River Lake Ltd

Processes driving periphyton growth in the Manawatu River and implications for wastewater treatment

Prepared for:

Palmerston North City Council

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Executive summary

Palmerston North City Council (PNCC) has consent to discharge treated wastewater to the Manawatu River from the Totara Road wastewater treatment plant (WWTP). Consent compliance monitoring in 2011 found significantly more periphyton at sites downstream of the discharge and a decline in the quantitative macroinvertebrate community index (QMCI). This led to concerns that the discharge may be in breach of condition 3 in its consent to not cause "significant adverse effects on aquatic life". A joint monitoring programme was undertaken in 2011/12 to identify the magnitude and causes of the effects from the discharge; it identified substantially more periphyton growth downstream of the discharge and identified a number of issues to better understand how this could be addressed. This report discusses the results of further investigations undertaken in 2012/13 to better understand river processes causing periphyton growth and the implications for discharges to the river.

Monitoring and investigations were undertaken in the river from November 2012 to April 2013. This included:

- Regular sampling of water quality and periphyton biomass in the Manawatu River;
- Using concrete substrates to measure the rate of periphyton growth at different times during the summer.
- Installing periphyton nutrient bioassay to identify which nutrient was limiting periphyton growth;
- Experiments to determine the potential for river sediments deposited on and trapped within periphyton mats to supply dissolved phosphorus to support periphyton growth;
- Experiments to assess potential changes in the dissolved phosphorus fraction of the effluent due to mixing with high pH river water and due to storage.

During periods of low flow (less than half median river flow) the wastewater treatment plant uses an alum treatment to reduce the amount of dissolved phosphorus (P) in the discharge to a very low concentration which helps control periphyton growth. However despite the treatment, periphyton grows prolifically downstream of the discharge. The investigations identified several reasons explaining rapid periphyton growth downstream of the discharge, these were:

- A portion of the particulate P in the discharge is released as dissolved phosphorus after mixing with the river under certain low flow conditions (i.e. low river flows, large pH fluctuations).
- Some of the particulate P associated with suspended solids in the river is trapped within periphyton mats and releases P directly to growing cells. An increase in pH was able to release some dissolved P at both the upstream and downstream sites.
- Some of the particulate P associated with suspended solids from the discharge is trapped within periphyton mats and releases P directly to growing cells. Increases in pH were more effective at releasing dissolved P from trapped sediments (from the river + discharge) at the downstream site compared to the sediment trapped (from the river only) by periphyton at the upstream site.

These processes for releasing dissolved P were controlled by pH within the river water and periphyton mats. Daily increases in pH are caused by the periphyton itself, and thus the effect of these processes was most apparent during periods of low river flow and when periphyton biomass was high. The potential supply of P increases as periphyton grows because more inorganic sediment is trapped in periphyton mats as periphyton biomass increases.

Under conditions of very low river flow (e.g. < 20-30 m^3/s) background concentrations of dissolved inorganic nitrogen (SIN) in the Manawatu River were very low and nitrogen became the key nutrient limiting periphyton growth in the river upstream of the discharge. Furthermore, as the flow continued to drop below 20 m^3/s, the dissolved P in the river increased – further reducing the ability for P to limit periphyton growth. This may reflect the combined effects of P released from river sediment trapped within the periphyton mat and a mature periphyton community with less net growth, more senescence and less net demand for nutrients.

These findings have significant implications for how to best treat the wastewater that is discharged into the river. Effluent treatment approaches that could be taken to help limit excessive periphyton growth include:

- Reducing the dissolved phosphorus concentration (as is currently done at <37 m^3/s);
- Reducing particulate phosphorus (P) concentration in the discharge in conjunction with the dissolved P treatment; and
- Reducing soluble inorganic nitrogen (SIN) concentration when river flow is very low (i.e. < 20-30 m^3/s).

Rivers are complex and it is possible that periphyton will still grow more quickly downstream of the WWTP discharge compared to upstream even if these actions are taken. However the rate of growth and the period of time guidelines values are exceeded would be expected to reduce.

We found the complex dynamics occurring in the Manawatu River which emphasises the need for site specific information when establishing resource consent conditions. In particular:

- Estimates of periphyton biomass downstream of the WWTP discharge differed depending on the method used. Chlorophyll *a* often over-estimate periphyton biomass at the downstream sites and AFDM over-estimated periphyton biomass at the upstream site. For sites downstream of the discharge, AFDM is a better measure for assessing periphyton cover against guideline values because the AFDM guideline value of 35 mg /m^2 corresponds to a decline in mayfly abundance in the river (as reported in Hamill 2012). Percent cover (e.g. weighted composite cover) provides complementary information that helps confirm biomass measures.
- There was evidence that some characteristics of the sewage stimulated periphyton growth in addition to the N and P. However the effect was small compared to the combined effect of N and P stimulating periphyton growth and of little practical consequence.
- Grazing by macroinvertebrates played an important role in controlling periphyton biomass at the upstream site.

- Measurement of dissolved reactive phosphorus differed depending on how long samples were stored prior to analysis. Filtering the samples in the field would ensure more accurate and consistent results. However it is acknowledged that field filtering is not always practical; where field filtering of effluent samples do not occur the samples should be transported and filtered in the laboratory as soon as possible after sampling.

1 Introduction

1.1 Background

In 2008 the Totara Road Wastewater Treatment Plant (WWTP), Palmerston North, 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 growth (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 in phosphorus concentrations in the discharge 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) 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 results of the 2012 joint monitoring programme are reported in Hamill (2012).

This report builds on that of the joint monitoring programme (Hamill 2012) and discusses the results of further investigations undertaken in 2012/13 to better understand river processes causing periphyton growth. The results are intended to inform decision makers on how to reduce the current effects of the discharge on the Manawatu River.

1.2 Major findings from joint monitoring programme

The joint monitoring programme (Hamill 2012) identified (among other things) that:

- The WWTP discharge stimulated periphyton growth and at high periphyton biomass there was a change in the composition of the aquatic macroinvertebrate community characterised by a decline in the abundance of mayfly. It was estimated that such a change in invertebrate community composition (indicating a decline in habitat quality) would occur on average 1.2

times per summer downstream of the discharge (corresponding to a duration of about 43 days per summer).

- Both nitrogen (N) and phosphorus (P) were identified as potentially limiting periphyton growth in the Manawatu River at different times; nutrient limitation experiments during April 2012 were inconclusive although P was found to exert some limitation.

- The dissolved reactive phosphorus (DRP) from the discharge was not sufficient to explain either DRP measured in the river or the rate of periphyton growth.

A number of questions were raised by the joint monitoring programme to further improve our understanding of the processes occurring in the Manawatu River and what aspects of the WWTP discharge should be improved. Additional monitoring and investigations were undertaken during the summer of 2012/13 in order to address these questions.

1.3 Overview of 2013 investigations

The following monitoring and investigations were undertaken in 2013:

- A trial of extending the flows at which the effluent was alum dosed of to reduced DRP.

- Manawatu river water quality and periphyton monitoring to provide background data and assess impact of trailing alum dosing for a longer period of time;

- Calculation of periphyton growth rates progressively during the summer to assess the benefits of increasing the period of time for which P is removed from the effluent;

- Periphyton bioassay to test limitation due to nitrogen, phosphorus and other factors in the discharge;

- Investigating the supply of dissolved phosphorus from river sediments;

- Measurement of changes in the dissolved phosphorus fraction of the effluent due to storage and mixing with river water.

The data collection and field surveys of the river were supported by Horizons Regional Council field staff and was integrated with other monitoring of the Manawatu River occurring during the summer. Monitoring of river water quality and periphyton was undertaken by Horizons RC and the data shared.

The summer of 2012/13 contrasted with that of 2011/12 and together they covered a range of river conditions. The summer of 2011/12 was wet with frequent floods, and many investigations were not possible until April; in contrast 2012/13 was very dry with long periods of periphyton accrual and record low flows.

Floods exert a major controlling influence over periphyton biomass and water quality (Biggs 2000) and results need to be interpreted in the context of river flow. All investigations were undertaken during a period of low river flows. In relation to the 2013 investigations, the most recent flood of magnitude over three times median flow was on 15 October 2012 and the largest flood during the monitoring

period was about two times median flow (145 m^3/s daily average) on 31 December 2012. A summary of when the different monitoring occurred with respect to flow during 2012/13 is shown in Figure 1.1

A Weight of Evidence (WOE) approach was used to draw conclusions and management implications from the various datasets and investigations. A weight of evidence approach combines the analysis of data (to determine patterns) and experimental hypothesis testing (to determine controlling mechanisms) to make management recommendations and predictions about their effectiveness.

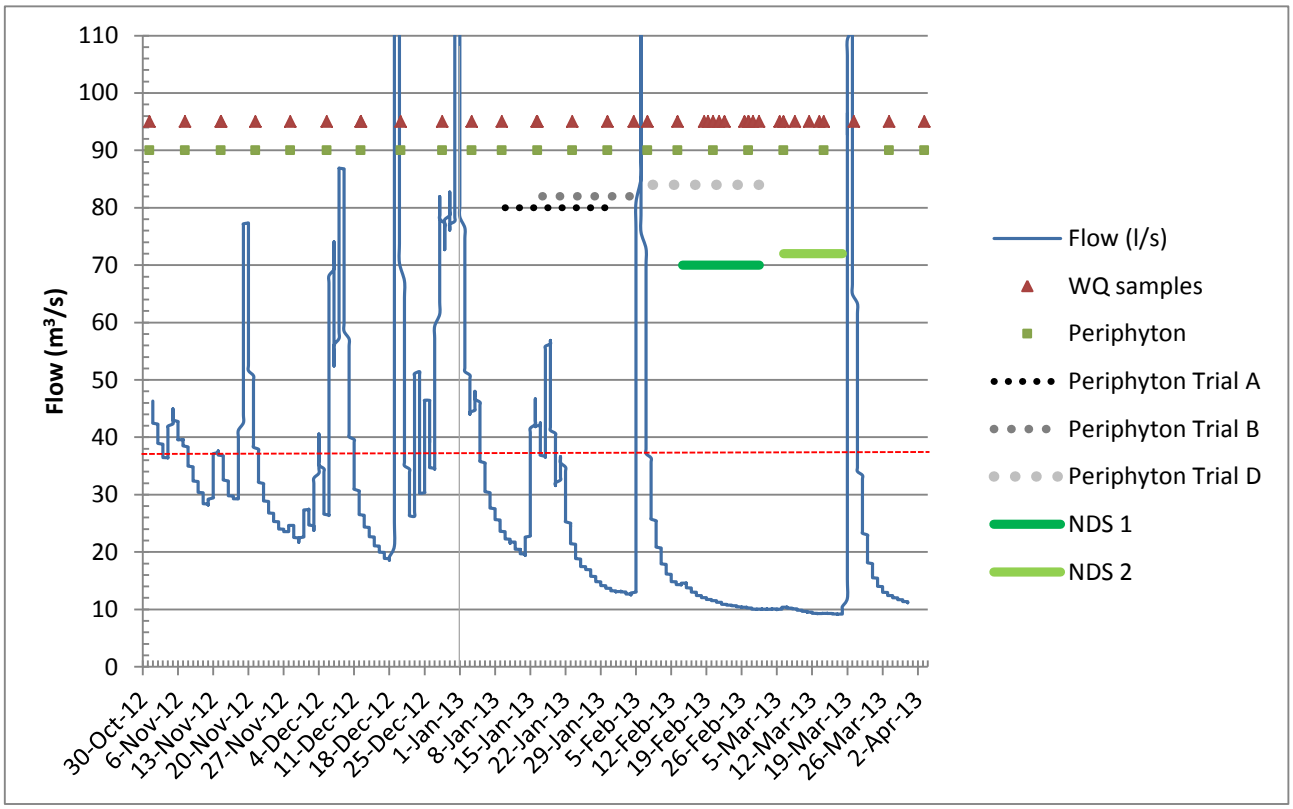

Figure 1.1: Timing of sampling in relation to flow in the Manawatu River (at Teachers College). The dashed red line indicates flows of 37 m^3/s (half median flow) below which the WWTP must be treating for phosphorus. Data are 24-hour averages of hourly measurements.

1.4 Statistical analysis

The statistical significance of results was usually determined using an equivalence test in the software 'TimeTrends'. Equivalence tests incorporate both testing of means (using a student t-test) and testing of a meaningful change (i.e. interval testing of 'equivalence' and 'inequivalence'). Equivalence tests are less sensitive to sample size than relying solely on parametric statistics. Increasing the sampling effort does not affect the likelihood that an equivalence hypothesis will be rejected, unlike parametric tests comparing mean values, where more data makes it more likely that the null hypothesis will be rejected.

Unless otherwise stated the equivalence tests were based on an interval of +/- 10% and a difference was only considered statistically significant if the *p*-value was < 0.05. The potential conclusions resulting from equivalence test analysis are shown in Table 1.1.

Table 1.1: Most common conclusions resulting from equivalent test analysis using TimeTrends software.

Conclusion of equivalence tests	**Null H0: no difference**	**Equivalence He: within limits**	**Inequivalence Hi: outside limits**
Strong evidence of a practically important difference	Reject	Reject	Accept
Moderate evidence of a practically important difference	Reject	Accept	Accept
No practically important difference (difference may be trivial compared to the limits)	Reject	Accept	Reject
Equivalent / No practically important difference	Fail to reject	Accept	Reject
Equivalent / Inconclusive	Fail to reject	Accept	Accept

In graphs where 95 percentile error bars are shown, these were calculated as two times the standard error (i.e. SE = SD / $\sqrt{n}$, where SE = standard error, SD = standard deviation and n = the number of samples).

2 Manawatu River water quality and periphyton monitoring 2012/13

2.1 Introduction

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.

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 increased after floods, and how much periphyton growth occurred before and after the discharge was treated for P.

2.2 Method

2.2.1 Water quality

Water quality samples were collected weekly by Horizons RC staff from 16 November 2012 to 10 April 2013. Additional samples were collected during February, increasing the sampling frequency to every 1-4 days. The sites sampled (Figure 2.1) were:

- Manawatu River about 1100m upstream of the discharge point on true right opposite Turitea Stream confluence and downstream of the riffle;
- Manawatu River 800m downstream of the discharge point (on true right)
- PNCC WWTP discharge after the wetland.

The unfiltered water samples were stored in a cool chilli-bin and sent to Eurofins ELS Laboratories to test for variables including: total nitrogen (TN), nitrate-nitrite nitrogen (NNN), total ammoniacal nitrogen (NH_4-N), total phosphorus (TP), total dissolved phosphorus (TDP), dissolved reactive phosphorus (DRP), turbidity, total suspended solids (TSS), *E. coli* bacteria. Acid soluble aluminium, dissolved boron, copper, iron, nickel, and zinc were also measured but the results are not reported in this report.

Laboratory methods and detection limits used for weekly water quality sampling are shown in Table 2.1. Data collected since October 2012 were reported and analysed as raw results. Where data was used prior to this period, any results that were less than the detection limit was given the value of half the detection limit prior to analysis.

In-stream field measurements were made for the following parameters in the Manawatu River: electrical conductivity (EC), dissolved oxygen (DO), pH, and temperature.

Table 2.1: Laboratory method and detection limits for key variables in weekly sampling

pH	Dedicated pH meter following APHA 21st Edition Method 4500 H.	<0.1
Turbidity	Turbidity Meter following APHA 21st Edition Method 2130 B.	<0.01 NTU
Infra Red Turbidity	Infrared Turbidity Meter following ISO7027:1999	<0.01 FNU
Inorganic Nitrogen	By Calculation - NNN plus Ammonia	<0.01 g/m³
Nitrate	Ion Chromatography following USEPA 300.0 (modified)	<0.005 g/m³
Nitrite-Nitrogen	Ion Chromatography following USEPA 300.0 (modified)	<0.005 g/m³
Ammonia Nitrogen	Flow Injection Autoanalyser following APHA 21st Edition Method 4500 NH3 H.	<0.01 g/m³
Total Dissolved Phosphorus	APHA 21st Edition Method 4500-P G. Persulphate digestion follows APHA 21st Edition 4500-P B.	<0.005 g/m³
Total Phosphorus	Flow Injection Autoanalyser following APHA 21st Edition Method 4500-P G. Persulphate digestion follows APHA 21	<0.005 g/m³
Dissolved Reactive Phosphorus	Flow Injection Autoanalyser following APHA 21st Edition Method 4500-P G.	<0.005 g/m³
Total Nitrogen	Flow Injection Autoanalyser following APHA 21st Edition Method 4500-NO3 I. Persulphate digestion follows APHA	<0.05 g/m³
E. coli	APHA 21st Edition,9223B:2005	<1 MPN/100mL

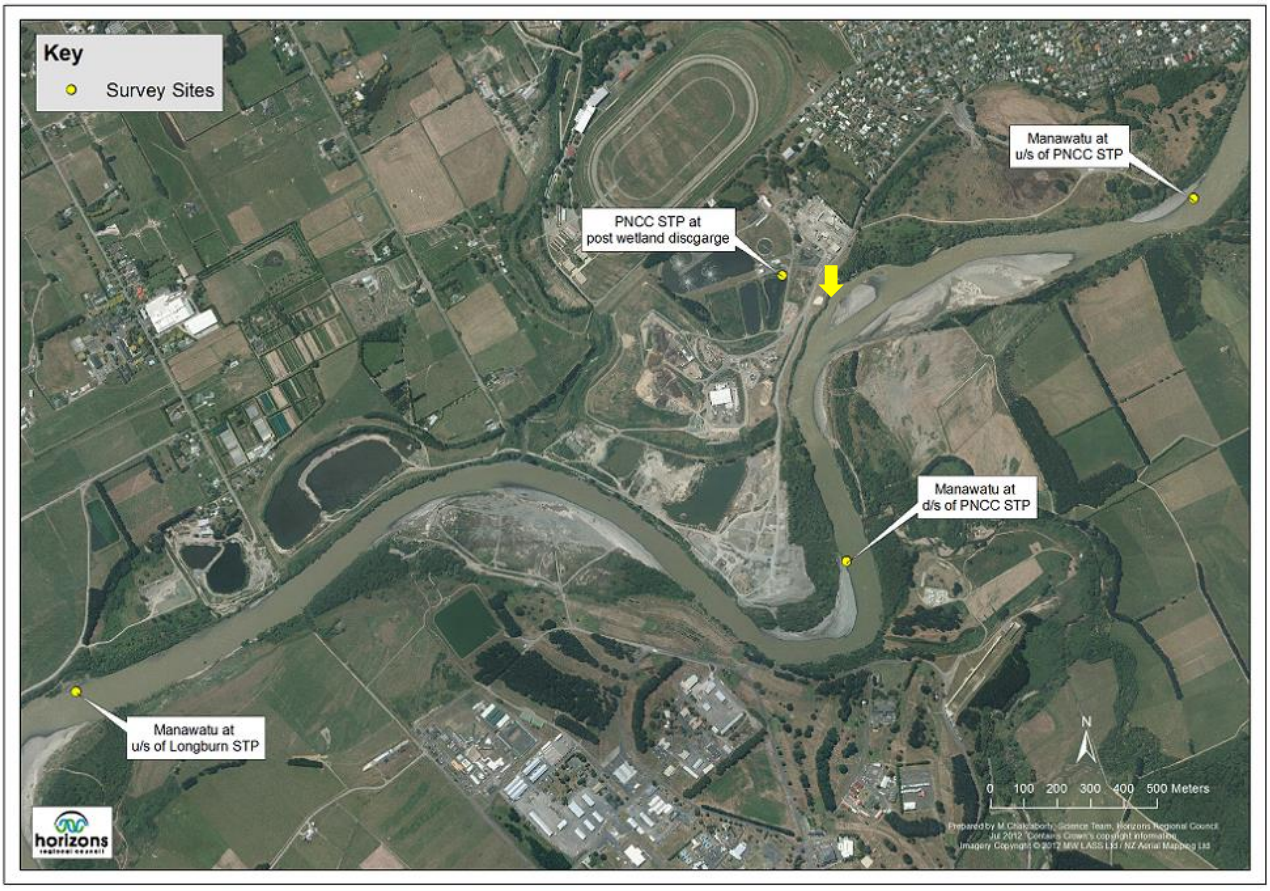

Figure 2.1: Location of water quality monitoring sites on the Manawatu River and the WWTP discharge. The location of the discharge is indicated with a yellow arrow

2.2.2 Periphyton

Horizons Regional Council staff carried out periphyton cover assessments and sample collection for biomass determination at the same time and at the same sites as river water quality sampling. All sites were at the upstream side of a gravel beach and had similar conditions in terms of lighting, clarity, water depth, water velocity. The upstream site had slightly smaller gravels that were less armoured.

The monitoring involved visual estimates of periphyton cover in runs and collecting a representative sample for analysis of chlorophyll *a* as follows:

1. Visual assessments of periphyton cover were made using an underwater viewer following the protocols outlined in Appendix 2 of "A periphyton monitoring plan for the Manawatu-Wanganui Region" (Kilroy et al. 2008). Five points were viewed across each of 8 transects encompassing run habitat and extending across the wadeable 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 about 5 metres apart.

2. The visual estimates reported percentage cover of the river bed in each view of the following categories of periphyton:

    - clean river bed (no algae);
    - film (typically diatoms) less than 0.3 cm thick;
    - loose 'sludge' (usually brown);
    - cohesive mats more (usually brown, don't fall apart when handled) ;
    - slimy filamentous algae (usually bright green but can be brown or dark coloured);
    - coarse filamentous algae (usually green or brown);
    - cyanobacteria mats (a subset of cohesive mats);
    - bacterial and/or fungal growths (sewage fungus) visible to the naked eye.

3. Quantitative periphyton samples were collected 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*. Analysis of periphyton samples followed the Biggs & Kilroy (2000) guidelines for chlorophyll *a* analysis.

4. Substrate type was assessed visually over the reach when periphyton biomass samples were collected.

Periphyton visual assessments were used to determine weighted composite cover (Peri WCC) was calculated as per Matheson et al. (2012) using the formula: Peri WCC = % cover filamentous periphyton + (% cover mats/2). Note that this calculation did not include the loose sludge.

2.3 Results and discussion

2.3.1 River water quality monitoring

The weekly sampling results from river water quality and periphyton monitoring are presented in Appendix 1. For the purpose of statistical analysis this data was filtered to isolate low flow periods (e.g. half median flow).

During the summer low flow pH, turbidity, electrical conductivity and *E. coli* bacteria did not differ between sites upstream and downstream of the WWTP discharge (see Table 2.1 and Table 2.2). In contrast, the concentration of nutrients (N and P) in the river were significantly higher downstream of the WWTP discharge (see Table 2.1 and 2.2).

The low flow concentration of total phosphorus was about 80% higher downstream of the discharge (Table 2.2 and Figure 2.2) and this differential was reasonably consistent throughout the summer (see Figure 2.4). The concentration of DRP was about 60% higher downstream (Table 2.2 and Figure 2.2) and on average was above the One Plan target to control periphyton growth both upstream and downstream of the discharge. The river DRP concentration varied considerably during the summer and dropped to very low concentrations during January, over which time the DRP measured at the downstream site was equal to or less than the upstream site (see Figure 2.5). The rise in upstream DRP as the summer progressed is consistent with the release of DRP from senescence of mature periphyton and perhaps some influence of periphyton deriving dissolved phosphorus from river sediments as discussed later in this report.

The discharge contributed a lot of nitrogen to the river, with total nitrogen (TN) about 380% higher downstream and soluble inorganic nitrogen (SIN) about 2 times higher downstream of the discharge (see Table 2.2 and Figure 2.3). On average during the summer SIN was below the One Plan target of 0.44 mg/L upstream of the discharge and above the target downstream (see Figure 2.6).

Total ammoniacal N was within ANZECC (2000) water quality guidelines for protection of aquatic life during the summer (i.e. <0.9 mg/L, see Table 2.1) applicable when sensitive species (e.g. freshwater mussel or freshwater clam (*Sphaerium* sp.) are minor components of the invertebrate community. The concentration of total ammoniacal N in the river downstream of the discharge increased during February and March as rivers levels dropped to record lows. The maximum recorded total ammoniacal N concentration during this period was 1.17 g/m^3. This is also less than the acute toxicity criterion set by the USEPA (2009) (i.e. 1.4 g/m^3 and 2.4 g/m^3 respectively when freshwater mussel are present and absent), assuming pH 8.5 and temperature of 22^{o}C which were the maximum pH and temperature recorded during routine monitoring. However, pH in the river late in the afternoon and close to the periphyton mat was typically higher than that recorded during routine weekly sampling (collected during the morning). Under these more extreme pH conditions the occasional spikes in total ammoniacal N during late February would have been close to the USEPA (2009) maximum criterion (e.g. 1.2 g/m^3 at pH 8.9 and 22^{o}C when freshwater mussel absent).

2.3.2 Phosphorus loads

A mass balance approach shows that the phosphorus load from the discharge explained the increase of TP measured downstream of the WWTP. This is demonstrated by comparing the TP load measured in

the Manawatu River downstream of the discharge with the sum of upstream load plus the load from the discharge on each sample occasion (i.e. the theoretical downstream load). The measured and calculated TP loads are similar (see Figure 2.11) which was consistent with results from 2012 (Hamill 2012). Measured TP was expected to be slightly higher than the calculated load since the sample site (800m downstream) is still within the mixing zone (see Rutherford et al. 1997, Hamill 2012)[1]. However, on most occasions it appeared to be slightly less than calculated values. The difference is greater for particulate P [2] and may indicate deposition or dissolving of some particulate P attached to alum floc (Figure 2.11) (also see discussion in Section 5).

Unlike the TP load, DRP in the WWTP discharge does not fully explain the increase of DRP measured downstream compared to upstream. Figure 2.11 shows that measured DRP downstream of the discharge was generally higher than could be explained by the discharge itself during early summer (Nov-mid December) and late summer (i.e. from early February), but less than could be explained by discharge during January. The lower values in January may be the result of periphyton uptake, while the high DRP values in February may reflect in river processes converting some of the alum bound particulate phosphorus into dissolved phosphorus, as discussed in Section 5 of this report. Modelling by MWH found the effect of pH on dissolving some of the particulate P fraction in the discharge was most apparent at low flows experienced in February and March (MWH 2013).

Table 2.1: Median values of key water quality variables in the Manawatu River and WWTP discharge when river flow was less than median flow (<73 m^3/s) (period 1 November 2012 to 10 April 2013)

Variable	pH	EC (uS/cm)	DRP HRC (g/m3)	TDP (g/m3)	Particulate P (g/m3)	TP (g/m3)	TN (g/m3)	SIN (g/m3)	NH4-N (g/m3)	NNN (g/m3)	Turbidity EPA (NTU)	E. coli (MPN/100mL)	Chla (mg/m2)
Mawatu u/s	7.9	222.0	0.012	0.013	0.0045	0.017	0.186	0.010	0.001	0.008	1.3	30	25
Mawatu d/s	7.9	234.3	0.018	0.019	0.011	0.032	0.832	0.630	0.473	0.092	1.3	30	212
PNCC STP discharage	7.3	818.5	0.06	0.096	0.883	0.99	37.037	36.849	36.7	0.103	5.4	25	

[1] However it is beyond the consented mixing zone which is 600m of total ammoniacal nitrogen and 400m for most other variables.
[2] Particulate P was calculated as total phosphorus - total dissolved phosphorus.

Table 2.2: Statistical comparison of variables in the Manawatu River upstream and downstream of the WWTP when river flow was less than half median flow (<37 m^3/s) (period 1 Nov 12 to 10 April 13). Refer to Table 1.1 for an explanation of the statistical method.

Manawatu River u/s vs d/s	equivalence analysis	t-test *p* -value	Bayesian posterior probability that difference is within limits	mean upstream	mean downstream	mean discharge
pH	No difference	0.14	100%	8.0	7.9	7.3
EC	Equivalent	0.15	77%	232.3	247.8	811.3
DRP (g/m^3)	Strong evidence	0.003	0.6%	0.0116	0.0182	0.0493
TDP (g/m^3)	Strong evidence	0.0003	0.1%	0.0136	0.0226	0.0975
Particulate P (g/m^3)	Strong evidence	0.0002	<0.1%	0.0040	0.0096	0.88
TP (g/m^3)	Strong evidence	<0.0001	<0.1%	0.017	0.031	0.974
TN (g/m^3)	Strong evidence	<0.0001	<0.1%	0.195	0.934	38.3
SIN (g/m^3)	Strong evidence	<0.0001	<0.1%	0.033	0.677	36.1
NH4-N (g/m^3)	Strong evidence	<0.0001	<0.1%	0.003	0.576	36
NNN (g/m^3)	Strong evidence	<0.0001	<0.1%	0.030	0.101	0.093
Turbidity (NTU)	Equivalent	0.7	43.0%	1.2	1.3	5.1
E. coli (MPN/100mL)	Equivalent	0.6	3.9%	146.2	277.3	190
Chorophyll *a* (mg/m^2)	Strong evidence	0.0001	<0.1	47	328	
Load DRP (kg/day)	Strong evidence	0.0009	0%	12.6	20.3	1.17
Load TP (kg/day)	Strong evidence	0.0001	<0.1	21.1	37.1	23.2

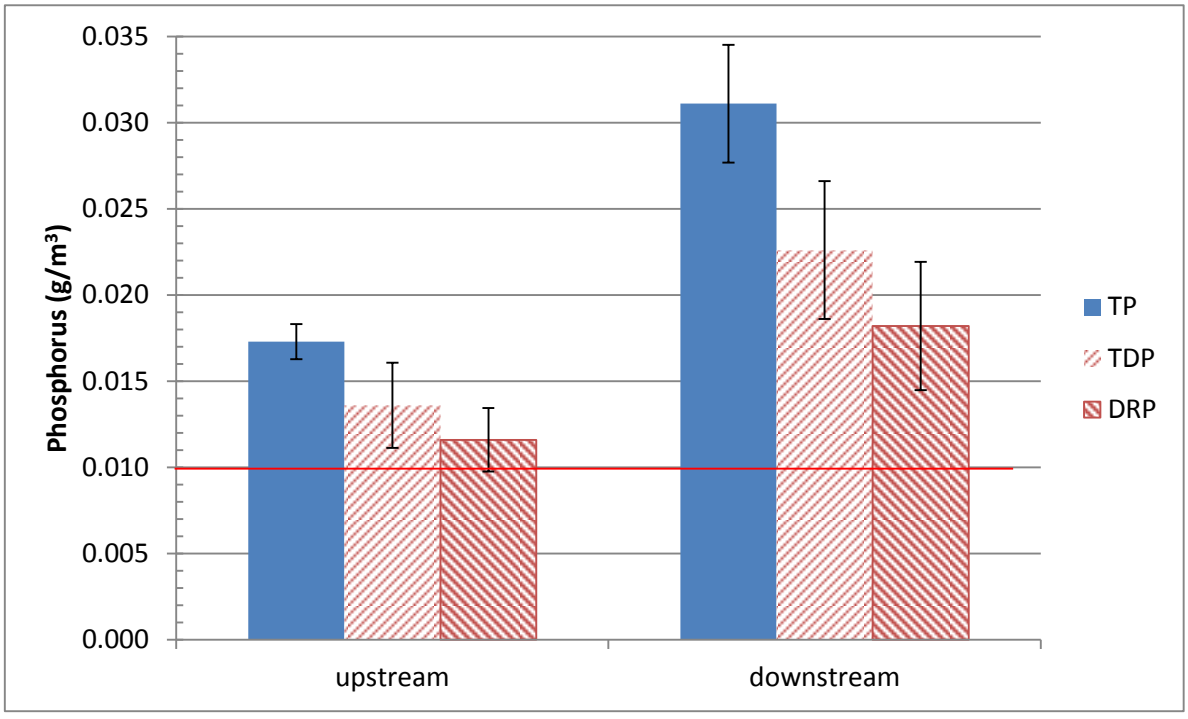

Figure 2.2: Average concentration of phosphorus fractions in the Manawatu River upstream and downstream of the WWTP discharge, when river flow was less than half median flow (<37 m^3/s) (period 1 November 2012 to 10 April 2013). Error bars are 95 percentiles. The red line = DRP One Plan target.

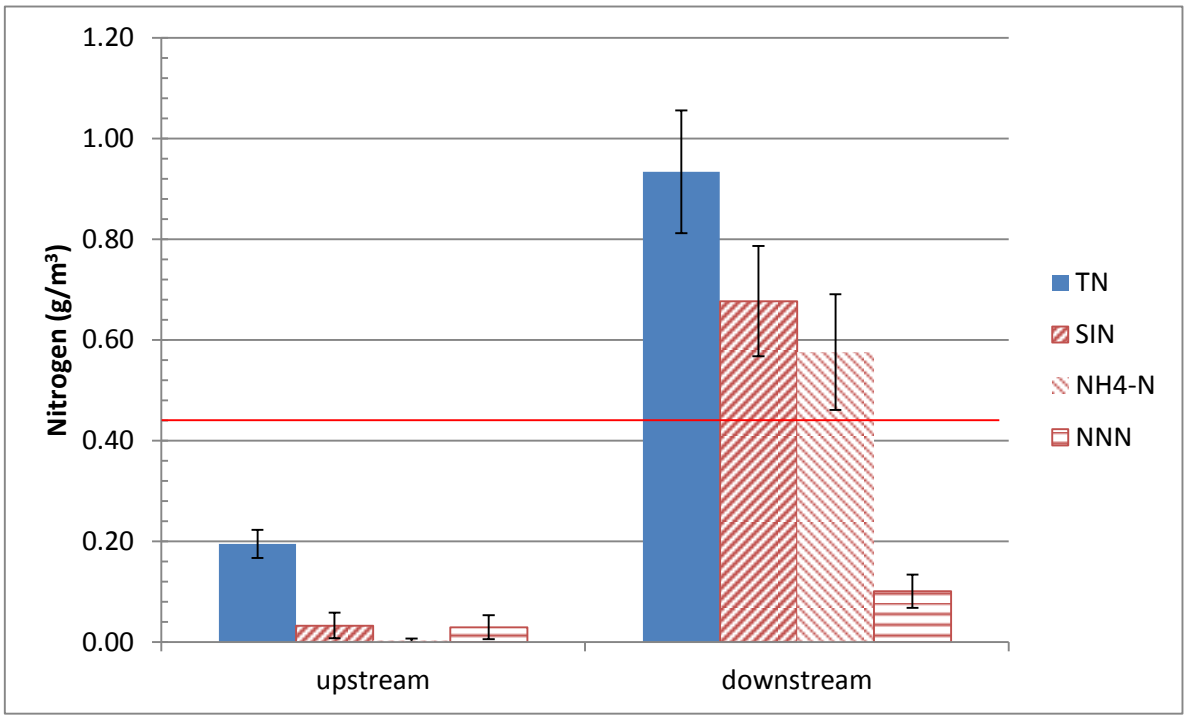

Figure 2.3: Average concentration of nitrogen fractions in the Manawatu River upstream and downstream of the WWTP discharge, when river flow was less than half median flow (<37 m^3/s) (period 1 November 2012 to 10 April 2013). Error bars are 95 percentiles. The red line = SIN One Plan target.

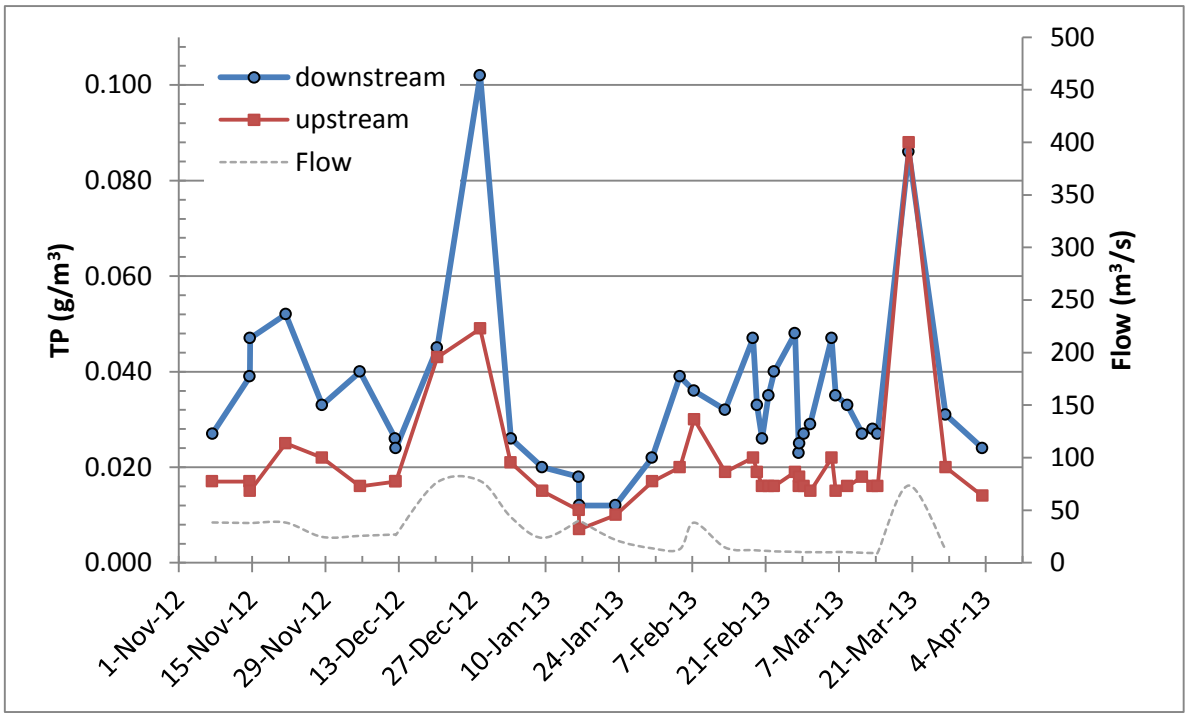

Figure 2.4: Total phosphorus concentration in the Manawatu River upstream and downstream of the WWTP discharge. Flow is daily average river flow on day of sampling. Note that spikes correspond with high river flow.

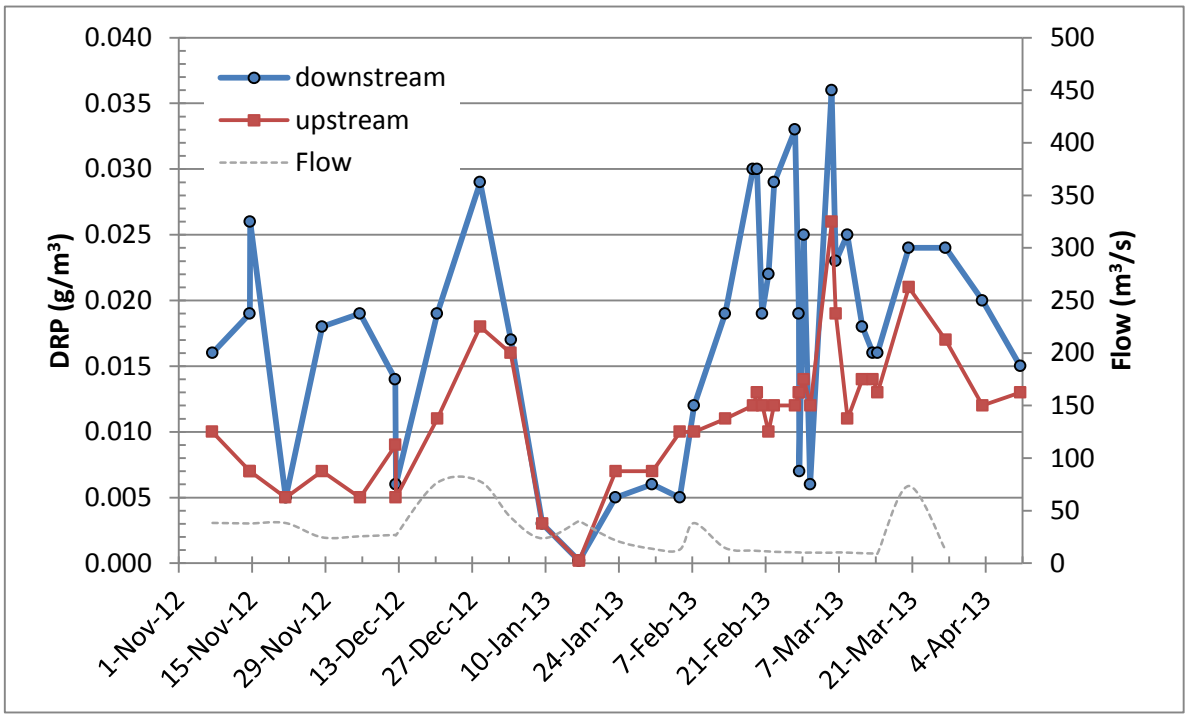

Figure 2.5: Dissolved reactive phosphorus concentration in the Manawatu River upstream and downstream of the WWTP discharge. Note that during January 2013 downstream DRP was equal to or less than upstream DRP.

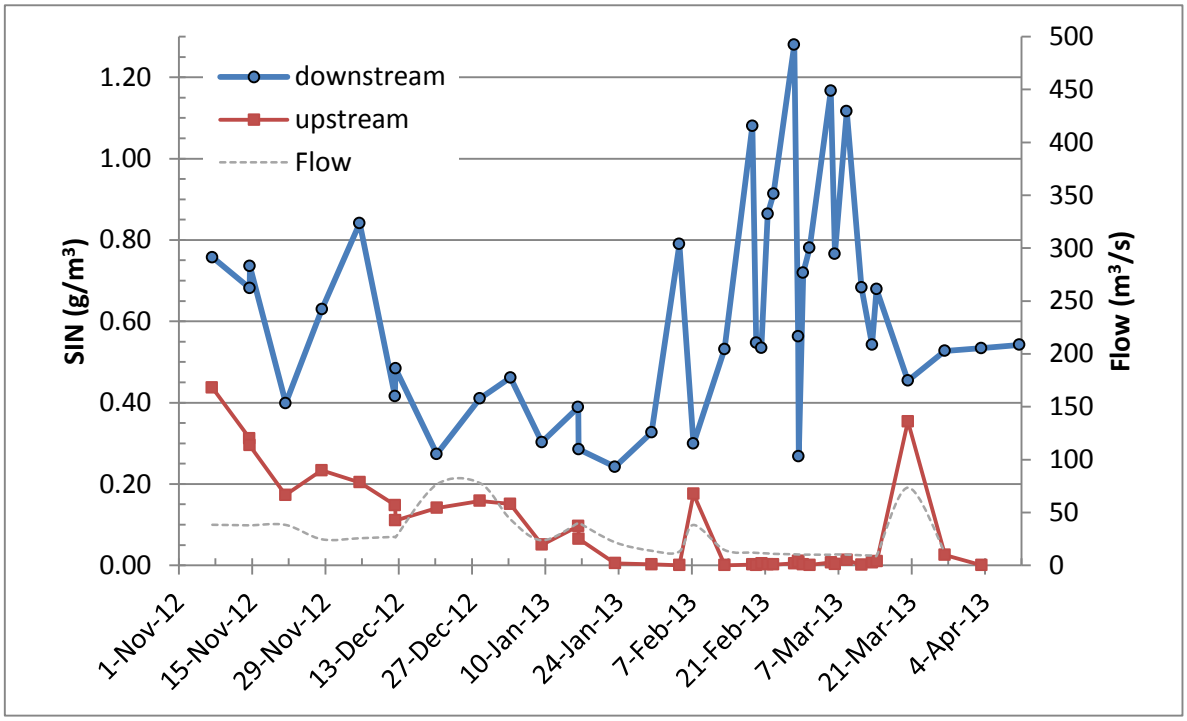

Figure 2.6: Soluble inorganic nitrogen concentration in the Manawatu River upstream and downstream of the WWTP discharge

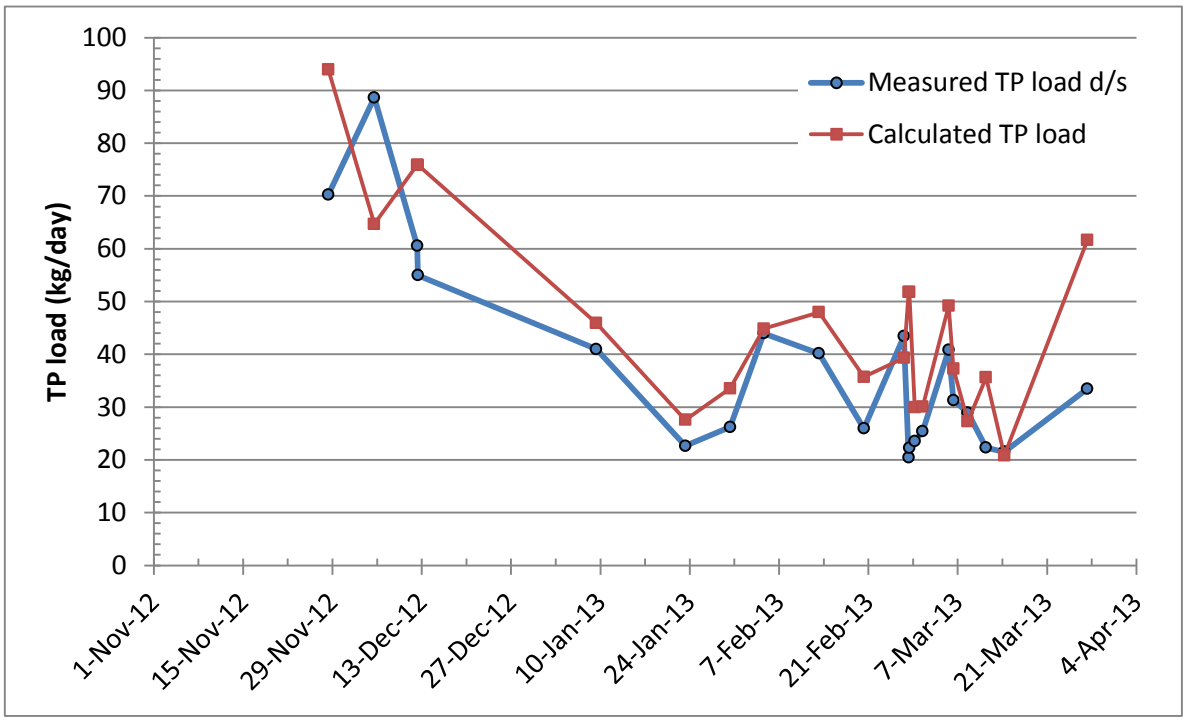

Figure 2.7: Total phosphorus load in the Manawatu River measured downstream of the discharge and a mass balance calculation of the loads upstream of the WWTP discharge + the discharge. Filtered for river flows < 37 m^3/s.

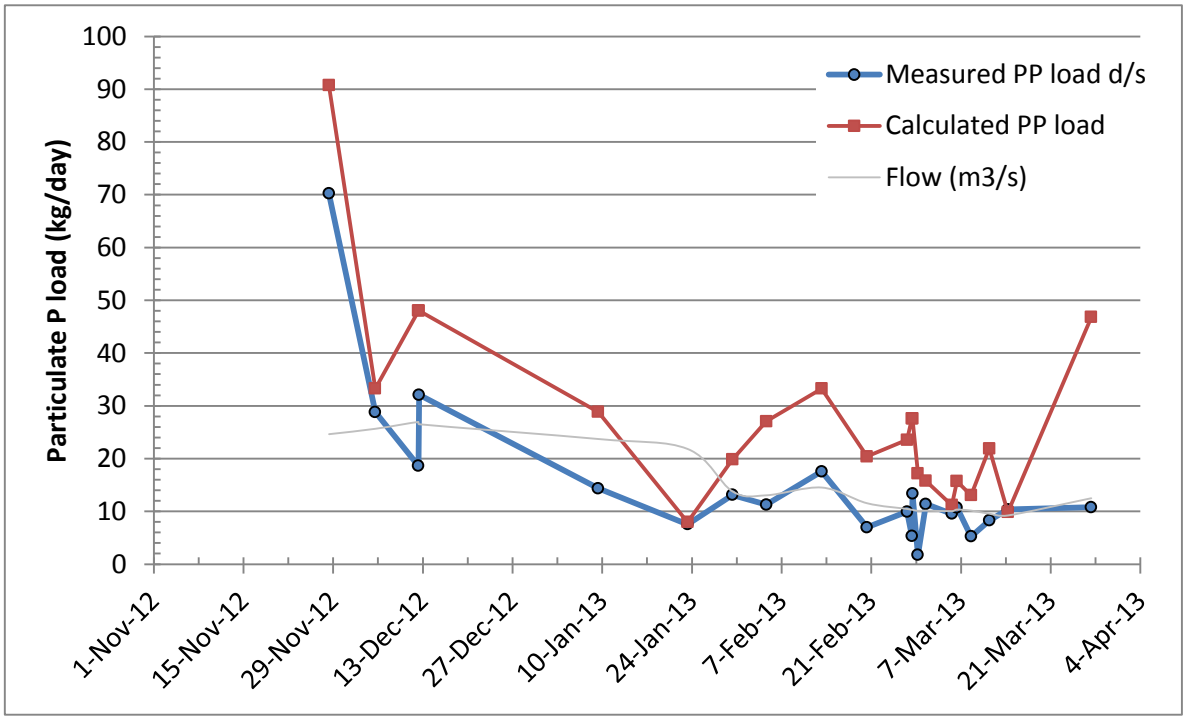

Figure 2.8: Particulate phosphorus load in the Manawatu River measured downstream of the discharge and a mass balance calculation of the loads upstream of the WWTP discharge + the discharge. Filtered for river flows < 37 m^3/s. Note that measured particulate P is consistently less than expected, suggesting that particles were settling out of the water.

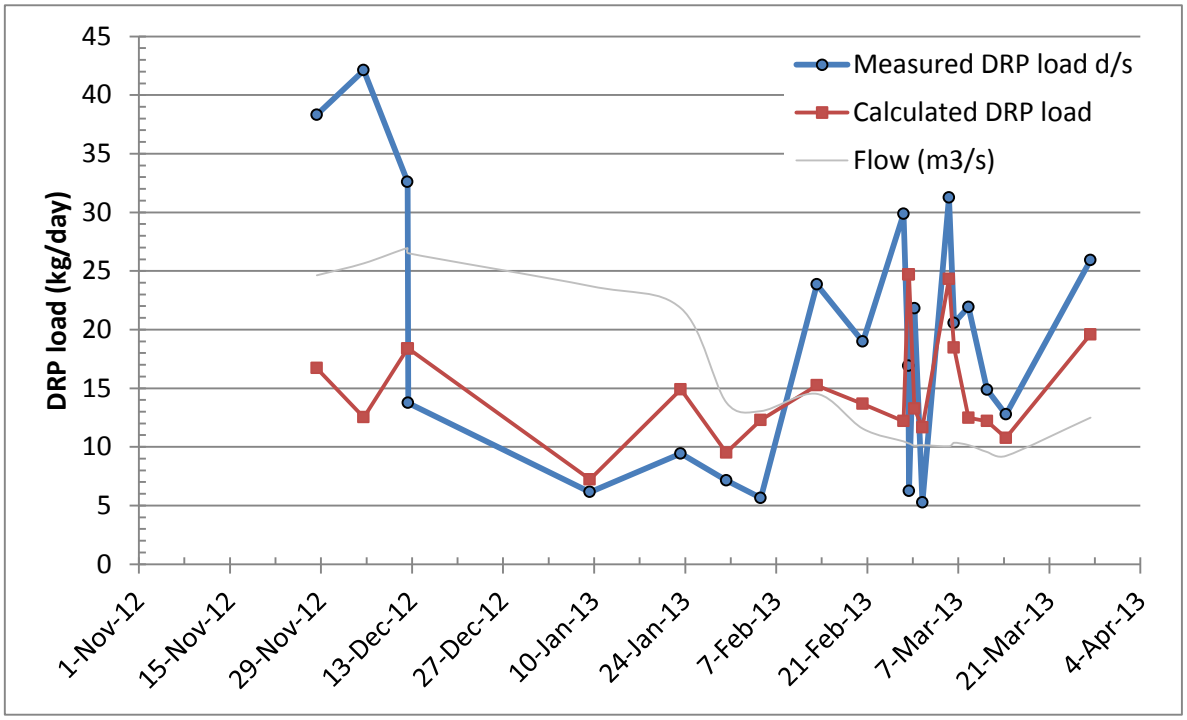

Figure 2.9: DRP load to the Manawatu River measured downstream of the discharge and a mass balance calculation of the loads upstream of the WWTP discharge + the discharge. Filtered for river flows < 37 m^3/s. Note that measured DRP was less than expected during January (corresponding to a period of rapid periphyton growth and nutrient demand) and more than expected in late summer - suggesting net release of dissolved P from a mature periphyton community.

2.3.3 Switch of potential nutrient limitation

In order to examine the potential for nutrient limitation of periphyton growth, the concentration of the dissolved nutrients SIN and DRP were expressed as equivalent periphyton biomass (chlorophyll *a*) using the equation in Biggs (2000) and assuming 21 days of accrual[3] (see Figure 2.10 and 2.11). This is not a prediction of the periphyton biomass (which will be affected by other factors such as flood events, grazing etc.), but allows us to express the river concentrations of SIN and DRP on a common scale relevant to periphyton growth.

The Manawatu River upstream of the WWTP showed a switch in the potential limiting nutrient from phosphorus to nitrogen as flow receded (Figure 2.11). Upstream of the WWTP, DRP was potentially limiting periphyton growth from 1 Nov to mid-Dec 2012 and again in January 2013. Background concentrations of DRP steadily increased above the median during February – March (Figure 2.5). SIN concentrations steadily declined over the summer and started exerting a strong control on periphyton growth from late December, interrupted only by small flood events which briefly elevated SIN.

[3] The accrual period is the period of time available for periphyton to grow between floods.

The gradual decline in SIN probably reflects a combination of reduced external loads (e.g. reduced soil drainage from the catchment) in addition to periphyton uptake and denitrification within river sediments (e.g. Partitt et al. 2007).

The changes in DRP probably reflect dynamics of internal river processes such as P uptake by growing periphyton, release by old periphyton cells and release from river sediments (see Section 5 for further discussion). McArthur et al. (2010) reported a similar situation of elevated DRP during late summer in the upper Manawatu; in this case DRP concentration during a low flow period in late March was higher than the annual median which was considered unexpected considering there are few mechanisms for P to reach waterways during dry conditions. This points towards an increase in internal P loads during these low flow conditions such as dissolved P release from sediments and senescence of algal cells.

Downstream of the WWTP the absolute concentration of DRP occasionally dropped to levels potentially limiting periphyton growth during December and throughout January (i.e. below the red line on Figure 2.11). SIN was continuously high at the downstream site and concentrations increased late in the summer due to dropping river flows (reducing dilution) and a constant SIN load from the WWTP.

Nutrient concentrations in the water reflect the residual of what is not being used by periphyton and it is apparent that even the low DRP concentrations during mid-January were not restricting periphyton growth (see Figure 2.11) – probably because periphyton was deriving P from sediment trapped in its mat (see Section 5).

In a review of Horizons RC periphyton monitoring data, Kilroy (2012) found that the percentage of periphyton biomass exceedance of One Plan targets was unrelated to mean DRP at the site, but there was a threshold response between exceedance and mean SIN concentration. The target guideline was rarely exceeded when mean SIN concentration was <0.1 g/m^3. This pattern suggested that periphyton biomass in the region may be more generally limited by SIN than by DRP.

The switch from potential P limitation to N limitation generally occurred when river flows were between 20 m^3/s to 30 m^3/s. This is illustrated in Figure 2.12 which shows the relationship between SIN:DRP ratio at the upstream site and river flow after filtering data for SIN<0.3 mg/L[4]. Provided absolute concentrations are sufficiently low a SIN:DRP ratio <7.2 indicates potential N limitation. When flows dropped below 30 m^3/s SIN concentrations reduced to <0.1 mg/L so as to allow either N or both N and P to be potential limiting. When flows were <20 m^3/s both the SIN concentration and the SIN:DRP ratio were low – indicating N as the primary limiting nutrient upstream of the discharge.

This switching from potential P limitation to N limitation at the upstream site as river flow receded has important implications for managing the WWTP discharge to avoid excessive periphyton growth. In the early stages of a flow recession P can control periphyton growth. As the flow continues to drop (e.g. to <30 m^3/s) N also becomes important for controlling periphyton growth, and as the flows drop further (e.g. to <20 m^3/s) N remains a controlling nutrient but P becomes less important due to increasing background concentrations (e.g. as flow fell below 15 m^3/s in February 2013).

[4] The data was filtered for low concentrations because a nutrient will only be limiting if the absolute concentrations are low. SIN is likely to become limiting in the range of 0.1 to 0.3 mg/L and DRP in the range 0.01 to 0.03 mg/L (corresponding to 120 to 200 mg/m^2 chlorophyll a after 21 days of accrual (Biggs 2000).

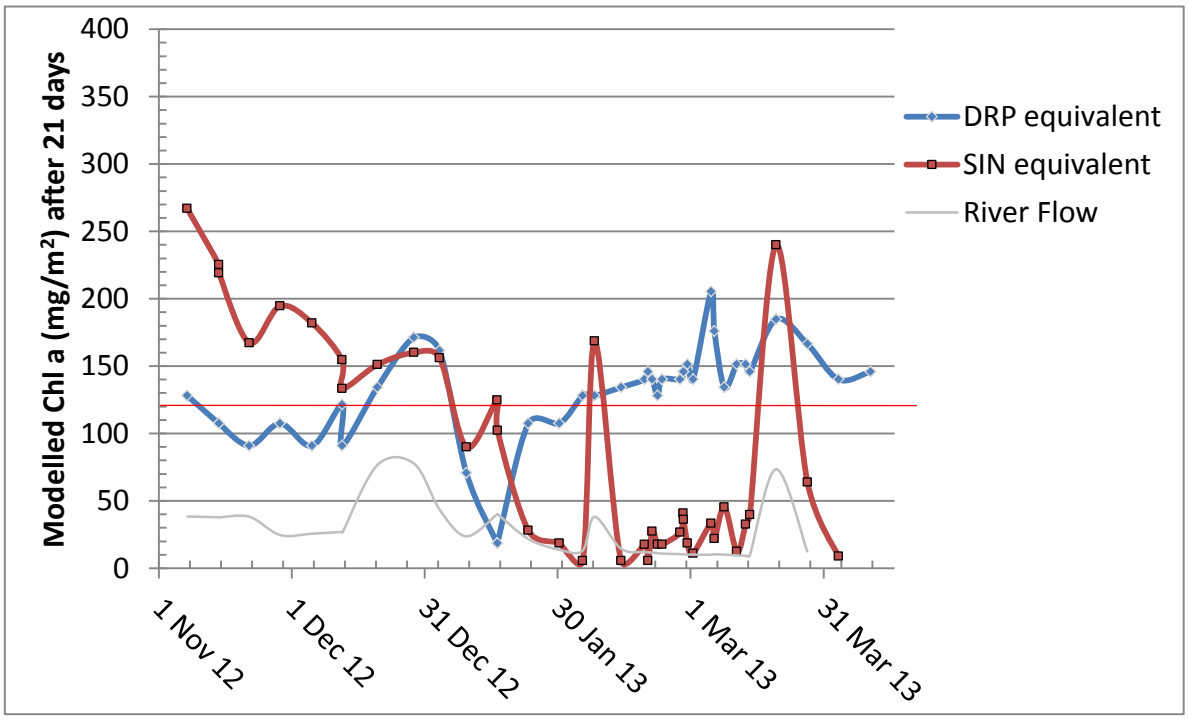

Figure 2.10: Modelled periphyton growth in response to dissolved reactive phosphorus (DRP) and soluble inorganic nitrogen (SIN) in the Manawatu River upstream of the WWTP discharge. The river is potentially N limited when the SIN line drops below the DRP line. The red line shows the One Plan target for periphyton chlorophyll *a* for this site.

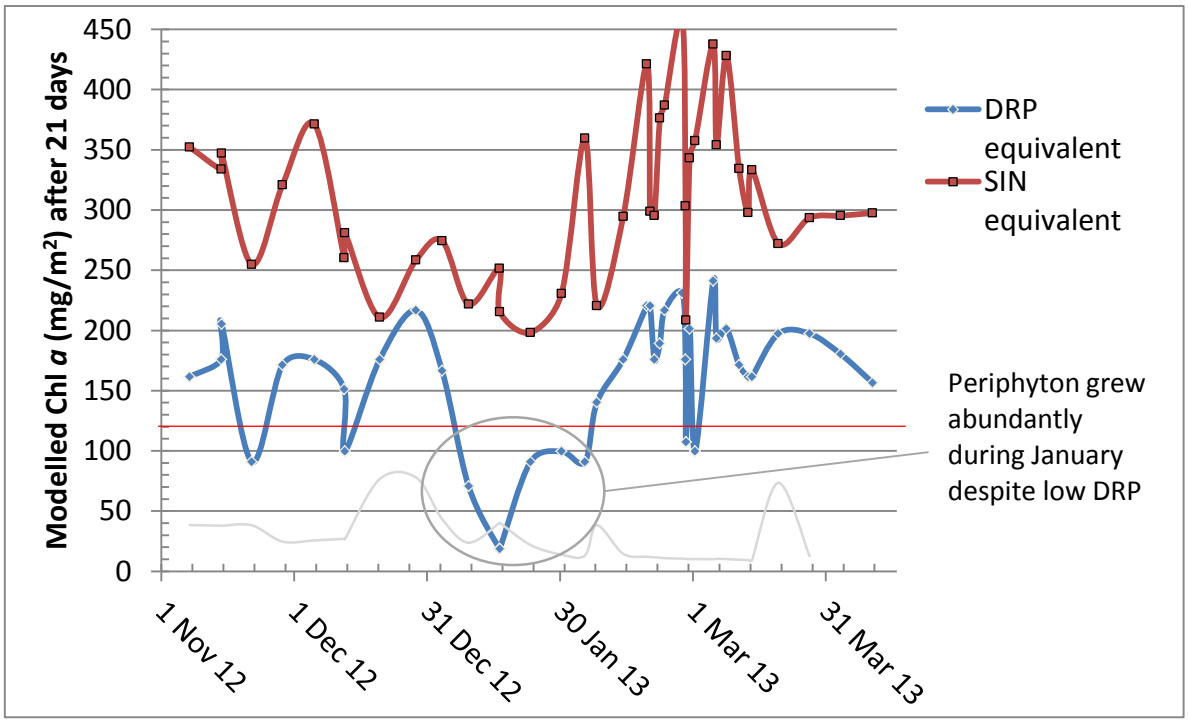

Figure 2.11: Modelled periphyton growth in response to DRP and SIN in the Manawatu River downstream of the WWTP discharge. The river is potentially P limited when the DRP line is below the SIN line. The red line shows the One Plan target for periphyton chlorophyll *a* for this site.

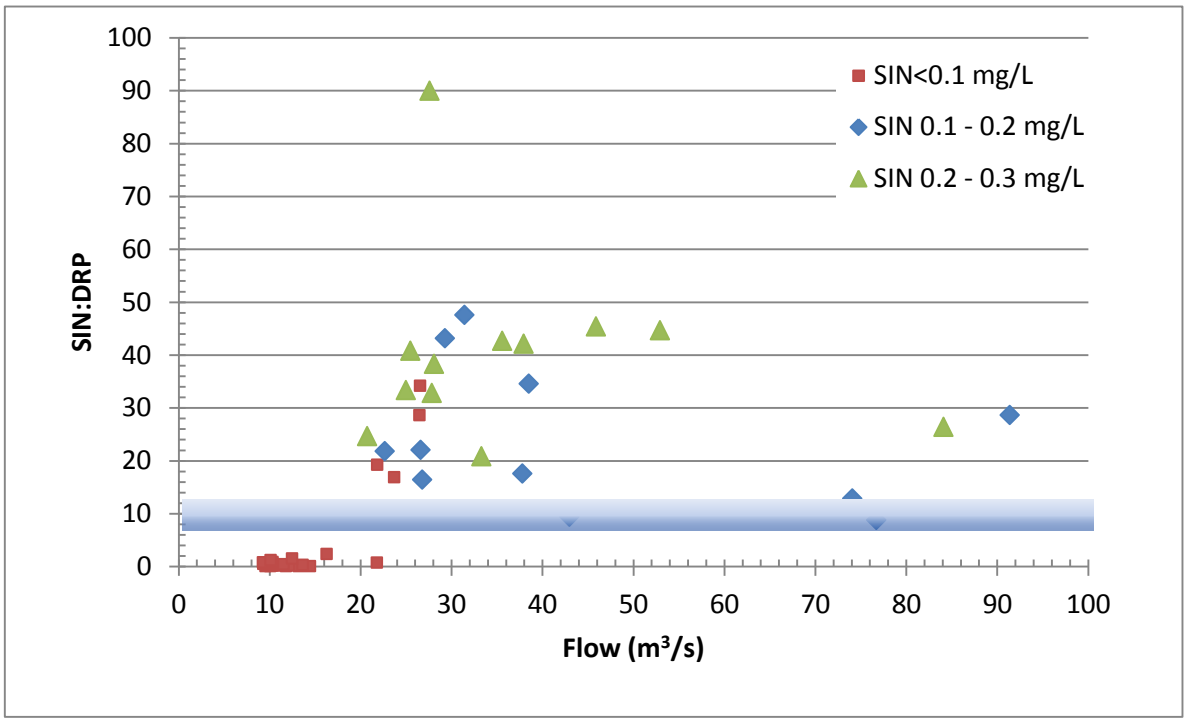

Figure 2.12: Ratio of SIN:DRP in Manawatu River upstream of the discharge filtered to only show records where SIN <0.3 mg/L (period March 2010 to April 2013). Values below the shaded blue line indicate potential nitrogen limitation (based on the Redfield ratio). The Redfield ratio is represented by a wide band because optimum N:P ratios can differ between species.

2.3.4 River periphyton monitoring

Weekly sampling of periphyton biomass in the Manawatu River found a substantially more biomass in the river downstream of the discharge compared to upstream (see Table 2.2 and Figure 2.13). Periphyton biomass at the upstream site initially increased in the one to two weeks after flood events after which it declined. Substantial grazing by macroinvertebrates was observed on substrates placed in the river. Therefore declines in biomass at the upstream site would be consistent with removal by macroinvertebrate grazing exceeding the periphyton growth rate.

The upstream site showed more response in terms of periphyton cover (expressed as Periphyton weighted composite cover (WCC))[5] compared to chlorophyll *a* (see Figure 2.14). This was particular evident during January. The decline in cover at the downstream site on 16 January may be due to a small fresh at the time causing sloughing.

There was spatial variability in periphyton cover at the upstream site. Periphyton cover was sparse in runs where regular sampling occurred, but the cover was relatively high in riffles (dominated by green filamentous alga *Cladophora* sp. and *Stigeoclonium* sp.). Periphyton (*Cladophora* sp.) from the riffle was used for sampling sediment trapped in periphyton mats (see Section 5).

[5] Peri WCC = % cover filamentous periphyton + (% cover mats/2) see Matheson et al. (2012).

Periphyton grew rapidly at the downstream site and attained high biomass in both runs and riffles, this was despite the DRP concentration in the river being very low during this period – suggesting that the periphyton had another source of phosphorus.

There was a positive correlation between periphyton biomass (measured as chlorophyll *a*) and periphyton weighted composite cover (WCC) (r^2 = 0.77 at the downstream site on log log transformed data) (see Figure 2.15).[6] If both periphyton cover and chl *a* biomass were used to assess compliance with the One Plan targets (based on Biggs 2000), then the biomass measure expressed as chlorophyll *a* would have exceed the target of 120 mg/m^2 more frequently than the equivalent 30% cover by filaments. Matheson et al. (2012) compared periphyton biomass and cover with QMCI and MCI scores and found that the boundary between 'good' and 'fair' ecological condition (i.e. MCI=100, QMCI=5) corresponded to chlorophyll *a* of about 200 mg/m^2 and Peri WCC of 40%. On this basis also the periphyton at the downstream site in the Manawatu River appears to have more relatively more chlorophyll *a* for a given cover and biomass measure using chlorophyll *a* may be over estimates (see also Section 3.3).

Kilroy et al. (2012) assessed the extent to which the One Plan target for periphyton biomass (i.e. 120 mg/m^2) corresponded to the One Plan target for periphyton cover (i.e. 30% and 60% cover by filaments and mats respectively). The analysis found that the cover thresholds were equivalent to about 53 mg/m^2 chlorophyll *a* provided the remaining cover had zero chlorophyll *a*. In practice there will be algae present in other sections of the stream and the biomass cover is likely to be more. Conversion factors derived for Canterbury indicated a chlorophyll *a* equivalent nearer 140 mg/m^2 for 30% filaments. The downstream site appears to have more chlorophyll *a* for a given percent cover than typical in other reaches. This may relate to the concentration of chlorophyll *a* in the cells (see Section 3.3.3).

During the summer of 2012/13 PNCC WWTP trialled increasing the period of time for which DRP is removed from the effluent, i.e. starting removal at about 60 m^3/s river flow. Because of the dry summer conditions this resulted in the discharge having low DRP in early December (interrupted by floods from 20 to 30 December) and almost continuously from January to March with the exception of flood events in 5 Feb and 19th March. These extended periods of alum dosing and low DRP in the discharge had no observable effect on reducing periphyton biomass in the river downstream. Periphyton biomass was higher in February and March compared to November and December (see Figure 2.13) despite the alum treatment having occurred for a longer period of time and very low DRP concentrations in the effluent (i.e. average DRP concentration in effluent during February March was 0.059 mg/L compared to about 3 mg/L when no alum dosing is occurring).

[6] A slightly better correlation was obtained when comparing cover with chlorophyll *a* + pheophytin, but pheophytin data was only available since November 2013.

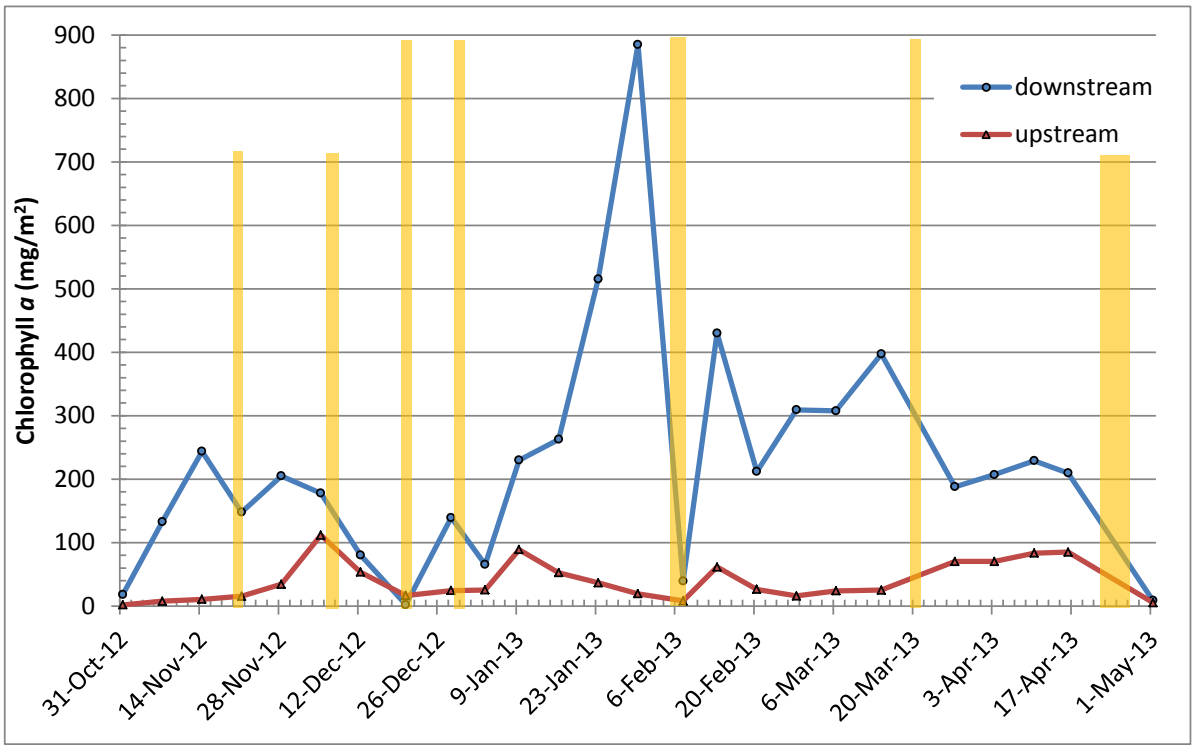

Figure 2.13: Periphyton biomass (measured by chl *a*) in the Manawatu River upstream and downstream of the WWTP. Periphyton biomass reduced after small floods as indicated by vertical lines on the graph (short line <100 m^3/s, long line >100 m^3/s).

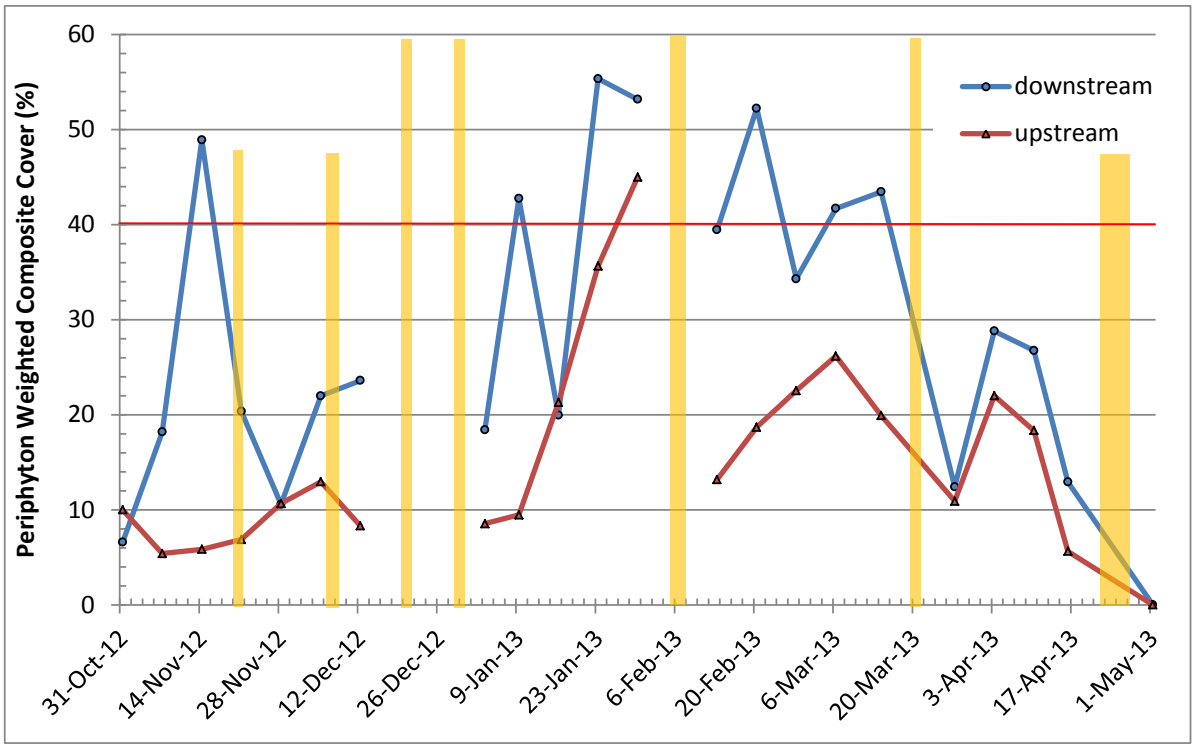

Figure 2.14: Periphyton weighted composite cover (WCC) in the Manawatu River upstream and downstream of the WWTP. Periphyton biomass reduced after small floods as indicated by vertical lines on the graph (short line <100 m^3/s, long line >100 m^3/s).

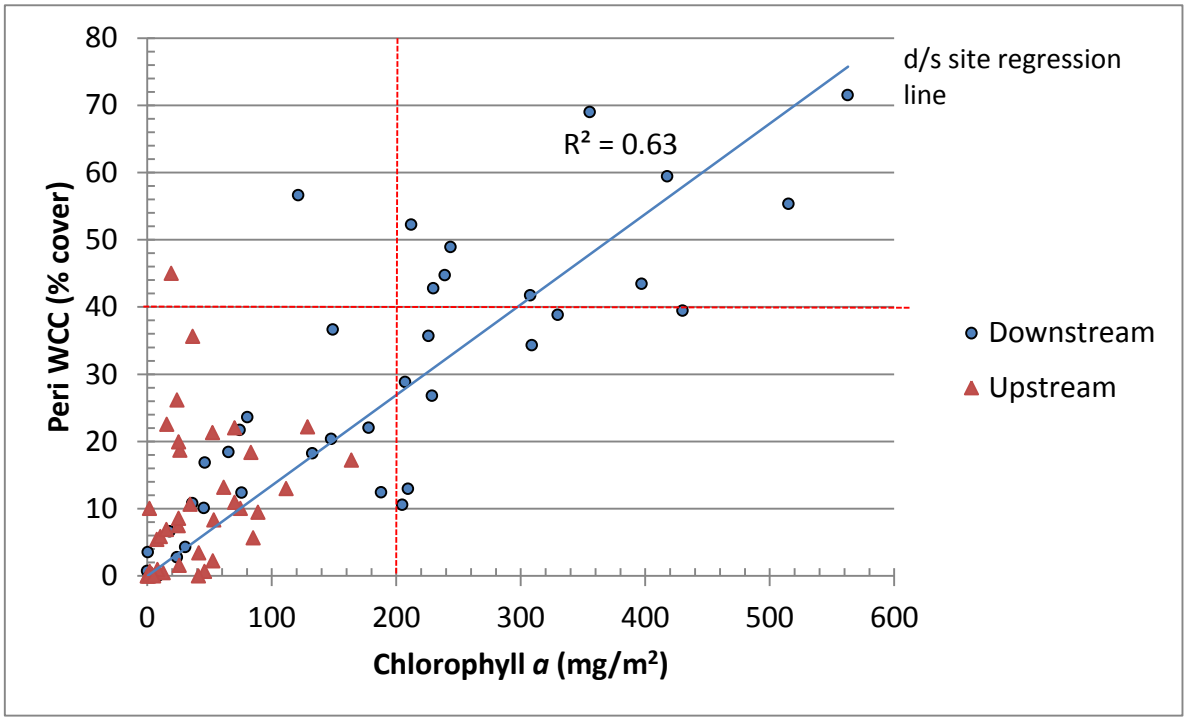

Figure 2.15: Correlation between chlorophyll *a* and periphyton weighted composite cover (WCC) in the Manawatu River upstream and downstream of the discharge (for summers 2011/12 and 2012/13). The red lines indicate values corresponding to 'good' water quality (from Matheson et al. 2012). An outlier of 885 mg/m^2 chlorophyll *a* was excluded from the regression.

2.4 Summary

The key messages from this chapter are:

- The concentrations of nutrients in the Manawatu River were, on average, higher downstream of the discharge compared to upstream.
- The river DRP concentration varied considerably during the summer and dropped to very low concentrations during January, over which time the DRP measured at the downstream site was equal to or less than the upstream site.
- DRP concentrations increased during late summer low flows to above the summer median at both upstream and downstream sites - suggesting the release of DRP from senescence of mature periphyton and perhaps some influence of periphyton deriving dissolved phosphorus from river sediments trapped in the periphyton mat.
- Downstream of the discharge there were periods when low DRP concentrations could potentially limit periphyton growth, but SIN continually remained high. During January, when DRP concentrations were very low, the periphyton downstream of the discharge was growing

rapidly and showed little evidence of nutrient limitation. This also suggests the periphyton was obtaining phosphorus from other sources (e.g. trapped sediments).

- There was less particulate P measured in the river than would be expected due to the discharge – suggesting settling out of particulate phosphorus.
- There was more DRP measured in the river than would be expected due to the discharge in early summer and late summer – but less DRP during January. This points to the interaction of different in-river processes such as periphyton uptake of DRP (in January) and net release of DRP in February, from senescence of mature periphyton and perhaps some influence of periphyton deriving dissolved phosphorus from river sediments.
- In the river upstream of the discharge, the potential limiting nutrient changed during the summer from potential P limitation to potential N limitation.
- The downstream site appeared to have relatively high concentrations of chlorophyll *a* for a given percent cover compared to the upstream site and typical values for other rivers.
- Long periods of removing DRP from the discharge by alum dosing (e.g. February and March) had no noticeable effect on downstream periphyton biomass compared to short periods (e.g. November and December).

3 Periphyton accrual rates over time on concrete tiles

3.1 Introduction

One possibility raised in Hamill (2012) to explain higher than expected dissolved phosphorus (e.g. TDP and DRP) concentrations in the river and faster than expected downstream periphyton growth was that river sediments may act as a buffer to store phosphorus when water concentrations are high (e.g. no alum treatment) and release phosphorus when river concentrations are low (e.g. when alum dosing is occurring). If this mechanism of phosphorus (P) release was occurring then one management response could be to start alum dosing at higher flows and for a longer period of time. This would be expected to reduce the period of time that DRP in the downstream river water could be sorbed by sediments and thus reduce the amount of P available for release during low flows (if this mechanism is occurring).

During the summer of 2012/13 PNCC WWTP trialled extending the period of time for which DRP was removed from the effluent. Between 1 November and 30 March the alum dosing was only interrupted by flood events on 19 November 2012, 6-9 December, 19-20 December, 28 December to 3 January, 5 -7 February and 19-20 March (the average DRP concentration in the effluent during the 1 Dec -31 March period was <0.1 mg/L [7]compared about 3 mg/L when not alum dosing. This presented an opportunity to test whether doing the current alum dosing for a longer period of time provided any benefit by, for example, depleting phosphorus that might be stored in river sediments as a result of a concentration gradient. As discussed in Chapter 2, long periods of removing DRP from the discharge by alum dosing (e.g. February and March) had no noticeable effect on downstream periphyton biomass compared to short periods (e.g. November and December).

The hypothesis that extending the period of alum dosing will help reduce periphyton growth (due to less DRP release from the sediment during low flows) was tested by growing periphyton on artificial substrates consecutively placed in the river at one to two week intervals. Specific growth rates for the periphyton (normalised for accrual period and for temperature) were estimated and compared. The hypothesis would be supported if specific growth rates at the downstream site were slower after river sediments were exposed to a longer period of low DRP concentrations in the water (i.e. a longer period of alum dosing).

Comparing estimates of specific growth rates for algae with theoretical maximum growth rates also tests the extent to which there is any limitation (e.g. by nutrients) on periphyton is growth (see Biggs 1990). For this purpose it is most effective to focus on measurements on the initial and exponential stages of periphyton growth.

3.2 Method

3.2.1 Sampling method

Periphyton accrual rates were assessed by measuring periphyton biomass growing on replicated artificial substrates (concrete paving tiles) placed in the river at one to three week intervals. Using

[7] Flow weighted composite sample.

artificial substrates reduces the influence of substrate type and armouring on periphyton development at different sites.

Ten concrete tiles (190x220mm) were placed in the river at monitoring sites: 800m upstream (true left bank) and 800m downstream (true right bank).

Three sets (trials) of tiles were placed in the river at one to six week intervals to allow comparison of different periods of alum treatment on periphyton accrual. The first trial (A) was installed on 10 January after 10 days of alum treatment, and subsequent trials were installed after 10 + 7 (B) and 10 + 14 (C) days of alum treatment (Table 3.1). Periphyton biomass samples were collected from artificial substrates every three to four days during a flow recession to allow up to 21 days of accrual. Table 3.1 shows the time at which substrates were placed in the river and sampled. Trial A was installed 10 days after the last flood, which was just under three times median flow.

Trial C was abandoned because of a small flood on 5th February 2013. These tiles were scrubbed clean of periphyton and used for Trial D, which was started on Friday 8 February. On this date additional tiles were also placed at three additional sites: 1.2 km downstream, 3.8km downstream (u/s Longburn) and 4.7 km downstream (d/s Longburn); replicate samples were collected from these sites on day 21 to enable a longitudinal comparison of periphyton.

Periphyton biomass was collected from the tiles using method QM-1b from the Stream Periphyton Monitoring Manual (Biggs & Kilroy 2000). Specifically this involved removing all periphyton from a 5.0 cm diameter area on the surface of ten (10) artificial substrates (one sample per concrete tile) and bulked (i.e. a total area sampled of 0.02 m^2). In order to allow a robust comparison of periphyton biomass between sites, replicate samples were collected on day 21 for Trial A and D. Instead of bulking all the samples, paired samples were bulked to produce five replicate samples from ten 5.0 cm diameter sample areas on the tiles (i.e. sampling 0.004 m^2 per replicate). Samples were frozen and sent to NIWA for analysis of chlorophyll *a* and Ash Free Dry Mass (AFDM) [8]. Analysis of periphyton samples followed the Biggs & Kilroy (2000) guidelines for AFDM and chlorophyll *a* analysis using ethanol extraction.

Chlorophyll *a* and AFDM provide complementary information on periphyton biomass. Chlorophyll *a* gives an indication of autotrophic organisms. AFDM is a measure of the total organic material in the sample and includes autotrophic and heterotrophic microorganisms, plus dead periphyton and detritus.

Water depth and velocity were recorded using the ruler method (see Harding et al 2009) above each tile (see Appendix 2).

Table 3.1: Timing of sampling from artificial substrates. Tiles were sampled every 3-4 days as recommended in Biggs and Kilroy (2000).

Date	Days since alum dosing	Lag since starting P treatment			
		Trial A (10 Jan)	**Trial B (17 Jan)**	**Trial C (24 Jan)**	**Trial D (8 Feb)**
10 Jan	7	installed			
17 Jan	14	X 7	installed		

[8] AFDM is also sometimes referred to as Ash Free Dry Weight (AFDW).

21 Jan	18	X 11		installed	
24 Jan	21	X 14	X 7		
28 Jan	18	X 18	X 11	X 7	
31 Jan	21	X 21 (replicates)	X 14	X 11	
4 Feb	25		X 18	X 14	
		170 m^3/s flood on 5th Feb (no alum dosing on 6-7 Feb)			
8 Feb	1				installed
12 Feb	5				
15 Feb	8				X 7
19 Feb	12				X 11
23 Feb	16				X 15
26 Feb	19				X 18
1 Mar	22				X 21 (replicates)
5 Mar	26				X 25

3.2.2 Net biomass accrual rate

The net rate of biomass accrual was calculated using the method in Biggs and Kilroy (2000). This method provides an estimate of periphyton growth rates in situations where loss due to invertebrate grazing and detachment are low.

The net accrual rate was calculated by regression using the equation:

$$B = a \exp(kT)$$

Where:

- B is the biomass measure per square metre at day T,
- a is the initial biomass concentration at T=0, and
- k is the net accrual rate during the exponential growth phase.

The net accrual rate (k) was calculated by $\log_e$ transforming the biomass data, plotting this against days accrual and calculating the slope of exponential phase of growth. A k value of < 0.1 /day is considered low and a k value of >0.35/day is considered high (Biggs and Kilroy 2000).

To reduce the effect of algal settlement rate on calculating growth rates the first seven days was omitted from the calculation unless otherwise stated (as per approach in Biggs 1990)

3.2.3 Specific growth rate

Specific growth rate (μ) was calculated by using the net accrual rate (k) and applying a correction to convert the value to $\log_2$: $\mu = k/0.693$. This equates to the number of cell divisions per day (Bothwell 1988).

The relative growth rate was calculated as: μ / μ_{max}

Where μ_{max} is the maximum specific growth rate for nutrient saturated algae.

The maximum specific growth rate (μ_{max}) was calculated using the model in Bothwell (1988) (based on P limiting conditions):

$$\mu_{max} = 0.189 + 0.0278\ t$$

where: t = temperature in degrees Celsius[9].

The relative growth rate adjusts for the effects of temperature on algae growth and thus isolates algae growth as a function of the limiting nutrient concentration. A low relative growth rate μ: μ_{max} of <0.3 indicates growth is limited by low nutrient (P) concentrations; 0.3-0.8 indicates slight nutrient deficiency, and a high relative growth rate μ: μ_{max} of >0.8 indicates that growth is not limited by nutrients (i.e. nutrients are replete) (Bothwell 1985, Biggs 1990).

These specific growth rate estimates assumed that losses from emigration, death and invertebrate grazing are minimal. This was not the case at the upstream site where there was evidence of considerable grazing from macroinvertebrates. Thus estimates for biomass accrual and specific growth rate at the upstream site were based on the maximum accrual rate measured between any sample dates and are considered minimum estimates.

In order to calculate specific growth rate from AFDM measurements the data was first converted into equivalent chlorophyll *a* values using the following formula from Biggs (2000):

$$\text{Ln Chlorophyll a (mg/m}^2\text{)} = 0.338 + 1.396 \times \text{Ln AFDM (g/m}^2\text{)}.$$

3.2.4 Autotrophic Index (AI)

The autotrophic index (AI) was calculated on sample results to indicate the extent of heterotrophic growth within the periphyton community. AI is the ratio of ash-free dry mass (AFDM) to chlorophyll *a*.

For in-stream periphyton communities unaffected by organic pollution, the AI ratio is normally between 100 and 200 (but the range can be from 50-250 and vary as a function of nutrient availability, light intensity and age of cells (McIntire and Phinney (1965))[10]. AI values greater than 400 are taken to be indicative of communities affected by organic pollution (Biggs and Kilroy 2000). AI is not accurate when the periphyton biomass is low i.e. < 2 g/m^2.

[9] Average of hourly monitoring data from Manawatu at Teaches College where available. An average temperature of 20.8°C and 21.1°C applied to trial A and B, and trial D respectively.

[10] Biggs (2000) provides regression equations to convert between AFDM and chlorophyll *a*. These are: Ln Chlorophyll a (mg/m^2) = 0.338 + 1.396 x Ln AFDM (g/m^2); and Ln AFDM (g/m^2) = 0.186 + 0.566 x Ln chlorophyll a (mg/m^2). Equivalent values for AFDM and Chl a in the periphyton guidelines (35 g/m^2 and 200 mg/m^2 respectively) imply a typical AI of 175.

3.3 Results and discussion

3.3.1 Periphyton accrual and growth rate

The results of periphyton biomass measurements from the artificial substrates are shown in Appendix 2. At the downstream sites the growth of periphyton showed a similar pattern on all the trials i.e. an accrual phase of initial colonisation and exponential growth followed by a loss phase with autogenic sloughing soon after the initial biomass peak. Trial D was long enough to see this followed again by a growth phase. The periphyton species composition on the concrete tiles changed over the three-four weeks of growth; *Stigeoclonium* sp. rapidly colonised the substrates, followed by cyanobacteria, stalked diatoms and filamentous algae such as *Cladophora* species (see Figure 3.1). This succession might be partially due to macroinvertebrate grazing pressure, for example *Cladophora* sp. is resistant to chironomid grazing.

At the downstream site the first biomass peak occurred progressively more quickly for trials later in the summer. In Trial A the initial peak biomass occurred after 18 days accrual, in Trial B it occurred after about 14 days accrual, and in Trial D it occurred between <7 to 11 days of accrual depending on which biomass measure was used (chlorophyll *a* or AFDM) (Figure 3.2 and 3.3), i.e. the sampling did not fully capture the first exponential growth phase.

Periphyton AFDM exceeded the 35 g/m^2 guideline for protection of trout habitat within 15 to 17 days (Figure 3.3). The equivalent guidelines in terms of chlorophyll *a* (i.e. 200 mg/m^2) was exceeded considerably sooner.

Periphyton biomass was supressed at the upstream site; probably due to macroinvertebrate grazing as chironomids tubes and caddis nets were abundant on the concrete tiles. Upstream results from the Trial A and B had very little biomass accrual and no exponential growth phase. During Trial D the upstream site had exponential growth from days 11 to 18. This would be consistent with macroinvertebrate sampling results National River Water Quality Network (NRWQN) invertebrate data for Teachers College (WA8) on 1 March 13 that showed moderate numbers of *Hydropsyche* (formerly *Aoteapsyche*) net-spinning caddis (1783/m^2) and *Tanytarsus* and *Orthoclad* chironomids (1429/m^2) along with moderate numbers of snails (*Potomopyrgus* 537/m^2), *Deleatidium* mayfly (676/m^2) and *Elmid* beetle larvae (439/m^2) (John Quinn pers. comm. 2013).

Net biomass accrual and specific growth rate was assessed on the period of exponential growth. Slightly different periods were used for measures of chlorophyll *a* and AFDM (see Table 3.2). The $\log_e$ adjusted AFDW values are shown in Figure 3.4.

Specific growth rates at the downstream site were fastest for Trial D followed by Trial A and Trial B (Table 3.2). Some of the differences between Trial A and B may be due to peak biomass falling between sampling intervals. During Trial B chlorophyll a accrued more slowly than AFDM, while in Trial A the rates of accrual estimated by the different measurements were similar and in Trial D the rate of chlorophyll *a* accrual was faster. Some nutrient limitation was suggested by chlorophyll *a* measurements of Trial B (i.e. a low relative specific growth rate of 0.24) but this was not supported by estimates using AFDM – suggesting other factors could have restricted chlorophyll *a* accrual (e.g. grazing). Relative specific growth rates from chlorophyll *a* data suggested that periphyton growth was nutrient replete during Trial D (i.e. specific growth rates were higher than theoretical maximum growth

rates). However the relative specific growth rates estimated from AFDM were considerably lower and suggested some limitation on growth (Table 3.2). The difference is partially due to a surprisingly low chlorophyll *a* value causing a high ratio of AFDM to chlorophyll *a* on Day 15 (23 February) (AI=743).

As discussed previously, the upstream site during Trial A and B had low periphyton biomass and very little growth after day 7 – probably due to grazing. However the burst of periphyton growth during days 15 to 18 of Trial D was very rapid and the growth rate similar (slightly faster even) than the downstream site over the same period. Biomass reached 198 mg/m^2 before rapidly declining again (Figure 3.2, Table 3.2).

Finding nutrient replete growth on upstream tiles, even over a short period of time, is surprising considering the low concentrations of DRP and particularly SIN in the water. However the short term increase (rapidly increasing and declining within a week) may reflect a temporal variation in grazing pressure and rapid recycling of nutrients, or patchiness of chlorophyll *a* rich species on the tiles. Note that photographs taken at the time support the data and show a mat of periphyton and patches of *Stigeoclonium* sp. on tiles at the upstream site (see Appendix 6).

Figure 3.1: Periphyton succession on a concrete tile in the Manawatu River downstream of the discharge (7 March 2013, day 27 of Trial D). Sections of the tile were sampled every 3 to 4 days in the order: top left, bottom left, top right, bottom right, top middle, bottom middle. *Stigeoclonium* sp rapidly colonised the substrates, followed by cyanobacteria (right hand side), stalked diatoms and filamentous algae such as *Cladophora* species.

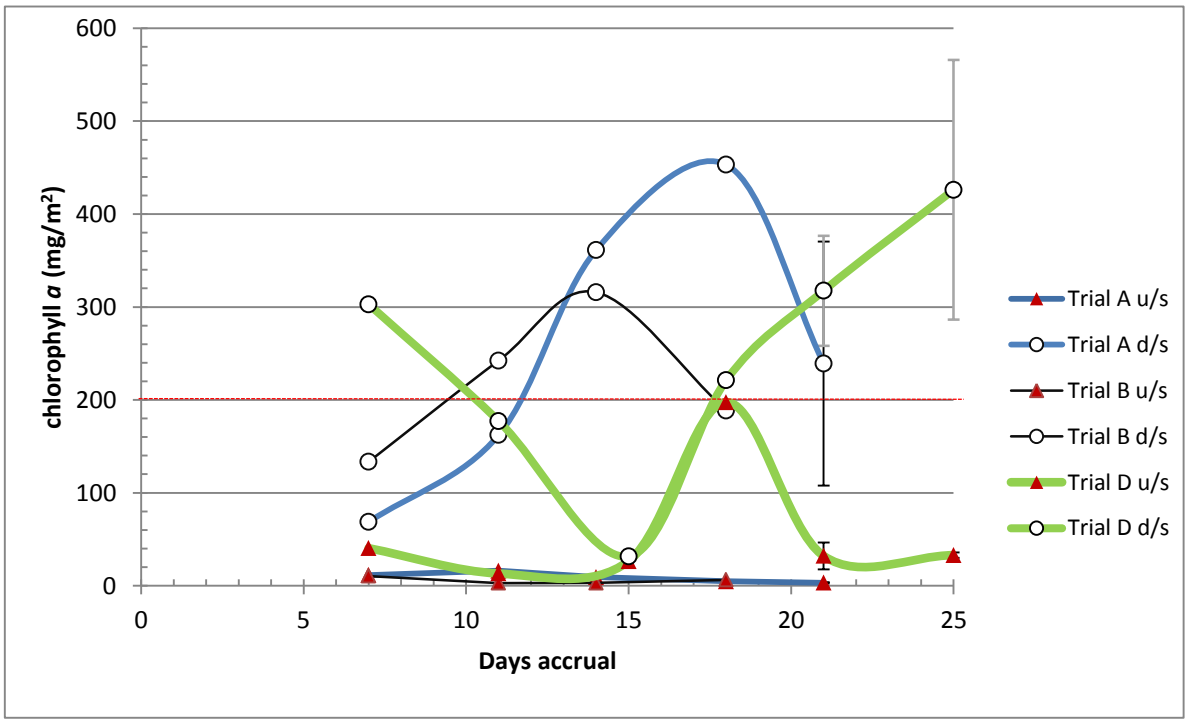

Figure 3.2: Periphyton accrual on artificial concrete substrates in the Manawatu River upstream and downstream of the WWTP discharge. Biomass assessed as chlorophyll *a*. The dashed red line indicates the NZ periphyton guideline value for trout habitat. Error bars are two standard errors.

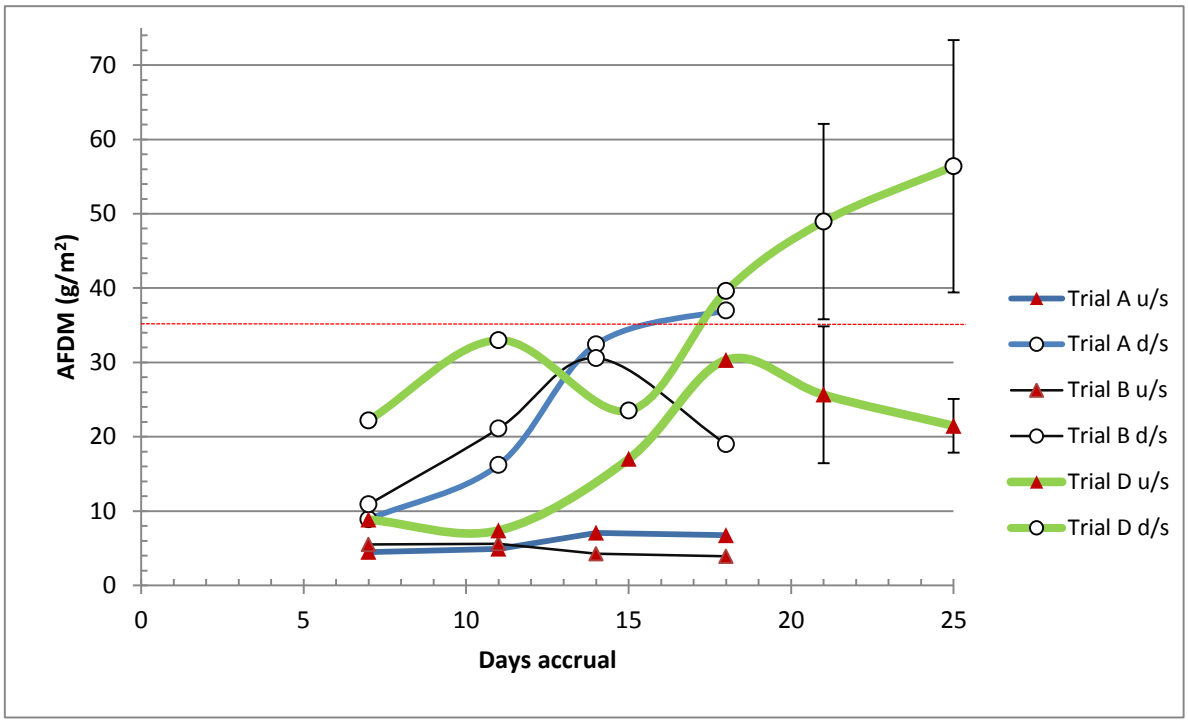

Figure 3.3: Periphyton accrual on artificial concrete substrates in the Manawatu River upstream and downstream of the WWTP discharge. Biomass assessed as AFDW. The dashed red line indicates the NZ periphyton guideline value for trout habitat. Error bars are two standard errors.

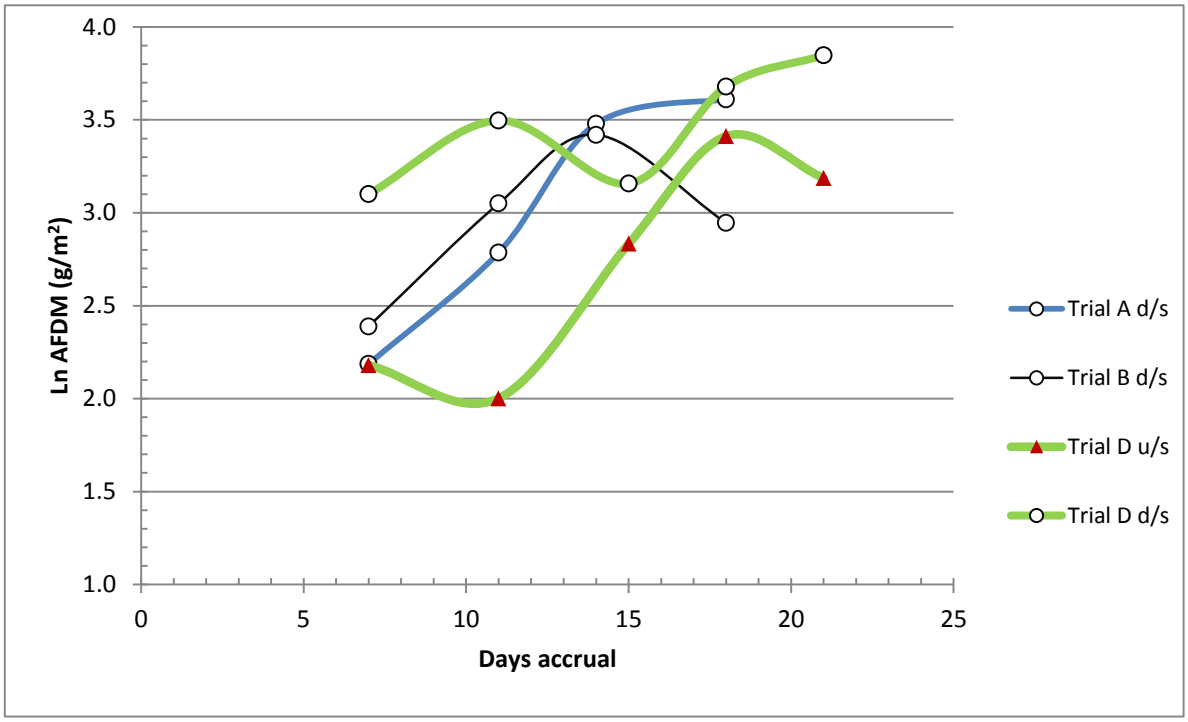

Figure 3.4: The natural log of periphyton accrual (AFDW) on artificial concrete substrates in the Manawatu River upstream and downstream of the WWTP discharge (days 0 - 21). Note the relatively consistent slope (i.e. *k* value) between each trial during growth phase.

Table 3.2: Periphyton biomass accrual (*k*, day^{-1}), specific growth rate (μ, divisions/day), P saturated specific growth rate (μ_{max}, divisions /day), and relative specific growth rates ($\mu:\mu_{max}$) for successive trials in the Manawatu River upstream and downstream of the WWTP discharge. AFDW values were first converted to equivalent chlorophyll *a* values before calculating *k*. A low relative growth rate $\mu: \mu_{max}$ of <0.3 indicates growth is limited by low nutrient concentrations, while $\mu: \mu_{max}$ of >0.8 indicates that growth is not limited by nutrients.

			Temp	Chlorophyll *a*				AFDM (converted to Chl *a*)			
Trial /site	**Dates**	**Days**	**(°C)**	***k***	**μ**	**μ_{max}**	**$\mu:\mu_{max}$**	***k***	**μ**	**μ_{max}**	**$\mu:\mu_{max}$**
Trial A d/s	17-24 Jan	7 to 14	20.8	0.24	0.35	0.77	0.46	0.26	0.37	0.77	0.48
Trial B d/s	24-31 Jan	7 to 11	20.8	0.13	0.19	0.77	0.24	0.21	0.30	0.77	0.39
Trial D d/s	23-26 Feb	15 to 18	21.1	0.65	0.94	0.78	**1.21**	0.24	0.35	0.78	0.45
Trial D u/s	23-26 Feb	15 to 18	21.1	0.67	0.97	0.78	**1.25**	0.27	0.39	0.78	0.50
Trial D u/s	19-26 Feb	11 to 18	21.1	0.39	0.56	0.78	**0.72**	0.28	0.41	0.78	0.52

3.3.2 Periphyton biomass between sites along the river

In order to allow a robust comparison of periphyton biomass between sites, replicate samples were collected on day 21 in Trials A and D and additional downstream sites sampled for Trial D. Chlorophyll *a* and AFDM showed a similar pattern of periphyton biomass changes longitudinally down the river. The sites 800m and 1200m downstream of the discharge had significantly more periphyton biomass than upstream, but biomass had declined to less than guideline values by 3.8km downstream (upstream of Longburn discharges), followed by an increase in biomass downstream of the Longburn discharges (4.7 km downstream of the Totara Road WWTP (see Figure 3.5 and Figure 3.6).

There was no difference in periphyton AFDM between Longburn (3.8 km downstream) and the site upstream of the WWTP discharge. At the upstream site chlorophyll *a* was considerably lower than AFDM compared to the periphyton guidelines. This was observed on all sample occasions with the exception of the chlorophyll *a* peak on day 18 during Trial D (see Figures 3.2, 3.3 and 3.5). In contrast, at the downstream sites chlorophyll *a* tended to exceed equivalent guideline values considerably earlier than AFDM; this was particularly apparent during Trials A and B (see Figure 3.2 and 3.3).

Periphyton cover was not measured in the river at Longburn, but it is apparent from photographs in Appendix 6 that there was considerably more periphyton cover at the Longburn sites compared to upstream of the discharge – suggesting that AFDM may be over-estimating periphyton biomass at the upstream site (possibly because it is sampling chironomid cases attached to the tiles).

A similar pattern of periphyton biomass change between sites along the river was found during periphyton sampling of river substrate two weeks later on 16 March 2013 (Figure 3.7). This sampling was done in conjunction with macroinvertebrate sampling and collected from riffle habitat rather than runs.

The sampling sites upstream and downstream of Longburn STP had lower water velocity than the other sites (i.e. 58 cm/s, 64 cm/s, 44 cm/s, 29 cm/s for sites upstream of the WWTP discharge, 800m downstream, 1200m downstream and upstream of the Longburn discharges), and this may explain some, but not all, difference in biomass between sites.

3.3.3 Assessing biomass using AFDM compared to chlorophyll *a*

The ratio of AFDM to chlorophyll *a,* i.e. the autotrophic index (AI), was extremely high at the upstream site with a median value of 650 (see Figure 3.8). At the downstream site there was a difference between trials, with periphyton during Trials A and B having a median value of 93 compared to periphyton during Trial D which had a median value of 158 (mean =171). The first sample from Trial D (day 7) is grouped with Trials A and B (to capture early colonising algae) and an equivalence test showed that the difference between the earlier samples and the later samples was statistically significant[11]. Another way to view this data is to compare the correlation of AFDM and chlorophyll *a* as shown in Figure 3.9. This shows a strong relationship between AFDM and chlorophyll *a* at the downstream sites during Trial A and B + day 7 of Trial D (r^2=0.94), but considerably more variability in AI during Trial D (days 11-25) (r^2=0.79).

[11] Student t-test *p*-value =0.04.

There was no evidence of organic pollution at the upstream site to cause the high AI values. Instead the high AI was likely to have been due to extensive grazing of periphyton by macroinvertebrates that both reduce periphyton accrual and turn this into insect biomass that contributes to AFDM. Jernakoff and Nielsen (1997) found that Gastropods reduced the ratio of chlorophyll *a* to ash-free dry weight of periphyton in seagrass meadows by 99%. On two sample occasions during late February and March a high percentage (73%) of 'sludge' was recorded at the upstream site during Horizon RC weekly assessments while chlorophyll *a* concentrations were relatively low (i.e. 16-70 mg/m^2), and it is likely that some of the 'sludge' was the residual of grazed periphyton, chironomids tubes and free-living caddis nets, both of which were abundant in the macroinvertebrate samples collected upstream of Fitzherbert Rd bridge on 1/3/13 in the NRWQN samples (1400 and 1800/m^2, respectively (Jon Quinn pers. comm. 2013)

The difference in the AFDM:chlorophyll *a* ratio between Trials A+B (10 January to 4 February) and Trial D (8 February to 5 March) probably reflects a change in the species composition of periphyton in the river and on the tiles. As discussed previously the species composition on the tiles changed over time with the filamentous green algae *Stigeoclonium* sp. being an early colonist, followed by *Cladophora* sp. stalked diatoms and cyanobacteria. The same sequence occurred in the river itself with *Cladophora* sp. and cyanobacteria being more common later in the summer and hence colonising substrates faster during Trial D. It appears that the early colonising periphyton (mostly *Stigeoclonium* sp.) are rich in chlorophyll *a* (a median AI of 93 compared to a typical AI of 175 implied in the NZ periphyton guidelines (i.e. 35,000 mg AFDM/m^2 / 200 mg chl *a*/m^2). The cyanobacteria *Phormidium* sp. was common at the downstream site late in the summer and is known to have very high concentrations of chlorophyll *a* (Kilroy et al. 2012). Sampling patches of *Phormidium* sp. on the tiles may explain the occasional low ratio of AFDW:chlorophyll *a* during Trial D.

There are several reasons why downstream periphyton had a high concentration of chlorophyll *a*; firstly *Stigeoclonium* sp. was the dominant species in early succession and it has a 'lean' structure with little cellulose. Secondly, the chlorophyll *a* density within cells may have been responding to high nitrogen concentrations in the downstream water. Nitrogen is a component of chlorophyll, and nitrogen can stimulate chlorophyll production without necessarily influencing growth (Menendez et al. 2002), conversely one of the first symptoms of nitrogen deficiency is a reduction in chlorophyll production (Meeks 1974 in White and Payne 1977).

One implication of downstream periphyton being rich in chlorophyll *a* is that measuring periphyton biomass using chlorophyll *a* will result in guideline values being exceeded while the AFDM periphyton biomass is within guideline values (as seen in trials A and B, Figures 3.2 and 3.3). Conversely, at the upstream site periphyton biomass may be over-estimated if measured using AFDM biomass because of the influence of chironomid grazers. So which measure of periphyton biomass is more appropriate for assessing the effects of the Totara Road WWTP discharge on the Manawatu River – chlorophyll *a*, AFDM or percent cover (e.g. Peri Weighted Composite Cover)?

Hamill (2012) found that the abundance of mayfly declined downstream of the discharge after chlorophyll *a* at the downstream sites increased to between 300 to 450 mg/m^2. This is considerably higher than the chlorophyll *a* values in the NZ periphyton guidelines (i.e. 120 mg/m^2 to 200 mg/m^2 depending on algae taxa). Using the AI value of 93[12] the range corresponds to 28 g/m^2 to 42 g/m^2 as

[12] This was the median AI for samples dominated by *Stigeclonium* sp.

AFDM. The middle of this range is 35 g AFDM/m^2, which, coincidentally, is the same as the NZ periphyton guidelines when expressed as AFDM. This suggests that AFDM is a more appropriate measure to use in the Manawatu River downstream of the discharge for comparing periphyton biomass against guideline values – particularly in the early stages of colonisation[13].

This conclusion is specific to assessing the effects of the Totara Road WWTP discharge to the Manawatu River. It does not imply changes are needed to regional monitoring programmes which have broader aims and for which chlorophyll *a* is often a more versatile measure of periphyton biomass (see discussion in Biggs and Kilroy 2000).

Measures of periphyton cover (e.g. Peri WCC) provide complementary information to biomass measures and can be used to test the validity of the results. Periphyton thickness, assessed as the settled volume of mixed samples, could be used as an inexpensive alternative comparable to AFDM (see Matheson et al. 2012).

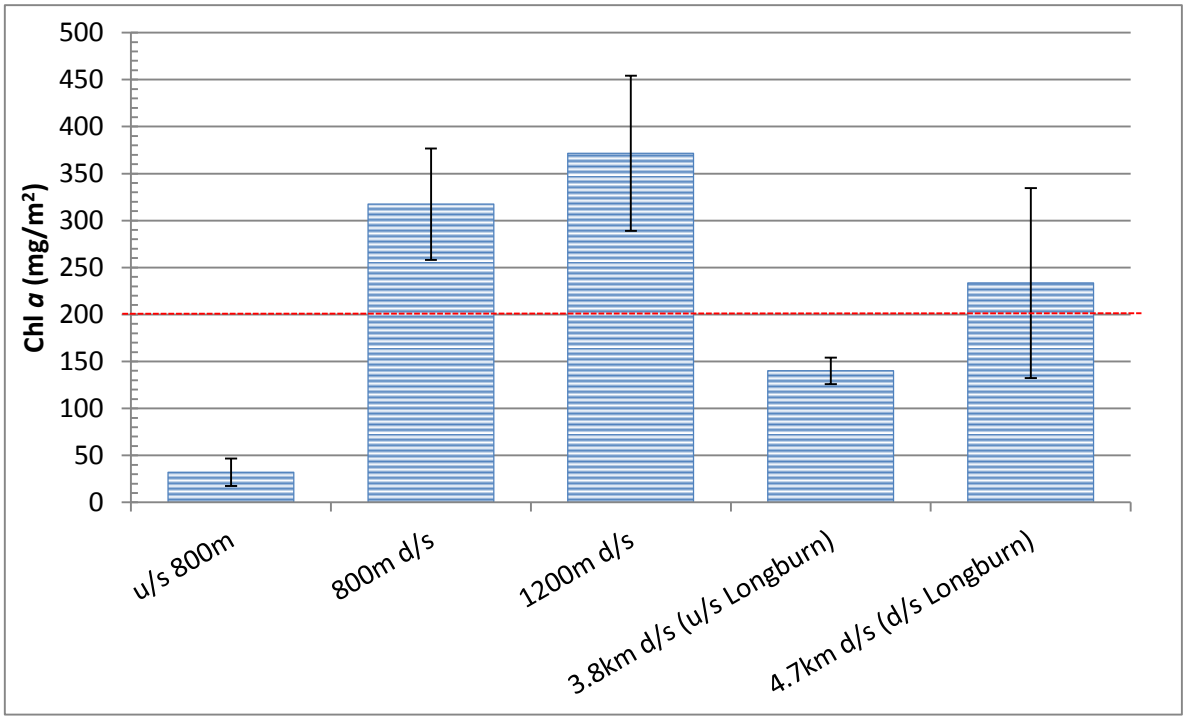

Figure 3.5: Mean periphyton biomass (estimated by chlorophyll *a*) on concrete tiles in the Manawatu River after 21 days accrual (1st March 2013). Error bars are 2 standard errors (i.e. 95% confidence). The red line is the NZ periphyton guideline for trout habitat (diatoms and cyanobacteria).

[13] Furthermore, the relative specific growth rates were much more consistent between trials when assessed using AFDM, which is closer to what would be expected between trials separated by only a few weeks (Table 3.2).

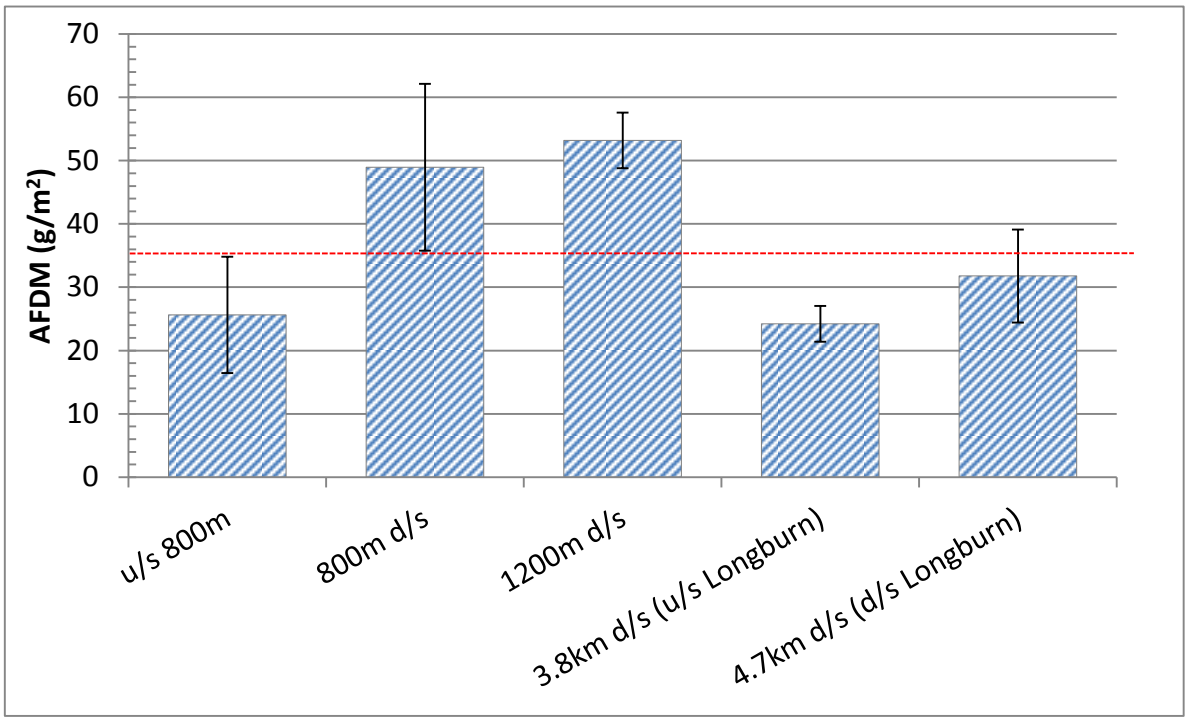

Figure 3.6: Mean periphyton biomass (estimated by AFDM) on concrete tiles in the Manawatu River after 21 days accrual (1st March 2013). Error bars are 2 standard errors. The red line is the NZ periphyton guideline for trout habitat.

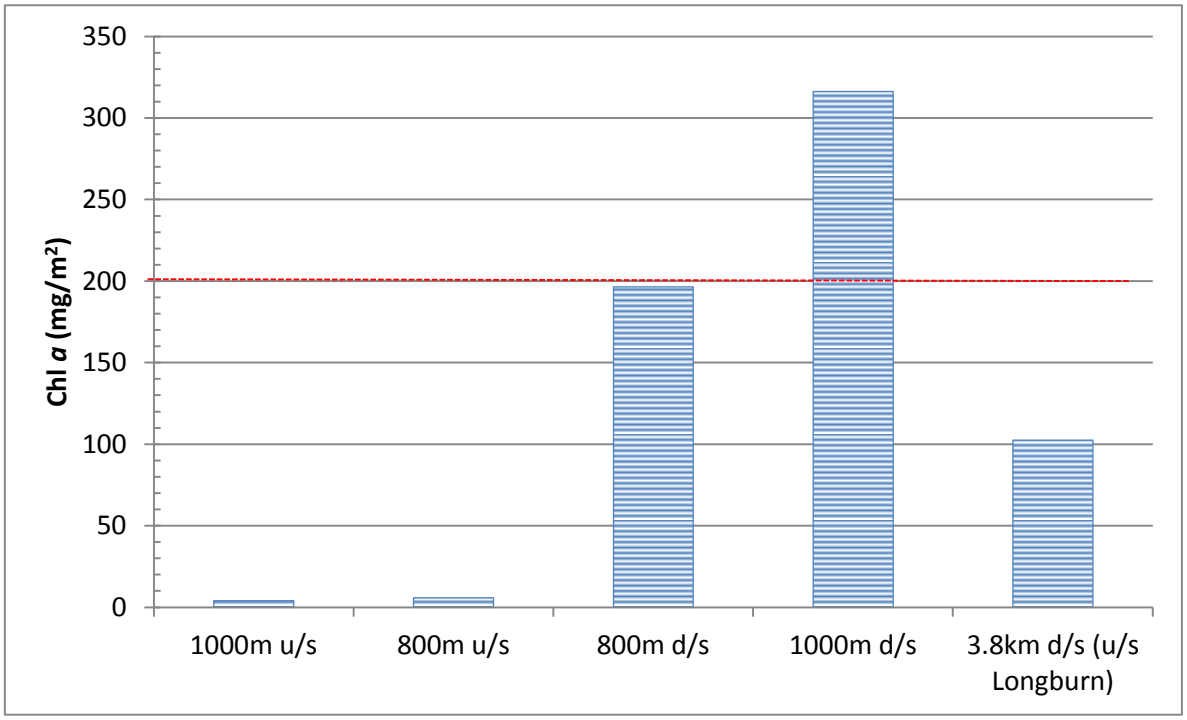

Figure 3.7: Periphyton biomass (as chlorophyll *a*) on Manawatu River cobbles collected from riffle habitat on 16 March 2013. The red line is the NZ periphyton guideline for trout habitat (diatoms and cyanobacteria).

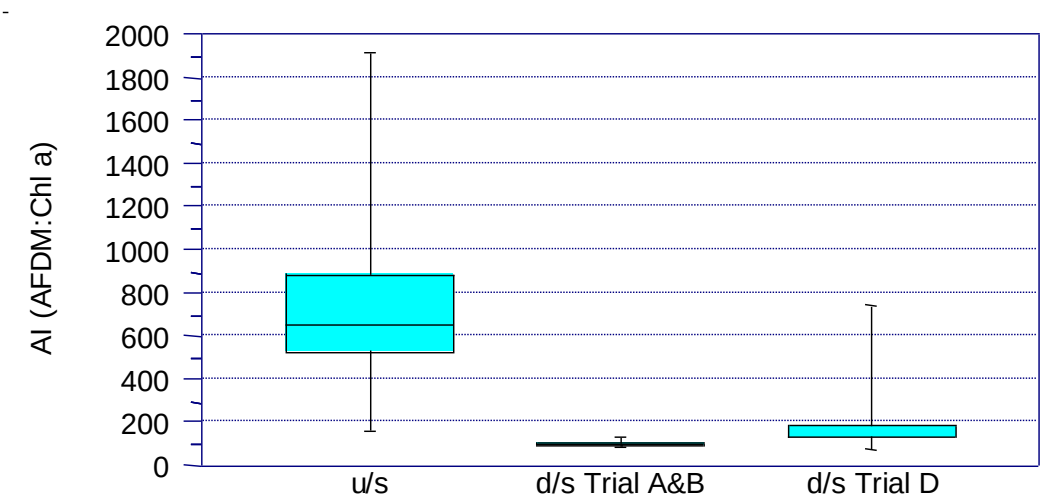

Figure 3.8: Comparison of Autotrophic Index (AI) between sites and trials. The difference between Trial A&B and Trial D was statistically significant.

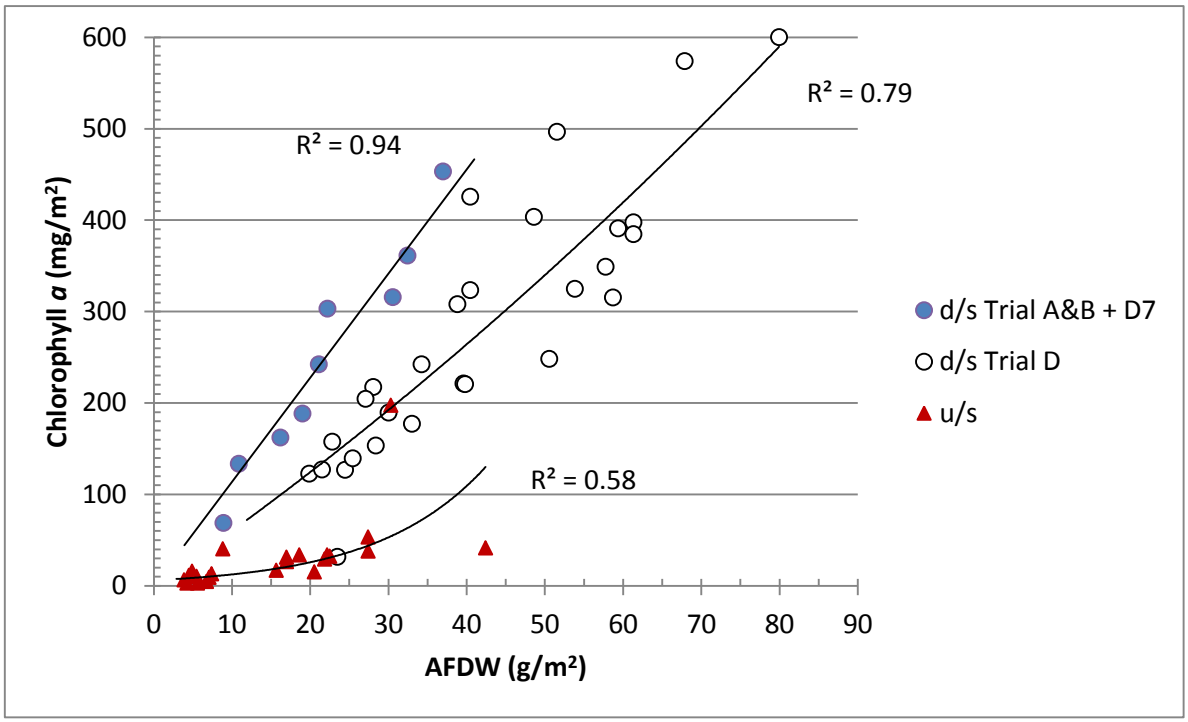

Figure 3.9: Relationship between AFDM and chlorophyll *a* comparing sites and trials. D7 refers to the sample from Trial D on day 7.

3.4 Summary

The key messages from this chapter are:

- While sediments are an important store of phosphorus, this experiment did not support a hypothesis that phosphorus release to the river was caused by phosphorus concentration gradients between the water and the sediments (that gradually decline over time). This was seen in:
    - The initial peak in maximum periphyton biomass occurred progressively earlier for trials later in the summer.
    - Periphyton growth rates measured on concrete tiles in Trial A were similar to growth rates on tiles place in the river a week later (Trial B) despite a longer period of alum dosing in late summer.
    - DRP concentrations in the river downstream of the discharge were very low during January – within a week of alum dosing recommencing following floods in late December, while later in the summer DRP concentrations increased.
    - Periphyton biomass in the river was higher in the period January to March compared to November and December despite sediments in late summer having been exposed to a longer period of relatively low DRP concentrations from alum dosing of the discharge (see previous chapter).
- There is no evidence that undertaking the alum dosing for a longer period of time with the current treatment system causes any control on downstream periphyton growth.
- The maximum periphyton growth rate at the downstream site during Trial D indicated little or no nutrient limitation, which is consistent with high nutrient concentration in the river during this period (see Section 2). There was a short period of time during Trial D when the periphyton growth rate at the upstream site was equivalent to downstream and near maximum.
- The stimulatory effect of the discharge on periphyton growth was most evident in the first 1.2 km downstream of the discharge and was considerably less near Longburn 3.8 km downstream.
- The periphyton biomass exceeded that 35 g/m^2 guideline for protection of trout habitat within 15 to 17 days based on AFDM measurements.
- Early colonising periphyton such as *Stigeoclonium* sp. appeared to be rich in chlorophyll *a* compared to AFDM. This caused guideline values to be exceeded earlier when measured using chlorophyll *a* compared to AFDM.
- Chlorophyll *a* often over-estimated periphyton biomass at the downstream site (associated with *Stigeoclonium* sp.). Conversely AFDM appeared to over-estimate periphyton biomass at the upstream site because much of the periphyton had been grazed by macroinvertebrates and AFDM also include biomass of chironomids and caddisfly within the sample).

- For sites downstream of the discharge, AFDM is a better measure for assessing periphyton cover against guideline values because the AFDM guideline value of 35 mg /m^2 corresponds to a decline in mayfly abundance in the river (as reported in Hamill 2012). Percent cover (e.g. weighted composite cover) provides complementary information that helps confirm biomass measures.

- Additional investigations that would help confirm some of these results includes:

    - Undertake specific sampling to assess the ratio of chlorophyll *a* to AFDM of specific periphyton species common in the Manawatu River e.g. *Stigeoclonium* sp, *Cladophora* sp, *Phormidium* sp.

4 Nutrient limitation

4.1 Introduction

Nutrient concentrations have a significant effect on the rate of periphyton growth. Both phosphorus and nitrogen are thought to have a controlling influence on 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 relies on alum dosing to remove dissolved phosphorus in order to limit the rate of periphyton growth in the river.

The results of a periphyton nutrient bioassay done in April 2012 were ambiguous – indicating some phosphorus limitation but also control by other factors. The periphyton bioassay was repeated in 2013 with some modifications to the method to include:

- A treatment of river water mixed with sewage effluent to test for possible effects of micronutrients, carbon or total ammoniacal N within the effluent, and
- Features to discourage and limit the effects of grazing by macroinvertebrates i.e. a Vaseline petroleum gel barrier around the edge of the trays and the use of felt covers rather than GFC filters.

Chapter 2 discussed the potential nutrient limitation based on SIN and DRP concentration in the river water; the experiment described in this chapter provides more definitive results.

4.2 Method

A periphyton nutrient bioassay was undertaken using the steel tray nutrient diffusing substrate (NDS) method described in Biggs and Kilroy (2000). Two trays were deployed at each site with one tray at each site containing a treatment of nitrogen+phosphorus+sewage. Each tray had five replicates of four different treatments. The treatments applied to each tray at each site were:

Trays A and C (downstream and upstream respectively)

i. agar with no enrichment as a control (C),
ii. agar enriched with nitrogen (N),
iii. agar enriched with phosphorus (P),
iv. agar enriched with nitrogen and phosphorus (N+P).

Trays B and D (downstream and upstream respectively)

i. agar with no enrichment as a control (C),
ii. agar enriched with nitrogen (N),
iii. agar enriched with phosphorus (P),

iv. agar enriched with nitrogen and phosphorus and sewage (N+P+S).

The agar treatments were made at CEL Laboratories using the recipe in Biggs and Kilroy (2000). Nitrogen treatments were made with 42.5 g/L sodium nitrate ($NaNO_3$) (about 7.0 g N/L); phosphorus treatments were made with 19 g/L trisodium orthophosphate ($Na_3PO_4.12H_2O$) (about 1.55 g P/L). For the sewage treatments the N and P were made up with a 1:1 mixture of WWTP sewage and river water, both collected on the day of preparation. The concentration of DRP and SIN in the 1:1 mix of sewage and river water was 0.23 mg/L and 17.4 mg/L respectively. Most of the SIN was in the form of ammoniacal nitrogen. The contribution of sewage to the overall concentration of N and P in the N+P+S treatment was negligible i.e. about 0.2% and 0.01% extra nutrients for N and P respectively.

The NDS method in Biggs and Kilroy (2000) was modified by: a) attaching felt batting as a substrate to grow periphyton above each nutrient treatment instead of hardened ashless filter paper, and b) smearing a layer of petroleum jelly around the edge of each tray. Both of these modifications help reduce the influence of macroinvertebrate grazers, the felt because it allowed periphyton to grow to some extent within the felt matrix and the petroleum jelly by deterring some grazers such as snails.

Trays were upstream and downstream of the discharge in similar locations as the concrete tiles (see Chapter 3), specifically:

- Manawatu River about 800m upstream of the discharge point on true left;
- Manawatu River about 880m below the discharge point on the true right.

At each site two trays were located side by side in areas with similar depth and velocity and partially dug into the river bed so that the top was elevated about 4 cm above the river substrate. The trays were secured to a single waratah via a rope (about 0.6m long). Water depth and velocity were measured at each side of the tray when they were installed and removed.

Two NDS experiments were placed in the river. The first experiment was installed on 14 February and removed on 1 March just prior to a small flood. This experiment used hardened ashless filter paper as per the method in Biggs and Kilroy (2000) and experienced considerable damage by birds to the extent that one tray had no useable replicates. The top layer of agar from these trays was removed and replaced with fresh treatment agar. The second experiment used felt substrate over the agar and was placed in the river on 6 March and removed on 18 March 2013 (i.e. 12 days for accrual).

Felt substrates were removed, stored in a dark chilli-bin, frozen and sent to NIWA for analysis of chlorophyll *a* using ethanol extraction method[14]. The results were converted to mg/m^2 based on each replicate having 6.5cm diameter (0.00332 m^2) of surface exposed for periphyton growth.

There was no difference in water velocity between the downstream trays but tray A was in slightly shallower water (see Table 4.1). There was a similar range of velocity across each of the downstream trays but at the upstream site the control treatment on tray D was in a zone of higher velocity than the control treatment of trays C. The nitrogen (N) treatment on tray C was also in a zone of slightly higher velocity than the N treatment of other trays. Velocity and depth measured when the trays were removed showed the same pattern across treatments and trays.

[14] Blend, filter, boil 5 min in ethanol @78°C, 24hr extraction, spectrophotometer measurement.

There was evidence of grazing by macroinvertebrates on the upstream trays when they were removed after the first experiment on 1 March (see Figure 4.1a). The second experiment had evidence of chironomids tubes on the downstream trays (see Figure 4.1b) but little evidence on the upstream trays.

Table 4.1: Water depth and velocity on the corner of each NDS tray when installed on 6 March 2013.

Site		**d/s B**				**d/s A**				
Treatment		**C**	**N+P**	**P**	**N**	**C**	**N+P+S**	**P**	**N**	TR bank
depth (cm)		22	←	←	21.5	15.5	←	←	15.5	
velocity (cm/s)		54	←	←	54	54	←	←	54	
Site	TL bank	**u/s C**				**u/s D**				
Treatment		**C**	**N+P+S**	**P**	**N**	**C**	**N+P**	**P**	**N**	
depth (cm)		18	←	←	17	16	←	←	15.5	
velocity (cm/s)		54	→	→	58	58	←	←	49	

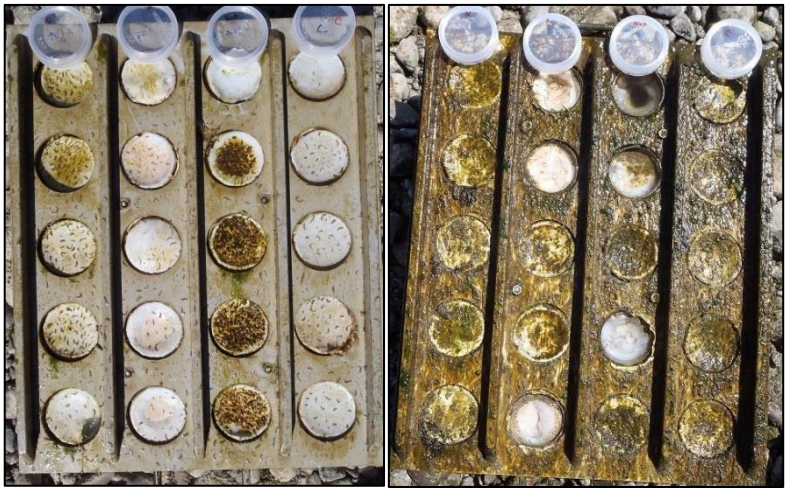

Figure 4.1a: Nutrient diffusing substrates (NDS) installed in the Manawatu River upstream (tray D, photo on left) and downstream (tray B, photo on right) of the discharge 14 February to 1 March 2013. Treatments are in order, from left to right, N, P, N+P and C. Evidence of chironomid tubes on upstream trays, and bird damage on downstream trays.

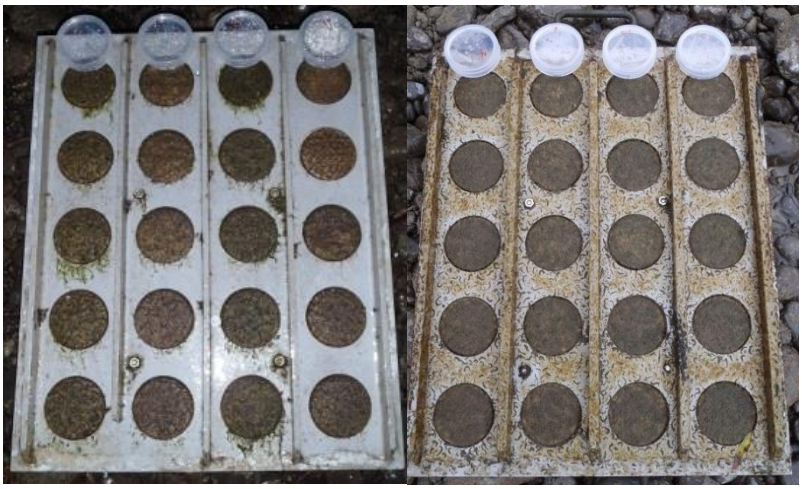

Figure 4.1b: Nutrient diffusing substrates (NDS) installed in the Manawatu River upstream (tray D, photo on left) and downstream of the discharge (Tray B, photo on right), on 6 March to 18 March 2013. Treatments are in order, from left to right, N, P, N+P and C. Evidence of chironomid tubes on downstream trays.

4.3 Results and discussion

4.3.1 Comparison between treatments

The nutrient bioassays indicated that periphyton in the Manawatu River upstream of the WWTP discharge was primarily limited by nitrogen with secondary phosphorus limitation exhibited when nitrogen was supplied in the N+P and N+P+S treatments. The greatest periphyton stimulation was caused by treatments with N+P (i.e. including N+P+S), followed by N (Figure 4.2 and Appendix 3 for all data). Both N+P and N treatments were statistically different from each other, the P treatment and the control. There was no statistically significant difference between the P treatment and the control (see Table 4.2).

Downstream of the discharge (trays A and B) there was an indication of dual limitation by N+P+S. No periphyton stimulation occurred with individual treatments of P, N or N+P, however the N+P+S treatment did stimulate periphyton growth and resulted in very similar biomass to that found on the upstream N+P+S treatment (tray C) (see Figure 4.3 and Table 4.2). The lack of response to enrichment by N at the downstream site is consistent with periphyton being sated by high SIN concentrations in the river downstream of the discharge (i.e. SIN 0.75 mg N/L, DRP 0.02 mg P/L). Despite the high concentration of SIN in the river downstream of the discharge the periphyton did not respond to the P treatment, perhaps reflecting the moderately high levels of DRP in the river (0.016-0.023 mg/L).

Faster water velocity can stimulate faster periphyton growth by increasing the diffusion of nutrients to growing cells. This effect occurs up to velocities of about 60 cm/s, after which periphyton biomass tends to reduce due to scouring (Horner et al. 1990) (although scouring is less common in the early stages of growth when 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. There were small differences in water velocity across the upstream trays, but the control treatment responded in the opposite way than would be expected due to velocity, i.e. there was a little less biomass on tray D. This suggests that the small velocity difference across and between the upstream trays were insignificant.

The results from the first bioassay (14 Feb-1 March) were consistent with those of the second, showing primarily nitrogen limitation and secondary phosphorus limitation. Damage to some trays and scouring of periphyton reduced replicates and increased variability from this experiment so the results are not presented in detail.

The results confirm that implied by water quality sampling as discussed in Section 2.3.3 i.e. N limitation at the upstream site but no nutrient limitation at the downstream site in late summer.

Stimulation by sewage + N+P

If there was a stimulatory effect of sewage separate from the nutrients N and P then it should show at the upstream site more strongly than at the downstream site[15]. In fact the opposite occurred. Upstream of the discharge there was no significant difference between the treatments N+P and N+P+S, while downstream of the discharge there was strong evidence of statistically significant difference (see Table 4.3 and Figure 4.4). The control treatment in tray C was slightly higher than the control in tray D and thus the difference between the treatments N+P and N+P+S was further reduced at the upstream site when statistical analysis was done on the residuals after subtracting the mean values from the control of each treatment.

The apparent response to the sewage treatment at the downstream site (but not upstream site) may reflect a poor response to the N+P treatment at the downstream site rather than some stimulation from the added sewage. The treatment N+P+S had a very similar periphyton biomass at both the upstream and downstream sites, but the treatment N+P grew more periphyton biomass at the upstream site (tray D) (150 mg/m^2 u/s compared to 112 mg/m^2 d/s, see Figure 4.3).

On the whole we cannot rule out the possibility of the sewage providing additional stimulation to periphyton growth independent of the N and P. Statistical analysis on the combined datasets from upstream and downstream indicate 'moderate evidence' of meaningful difference between the N+P and N+P+S treatments (Table 4.3).

There are a number of possible reasons for this effect including supply of micronutrients (e.g. cobalt or molybdenum) as discussed in (Hamill 2012). Hamill (2012) compared the relative composition of dissolved nutrients in the river (normalised relative to phosphorus) compared to the relative composition of algae (from Heckey and Kilham 1988). This found nitrogen to be the next most potentially limiting nutrient after phosphorus, but no sample data was available for cobalt, molybdenum or silica. River and effluent samples were tested for a suite of micronutrients in December 2012 and January 2013, and the analysis updated (see Table 4.4). Silica was clearly not limiting diatom growth. The algal requirements for cobalt and molybdenum are so low that laboratory detection limits were too high to confirm if there might be potential limitation. Raw data from cobalt suggested it was getting close to concentrations that might limit periphyton growth more strongly than P. No

[15] As discussed in the methods, the sewage treatment added negligible additional N and P to the treatments.

molybdenum was detected in river water samples. Molybdenum was detected in the discharge at a mean concentration of 0.0013 mg/L but cobalt was not detected in the discharge (<0.0005 mg/L).

While we cannot rule out the possibility of the sewage providing additional stimulation to periphyton growth separate from N and P, even if the effect is real, it remains small compared to the combined effect of N and P stimulating periphyton growth, and probably of little practical consequence.

Table 4.2: Results of statistical tests comparing treatments at each site. Shaded cells indicate sites with a statistically significant difference between treatments.

Site	C vs P	C vs N	C vs N+P(+S)	P vs N	P vs N+P(+S)	N vs N+P(+S)
Downstream (grouped)	Equivalent	Equivalent	see below	Equivalent	see below	see below
Upstream (grouped)	Equivalent	Strong evidence	see below	Strong evidence	see below	see below
ds A TR (bank) N+P+S	Equivalent	Equivalent	Strong evidence	Equivalent	Strong evidence	Strong evidence
ds B TL (centre)	Equivalent	Equivalent	Equivalent	Equivalent	Equivalent	Equivalent
us C TL (bank) N+P+S	Equivalent	Strong evidence	Strong evidence	Equivalent [1]	Strong evidence	Strong evidence
us D TR (centre)	Equivalent	Strong evidence	Strong evidence	Strong evidence	Strong evidence	Strong evidence
t-test *p*-values						
ds A TR (bank) N+P+S	0.6	0.8	0.0007	0.8	0.0004	0.006
ds B TL (centre)	1	0.4	0.6	0.2	0.5	0.2
us C TL (bank) N+P+S	0.4	0.006	0.0006	0.06	0.003	0.04
us D TR (centre)	0.7	0.008	0.0002	0.004	0.0001	0.005

Note: Equivalent = t-test *p*-value >0.05, inequivalence test was accepted and equivalence test was accepted.
1 = In this case the bayesian posterior probability that difference is within limits = 4%,
and there was moderate evidence of a difference on log transformed data (p=0.05).

Table 4.3: Results of statistical tests comparing treatments N+P with N+P+S at the upstream and downstream site. Shaded cells indicate sites with a statistically significant difference between treatments.

N+P vs N+P+S	equivalence analysis	t-test *p*-value	Bayesian posterior probability that difference is within limits	mean N+P (mg/m^2)	mean N+P+S (mg/m^2)
Upstream	Equivalent	0.4	37%	150	170
Downstream	strong evidence	0.02	3.3%	112	168
Combined u/s and d/s	moderate evidence	0.02	9.2%	131	169

Note: Equivalent = t-test *p*-value >0.05, inequivalence test was accepted and equivalence test was accepted.
Moderate evoidence = t-test p-value <0.05, inequivalence test was accepted and equivalence test was accepted.

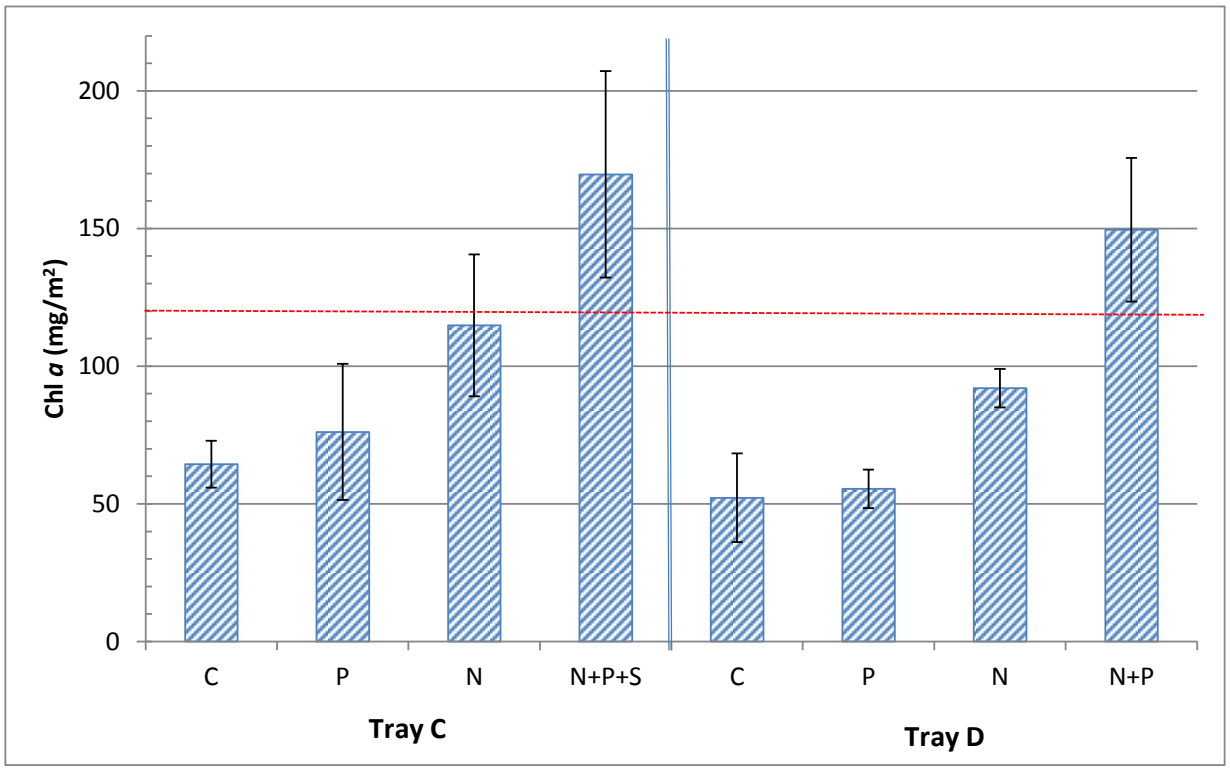

Figure 4.2: Periphyton biomass (measured as chlorophyll *a*) on NDS at upstream site after 12 days of accrual (6 March to 18 March 2013). Error bars are 95 percentiles. The dashed red line is the One Plan periphyton target, included to assist comparison between sites.

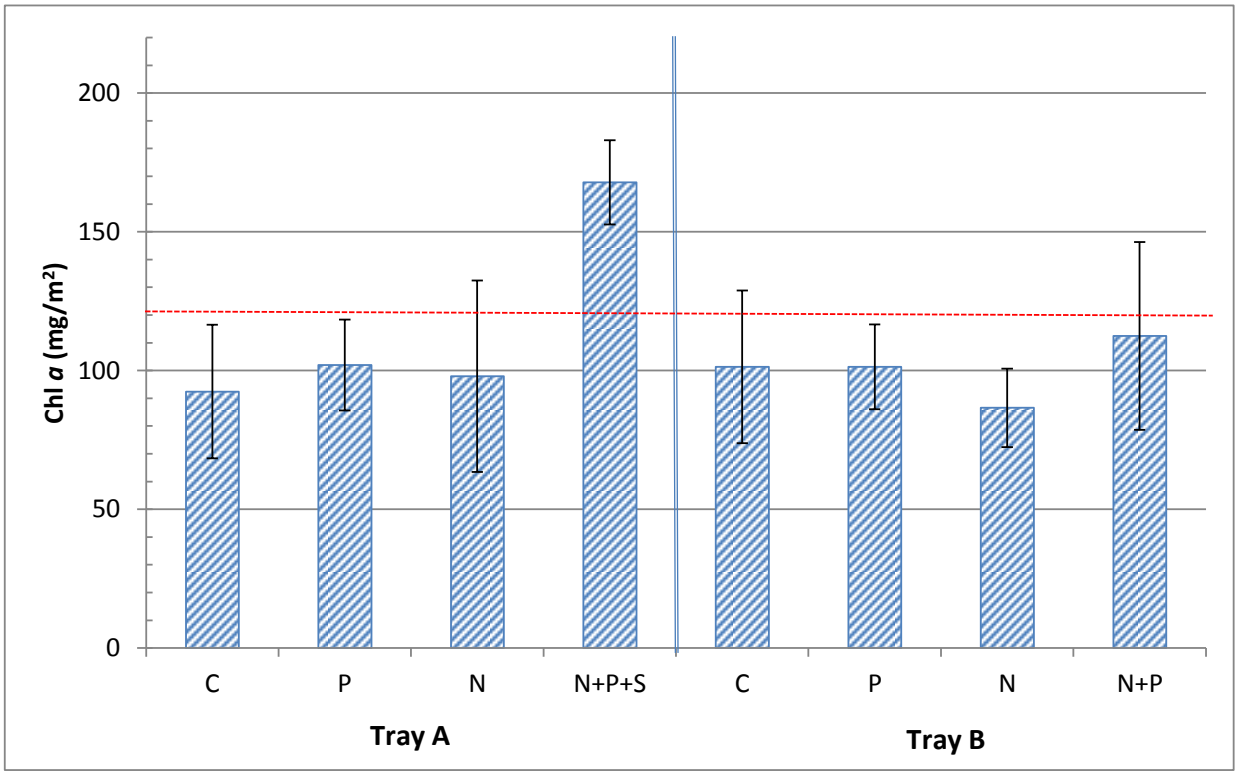

Figure 4.3: Periphyton biomass (measured as chlorophyll *a*) on NDS at downstream site after 12 days of accrual (6 March to 18 March 2013). Error bars are 95 percentiles. The dashed red line is the One Plan periphyton target, included to assist comparison between sites.

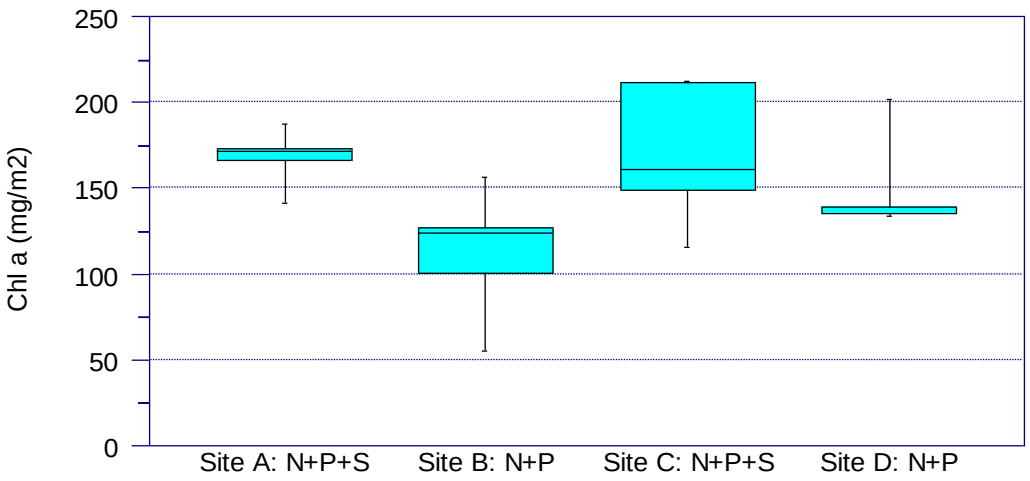

Figure 4.4: Comparison of treatment N+P and N+P+S between trays and sites (6-18 March). The difference between trays A & B (downstream) was statistically significant, but the difference between trays C & D (upstream) was not. The shaded boxes contain 50% of the data; lines in the boxes are median values and whiskers show minimum and maximum values.

Table 4.4: Relative elemental composition of algae (normalised to total dissolved P on a molar basis) compared to the relative mean concentration of dissolved constituents in the river on 20 Dec, 3 Jan, 9 Jan and 16 Jan 2013. Bold values are similar to algal requirements (adapted from Hecky and Kilham 1988).

Element	River u/s (mg/L)	molar mass	River u/s (mols/L)	Composition relative to P	
				River u/s	Algal
N	0.12	14.01	0.0086	**26.53**	11.1
Si	4.45	28.09	0.1584	490.62	96
K	1.2	39.1	0.0307	95.05	1.3
P	0.01	30.97	0.00032	**1.00**	1
Na	8.18	22.99	0.3558	1101.93	0.74
Mg	2.4	24.31	0.0987	305.75	0.66
Ca	15.4	40.08	0.3842	1189.97	0.63
S	2.77	32.07	0.0864	267.50	0.54
Fe	0.156	55.85	0.0028	8.65	0.32
Zn	0.00075	65.39	0.000011	**0.04**	0.012
B	0.022	10.81	0.0020	6.30	0.008
Cu	0.0006	63.55	0.000009	0.03	0.004
Mn	0.0103	54.94	0.00019	0.58	0.003
Co	0.00015	58.93	0.00000	**0.008**	0.003
Mo	<0.0001	95.94	0.000001	<0.003	0.00002

Typical algal composition from Heckey and Kilham (1988)
River concentrations are for dissolved, N = DIN, P = total dissolved P
Cobalt value is median of raw data and error may be >50%.

4.3.2 Comparison between sites

Periphyton grew significantly faster on control and phosphorus treatments at the downstream site compared to control treatments at the upstream site (see Figure 4.5 and Table 4.5). There was no difference in periphyton chlorophyll *a* between sites for N, N+P or N+P+S treatments (see Table 4.5 and Figure 4.4). At the downstream site the chlorophyll *a* was elevated on the C and P treatments to be similar to that of the N treatment – reflecting the supply of nitrogen from the discharge.

The phosphorus and nitrogen regression models described in Biggs (2000) was used to predict periphyton biomass that would be expected due to river nutrient concentrations (SIN and DRP) over the 12-day accrual period of the two NDS bioassays. 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 is used here as a tool to examine the effects of nutrients.

Mean chlorophyll *a* measured on the control treatments was compared with predictions using SIN at the upstream site and DRP at the downstream site (see bold values in Table 4.6). At the upstream sites the NDS controls had less chlorophyll *a* than predicted due to SIN on for the first experiment and more

than predicted for the second experiment. At the downstream site the NDS controls had considerably more periphyton than expected due to DRP for both experiments.

There are a number of possible explanations for the difference between modelled and actual periphyton growth at the upstream site. Periphyton biomass is likely to have been reduced by macroinvertebrate grazing during the first experiment and chironmids tubes were seen on the substrates (see Figure 4.1). In the second experiment, the felt substrates with the petroleum jelly barrier would have restricted the effects of grazing, the felt also appeared to hold algae fragments drifting in the water. Therefore some of the chlorophyll *a* could reflect recruitment from algae drift rather than algae growth. The higher than expected growth at the downstream site could also reflect recruitment of algae drifting in the water; alternatively the DRP concentrations in water samples may be under-estimating the true nutrients available, i.e. periphyton could be obtaining DRP from sediment trapped in the periphyton mat so the control and N treatments were not really P limited (as discussed earlier in this report). This possibility is explored in more detail in Section 5.

When periphyton growth rates are low, grazing by macroinvertebrates can be a very significant factor restricting the accumulation of periphyton biomass. This appeared to be the case during the first experiment in which very little chlorophyll *a* accumulated at the upstream site compared to in the second experiment using felt substrates (i.e. an average biomass at the downstream site of 3.3 mg/m^2 compared to 58 mg/m^2 for the control of the first and second experiment respectively, corresponding to an average chlorophyll *a* accumulation of 0.22 mg/m^2/day and 4.86 mg/m^2/day respectively).

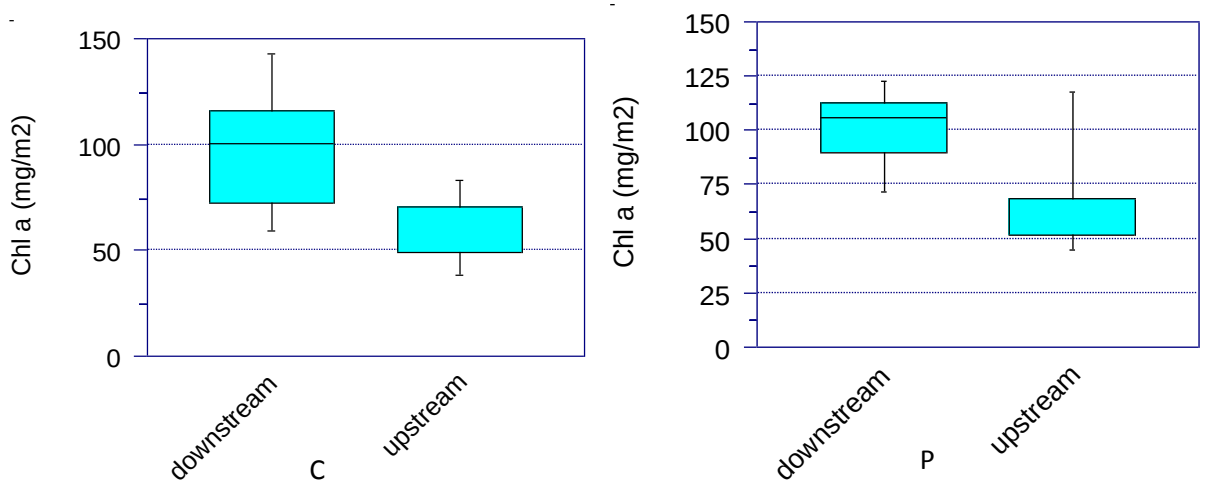

Figure 4.5: Comparison between downstream (trays A & B) and upstream (tray C & D) sites for control treatment (left) and phosphorus treatment (right). The shaded boxes contain 50% of the data; lines in the boxes are median values and whiskers show minimum and maximum values.

Table 4.5: Results of statistical tests comparing upstream and downstream at each treatment. Shaded cells indicate treatments with a statistically significant difference between upstream and downstream.

u/s vs d/s grouped	equivalence analysis	t-test *p*-value	Bayesian posterior probability that difference is within limits	mean upstream (mg/m^2)	mean downstream (mg/m^2)
Control	Strong evidence	0.001	0.5%	58	97
Phosphorus	Strong evidence	0.0007	0.4%	66	102
Nitrogen	Equivalent	0.4	38%	103	92
N+P	Equivalent	0.12	10%	150	112
N+P+S	Equivalent	0.9	56%	170	168

Note: Equivalent = t-test *p*-value >0.05, inequivalence test was accepted and equivalence test was accepted.

Table 4.6: Predicted and actual chlorophyll *a* on NDS control treatment for each period. Chlorophyll *a* concentrations modelled using the equation in Biggs (2000) and the measured concentrations of SIN and DRP. Water quality would predict SIN as most limiting at the upstream site and DRP at the downstream site.

Experiment	site	accrual period	DRP (mg/m^3)	SIN (mg/m^3)	DRP Predicted max Chl *a* (mg/m^2)	SIN Predicted max Chl *a* (mg/m^2)	Measured Chl *a* (mg/m^2)
NDS 14 Feb -1 March 13	u/s	15	12.3	3.74	72	**13**	3.3
NDS 14 Feb -1 March 13	d/s	15	22	755	**96**	182	159.4
NDS 6 March -18 March 13	u/s	12	14	6.8	46	**10**	58.3
NDS 6 March -18 March 13	d/s	12	19.6	757	**54**	112	96.9

Note: SIN and DRP = mean of data for period. Period 14 Feb to 1 March n=10; period 6 March to 18 March n=5.

Pheophytin – degradation

Pheophytin is a degradation product of chlorophyll *a* and the ratio of Phe to Chl *a* can indicate the physical condition for algae. Pheophytin concentration and the percentage of pheophytin to chlorophyll *a* showed the opposite pattern across treatments to that of chlorophyll *a*. This reflected a negative correlation between pheophytin and chlorophyll *a* (see Figure 4.6). There was a negative correlation between log Phe vs. log Chl *a* at the upstream site (r^2=0.54) but not at the downstream site (r^2=0.03). [16]

Samples from concrete tiles had a positive correlation between pheophytin and chlorophyll *a*, so it is surprising that the correlation on NDS substrates was negative. It may indicate the influence of macroinvertebrate grazing at the upstream site removing fresh algae biomass and excreting detritus; the influence of macroinvertebrate grazing would be relatively stronger at low periphyton biomass.

The laboratory found that all samples from trays C and D (18 March) had something interfering with analysis of acidified samples. This may result from the presence of chlorophyll *b* or chlorophyll *c* from

[16] Note that the equivalent data from the tiles showed a weak positive correlation between Phe vs Chl *a*.

particular algae species interfering with the acidification method for calculating chlorophyll *a* and pheophytin (see Stich and Brinker 2005), although it is doubtful that the substrates would have significantly different species composition between upstream and downstream.

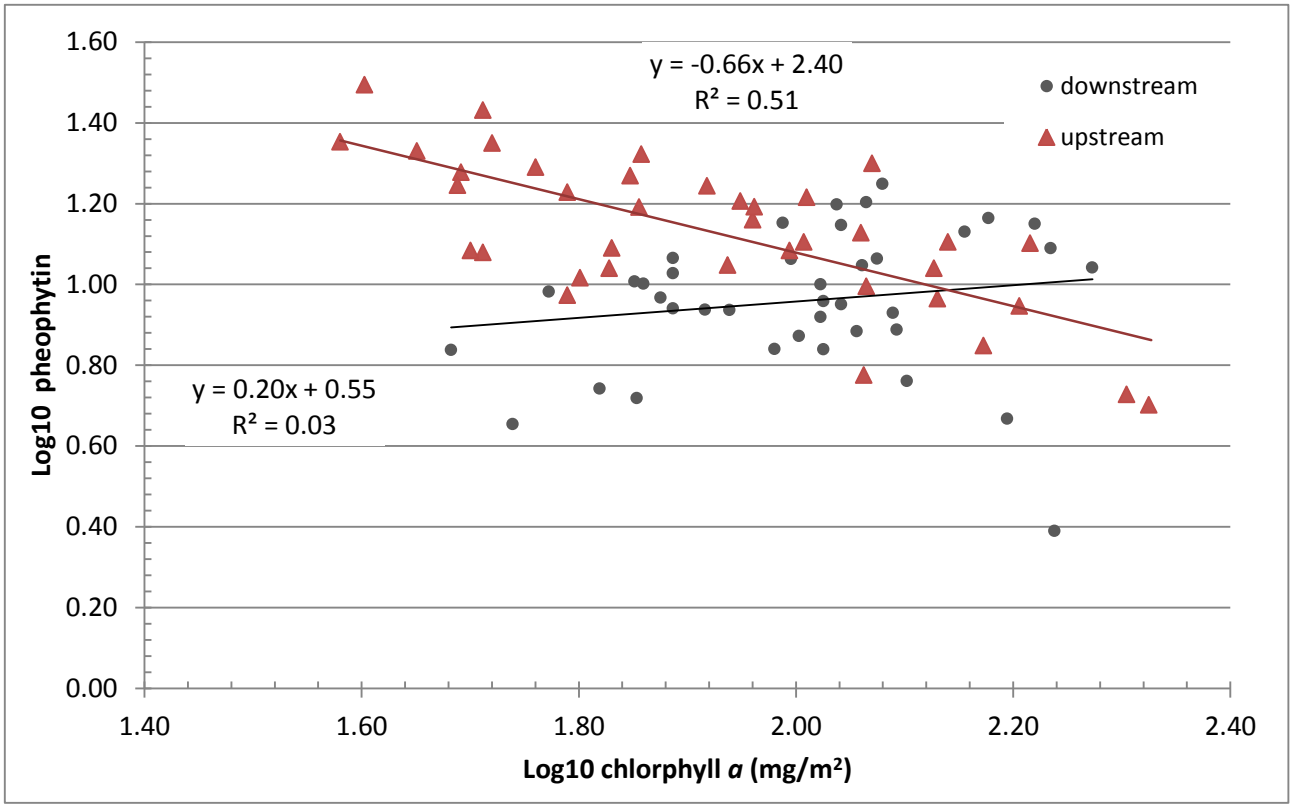

Figure 4.6: Negative correlation between chlorophyll *a* and pheophytin. R^2=0.51 on log transformed data.

4.4 Summary

The key messages from this chapter are:

- The nutrient bioassays indicated that periphyton in the Manawatu River upstream of the WWTP discharge from mid-February to mid-march was primarily limited by nitrogen with secondary phosphorus limitation exhibited when nitrogen was supplied in the N+P and N+P+S treatments. This is consistent with the results of water quality analysis in Chapter 2.3.3.
- Periphyton growth in the river downstream of the WWTP showed a small amount of dual limitation by N+P.
- There was evidence that some characteristic of the sewage stimulated periphyton growth in addition to the N and P; however the effect was small compared to the combined effect of N and P stimulating periphyton growth and of little practical consequence.

- Despite high concentrations of SIN in the river downstream of the discharge, the P treatment did not stimulate periphyton growth – indicating that periphyton was obtaining sufficient DRP from the water and/or by extracting P from sediments trapped in their mat (see next section).

- Periphyton grew significantly faster on control treatments at the downstream site compared to control treatments at the upstream site. The amount of periphyton biomass at the downstream site was more than expected due to DRP in the river water – suggesting either recruitment of drifting periphyton fragments or water samples under-estimating the true nutrients available, e.g. from sediment trapped in the periphyton mat.

- Grazing by macroinvertebrate plays an important role in controlling periphyton biomass. This was highlighted by periphyton accumulation at the upstream site on felt substrates that limited the impact of macroinvertebrate grazing.

- The nitrogen limitation found during experiments in February-March 2013 contrasted with results from April 2012 which found evidence of phosphorus limitation. These different results largely reflect the different river conditions and background concentrations of N and P in the river.

5 Supply of dissolved phosphorus from river sediments

5.1 Introduction

Previous investigations in the Manawatu River have found more DRP in the river than could be explained by loads from the WWTP discharge, furthermore the periphyton growth downstream of the discharge was more rapid than could be explained by measured concentrations of DRP in the water. Hamill (2012) speculated that this could be due to the storage and release of P from river sediments. A number of studies have shown that river sediments can store and release phosphorus (e.g. Stutter et al. 2010) due to changing phosphorus concentrations from sewage discharges. This biogeochemical process can be driven by concentration gradients as well as biological activity and changes in oxygen and pH at the sediment interface (e.g. Afsar et al. 2012)

Extensive and actively growing periphyton can cause substantial diurnal fluctuations in river water pH and dissolved oxygen – increasing during the day and decreasing at night. The magnitude of these fluctuations is greater near and within the periphyton mats, where pH can range up to pH 9.5 or 10. Even under oxygenated conditions high pH can cause phosphorus bound to iron or aluminium within sediments to dissolve and become available for periphyton uptake or release to the water column.

Initial measurements in the Manawatu River (January 2013) found dissolved oxygen in river pore water to be reasonably well oxygenated (e.g. about 5 mg/L), so the focus of our investigations have been on the effect of pH fluctuations on release of dissolved phosphorus. This chapter presents the results of three simple investigations:

- Spatial change in stream pH and nutrients between the periphyton interface and flowing river water;
- Sampling water quality of pore water amongst the river gravels;
- The effect of pH on the release of dissolved phosphorus from sediment deposited on and trapped within the periphyton mat.

5.2 Methods

5.2.1 Phosphorus and pH at periphyton interface

To assess the extent to which periphyton can change pH in their immediate environment, field measurements of pH and dissolved oxygen (DO) were taken in the Manawatu River 800 to 1000m downstream of the WWTP discharge during mid-afternoon on 10 January and 29 January 2013. The measurements were made in a range of habitats including runs, riffles and directly adjacent to the periphyton mats.

DRP and pH in water samples collected from immediately above/within periphyton mats were compared with that in samples collected at the same time from the river. It was hypothesised that a higher DRP concentration at the periphyton interface would indicate release of nutrients from the

sediment to the water column while a lower concentration would indicate periphyton were 'hungry' for nutrients from the water column and possibly also the sediment.

Water samples were collected at the site about 800m downstream of the discharge on 29 January 2013 during a period of low flows and high periphyton cover. A large gauge syringe was used to collect six (6) replicate samples from immediately above and amongst periphyton mats, and two (2) replicate samples from the over lying river water at about 30 to 40cm depth. Sampling occurred about 3:15pm on a fine day to coincide with a high photosynthetic activity. The periphyton sampled was dominated by either *Phormidium* sp. or *Stigeoclonium* sp.

Water samples were stored in a chilli-bin and transported to CEL Laboratories for analysis of dissolved reactive phosphorus and pH. Field measurements were made of pH and dissolved oxygen.

5.2.2 Water quality of river sediment pore-water

The Manawatu River has zones of down-welling where some of the river water flows through the river gravels and zones of upwelling where it returns back to river. As the water flows through river gravels there is potential for both nutrient removal (e.g. through denitrification) or increase of nutrients as some dissolved nutrients in pore-water are carried back to the river.

The pore-water amongst river gravels was sampled at the sites 1000m upstream of the discharge (TR) and 800m downstream of the discharge (TR) and the water quality compared with samples collected at the same time from the overlying river water[17]. Samples were collected in the early afternoon on 7 March 2013.

Pore-water samples were collected from a depth of 25-28cm below the river bed using a stainless steel piezometer rammed into the river bed. The piezometer was purged using a hand pump by removing at least four times the volume of the tube prior to collecting the sample. Overlying water above the sample points was between 15 and 25 cm deep.

Five replicate samples were collected from the downstream site but only one sample was collected from the upstream site due to equipment failure. Duplicate river water samples were collected from both sites before and after completing the pore water sampling.

Prior to sampling it was confirmed that there were zones of down-welling in sections of the Manawatu River above riffles at both the upstream and downstream site. This was done using a simple test of digging a hole about 0.5m from the water's edge, allowing time (about 5-10 minutes) for water level in the holes to stabilise and measuring the difference in water level compared to that of the river[18]. Above riffles, the water in holes at the river edge was about 5-15cm lower than the river water – suggesting a zone of down-welling water; below riffles and along runs there was little difference in water level.

[17] Note that the upstream site was 200m upstream of where concrete tiles had been place.
[18] The method assumes a strong connection between the between shallow groundwater and surface water through the gravel/cobble riverbed. This seemed reasonable because of their water drained quickly out of the holes.

5.2.3 Release of phosphorus from sediment trapped within the periphyton mat

Sediment trapped within the periphyton mat itself was sampled to assess the extent to which this trapped sediment released dissolved phosphorus in response to pH changes. This provided an estimate of phosphorus release from sediment in direct contact with periphyton cells.

Prior to undertaking this experiment (on 6 March 2013) a pilot experiment had been carried out on 10 January 2013) by collecting a suspension of deposited sediments from the stream bed (using the Quorer sampling technique (Harding et al. 2009)) and assessing the effects of pH changes on dissolved nutrients. The sampling method avoided sediment trapped within the periphyton mat. Therefore the extent of pH fluctuations experienced by collected sediment was uncertain. The results are not presented in this report, but were similar to the results of sediment trapped within the periphyton mat, i.e. a pH increase released more dissolved P and dissolved aluminium and the effect was strongest at the downstream site.

Sediment samples were collected on 6 March 2013 from the sites 1000m upstream of the discharge (TR bank at 11am) and 800m downstream of the discharge (TR bank at 8:30am). Five (5) replicate samples were collected from each site by selecting cobbles covered in periphyton and using distilled water to flush the fine sediments out of the periphyton mats, through a 500µm net (to exclude coarse material or invertebrates) and into a sample container. Sediment from one to three cobbles were bulked for each replicate forming a total sample area for each replicate of between 160cm^2 to 295 cm^2 (see Figure 5.1 for example of cobbles sampled). Gloves were worn to minimise potential contamination.

All cobbles were collected from flowing water 5cm to 30cm deep. The dominant periphyton species on the cobbles sampled at both upstream and downstream sites was the filamentous green algae *Cladophora* sp. (generally about 1-2cm length).

The dimensions and shape of each cobble were recorded to allow the sample area to be estimated using an online polygon area calculator (http://www.mathsisfun.com/geometry/area-polygon-drawing.html). This allowed the results to be expressed in terms of square metres.

Samples were stored in a cool, dark chilli-bin and transported to CEL Laboratories. At the laboratory, samples of sediment suspension were mixed and analysed for: total suspended solids (TSS), volatile suspended solids, total aluminium (Al), dissolved Al, total iron (Fe), dissolved Fe, total calcium (Ca), dissolved Ca, total phosphorus (TP), dissolved reactive phosphorus (DRP), nitrate-nitrite nitrogen (NNN), total ammoniacal nitrogen, total nitrogen (TN), pH, dissolved oxygen and electrical conductivity.

Sub-samples were taken of the suspensions of trapped sediment and treated as follows: a) adjust to pH 8.5, b) adjust to pH 9.5. The pH was adjusted using a sodium hydroxide solution[19], were mixed and left for half an hour, than sampled, filtered (0.45 micron filter) and analysed for: pH, dissolved Al, dissolved Fe, dissolved Ca, and DRP.

[19] Adjusting pH using bicarbonate would have probably better represented processes influencing pH in the river but sodium hydroxide allowed a much more stable pH adjustment.

Figure 5.1: Selected cobbles from which sediment deposited within the periphyton mat were sampled (upstream site on left, downstream site on right). The dominant periphyton species on these cobbles was *Cladophora* sp.

5.3 Results and discussion

5.3.1 Phosphorus and pH at periphyton interface

On 10 January 2013 pH and DO in the river water close to the surface of periphyton mats were 8.4 and 131% respectively in the mid-afternoon compared to 7.4 and 87% respectively in the early morning (7am). These measurements indicated the extent of pH and DO shifts caused by photosynthetic activity. Measurements on 29 January indicated a high level of photosynthetic activity with high pH (up to 9.6) and high DO (up to 125%) especially in shallow water and close to the surface of periphyton mats (Table 5.1).

Water samples collected 29 January) showed lower DRP concentrations within and immediately above the periphyton mat than in the overlying river water (see Figure 5.2, median of 0.004 mg/L in the mat and 0.017 mg/L in the river water[20]). The samples from the periphyton interface also had slightly lower pH (pH 8.1 compared to 8.4), which is surprising considering samples were collected about 3pm in the afternoon and close to peak photosynthetic activity. The difference was less than the standard deviation and may have been caused by sediment within the algae mat being entrained with the sample.

The results indicate that, during this period, the periphyton at the downstream site was taking dissolved phosphorus from the river water rather than releasing P to the water column, i.e. the periphyton was hungry for P. This is consistent with observations during January 2013 of periphyton increasing in biomass (actively growing) and generally low DRP concentration in the surrounding river water (see Chapter 2).

[20] River samples at the downstream site on the following day had lower DRP, i.e. 0.006 mg/L.

Table 5.1: *In-situ* field measurements of pH, dissolved oxygen and temperature in the Manawatu River and amongst periphyton on 29 January 2013, 3:30pm.

Manawatu River d/s WWTP	Depth (cm)	pH	DO %	DO (mg/L)	temperature (°C)
main river flow 800m d/s	35	8.5	122.0	10.21	22.8
main current d/s riffle 1000m d/s	30	8.85	136.2	11.7	23.2
over riffle 950m d/s	20	9.11	140.0	11.82	24.2
among periphyton	10	9.5	142.0	12.2	25.3
backwater at periphyton interface	10	9.61	124.5	10.12	26.7
Periphyton interface	20	9.36	160.5	13.7	23.5

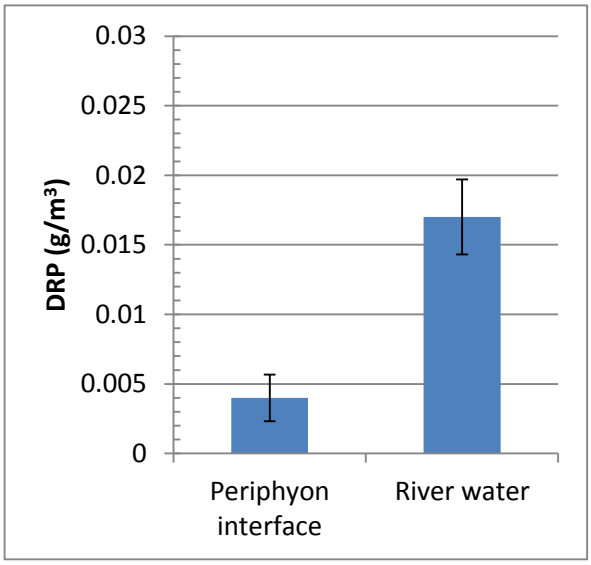

Figure 5.2: Comparison of DRP at the periphyton interface and overlying river water (29 January 2013). Error bars are two standard errors for the periphyton interface and the range for river water samples.

5.3.2 Water quality of river sediment pore-water

The pore-water of river sediments at the upstream site had more SIN and DRP than in the river as would be expected (Table 5.2). At the downstream site pore water had similar DRP but less SIN compared to river water - suggesting some attenuation or denitrification may be occurring in the river sediments. The water quality of pore-water reflected that of the river water with higher concentrations of dissolved nutrients at the downstream site compared to upstream (Table 5.2). The pore-water pH was neutral (7.6 and 7.3 at the upstream and downstream site respectively) and reasonably well oxygenated (DO = 5.9 mg/L).

The results suggest that down-welling river water flowing through the gravel substrate at the upstream site could pick up some additional SIN and DRP to support periphyton growth when the water returns to the river. However there was no evidence of pore water contributing to dissolved nutrients in the river at the downstream site. Instead the there is some evidence of possible denitrification occurring in

the river sediments at the downstream site. These results are only indicative because of the lack of replicate samples at the upstream site.

Table 5.2: Median water quality in pore water of river gravels (6 March 2013).

Site	DO (g/m3)	pH	NH_4-N (g/m3)	NNN (g/m3)	SIN (g/m3)	Total N (g/m3)	DRP (g/m3)	TP (g/m3)	Al diss (g/m3)	Al Total (g/m3)	Fe diss (g/m3)	Fe Total (g/m3)
Pore water downstream	5.9	7.3	0.457	0.185	0.763		0.0307	1.253				
Pore water upstream		7.6	0.000	0.042	0.042		0.0180	0.928				
River downstream		7.8	0.801	0.069	0.870	1.176	0.0288	0.037	0.075	0.115	0.034	5.50
River upstream		7.7	0.000	0.008	0.008	0.089	0.0079	0.017	0.012	0.029	0.042	6.26
Pore water downstream 2x standard error		0.213	0.290	0.212	0.255		0.0043	0.633				

n=5 for downstream porewater, n=2 for river water, n=1 for upstream porewater.

5.3.3 Release of phosphorus from sediment trapped within the periphyton mat

Characteristics of trapped sediment at each site

The water quality results from replicate samples of trapped sediment before and after adjusting pH upward are shown in Appendix 4.

The downstream site was characterised by slightly less sediment than upstream and a sediment quality less rich in iron. Organic matter (VSS) comprised about 9% of the sampled sediment at both sites which gives confidence that responses to pH changes were being caused by inorganic sediment fractions (see Table 5.3 and 5.4). There was evidence of effects of the WWTP discharge on sediment quality through higher concentrations of total ammoniacal N and slightly lower pH. Median total phosphorus was higher downstream but the difference was not statistically significant (Table 5.3 and 5.4).

Although there was similar or more total phosphorus in the trapped sediment at the downstream site, there was considerably less DRP and a lower ratio of DRP:TP (see Figure 5.3 and Figure 5.4). Even after the pH was increased to 8.5 and 9.5 there was less DRP at the downstream site compared to the unadjusted samples upstream (Figure 5.4). Periphyton growth at the upstream site was limited by low concentrations of SIN (see discussion on nutrient limitation in chapter 4), which would account for why the DRP within the periphyton mat at the upstream site not being utilised. In contrast the downstream periphyton had no N limitation, allowing faster periphyton growth and more demand for DRP which would be extracted from the surrounding water.

The upstream site had more total aluminium (Al) and there was no significant difference in the concentration of dissolved aluminium between sites, suggesting that Al associated with natural river sediments (e.g. clay) had more effect on sediment quality than any residual alum from the discharge (Table 5.4).

Table 5.3: Median water quality data from suspension of trapped sediment before and after pH adjustment. Note that all concentrations are per unit area (m2) of river bed.

Site & treatment	pH	sample area (cm2)	Al Total (mg/m2)	Fe Total (mg/m2)	Ca Total (mg/m2)	Al dissolved (mg/m2)	Fe dissolved (mg/m2)	Ca dissolved (mg/m2)	DRP (mg/m2)	TP (mg/m2)	NH4-N (mg/m2)	SIN (mg/m2)	TSS (mg/m2)	%VSS
d/s original	6.75	227.6	1210.5	1750.6	982.0	2.47	1.46	180.8	0.395	60.72	13.05	16.68	161262	8.6%
d/s pH 8.5	8.52	227.6				4.45	1.54	194.2	0.800					
d/s pH 9.5	9.48	227.6				9.78	1.69	131.9	1.358					
u/s original	6.97	175.4	2525.5	4287.6	1349.3	1.95	3.33	153.9	2.240	53.42	5.42	5.93	186354	9.2%
u/s pH 8.5	8.51	175.4				2.78	3.88	130.7	2.155					
u/s pH 9.5	9.51	175.4				3.11	3.68	113.0	2.828					

Site & treatment	Al:TSS	Ca:TSS	Fe:TSS	TP:TSS	DRP:TSS	DRP:TP	DRP released (mg/m2)	diss Al released (mg/m2)	diss Ca released (mg/m2)	diss Fe released (mg/m2)	% change DRP	% change diss Al	% change diss Ca	% change diss Fe
d/s original	0.911%	0.0073	1.38%	0.036%	0.0003%	0.651%								
d/s pH 8.5							0.376	1.977	-4.71	0.089	102.2%	80.6%	-3.8%	8.7%
d/s pH 9.5							0.702	7.304	-40.06	0.098	243.3%	295.2%	-22.9%	3.6%
u/s original	1.175%	0.0066	1.99%	0.025%	0.0012%	4.749%								
u/s pH 8.5							-0.436	0.258	-16.26	0.221	-23.5%	16.1%	-11.1%	8.2%
u/s pH 9.5							0.481	0.950	-33.26	0.078	12.5%	35.2%	-25.5%	2.0%

Table 5.4: Strength of evidence of a statistical difference in trapped sediment chemistry between sites upstream and downstream of the discharge. Shaded cells show some evidence of a statistically significant difference.

Variable	raw data	pH 8.5	pH 9.5
pH	Moderate evidence ds<us [1]	equivalent	equivalent
TP	equivalent	na	na
DRP	Strong evidence ds<us [note 3]	equivalent	equivalent
SIN	Strong evidence ds>us	na	na
TSS	equivalent	na	na
VSS	equivalent	na	na
Al dissolved	equivalent	Strong evidence ds>us	Strong evidence ds>us
total Al	Strong evidence ds<us	na	na
dissoved Ca	equivalent	equivalent	equivalent
total Ca	Strong evidence ds<us	na	na
dissolved Fe	Strong evidence ds<us	Strong evidence ds<us	Strong evidence ds<us
total Fe	Strong evidence ds<us	na	na
Al:TSS	equivalent	na	na
Ca:TSS	equivalent	na	na
Fe:TSS	Strong evidence ds<us	na	na
TP:TSS	equivalent	na	na
DRP:TP	Strong evidence ds<us	na	na
DRP released	na	Strong evidence ds>us	equivalent [note 2]
Al released	na	Strong evidence ds>us	Strong evidence ds>us
Fe released	na	equivalent	equivalent
Ca released	na	equivalent	equivalent

1. pH difference was only meaningful at 5%
2. DRP released, bayesian posterior probability that differnence was within limits was <4%
3. A significant difference found on square root transformed data only.

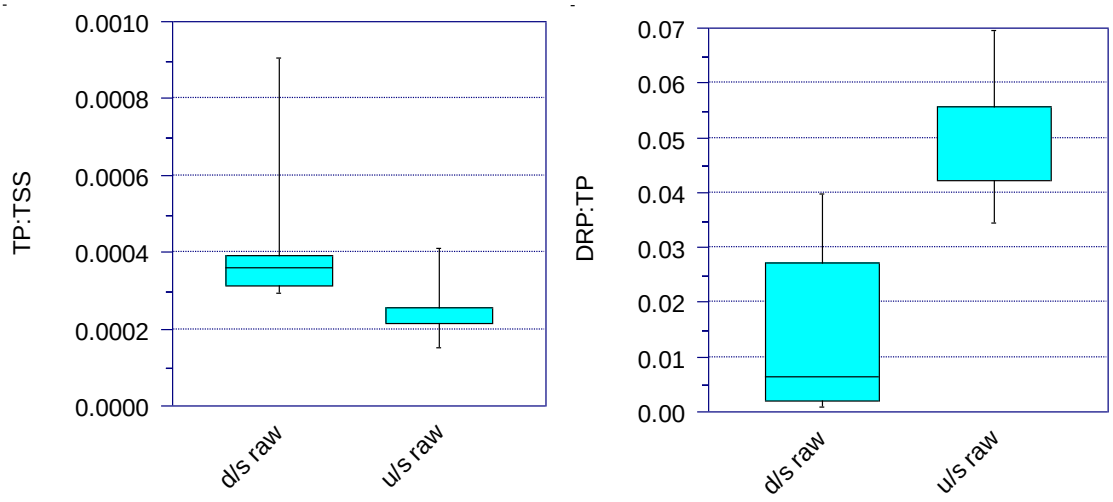

Figure 5.3: Box plot showing ratio of TP:TSS and DRP to TP in the suspensions of trapped sediment comparing sites and treatments. The shaded boxes contain 50% of the data; lines in the boxes are median values and whiskers show minimum and maximum values.

Effect of pH on dissolved nutrients from trapped sediment

There was considerable variation in DRP and dissolved metals between replicates at each site (see Figure 5.4 to 5.7), so the effect of pH treatments is most easily seen by pairing treatments for each replicate and comparing the differences (see Figure 5.8 to 5.12). Statistical analysis compared paired samples of the square root transformed data and the binomial probability statistic was calculated based on whether the pH treatment caused an increase or decrease in concentration (assuming the probability of no effect was 50:50) (see Table 5.5).

Increasing the pH of trapped sediment from the downstream site to 8.5 caused a significant increase in DRP and dissolved aluminium (Al). An increase to pH 9.5 caused more to be released (see Figure 5.8 to 5.10 and Table 5.5). The effect of pH on dissolved Fe and Ca in downstream sediment was equivocal, with more iron released at pH 8.5 but not significantly more at pH 9.5. Dissolved calcium declined as pH increased to 9.5 (Figure 5.11 – 5.12).

Trapped sediment from the upstream site showed a different response to increases in pH. There was no significant increase in DRP or Al at pH 8.5, but there was a response at pH 9.5 (Figure 5.8 to 5.10, Table 5.5). The absolute amount of DRP released from upstream sediment at pH 9.5 was similar to that released from downstream sediment at pH 8.5, but the percent increase was very small (a 243% increase at the downstream site compared to only a 13% at the upstream site), perhaps because DRP within the upstream mats was already high (Figure 5.8 and 5.9).

Dissolved iron did not respond to the pH increases at the upstream site and dissolved calcium declined as pH increased to 9.5.

The downstream sample with the highest DRP (and TSS) came from a backwater where the surrounding cover of periphyton was relatively sparse. The percentage of DRP:TP in this replicate was 4% which was

closer to the upstream percentage (median 4.7%) than the downstream (median 0.7%). This sample also had the greatest release of DRP due to pH which suggests that the strong response to pH at the downstream site was due to characteristics of the sediment (e.g. the influence of alum floc) rather than being related to a lower initial DRP concentration.

Periphyton is known to use P from sediment on the river bed. Both the surface-bound organic-P and inorganic-P are readily taken up by algae that are in contact with sediment. Stream bed sediments are also know to release a small fraction (about 2%) of PP into the river water as DRP by mineralisation, desorption and/or reducing conditions (Hedley 1978 in Parfitt et al. 2007). The pH driven response caused by our experiment is consistent with this finding (i.e. releasing 1-2% of the particulate P as DRP).

The weak response of sediment at the upstream site to releasing DRP compared to the downstream site may reflect the ability of natural river sediments to bind phosphorus more tightly. P in sediment derived from stream bank material is less available to algae than P in sediment from farm runoff (McDowell and Wilcock 2007, Hedley 1978). Afsar et al. (2012) found that freshly sorbed phosphates can be readily desorbed from soil colloids, and the same is likely from residual alum floc discharged in the effluent.

Extent to which trapped sediment meet periphyton growth requirements

This experiment was not designed to estimate the flux of P from trapped sediments. Nevertheless calculations indicate that the quantity of DRP released during a half hour extraction by solely elevating pH was in the ball park to account for a large portion of the periphyton growth requirements. Our experiment found up to 0.7 mg P/m^2 was released from trapped sediments during a half hour extraction. This source of P might be available for about 8 hours a day when photosynthesis is causing high pH. Recent experiments in the Tukituki River have found that periphyton (incl. *Stigeoclonium* sp. and *Cladophora* sp.) had DRP uptake rates typically less than 0.62 mg/m^2/hr (90^{th} percentile of chamber experiments) and that the DRP uptake rate was similar during both day and night (John Quinn pers. comm. 2013). Thus the release from trapped sediment appears to be sufficient to meet day time growth requirements. This assumes that longer exposures to high pH will continue to extract DRP from the sediment, but this assumption seems reasonable considering that the trapped sediments were not 'fresh' and had already been exposed to diurnally high pH in the periphyton mats which may have depleted the supply of extractable phosphorus prior to collection. Further work is required to better understand the kinetics of P release (e.g. experiments using a time series of pH adjustments).

Another way to assess whether the magnitude of what is supplied by the sediment is sufficient to support periphyton growth is to convert the areal concentration (mg/m^2) to a volume concentration (mg/m^3), and compare with periphyton nutrient requirements. Assuming a depth of 3cm of overlying water influencing the periphyton then the values in Table 5.3 can be multiplied by 12 to convert to mg/m^3. At the downstream site this gives a theoretical initial concentration of SIN and DRP of 200 mg/m^3 and 4.7 mg/m^3 respectively, i.e. indicative of potential DRP limitation[21] but minor SIN limitation. At the upstream site this calculation gives a theoretical initial concentration of SIN and DRP of 71 mg/m^3 and 27 mg/m^3 respectively, i.e. indicative of potential N limitation but not DRP limitation.

[21] Note that at a cellular level much lower concentrations of DRP may be need to for P saturated growth e.g. 2-4 mg/m^3 (Bothwell 1989).

Table 5.5: Strength of evidence of a statistical difference between raw data and pH adjusted treatments of sediment trapped by periphyton. Shaded cells show some evidence of a statistically significant difference. The results are for a paired sample equivalence test on square root transformed data, and from a binomial probability test.

Paired sample equivalence test on square root transformed date				
	raw vs pH8.5	**raw vs pH8.5**	**raw vs pH9.5**	**raw vs pH9.5**
Variable	**d/s**	**u/s**	**d/s**	**u/s**
DRP	strong evidence raw<pH8.5	equivalent	strong evidence raw<pH9.5	equivalent
Dissolved Al	strong evidence raw<pH8.5	equivalent	strong evidence raw<pH9.5	strong evidence raw<pH9.6
Dissolved Fe	equivalent	equivalent	equivalent	equivalent
Dissolved Ca	equivalent	equivalent	strong evidence raw>pH9.5	strong evidence raw>pH9.6
Binomial probability test				
DRP	**0.03**	0.16	**0.03**	**0.03**
Dissolved Al	**0.03**	0.16	**0.03**	**0.03**
Dissolved Fe	**0.03**	0.3	0.3	0.16

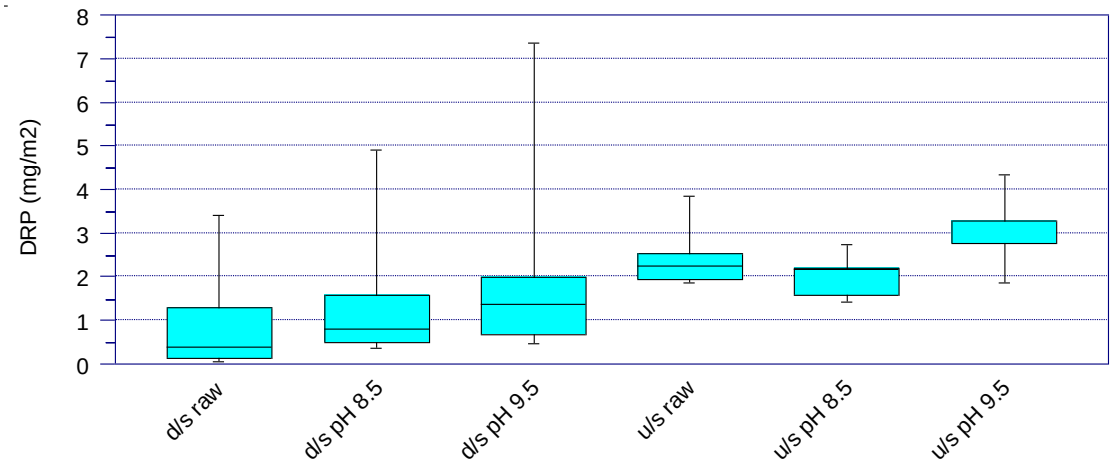

Figure 5.4: Box plot of DRP in the suspensions of trapped sediment comparing sites and treatments. The shaded boxes contain 50% of the data; lines in the boxes are median values and whiskers show minimum and maximum values.

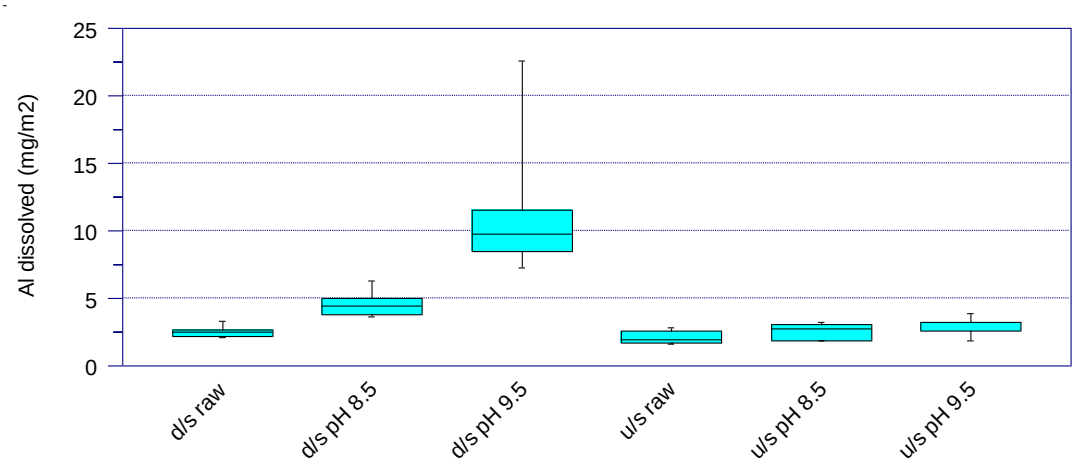

Figure 5.5: Box plot of dissolved aluminium in the suspensions of trapped sediment comparing sites and treatments.

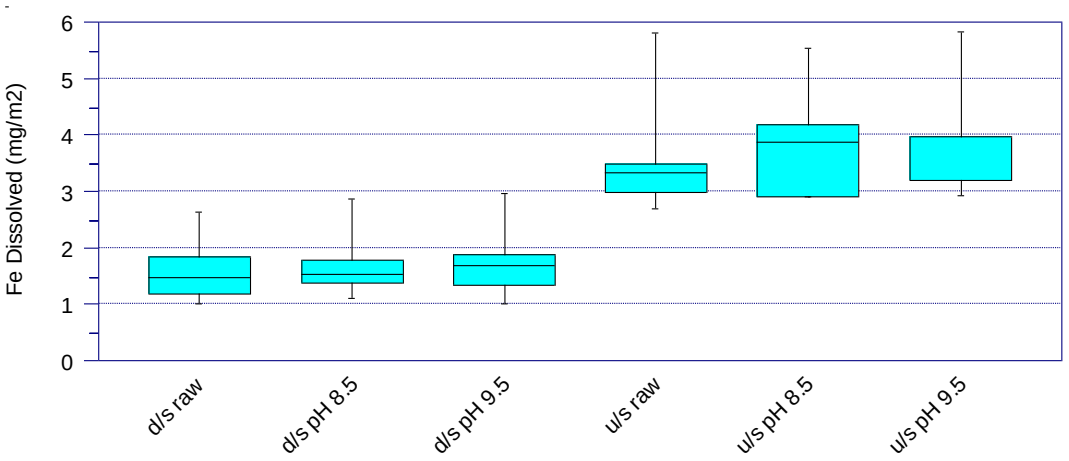

Figure 5.6: Box plot of dissolved iron in the suspensions of trapped sediment comparing sites and treatments.

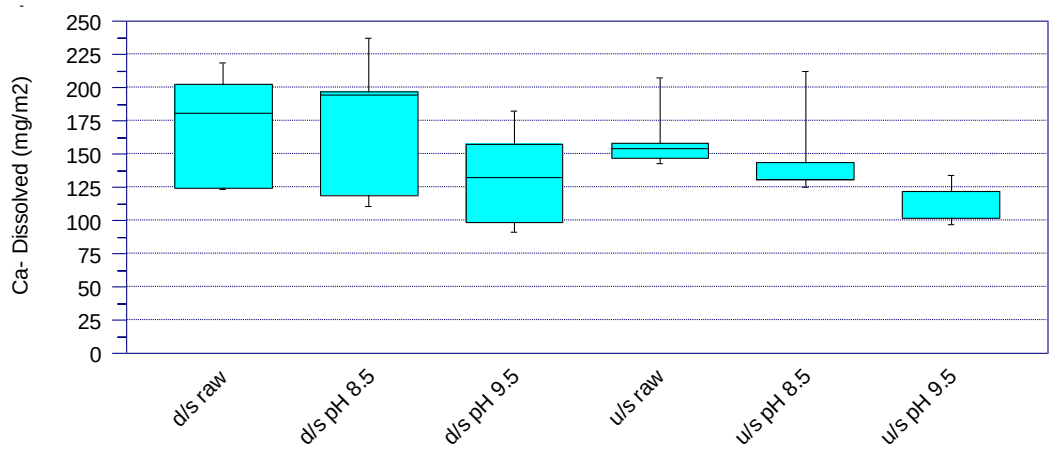

Figure 5.7: Box plot of dissolved calcium in the suspensions of trapped sediment comparing sites and treatments.

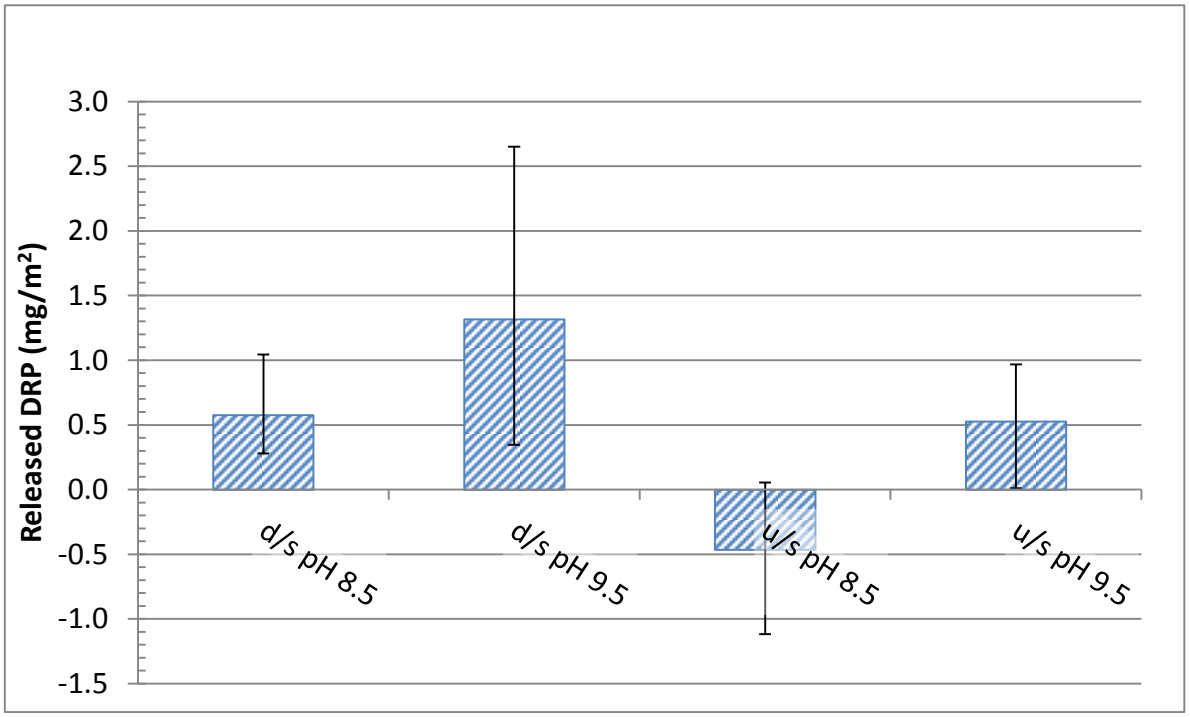

Figure 5.8: Average change in DRP in the suspensions of trapped sediment due to increasing the pH. Upper error bar is two times the standard error, lower error bar is minimum value. Note that these average values are larger than the median values presented in Table 5.3.

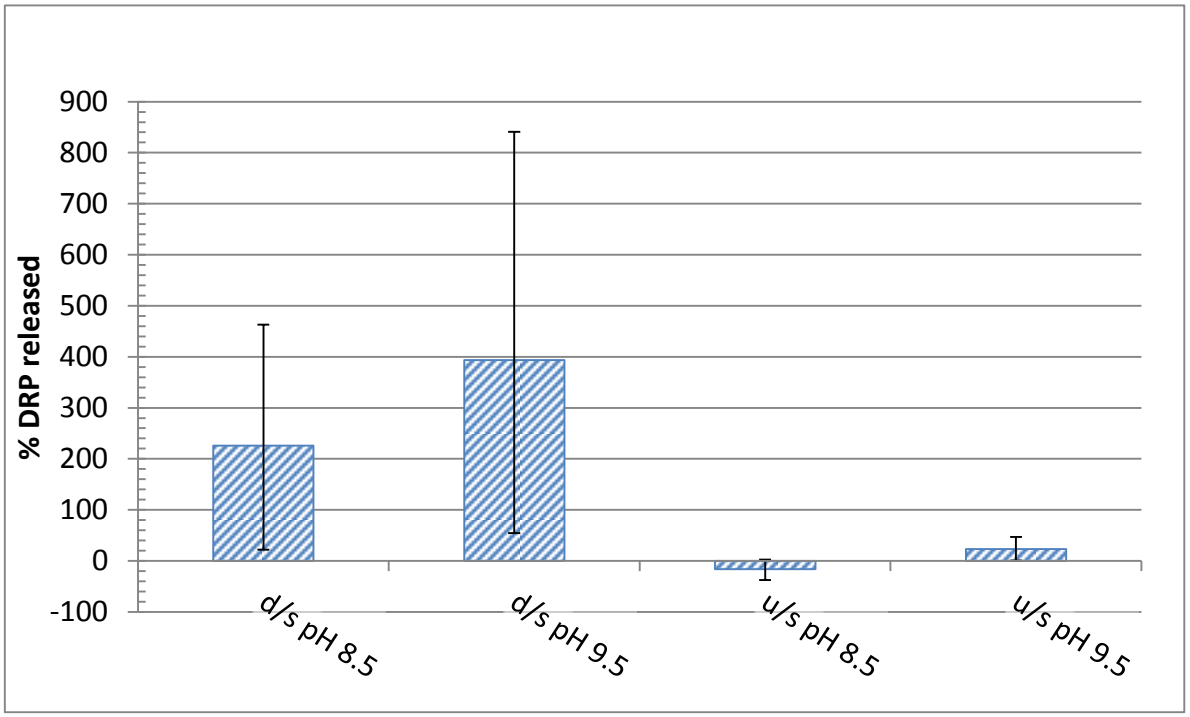

Figure 5.9: Average percent change in DRP in the suspensions of trapped sediment due to increasing the pH. Upper error bar is two times the standard error, lower error bar is minimum value. Note that these average values are larger than the median values presented in Table 5.3.

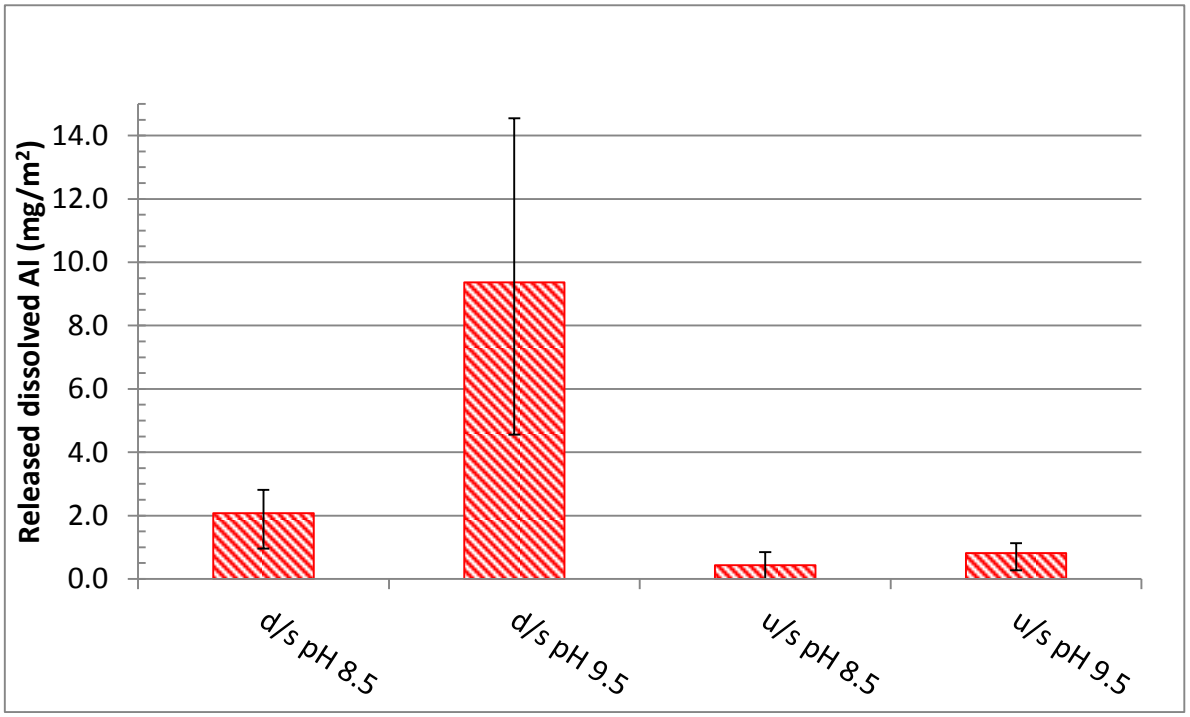

Figure 5.10: Average change in dissolved aluminium in the suspensions of trapped sediment due to increasing the pH. Error bars and two times the standard error. Note that these average values are larger than the median values presented in Table 5.3.

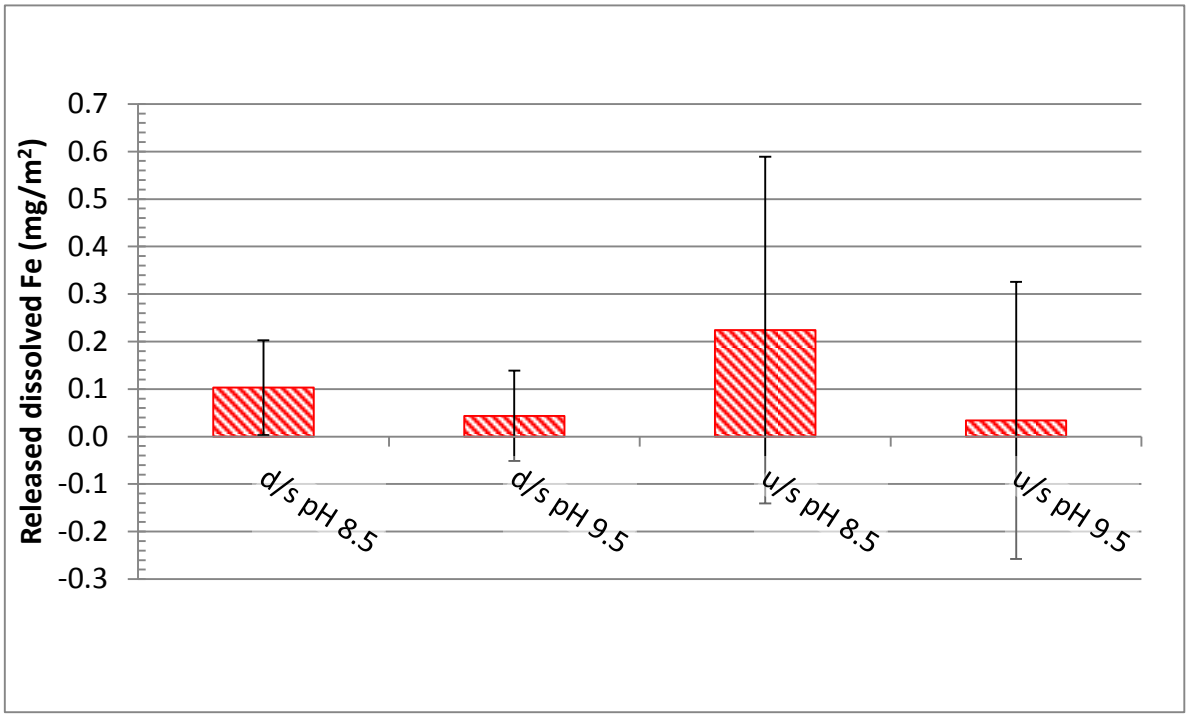

Figure 5.11: Average change in dissolved iron in the suspensions of trapped sediment due to increasing the pH. Error bars and two times the standard error. Note that these average values are larger than the median values presented in Table 5.3.

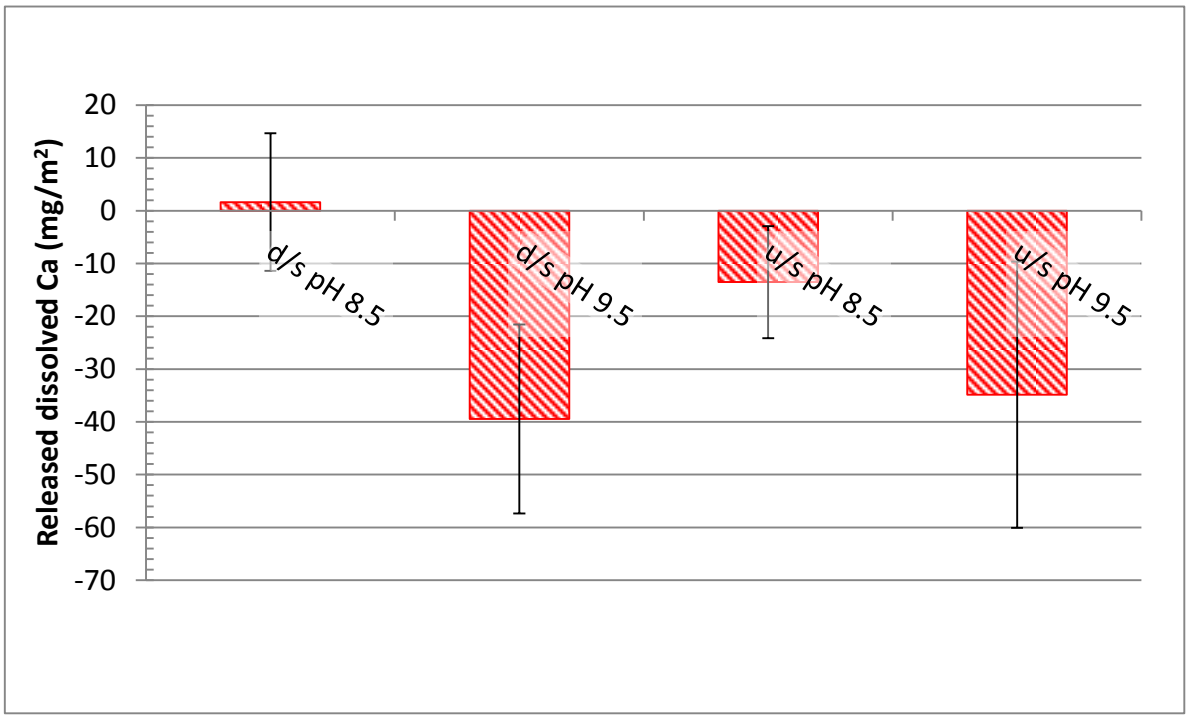

Figure 5.12: Average change in dissolved calcium in the suspensions of trapped sediment due to increasing the pH. Error bars and two times the standard error.

Periphyton ability to capture sediment

It is well known that periphyton is effective at trapping fine sediment within their mats. Davies-Colley et al. (1992) found that periphyton were effective at trapping fine suspended clay despite the clay's extremely low settling velocities (<1 µm/s). This decreased the organic content of the periphyton from 19% to 8.5%. It is probably that fine floc from alum that is released with the WWTP discharge may be trapped in the periphyton mat. Although there was no significant difference in turbidity in the river upstream and downstream of the WWTP (see Table 2.2), there is indirect evidence that more inorganic sediment was being trapped by periphyton and settling on the river bed downstream of the WWTP discharge.

Firstly, more fine sediment was observed to have accumulated inside the nutrient diffusing substrate trays at the downstream site (about 10mm) compared to the upstream site (about 2mm) (L. Brown pers. comm. 2013).

Secondly, although there was no noticeable difference in the organic matter content of periphyton on tiles upstream and downstream of the discharge (a mean of 13 % at both sites, see Appendix 2), when the data were filtered to compare similar amounts of biomass between sites (i.e. AFDM between 10 and 40 g/m^2) there was significantly[22] lower % organic content at the downstream site (i.e. a mean of 17.2 and 12.1 g/m^2 respectively upstream and downstream). This was a localised effect and the organic content in periphyton 3.8km downstream near Longburn was similar to that at the upstream site. This

[22] Equivalence test = strong evidence of a practically important difference; t-test *p*-value = 0.002.

suggests that periphyton within about 1km of the discharge was trapping more inorganic sediment for a given biomass compared to upstream.

Thirdly, the trapped within periphyton mats at the downstream site had a lower pH compared to that upstream which would be consistent with an influence of alum floc from the effluent discharge.

During the initial phases of growth periphyton require only very low nutrient concentrations (e.g. 2-4 mg/m^3 for diatom growth in Canterbury Biggs 1990). As the periphyton mat becomes thicker cells within the mat become limited and higher concentrations of phosphorus in the water is required to maintain the growth rate of those deeper in the matrix by increasing the supply rate (Bothwell 1989). Thus the phosphorus guidelines to prevent the excessive growth of periphyton mats are many times higher than what is needed for thin layers of individual cells. Periphyton will be much less reliant on river phosphorus concentrations if it can trap sediment within its mat as it grows and then extract dissolved P from the sediment.

The thicker periphyton mats grow the more sediment they trap. This was evidenced by the inorganic content of periphyton mat (i.e., the ash mass in Appendix 2) increasing with periphyton biomass (AFDM) (r^2=0.58, n=22 at the downstream site). The percent organic content also increased with biomass (AFDM) (r^2 =0.4 and 0.3 at for upstream and downstream sites respectively). This explains why there was no noticeable difference in the organic matter content of periphyton on tiles upstream and downstream of the discharge (a mean of 13 % at both sites) if the data were not first filtered.

5.4 Summary

The key messages from this chapter are:

- Periphyton can cause large pH fluctuations in surrounding river water (measured up to pH 9.6 close to the periphyton mat). During January 2013 the periphyton was actively growing and extracting DRP from the overlying river water – i.e. it was hungry for phosphorus.
- The pore water amongst the river gravels does not appear to be a source of dissolved nutrients at the downstream site.
- It is possible that river sediment at the downstream site is attenuating or removing some nitrogen. This is indicated by lower SIN in the sediment pore water compared to the overlying river water.
- Periphyton can trap fine sediment within their mat. There was evidence that more fine sediment is trapped in periphyton mats downstream of the WWTP once data was adjusted for biomass. The lower pH and higher dissolved Al suggests some contribution of alum floc.
- There was more DRP in trapped sediment at the upstream site which reflects periphyton utilising less DRP compared to downstream - probably due to N limiting periphyton growth upstream of the discharge.

- Increasing the pH of trapped sediment from the downstream site caused a significant increase in DRP, i.e. pH 8.5 = 102% increase, pH 9.5 = 243% increase. The response to pH at the upstream site was much weaker with no significant increase in DRP at pH 8.5 and a 13% increase at pH 9.5. The different response to pH was attributed to the WWTP discharge.

- Release of dissolved Al from sediments showed a similar pattern to DRP, responding strongly to pH increases at the downstream site but more weakly at the upstream site – pointing to the different responses being due to deposition of alum floc.

- Periphyton can trap more fine sediment as it grows thicker. The sediment continually trapped within the mats becomes a potential source of phosphorus that is released when photosynthesis causes a sufficient increase in pH. Our results indicate that this mechanism for periphyton to obtain P is particularly strong downstream of the WWTP discharge, possibly because the P attached to the fine alum floc is weakly bound.

- The results of these investigations could be further confirmed by:

    - Regular sampling of pH and nutrients at the periphyton interface and overlying river water throughout a summer period covering a periphyton accrual phase and a loss phase.

    - Confirm evidence of more fine sediment settling downstream of the WWTP by installing sediment traps in the river during a period of low flow and measuring the quantity and quality of fine sediment collected.

    - Repeat sampling of sediment trapped in the periphyton mat upstream and downstream of the discharge and during a period of low and high background SIN concentrations. Use a longer period of time for extraction from sediments e.g. 4 hours to better estimate total amount of P able to be delivered.

    - Run an experiment to estimate the daily dissolved P flux from the sediments as a result of diurnal pH increases (i.e. mg P/m^2/day) (i.e. a time series of pH adjustment experiment).

6 Change in dissolved phosphorus fraction due to storage and mixing of effluent with river water

6.1 Introduction

Hamill (2012) found that during periods of low flow (< half median flow) there was more dissolved phosphorus and dissolved aluminium in the downstream river water than could be explained by the WWTP discharge but total phosphorus (TP) concentrations in the river water reduced as would be expected due to dilution of the effluent. One process that might explain this is that after the effluent enters the river some of the particulate phosphorus bound to alum dissociates to a dissolved form due to the elevated pH of the river. The effectiveness of aluminium hydroxides (i.e. alum) and iron (III) at adsorbing P is controlled by pH; optimum absorption at about pH 6.5, and as pH decreases below 6 or increases above 8 soluble intermediary compounds become more dominate and bound P is released (Malecki-Brown et al. 2007, Ebeling et al. 2003).

There is also the possibility that the fraction of dissolved phosphorus in effluent samples changed within the bottles between sampling and analysis. Discrepancies have been observed in DRP data from samples collected from the same sites, on the same day, analysed using the same methods but by different laboratories after different time periods. This chapter discusses the results of two simple experiments investigating how the fraction of dissolved phosphorus in effluent samples is influenced by pH changes on mixing with river water and by the time between sampling and laboratory analysis.

6.2 Method

6.2.1 Effect of mixing with river water and pH increases

The effect of pH changes due to mixing with river water on the fraction of dissolved phosphorus was tested for effluent and river water samples.

Samples were collected from the Manawatu River 800m upstream, 800m downstream and from the WWTP effluent (post wetland) on three occasions during a period of low flow when the WWTP was dosing effluent with alum to remove DRP, i.e. 11 December 2012, 9 January 2013, and 15 March 2013.

In the laboratory the effluent samples were diluted by mixing effluent and river water at a ratio of 1:100 by volume. Subsamples were collected and the pH adjusted to 7.0, 8.5, 8.8, 9.0 and 9.5. These were allowed to sit for 20 minutes[23] before raw samples and pH-adjusted samples were analysed for the following variables: total phosphorus (TP), total dissolved phosphorus (TDP), dissolved reactive phosphorus (DRP), total aluminium (Al), dissolved aluminium, pH, and electrical conductivity (EC).

Laboratory analysis methods and detection limits are show in Table 6.1. All data were reported and analysed as raw results without censoring even if below the detection limit.

[23] This is about the time it takes for effluent to travel to the 800m downstream sample site.

Table 6.1: CEL laboratory methods and detection limits

variable	Lab method	Detection limit
pH	pH	
dissolved Aluminium	Aluminium - Dissolved	0.016 mg/L
total Aluminium	Aluminium - Total	0.016 mg/L
dissolved iron	Iron - Dissolved	0.002 mg/L
Total iron	Iron - Total	0.002 mg/L
Total ammoniacal nitrogen	Nitrogen - Ammonia (colorimetric)	0.005 mg/L
nitrate	Nitrite - Ion Chromatography CS	0.005 mg/L
nitrite	Nitrate - Ion Chromatography CS	0.005 mg/L
Dissolved oxygen	Oxygen - Dissolved Electrode	1 mg/L
Dissolved reactive phosphorus	Phosphorus - Dissolved Reactive (colorimetric)	0.002 mg/L
Total dissolved phosphorus	Phosphorus - Total Dissolved (colorimetric)	0.01 mg/L
Total phosphorus	Phosphorus - Total (colorimetric)	0.01 mg/L

6.2.2 Effect of time

The effect of storage time in sample bottles on the fraction of dissolved phosphorus was tested using effluent and river water samples.

Samples were collected from the Manawatu River 800m upstream, 800m downstream and from the WWTP effluent (post wetland) on two occasions during a period of low flow when the WWTP was dosing effluent with alum to remove DRP, i.e. 11 December 2012 and 9 January 2013.

Samples were collected in order upstream to downstream and time of collection was noted on the field sheet. All samples were stored in a cool, dark chilli-bin and taken to CEL laboratory within an hour. At the laboratory samples were stored in the chilli-bin filtered (0.45 micron) prior to analysis and sub-sampled as follows:

- Within one hour of sample collection;
- Six hours after sample collection (stored in chilli-bin);
- 12 hours after sample collection;
- 24 hours after sample collection.

Samples were analysed for: total phosphorus, total dissolved phosphorus, dissolved reactive phosphorus, total aluminium, dissolved aluminium, pH, and electrical conductivity. Laboratory analysis methods and detection limits are show in Table 6.1.

6.3 Results and discussion

6.3.1 Effect of changing pH

The effect of manipulating the pH of mixed effluent/river samples differed between samples and indicated a complex chemistry influencing the transformation and precipitation of river phosphorus and

aluminium. Samples from December 2012 and January 2013 showed an increase in DRP, total dissolved P and dissolved aluminium (Al) as the pH of the mixture was increased. The sample from March showed a more complex interaction, with an initial increase in DRP from pH 7.8 to 8.0, followed by a decline as pH was further increased (see Figure 6.1 and 6.2). The concentration of total phosphorus should have, theoretically, remained constant for different pH treatments but instead declined with increasing pH (Figure 6.3). Total Al showed a similar pattern to TP, but was more consistent for the March samples.

Some of the differences between samples may be explained by differences in the concentration of particulate P in the effluent, which was 1, 0.6 and 0.5 mg/L in samples from December, January and March respectively. More particulate P in the unadjusted sample from January gave greater opportunity for releasing some as dissolved P as pH increased. There may also be competing interactions for dissolved phosphorus between different elements. A dominance of calcium-bound phosphorus after mixing with river water might explain why DRP in the March sample decline as pH was increased.

Simply mixing the effluent with river water appeared to cause changes in the form of phosphorus. For the March sample, the DRP of the laboratory-mixed sample was 0.0046 mg/L while DRP in the river and effluent were 0.0087 and 0.0089 mg/L respectively. In contrast total dissolved P increased with mixing i.e. 0.02 mg/L in the mixed sample compared to 0.013 mg/L and 0.014 mg/L in the river and effluent respectively.

The concentrations of DRP and dissolved Al in mixed samples from January and March were very low and close to the detection limit of 0.002 mg/L and 0.016 mg/L respectively. Analytical results have considerably more error at low concentrations, and some caution is needed in using the results from January and March. Ideally the samples should have had less dilution. A 100:1 dilution was characteristic of actual dilution of the effluent in the river during December, but during January and March the river provided less than 30 times dilution.

Despite the complex nature of some of the results this work indicates that under some situations mixing of treated effluent with river water of higher pH would cause release of dissolved phosphorus from the particulate P in the effluent. This is likely to be most apparent when particulate P in the effluent is high and dilution with the river is low. Under low flow conditions the pH of the Manawatu River varies diurnally from about 7.1 to over 8.7. The December samples found that increasing the pH of the mixture from 7.2 to 8.7 caused a 50% increase in DRP concentration and a 60% increase in TDP concentration (see Figure 6.1).

This is consistent with results of modelling the water quality effects of the effluent using the Biowin model (MWH 2013). MWH (2013) applied the Biowin model to assess the effect of the discharge on DRP concentrations under conditions of diurnally fluctuating river pH. The results showed diurnal fluctuations in DRP downstream of the discharge as some particulate phosphorus becomes soluble with diurnal increases in pH. The effect was more apparent when there is less dilution, large pH fluctuations (i.e. low river flow) and high particulate P in the discharge.

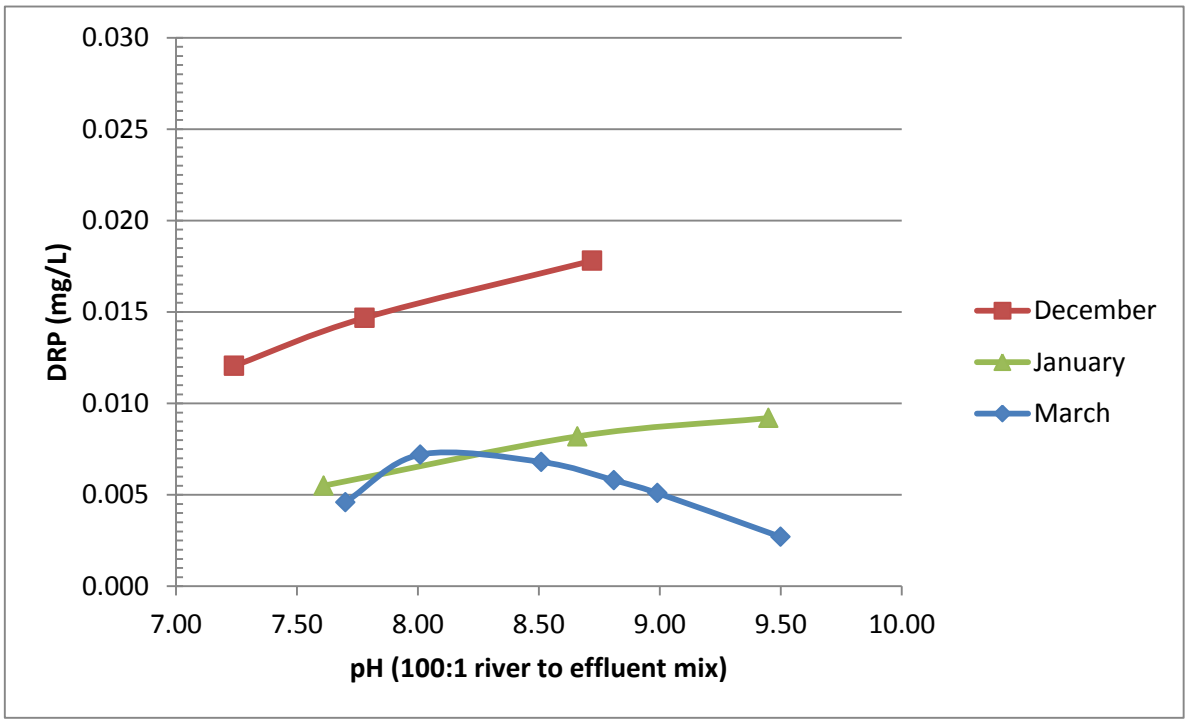

Figure 6.1: Change in DRP due to pH in effluent diluted 100:1 with river water. Particulate P in the effluent was 1 mg/L, 0.6 mg/L and 0.5 mg/L in samples from December, January and March respectively.

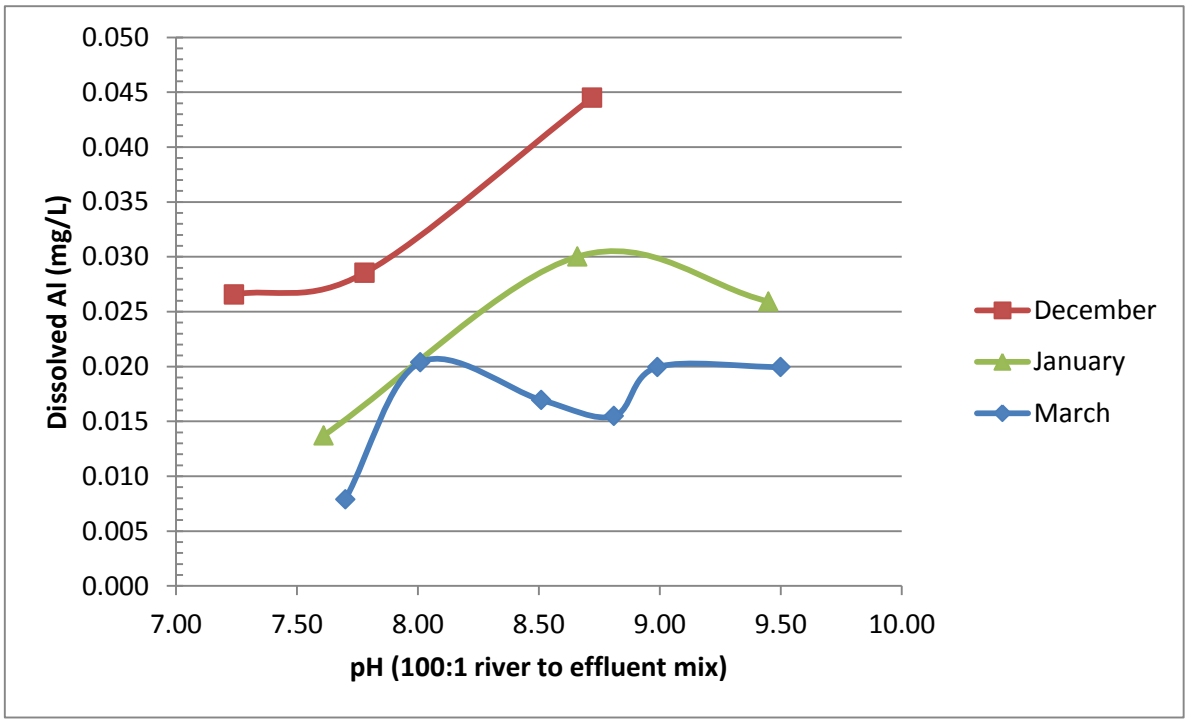

Figure 6.2: Change in dissolved aluminium due to pH in the effluent diluted 100:1 with river water.

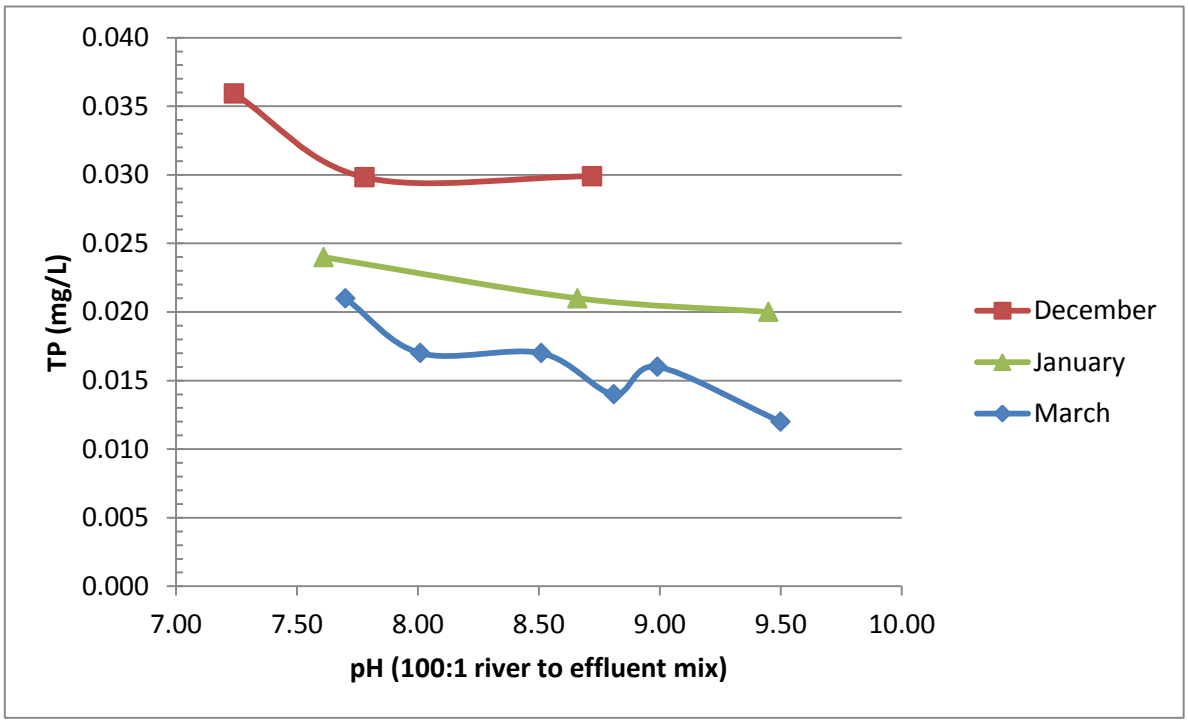

Figure 6.3: Change in total phosphorus due to pH in the effluent diluted 100:1 with river water.

6.3.2 Changes within bottles over time

Samples were collected in December and January to assess the effect of delays in sample analysis on analytical measurements of DRP. DRP results from treated effluent samples were lowest in samples analysed within about 1 hour and generally increased as the storage time increased (Table 6.2, Figure 6.4). The DRP in effluent samples analysed after about 24 hours was 71-78% higher than in samples analysed within an hour (Figure 6.5).

A similar pattern was observed for total dissolved P and dissolved Al, with a higher concentration found in samples analysed after a longer period of samples being stored in the bottles (Figure 6.6). As expected the concentration of TP and total Al remained relatively constant (Table 6.2).

The effect of delayed analysis was much more apparent in effluent samples compared to river water samples (Figure 6.5), and the variability seen in upstream samples probably reflects the variability due to values being close to analytical detection limits.

Delays in analysis of effluent samples were positively correlated with pH changes in the bottles (Figure 6.7), and small increases in pH (i.e. 0.4 units) are likely to be driving the increases in dissolved P and dissolved Al.

The results suggest that the chemistry of discharged effluent is relatively unstable and phosphorus readily shifts from a particulate form to a dissolved form in response to pH changes in the bottles. One implication is that samples may need to be filtered in the field in order to obtain accurate results of dissolved phosphorus from effluent samples.

Increases in DRP as a result of storage in the bottles (and corresponding pH increases) may explain the differences in results observed between 24 hour integrated samples (collected by PNCC) and spot samples collected on the same day for the joint monitoring programme (see Hamill 2012). The results from the time integrated sampler were consistently higher than spot samples on the same day. Additional sampling has not found a diurnal fluctuation in DRP, but the difference is consistent with an increasing fraction of DRP due to longer delays between collection and analysis.

Table 6.2: Effect of delays in sample analysis on samples collected from the WWTP discharge, the Manawatu River upstream and the Manawatu River downstream.

Site	sample date	Hours since sampling	DRP (mg/L)	TDP (mg/L)	TP (mg/L)	Particulate P (mg/L)	Al Total (mg/L)	Al dissolved	EC (µS/cm)	pH	DRP:TP	% change DRP	% change TDP
Downstream 800 m	11-Dec-12	0.95	0.013	0.019	0.032	0.013	0.188	0.017	182	7.51	0.42		
Downstream 800 m	11-Dec-12	4.45	0.015	0.022	0.032	0.010	0.170	0.038	177.2	7.51	0.47	13.0	16.7
Downstream 800 m	11-Dec-12	8.45	0.017	0.017	0.022	0.005	0.182	0.037	180.2	7.76	0.78	25.9	-9.1
Downstream 800 m	11-Dec-12	24.95	0.016	0.018	0.031	0.012	0.168	0.040	180.9	7.72	0.53	22.5	-3.2
Upstream 800 m	11-Dec-12	1.25	0.008	0.011	0.022	0.010	0.159	0.014	178.6	7.49	0.36		
Upstream 800 m	11-Dec-12	4.75	0.008	0.010	0.019	0.009	0.172	0.015	174.2	7.51	0.41	0.8	-15.3
Upstream 800 m	11-Dec-12	8.75	0.008	0.013	0.019	0.006	0.150	0.023	175.3	7.72	0.42	3.6	15.1
Upstream 800 m	11-Dec-12	25.25	0.009	0.010	0.015	0.005	0.136	0.020	176.4	7.59	0.57	11.5	-10.0
Wetland outfall	11-Dec-12	1.05	0.129	0.206	1.211	1.004	3.406	0.309	693	7.16	0.11		
Wetland outfall	11-Dec-12	4.55	0.142	0.215	1.287	1.072	3.230	0.357	689	7.32	0.11	9.9	4.1
Wetland outfall	11-Dec-12	8.55	0.167	0.241	1.242	1.001	3.344	0.339	686	7.41	0.13	30.0	16.8
Wetland outfall	11-Dec-12	25.05	0.220	0.304	1.239	0.934	3.187	0.519	688	7.58	0.18	70.8	47.6
Downstream 800 m	9-Jan-13	0.83	0.006	0.010	0.017	0.007	0.087	0.062	203	7.69	0.36		
Downstream 800 m	9-Jan-13	4.83	0.008	0.011	0.018	0.007	0.070	0.035	203	7.65	0.46	32.3	10.0
Downstream 800 m	9-Jan-13	6.25	0.008	0.016	0.018	0.002	0.078	0.033	201	7.66	0.44	29.0	60.0
Downstream 800 m	9-Jan-13	23.6	0.009	0.010	0.017	0.007	0.078	0.079	201	7.68	0.50	37.1	0.0
Upstream 800 m	9-Jan-13	1.08	0.005	0.008	0.013	0.005	0.076	0.025	202	7.69	0.35		
Upstream 800 m	9-Jan-13	5.08	0.007	0.008	0.016	0.008	0.066	0.014	197	7.5	0.41	41.3	0.0
Upstream 800 m	9-Jan-13	6.5	0.005	0.008	0.013	0.005	0.074	0.029	197.3	7.57	0.36	2.2	0.0
Upstream 800 m	9-Jan-13	23.85	0.005	0.006	0.012	0.006	0.066	0.030	198.7	7.67	0.41	6.5	-25.0
Wetland outfall	9-Jan-13	1	0.051	0.118	0.705	0.587	1.841	0.127	709	7.22	0.07		
Wetland outfall	9-Jan-13	5	0.073	0.150	0.684	0.534	1.873	0.164	707	7.28	0.11	44.6	27.1
Wetland outfall	9-Jan-13	6.4	0.068	0.134	0.700	0.566	1.643	0.138	706	7.35	0.10	33.1	13.6
Wetland outfall	9-Jan-13	23.8	0.090	0.158	0.689	0.531	1.745	0.188	704	7.49	0.13	78.3	33.9

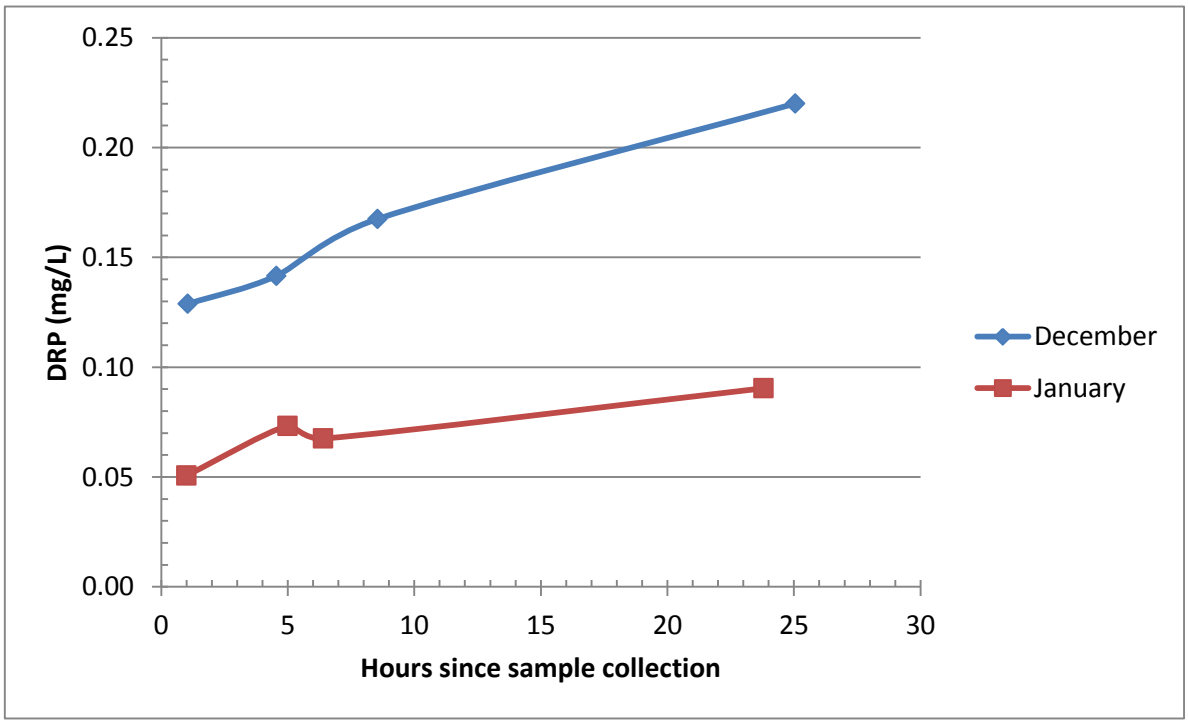

Figure 6.4: DRP variation in stored effluent samples due to timing of laboratory analysis.

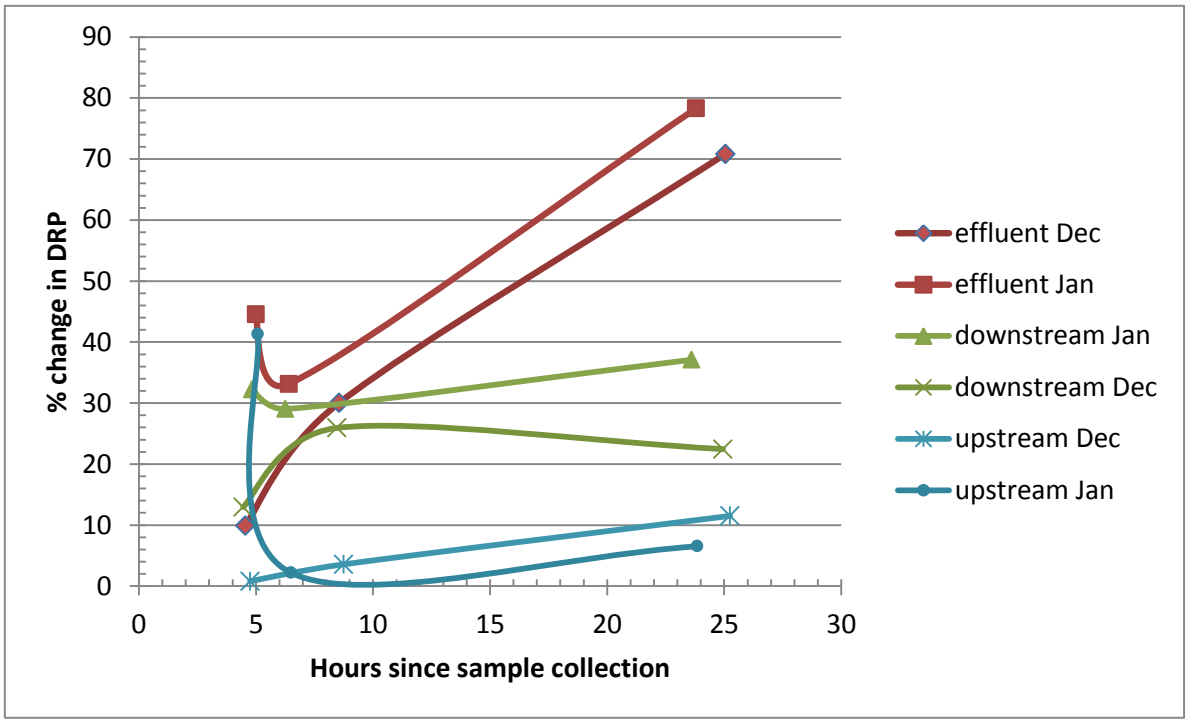

Figure 6.5: Percent change in DRP due to timing of laboratory analysis in stored effluent samples from effluent, upstream river samples and downstream river samples.

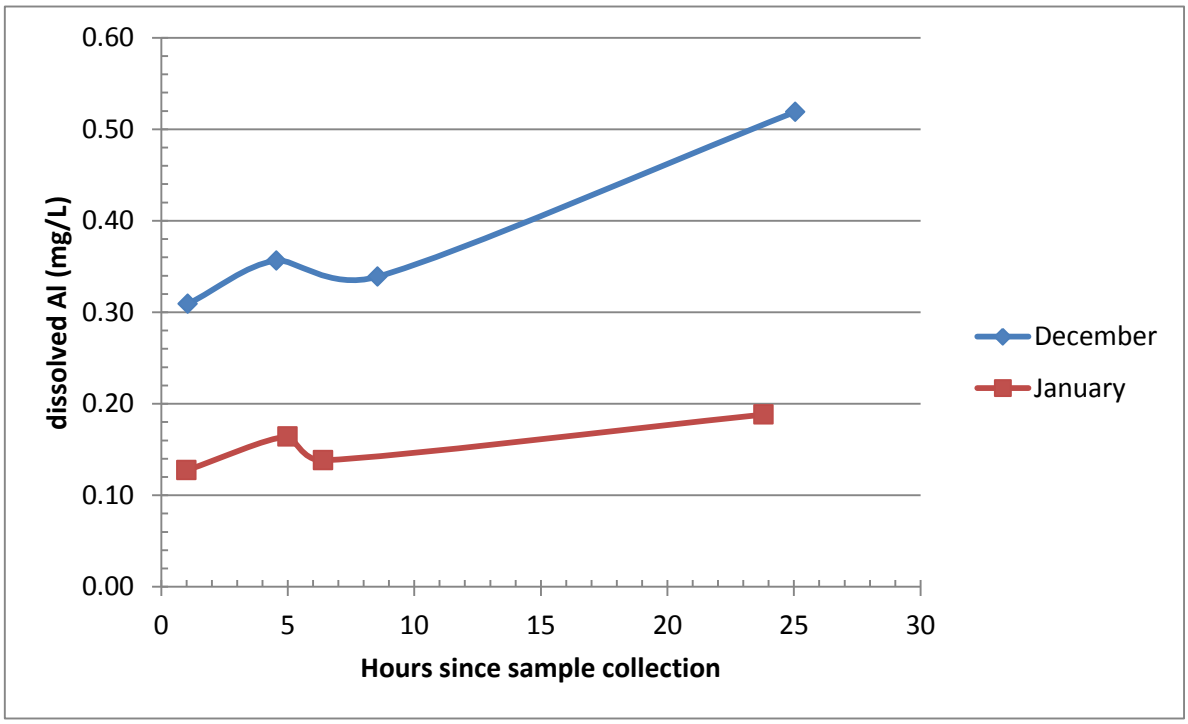

Figure 6.6: Dissolved aluminium variation in stored effluent samples due to timing of laboratory analysis.

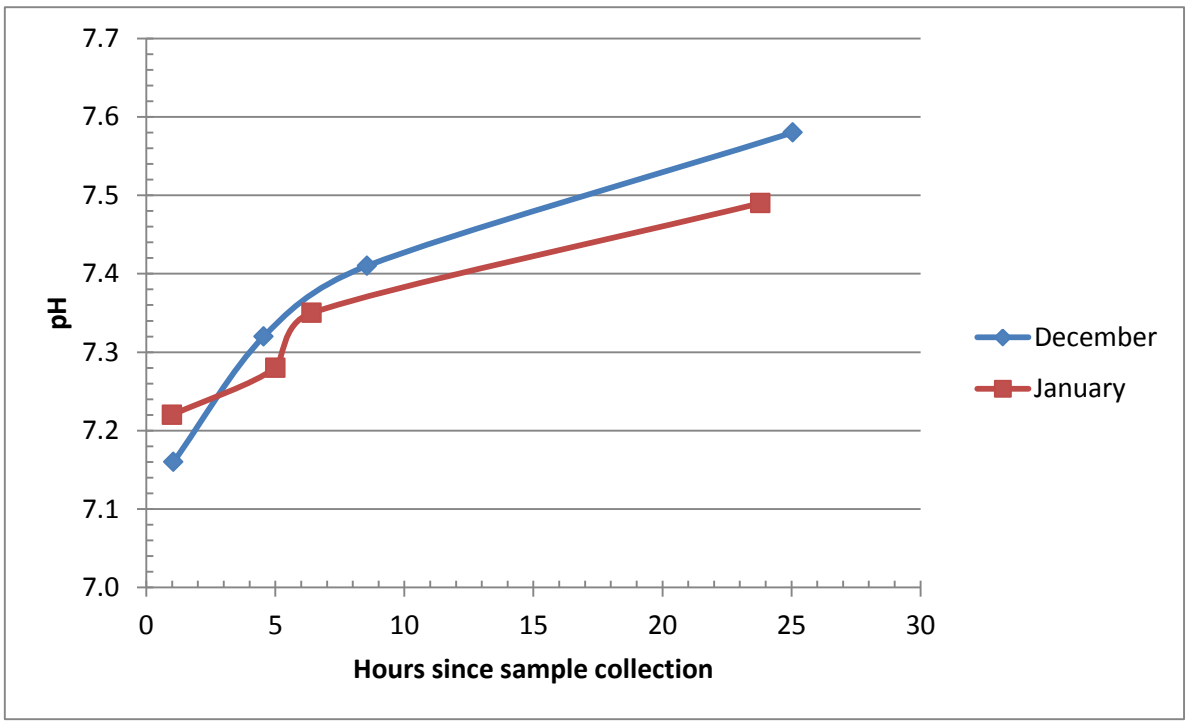

Figure 6.7: pH variation in stored effluent samples due to timing of laboratory analysis.

6.4 Summary

The key messages from this chapter are:

- The effect of manipulating the pH of mixed effluent/river samples differed between samples and indicated a complex chemistry influencing the dissolving and precipitation of river phosphorus and aluminium, nevertheless there was a general pattern of an increase in pH causing an increase in DRP within effluent samples.
- The results of the present experiments supported model outputs (MWH 2013) that indicating during periods of low flow and when the treated effluent has high particulate P, diurnal pH increases in the river water can release dissolved phosphorus from the particulate P in the effluent.
- The analytical results from analysing samples for dissolved reactive phosphorus from the WWTP discharge showed considerable variability depending on the number of hours the sample was in storage prior to analysis.
- To improve the accuracy of measurements it is recommended that where appropriate effluent samples are filtered in the field prior to transporting to the laboratory. It is acknowledged that field filtering is not always practical; where field filtering of effluent samples do not occur the samples should be transported and filtered in the laboratory as soon as possible after sampling.

7 Synthesis and Conclusions

Investigations carried out for this report have found that the Manawatu River is a highly complex natural system with significant changes in nutrient concentrations in the river during periods of prolonged low flow. This has implications for how wastewater discharges might be treated in order to minimise effects on periphyton growth.

7.1 Impact of the wastewater discharge

During periods of low river flow (i.e. less than half median flow) the wastewater treatment plant uses alum treatment to reduce the amount of dissolved phosphorus (DRP) in the discharge to a very low concentration and occasionally during January 2013 it was not possible to detect any elevation in DRP due to the discharge. Hamill (2012) found that reducing DRP in the discharge was helping to control periphyton growth; nevertheless periphyton still grew prolifically downstream of the discharge.

In 2013 the periphyton biomass downstream of the discharge exceeded the 35 g/m^2 AFDM guideline for protection of trout habitat within 15 to 17 days. This stimulatory effect of the discharge on periphyton growth was most evident at the sample sites with 1.2 km downstream of the discharge and was considerably less at the sample site 3.8 km downstream (i.e. upstream of Longburn). The smaller effect observed near Longburn may reflect the assimilation and removal (e.g. by denitrification) of nutrients by algae and within the riverbed.

The very low DRP concentrations recorded in January 2013 should theoretically have been limiting periphyton growth, despite high nitrogen concentrations downstream of the discharge. However, the periphyton grew rapidly and showed little evidence of nutrient limitation. This suggested that the periphyton was obtaining phosphorus from other sources (e.g. trapped sediments).

7.2 Reasons why periphyton grows fast downstream of the discharge

The investigations identified several reasons explaining rapid periphyton growth downstream of the discharge, these were:

- Particulate P in the discharge released dissolved phosphorus to the river under low flow conditions when pH in the river has large diurnal fluctuations. The downstream load of DRP was often higher than could be explained by DRP in the discharge. Experiments found a complex chemistry influencing the form of phosphorus. Nevertheless, the results supported model outputs (MWH 2013) that diurnal pH increases in the river water can release dissolved phosphorus from the particulate P in the effluent, especially when the treated effluent has high particulate P and during periods of low flow.

- Particulate P associated with suspended sediment in the river was trapped within periphyton mats and released P in direct vicinity to algal cells. An increase in pH was able to release some dissolved P at both the upstream and downstream sites.

- Particulate P released in the discharge was trapped within periphyton mats and released P in direct vicinity to algal cells. Increases in pH were more effective at releasing dissolved P from trapped sediments at the downstream site compared to the sediment trapped by periphyton at

the upstream site. At the downstream site, increasing the pH of trapped sediments to 8.5 and 9.5 caused a 102% and 243% increase in DRP respectively; while at the upstream site it caused no change and a 13% increase respectively. This mechanism for periphyton to obtain P from sediment is particularly strong downstream of the WWTP discharge, which may be because some of the trapped sediment is residual alum floc to which P is more weakly bound compared to river sediments.

There was no evidence that undertaking the alum dosing for a longer period of time with the current treatment system would reduce downstream periphyton growth; instead:

- The initial peak in maximum periphyton biomass occurred progressively earlier for periphyton trials started later in the summer – suggesting periphyton grew more rapidly rather than more slowly after longer periods of alum dosing;
- Periphyton growth rates measured on concrete tiles early in summer when alum dosing occurred for short periods were similar to those placed in the river later in the summer after a longer period of alum dosing;
- Long periods of removing DRP from the discharge by alum dosing (e.g. February and March) had no noticeable effect on downstream periphyton biomass compared to short periods (e.g. November and December).

These processes for releasing dissolved P were controlled by pH within the river water and periphyton mats. Daily increases in pH are caused by the periphyton itself, and thus the effect of these processes is most apparent during periods of low river flow, when periphyton biomass is high, and within the periphyton mat (i.e. measured up to pH 9.6). The periphyton downstream of the discharge was 'hungry' for P with lower concentrations close to the mat compared to overlying water.

The potential supply of P increases as periphyton biomass increases because more inorganic sediment is trapped in periphyton mats. There was also evidence that more fine sediment was trapped in periphyton mats downstream of the WWTP.

During the summer the potential limiting nutrient upstream of the discharge changed from P limitation to N limitation. This switch to nitrogen limitation occurred after an extended period of low flow (i.e. river flow < 20-30 m^3/s) due to a drop in the background concentration of dissolved inorganic nitrogen (SIN) in the Manawatu River.

Furthermore as the flow continued to drop to below 20 m^3/s; the dissolved P in the river increased to above the summer median – further reducing the potential for P to limit periphyton growth. This increase in dissolved P may have been caused by the combined effects of P released from river sediment trapped within the periphyton mat and a mature periphyton community with less net growth, more senescence and less net demand for nutrients.

Nitrogen limitation was confirmed by periphyton nutrient bioassays during February-March 2013. These indicated that periphyton in the Manawatu River upstream of the WWTP discharge was primarily limited by nitrogen with secondary phosphorus limitation. Periphyton growth in the river downstream of the WWTP showed a small amount of dual limitation by N+P.

Despite high concentrations of SIN in the river downstream of the discharge, the P treatment did not stimulate periphyton growth compared to that on the control and N treatments – adding weight to evidence that periphyton was extracting P from sediments trapped within their mat.

The nitrogen limitation found in 2013 contrasted with results from April 2012 which found possible phosphorus limitation. These different results are consistent with the different river flow conditions and background concentrations of N and P in the river during the wet summer of 2011-12 and the drought of 2012-13.

7.3 Implications for the WWTP discharge

These findings have significant implications for how to best treat the wastewater that is discharged into the river. Effluent treatment approaches that could be taken to help limit excessive periphyton growth include:

- Reducing dissolved phosphorus concentrations (as is currently done);
- Reducing particulate phosphorus (P) concentration in the discharge in addition to the dissolved P; and
- Reducing soluble inorganic nitrogen (SIN) concentration when river flow is very low (e.g. <20-30 m^3/s).

River are complex and even if these actions are taken it is possible that periphyton will grow more quickly downstream of the WWTP discharge compared to upstream, however the rate of growth and the period of time guidelines values are exceeded will reduce.

7.4 Complex river dynamics

A number of findings highlighted the complex dynamics occurring in the Manawatu River and emphasises the need for site specific information when establishing resource consent conditions, these included:

- Estimates of periphyton biomass downstream of the WWTP discharge differed depending on the method used. Chlorophyll *a* appeared to over-estimate periphyton biomass at the downstream sites and AFDM over-estimated periphyton biomass at the upstream site. For sites downstream of the discharge, AFDM is a better measure for assessing periphyton cover against guideline values because the AFDM guideline value of 35 mg /m^2 corresponds to a decline in mayfly abundance in the river (as reported in Hamill 2012). Percent cover (e.g. weighted composite cover) provides complementary information that helps confirm biomass measures.
- There was evidence that some characteristics of the sewage stimulated periphyton growth in addition to the N and P; however the effect was small compared to the combined effect of N and P stimulating periphyton growth and of little practical consequence.
- Grazing by macroinvertebrates appeared to play an important role in controlling periphyton biomass at the upstream site. This was highlighted by the periphyton accumulation on substrates that resisted the effects of periphyton grazing.

- Measurement of dissolved reactive phosphorus differed depending on how long samples were stored prior to analysis. Filtering the samples in the field would ensure more accurate and consistent results. However it is acknowledged that field filtering is not always practical; where field filtering of effluent samples do not occur the samples should be transported and filtered in the laboratory as soon as possible after sampling.

7.5 Further investigations

This report has taken a weight of evidence approach to understand river processes causing excessive periphyton growth downstream of the WWTP discharge. While the studies provide confidence to make recommendations regarding upgrades to the WWTP discharge quality, a number of additional investigations have been identified that would improve understanding of the periphyton dynamics, these might include:

- Estimate the rate at which periphyton accumulates fine sediment at sites upstream and downstream of the WWTP discharge. This could be done by installing sediment traps (e.g. Astroturf matting) in the river during a period of low flow and measuring the quantity and quality of fine sediment collected. This would help establish the extent to which a rain of sediments provide a continual source of P and help confirm evidence of more fine sediment settling downstream of the WWTP as a result of alum floc.

- Estimate the total amount of P able to be delivered by trapped sediment. This could be done by sampling of sediment trapped in the periphyton mat upstream and downstream of the discharge and extracting P using a time series of pH adjustments, repeated over multiple time periods.

- Estimate the daily flux (i.e. mg P/m^2/day) of dissolved N and P from sediment trapped in periphyton mats due to diurnal pH increases (i.e. light + dark conditions). An experiment could place cobbles covered in periphyton in benthic chambers within the river to measure changes in N, P, dissolved oxygen and pH. Paired chambers could test P uptake from the water by periphyton with and without sediment trapped in the mat, as well as testing the effect of different types of sediment quality. Periphyton obtaining P from sediments would use less P from the water.

- Better understand the cause of late summer increase in DRP within the river by regular sampling of pH and nutrients at the periphyton interface and overlying river water throughout a summer period covering a periphyton accrual phase and a loss phase.

- Undertake specific sampling to assess the ratio of chlorophyll *a* to AFDM of specific periphyton species common in the Manawatu River e.g. *Stigeoclonium* sp., *Cladophora* sp., *Phormidium* sp.

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Appendix 1: Water quality results for summer 2012/2013

Site 1	Date	Flow (m3/s	Discharge eff (m3/	Temperature (oC)	DO (g/m	pH (lab/fiel	Field EC (uS/cn	DRP HRC (g/m3	TDP (g/m3	TP (g/m3	TN (g/m3	SIN (g/m3	NH4-N (g/m3	NNN (g/m3	Turbidity EPA (NTU	*E. coli* (MPN/100m	Chl *a* (mg/m2)	Phe (mg/m2)	PeriWCC (%)
Manawatu d/s	31/10/2012 8:30	46.8		16.2		8	201	0.033	0.037	0.064	0.931	0.7114	0.195	0.5164		118	17.9	8.0	6.6
Manawatu d/s	7/11/2012 10:05	38.4		13.9	10.47	7.97	212.5	0.016	0.019	0.027	0.895	0.7572	0.312	0.4452		12	132.7	69.4	18.2
Manawatu d/s	14/11/2012 13:00	37.9		14.4	12.29	8.54	202.6	0.019	0.013	0.039	0.758	0.6817	0.32	0.3617	2.71	953	243.8	202.8	48.9
Manawatu d/s	14/11/2012 14:24	37.7		14.6	12.13	8.37	234.3	0.026	0.024	0.047	0.884	0.7356	0.424	0.3116		38			
Manawatu d/s	21/11/2012 10:02	38.2		16.3	9.97	7.88	190.6	0.005	0.023	0.052	0.832	0.3985	0.168	0.2305	3.01	70	147.9	93.6	20.3
Manawatu d/s	28/11/2012 8:37	24.6		17.2	9.12	7.66	228.6	0.018		0.033	0.825	0.6299	0.361	0.2689	2.04	64	205.0	33.8	10.6
Manawatu d/s	5/12/2012 13:25	25.6		19.6	11.27	8.1	207.8	0.019	0.027	0.04	0.941	0.8413	0.634	0.2073	1.08	39	178.0	74.7	22.0
Manawatu d/s	12/12/2012 7:11	27.0		16.9	9.28	7.7	193.6	0.014	0.018	0.026	0.633	0.416	0.197	0.219	1.72	49	80.4	19.4	23.6
Manawatu d/s	12/12/2012 10:00	26.5		18.1	10.31	7.84	199.1	0.006	0.01	0.024	0.528	0.4838	0.093	0.3908	5.19	63			
Manawatu d/s	20/12/2012 7:09	76.6		17.7	8.56	7.47	107.9	0.019	0.021	0.045	0.421	0.2735	0.112	0.1615	29.3	582	2.1	0.7	
Manawatu d/s	28/12/2012 12:06	77.9		18.4	8.87	7.8	151.3	0.029	0.033	0.102	0.747	0.41	0.195	0.215	13.3	447	139.5	3.4	
Manawatu d/s	3/01/2013 9:10	43.4		18	8.52	7.66	167.7	0.017	0.017	0.026	0.761	0.4616	0.344	0.1176	3.53	265	65.4	11.6	18.4
Manawatu d/s	9/01/2013 8:42	23.7		16.8	8.5	7.63	205.7	0.003	0.013	0.02	0.539	0.3027	0.218	0.0847	1.48	53	229.9	117.4	42.8
Manawatu d/s	16/01/2013 7:26	40.1		18.7	8.1	7.49	140.6	0.0002	0.01	0.018	0.615	0.3888	0.238	0.1508	7.06	222			20.0
Manawatu d/s	16/01/2013 9:55	40.2		19.2	9.25	7.67	150.2	0.0002	0.009	0.012	0.535	0.2855	0.136	0.1495	5.53	138	262.6	112.5	
Manawatu d/s	23/01/2013 8:00	21.8		20.8	7.64	7.6	185.1	0.005	0.008	0.012	0.45	0.2422	0.202	0.0402	1.16	16	515.2	57.3	55.3
Manawatu d/s	30/01/2013 8:00	13.8		20.4	8.11	7.8	225.7	0.006	0.011	0.022	0.583	0.3266	0.276	0.0506	1.43	8	885.1	105.2	53.2
Manawatu d/s	4/02/2013 14:47	13.0		22.5	8.13	8	292.7	0.005	0.029	0.039	1.186	0.7897	0.691	0.0987	0.92	6212			
Manawatu d/s	7/02/2013 7:50	38.2		16	9.36	7.66	173.7	0.012	0.019	0.036	0.576	0.2992	0.1	0.1992	5.77	373	39.3	1.7	
Manawatu d/s	13/02/2013 6:23	14.5		20	7.47	7.51	226.9	0.019	0.018	0.032	0.7	0.5315	0.464	0.0675	1.92	80	430.2	38.5	39.4
Manawatu d/s	18/02/2013 14:14	12.1		21.9	10.43	8.1	250.6	0.03	0.036	0.047	1.269	1.0806	0.943	0.1376	1.29	25			
Manawatu d/s	19/02/2013 8:05	11.8		19.9		8	258	0.03	0.041	0.033	0.797	0.5468	0.484	0.0628	1.34	21			
Manawatu d/s	20/02/2013 7:35	11.6		20.5	7.33	7.6	245.3	0.019	0.019	0.026	0.689	0.5345	0.473	0.0615	0.73	25	212.0	32.3	52.2
Manawatu d/s	21/02/2013 12:56	11.3		22.3		8.1	249	0.022	0.027	0.035	1.15	0.8641	0.768	0.0961	1.18	30			
Manawatu d/s	22/02/2013 14:48	10.9		21.3	10.24	8.1	304.9	0.029	0.031	0.04	1.22	0.9135	0.822	0.0915	0.68	118			
Manawatu d/s	26/02/2013 14:02	10.5		21.3		8	261	0.033	0.037	0.048	1.553	1.28	1.168	0.112	0.73	28			
Manawatu d/s	27/02/2013 7:51	10.3		19.9	9.08	7.57	263.2	0.019	0.017	0.023	0.707	0.5629	0.495	0.0679	1.24	21	309.2	5.2	34.3
Manawatu d/s	27/02/2013 10:10	10.3		21.4	11.96	8.49	256.4	0.007	0.01	0.025	0.769	0.2676	0.197	0.0706	1.16	4			
Manawatu d/s	28/02/2013 6:26	10.1		19.4	7.16	7.7	267.3	0.025	0.04	0.724	37.501	0.7195	0.64	0.0795	0.81	21			
Manawatu d/s	1/03/2013 12:00	10.1		21.7	9.56	8	267.9	0.006	0.016	0.029	1.184	0.7805	0.748	0.0325	0.75	21			
Manawatu d/s	5/03/2013 13:55	10.0		19.8	10.64	8.1	274.6	0.036	0.036	0.047	1.48	1.1667	1.106	0.0607	0.71	25			
Manawatu d/s	6/03/2013 8:12	10.3		17.6	8	7.8	268.9	0.023	0.023	0.035	1.008	0.766	0.685	0.081	0.68	30	307.7	52.2	41.7
Manawatu d/s	8/03/2013 13:56	10.1		21.4	10.72	8.1	276	0.025	0.027	0.033	1.511	1.1166	1.069	0.0476	0.65	21			
Manawatu d/s	11/03/2013 10:15	9.6		19.6	9.51	8	271.3	0.018	0.017	0.027	1.033	0.683	0.622	0.061	0.81	8			
Manawatu d/s	13/03/2013 10:55	9.3		18.4	9.97	8.27	270.4	0.016	0.019	0.028	0.858	0.5425	0.5	0.0425	1.34	21			
Manawatu d/s	14/03/2013 7:40	9.2		17.6		7.9	258	0.016	0.014	0.027	0.959	0.6791	0.621	0.0581	0.85	30	397.2	4.2	43.4
Manawatu d/s	20/03/2013 7:03	73.5		15.3	8.61	7.6	159.4	0.024	0.022	0.086	0.91	0.4539	0.127	0.3269	39.7	2634			
Manawatu d/s	27/03/2013 7:46	12.5		17.6	7.95	7.3	233.6	0.024	0.021	0.031	0.753	0.5273	0.501	0.0263	1.36	176	188.0	0.0	12.4
Manawatu d/s	3/04/2013 8:14					7.7	266	0.02	0.022	0.024	0.739	0.5339	0.472	0.0619	1.02	146	207.2	35.8	28.8
Manawatu d/s	10/04/2013 14:23					8.2	217.6	0.015				0.542	0.542		3.29	593.2	228.9	57.7	26.8
Manawatu d/s	16/04/2013																209.6	63.8	12.9
Manawatu d/s	1/05/2013																8.8	0.9	0.0

Site 1	Date	Flow (m3/s)	Discharge eff (m3/s)	Temperature (oC)	DO (g/m3)	pH (lab/field)	Field EC (uS/cm)	DRP HRC (g/m3)	TDP (g/m3)	TP (g/m3)	TN (g/m3)	SIN (g/m3)	NH4-N (g/m3)	NNN (g/m3)	Turbidity EPA (NTU)	*E. coli* (MPN/100mL)	Chl *a* (mg/m2)	Phe (mg/m2)	PeriWCC (%)
Manawatu u/s	31/10/2012 7:41	47.0		16.1		7.9	198	0.014	0.015	0.024	0.665	0.5193	0.002	0.5173		64	1.9	0.3	10.0
Manawatu u/s	7/11/2012 9:09	38.6		13.6	9.94	7.88	207.1	0.01	0.013	0.017	0.544	0.4371	0.0001	0.4371		93	7.7	2.1	5.4
Manawatu u/s	14/11/2012 12:35	37.9		14.7	11.44	8.29	195.4	0.007	0.006	0.017	0.379	0.3121	0.0001	0.3121	3.29	39	10.7	2.5	5.8
Manawatu u/s	14/11/2012 13:26	37.9		14.4	11.58	8.3	193.6	0.007	0.007	0.015	0.376	0.2951	0.001	0.2941		30			
Manawatu u/s	21/11/2012 9:26	38.5		16.1	10.05	7.89	184.7	0.005	0.01	0.025	0.47	0.1729	0.002	0.1709	3.14	92	15.5	4.0	6.9
Manawatu u/s	28/11/2012 7:45	25.0		17.2	8.99	7.7	263	0.007		0.022	0.441	0.2334	0.001	0.2324	1.86	29	34.4	6.2	10.7
Manawatu u/s	5/12/2012 14:05	25.5		19.6	11.36	8.3	197.7	0.005	0.013	0.016	0.206	0.2041	0.052	0.1521	1.16	34	111.7	18.5	13.0
Manawatu u/s	12/12/2012 8:07	26.8		16.9	10.02	7.8	189.1	0.009	0.013	0.017	0.343	0.1478	0.007	0.1408	1.69	39	53.5	7.3	8.3
Manawatu u/s	12/12/2012 9:30	26.6		17.7	10.98	7.92	193.4	0.005	0.008	0.017	0.313	0.1104	0.0001	0.1104	1.62	16			
Manawatu u/s	20/12/2012 7:45	74.1		17.8	8.53	7.45	108.2	0.011	0.013	0.043	0.3	0.1413	0.014	0.1273	26.3	524	16.7	1.4	
Manawatu u/s	28/12/2012 12:48	76.7		18.4	8.92	7.8	149.5	0.018	0.022	0.049	0.459	0.1584	0.016	0.1424	10.1	576	24.5	5.7	
Manawatu u/s	3/01/2013 7:43	43.0		17.9	8.44	7.59	162.7	0.016	0.021	0.021	0.383	0.1509	0.007	0.1439	3.86	208	25.3	9.9	8.5
Manawatu u/s	9/01/2013 9:31	23.7		16.8	8.95	7.72	201	0.003	0.007	0.015	0.209	0.0505	0.0001	0.0505	1.78	61	88.9	29.1	9.5
Manawatu u/s	16/01/2013 8:35	40.5		18.9	8.54	7.71	136.8	0.0002	0.008	0.011	0.282	0.0966	0.003	0.0936	6.96	154	52.5	29.1	21.3
Manawatu u/s	16/01/2013 9:35	40.5		19	9.08	7.89	141.5	0.0002	0.008	0.007	0.249	0.0652	0.0001	0.0652	5.68	158			
Manawatu u/s	23/01/2013 8:55	21.8		21.7	8.93	7.84	181.6	0.007	0.009	0.01	0.145	0.005	0.001	0.004	1.39	4	36.6	13.2	35.6
Manawatu u/s	30/01/2013 8:50	13.7		20.7	8.52	7.86	220.6	0.007	0.01	0.017	0.176	0.0022	0.001	0.0012	1.28	8	19.4	16.0	45.0
Manawatu u/s	4/02/2013 15:38	13.2		22.5	8.61	8.1	67	0.01	0.014	0.02	0.207	0.0002	0.0001	0	1.34	2908			
Manawatu u/s	7/02/2013 8:20	37.8		16.2	9.55	7.81	171.5	0.01	0.014	0.03	0.416	0.1757	0.0001	0.1757	3.66	310	8.2	1.5	
Manawatu u/s	13/02/2013 7:11	14.5		19.6	7.93	7.63	220.5	0.011	0.01	0.019	0.163	0.0002	0.0001	0	1.67	34	61.4	10.5	13.2
Manawatu u/s	18/02/2013 14:47	12.1		22.6	10.62	8.2	281.6	0.012	0.013	0.022	0.162	0.002	0.002	0	1.53	21			
Manawatu u/s	19/02/2013 8:50	11.8		19.9		8	249	0.013	0.016	0.019	0.168	0.0002	0.0001	0	1.21	25			
Manawatu u/s	20/02/2013 8:33	11.6		20.4	8.27	7.78	234.8	0.012	0.013	0.016	0.157	0.0048	0.001	0.0038	0.87	21	26.2	6.6	18.7
Manawatu u/s	21/02/2013 12:46	11.3		22.4		8.2	234	0.01	0.011	0.016	0.173	0.002	0.0001	0.002	1.14	16			
Manawatu u/s	22/02/2013 14:08	10.9		21.4	10.42	8.2	285.9	0.012	0.012	0.016	0.159	0.002	0.0001	0.002	0.64	281			
Manawatu u/s	26/02/2013 15:00	10.5		27.9		8.3	243	0.012	0.015	0.019	0.182	0.0045	0.0001	0.0045	0.72	12			
Manawatu u/s	27/02/2013 8:42	10.3		20.2	9.96	7.75	254.4	0.013	0.013	0.016	0.15	0.0106	0.007	0.0036	1.55	12	15.8	4.9	22.5
Manawatu u/s	27/02/2013 9:47	10.3		20.6	9.5	7.89	255.3	0.013	0.012	0.018	0.152	0.0083	0.003	0.0053	2.53	44			
Manawatu u/s	28/02/2013 6:50	10.1		19.2	7.59	7.8	255.2	0.014	0.013	0.016	0.167	0.0022	0.0001	0.0022	0.74	8			
Manawatu u/s	1/03/2013 13:10	10.1		22.8	9.8	8.2	252.6	0.012	0.014	0.015	0.142	0.0008	0.0001	0.0008	0.63	21			
Manawatu u/s	5/03/2013 14:48	10.1		20.5	10.73	8.3	256.7	0.026	0.04	0.022	0.17	0.007	0.007	0	0.68	16			
Manawatu u/s	6/03/2013 9:00	10.3		17.9	8.73	7.9	257.6	0.019	0.021	0.015	0.158	0.0031	0.001	0.0021	0.86	4	24.0	9.9	26.2
Manawatu u/s	8/03/2013 14:49	10.1		22.1	10.75	8.4	257.7	0.011	0.011	0.016	0.189	0.013	0.001	0.012	0.62	25			
Manawatu u/s	11/03/2013 10:36	9.6		19.7	9.34	8.2	257.5	0.014	0.015	0.018	0.176	0.001	0.001	0	0.85	4			
Manawatu u/s	13/03/2013 10:40	9.3		18.6	9.64	8.12	260.6	0.014	0.011	0.016	0.141	0.0067	0.002	0.0047	1	12			
Manawatu u/s	14/03/2013 9:00	9.3		18.3		8.1	246	0.013	0.013	0.016	0.136	0.01	0.0001	0.01	0.84	21	25.1	9.5	19.9
Manawatu u/s	20/03/2013 7:35	72.2		15.3	8.78	7.7	155.7	0.021	0.021	0.088	0.808	0.3539	0.018	0.3359	35.4	393			
Manawatu u/s	27/03/2013 8:36	12.5		17.4	8.64	7.5	223.3	0.017	0.012	0.02	0.276	0.0256	0.0001	0.0256	1.31	127	70.2	0.0	10.9
Manawatu u/s	3/04/2013 9:06					7.9	258.8	0.012	0.013	0.014	0.193	0.0005	0.0001	0.0005	0.768	48	70.2	26.4	22.0
Manawatu u/s	10/04/2013 15:21					8.5	204.9	0.013					0.005		2.97	129.2	83.3	0.0	18.3
Manawatu u/s	16/04/2013																85.1	0.0	5.6
Manawatu u/s	1/05/2013																5.3	1.5	0.0

Site 1	Date	Flow (m3/s)	Discharge eff (m3/s)	Temperature (oC)	DO (g/m3)	pH (lab/field)	Field EC (uS/cm)	DRP HRC (g/m3)	TDP (g/m3)	TP (g/m3)	TN (g/m3)	SIN (g/m3)	NH4-N (g/m3)	NNN (g/m3)	Turbidity EPA (NTU)	*E. coli* (MPN/100mL)	Chl *a* (mg/m2)	Phe (mg/m2)	PeriWCC (%)
PNCC STP discharage	7/11/2012 11:08	38.3	0.303					0.044	0.092	1.72	38.372	36.1739	36.123	0.0509		395			
PNCC STP discharage	14/11/2012 14:05	37.7	0.279					0.095	0.157	2.228	39.423	36.3286	36.3	0.0286		95			
PNCC STP discharage	21/11/2012 10:45	38.0	0.299					0.012		1.515	36.732	35.7261	35.715	0.0111	5.86	54			
PNCC STP discharage	28/11/2012 9:26	24.7	0.290					0.064	0.129	1.855	39.827	36.6545	36.624	0.0305		25			
PNCC STP discharage	5/12/2012 14:40	25.4	0.277			7.2	817	0.063	0.118	1.231	36.623	38.0281	37.991	0.0371	5.8	25			
PNCC STP discharage	12/12/2012 8:50	26.6	0.398			7.2	718	0.065	0.106	1.068	33.677	33.4755	33.391	0.0845	4.24	39			
PNCC STP discharage	20/12/2012 8:00	73.2	0.432				745	3.217	3.219	3.52	32.36	35.3271	35.306	0.0211	16.9	384			
PNCC STP discharage	3/01/2013 10:23	43.6	0.234				580	0.018	0.043	0.26	30.416	29.1119	28.89	0.2219	1.92	85			
PNCC STP discharage	9/01/2013 10:15	23.7	0.249				707	0.05	0.126	0.704	37.83	31.6429	31.433	0.2099	3.2	115			
PNCC STP discharage	16/01/2013 8:20	40.5	0.361				733	0.08	0.134	1.058	32.631	33.7834	33.6	0.1834	6.54	1252			
PNCC STP discharage	23/01/2013 9:30	21.5	0.255				728	0.077	0.119	0.396	32.402	34.4668	34.258	0.2088	3.87	20			
PNCC STP discharage	30/01/2013 9:28	13.8	0.273				839	0.053	0.08	0.57	37.1	38.1464	38.038	0.1084	5.8	25			
PNCC STP discharage	4/02/2013 14:40	13.3	0.228			7.5	788	0.044	0.092	1.12	36.385	34.7242	34.548	0.1762	5.36	826			
PNCC STP discharage	7/02/2013 7:34	38.3	0.293				701	0.039	0.073	0.778	31.181	29.7055	29.57	0.1355	4.79	144			
PNCC STP discharage	13/02/2013 7:45	14.5	0.275				827	0.063	0.093	1.02	36.715	37.0352	36.783	0.2522	6.02	74			
PNCC STP discharage	20/02/2013 9:15	11.6	0.271				820	0.072	0.1	0.845	36.852	36.8	36.498	0.302	2.8	25			
PNCC STP discharage	26/02/2013 14:38	10.5	0.260			7.2	815	0.058	0.096	0.983	38.696	38.6671	38.5	0.1671	4.09	20			
PNCC STP discharage	27/02/2013 9:30	10.3	0.288				817	0.061	0.079	0.865	36.973	37.6854	37.5	0.1854	5.32	12			
PNCC STP discharage	28/02/2013 7:10	10.1	0.256			7.3	824	0.047	0.064	0.027	0.959	38.6172	38.491	0.1262	3.89	25			
PNCC STP discharage	1/03/2013 12:55	10.1	0.258			7.3	861	0.053	0.094	0.762	41.941	40.9549	40.857	0.0979	4.5	12			
PNCC STP discharage	5/03/2013 13:40	10.0	0.259			7.3	881	0.073	0.139	1.341	45.124	42.5462	42.49	0.0562	5.92	4			
PNCC STP discharage	6/03/2013 9:50	10.5	0.249			7.3	874	0.068	0.128	1.109	44.876	44.6137	44.559	0.0547	6.35	12			
PNCC STP discharage	8/03/2013 14:20	10.1	0.268			7.2	885	0.122	0.198	0.572	41.282	41.3727	41.258	0.1147	4.86	64			
PNCC STP discharage	11/03/2013 11:05	9.6	0.258			7.3	863	0.028	0.059	0.931	41.03	37.6026	37.56	0.0426	7.39	12			
PNCC STP discharage	14/03/2013 9:55	9.3	0.166			7.3	889	0.024	0.038	0.558	38.692	36.8971	36.86	0.0371	6.33	12			
PNCC STP discharage	20/03/2013 7:45	71.9	0.545			7.3	707	0.078	0.117	0.996	33.974	31.4317	31.414	0.0177	5.47	189			
PNCC STP discharage	27/03/2013 9:20	12.5	0.274			7.2	830	0.052	0.08	1.693	42.114	39.2846	39.264	0.0206	6.24	16.4			
PNCC STP discharage	3/04/2013 10:00		0.293			7.2	827	0.071	0.11	1.045	42.174	40.1469	40.06	0.0869	4.47	16			
PNCC STP discharage	10/04/2013 14:11		0.744			7.1	654	0.021					27.95		3.66	1312.8			

Appendix 2: Periphyton growth on artificial substrate upstream (u/s) and downstream (d/s) of WWTP

Trial	Site (u/s d/s)	Day	Chl a (mg/m2)	Pheo (mg/m2)	% Phe:Chla	Dry Mass (g/m2)	AFDM (g/m2)	Ash Mass (g/m2)	AFDM:Chl a (AI)	% organic (AFDM:dryM)	velocity (cm/s)
A	u/s	7	11.52	0.13	1%	41.0	4.5	36.5	390.7	11.0%	
A	u/s	11	16.05	1.28	8%	44.8	4.9	39.9	306.3	11.0%	
A	u/s	14	9.31	0.54	6%	77.1	7.1	70.0	759.3	9.2%	
A	u/s	18	4.77	1.01	21%	159.8	6.8	153.1	1415.2	4.2%	
A	u/s	21	3.41	0.00	0%						49.2
A	u/s	21	3.12	0.00	0%						58.4
A	u/s	21	4.02	0.00	0%						49.2
A	u/s	21	2.74	0.00	0%						54.2
A	u/s	21	2.46	0.00	0%						49.2
A	d/s	7	68.54	0.00	0%	110.2	8.9	101.3	129.8	8.1%	
A	d/s	11	162.22	22.36	14%	114.0	16.2	97.8	100.0	14.2%	
A	d/s	14	361.24	127.52	35%	192.4	32.4	160.0	89.8	16.9%	
A	d/s	18	453.10	62.87	14%	255.6	37.0	218.6	81.6	14.5%	
A	d/s	21	103.86	6.67	6%						49.2
A	d/s	21	109.78	7.96	7%						58.4
A	d/s	21	440.05	57.51	13%						66.3
A	d/s	21	337.10	52.22	15%						73.3
A	d/s	21	204.99	19.02	9%						79.8
B	u/s	7	10.47	0.00	0%	163.5	5.5	158.0	525.3	3.4%	
B	u/s	11	2.93	0.49	17%	163.6	5.6	158.0	1910.2	3.4%	
B	u/s	14	3.02	0.33	11%	187.4	4.3	183.2	1405.1	2.3%	
B	u/s	18	6.28	0.25	4%	138.8	3.9	134.9	620.8	2.8%	
B	d/s	7	133.32	0.00	0%	96.0	10.9	85.1	81.8	11.4%	
B	d/s	11	242.35	14.23	6%	192.3	21.1	171.2	87.1	11.0%	
B	d/s	14	315.80	21.45	7%	280.2	30.6	249.7	96.8	10.9%	
B	d/s	18	188.44	1.77	1%	266.8	19.0	247.8	100.9	7.1%	
D	u/s	7	40.41	2.55	6%	45.6	8.9	36.8	219.0	19.4%	
D	u/s	11	13.12	1.35	10%	45.7	7.4	38.3	563.9	16.2%	
D	u/s	15	26.52	2.62	10%	159.3	17.0	142.3	640.9	10.7%	
D	u/s	18	197.53	14.21	7%	367.3	30.3	337.0	153.6	8.3%	
D	u/s	21	15.03	2.35	16%	126.3	20.6	105.7	1367.5	16.3%	54.2
D	u/s	21	33.71	2.37	7%	143.9	22.2	121.7	658.3	15.4%	62.6
D	u/s	21	41.45	5.12	12%	206.9	42.4	164.5	1023.3	20.5%	62.6
D	u/s	21	16.85	0.86	5%	67.5	15.7	51.9	929.3	23.2%	57.1
D	u/s	21	53.30	6.72	13%	137.7	27.4	110.3	514.3	19.9%	54.2
D	u/s	25	31.43	3.33	11%	102.5	17.0	85.5	539.8	16.6%	49.2
D	u/s	25	37.81	1.88	5%	141.0	27.4	113.6	725.0	19.4%	53.4
D	u/s	25	32.34	1.77	5%	107.0	22.5	84.5	696.2	21.0%	54.2
D	u/s	25	33.71	4.99	15%	81.6	18.6	63.0	551.8	22.8%	54.2
D	u/s	25	29.15	3.97	14%	135.7	21.9	113.9	749.9	16.1%	58.4
D	d/s	7	302.93	15.09	5%	210.6	22.2	188.4	73.3	10.5%	
D	d/s	11	177.29	11.47	6%	380.0	33.0	347.0	186.1	8.7%	
D	d/s	15	31.64	2.36	7%	187.0	23.5	163.5	742.7	12.6%	
D	d/s	18	221.13	32.97	15%	494.2	39.6	454.6	179.1	8.0%	
D	d/s	21	217.29	4.43	2%	180.5	28.1	152.4	129.2	15.6%	34.2
D	d/s	21	397.69	34.60	9%	342.3	61.3	281.0	154.3	17.9%	62.6
D	d/s	21	307.94	93.19	30%	281.3	38.8	242.5	126.1	13.8%	73.3
D	d/s	21	348.94	90.23	26%	396.5	57.8	338.7	165.5	14.6%	73.3
D	d/s	21	315.23	47.52	15%	436.6	58.7	377.9	186.3	13.5%	76.4
D	d/s	25	242.35	7.25	3%	280.3	34.3	246.0	141.4	12.2%	49.2
D	d/s	25	323.43	24.89	8%	289.1	40.5	248.7	125.1	14.0%	54.2
D	d/s	25	599.95	41.60	7%	467.3	79.9	387.3	133.3	17.1%	73.3
D	d/s	25	573.98	43.95	8%	383.4	67.9	315.5	118.3	17.7%	57.1
D	d/s	25	390.85	68.99	18%	365.1	59.4	305.8	151.9	16.3%	76.4

Trial	Site (u/s d/s)	Day	Chl a (mg/m2)	Pheo (mg/m2)	% Phe:Chla	Dry Mass (g/m2)	AFDM (g/m2)	Ash Mass (g/m2)	AFDM:Chl a (AI)	% organic (AFDM:dryM)	velocity (cm/s)
D	d/s 1200m	21	496.54	56.78	11%	247.0	51.6	195.5	103.8	20.9%	19.8
D	d/s 1200m	21	403.61	72.30	18%	234.6	48.6	186.0	120.5	20.7%	31.3
D	d/s 1200m	21	384.48	52.73	14%	325.3	61.3	264.0	159.6	18.9%	54.2
D	d/s 1200m	21	248.27	44.95	18%	267.3	50.6	216.7	203.7	18.9%	54.2
D	d/s 1200m	21	324.80	37.63	12%	323.7	53.8	269.9	165.8	16.6%	62.6
D	u/s longburn	21	153.52	20.97	14%	86.8	28.4	58.4	184.9	32.7%	31.3
D	u/s longburn	21	122.54	10.95	9%	124.3	19.9	104.4	162.4	16.0%	28.0
D	u/s longburn	21	126.64	11.44	9%	167.4	24.5	142.9	193.3	14.6%	24.2
D	u/s longburn	21	157.62	14.25	9%	130.9	22.8	108.0	144.9	17.5%	28.0
D	u/s longburn	21	139.40	9.84	7%	180.8	25.5	155.3	182.6	14.1%	31.3
D	d/s longburn	21	127.10	14.60	11%	168.1	21.5	146.5	169.5	12.8%	19.8
D	d/s longburn	21	220.48	20.92	9%	334.1	39.8	294.3	180.6	11.9%	28.0
D	d/s longburn	21	204.54	15.54	8%	200.7	27.1	173.6	132.4	13.5%	19.8
D	d/s longburn	21	189.50	19.10	10%	170.3	30.0	140.3	158.4	17.6%	28.0
D	d/s longburn	21	425.47	57.33	13%	234.3	40.5	193.8	95.1	17.3%	37.0

Appendix 3: Nutrient diffusing substrate results (18 March 2013)

Site1	Site2	Treatment	Client ID	Chl a (mg/m2)	Pheophytin (mg/m2)	Phe/Chl a
downstream	d/s A	C	Site A: Control	95.6	6.9	7.2%
downstream	d/s A	C	Site A: Control	120.2	17.7	14.8%
downstream	d/s A	C	Site A: Control	59.2	9.6	16.2%
downstream	d/s A	C	Site A: Control	71.0	10.2	14.3%
downstream	d/s A	C	Site A: Control	116.1	16.0	13.7%
downstream	d/s A	P	Site A - Phosphorus	118.8	11.6	9.7%
downstream	d/s A	P	Site A - Phosphorus	99.0	11.6	11.7%
downstream	d/s A	P	Site A - Phosphorus	86.8	8.6	10.0%
downstream	d/s A	P	Site A - Phosphorus	82.5	8.7	10.5%
downstream	d/s A	P	Site A - Phosphorus	122.9	8.5	6.9%
downstream	d/s A	N	Site A - Nitrogen	106.0	9.1	8.6%
downstream	d/s A	N	Site A - Nitrogen	110.1	14.0	12.7%
downstream	d/s A	N	Site A - Nitrogen	150.5	14.6	9.7%
downstream	d/s A	N	Site A - Nitrogen	75.1	9.3	12.4%
downstream	d/s A	N	Site A - Nitrogen	48.1	6.9	14.3%
downstream	d/s A	N+P+S	Site A: N+P+S	187.5	11.0	5.9%
downstream	d/s A	N+P+S	Site A: N+P+S	171.7	12.3	7.2%
downstream	d/s A	N+P+S	Site A: N+P+S	141.0	0.0	0.0%
downstream	d/s A	N+P+S	Site A: N+P+S	173.0	2.5	1.4%
downstream	d/s A	N+P+S	Site A: N+P+S	165.9	14.1	8.5%
downstream	d/s B	C	Site B - Control	105.4	10.0	9.5%
downstream	d/s B	C	Site B - Control	77.1	8.7	11.3%
downstream	d/s B	C	Site B - Control	115.1	11.1	9.7%
downstream	d/s B	C	Site B - Control	143.1	13.5	9.4%
downstream	d/s B	C	Site B - Control	66.0	5.5	8.4%
downstream	d/s B	P	Site B - Phosphorus	71.4	5.2	7.3%
downstream	d/s B	P	Site B - Phosphorus	110.1	8.9	8.1%
downstream	d/s B	P	Site B - Phosphorus	105.4	8.3	7.9%
downstream	d/s B	P	Site B - Phosphorus	106.0	6.9	6.5%
downstream	d/s B	P	Site B - Phosphorus	113.8	7.6	6.7%
downstream	d/s B	N	Site B - Nitrogen	97.3	14.2	14.6%
downstream	d/s B	N	Site B - Nitrogen	109.1	15.8	14.4%
downstream	d/s B	N	Site B - Nitrogen	77.1	10.6	13.8%
downstream	d/s B	N	Site B - Nitrogen	77.1	11.6	15.1%
downstream	d/s B	N	Site B - Nitrogen	72.4	10.0	13.9%
downstream	d/s B	N+P	Site B: N+P	126.6	5.8	4.6%
downstream	d/s B	N+P	Site B: N+P	100.6	7.4	7.4%
downstream	d/s B	N+P	Site B: N+P	123.9	7.7	6.2%
downstream	d/s B	N+P	Site B: N+P	156.5	4.6	3.0%
downstream	d/s B	N+P	Site B: N+P	54.9	4.5	8.2%

Site1	Site2	Treatment	Client ID	Chl a (mg/m2)	Pheophytin (mg/m2)	Phe/Chl a
upstream	u/s C	C	Site C - Control	71.7	15.6	21.7%
upstream	u/s C	C	Site C - Control	49.1	19.0	38.6%
upstream	u/s C	C	Site C - Control	61.6	9.4	15.3%
upstream	u/s C	C	Site C - Control	67.7	12.3	18.2%
upstream	u/s C	C	Site C - Control	72.0	21.0	29.2%
upstream	u/s C	P	Site C - Phosphorus	70.3	18.6	26.4%
upstream	u/s C	P	Site C - Phosphorus	117.5	19.9	17.0%
upstream	u/s C	P	Site C - Phosphorus	88.9	16.1	18.1%
upstream	u/s C	P	Site C - Phosphorus	52.5	22.4	42.6%
upstream	u/s C	P	Site C - Phosphorus	51.5	27.0	52.5%
upstream	u/s C	N	Site C - Nitrogen	91.2	14.4	15.8%
upstream	u/s C	N	Site C - Nitrogen	114.8	13.4	11.7%
upstream	u/s C	N	Site C - Nitrogen	164.3	12.7	7.7%
upstream	u/s C	N	Site C - Nitrogen	101.6	12.7	12.5%
upstream	u/s C	N	Site C - Nitrogen	102.3	16.4	16.1%
upstream	u/s C	N+P+S	Site C: N+P+S	160.6	8.8	5.5%
upstream	u/s C	N+P+S	Site C: N+P+S	211.4	5.0	2.4%
upstream	u/s C	N+P+S	Site C: N+P+S	115.4	6.0	5.2%
upstream	u/s C	N+P+S	Site C: N+P+S	212.4	0.0	0.0%
upstream	u/s C	N+P+S	Site C: N+P+S	148.8	7.1	4.7%
upstream	u/s D	C	Site D - Control	82.8	17.5	21.2%
upstream	u/s D	C	Site D - Control	38.0	22.6	59.3%
upstream	u/s D	C	Site D - Control	51.5	12.0	23.3%
upstream	u/s D	C	Site D - Control	40.1	31.2	77.9%
upstream	u/s D	C	Site D - Control	48.8	17.6	36.1%
upstream	u/s D	P	Site D - Phosphorus	50.2	12.1	24.2%
upstream	u/s D	P	Site D - Phosphorus	61.6	16.9	27.5%
upstream	u/s D	P	Site D - Phosphorus	44.8	21.4	47.8%
upstream	u/s D	P	Site D - Phosphorus	63.3	10.4	16.4%
upstream	u/s D	P	Site D - Phosphorus	57.6	19.5	33.9%
upstream	u/s D	N	Site D - Nitrogen	116.1	9.9	8.5%
upstream	u/s D	N	Site D - Nitrogen	67.3	11.0	16.3%
upstream	u/s D	N	Site D - Nitrogen	86.5	11.2	12.9%
upstream	u/s D	N	Site D - Nitrogen	98.6	12.1	12.3%
upstream	u/s D	N	Site D - Nitrogen	91.6	15.6	17.0%
upstream	u/s D	N+P	Site D: N+P	135.0	9.2	6.8%
upstream	u/s D	N+P	Site D: N+P	138.0	12.7	9.2%
upstream	u/s D	N+P	Site D: N+P	134.0	11.0	8.2%
upstream	u/s D	N+P	Site D: N+P	139.3	0.0	0.0%
upstream	u/s D	N+P	Site D: N+P	201.6	5.3	2.7%

Appendix 4: Sediment trapped within periphyton mat

us ds	Site treatment rep	pH	sample area (cm2)	Al dissolved (mg/m2)	Al Total (mg/m2)	Ca-Dissolved (mg/m2)	Ca Total (mg/m2)	Fe Dissolved (mg/m2)	Fe Total (mg/m2)	NH4-N (mg/m2)	NNN (mg/m2)	SIN (mg/m2)	DRP (mg/m2)	TP (mg/m2)	TSS (mg/m2)	VSS (mg/m2)	%VSS	Al:TSS	Ca:TSS	Fe:TSS	TP:TSS
ds	d/s original 1	6.58	228.8	2.67	855	124.2	700	1.85	1172	13.05	3.628	16.68	0.122	63.8	70,367	10,052	14.3%	1.22%	0.99%	1.67%	0.091%
ds	d/s original 2	6.78	227.6	2.20	1481	180.8	1221	1.01	2239	7.23	1.538	8.77	0.048	58.3	162,566	14,060	8.6%	0.91%	0.75%	1.38%	0.036%
ds	d/s original 3	6.78	227.6	2.47	1204	218.8	1138	1.46	1751	13.75	3.779	17.53	0.395	60.7	155,097	13,620	8.8%	0.78%	0.73%	1.13%	0.039%
ds	d/s original 4	6.75	225.1	2.08	1211	123.6	565	1.19	1733	8.25	2.976	11.23	1.288	47.4	161,262	13,327	8.3%	0.75%	0.35%	1.07%	0.029%
ds	d/s original 5	6.64	192.9	3.30	2523	202.5	982	2.63	3827	17.00	9.746	26.75	3.401	85.6	273,717	22,291	8.1%	0.92%	0.36%	1.40%	0.031%
ds	d/s pH 8.5 1	8.58	228.8	3.63		110.9		1.79					0.498								
ds	d/s pH 8.5 2	8.48	227.6	4.97		197.1		1.10					0.365								
ds	d/s pH 8.5 3	8.51	227.6	4.45		236.9		1.54					0.800								
ds	d/s pH 8.5 4	8.53	225.1	3.75		118.9		1.37					1.568								
ds	d/s pH 8.5 5	8.52	192.9	6.29		194.2		2.86					4.909								
ds	d/s pH 9.5 1	9.52	228.8	7.23		98.3		1.88					0.468								
ds	d/s pH 9.5 2	9.48	227.6	8.50		157.1		1.00					0.663								
ds	d/s pH 9.5 3	9.47	227.6	9.78		182.6		1.69					1.358								
ds	d/s pH 9.5 4	9.5	225.1	11.50		90.9		1.34					1.990								
ds	d/s pH 9.5 5	9.46	192.9	22.54		131.9		2.96					7.356								
us	u/s original 1	7.13	294.6	1.60	1948	146.6	1140	2.98	3659	5.13	0.441	5.57	2.240	40.3	186,354	15,614	8.4%	1.05%	0.61%	1.96%	0.022%
us	u/s original 2	7.08	160.2	2.85	1704	158.3	1140	5.80	2909	6.97	0.499	7.47	3.851	55.4	135,456	13,733	10.1%	1.26%	0.84%	2.15%	0.041%
us	u/s original 3	6.95	211	1.67	2633	143.1	1349	2.69	4832	3.52	0.379	3.90	1.858	44.0	171,090	16,588	9.7%	1.54%	0.79%	2.82%	0.026%
us	u/s original 4	6.97	140.5	1.95	3443	207.4	1764	3.49	6224	5.42	1.210	6.63	1.936	56.1	367,972	32,028	8.7%	0.94%	0.48%	1.69%	0.015%
us	u/s original 5	6.94	175.4	2.55	2526	153.9	1420	3.33	4288	5.47	0.456	5.93	2.537	53.4	214,937	17,104	8.0%	1.18%	0.66%	1.99%	0.025%
us	u/s pH 8.5 1	8.52	294.6	1.86		130.4		2.90					2.155								
us	u/s pH 8.5 2	8.51	160.2	2.78		130.7		5.53					2.734								
us	u/s pH 8.5 3	8.56	211	1.83		125.0		2.91					1.422								
us	u/s pH 8.5 4	8.47	140.5	3.05		211.8		4.19					2.206								
us	u/s pH 8.5 5	8.46	175.4	3.24		143.8		3.88					1.585								
us	u/s pH 9.5 1	9.52	294.6	1.87		97.1		2.93					2.766								
us	u/s pH 9.5 2	9.51	160.2	3.85		113.0		5.82					4.332								
us	u/s pH 9.5 3	9.49	211	2.62		121.7		3.19					1.867								
us	u/s pH 9.5 4	9.49	140.5	3.11		134.2		3.68					3.267								
us	u/s pH 9.5 5	9.54	175.4	3.24		101.4		3.96					2.828								

Appendix 6: Photos from artificial substrates Trial A, B and D.