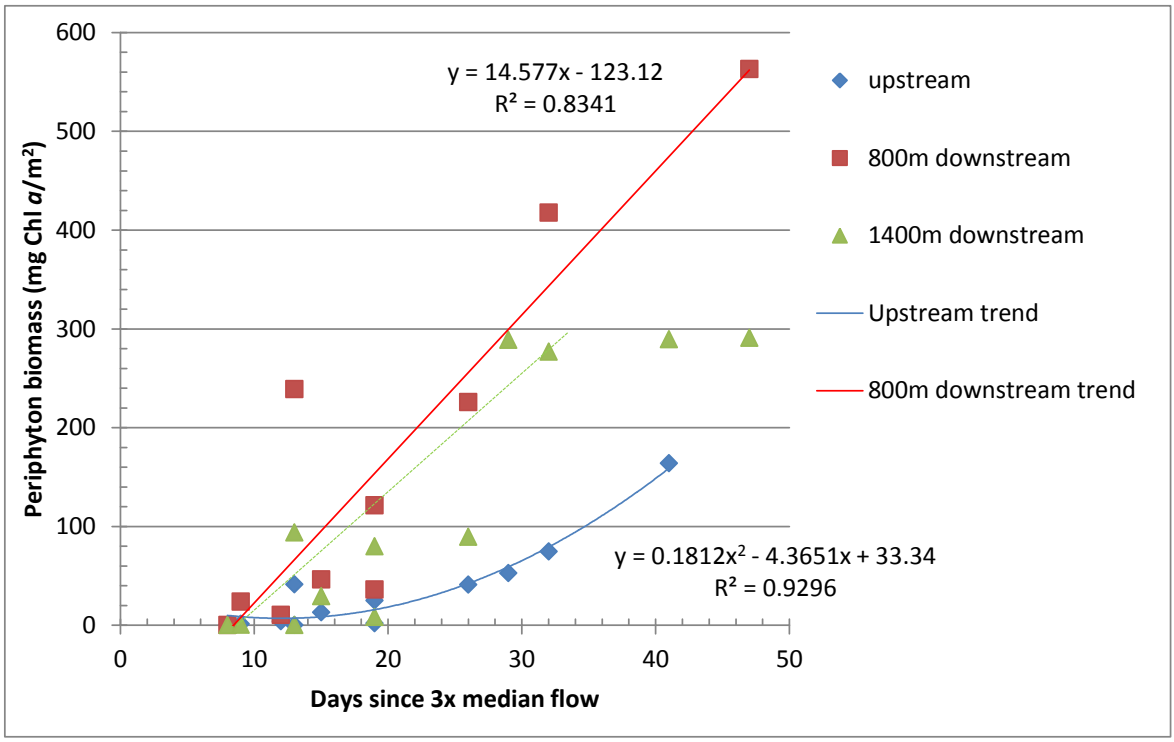


Figure 4.5: Periphyton biomass increasing with increasing number of days of accrual. Data limited to periods of accrual.

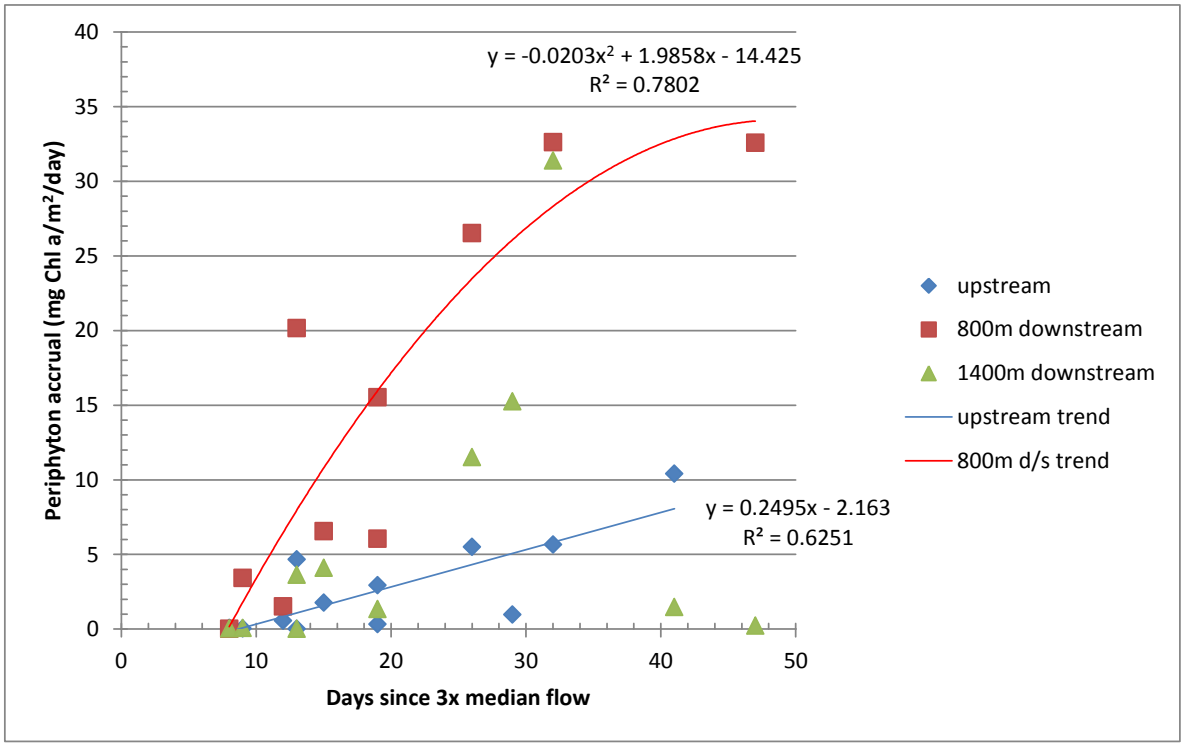


Figure 4.6: Periphyton accrual rate increasing with the number of days of accrual. Data restricted to periods of accrual and positive accrual rates.

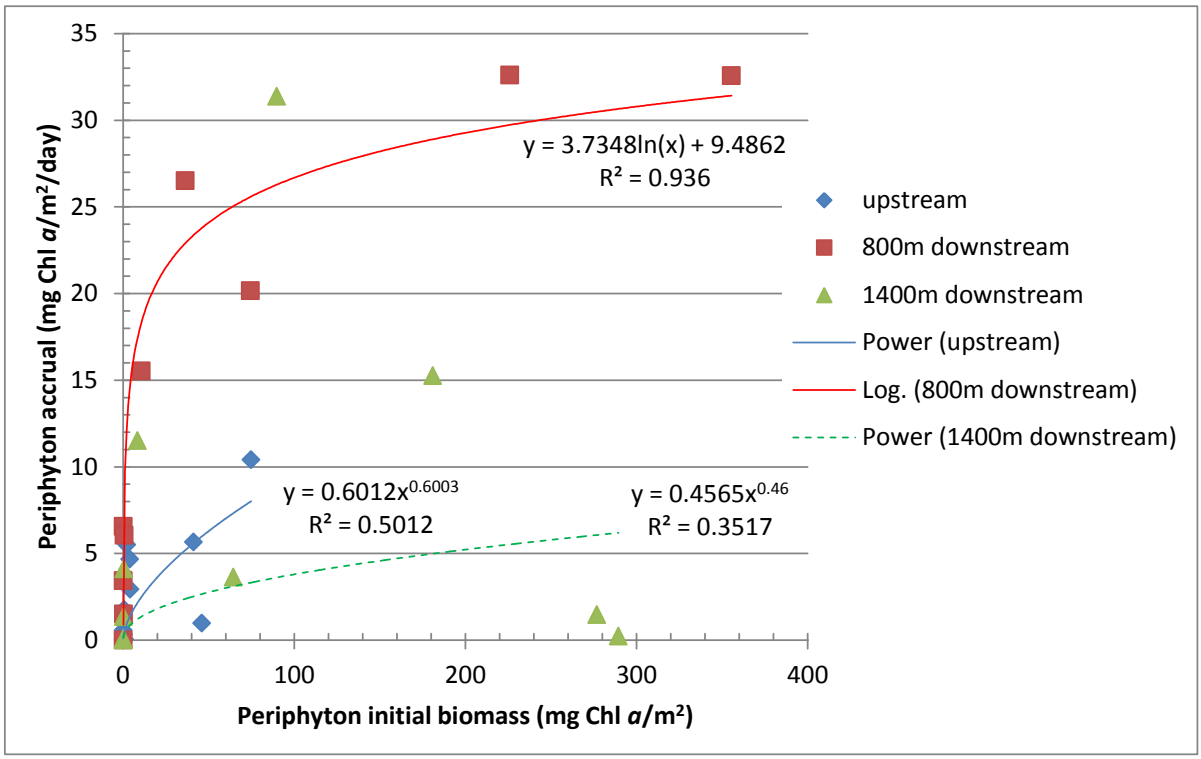


Figure 4.7: Periphyton accrual rates compared to initial biomass in the Manawatu River

4.3.3 Frequency of exceeding periphyton biomass guidelines

The relationships derived from Figure 4.5 can be used to estimate the number of days since a 3x median flow flood for periphyton to exceed the diatom guideline value of 200 mg chl *a*/m^2 in the Manawatu River. On average it took about 44 days, 22 days and 25 days to exceed guideline value for the upstream site, 800m downstream site and 1400m downstream site respectively. Flow data from the Manawatu River at Teachers College from a 21 year period (1987-2008) was used to estimate the frequency of flood events and accrual periods during the summer (1 November to 30 April) (for details see McConchie 2009). Over this 21 year period the frequency of accrual periods exceeding 22, 25 and 44 days during the summer were respectively 1.65 times per summer, 1.51 times per summer and 0.78 times per summer. The median length of time for which the accrual period exceeded 22 days per summer, 25 days per summer and 44 days per summer were respectively 19 days, 24 days and 34 days.

Thus, using the relationship between periphyton biomass and days accrual since the last flood (3 times median flow) it was estimated that the 200 mg/m^2 guideline for periphyton biomass would (on average) be exceeded at the upstream site 0.78 times per summer for a median period of 34 days; at the 800m downstream site 1.65 times per summer for a median period of 19 days; and at the 1400m downstream site 1.51 times per summer for a median period of 24 days.

The One Plan sets a periphyton biomass guideline for the Manawatu River of 120 mg/m^2 – equivalent to the filamentous algae guideline. It took 37 days, 17 days and 19 days to exceed this biomass for the upstream site, 800m downstream site and 1400m downstream site respectively. This corresponds to an exceedance frequency of 1.06 times per summer, 1.93 times per summer and 1.70 times per summer for the upstream site, 800m downstream site and 1400m downstream site respectively.

It should be noted that periphyton biomass can be reduced by flows much less than a three times median flow flood. The relationship between periphyton biomass and days accrual since 3x median flow flood excluded data where smaller floods cause a reduction in periphyton biomass, extending the method to estimate a total number of days guidelines are exceeded is likely to be an over-estimate.

Francis and Death (2001) monitored periphyton weekly during low flows during the summers of 1999/2000 and 2000/2001. They found rapid periphyton growth downstream of the WWTP discharge which exceeded guideline values after only 7 to 10 days of accrual, while the upstream sites took 25 to 40 days to exceed the 120 mg/m^2 guideline value. David Cameron (in evidence presented for the Totara Road WWTP hearing) used this data to estimate that after upgrading the WWTP to remove P during low flow periods (median DRP of 0.015 g/m^3 in the discharge) periphyton would require 18 days to exceed the guideline. This is very similar to the mean accrual period of 17 to 19 days for the downstream sites (Figure 4.5), suggesting that evidence presented about the frequency and duration of effects on periphyton was reasonable.

4.3.4 Periphyton growth rates during the April low flow period

During the six months of monitoring the only extended period of low flow occurred in April and into May. Changes in periphyton biomass, biomass accrual and percent accrual are shown in Figures 4.8 and 4.9. A large flood (607 m^3/s) occurred on 21st March with flow dropping below three times median flow on 23rd March. Turbidity in the river remained high for a number of days and dropped below 12 FTU (about 38cm water clarity[16]) on 28th March. The Manawatu River flow at this time was about 60 m^3/s. Another small flood occurred on 6th April elevating the turbidity for four days from 4th to 8th of April. After this the flow steadily receded until a small fresh on 29th April elevated flows to 62 m^3/s (less than median flow), and another on 15th May (about 2 times median flow). The WWTP was removing P from the effluent from 1st to 3rd April, 11th-12th April, 14th to 30th April and 2nd to 14th May. The mean nutrient concentration in the stream during the March-May low flow period is shown in Table 4.1.

The increase in periphyton biomass (Figure 4.8) followed a sigmoid curve at all sites, with little initial change followed by a period of rapid accumulation with the rate of accumulation then reducing at over time. The downstream sites showed substantially more biomass accrual compared to upstream. The greatest increase in periphyton biomass occurred at the site 800m downstream, followed by 1400m downstream. Photos in Appendix 1 illustrate this change in cover over the period of April to May.

Graphs of biomass accrual in Figure 4.9 can be understood as showing the 'velocity' periphyton growth with a steeper gradient indicating more 'acceleration' in the growth rate. The rate of biomass accrual steadily increased over the period until the small fresh on 29th April which probably increased sloughing of algae from the two downstream sites that had high biomass. In terms of percent change the highest accrual rates at the downstream sites occurred early in the growth period between 5th and 11th of April (see Figure 4.9).

Although the maximum rate of periphyton accumulation was similar between the sites 800m downstream and 1400m downstream; the initial phase of periphyton growth (before 11th April) was much more rapid at the site 800m downstream compared to the sites 1400m downstream and upstream. This suggests some factor was stimulating ("kick-starting") the initial phases of periphyton growth so that it entered a rapid growth phase about a week before the other sites.

Fungus was found scattered through the 800m downstream sample on 28th March and it is possible that labile organic carbon in the discharge promoted more rapid development of heterotrophic biofilms (consisting of fungus and bacteria) at the site 800m downstream and that 'conditioned' the substrate for more rapid periphyton growth. Fungus can promote the release of phosphorus from sediments (Palmer-Felgate et al. 2010). Ardón and Pringle (2007) found that labile organic carbon stimulated the development of biofilms in streams and magnified the stimulatory effect of phosphorus enrichment on biofilm activity.

[16] Black disk water clarity was calculated from 15 minute turbidity measurements at the Teachers College site. A correlation between black disk and turbidity (in FTU) was derived using data from 2006 to 2012, where: black disk distance (m) = 2.2143 $[turbidity]^{-0.706}$. R^2=0.91

The change in periphyton biomass on the bioassay is also shown in Figure 4.8. Although shown as a straight line periphyton growth on the bioassays would have also followed a sigmoid curve[17]. The lines labelled "a" shows the gradient equal to the change in periphyton biomass on the bioassay site "d/s D". The rate of change of the downstream bioassay over two weeks was similar to that observed for the first two weeks of growth at the site 800m downstream (assuming that periphyton accumulation started about 4th April[18]). Interestingly the bioassay at the upstream site had a considerably higher rate of growth compared to that observed for the first two weeks of growth in the stream.

Table 4.1: Mean nutrient concentrations during the period 28th March to 25 May 2012 (n=8).

Site	All flows		River flow >40 m^3/s		River flow <40 m^3/s	
	DRP (g/m^3)	**SIN (g/m^3)**	**DRP (g/m^3)**	**SIN (g/m^3)**	**DRP (g/m^3)**	**SIN (g/m^3)**
Upstream	0.0089	0.254	0.011	0.31	0.0075	0.22
800m d/s	0.0137	0.467	0.020	0.44	0.0097	0.483
1400m d/s	0.012	0.391	0.015	0.39	0.01	0.393
Effluent post-wetland	1.17	32.5	2.7	30.3	0.025	34.1

[17] The five day bioassay had less than 2 mg/m^2 after five days.

[18] Periphyton biomass at 800m downstream was 0.6 mg/m^2 on 5th April. This is similar to biomass on the bioassay after 5 days of growth (suggesting growth started about 31st March but algae growth would have been delayed about four days by elevated turbidity from 4th to 8th of April.

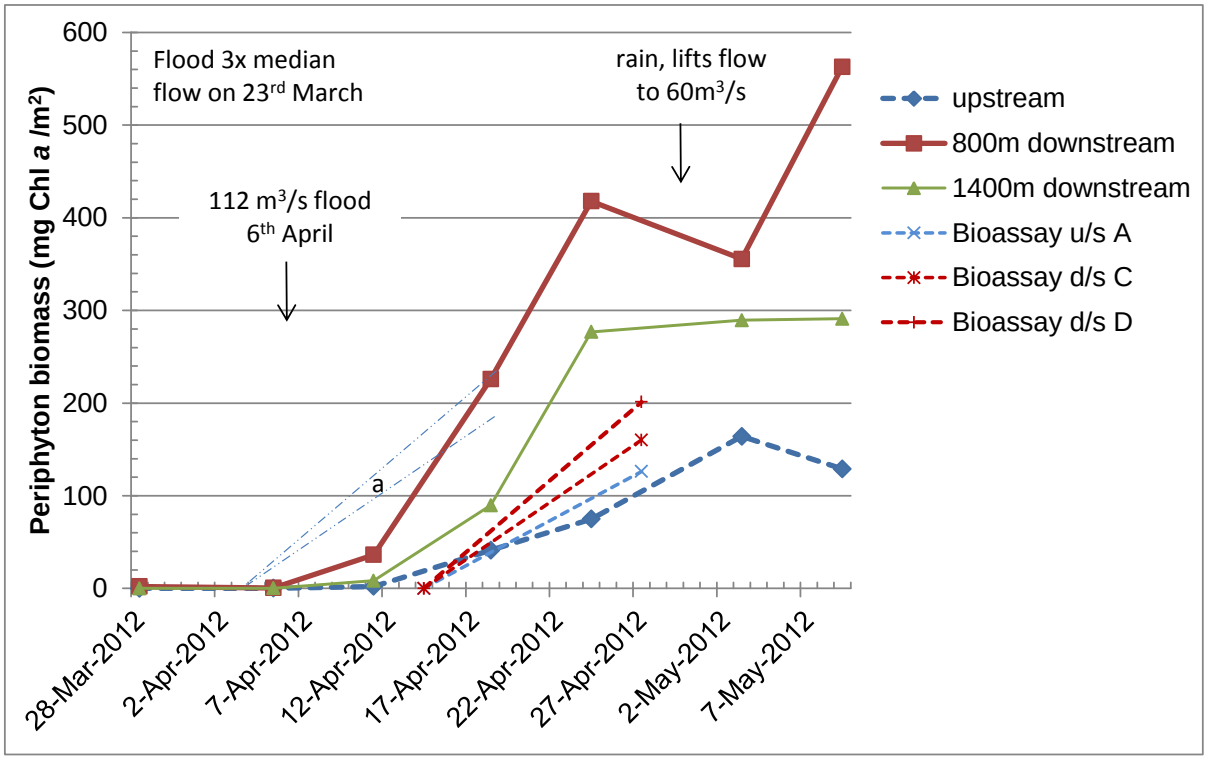


Figure 4.8: Periphyton biomass in the Manawatu River during receding river flows in April 2012. Line "a" shows the gradient equal to the change in periphyton biomass on the bioassay sites "d/s D".

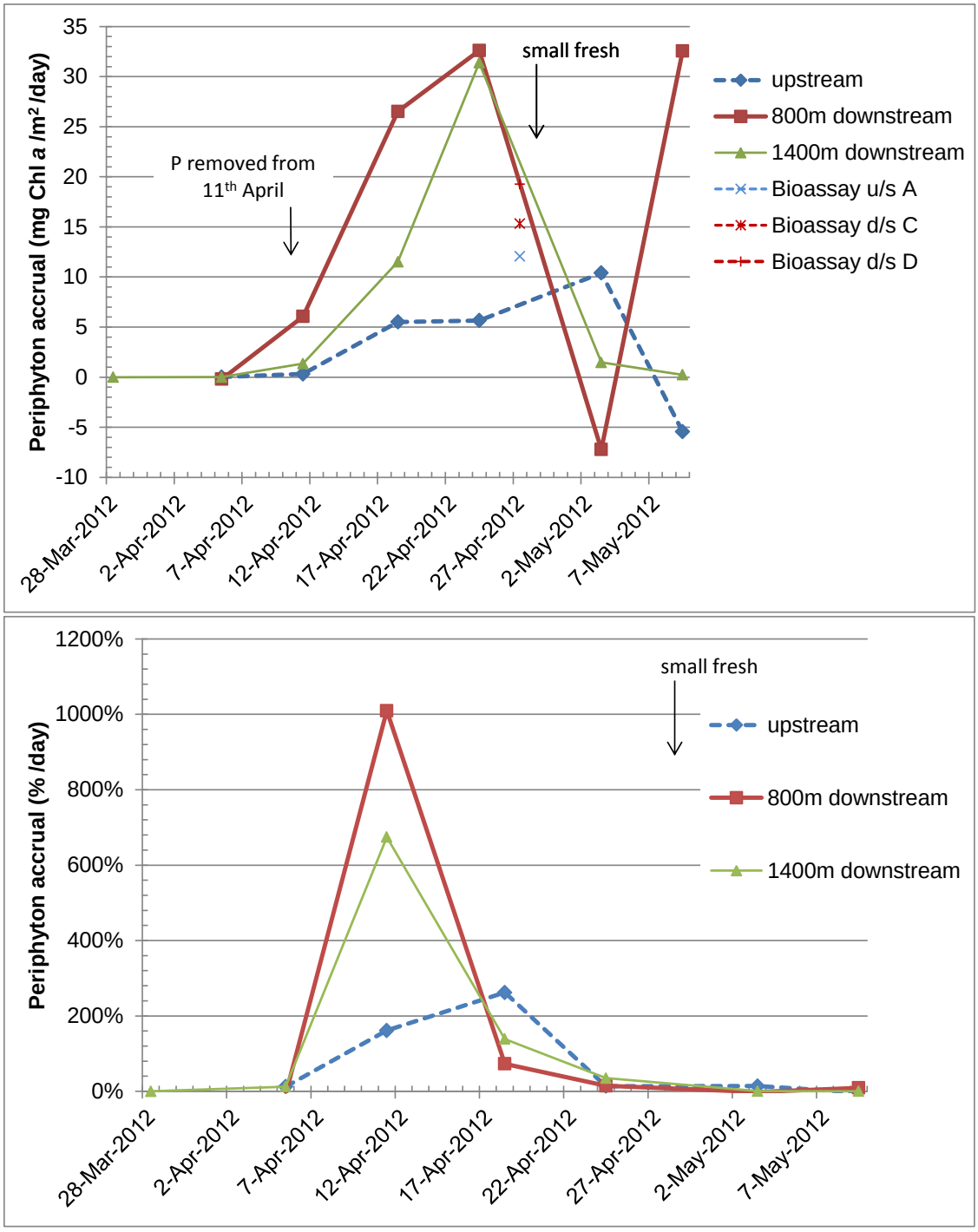


Figure 4.9: Periphyton biomass accrual and % accrual in the Manawatu River during receding river flows in April 2012.

4.3.5 Comparison of April periphyton growth and model predictions

The phosphorus regression model described in Biggs (2000) was applied to the data for the purpose of examining the possible reasons for elevated periphyton growth at the downstream site. The regression model predicts maximum chlorophyll *a* concentrations as a function of mean days of accrual and mean monthly DRP using data from 30 New Zealand rivers. This model will, at best, be approximate when applied to a specific river, but it is helpful for examining the effects of DRP and extending the number of day of accrual. It is also helpful to apply because it forms the basis of the nutrient guidelines for periphyton in Biggs (2000).

The results of applying different scenarios to the model are shown in Table 4.2. The first scenario (i.e. April DRP, accrual from flood event) applies the model using the most realistic data for the March-April period. It uses mean DRP concentrations for the period 28th March to 25th May and an accrual period based on time elapsed from the end of the flood event that exceeded more than three times median flow. This is the definition of accrual period used for data used for developing the model (Biggs 2000 b).

The results show that for the growth period up to 24th April, the upstream sites had considerably less periphyton biomass compared to what was predicted by the model, the 800m downstream site had considerably more periphyton and the 1400m downstream site was similar to the predicted periphyton biomass.

A shorter, 20 day accrual period (starting 4th April) reflects a best estimate of when accrual actually did start in the section of the river being monitored considering the constraints of elevated turbidity and the low flow DRP is based on the median DRP concentration during low flow periods during the summer. Although not the most appropriate period or concentration to apply to the model, it allows us to investigate the sensitivity of periphyton growth to a shorter accrual period and a lower DRP concentration. Under each of these scenarios the periphyton biomass at the upstream site remained less than model predictions, suggesting that some factor other than DRP concentrations was controlling periphyton growth at the upstream site.

The scenarios with high DRP concentrations used the mean DRP during the monitoring period when flows were between 40 m^3/s and 110 m^3/s. This reflects concentrations when there was no phosphorus removal and over-estimates the actual DRP concentrations during the April growth period. Under these scenarios the model continued to under-estimate the actual periphyton biomass at the site 800m downstream but approximately predicted measured biomass at the site 1400m downstream (assuming a 30 day accrual period). This adds weight to the previous observation that some factor(s) were either stimulating or kick-starting periphyton growth at the site 800m downstream. The magnitude of this effect is sufficiently strong that we were measuring more periphyton than could be explained by the model if no P treatment was occurring.

Table 4.2: Predicted verses actual chlorophyll *a* concentrations for different assumptions of accrual period and DRP. Chlorophyll *a* was modelled using the equation in Biggs (2000).

Scenario	site	Date	Measured Chl *a* (mg/m^2)	Predicted max Chl *a* (mg/m^2)	accrual period	DRP (mg/m^3)
April DRP, accrual from flood event	**u/s**	24-Apr	75	222	30	8.9
April DRP, accrual from flood event	**800 d/s**	24-Apr	418	275	30	13.7
April DRP, accrual from flood event	**1400 d/s**	24-Apr	277	257	30	12
April DRP, 20 day accrual	**u/s**	24-Apr	75	110	20	8.9
April DRP, 20 day accrual	**800 d/s**	24-Apr	418	136	20	13.7
April DRP, 20 day accrual	**1400 d/s**	24-Apr	277	128	20	12
Low flow DRP, 30 day accrual	**u/s**	24-Apr	75	183	30	6
Low flow DRP, 20 day accrual	**u/s**	24-Apr	75	91	20	6
High DRP, 20 day accrual	**800 d/s**	24-Apr	418	187	20	**26**
High DRP, 20 day accrual	**1400 d/s**	24-Apr	277	143	20	**15**
High DRP, 30 day accrual	**800 d/s**	24-Apr	418	377	30	**26**
High DRP, 30 day accrual	**1400 d/s**	24-Apr	277	287	30	**15**

30 day accrual period based on accrual starting after flood ending 25th March.
April DRP concentrations based on 8 measurements between 28 March - 25 May.
High DRP based on average if no treatment (using flow 40 m^3/s > 110 m^3/s)
Low DRP based on median DRP during summer when flows < 40 m^3/s

4.4 Summary

- During periods of low flow periphyton biomass and cover were higher downstream of the discharge than upstream and exceeded guidelines values.
- There was more cyanobacteria (*Phormidium* sp.) cover at the 800m downstream site than at the upstream and 1400m downstream sites, but overall there was very little cyanobacteria present (less than 3% cover at the site 800m downstream).
- Periphyton cover was strongly controlled by flow in the Manawatu River. Flow of 2 to 3 times median flow were generally sufficient to reduce periphyton biomass to very low levels – particularly if they occurred for an extended period of time.
- The rate of periphyton growth increased with the length of time it had to grow up to the point where periphyton biomass exceeded about 200 to 300 mg/m^2 so that loss due to sloughing balanced (or exceeded) in growth.
- On average it took about 37 days, 17 days and 19 days to exceed the 120 mg/m^2 filamentous algae guideline values for the upstream site, 800m downstream site and 1400m downstream site respectively. When compared to 21 years of flow data during summer (1 November to 30 April) these correspond to frequencies of: 1.1 times per summer; 1.9 times per summer; and 1.7 times per summer for each site respectively.

- The accrual period at the downstream sites are similar to those predicted by in evidence presented at the hearing for the WWTP current consent.

- During the April low flow period the downstream sites showed substantially more biomass accrual compared to upstream. The greatest increase in periphyton biomass occurred at the site 800m downstream. Periphyton growth at all sites followed a sigmoid curve.

- The initial phase of periphyton growth (while biomass was still relatively low) was much more rapid at the site 800m downstream compared to the other sites. This would be consistent with more rapid development of heterotrophic biofilms stimulating the initial phases of periphyton growth allowing more rapid growth to occur sooner and possibly increasing nutrient cycling.

- A simple regression model (from Biggs 2000) was used to predict periphyton biomass in April and examine sensitivity to changes in nutrients and accrual period. Upstream periphyton biomass was considerably less than those predicted by the model, even when accrual period and DRP concentrations were reduced. This suggested that some factor other than DRP concentrations was controlling periphyton growth at the upstream site.

- The 800m downstream site had considerably more periphyton biomass than predicted by the model and the 1400m downstream site had a similar biomass. A scenario assuming high DRP concentrations typical of no phosphorus treatment still underestimated the periphyton biomass at the site 800m downstream. This would be consistent with the theory that dissolved organic carbon form the discharge may be stimulating development of heterotrophic biofilms which condition substrate for periphyton colonisation thus reducing the initial accrual period.

- Faster growth rates at the site 800m downstream might also be explained by periphyton experiencing higher DRP concentrations than measured in the water due as DRP enters the river from groundwater seepage or desorption at the sediment-water interface (see previous chapter).

- The periphyton bioassay at the upstream site had a faster growth rate compared to the first two weeks of periphyton growth in the river; however the downstream bioassay had a similar growth rate compared to that observed in the river.

5 Nutrient limitation

5.1 Aim

Nutrient concentrations have a significant effect on the rate of periphyton growth. Both phosphorus and nitrogen are thought to have a controlling influence of periphyton growth in the Manawatu River with the potential controlling nutrient varying in space and time and being highly influenced by river flow (McArthur *et al*. 2010). The WWTP treats for phosphorus with the aim of reducing any stimulation of periphyton growth. A periphyton nutrient bioassay was undertaken and measurements of periphyton cellular nutrient concentrations were made in order to:

- Confirm which if any nutrient is limiting periphyton growth upstream and downstream of the discharge;
- Inform any decision to refine the timing and extent of wastewater phosphorus removal in order to limit downstream periphyton growth.

5.2 Method

5.2.1 Cellular nutrients

Cellular nutrients provide a measure of nutrient limitation. The Redfield ratio (i.e. TN:TP of 7.2:1 by weight) is typically regarded as the optimum ratio of nutrient growth with a higher ration indicating possible P limitation. There is some evidence that not all periphyton species follow the Redfield ratio so the optimum cellular N:P ratio was based on the ratio in periphyton growing with excess N and P (e.g. downstream of the discharge with no P treatment). If treatment of P by the WWTP is being effective at limiting periphyton growth we would expect to see an increase in the TN:TP ratio beyond the optimum ratio.

Estimates of periphyton cover were extended to measure replicate cellular N, cellular P concentrations and relative abundance of periphyton species on the following three occasions.

- 25 January 2012 after about two weeks of receding water flow and just before commencement of phosphorus treatment (i.e. flow just above half median flow).
- 21 April 2012, after 9 days of the WWTP treating for phosphorus.
- 28 April 2012 after 16 days of the WWTP treating for phosphorus.

Samples were collected from the same upstream and downstream sites as used for assessing periphyton cover, i.e. Manawatu River about 1000m above the discharge point; and Manawatu River about 800m below the discharge point (see Figure 3.1).

On each sample occasion six (6) replicate samples were collected at each site, using method QM-1b from the Stream Periphyton Monitoring Manual (Biggs & Kilroy 2000). Each replicate consisted of up to five samples bulked to obtain a minimum of five grams. For each replicate the following variables were analysed: cellular N and cellular P.

A separate sample from each replicate rock was scrapped and collected and sent to NIWA for analysis of periphyton identification and relative abundance. Subsamples are examined using an inverted microscope at magnifications up to 400x, and algal taxa present identified and listed. The relative abundance of each taxon was assessed following the method in Biggs and Kilroy (2000) on a scale of: 8 =dominant, 7 =abundant; 6 =common-abundant, 5 = common, 4 = occasional-common, 3 = common, 2 = rare-occasional, 1 =rare. For simplicity we have only listed the most abundant taxa in the results tables (scores 6 to 8) in order of dominant (8) to common-abundant (6).

Since optimum N to P ratios can vary between periphyton species, attention was given to sampling the periphyton type that was dominant at the site downstream of the discharge as opposed to collecting a representative sample. In practice this meant collecting five replicates of filamentous algae mats and a single sample of cyanobacteria mats if they were present.

5.2.2 Periphyton nutrient limitation bioassay

A periphyton nutrient bioassay was undertaken using the steel tray nutrient diffusing substrate method described in Biggs and Kilroy (2000). This used five replicates of four different treatments:

a) agar with no enrichment (control),

b) nitrogen enriched agar,

c) phosphorus enriched agar, and

d) nitrogen and phosphorus enriched agar.

The agar and nutrient solution was made and stored in a fridge for several weeks prior to using the trays in the river. They were originally put in the river in late March but had to be removed on 3 April (after five days) due to a predicted flood event. Just prior to applying the GFC filter papers and installing in the river for the second time on 14th April, the top layer of agar was removed and the bottles topped up with fresh agar /nutrient solution.

Two trays were deployed upstream and two downstream of the discharge. The trays were located close to the upstream and downstream sites as used for assessing periphyton cover and water quality sampling. One of the upstream trays was stolen from the river during the experiment so the experiment had data from:

- Manawatu River about 1000m above the discharge point;
- Manawatu River about 800m below the discharge point;
- Manawatu River about 880m below the discharge point.

The bioassay was put in the river on 14th April 2012 (when the river reached half median flow and the WWTP started treating for P) and removed on 27th April 2012 (13 days).

The samples from the artificial substrates were sampled for chlorophyll *a* concentration (converted to mg/m^2).

Before and after installing the bioassays measurements were made of water depth, water velocity, and the abundance of any snails on substrates. All sites were exposed and had limited impacts from riparian shading.

Interpretation of the results was complicated by the trays containing the nutrient diffusing substrates being installed perpendicular to the water flow rather than parallel to the water flow as described in Biggs and Kilroy (2000). In each case the most upstream treatment was the N treatment, followed by P, N+P and the control. This error caused the possibility of the N treatment interfering with the P treatment and both the N and the P treatment interfering with the control. It also caused the possibility of laminar flow over the trays causing higher velocities and more turbulence at the downstream end – closest to the control treatments. Despite these complications useful conclusions can be made from the results by focusing on comparisons with the N and P treatments and difference between sites.

5.3 Results and discussion

5.3.1 Cellular nutrients

The results of cellular nutrient analysis are summarised in Table 5.1 with statistical comparisons shown in Table 5.2. Results from all replicates are shown in Table 5.3. Replicates dominated by (or containing significant amounts) of cyanobacteria (i.e. *Phormidium* sp) were excluded from the analysis because these samples responded differently to nutrients from the discharge compared to samples dominated by green filamentous algae (i.e. *Stigeoclonium*) or diatoms (e.g. *Diatoma tenuis* or *Melosira varians*). Samples containing *Phormidium* had higher TN:TP ratios at the downstream site compared to non-cyanobacteria samples because of a disproportionately greater increase in cellular N.

The optimum ratio of TN:TP for the periphyton community is indicated by results from downstream samples, and particularly those collected in January which was a period when the WWTP was not removing P from the discharge. The results indicate an optimum TN:TP ratio of 6.3 - 6.4, which is similar to the Redfield ratio of 7.2 (by weight).

The upstream site had a significantly higher TN:TP ratio compared to the downstream sites on all sample occasions (see Figure 5.1 and Table 5.2) suggesting that periphyton cells were potentially phosphorus limited. The TN:TP ratio was significantly higher on the 28th April (9 days of P treatment) compared to the 21st April (16 days of P treatment), suggesting that the degree of potential P limitation was increasing at the upstream site as periphyton biomass increased (estimated to change from about 50 mg/m^2 to about 120 mg/m^2). DRP concentrations in the Manawatu River upstream of the discharge was low (0.005 mg/l to 0.006 mg/l) in the period 18th to 24th April).

In contrast, the downstream site had no significant difference in the cellular TN:TP ratio between sampling dates (see Figure 5.1) – suggesting that neither N or P was controlling periphyton growth at the downstream site even after the two weeks of P removal from the effluent.

The increase in TN:TP ratio observed at the upstream site was due to a disproportionate reduction in cellular P compared to cellular N (see Figure 5.1, Tables 5.1 and 5.2). The reduction in the absolute concentration of cellular TP and (to a lesser extent) TN in between 21st April and 28th April could be due to a number of factors, for example Hill and Fanta (2008) found cellular nutrient concentrations to be highly positively correlated with specific growth rate.

Table 5.1: Median cellular nutrient concentrations (excluding replicates with *Phormidium*) and river water quality grab samples. The WWTP was treating for P during April but not during January sampling.

Date *	Site	TP (mg/kg)	TN (mg/kg)	TN/TP	River DRP (mg/l)	River DIN (mg/l)
25-Jan-12	upstream	1690	15500	8.5	0.007	0.360
25-Jan-12	downstream	3400	26000	6.3	0.012	0.960
21-Apr-12	upstream	4700	36000	7.9	0.007	0.230
21-Apr-12	downstream	5200	33000	6.4	0.018	0.910
28-Apr-12	upstream	2500	32000	12.0	<0.005	0.470
28-Apr-12	downstream	4500	31000	6.4	0.011	0.540

* Water qualtiy grab samples were collected on 25th Jan, 19th April and 26th April.

Table 5.2: Results of statistical analysis for samples excluding those containing the blue-green algae *Phormidium*. The WWTP was treating for P during April but not during January sampling.

		Mann-Whitney p-values			Equivalence test - strength of evidence		
Group	Comparison	TP	TN	TN/TP	TP	TN	TN/TP
25-Jan-12	u/s vs d/s	0.009	0.047	0.009	Strong	Strong	Moderate
21-Apr-12	u/s vs d/s	0.14	0.5	0.009	Inconclusive	Inconclusive	Strong
28-Apr-12	u/s vs d/s	0.016	0.5	0.009	Strong	Inconclusive	Strong
u/s site	21st vs 28th	0.028	0.5	0.009	Strong	Inconclusive	Strong
d/s site	21st vs 28th	0.5	0.6	0.6	Inconclusive	Inconclusive	Inconclusive

Equivalence test assumed +/-10% and a *p*-values statistically significant if <0.05

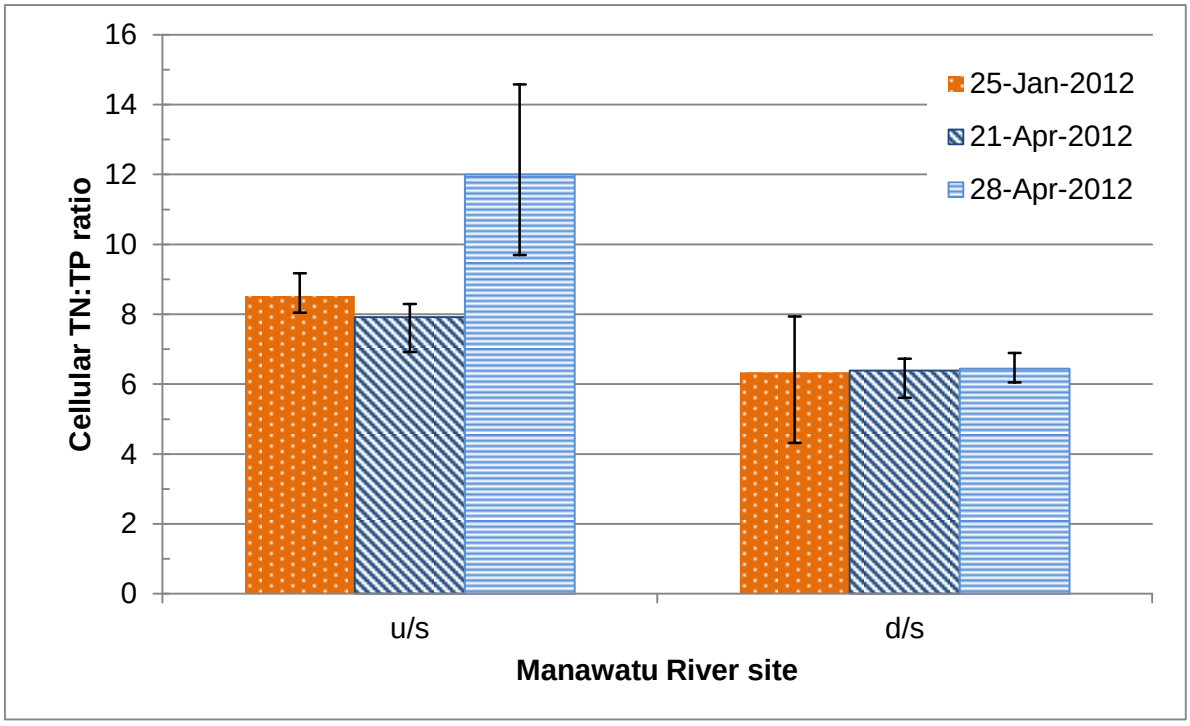


Figure 5.1: Ratio of TN:TP in periphyton cells (excluding replicates dominated by *Phormidium*) . The error bars show the extreme values (minimum and maximum).

Table 5.3: Results of cellular nutrient analysis. The WWTP was treating for P during April but not during January sampling.

Date	us or ds	site / replicate	TP (mg/kg)	TN (mg/kg)	TN/TP	Dominant periphyton species
25-Jan-12	us	us 1a	1830	15600	8.5	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	us	us 1b	1690	15500	9.2	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	us	us 1c	2100	16900	8.0	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	us	us 1d	1210	9900	8.2	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	us	us 1f	1340	11700	8.7	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	us	us 1e	1500	9600	6.4	*Stigeoclonium, Melosira varians, Gomphonema sp., Phormidium*
25-Jan-12	ds	ds 1a	3400	27000	7.9	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	ds	ds 1b	3100	19600	6.3	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	ds	ds 1d	3100	13400	4.3	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	ds	ds 1e	3900	29000	7.4	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	ds	ds 1f	4100	26000	6.3	*Stigeoclonium, Melosira varians, Gomphonema sp.*
25-Jan-12	ds	ds 1c	2800	21000	7.5	*Phormidium, Gomphonema sp., Stigeoclonium,*
21-Apr-12	us	us 2a	4800	38000	7.9	*Stigeoclonium, Diatoma cf. tenuis, Gomphonema sp.*
21-Apr-12	us	us 2b	3200	26000	8.1	*Stigeoclonium, Diatoma cf. tenuis, Gomphonema sp.*
21-Apr-12	us	us 2d	4700	39000	8.3	*Stigeoclonium, Diatoma cf. tenuis, Gomphonema sp.* (small)
21-Apr-12	us	us 2e	4100	32000	7.8	*Diatoma cf. tenuis, Stigeoclonium*
21-Apr-12	us	us 2f	5200	36000	6.9	*Stigeoclonium, Diatoma cf. tenuis, Diatoma vulgaris / Gomphonema sp.*
21-Apr-12	us	us 2c	1560	8000	5.1	*Phormidium > Navicula cf. lanceolata > Melosira varians*
21-Apr-12	ds	ds 1a	4800	31000	6.5	*Stigeoclonium, Diatoma vulgaris, Gomphonema sp.*
21-Apr-12	ds	ds 1b	6100	39000	6.4	*Stigeoclonium, Diatoma cf. tenuis/Gomphonema sp.*
21-Apr-12	ds	ds 1d	4100	23000	5.6	*Stigeoclonium, Diatoma cf. tenuis, Gomphonema sp./ Melosira varians/Navicula cf. lanceolata*
21-Apr-12	ds	ds 1e	5200	35000	6.7	*Stigeoclonium, Diatoma cf. tenuis, Gomphonema sp.*
21-Apr-12	ds	ds 1f	5300	33000	6.2	*Stigeoclonium > Diatoma cf. tenuis, Melosira varians/Diatoma vulgaris*
21-Apr-12	ds	ds 1c	5100	40000	7.8	*Phormidium, Stigeoclonium, Diatoma cf. tenuis, Diatoma vulgaris, Navicula cf. lanceolata*
28-Apr-12	us	us 2a	2400	35000	14.6	*Diatoma cf. tenuis > Stigeoclonium / Diatoma vulgaris/Gomphonema sp*
28-Apr-12	us	us 2b	2000	24000	12.0	*Diatoma cf. tenuis > Stigeoclonium*
28-Apr-12	us	us2d	2500	31000	12.4	*Diatoma cf. tenuis, Stigeoclonium*
28-Apr-12	us	us 2e	3700	40000	10.8	*Diatoma cf. tenuis, Stigeoclonium, Melosira varians*
28-Apr-12	us	us 2f	3300	32000	9.7	*Diatoma cf. tenuis, Stigeoclonium*
28-Apr-12	us	us 2c	1540	8500	5.5	*Phormidium, > Diatoma cf. tenuis /Gomphonema sp.*
28-Apr-12	ds	ds 1a	5900	38000	6.4	*Diatoma cf. tenuis, Stigeoclonium, Melosira varians*
28-Apr-12	ds	ds 1b	4500	31000	6.9	*Stigeoclonium, Diatoma cf. tenuis ,*
28-Apr-12	ds	ds 1d	5500	37000	6.7	*Diatoma cf. tenuis > Stigeoclonium*
28-Apr-12	ds	ds 1e	3500	22000	6.3	*Diatoma cf. tenuis, Stigeoclonium / Gomphonema sp. / Melosira varians*
28-Apr-12	ds	ds 1f	3800	23000	6.1	*Diatoma cf. tenuis, Stigeoclonium, Gomphonema sp. / Navicula cf. lanceolata*
28-Apr-12	ds	ds 1c	4600	40000	8.7	*Phormidium, > Diatoma cf. tenuis /Gomphonema sp.*

Periphyton taxa listed in order of 'dominant' to those that were 'common abundant'.

Relicates dominated by cyanobacteria are coloured blue and were excluded from our analysis.

5.3.2 Periphyton bioassays

Comparison of sites

The results of the periphyton bioassays 14th - 27th April (13 days) and the much shorter bioassay 29th March – 3rd April (5 days) are shown in Appendix 5. Both the 5 day bioassay and the 13 day bioassay showed more periphyton growth at the downstream sites compared to the upstream site (see Figures 5.2 and 5.3). The difference between sites was most apparent (and least variable) when looking at the N treatment only; this can be seen by comparison of *p*-values from a Mann-Whitney test in Tables 5.4 and 5.5.

Mean chlorophyll *a* at the combined downstream sites was about 66% higher than combined upstream sites after the 5 day bioassay, but the absolute biomass at all sites was very low – indicative of early stages of periphyton colonising the substrates. After the 13 day bioassay there was considerably more periphyton at all sites and about 36% more chlorophyll *a* at the downstream sites – pushing the mean chlorophyll *a* values at site D above the diatom biomass guideline for maintaining trout habitat (i.e. 200 mg/m^2) (Biggs and Kilroy 2000). This shows that the higher periphyton biomass observed in the river downstream was caused by factors unrelated to luxury uptake of phosphorus from periods when the WWTP is not treating for P (although it does not rule out the possibility that luxury uptake of P also occurs)[19].

During the 13 day bioassay the periphyton biomass was significantly higher at the downstream sites for the N treatment, P treatment and N+P treatment. This shows that some factor other than nitrate or phosphate (or N+P) was stimulating periphyton growth during the period when the WWTP was treating for phosphorus.

During the 5 day bioassay the periphyton biomass was significantly higher at the downstream sites only for the N treatment. This shows that some factor other than nitrate was stimulating periphyton growth but it does not rule out the possibility of P limitation during this initial growth phase.

The 13 day bioassay had about 25% more chlorophyll *a* at site D compared to site C (see Figure 5.2). These downstream sites were about 80m apart and the difference between them might be attributed to difference in faster water velocity at Site D. Table 5.6 shows the water depth and water velocity above each tray when they were installed and removed. Initial water velocity was faster at sites A and D (99 cm/s and 94 cm/s respectively compared to 77 cm/s at sites B and C), but declined as the river receded to be about 44 cm/s at all sites by the end of the experiment. Nutrient uptake rates are enhanced by faster water velocities that increase the rate of nutrient diffusion to growing cells. Velocities over about 60 cm/s have been shown to reduce periphyton biomass due to scouring (Horner *et al.* 1990), but the effects of scouring will be limited during the early stages of growth when

[19] Luxury uptake is not supported by comparison of periphyton growth rates at the downstream site in the river compared to that on the bioassay. The growth rate in the river was 13.4 mg chl *a* /m^2/day over 31 days between a flood on 24 March and 24th April, the bioassay periphyton had a growth rate of 13.2 mg chl *a* /m^2/day over 13 day period. Growth was much faster after the first 5 to 10 days. At the upstream site the periphyton appeared to have a slightly slower growth rate compared to the bioassay (3.9 mg chl *a*/m^2/day compared to 9.7 mg chl *a* /m^2/day).

periphyton biomass is low. Horner *et al.* (1990) found the positive effect of velocity to be more evident at low (<0.0075 mg/l) DRP concentrations.

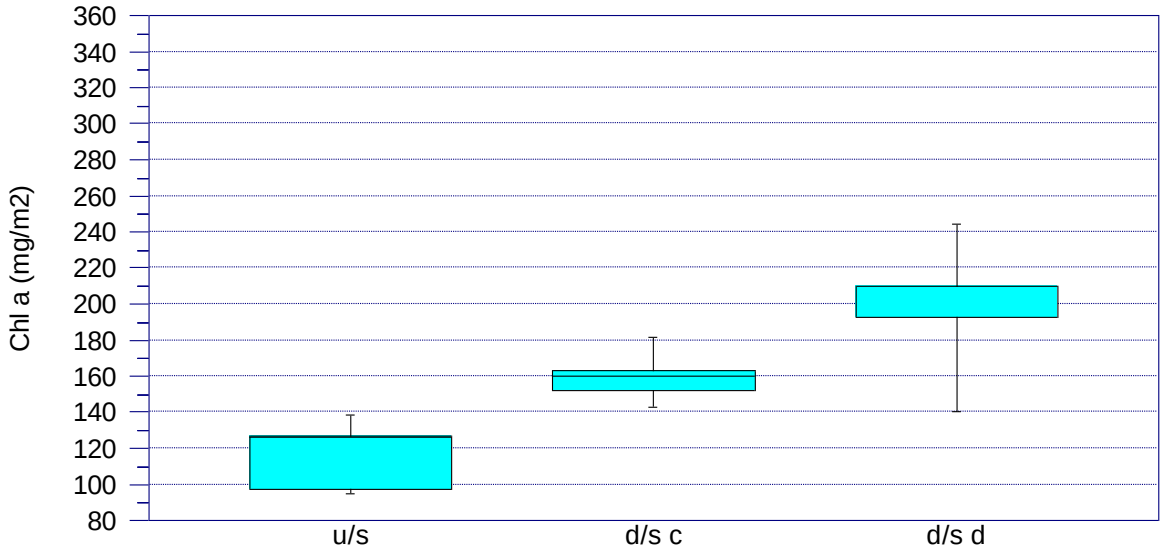


Figure 5.2: Comparison of periphyton biomass between sites on 27th April (N treatments). The box shows the median and quartiles, the whiskers show the extreme values.

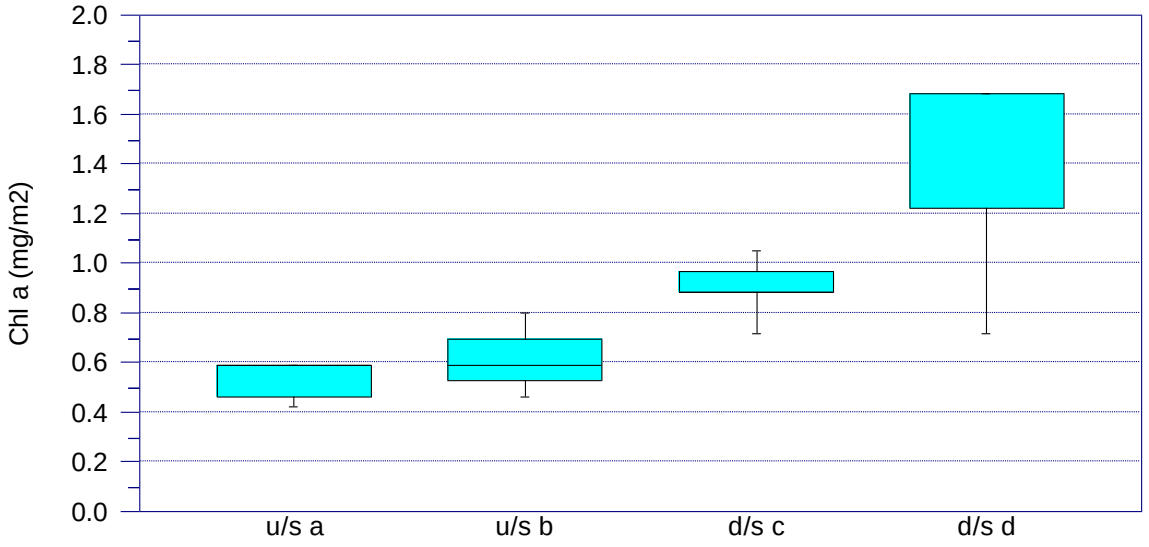


Figure 5.3: Comparison of periphyton biomass between sites on 3rd April (N treatments only). The box shows the median and quartiles, the whiskers show the extreme values.

Table 5.4: Statistical comparison between sites for periphyton bioassay, 27th April 2012

Mann-Whitney, *p*-value				
Treatment	**us vs ds (combined)**	**u/s vs d/s c**	**u/s vs d/s d**	**d/s c vs d/s d**
N	0.002	0.009	0.009	0.11
P	0.007	0.03	0.016	0.75
N+P	0.01	0.075	0.009	0.047
control	0.027	0.25	0.009	0.028
all treatments grouped	**<0.001**	**<0.001**	**<0.001**	**0.002**
Equivalence test, +/- 10%				
N	strong	strong	strong	inconclusive
P	strong	strong	strong	inconclusive
N+P	strong	moderate	strong	moderate
control	strong	inconclusive	strong	strong
all treatments grouped	**strong**	**strong**	**strong**	**strong**

Table 5.5: Statistical comparison between sites for periphyton bioassay, 3rd April 2012

Mann-Whitney, p-value	
Treatment	**us vs ds**
N	**0.001**
P	0.14
N+P	0.3
control	0.9
all treatments grouped	**0.05**
Equivalence test, +/- 10%	
N	**strong**
P	inconclusive
N+P	inconclusive
control	inconclusive
all treatments grouped	inconclusive

Table 5.6: water depth and velocity at each site for the 13 day bioassay.

	14th April		27th April	
site	**Depth (m)**	**Velocity (cm/s)**	**Depth (m)**	**Velocity (cm/s)**
u/s A	42	99.0	25	44.3
u/s B	46	76.7		
d/s C	46	76.7	20	44.3
d/s D	41.5	93.9	26	44.3

Comparison between treatments

A comparison of treatments for upstream and downstream sites during the 13 day bioassay is shown in Figures 5.4 to 5.6. A statistical comparison of the treatments is provided in Table 5.7. The results show that neither N (nitrate), P nor N+P were limiting periphyton growth at the upstream site or downstream Site D. There was weak evidence of P limitation at downstream Site C but the results were not statistically significant and not confirmed by any corresponding biomass increase on the N+P treatment.

This evidence of P limitation at site C suggests that P could be controlling periphyton growth in areas of slower velocity once other limiting factors have been removed.

Surprisingly the control treatment was significantly elevated at the downsteam Site D compared to the N or N+P treatments. The reason for this is not clear; it could be due to the leaking of nutrients from other treatments interfering with the control treatment in combination with higher water velocities stimulating algae growth at the downstream side of the trays used for the experiment. However, visual observation of the trays after they were removed showed less periphyton on the downstream end of the tray (presumably from turbulence causing more scouring), which is not consistent with higher biomass on the control treatments.

There are a number of possible explanations for the 13 day bioassay not showing any nutrient limitation at the upstream sites. These include upstream periphyton biomass being controlled by:

- The total ammonia concentration rather than nitrate concentration with this control being released by ammonia in the discharge.
- Micronutrients (e.g. molybdenum, cobalt, silica, zinc,) with this control being released by the micronutrients in the discharge.
- Aquatic macroinvertebrate grazers (e.g. mayfly) exerting partial control. Rapid growth in periphyton at the downstream site could create a positive feedback loop whereby more periphyton reduces habitat suitability for mayfly, which reduces grazing pressure.
- Some combination of these effects in combination with additional phosphorus due to the WWTP discharge and DRP entering via groundwater

The N and N+P treatment of the bioassay used sodium nitrate. Some studies have found a periphyton response to ammonium but not to nitrate. Pan (1993) found algae community growth was significantly stimulated by either nitrate or ammonia under phosphate enriched conditions, but the diatoms *Gomphonema parvulum* and *Nitzschia palea* strongly preferred ammonium as an inorganic nitrogen source and showed no significant response to nitrate. However ammonium limitation is unlikely because cellular nutrient analysis indicated potential P limitation in periphyton at the upstream site.

Some studies have found periphyton to be limited by micronutrients rather than nitrogen or phosphorus. Pringle *et al.* (1986) found that during *in situ* periphyton bioassays neither N or

P were limiting periphyton growth but a micronutrient treatment (Fe, B, Mn, Zn, Co, Mo, EDTA) supplemented with and without N and P had significantly more chlorophyll *a* accrual compared to all other treatments. Figure 5.7 shows the relative composition of algae compared to the relative concentration of dissolved nutrients in the river (normalised relative to phosphorus). Of the variables we measured only nitrogen and zinc were close to being limiting relative to phosphorus (both we elevated below the discharge). It is also possible that limitation occurred from a micronutrient that we didn't test i.e. silica (which might control diatom growth), cobalt, and molybdenum.

A number studies have shown periphyton communities to be controlled grazing by aquatic macroinvertebrates (e.g. Pan 1993). Welch *et al*. (1992) found lower than expected periphyton biomass associated with high macroinvertebrate grazer density, riparian shading and unsuitable substrate. Grazing by macroinvertebrates has been found to remove 11-29 mg chl *a* /m^2/day (Walton 1990 in Welch *et al*. 1992) and 37 mg chl *a* /m^2/day (Jacoby 1987 in Welch et al. 1992). This is higher than the measured periphyton growth rate at the downstream site in the river (22 mg chl *a* /m^2/day over 19 day period of 5th to 24th April) and on the bioassay (13.2 mg chl *a* /m^2/day over the 13 day period).

The actual rate of grazing will depend on grazer density, type and temperature. The macroinvertebrate survey found that on 20th April (six days into the bioassay and the day before cellular nutrients were collected) the grazer density[20] at sites 1000m u/s and 800m d/s was 246/m^2 and 532/m^2 respectively. By 27th April (the day bioassays were removed), the grazer density had increased to 486/m^2 (range 170-740) at the upstream site, but had reduced to 116/m^2 (range 30-250) at the 800m downstream site. Thus, during the second week of the bioassay grazers at the downstream site reduced in density to well below densities needed to control periphyton biomass. The decline is likely to have been caused by excess periphyton biomass reducing the suitability of the habitat for mayfly.

The main grazer was *Deleatidium* mayfly. The densities of *Deleatidium* at the upstream site were typical of rivers in the National River Water Quality Network (NRWQN). The NRWQN site on the Manawatu River at Teaches College had a median *Deleatidium* density of 161 /m^2 – suggesting that the April low flow period had higher densities compared to long term sampling at Teaches College (John Quinn *pers. Comm*. 2012). We speculate that the macroinvertebrate grazers may exert partial control of periphyton accrual rates but are not expected to prevent dense periphyton development because grazing rates are expected to be less than growth rates.

There is no data to suggest that mayfly or caddisfly density was reduced by any toxic effects. No variable was found in the effluent in sufficient concentrations to cause chronic toxicity to macroinvertebrates after mixing. Whole effluent toxicity tests were undertaken in 2000 as part of the consent application. Waste water samples were tested for freshwater algae (using 72 hour cell growth), microtox (using 15 minute light output) and 48 hour survival of Cladocera (*D. magna*). The results indicated a 5.5 fold dilution of the wastewater was required for no toxicity (evidence by D. Cameron, 2003) – which occurs after a very short distance.

[20] Grazers were defined as mayflys + *Aoteapsyche* caddisfly, + Elmid beetle lavae + snails.

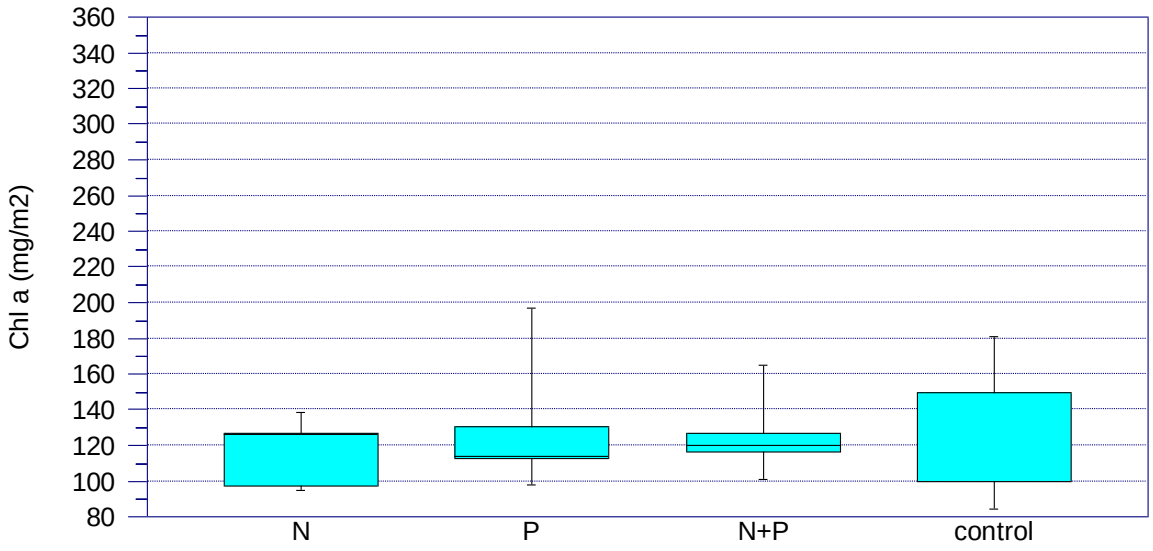


Figure 5.4: Comparison of treatments at the upstream Site A, 27th April 2012. The box shows the median and quartiles, the whiskers show the extreme values.

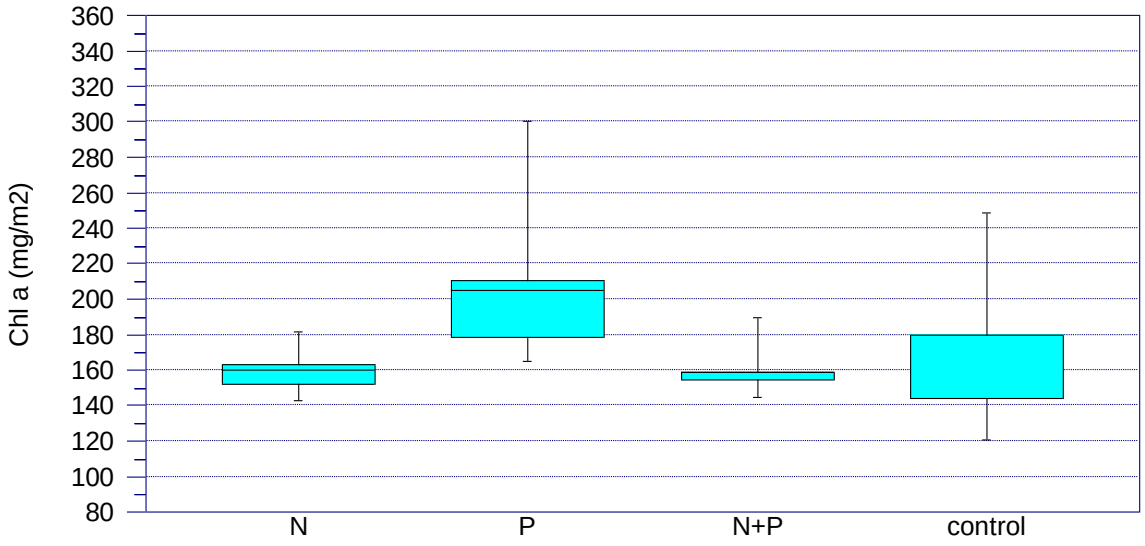


Figure 5.5: Comparison of treatments at downstream Site C, 27th April 2012. The box shows the median and quartiles, the whiskers show the extreme values.

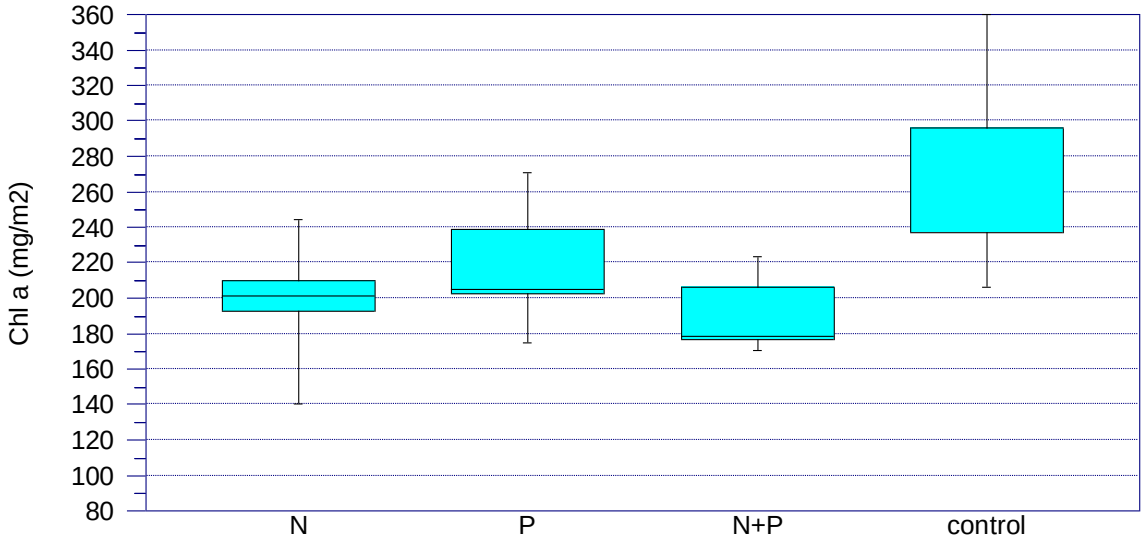


Figure 5.6: Comparison of treatments at downstream Site D, 27th April 2012. The box shows the median and quartiles, the whiskers show the extreme values.

A comparison of treatments for grouped upstream and downstream sites during the 5 day bioassay is shown in Figures 5.7 and 5.8. A statistical comparison of the treatments is provided in Table 5.9. Consistent with the 13 day bioassay there is no indication of nitrogen elevating periphyton growth at either the upstream or downstream sites. However there was evidence that either P or N+P was elevating periphyton growth at the upstream sites but not at the downstream sites. This indication of possible P limitation is not conclusive because the control treatment is also elevated above N treatment but this could be due to interference between the treatments.

The difference between the 5 day bioassay and the 13 day bioassay may be due to different water quality conditions during the experiments (e.g. there was higher nitrate and total ammonia concentrations during the 5 day bioassay compared to the 13 day bioassay). Alternatively it could be due to different requirements at different stages of growth and as the periphyton mat becomes thicker. A third option is that if the periphyton is limited by grazing by aquatic macroinvertebrates. Grazer density takes some time to build after a flood event so it is possible that the 5 day bioassay reflects a situation where the density of macroinvertebrate grazers (mostly mayflies) is too low to control periphyton biomass and the 13 day bioassay reflects a situation where grazer density has increased sufficiently to be a primary control on periphyton biomass at the upstream site.

Grazing by aquatic macroinvertebrates might also explain the higher periphyton biomass found at some of the control treatments, because more turbulence at the downstream end of trays (near the control treatments) might reduce grazer abundance in this section of the trays.

Table 5.7: Mann-Whitney statistical comparison of treatments on each bioassay, 27th April 2012

Treatment	site			
	u/s a	d/s c	d/s d	d/s combined
N vs control	ns	ns	**0.047**	0.2
N+P vs control	ns	ns	**0.016**	0.17
P vs control	ns	0.17	0.12	ns
N vs P	ns	**0.03**	ns	**0.04**
N vs N+P	ns	ns	ns	ns
N+P vs P	ns	**0.03**	ns	**0.028**

ns = not statistically significant (and *p*-value >0.2)
Cells in bold = statistically significant (*p*-value <0.05)

Table 5.8: Relative elemental composition of algae (normalised on total dissolved P on a molar basis) compared to the relative concentration of dissolved constituents of river water on 18th and 26th April 2012. Bold values are similar to algal requirements (adapted from Hecky and Kilham 1988)

	mg/l	mols	Composition relative to P	
Element	River u/s	River u/s	River u/s	Algal
N	0.1445	0.0103	**39.9**	11.1
Si				96
K	1.65	0.0422	163	1.3
P	0.008	0.00026	**1.0**	1
Na	14	0.6090	2357	0.74
Mg	3.3	0.1357	525.5	0.66
Ca	24.5	0.6113	2366	0.63
S	3.45	0.1076	416	0.54
Fe	0.095	0.0017	6.6	0.32
Zn	0.00095	0.000015	**0.056**	0.012
B	0.022	0.0020	7.9	0.008
Cu	0.000405	0.000006	0.025	0.004
Mn	0.00525	0.00010	0.370	0.003
Co				0.003
Mo				0.00002

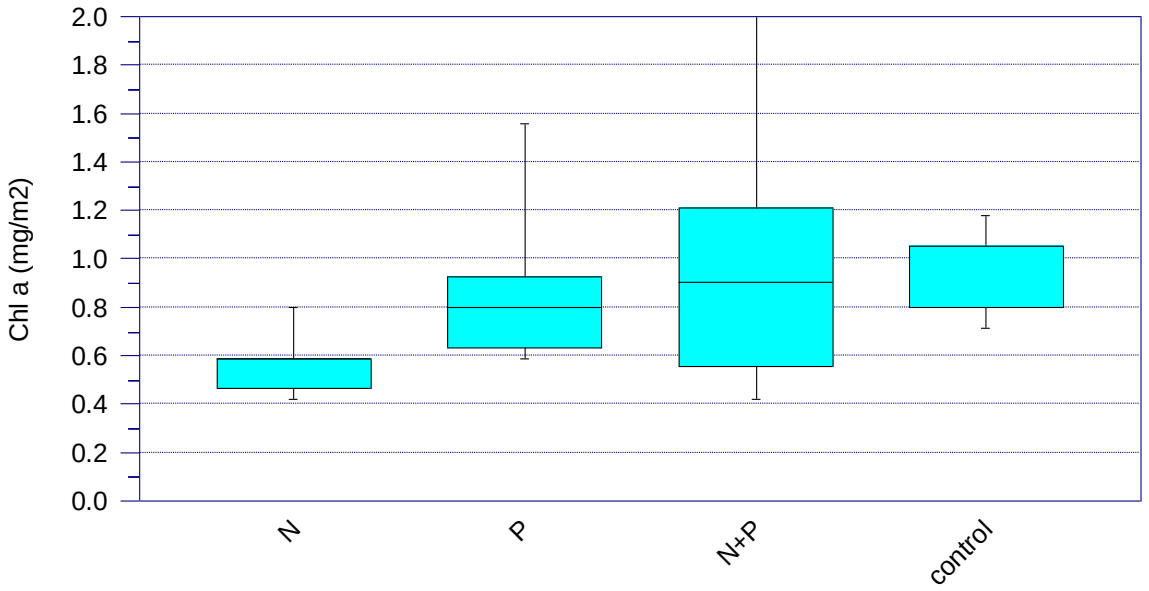


Figure 5.7: Comparison of treatments at the upstream Sites A & B on 3rd April 2012. The box shows the median and quartiles, the whiskers show the extreme values.

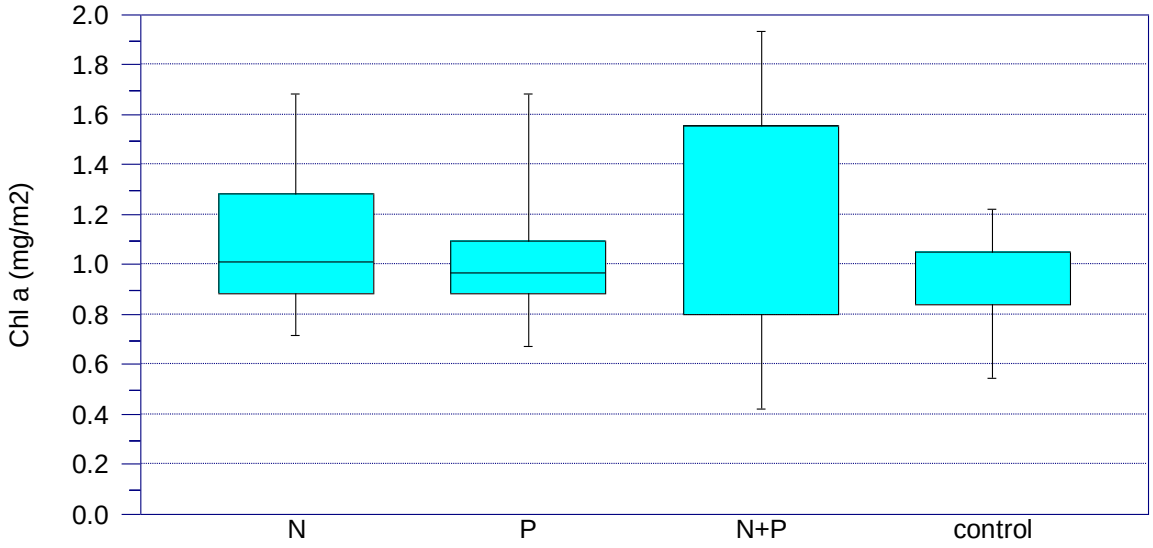


Figure 5.8: Comparison of treatments at downstream Sites C and D, 3rd April 2012. The box shows the median and quartiles, the whiskers show the extreme values.

Table 5.8: Mann-Whitney statistical comparison of treatments on each bioassay, 3rd April 2012

Treatment	site					
	u/s a	**us b**	**d/s c**	**d/s d**	**u/s combined**	**d/s combined**
N vs control	**0.008**	0.1	ns	ns	**0.001**	ns
N+P vs control	ns	ns	ns	**0.01**	ns	0.12
P vs control	**0.08**	ns	ns	0.14	0.2	ns
N vs P	ns	**0.05**	ns	ns	**0.007**	ns
N vs N+P	**0.01**	ns	ns		0.07	ns
N+P vs P	ns	0.11	ns	0.13	ns	ns

ns = not statistically significant (and *p*-value >0.2)
Cells in bold = statistically significant (*p*-value <0.05)

5.4 Summary

Cellular nutrient analysis

- Analysis of cellular nutrients identified that an optimal TN:TP ratio for the periphyton community (excluding cyanobacteria) was about 6.3.
- Cellular nutrient analysis showed potential phosphorus limitation of periphyton growth at the upstream site but not at the downstream site even after two weeks of the WWTP treating for P. This was indicated by:
    - An elevated ratio of TN:TP at the upstream site;
    - An increase in the TN:TP ratio at the upstream site during the week of periphyton growth with no change at the downstream site;
    - No significant difference in the TN:TP ratio during a period when the WWTP wasn't treating for P (25 January) and a period when it was (during April).
- Cellular nutrient analysis and bioassays assess potential nutrient limitation at a particular point in time and do not rule out the possibility of nitrogen becoming limiting under different circumstances.

Periphyton nutrient bioassay

- Periphyton biomass was statistically significantly higher at the downstream site compared to the upstream site.
- The higher periphyton biomass downstream is caused by factors that occur while the WWTP is treating for P and is thus unrelated to periphyton using P stored from any previous luxury uptake of P from before treatment started.

- During the 13 day bioassay some factor other than concentrations of nitrate and/or phosphorus was primarily limiting periphyton growth upstream and stimulating periphyton growth downstream. This was indicated by two lines of evidence:
    - The treatments of N, P and N+P all showing a statistically significant increase in periphyton biomass between the upstream and downstream sites.
    - There was no statistically significant difference between any of the treatments at any site.
- During the 13 day bioassay there was weak evidence (not statistically significant) of P limitation at downstream Site C. This suggests that P might be controlling periphyton grow in areas of slower velocity once other limiting factors have been removed.
- During the 5 day bioassay there was some evidence of P limiting periphyton growth at the upstream sites but not at the downstream sites. The result was not definitive due to the control treatment also having high biomass but was nevertheless indicated by:
    - Significantly higher periphyton biomass at the downstream sites for the N treatment but not for the treatments of P or N+P.
    - The upstream sites having significantly more periphyton biomass on the P and N+P treatments compared to the N treatment.
- P appears to be a secondary limiting factor on periphyton growth but something other than N or P is the primary limiting factor. There are several possible factors that could account for downstream stimulation of periphyton growth other than nitrate or phosphorus. These include:
    - Stimulation of growth by micronutrients (e.g. silica, cobalt, molybdenum, zinc, soluble sugars) in the discharge and possibly groundwater sources.
    - Dissolved carbon from the discharge promoting heterotrophic growths which in turn enhance periphyton colonisation, nutrient availability and a growth.
    - Stimulation of growth by total ammonia in the discharge – but not by nitrate which was used in the bioassay. This is unlikely because cellular nutrient analysis indicated potential P limitation in periphyton at the upstream site.
    - Rapid growth in periphyton at the downstream site creating a positive feedback loop whereby more periphyton reduces habitat suitability for mayfly, which reduces grazing pressure.
    - Some combination of these effects in combination with additional phosphorus due to the WWTP discharge and DRP entering via groundwater.

- The results of bioassays and cellular nutrient analysis do not rule out the possibility of nitrogen limitation of green algae (*Stigeoclonium* sp.) at the upstream site late in the succession.

6 Aquatic macroinvertebrates and periphyton cover

6.1 Aim

Aquatic macroinvertebrate sampling was undertaken to clarify the extent to which the PNCC WWTP discharge was affecting periphyton growth and the aquatic macroinvertebrate community in the Manawatu River. Sampling occurred on two sequential occasions during a period of low flow when the WWTP was treating for phosphorus to distinguish the potential impact of the WWTP discharge before and after treating for P.

6.2 Method

6.2.1 Macroinvertebrates

A macroinvertebrate assessment was undertaken on two occasions over a receding flow, a third sample was planned but a flood event prevented this being practical. Samples were undertaken on:

- 20th April 2012, 28 days since the last flood >3x median flow (on 23rd March) and after 8 days of the WWTP treating for P;
- 27th April 2012, 35 days since the last flood >3x median flow and after 15 days of the WWTP treating for P

Samples were collected from five sites in the Manawatu River:

- 1000m above the discharge point;
- 800m above the discharge point;
- 800m below the discharge point;
- 1000m below the discharge point;
- Upstream of Longburn STP discharge (about 1.4km downstream of Mangaone Stream confluence).

Sampling locations are shown on Figure 6.1.

The site upstream of Longburn STP was added to see if any change was evident further downstream.

The macroinvertebrate sampling followed Protocols C3 (Hard-bottomed quantitative) from the Ministry for the Environment's "protocols for sampling macroinvertebrates in wadeable streams" (Stark *et al*. 2001). This involved collecting 5 replicate 0.1 m^2 Surber samples approximately located near separate transects used for the periphyton assessment of cover (at least 5 metres apart).

The following measurements were recorded for each Surber sample:

- Velocity (head rod);
- Depth
- Periphyton cover and type as per periphyton assessment above,

Making precise measurements for each replicate sample was intended to help confirm the link between the macroinvertebrate indices and periphyton cover.

Samples were sent to Stark Environmental to be identified and counted using Protocol P3 (full count) of Stark *et al.* (2001). Macroinvertebrate taxa was identified to the taxonomic resolution level specified for use of the Macroinvertebrate Community Index (MCI).

The following ecological indices were used to assess the biological health of the river and the effects of the discharge on the stream ecology:

Taxa Richness: This is a measure of the types of invertebrate taxa present in each sample. Generally in streams, the greater the numbers of taxa present, the higher the quality of the environment.

EPT richness and % EPT abundance (Ephemeroptera-Plecoptera-Trichoptera). This measures the number of pollution sensitive mayfly, stonefly and caddisfly (EPT) taxa in a sample excluding the tolerant *Oxyethira* and *Paroxyethira*. A high EPT number is indicative of good water and habitat quality.

Macroinvertebrate Community Index (MCI). The MCI is an index for assessing the water quality and 'health' of a stream using the presence/absence of benthic macroinvertebrates (Stark 1985).

Quantitative MCI (QMCI). The Quantitative MCI is similar to the MCI but utilises quantitative data. The QMCI is based on the relative sensitivity of different taxa in a sample to changes in water quality. The QMCI is designed to be particularly sensitive to changes in the relative abundance of individual taxa within a community (Stark 1993, Stark 1998).

The MCI and QMCI reflect the sensitivity of the macroinvertebrate community to pollution, with higher scores indicating higher water quality. Generally accepted water quality classes for different MCI and QMCI scores and soft-bottomed version are shown in Table 6.1.

Table 6.1: Quality thresholds for interpretation of the MCI & QMCI.

Quality Class	Stark (1998) descriptions	MCI	QMCI
Excellent	Clean water	> 120	> 6.0
Good	Doubtful quality or possible mild pollution	100 – 120	5.0 - 6.0
Fair	Probable moderate pollution	80 – 100	4.0 – 5.0
Poor	Probable severe pollution	< 80	< 4.0

Figure 6.1: Location of aquatic macroinvertebrate sampling.

6.2.2 Periphyton cover

Visual periphyton measures were made at the same time as aquatic macroinvertebrate sample collections, using the same method as described above for 'periphyton dynamics'. Assessments were made within each of the replicates defined by the Surber sampler to improve comparability of the results.

6.2.3 Statistics

Tests for equivalence and inequivalence were based on +/- 20% change compared to the upstream control sites. A 20% change corresponds to water quality target in the One Plan that "*Discharges to water to cause no more than a 20 % reduction in QMCI score between appropriately matched habitats upstream and downstream of discharges to water*." Allowing up to a 20% change recognises that habitats can seldom be perfectly matched and even small changes in substrate size, flow and location can impact on macroinvertebrate composition to some extent.

6.3 Results

6.3.1 Aquatic macroinvertebrates

The summary results of aquatic macroinvertebrate sampling are shown in Table 6.2 and the full table of results for 20th and 27th April are in Appendix 2. MCI scores (graphed in Figure 6.2) indicate that water quality in the Manawatu River is on the boarder of categories 'fair' to 'good'. Only one site (800m upstream on 20th April) had an MCI score that complied with the target set in the Proposed One Plan (POP) of exceeding 100. A week later on 27th April there had been a reduction in MCI scores at two sites (800m u/s and 100m d/s) and no site had an MCI score exceeding 100.

Statistical differences were analysed by grouping the two upstream sites (1000m u/s and 800m u/s) and the first two downstream sites (800m d/s and 1000m d/s). A summary of the results of a non-parametric Mann-Whitney test is shown in Table 6.3 and results of an equivalence test (with a practically important difference set at +/- 20%) is shown in Table 6.4. A Mann-Whitney test found no statistical difference in MCI scores between upstream and downstream on either sample dates, and it found no statistical difference in MCI scores between sample dates for either upstream or downstream sites. The results of the equivalence test were inconclusive.

QMCI scores (graphed in Figure 6.3) and % EPT abundance[21] (Figure 6.4) were a more responsive indicator of the effects of the discharge. On both sample dates there were statistically significant lower QMCI scores at downstream site compared to upstream sites, this difference was stronger on the second sample date (on 27th April and after 15 days of the WWTP treating for P). The equivalence test found 'moderate evidence' of a >20% difference in QMCI between upstream and downstream on 20th April and 'strong evidence' on 27th April.

Comparing the change in QMCI scores in the week between sample dates found no statistically significant difference in QMCI at the upstream sites, but strong evidence of a decline in QMCI during the week at the downstream sites. The equivalence test showed strong evidence of a decline in QMCI during the week up to a practical difference of +/- 29%.

Interestingly, the site above Longburn (below Mangaone Stream) had similar QMCI scores between sample dates suggesting that the site was less sensitive to any impact of the WWTP discharge during this period. This may be due to the WWTP discharge (and other possible inputs) not being fully mixed until 1400m downstream of the discharge point.

The abundance and composition of macroinvertebrate taxa groups is summarised in Figure 6.5 and Figure 6.6 for sampling on 20th April and 27th April respectively. This shows that on 20th April there was similar abundance of mayfly and EPT taxa at the two downstream and upstream sites (statistical analysis were inconclusive due to the large variation). There were also more Diptera (predominantly Orthocladiinae) at the downstream sites and this is what drove the decline in QMCI and %EPT taxa between the upstream and downstream

[21] There was negligible difference between % EPT abundance and % EPT abundance (excluding Hydroptilidae taxa).

sites. One week later, on 27th April, the abundance mayfly (and to a lesser extent true fly) had increased at the upstream sites (probably reflecting a continued recovery from the large flood on 23rd March); however at the two sites immediately downstream there was an increase in true fly abundance and a substantial decline in mayfly abundance (a 2 way ANOVA on normalised mayfly data found the difference to be statistically significant).

Table 6.2: Median results of five replicate Surber samples at sites in the Manawatu River upstream and downstream of the WWTP (20th April 2012 and 27th April 2012).

site	Date	Number of taxa	No individuals	MCI	QMCI	%EPT richness	%EPT abundance	EPT abundance	Mayfly (No./m^2)
1000m u/s	20-Apr	5	30	92	6.4	40.0	78.9	30.0	230
800m u/s	20-Apr	5	28	116	6.8	60.0	96.3	28	230
800m d/s	20-Apr	8	109	90	4.4	50.0	45.0	109	300
1000m d/s	20-Apr	5	50	100	6.1	33.3	62.5	50	280
d/s Mangaone	20-Apr	8	41	93	4.0	50.0	36.6	41	70
1000m u/s	27-Apr	6	75	90	6.5	40.0	73.9	75	350
800m u/s	27-Apr	7	63	93	6.7	55.6	84.2	63	500
800m d/s	27-Apr	8	76	91	2.9	44.4	13.6	76	60
1000m d/s	27-Apr	5	28	80	2.6	20.0	7.1	28	10
d/s Mangaone	27-Apr	6	50	93	3.8	50.0	38.7	50	100

EPT abundacne excludes Hydroptilidae and is the sum of the 5 replicates.

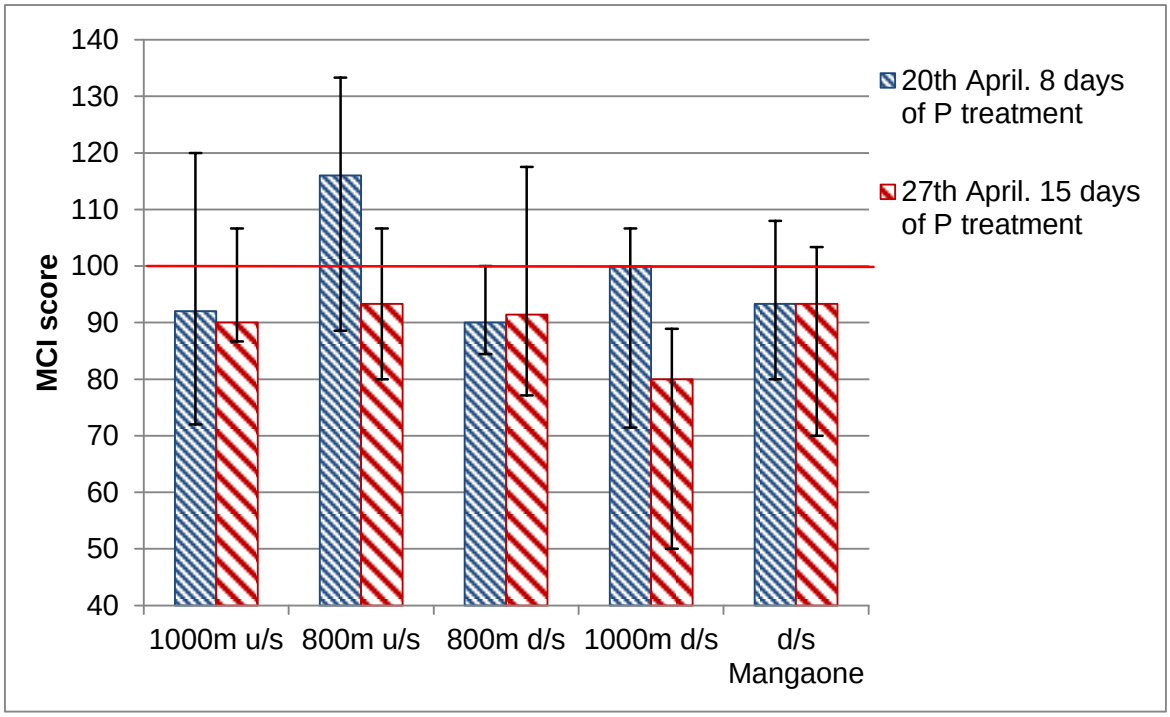


Figure 6.2: Median MCI scores in the Manawatu River upstream and downstream of the WWTP during a low flow period. Error bars show the full range of five replicate samples. The red line indicates the Proposed One Plan target of this section of the Manawatu River.

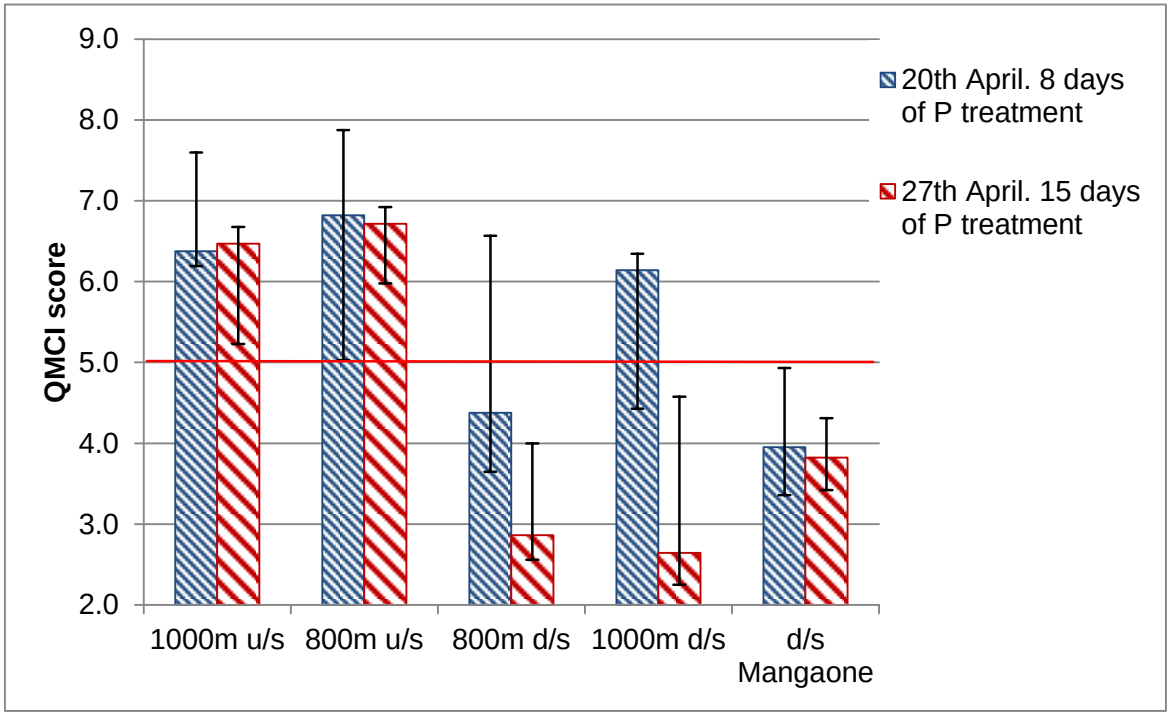


Figure 6.3: Median QMCI scores in the Manawatu River upstream and downstream of the WWTP during a low flow period. Error bars show the full range of five replicate samples. The red line indicates the boundary between "good" and "fair" water quality.

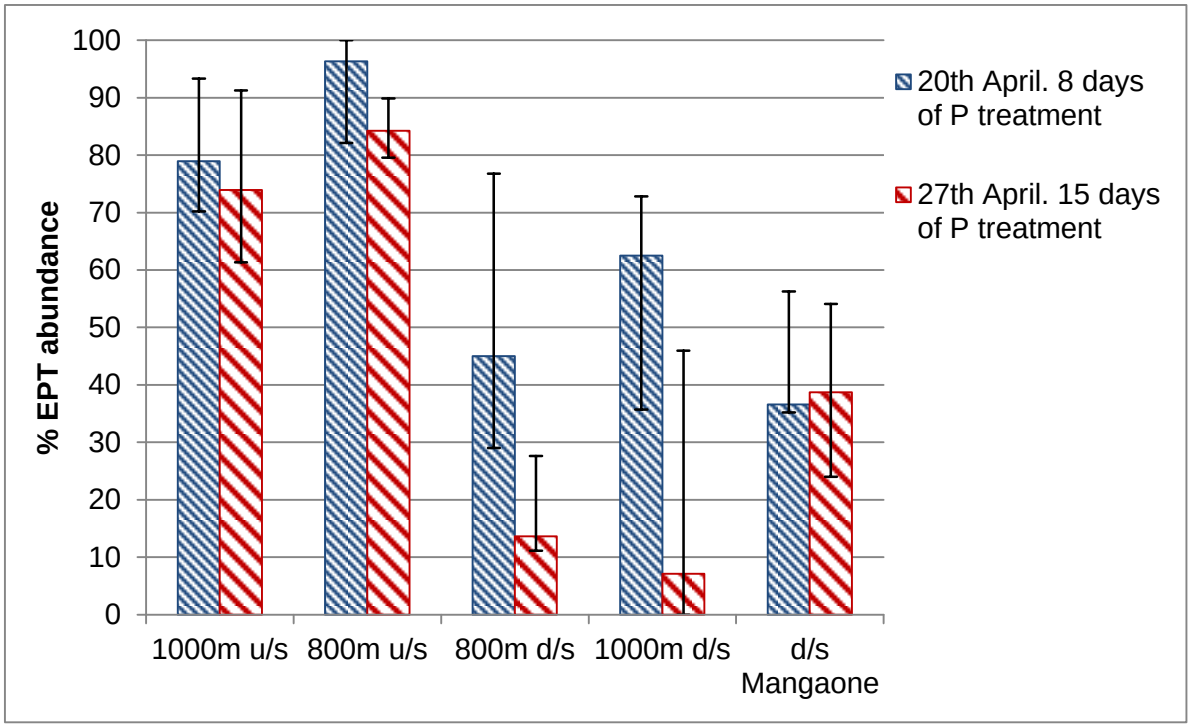


Figure 6.4: Median % EPT abundance in the Manawatu River upstream and downstream of the WWTP during a low flow period. Error bars show the full range of five replicate samples.

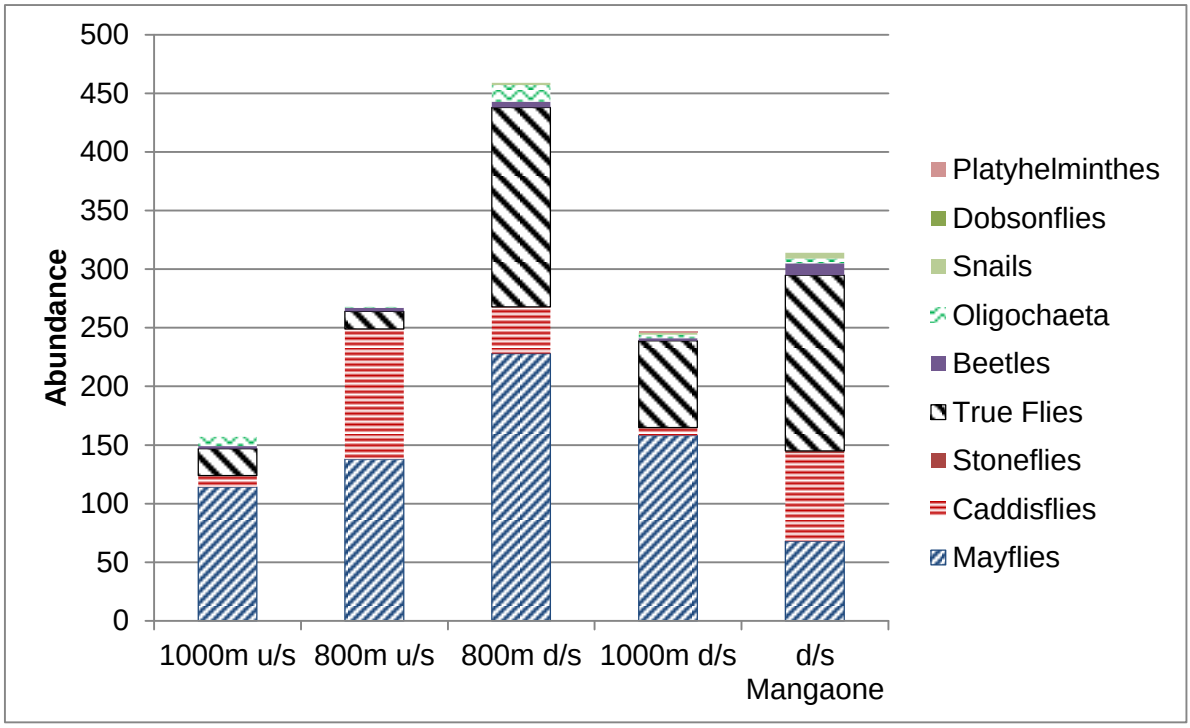


Figure 6.5: Macroinvertebrate composition in the Manawatu River upstream and downstream of the WWTP during a low flow period on 20th April 2012. Double these abundances to express as number per m^2.

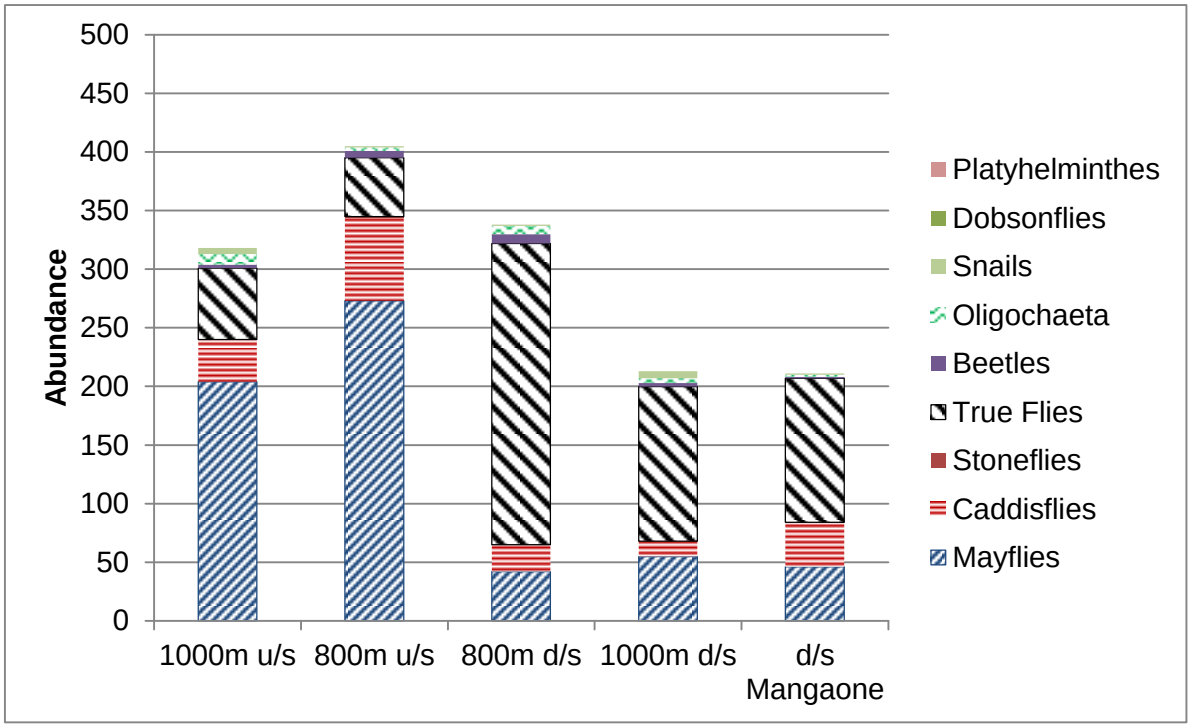


Figure 6.6: Macroinvertebrate composition in the Manawatu River upstream and downstream of the WWTP during a low flow period on 27th April 2012. Double these abundances to express as number per m^2.

Table 6.3: Summary *p*-values of Mann-Whitney test between upstream (and downstream sites (grouped excluding Longburn site) and between dates. The *p*-value were adjusted for multiple comparisons and considered statistically significant if <0.05.

Index	u/s vs d/s 20th April	u/s vs d/s 27th April	20th vs 27th at u/s sites	20th vs 27th at d/s sites
Number of taxa	0.08	0.90	0.25	0.97
Number individuals	0.16	0.31	0.15 *	0.60
MCI	0.16	0.14	0.25	0.42
QMCI	**0.01**	**<0.001**	0.71	**0.002**
%EPT richness	**0.05**	0.14	0.25	0.51
%EPT abundance	**0.002**	**<0.001**	0.25	**0.002**
EPT abundance	0.160	0.260	0.15 *	0.5

* shows results where *p*-value = 0.05 before adjusting for multiple comparisons.

Table 6.4: Summary results of an equivalence test between upstream and downstream sites (grouped) and between dates. The equivalence test used a significance level of *p*-value <0.05 and a practically important difference set at +/- 20%.

Index	u/s vs d/s 20th April	u/s vs d/s 27th April	20th vs 27th at u/s sites	20th vs 27th at d/s sites
Number of taxa	Inconclusive	Inconclusive	Inconclusive	Inconclusive
Number individuals	Inconclusive	Inconclusive	Inconclusive	Inconclusive
MCI	Inconclusive	Inconclusive	Inconclusive	Inconclusive
QMCI	**Moderate evidence**	**Strong evidence**	No practical difference	**Strong evidence**
%EPT richness	**Moderate evidence**	**Moderate evidence**	Inconclusive	Inconclusive
%EPT abundance	**Strong evidence**	**Strong evidence**	No practical difference	**Strong evidence**
EPT abundance	Inconclusive	Inconclusive	Inconclusive	Inconclusive

'Moderate' = moderate evidence of a >20% difference.

'Strong' = strong evidence of a >20% difference.

6.3.2 Periphyton cover

The periphyton cover within areas sampled for macroinvertebrates is summarised in Table 6.5 and statistical comparisons are shown in Table 6.6. The cover of thick diatoms (>3mm thick) and long filamentous algae was higher at the downstream sites and exceeded the guidelines set for protection of aquatic habitat (i.e. upper level of 30% cover by long filamentous algae and 60% cover of thick diatom mats or cyanobacteria (Biggs 2000).

There was statistically significantly more cover of “slimy” green filamentous algae at the downstream sites on both sample occasions (Figure 6.7). The variation in periphyton cover between replicates was reduced when that cover data was aggregated into the Periphyton Slimyness Index (PSI) which weights different thickness categories (see Figure 6.8).

The upstream sites showed no significant difference in periphyton cover during the week between the two sample occasions. However, the downstream sites (800m and 1000m d/s) had significantly more periphyton cover. These patterns are generally consistent with the results of QMCI scores from macroinvertebrate monitoring.

There was a strong negative relationship between periphyton cover (as measured by PPI) and QMCI scores, i.e. more periphyton was associated with lower QMCI scores. Figure 6.8 shows that the relationship became stronger on 27th April and upstream and downstream sites became more distinct groups (all downstream sites, for both dates, are included within the grey line on the graph). On 20th April the r^2 was 0.46 and on 27th April the r^2 was 0.64.[22]

[22] The equation describing the relationship on 20th April was: QMCI = -0.035 PPI + 6.784. And on 27th April was: QMCI = -1.356 ln(PPI) + 9.3434.

The mayfly (*Deleatidium* sp.) was the most common macroinvertebrate grazer found on the in the river. This species showed a different pattern with periphyton growth compared to the QMCI. The biomass of mayfly increased with PPI up to a PSI value of about 40-50% – after which it declined again.

Between the sampling dates of 20th April and 27th April the site 800m downstream had a substantial decline in QMCI scores (4.4 to 2.9) and mayfly abundance (300/m^2 to 60/m^2). This corresponded to an increase in periphyton cover from a PPI of 55% to 80% and an increase in periphyton biomass from about 300 mg/m^2 to 450 mg/m^2 (estimated from Figure 4.8). If it is assumed that the decline in mayfly abundance was caused by the increasing periphyton biomass then the frequency of this event occurring could be estimated using the same method as used for periphyton in Section 4. It took about 29 days for the site 800m downstream to exceed a biomass of 300 mg/m^2 (Figure 4.5), which corresponded to about 1.24 times per summer.

Table 6.5: Median periphyton cover and hydrology over the areas sampled for macroinvertebrates.

site	Date	Depth (cm)	Velocity (cm/s)	Mats <3mm	Mats >3mm	Slimy green algae >2cm	Coarse green algae >2cm	PPI	PSI
1000m u/s	20-Apr	22	77	90	0	10	0	10	26
800m u/s	20-Apr	20	83	90	0	5	0	10	24
800m d/s	20-Apr	24	63	45	0	50	0	55	52
1000m d/s	20-Apr	15	70	70	10	20	0	30	34
d/s Mangaone	20-Apr	25	77	30	20	30	10	70	52
1000m u/s	27-Apr	28	54	90	0	10	0	10	26
800m u/s	27-Apr	26	70	85	0	10	0	15	29
800m d/s	27-Apr	28	77	20	20	60	0	80	60
1000m d/s	27-Apr	25	99	40	10	50	0	60	52
d/s Mangaone	27-Apr	21	63	10	5	70	0	90	70

PPI = Periphyton Proliferation Index = percent cover of long filaments + thick mats

PSI = Periphyton Slimyness Index = cover of weighted thickness category

Table 6.6: Summary *p*-values of statistical test between upstream (and downstream sites (grouped excluding Longburn site) and between dates. The *p*-value were adjusted for multiple comparisons and considered statistically significant if <0.05

Index	u/s vs d/s 20th April	u/s vs d/s 27th April	20th vs 27th at u/s sites	20th vs 27th at d/s sites
Depth (m)	ns	ns	ns	0.08
Velocity (cm/s)	ns	**0.022**	0.07	0.18
Slimy filamentous >2cm	**0.004**	**<0.001**	ns	**0.05**
PPI	**0.002**	**<0.001**	ns	**0.007**
PSI	**0.002**	**<0.001**	ns	**0.007**

ns = not significant

Equivalence test - strength of evidence

Index	u/s vs d/s 20th April	u/s vs d/s 27th April	20th vs 27th at u/s sites	20th vs 27th at d/s sites
Depth (m)	Inconclusive	Inconclusive	Inconclusive	Moderate
Velocity (cm/s)	Inconclusive	Moderate	Inconclusive	Inconclusive
Slimy filamentous >2cm	Strong	Strong	Inconclusive	Moderate
PPI	Strong	Strong	Inconclusive	Strong
PSI	Strong	Strong	Inconclusive	Moderate

Equivalence test assume +/- 20% difference and *p*-values significant if <0.05

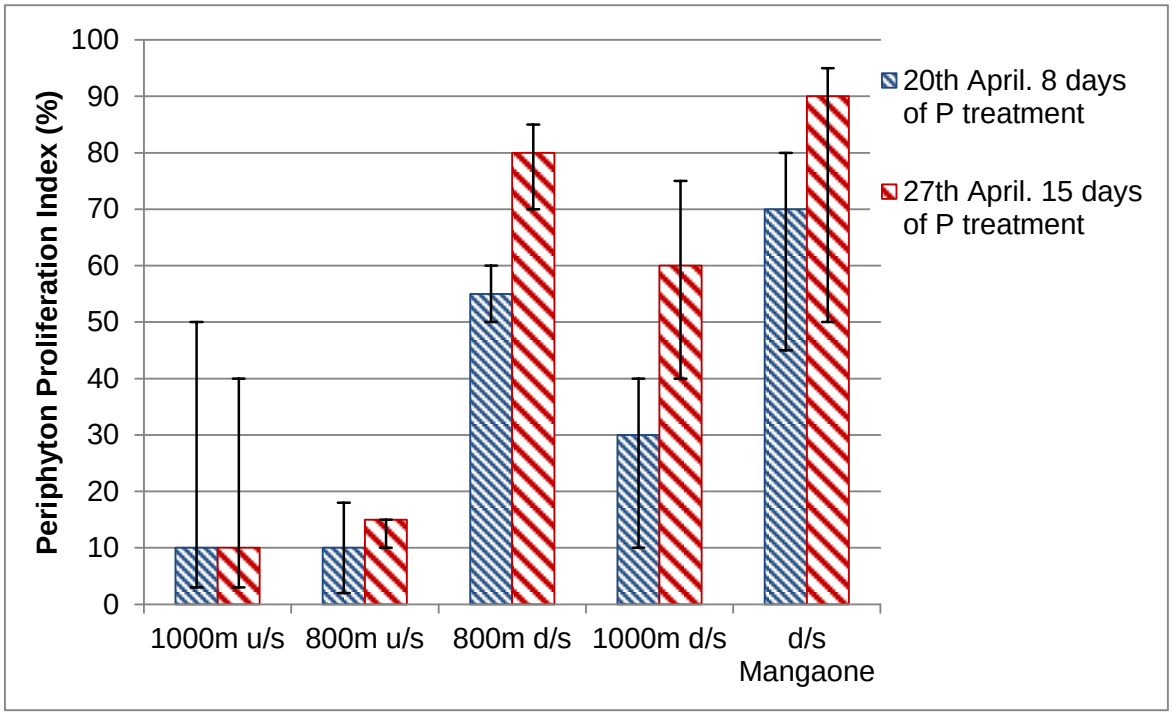


Figure 6.7: Median periphyton proliferation index in the Manawatu River upstream and downstream of the WWTP during a low flow period. Error bars show the full range of five replicate samples.

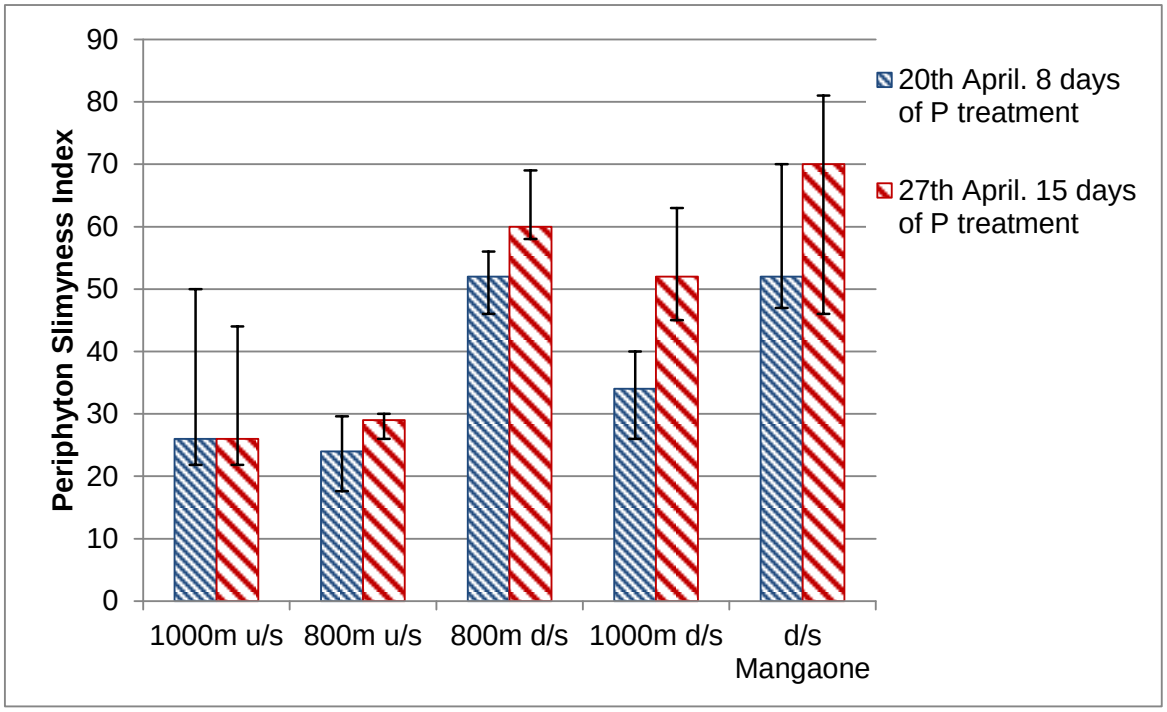


Figure 6.8: Median Periphyton Slimyness Index for sites in the Manawatu River upstream and downstream of the WWTP during a low flow period. Error bars show the full range of five replicate samples.

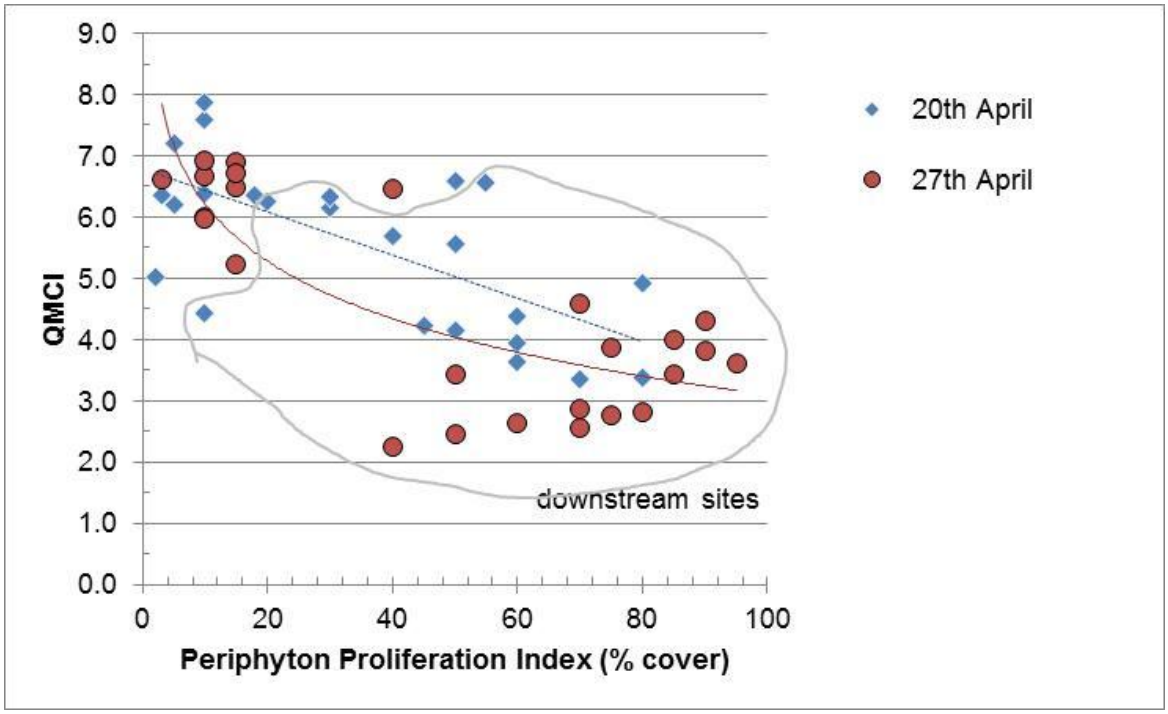


Figure 6.8: Relationship between the QMCI and the Periphyton Slimyness Index for sites in the Manawatu River upstream and downstream of the WWTP. Downstream sites are incorporated within the grey circle.

6.3.3 Summary of macroinvertebrate survey

- Aquatic macroinvertebrates were sampled upstream and downstream of the WWTP discharge on 20th April, 8 days after the WWTP starting treatment for P and on 27th April, 15 days after the WWTP starting treatment.
- There was no statistically significant change in MCI scores between upstream and downstream of the discharge and between sample occasions.
- QMCI and % EPT taxa were a sensitive indicator of changes in aquatic macroinvertebrate community downstream of the discharge and over time. On both sample dates there were statistically significant lower QMCI scores at downstream site compared to upstream sites.
- In the week between sample dates QMCI scores did not significantly change at the upstream sites, but there was strong evidence that QMCI scores at the two downstream sites reduced by about 29% during the week.
- On the 20th of April there was a greater abundance of mayfly and EPT at the two immediately downstream sites. A week later on 27th April the number of EPT taxa had increased at the upstream site but decreased at the two downstream sites (although none of these changes were statistically significant). The number of diptera had increased at all sites except downstream of Mangaone Stream.

- The decline in QMCI scores observed downstream of the WWTP on 27th April discharge did not persist down the river to the site downstream of Mangaone Stm (upstream of Longburn).
- There was a strong negative correlation between periphyton cover and QMCI.
- Periphyton cover showed similar patterns to the QMCI, i.e. more cover at all the downstream sites and an increase between the two sample occasions between sites 800m downstream and 100m downstream (but not at the other sites).
- The decline in mayfly abundance observed on the 27th April represents a significant change in community structure. This degree of impact was predicted to occur 1.24 times per summer for the site 800m downstream (based on 29 days to exceed a biomass of 300 mg/m^2).

7 Synthesis and Conclusions

This report has described the results of a joint work programme for monitoring the Manawatu River from November 2011 to May 2012. The key purposes of this monitoring were to address:

- The effects that are occurring in the periphyton and aquatic macroinvertebrate communities downstream of the WWTP; including identifying the extent to which the WWTP discharge is affecting periphyton growth and the aquatic macroinvertebrate community in the Manawatu River.
- The causes of these effects; including other sources of nutrients in the vicinity of the WWTP, and which (if any) nutrient is controlling periphyton growth.
- What future management actions may be required for the treatment processes to lessen adverse effects on the river environment, including refining the timing and extent of phosphorus removal from the wastewater in order to limit downstream periphyton growth.

This section synthesises the result of monitoring to address each of these questions.

7.1 Effects of the WWTP discharge on periphyton growth and aquatic macroinvertebrate communities.

- The WWTP and other contaminants entering the river in the vicinity of the WWTP caused a noticeable increase in periphyton growth and a decline in the quality of the aquatic macroinvertebrate community during periods of extended low flow.
- The adverse effects on biota only became evident during periods of low flow. This is typical of river dynamics because periphyton biomass is strongly influenced by the period of time since the last flood that removed periphyton biomass. In practice this meant that periphyton cover and biomass remained low (i.e. <30 mg/m^2 and PPI <10%) when Manawatu River flows were above about 50 m^3/s.
- During low flow periods the periphyton at sites downstream of the WWTP grew faster than at upstream sites and exceeded guideline trigger values sooner. The periphyton biomass exceeded filamentous periphyton guidelines for trout habitat (120 mg/m^2) after about 37 days, 17 days and 19 days of growth for sites upstream, 800m downstream and 1400m downstream respectively. This corresponded to respective exceedence frequencies of 1.1 times per summer, 1.9 times per summer and 1.7 times per summer.
- The site 800m downstream of the WWTP showed faster periphyton growth compared to the site 1400m downstream, probably because the effluent was not fully mixed until after 1000 - 1200m downstream and concentrations of nutrients and were higher.
- The periphyton community was usually dominated by diatom species and the green algae *Stigeoclonium* sp. There was more cyanobacteria cover at the site 800m

downstream but only small amounts (3% cover) and this effect didn't persist far downstream.

- The increase in periphyton biomass was associated with a decline in the quality of the macroinvertebrate community (as measured by QMCI scores) at the downstream sites during periods of low flow.

- At the downstream sites periphyton continued to accumulate and aquatic macroinvertebrates continued to decline even during a period when the WWTP was treating for P. A reduction in the abundance of mayfly (300/m^2 to 60/m^2) at the 800m downsteam site corresponded with an increase in periphyton cover and biomass (from about 300 mg/m^2 to 450 mg/m^2). Using a 21 year flow record it was estimated that on average this degree of change might occur 1.24 times per summer.

- The macroinvertebrate community at the site upstream of Longburn (about 1.4 km downstream of Mangaone Stream confluence) had lower QMCI and %EPT abundance than at the upstream of WWTP sites but did not change over time.

7.2 What is causing the effects observed in the Manawatu River downstream of the WWTP plant

- An increase in periphyton biomass and cover was, at least partially, driving a decline in the quality of the macroinvertebrate community (e.g. QMCI) both spatially (downstream of the WWTP) and temporally as periphyton biomass accumulated during periods of low flow. A decline in the density of macroinvertebrate grazers will create a positive feedback allowing periphyton biomass to increase even further.

- During low flow in April, when the WWTP was alum dosing for phosphorus (P), the discharge was estimated to increase bioavailable phosphorus (DRP) by 7% to 23% (ca. 0.0005 to 0.0016 g/m^3) above background concentrations at site 800m d/s. However, measured concentrations of DRP in the river were about 160% (ca. 0.011 g/m^3) above background concentrations (and about 2.4 times higher than what might be attributed to the WWTP). This indicates another external (e.g. groundwater) or internal (e.g. desorption of dissolved P from river sediments) source of P to the river during periods of low flow.

- The additional source of DRP means that the full effects of the WWTP removing phosphorus (P) from the discharge were not often being realised in the river.

- There was evidence of groundwater seepage to the river about 400m to 600m downstream of the discharge. The precise location of groundwater inputs may change with river level.

- Despite the influence of other DRP sources, when the WWTP was removing P from the effluent, the DRP concentrations in the river water downstream were still reduced to low levels (i.e. a low flow mean of 0.008 g/m^3) which should have been sufficient to limit the rate of periphyton growth. The higher than expected periphyton growth at the 800m downstream site might be explained by periphyton experiencing higher DRP concentrations at the sediment surface than measured in the water as DRP enters the river from groundwater seepage and/or desorption from sediments.

- During low flows periphyton biomass in the Manawatu River upstream of the WWTP discharge was limited by something other than the macronutrients, nitrate nitrogen or phosphorus. This was indicated by:

    - The nutrient bioassay which supplied excess phosphorus for periphyton growth at the upstream site but still had lower biomass accrual than the downstream site.

    - Comparison of measured periphyton biomass in April with predictions from a regression model (Biggs 2000) which found measured periphyton biomass was lower than those predicted under a range of scenarios.

- Nevertheless, phosphorus did appear to be a secondary limiting factor for periphyton growth upstream of the WWTP. This was indicated by increasing cellular ratios of TN:TP during a growth period and compared to the downstream site.

- While the evidence pointed to possible P limitation (in combination with something else), we cannot rule out the possibility of duel limitation of N and P also occurring. For example, during low flow periods soluble inorganic nitrogen (SIN) was at times within concentrations that might reduce the periphyton growth rate.

- Whatever was limiting periphyton growth upstream was not limiting it downstream. Downstream of the WWTP neither N or P were limiting periphyton growth, allowing periphyton on the bioassays to quickly attain high biomass and coverage (about 66% more than upstream). There was weak evidence of phosphorus limitation in areas of slower water, but biomass was still about 33% higher than the upstream site).

- At this stage we can only speculate as to what is the primary control on periphyton biomass (and hence the primary cause of d/s periphyton growth). Possible causes include:

    - Micronutrients (e.g. silica, cobalt, molybdenum, zinc) controlling periphyton growth upstream, and the discharge providing these nutrients.

    - Grazing by macroinvertebrates (e.g. mayflies) partially controlling periphyton biomass upstream. While at the downstream sites periphyton growth rates exceed removal rates by grazers.

- In addition to this, it appears that the river substrate (gravels and cobbles) at the site 800m downstream are being ‘conditioned’ by heterotrophic biofilms which might reduce the period of time after a flood for the early stages of periphyton growth, allowing more rapid growth sooner. This was indicated by:

    - Comparative growth rates between sites during April; and

    - Comparison of measured periphyton biomass in April with predictions from a regression model which found higher periphyton biomass in the river than those predicted even when unrealistically high DRP concentrations were assumed.

    - The presence of fungus filaments in some of the periphyton samples.

One possible explanation for higher initial growth rates downstream is that dissolved organic carbon from the discharge (part of the BOD load) stimulates heterotrophic biofilms, which conditions the substrate for periphyton to more quickly colonize. Heterotrophic biofilms might also increase the rate of nutrient cycling within the periphyton community.

7.3 Potential management actions

- Removing phosphorus (P) from the effluent during periods of low flow is reducing DRP concentrations. It is important to continue treating for P during low flow periods because this is providing some control on periphyton growth. Furthermore, discharging more P could cause a change in the type of periphyton e.g. a relative increase in green filamentous algae.

- Further reducing the concentration of P in the discharge during periods of low flow (when treatment is currently occurring) is unlikely to provide significant benefit to the river because there appears to be another source of P entering during low flow periods.

- One possible management action is to start removing P from the effluent at flows higher than half median flow. This should be seriously considered if dissolved phosphorus is being released from river sediments during periods of low flow when the discharge is being treated for P. If this mechanism is not occurring to any great extent, then treating earlier would not stop the more rapid periphyton growth at the downstream sites, although it still might slow the initial phases of periphyton development at the site 800m downstream (but apparently not at the site 1400m downstream).

- Sometimes a period of low flow is interrupted with small rain events sufficient to raise the river above 40 m^3/s but not sufficient to remove periphyton cover. In these situations (where periphyton is already present) the extent of periphyton biomass at the downstream sites might be reduced by continuing P treatment until periphyton cover/biomass is reduced (e.g. to <30 mg/m^2 or PPI <10%) – this might be approximated using a flow statistic.

- This study has answered a number of questions regarding why there is more periphyton downstream of the WWTP discharge. There are factors controlling the rate of periphyton growth at the upstream site as well as factors stimulating periphyton growth at the downstream site that cannot be simply explained by phosphorus and nitrate nitrogen in the discharge. The results provide some clear pointers for further investigations that would help unravel the dynamics of periphyton growth in the Manawatu River near Palmerston North and refine the most appropriate management actions to reduce effects of the discharge. These investigations should include:

    i. Investigate the possibility of river sediments desorbing DRP to river water during periods when the WWTP is treating for P by testing the sorption capacity of river sediments. If river sediments are found to sorb and desorb dissolved P than a management response might be to start treating for P sooner. If not then it becomes more important to identify sources of dissolved P to groundwater.

    ii. Investigate whether heterotrophic biofilms stimulate periphyton growth and/or enhance P release at the site 800m downstream? If this is that case than reducing the amount of BOD in the discharge might reduce periphyton growth rates in the river prior to full mixing.

iii. Investigate whether micronutrients or total ammonia is controlling/stimulating periphyton growth in the Manawatu River. This could involve repeating a periphyton nutrient bioassay to include treatments with micronutrients (e.g. silica, cobalt, molybdenum, zinc, soluble sugars). Nitrogen should be in the form of ammonium.

iv. Investigate the sources of contaminants to groundwater seeping to the river during periods of low flow. Potential source of DRP might include ponds from the sewage treatment plant or possibly the landfill. Some work is already being done by Horizons RC that might further clarify the possible sources and magnitude of this contamination.

v. Investigate the extent to which macroinvertebrate grazers are controlling periphyton growth at the upstream and downstream sites. This could be done by undertaking a grazer exclusion experiment.

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Appendix 1: Photographs of samples sites

Appendix 2: Result of aquatic macroinvertebrate sampling in Manawatu River, 20th April 2012 and 27th April 2012

Manawatu River replicate		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
site	MCI	1000m u/s	1000m u/s	1000m u/s	1000m u/s	1000m u/s	800m u/s	800m u/s	800m u/s	800m u/s	800m u/s	800m d/s	800m d/s	800m d/s	800m d/s	800m d/s	1000m d/s	1000m d/s	1000m d/s	1000m d/s	1000m d/s	d/s Mangaone	d/s Mangaone	d/s Mangaone	d/s Mangaone	d/s Mangaone
PO28819. Protocols C3, P3, QC3.	TV	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr	20-Apr
Mayflies																										
Austroclima sp.	9	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deleatidium sp.	8	18	27	13	33	23	23	26	11	20	57	6	66	113	13	30	5	34	3	88	28	42	7	3	9	7
Stoneflies																										
Acroperla trivacuata	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Zelandobius furcillatus group	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Zelandoperla decorata	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Dobsonflies																										
Beetles																										
Elmidae	6	1	-	-	1	-	-	2	-	1	-	-	-	2	-	3	1	-	-	-	1	8	-	-	1	1
True Flies																										
Aphrophila neozelandica	5	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Austrosimulium spp.	3	-	-	-	1	-	-	1	-	-	-	-	-	1	-	1	-	-	-	-	-	2	-	-	-	2
Eriopterini	9	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Orthocladiinae	2	2	2	4	6	4	-	-	-	4	3	17	25	18	12	52	2	13	6	20	16	17	30	18	17	12
Tanytarsus spp.	3	2	-	-	-	2	-	1	-	-	6	4	12	9	6	11	-	3	2	9	3	24	11	7	8	1
Caddisflies																										
Aoteapsyche spp.	4	6	-	-	-	1	-	76	3	2	23	1	13	5	3	9	-	-	-	3	-	30	15	10	4	10
Costachorema xanthopterum	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Hydrobiosis copis	5	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	-
Hydrobiosis frater	5	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrobiosis spp.	5	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrobiosis umbripennis	5	-	-	1	-	-	-	-	-	-	-	1	-	-	-	2	-	-	-	-	-	-	-	-	-	1
Olinga spp.	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Psilochorema leptoharpax	8	-	1	1	-	-	-	1	1	-	1	-	-	-	1	-	-	-	2	-	1	-	-	-	-	1
Pycnocentria evecta	7	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Pycnocentrodes sp.	5	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Oligochaeta	**1**	-	-	-	6	2	-	-	-	-	1	-	5	5	4	-	-	-	-	3	-	2	2	-	-	-
Platyhelminthes	**3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Snails																										
Potamopyrgus antipodarum	4	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	1	-	2	3	-	-	-
Number of taxa		5	3	4	5	5	2	7	3	5	7	7	8	9	7	8	3	3	5	7	6	9	9	5	6	8
Number of individuals		29	30	19	47	32	24	109	15	28	92	31	124	155	40	109	8	50	14	125	50	128	71	39	41	35
MCI		92	120	115	80	72	130	106	133	116	89	90	90	84	89	100	107	87	100	71	107	80	87	108	93	98
QMCI		6.3	7.6	6.6	6.2	6.4	7.9	5.0	7.2	6.8	6.4	3.6	5.6	6.6	4.4	4.2	6.3	6.1	4.4	6.3	5.7	4.9	3.4	3.4	4.0	4.2
EPT abundance (excl Hydroptilidae)		29	30	19	47	32	24	109	15	28	92	31	124	155	40	109	8	50	14	125	50	128	71	39	41	35
%EPT richness (excl. Hydroptilidae)		40.0	66.7	75.0	20.0	40.0	100.0	57.1	100.0	60.0	57.1	57.1	37.5	33.3	57.1	50.0	33.3	33.3	40.0	28.6	50.0	22.2	55.6	60.0	50.0	50.0
%EPT abundance		82.8	93.3	78.9	70.2	75.0	100.0	96.3	100.0	82.1	89.1	29.0	64.5	76.8	45.0	38.5	62.5	68.0	35.7	72.8	60.0	56.3	35.2	35.9	36.6	54.3
%EPT abundance (excl. Hydroptilidae)		82.8	93.3	78.9	70.2	75.0	100.0	96.3	100.0	82.1	89.1	29.0	64.5	76.8	45.0	38.5	62.5	68.0	35.7	72.8	60.0	56.3	35.2	35.9	36.6	54.3

Manawatu River replicate		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
site	MCI	1000m u/s	1000m u/s	1000m u/s	1000m u/s	1000m u/s	800m u/s	800m u/s	800m u/s	800m u/s	800m u/s	800m d/s	800m d/s	800m d/s	800m d/s	800m d/s	1000m d/s	1000m d/s	1000m d/s	1000m d/s	1000m d/s	d/s Mangaone	d/s Mangaone	d/s Mangaone	d/s Mangaone	d/s Mangaone
PO29010. Protocols C3, P3, QC3.	TV	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr	27-Apr
Mayflies																										
Coloburiscus humeralis	9	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deleatidium sp.	8	57	35	17	64	30	30	95	50	45	52	18	1	6	16	1	3	-	1	-	51	10	4	6	16	10
Stoneflies																										
Zelandobius furcillatus group	5	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Beetles																										
Elmidae	6	-	1	1	1	-	-	1	-	2	3	2	1	-	4	1	1	-	-	1	1	1	-	-	-	-
True Flies																										
Aphrophila neozelandica	5	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Austrosimulium spp.	3	-	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eriopterini	9	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-
Orthocladiinae	2	7	23	5	12	3	-	10	9	7	13	56	37	45	34	15	17	3	21	9	60	25	6	17	21	31
Polypedilum spp.	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-
Tanytarsus spp.	3	-	4	-	3	2	3	3	-	2	2	23	10	17	16	2	3	1	8	2	7	3	4	1	6	7
Caddisflies																										
Aoteapsyche spp.	4	16	11	-	5	2	2	35	4	4	21	5	5	1	3	1	-	-	-	1	10	10	1	4	16	2
Costachorema spp.	7	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-
Hydrobiosis copis	5	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Hydrobiosis frater	5	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrobiosis spp.	5	-	-	-	-	-	-	1	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	2	-	-
Hydrobiosis umbripennis	5	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2	-	-	-	-
Oxyethira albiceps	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Psilochorema leptoharpax	8	-	-	-	-	1	-	1	-	1	-	-	-	-	1	1	-	-	-	-	1	-	-	-	-	-
Pycnocentrodes sp.	5	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oligochaeta	**1**	-	-	-	1	8	2	-	-	-	1	2	-	4	-	1	4	-	-	-	-	-	2	-	-	-
Snails																										
Physa sp.	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
Potamopyrgus antipodarum	4	-	1	-	4	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	4	-	-	-	1	-
Number of taxa		3	6	3	10	6	5	9	3	8	7	9	5	9	8	7	5	2	3	5	9	6	6	6	6	4
Number of individuals		80	75	23	94	46	38	148	63	63	93	109	54	77	76	22	28	4	30	14	137	51	18	31	61	50
MCI		93	90	107	90	87	80	107	93	95	83	85	92	77	118	91	80	50	87	72	89	93	70	103	93	85
QMCI		6.7	5.2	6.6	6.5	6.0	6.9	6.5	6.9	6.7	6.0	3.4	2.6	2.8	4.0	2.9	2.8	2.3	2.5	2.6	4.6	3.8	3.6	3.9	4.3	3.4
EPT abundance (excl Hydroptilidae)		80	75	23	94	46	38	148	63	62	93	109	54	77	76	22	28	4	30	14	136	51	18	31	61	50
%EPT richness (excl. Hydroptilidae)		66.7	33.3	33.3	40.0	50.0	40.0	55.6	66.7	50.0	42.9	44.4	40.0	55.6	50.0	42.9	20.0	0.0	33.3	20.0	33.3	50.0	33.3	50.0	50.0	50.0
%EPT abundance		91.3	61.3	73.9	75.5	71.7	84.2	89.9	85.7	82.5	79.6	22.9	11.1	13.0	27.6	13.6	10.7	0.0	3.3	7.1	46.0	43.1	27.8	38.7	54.1	24.0
%EPT abundance (excl. Hydroptilidae)		91.3	61.3	73.9	75.5	71.7	84.2	89.9	85.7	81.0	79.6	22.9	11.1	13.0	27.6	13.6	10.7	0.0	3.3	7.1	45.3	43.1	27.8	38.7	54.1	24.0

Appendix 3: Results of synoptic survey 19th April and 26th April 2012.

Appendix 4: Results of water quality monitoring in the Manawatu River upstream and downstream of the Totara Road wastewater treatment plant (Nov 2011 to May 2012). The estimates of periphyton cover are the mean of all replicate measurements.

Appendix 5: Results of nutrient diffusing substrate periphyton bioassay

Site and treatment	Chl a (mg/m^2)	Pheophytin (mg/m^2)	Chl a (mg/m^2)	Pheophytin (mg/m^2)	*Site and treatment*	Chl a (mg/m^2)	Pheophytin (mg/m^2)	Chl a (mg/m^2)	Pheophytin (mg/m^2)
Date Collected	27/04/2012		3/04/2012		*Date Collected*	27/04/2012		3/04/2012	
Site A - Nitrogen	96.9	13.1	0.6	0.0	Site C - Nitrogen	151.8	0.0	1.1	0.0
Site A - Nitrogen	126.6	5.3	0.6	0.0	Site C - Nitrogen	162.9	0.0	0.7	0.0
Site A - Nitrogen	94.9	18.7	0.5	0.0	Site C - Nitrogen	142.7	17.2	0.9	0.1
Site A - Nitrogen	126.2	20.2	0.4	0.1	Site C - Nitrogen	160.2	8.2	1.0	0.0
Site A - Nitrogen	138.7	13.5	0.6	0.0	Site C - Nitrogen	181.8	10.9	0.9	0.1
Site A - Phosphorus	196.9	0.0		0.0	Site C - Phosphorus	210.4	0.0	0.7	0.0
Site A - Phosphorus	112.4	0.0	0.9	0.0	Site C - Phosphorus	164.9	0.0	0.9	0.1
Site A - Phosphorus	130.3	0.0	0.7	0.1	Site C - Phosphorus	204.6	0.0	1.0	0.0
Site A - Phosphorus	113.8	0.0	0.8	0.0	Site C - Phosphorus	300.6	0.0	0.9	0.0
Site A - Phosphorus	97.6	0.0	0.9	0.0	Site C - Phosphorus	178.7	2.1	0.9	0.0
Site A - N + P	164.9	6.2	0.5	0.1	Site C - N + P	154.2	0.0	0.4	0.0
Site A - N + P	100.6	0.0	0.4	0.2	Site C - N + P	144.7	0.0	0.8	0.0
Site A - N + P	116.5	0.0	0.9	0.0	Site C - N + P	189.8	0.0		0.0
Site A - N + P	120.2	0.0	0.5	0.0	Site C - N + P	158.5	0.0	0.8	0.0
Site A - N + P	126.6	0.0	1.2	0.0	Site C - N + P	158.5	0.0	1.6	0.0
Site A - Control	180.7	6.1	1.0	0.2	Site C - Control	120.5	8.7	1.2	0.0
Site A - Control	99.6	1.9	1.2	0.0	Site C - Control	158.2	0.8	0.5	0.1
Site A - Control	127.6	0.0	0.7	0.1	Site C - Control	143.7	15.3	0.8	0.1
Site A - Control	149.4	0.0	1.0	0.1	Site C - Control	248.4	31.3	0.7	0.1
Site A - Control	84.5	10.8	1.1	0.0	Site C - Control	179.4	2.6	1.1	0.1
Site B - Nitrogen			0.5	0.0	Site D - Nitrogen	140.0	0.0	1.7	0.0
Site B - Nitrogen				0.0	Site D - Nitrogen	192.9	0.0	1.7	0.0
Site B - Nitrogen			0.8	0.0	Site D - Nitrogen	201.3	0.0	1.2	0.1
Site B - Nitrogen				0.0	Site D - Nitrogen	244.4	16.2	0.7	0.0
Site B - Nitrogen			0.6	0.1	Site D - Nitrogen	209.7	0.0	1.3	0.0
Site B - Phosphorus			0.6	0.0	Site D - Phosphorus	202.6	0.0	1.0	0.0
Site B - Phosphorus			0.6	0.0	Site D - Phosphorus	239.0	0.0	1.1	0.0
Site B - Phosphorus			0.6	0.0	Site D - Phosphorus	205.0	0.0	1.7	0.0
Site B - Phosphorus			1.1	0.0	Site D - Phosphorus	175.0	0.0	1.2	0.0
Site B - Phosphorus			1.6	0.0	Site D - Phosphorus	271.0	0.0		0.0
Site B - N + P			3.8	0.0	Site D - N + P	178.4	0.0	1.6	0.0
Site B - N + P			0.9	0.3	Site D - N + P	170.3	0.0	1.6	0.0
Site B - N + P			1.2	0.0	Site D - N + P	206.0	0.0	1.6	0.0
Site B - N + P			1.9	0.0	Site D - N + P	176.4	0.0	1.3	0.0
Site B - N + P			0.7	0.0	Site D - N + P	223.2	0.3	1.9	0.0
Site B - Control			1.1	0.0	Site D - Control	360.1	0.0	1.1	0.0
Site B - Control			0.8	0.1	Site D - Control	237.0	0.0	1.0	0.0
Site B - Control				0.0	Site D - Control	262.2	10.7	1.0	0.0
Site B - Control			0.9	0.0	Site D - Control	295.9	8.8	0.9	0.0
Site B - Control			0.8	0.3	Site D - Control	206.3	22.9		0.0
Median for sites Upstream A and B					**Median for sites Downstream C and D**				
N	126.2	13.5	0.6	0.0	N	172.3	0.0	1.0	0.0
P	113.8	0.0	0.8	0.0	P	204.8	0.0	1.0	0.0
N+P	120.2	0.0	0.9	0.0	N+P	173.3	0.0	1.6	0.0
Control	127.6	1.9	1.0	0.0	Control	221.6	8.7	1.0	0.0

Note: missing values due to damaged filter papers

Tray B stollen during 27th April bioassay.

Based on a 65cm diameter exposed filter paper area = 0.003318 m^2.

Appendix 6: Impact of the treatment wetland on the Totara Road WWTP discharge