Tutaekuri and Ngaruroro Catchments

Nutrient Limitation of Algal Growth

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Environmental Science - Water Quality and Ecology

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Executive summary

This report summarises a study that was conducted to inform the process of setting nutrient management strategies in a proposed plan change for the Tutaekuri, Ahuriri, Ngaruroro and Karamu (TANK) catchments. The study provides information on nutrient limitation dynamics that affect algal growth rates in the gravel dominated rivers Ngaruroro and Tutaekuri and in the Waitangi Estuary. The following conclusions could be drawn from the results of this study:

- Long dry spells typical of Hawke's Bay increase the risk of high algal biomass occurring in our rivers and streams, even at relatively pristine sites.
- Any increase in N and P above current levels will result in increased algal growth rates and also higher potential algal biomass and algal cover of a river.
- Although nutrient concentrations increase from source to sea in the Ngaruroro River, they remain low for its length and remain below ANZECC guideline levels.
- Despite nutrient concentrations remaining relatively low, based on a periphyton cover index, algal growth rates double between Whanawhana and Fernhill.
- Nutrients in the Tutaekuri River are higher than in the Ngaruroro River. Algal growth rates are elevated accordingly.
- Algal growth rates are predominantly co-limited in the Ngaruroro River and co-limited to N-limited in the Tutaekuri River.
- The Waitangi estuary is a highly valued estuarine ecosystem that is co-limited. Any increase of N or P being discharged to rivers in the upstream catchments risks increased eutrophication of the estuary.

1 Introduction

Hawke's Bay Regional Council is working through a proposed plan change for the Tutaekuri, Ahuriri, Ngaruroro and Karamu (TANK) catchments to meet the requirements of the National Policy Statement for Freshwater Management (MfE 2014). To support this process - and the requirement under the NPS to set water quality limits to protect in-stream community values, information on nutrient limitation dynamics of algal growth is required.

Algae are found in many locations in rivers. They may drift in the water column in both rivers and lakes, but they are called periphyton when they are attached to objects underwater such as the streambed or underwater material such as branches or logs.

Low levels of algal growth occur naturally in healthy riverine ecosystems. Algae are necessary to support invertebrate and fish productivity and diversity. But when periphyton cover increases to thick growths it can detrimentally affect ecosystem health and recreational values. Algal growth is controlled by several biotic and abiotic factors. Biotic factors include grazing by invertebrates and abiotic factors are nutrient availability, available light, and the time available for algae to grow between flood flows that scour algae off the river bed (known as the 'accrual' period).

Regional climate determines the frequency of floods capable of resetting algae to low levels, thereby starting a new accrual period. Catchments in dryer climates generally accumulate more algae between infrequent floods than those in regions with wetter climate and higher flood frequencies (Snelder et al. 2014).

The three abiotic factors (nutrient availability, light, accrual period) can be influenced by human activities. For example, land use changes may result in water abstractions that reduce flood peaks and thereby increase accrual periods. Land use change and shifts in farming practices may alter nutrient runoff and change the amount of riparian shade (Snelder et al. 2014).

Knowing which nutrient restricts algal growth is an important step in setting concentration limits and identifying appropriate nutrient management strategies to limit excessive periphyton growth (Death et al. 2007). Algae require a wide range of chemical elements for growth (Larned 2010). River water normally has adequate supplies of most elements, so their relative abundance is not limiting. However, periphyton growth is often limited by how much of the nutrient elements nitrogen (N) and phosphorus (P) are available. Because land use changes can alter the supply of N and P to streams, these nutrients are most often the focus for management strategies. Identification of the growth limiting nutrient (either N or P) will allow the most effective nutrient management strategies to be identified.

To understand nutrient dynamics and how nutrients limit algal growth in the Ngaruroro and Tutaekuri catchments, study sites have been chosen with a range of nutrient enrichment levels in both rivers. A site in the Mangaone River, a large tributary of the Tutaekuri River was also included. Additionally, two sites in the Waitangi estuary downstream of the confluence of the Ngaruroro and Tutaekuri rivers were included in the study. The Waitangi estuary is the downstream receiving environment of both the Tutaekuri and Ngaruroro rivers before they discharge to the ocean.

In the past, nutrient limits have been set only for freshwater environments (Biggs 2000). Limits suitable for rivers may not be appropriate for estuaries. Understanding the nutrient dynamics of estuaries is essential to fully understand how estuarine ecology is affected by nutrient limits set in the freshwater environment.

1.1 Catchment descriptions

The Ngaruroro and Tutaekuri catchments have varying land uses from their upper catchments to the coast. The upper catchments are mostly native forest; the middle catchments are dominated by dry stock farming with some dairy operations; and the lower parts of the catchments are characterised by a more diverse mix of land use, including vineyards, orchards and perennial crops alongside the (still predominant) high producing grassland (Figure 2-4).

The Waitangi estuary is an important ecological area in the wider Hawke's Bay context, and is classified as one of the top ten 'best' wetlands in Hawke's Bay (Adams 1995). Despite having undergone significant modification due to channelling and stop bank construction, the estuary remains an important area for native birds, including migrant waders, nesting seabirds and resident species (Walls 2005). It is also an important habitat for fish and is used as a migratory path, breeding, or feeding ground by many species. Two inanga spawning sites have been identified and protected in the estuary and juveniles of many other species have been caught whilst beach seining (HBRC, unpublished data). The estuary supports a productive recreational and customary fishery for flounder, mullet, kahawai and inanga.

The estuary is the downstream receiving environment for all water and associated nutrients and sediment being discharged from the Karamu/ Clive, Tutaekuri, and Ngaruroro rivers. This means it is affected by land use effects on water quality upstream of the estuary. These land use effects can increase concentrations of nutrients entering the estuary, which can cause eutrophication and other negative effects. Eutrophication of estuarine systems takes the form of excessive phytoplankton and macroalgal growth. Large levels of algal growth can reduce light levels and smother benthic species. Algal growth can also cause large diurnal fluctuations in dissolved oxygen levels, which can affect benthic algae and invertebrate communities, and subsequently affect fish and bird distributions.

Some tributaries in the Ngaruroro catchment discharge water with relatively high concentrations of dissolved nutrients into the Ngaruroro main stem, in which nutrient concentrations increase from upstream to downstream towards the coast. Although the nutrient concentrations increase in the Ngaruroro main stem they remain well below the ANZECC (2000) guidelines for lowland streams and periphyton 20-day accrual guidelines (Biggs 2000) because they're diluted by a high volume of water from upper catchments in native forest, with low nutrient levels (refer to the TANK catchments State and Trend Report (HBRC in prep.).

The tributaries of the Tutaekuri also contribute water with higher levels of dissolved nutrients to the main stem of the river. There is a smaller forested upper catchment in the Tutaekuri compared to the Ngaruroro, so the nutrients from the tributaries are not diluted as much by water from the upper catchments. This means that nutrient concentrations in the main stem of the Tutaekuri increase from the mountains to the sea. In some cases nutrient levels in the lower Tutaekuri catchment exceed ANZECC (2000) guidelines and periphyton accrual guidelines.

Algal growth was higher in the middle to lower main stem of the Ngaruroro River than at Whanawhana in the upper catchment. However, algal growth usually remained below the periphyton biomass guideline of 120 mg/m^2 chlorophyll *a* for aesthetic/recreational values (Biggs 2000), although occasional blooms occur in the middle to lower main stem. The same pattern was observed for periphyton cover on the stream bed: Periphyton weighted composite cover (PeriWCC) - which is an index calculated from % cover data(Matheson et al. 2012) (Matheson et al. 2012) – remained generally below 30% PeriWCC at all sites (TANK Catchments State and Trend Report, (HBRC in prep.). Algal biomass in the Tutaekuri main stem increases steadily from upstream to downstream. At the lowest site at Brookfields Bridge algal biomass levels are above the periphyton biomass guideline for aesthetic/recreational values. *Phormidium*, a blue-green alga (cyanobacteria) that can become toxic at times, was recorded more often and in higher abundances in the Tutaekuri catchment than in the Ngaruroro catchment. Cyanobacteria mats exceeded 10% benthic cover at only a few of the monitored sites, including:

- In the Tutaekuri River at Dartmoor, Puketapu and Brookfields Bridge *Phormidium* cover on the river bed was always less than 15%
- In the Mangatutu tributary the cyanobacteria was observed more often and cover was significantly higher, reaching more than 80% at one observation in January 2013.
- In the Ngaruroro catchment *Phormidium* mats were observed periodically at Whanawhana and in the Waitio tributary at Ohiti (between 10 and 20% cover), and in the Ngaruroro downstream of Hawke's Bay Dairies (with maximum cover of 30%).

2 Methods

2.1 Identification of the nutrient most likely to control algal growth

At least two methods exist to identify the nutrient most likely to control algal growth:

- Nutrient diffusing substrate (NDS) assays, which is the most rigorous method for assessing periphyton response to nutrients (Wilcock et al. 2007).
- Assessment of the soluble N:P ratios and concentrations, which also provides insights regarding the nutrient most likely to be growth limiting (Wilcock et al. 2007).

Figure 2-1: Nutrient diffusing substrate tray in the Ngaruroro River at Whanawhana. Five replicates of treatments from left to right: Nitrogen added, phosphorus added, nitrogen and phosphorus added and control without nutrient addition.

NDS assays involve direct measurement of N- or P-limitation. The method used for nutrient diffusing substrates is described in detail in Death et al. (2007). The method places jars of agar onto the river bed and measures the algal growth response to control treatments (without nutrients), and to treatments where nutrients are added. The assays have four treatments:

1. A control treatment without any nutrient added (termed 'Control').
2. A treatment with nitrogen added (termed '+N').
3. A treatment with phosphorus added (termed '+P').
4. A treatment with both N and P added (termed '+N+P').

A filter paper is placed over each jar, through which the nutrient diffuses ('bleeds'). The in situ response of the algae to the nutrient condition created by each treatment is observed as a relative stimulation of growth (Figure 2-1).

For each treatment there are generally five replicates, giving a total of 20 samples per tray. These trays (1 tray of 5 replicates for each of the 4 treatments) are normally deployed for two weeks, allowing sufficient time for algae to grow on the filter papers before being removed and sent to the laboratory for analysis. Algal growth response is then estimated by measuring the amount of chlorophyll *a* on each filter paper (Figure 2-2).

The method can indicate 5 potential limitations to growth (Tank and Dodds 2003). This assumes that conditions are not unlimited, which would be indicated by increased growth on all filter papers, including the control, and no difference in algal growth between treatments. The five potential limitations to growth (Figure 1-2) are:

a) **N-limitation**, indicated by increased algal growth on the +N and +N+P treatment (Figure 2-2 a)

b) **P-limitation**, indicated by increased algal growth on the +P and +N+P treatment (Figure 2-2 b)

c) **Co-limitation**, indicated by increased algal growth only on the +N+P treatment (Figure 2-2 c)

d) **N primary limiting nutrient, but P-limited when more N available**. This is indicated by increased algal growth on the +N treatment and a further significant growth increase on the +N+P treatment. Although N is the primary limiting nutrient (because it produces a significant growth response on its own), the higher growth response on the +N+P treatment indicates that P becomes limiting when more N is available (and P doesn't produce a response on its own) (Figure 2-2 d).

e) **P primary limiting nutrient, but N-limited when more P available**. This is indicated by increased algal growth on the +P treatment and a further significant growth increase on the +N+P treatment. Although P is the primary limiting nutrient, the higher growth response on the +N+P treatment indicates that N becomes limiting when more P is (Figure 2-2 e).

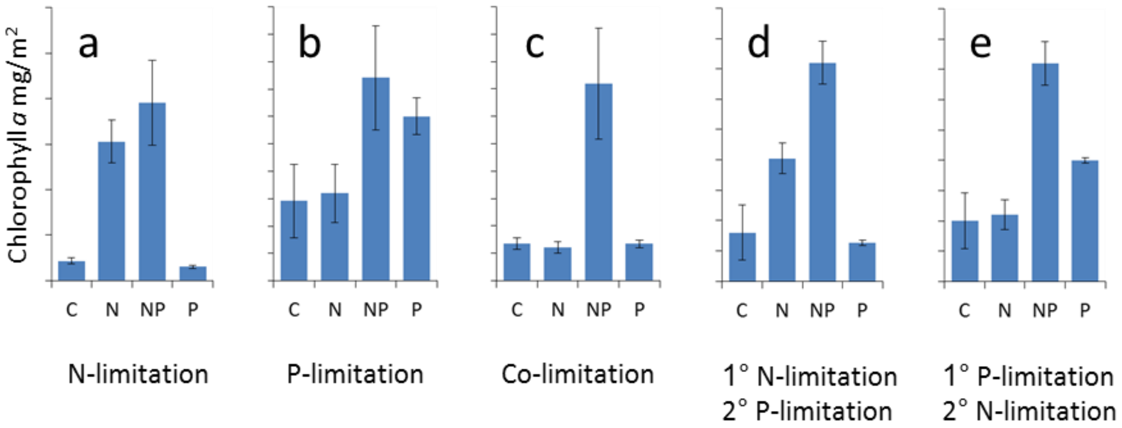

Figure 2-2: Examples for NDS results. Boxes show mean algal growth response for each treatment measured in chlorophyll *a* (mg/m^2). Whiskers: Means ± standard errors (SE).

Wilcock et al. (2007) acknowledged the value of directly measuring and identifying limiting nutrients through the deployment of Nutrient Diffusing Substrate (NDS) assays, and identified this technique as being the most robust method available at the time for assessing nutrient limitation of periphyton.

For statistical analysis of the NDS trial results a two-factor analysis of variance (ANOVA) were used as described in Tank and Dodds (2003). Two factors with N (presence or absence) and P (presence or absence) were used to test whether the trial had a significant response to a substrate treatment with N, with P, and/or an interaction between the two treatments. Following a significant ANOVA (<0.05), *post-hoc* least squares means (LSM) was used to distinguish between biomass means.

The second option for identifying the nutrient likely to be growth limiting is to look at soluble N and P concentrations and N:P ratios in river water. Redfield (1958) published a theoretical nutrient N:P ratio of 16:1 (by atoms) (which is the N:P ratio of 7:1 by weight) required for growth of marine algae. The ratio at which a nutrient becomes limiting for growth has been tested in various ecosystems including freshwater streams and lakes, and N- and P-limitation was observed at very variable N:P ratios. Consequently, nutrient ratios in river water can only be seen as rules-of-thumb for roughly gauging nutrient limitation (Francoeur et al. (1999); Tank and Dodds (2003); Larned et al. (2011); Keck and Lepori (2012)).

The N:P ratios (by weight) of 7:1 for N- limitation and 15:1 for P-limitation used in this study are not definitive thresholds for determining N- and P-limitation, but suggest whether a nutrient is likely to become limiting. The N:P ratios in this study are used as complementary data to support the interpretation of NDS results.

McDowell et al. (2009) identified how weight-ratios of nitrogen to phosphorus may indicate limitation of periphyton growth by one or other of the nutrients, as follows:

- **N-limitation** was likely when N and P were present at ratios <7:1 (N:P),
- **Co-limitation** (either nutrient may limit growth) when N and P were present at ratios between 7:1 and 15:1 (N:P).
- **P-limitation** was likely when N and P were present at ratios >15:1 (N:P).

The ratios represent the relative abundance of the nutrients, not their absolute availability. For example if both dissolved inorganic nitrogen (DIN) and dissolved reactive phosphorus (DRP) concentrations are high, then both may be present at concentrations that exceed biological requirements and neither may be limiting. Nutrient ratios are useful for identifying the nutrient that is in excess, as opposed to absolute concentration thresholds.

In-stream nutrient concentrations and DIN:DRP ratios at the time of the NDS studies were assessed. Nutrient concentrations measured in the Tutaekuri and Ngaruroro rivers were often very low, and lower than the laboratory's reporting level. These values are reported as 'less than' values, also called 'censored' values because they are lower than the levels that the laboratory is confident to report on. These 'uncensored' values may be less precise and potentially lead to a bias in the ratios, when used.

A third way to identify the potentially limiting nutrient(s) is to examine long-term data for in-stream nutrient concentrations, and to relate those concentrations to guidelines for limiting periphyton growth.

The plots indicate which nutrient is potentially limiting, as well as the absolute availability of dissolved nutrients. The DIN and DRP concentrations are plotted against each other, together with guidelines for nutrient limitations on periphyton growth (Biggs 2000). These guidelines are DIN less than 0.295 mg/l and DRP less than 0.01 mg/l (Figure 2-3).

The green shaded area shows samples when both DIN and DRP concentrations are below guideline levels. The orange shaded area shows samples where DIN concentrations are low and the grey shaded area shows samples with low DRP concentrations (<0.01 mg/l). The red area shows samples with nutrient concentrations of both DIN and DRP higher than guideline levels.

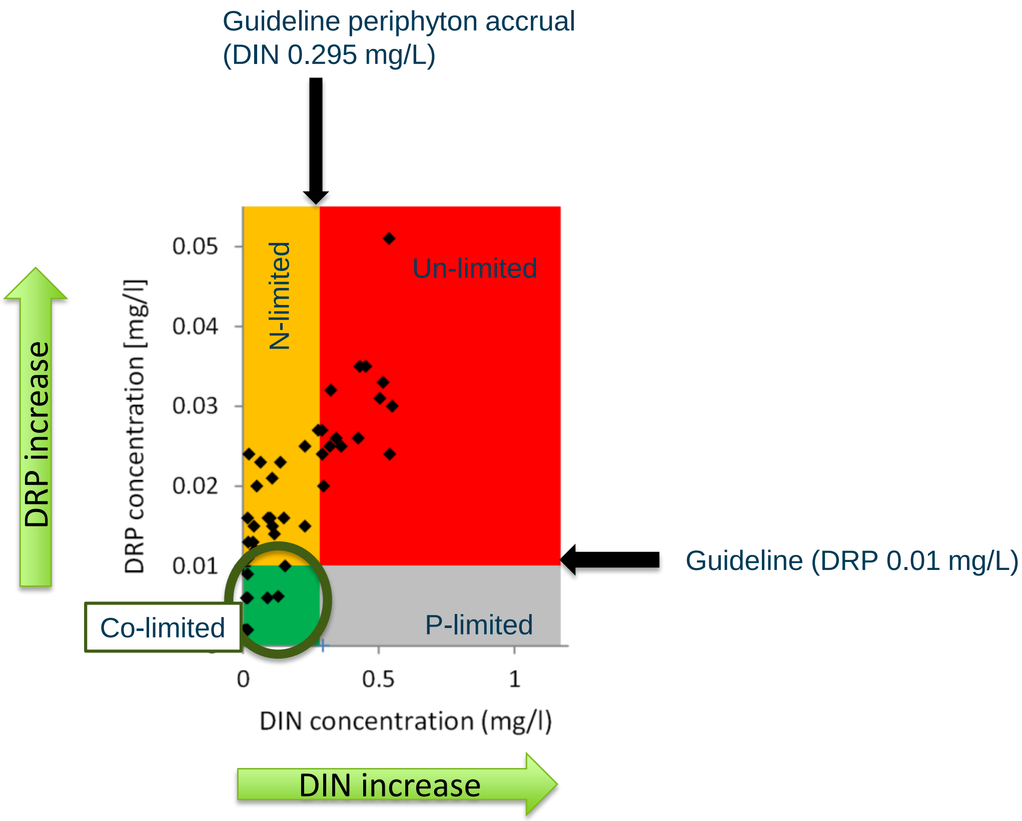

Figure 2-3: Example scatterplot showing DIN and DRP concentrations in nutrient limitation categories. Thresholds for limitation categories are guidelines recognised as limiting periphyton growth (Biggs 2000).

In this chapter results of NDS studies will be discussed in the context of long term DIN and DRP concentrations obtained from 10 years of SoE data. Nutrient concentrations observed when the agar trays were deployed are also reported. All result are presented in the following format:

- The NDS results for each site are presented in bar graphs showing mean Chlorophyll *a* for each treatment, with error bars.
- The long-term nutrient concentrations at all flows are presented as scatterplots next to the NDS results.
- The N:P ratios at the time of NDS deployment are presented in a table with the potentially limiting nutrient derived from the ratio.

2.2 Data analysis

Data from each NDS trial were analysed using 2-way ANOVA, with N addition and P addition as factors. This analysis tests for differences between N and P treatments and the control, and for any interaction between N and P (i.e., N x P interaction). Significant interactions could indicate co-limitation (i.e., both N and P are limiting growth) or primary limitation by one nutrient, with growth stimulated further when the second nutrient is added (secondary limitation).

2.3 Study Area and NDS sites

The upper zone of the Ngaruroro catchment – which includes the Ngaruroro from its source in the Kaimanawa and Kaweka ranges to Whanawhana, and the Taruarau River, which is a major tributary – is 84% native forest vegetation, with only 10% pasture in the Taruarau catchment.

Land use in the middle zone between Whanawhana and Fernhill is 68% high producing grassland, which is mainly dry stock farming and two large dairy operations downstream of Whanawhana, 9% plantation forestry and 17% native vegetation.

In the lowest catchment zone, from Fernhill to the coast, the Ngaruroro flows through low hill country and plains with a mix of land uses including pasture, viticulture and cropping.

The upper Tutaekuri catchment from its source in the Kaweka Ranges to Lawrence Hut is dominated by native vegetation, with some small areas of commercial pine forest.

The middle catchment between Lawrence Hut and Puketapu is dominated by dry stock farming and areas in dairy farming around Patoka.

The lower catchment, downstream of Puketapu, is dominated by pastoral farming, but orchards, vineyards and cropping add to a more varied land use (Figure 2-4).

Study sites were chosen to represent a gradient in land use and riverine conditions, to see if nutrient limitation changed spatially across an eutrophication risk gradient. At some sites, several NDS trials were carried out during 2013 and 2014 to see if nutrient limitation changed over time. The sites ranged from relatively pristine ‘reference’ sites in near natural condition, such as the Tutaekuri River at Lawrence Hut, to those with little influence from land use, such as the Ngaruroro at Whanawhana. Sites in the lower catchments with moderate levels of eutrophication risk were examined, such as the Ngaruroro River site at Fernhill and even higher eutrophication levels, such as the Tutaekuri River at Brookfields Bridge and Puketapu.

The Mangaone River at Rissington was chosen because it is a tributary with high nutrient levels and eutrophication risk. To understand the potential eutrophication risk of the Waitangi estuary, NDS trays were also deployed in the Waitangi Estuary at Site 1 in late January 2013 and at Sites 1 and 2 (Figure 2-5) in March 2014 at the same time as the NDS trays were deployed upstream in the rivers. The sites were located to effectively represent the dynamics of the estuary with site 1 exposed more to the influence of the Ngaruruo and Tutaekuri rivers and site 2 exposed to the influence of the Clive/ Karamu river.

Site locations for NDS trays were selected to have similar flow velocities and depth, to facilitate comparison of sites. The trays were left in the rivers for two weeks in January 2013, and January, February and March 2014 (Table A-1).

Additional water quality data was collected during the 2014 trials at the time of deployment, after one week, and after two weeks when the trays were retrieved. In-stream algal biomass was measured at each site one week after deployment. At the freshwater riverine sites, water samples were taken during each sampling run for:

- Analysis of DIN and DRP concentrations
- Water quality meter readings for instantaneous temperature, oxygen and conductivity
- An algal visual assessment

Monthly water quality data was collected in the Waitangi estuary, adjacent to site Waitangi 1. Algal visual assessments were not conducted because this protocol is not appropriate for estuaries. Instead water-borne chlorophyll *a* is used as an indicator of phytoplankton concentrations.

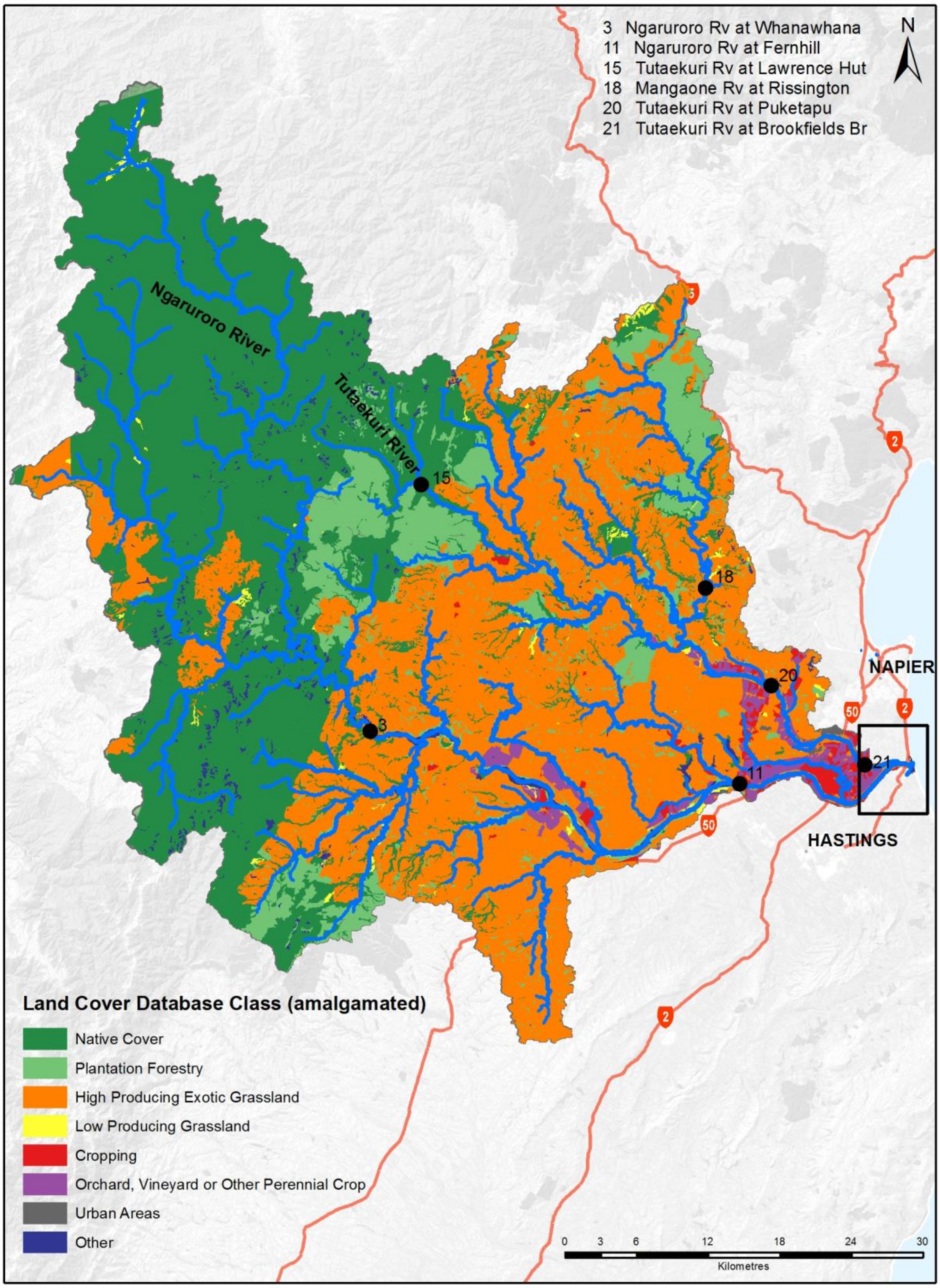

Figure 2-4: Nutrient Limitation Study sites and SoE sites in the Tutaekuri and Ngaruroro catchments. Amalgamated land-use categories from the LDCB 4 database are shown in the Ngaruroro and Tutaekuri River catchments. Black dots are NDS deployment sites, with SoE site numbering. Black rectangles are estuary deployment sites, shown in the enlarged map in Figure 2-5.

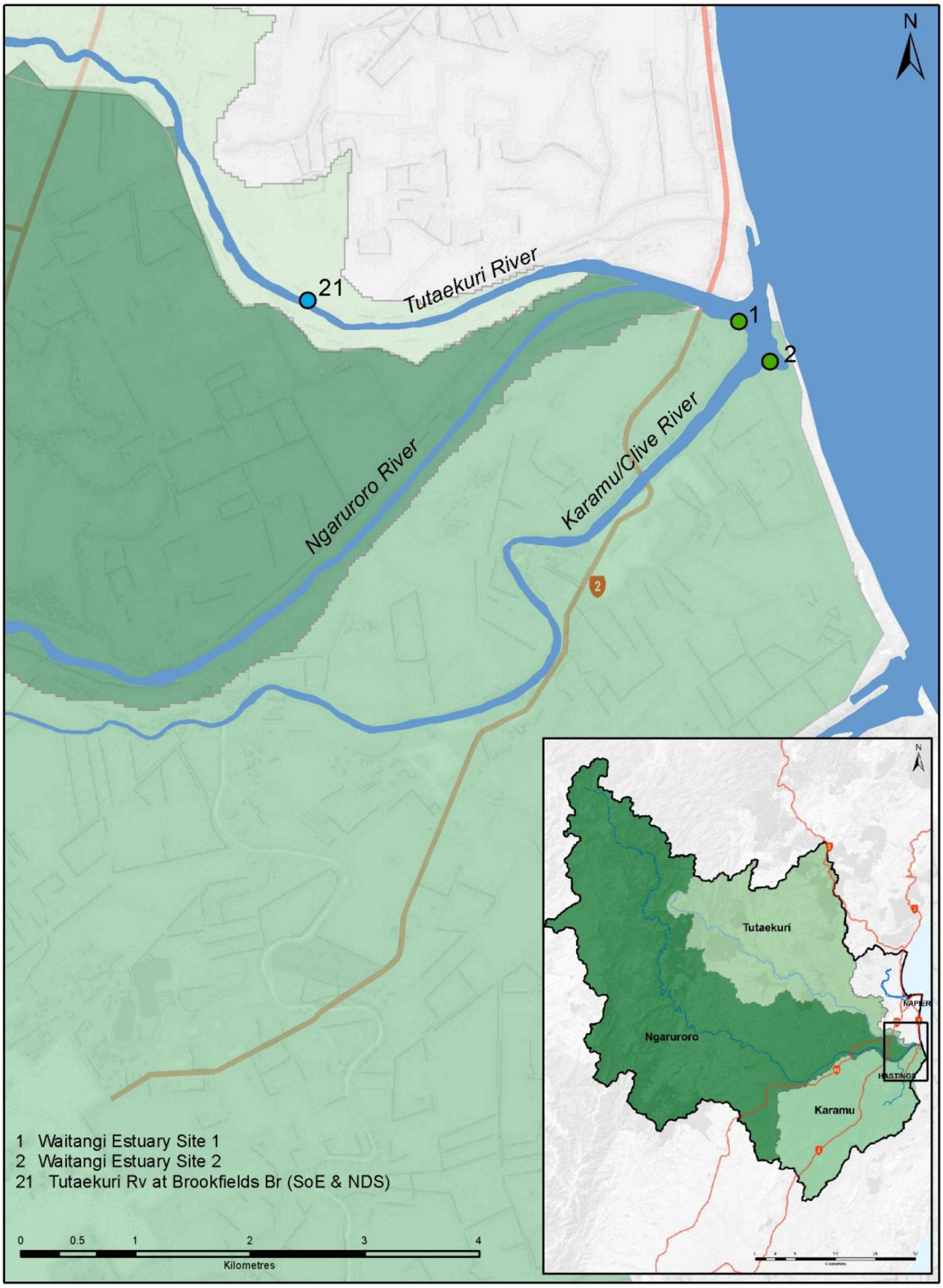

Figure 2-5: Map of Nutrient Diffusing Study sites in the estuary. Sites 1 and 2 are NDS sites in the Waitangi Estuary. Site 21 is the NDS and SoE site Tutaekuri at Brookfields Bridge.

Due to vandalism or excessive debris getting repeatedly caught on the NDS trays, some sites were shifted to more suitable locations between the four deployment periods and other study sites had to be dropped. The tray in the Mangaone River was repeatedly damaged, the site downstream of Hawke's Bay Dairies was unsuitable and the Tutaekuri River site at Brookfields Bridge had to be shifted 12 km upstream to Puketapu. The new location had similar flow and nutrient concentrations.

The 4th deployment series in March 2014 had to be shortened due to an approaching severe weather front (Cylone Lusi 15/03/2014), and all trays were retrieved after 8 days of deployment. Refer to Appendix A for details of deployment locations and dates.

Table 2-1: Photographs of the Nutrient Limitation Study sites.

	
Tutaekuri River at Lawrence Hut	Tutaekuri River at Puketapu
	
Mangaone River at Rissington	Tutaekuri River at Brookfields Bridge

	
Ngaruroro River at Whanawhana	Ngaruroro River at Fernhill
	
Waitangi Estuary Site 1	Waitangi Estuary Site 2

3 Results

3.1 Tutaekuri catchment NDS results summary

The upper Tutaekuri site at Lawrence Hut within the Kaweka Forest Park is a near-pristine site, where almost all of the upstream catchment is in native vegetation. Some small areas are in softwood production forestry. The largest of these is in the Gold Creek tributary, which joins the Tutaekuri 2 km upstream of this sampling site. Long term nutrient sampling showed the upstream catchment usually leaches extremely low levels of nutrients. Both DIN and DRP were generally well below growth limiting thresholds such as periphyton 20 day accrual guidelines (Biggs 2000) (scatterplot in Figure 3-1).

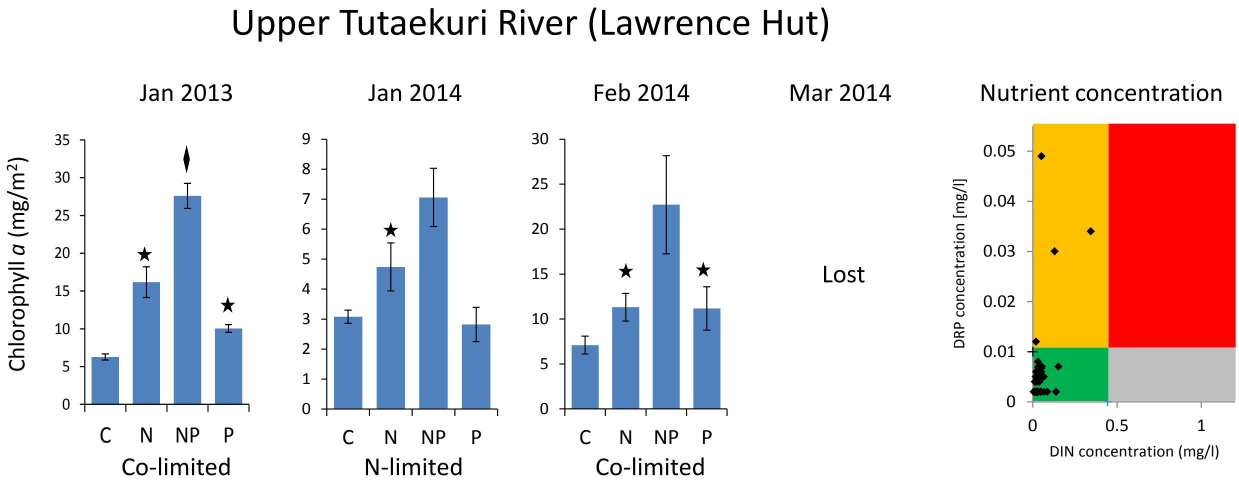

Figure 3-1: Nutrient Limitation Study results for the upper Tutaekuri River at Lawrence Hut. Boxplots show NDS treatment results in 2013 and 2014 with standards errors (whiskers); C=Control, N=nitrogen addition, NP= nitrogen and phosphorus addition, P=phosphorus addition. Asterisks (*) above bars indicate a significant N effect, P effect, and (I) represents a significant interaction term as determined by 2-way ANOVA ($p < 0.05$) for positive responses to nutrient addition. The scatterplot displays long-term DIN and DRP concentrations (2003-2013, nutrient concentration at all flows). Box colours represent red= likely unlimited conditions, yellow=likely N-limited, grey=likely P-limited, green=likely co-limited with box boundaries representing periphyton 20-day accrual guidelines for DIN and DRP (Biggs 2000).

The January 2013 and February 2014 NDS trials in the Tutaekuri at Lawrence Hut showed that adding both N and P resulted in significantly higher periphyton biomass than the control, which indicates that both nutrients were limiting algal growth. In January 2014 addition of N alone increased the growth rate, while P did not, indicating N-limitation at the time. Long-term nutrient concentration data as shown in the scatterplot in Figure 3-1 confirms the predominant co-limitation at this site (data points in the green box).

During the time period NDS studies were undertaken in 2014, DRP concentrations were less than 0.005 mg/l in all surface water samples, and DIN was always below 0.018 mg/l. The low concentration of dissolved nutrients in the water is likely to limit algal growth rates. The DIN:DRP ratios indicate that N could potentially become limiting, which contradicts the NDS results (co-limitation) at first sight, but can be explained by the uncertainty of N:P ratio thresholds (see Methods) and the extremely low nutrient concentrations that were often below detection limits. This may lead to over- or under-estimating the nutrient concentration, as discussed in Chapter 2.

Table 3-1: Nutrient concentrations and DIN:DRP ratios at the Nutrient Limitation Study site in the upper Tutaekuri River at Lawrence Hut. DIN:DRP ratios (by weight) indicate N-limitation at <7:1 (N:P), P-limitation >15:1 (N:P) and co-limitation between 7 and 15 (McDowell et al. 2009). Bold italicised numbers: uncensored values below Level of Detection (LOD).

		Nutrient ratio during time of deployment			
	Date	DIN (mg/L)	DRP (mg/L)	DIN/DRP	Ratio indication
Lawrence Hut					
Jan 2013	30/01/13	0.018	0.005	3.60	N-limited
	04/02/13	***0.007***	***0.001***	*4.86*	*N-limited*
Jan 2014	08/01/14	0.016	0.004	4.00	N-limited
	17/01/14	0.016	0.004	4.00	N-limited
	20/01/14	0.014	0.005	2.80	N-limited
Feb 2014	29/01/14	0.016	***0.004***	*4.32*	*N-limited*
	04/02/14	0.015	***0.003***	*4.84*	*N-limited*
	12/02/14	0.015	***0.004***	*4.11*	*N-limited*

In the lower catchment of the Tutaekuri River the land use changes from predominantly native forest and little plantation forest (at Lawrence Hut) to predominantly pastoral farming including some dairying upstream of the study sites at Puketapu and Brookfields Bridge. Long-term nutrient data shows that the water in the lower catchment is enriched in both DIN and DRP and often exceeds thresholds for limiting periphyton growth (scatterplot in Figure 3-2 for Brookfields Bridge, and for Puketapu refer to the State and Trends report, Tutaekuri and Ngaruroro catchments (HBRC in prep.). At other times low in-stream DIN concentrations and elevated DRP concentrations (data points in the yellow box in the scatterplot in Figure 3-2) indicate that N could potentially become the limiting nutrient.

More periphyton biomass accumulated on the nutrient-diffusing substrates at Brookfields Bridge and Puketapu compared to the upper Tutaekuri site at Lawrence Hut over the same deployment period of two weeks (bar graphs in Figure 3-2). This indicates that growth rates for periphyton were higher in the lower Tutaekuri catchment sites than at Lawrence Hut. The NDS trials in January and February 2014 showed that the addition of nitrogen alone resulted in a significant increase in algal growth compared to the control. In fact, this was the second highest growth response in January 2014 (and similarly but less pronounced in February 2014) at Puketapu. Adding N and P together had the greatest growth increase, which indicates availability of P is the secondary limitation. Together these results indicate that P does not produce a response on its own, but becomes limiting when more N is available. This is known as N-limitation with secondary P-limitation (Tank and Dodds 2003).

Summarised, long-term periphyton growth rates at the lower Tutaekuri sites are N-limited and tend to be secondary P-limited at times.

The March 2014 trial did not show a clear difference across treatments and chlorophyll-*a* concentrations were low. As mentioned earlier, the March 2014 deployment was cut short because impending inclement weather and associated high flows were likely to destroy the experiments. The short deployment time of 8 days resulted in low algal biomass growth of around 7 mg/m^2, and no visible difference between treatments. A longer deployment period of 2 weeks (as the other trials) would have allowed for additional growth and variation between treatments may have been more pronounced.

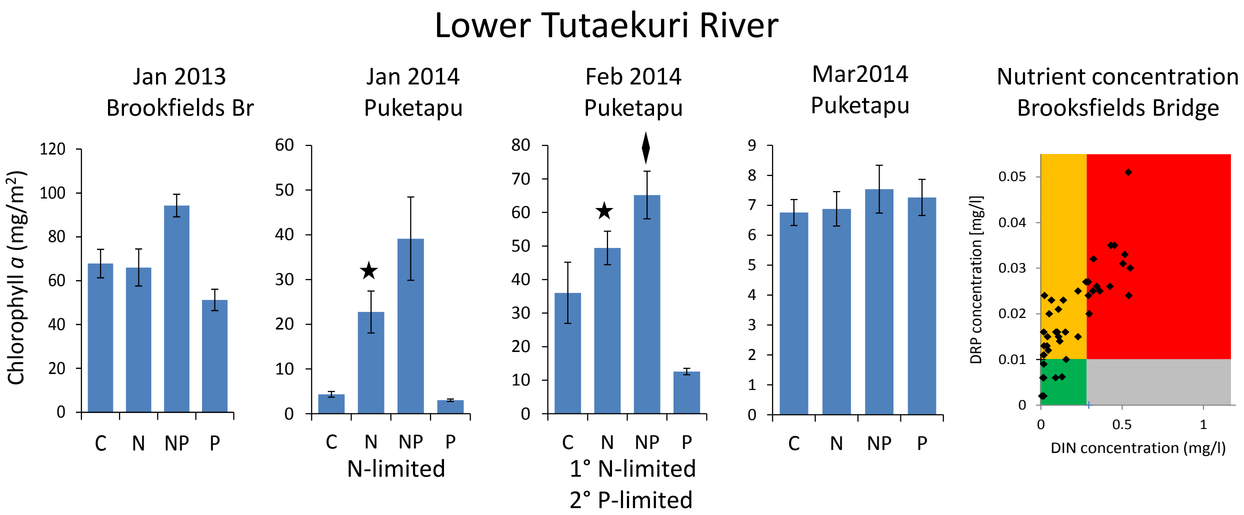

Figure 3-2: Nutrient Limitation Study results for the lower Tutaekuri River at Puketapu and Brookfields Bridge. Boxplots show NDS treatment results in 2013 and 2014 with standards errors (whiskers); C=Control, N=nitrogen addition, NP= nitrogen and phosphorus addition, P=phosphorus addition. Asterisks (*) above bars indicate a significant N effect, P effect, and (I) represents a significant interaction term as determined by 2-way ANOVA ($p < 0.05$) for positive responses to nutrient addition. The scatterplot displays long-term DIN and DRP concentrations (2003-2013, nutrient concentration at all flows). Box colours represent red= probably unlimited conditions, yellow= probably N-limited, grey= probably P-limited, green= probably co-limited, with box boundaries representing periphyton 20-day accrual guidelines for DIN and DRP (Biggs 2000) .

Both DIN and DRP concentrations were low at Brookfields Bridge at the time of the NDS deployment in January 2013. The concentrations were substantially lower than the 0.295 mg/l periphyton accrual threshold for DIN, and the 0.01 mg/l threshold for DRP (Table 3-2). This is consistent with the result of the NDS study that both nutrients were needed for a significant increase in periphyton growth. The DIN:DRP ratio at this time was <7:1 and reflects possible N-limitation which is not consistent with the NDS result. As explained in Methods this may be due to two reasons: 1) The uncertainty in the thresholds for nutrient limitation categories; and 2) The low in-stream DRP concentration at and below the limits of detection, which cause uncertainty in calculating N:P ratios.

In 2014 at Puketapu DIN concentrations were well below the 0.295 mg/l periphyton growth threshold value, whereas DRP was generally above 0.01 mg/l for the DRP threshold. Therefore relatively more phosphorus was available than nitrogen for algal growth. DIN:DRP ratios of generally less than 1.5:1 at the time NDS deployment indicated N-limitation.

Table 3-2: Nutrient concentrations and calculated DIN:DRP ratios at the NDS study site in the lower Tutaekuri River. DIN:DRP ratios indicate N-limitation at <7:1 (N:P), P-limitation >15:1 (N:P) and co-limitation between 7 and 15 (McDowell et al. (2009). Bold italicised numbers: uncensored values below Level of Detection (LOD).

		Nutrient ratio during time of deployment			
	Date	DIN (mg/L)	DRP (mg/L)	DIN/DRP	Ratio indication
Brookfields Bridge					
Jan 2013	30/01/13	0.017	0.006	2.83	N-limited
	19/02/13	0.015	***0.003***	5.77	N-limited
Puketapu Bridge					
Jan 2014	08/01/14	0.028	0.017	1.65	N-limited
	16/01/14	0.016	0.015	1.07	N-limited
	20/01/14	0.014	0.014	1.00	N-limited
Feb 2014	29/01/14	0.014	0.016	0.88	N-limited
	04/02/14	0.014	0.012	1.17	N-limited
	12/02/14	0.014	0.011	1.27	N-limited
Mar 2014	06/03/14	0.014	0.011	1.27	N-limited
	14/03/14	0.014	0.009	1.56	N-limited

Periphyton on the NDS tray in the Mangaone River at Rissington reached the highest biomass of all study sites in January 2013, and growth was similar on all treatments, including the control treatment (without additional nutrients) (Figure 3-3). This indicates that neither N nor P were limiting algal growth, and the supply of nutrients for algal growth is unlimited or 'saturated'. The nutrient limitation scatterplot in Figure 3-3 shows that nutrient concentrations of both DIN and DRP in the Mangaone River are higher than any other sites in this study, confirming that nitrogen and/or phosphorus rarely limit algal growth rates in this large tributary.

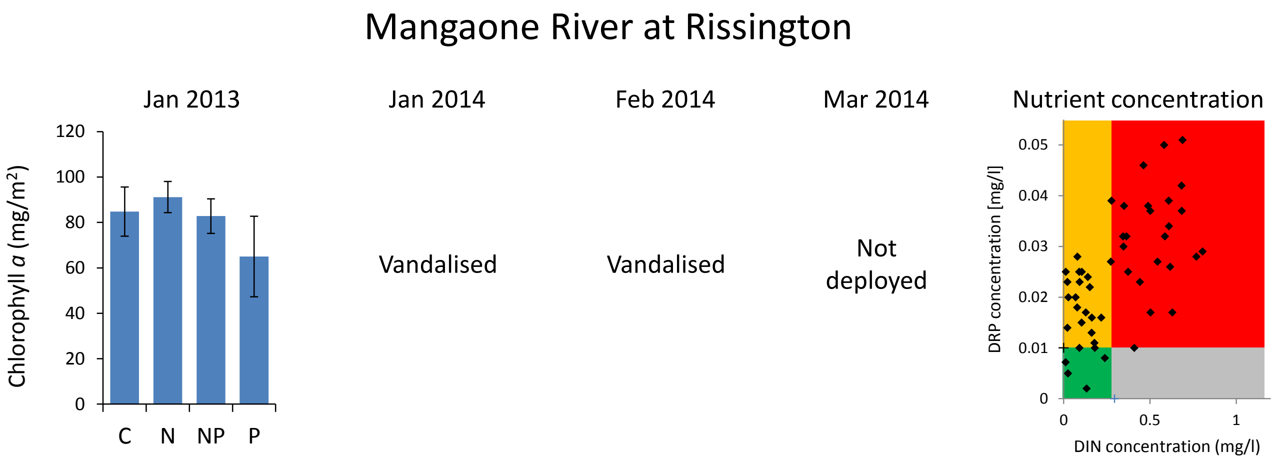

Figure 3-3: Nutrient Limitation Study results for the Mangaone River at Rissington. Boxplots show NDS treatment results with standards errors (whiskers); C=Control, N=nitrogen addition, NP= nitrogen and phosphorus addition, P=phosphorus addition. Asterisks (*) above bars indicate a significant N effect, P effect, and (I) represents a significant interaction term as determined by 2-way ANOVA ($p < 0.05$) for positive responses to nutrient addition. The scatterplot displays long-term DIN and DRP concentrations (2003-2013, nutrient concentration at all flows). Box colours represent red= probably unlimited conditions, yellow=probably N-limited, grey=probably P-limited, green=probably co-limited; with box boundaries represent periphyton 20 day accrual guidelines for DIN and DRP (Biggs 2000).

3.2 Ngaruroro catchment NDS results summary

The very low levels of in-stream nutrient concentrations over an assessment period of 10 years (scatterplot in Figure 3-4) reflect the minimal land use impact upstream of the Ngaruroro River at Whanawhana site. The upper catchment is mainly native vegetation, with only a small area of approximately 10% used for dry stock farming in the Taruarau tributary, which flows into the Ngaruroro upstream of Whanawhana. Areas in native vegetation leach very low levels of nitrogen and phosphorus and typically result in very low in-stream nutrient concentrations.

The NDS results showed that only the addition of both nitrogen and phosphorus together increased the periphyton growth rate significantly (Figure 3-4). NDS results indicated that periphyton growth was co-limited by nitrogen and phosphorus across all trials in 2013 and 2014. This conclusion is consistent with the long-term P and N concentrations, which were both well below threshold levels for periphyton accrual at all times and all samples were in the range of values indicating co-limitation (scatterplot in Figure 3-4: green box).

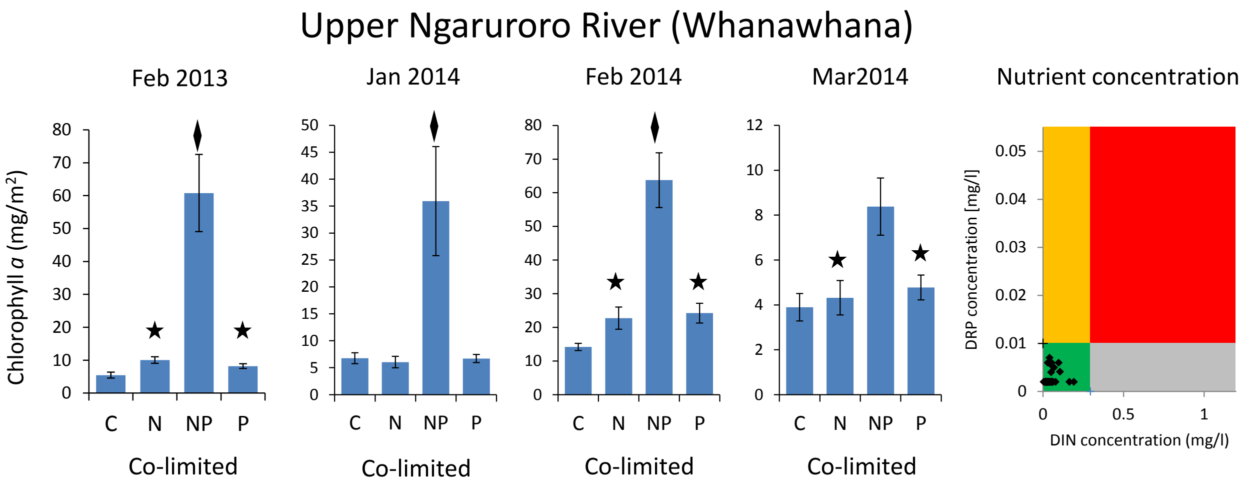

Figure 3-4: Nutrient Limitation Study results for the upper Ngaruroro River at Whanawhana. Boxplots show NDS treatment results with standards errors (whiskers); C=Control, N=nitrogen addition, NP= nitrogen and phosphorus addition, P=phosphorus addition. Asterisks (*) above bars indicate a significant N effect, P effect, and (I) represents a significant interaction term as determined by 2-way ANOVA ($p < 0.05$) for positive responses to nutrient addition. The scatterplot displays long-term DIN and DRP concentrations (2003-2013, nutrient concentration at all flows). Box colours represent red= probably unlimited conditions, yellow=probably N-limited, grey=probably P-limited, green=probably co-limited; with box boundaries represent periphyton 20 day accrual guidelines for DIN and DRP (Biggs 2000).

In-stream DIN and DRP concentrations were very low during the NDS deployments (Table 3-3). All DRP concentrations were below the laboratory's Limit of Detection of 0.004 mg/l, so uncensored values were used for the calculation of the DIN:DRP ratio. DIN never exceeded 0.017 mg/l over this period. The ratios ranged from DIN:DRP of 1.25:1 to 25:1, so nutrient ratios indicated N-, Co-, and P-limitation at various times. This could be an artefact of very low concentrations approaching laboratory detection limits, or it could be a higher uncertainty in predicting the true limiting status by DIN:DRP ratios close to the 7:1 balance. Nevertheless, nutrient concentrations were extremely low during the times of NDS deployments, suggesting strong periphyton growth limitation by both N and P.

Table 3-3: Nutrient concentrations and calculated DIN:DRP ratios at the NDS study site in the upper Ngaruroro River at Whanawhana. DIN:DRP ratios indicate N-limitation at <7:1 (N:P), P-limitation >15:1 (N:P) and co-limitation between 7 and 15 (McDowell et al. (2009). Bold italicised numbers: uncensored values below Level of Detection (LOD).

	Date	Nutrient ratio during time of deployment DIN (mg/L)	DRP (mg/L)	DIN/DRP	Ratio indication
Whanawhana					
Feb 2013	18/02/13	***0.008***	***0.001***	8.37	Co-limited
	04/03/13	***0.001***	***0.001***	1.25	N-limited
	05/03/13	0.014	***0.002***	8.00	Co-limited
Jan 2014	08/01/14	0.015	***0.003***	4.76	N-limited
	16/01/14	0.014	***0.002***	7.37	Co-limited
	20/01/14	0.017	***0.002***	11.33	Co-limited
Feb 2014	29/01/14	0.014	***0.001***	10.77	Co-limited
	04/02/14	0.015	***0.002***	7.89	Co-limited
	12/02/14	0.017	***0.001***	22.67	P-limited
Mar 2014	06/03/14	0.014	***0.001***	20.00	P-limited
	14/03/14	0.014	***0.001***	25.45	P-limited

Since human settlement, land cover upstream of the lower Ngaruroro at Fernhill has changed from native forest and scrub to high producing grassland and about 6% in production forestry.

The Ngaruroro River at Fernhill (Figure 3-5) in February 2013 was N-limited. In January 2014 the same location was P-limited. Although adding N and P together had an even greater growth response, secondary N-limitation was statistically not significant. In March 2014 the addition of N and P resulted in significantly higher periphyton growth rates compared to the control without added nutrients, showing the site was co-limited at the time.

Results from February 2014 should be treated with caution since filamentous algae accumulated on the edges of the tray, smothering the substrates for an undetermined period of time in the second week of the trial. The resulting shade on parts of the tray introduced additional variation across treatments, confounding the experiment.

The results for the lower Ngaruroro River at Fernhill show nutrient limitation varies between N-, P- and co-limited conditions.

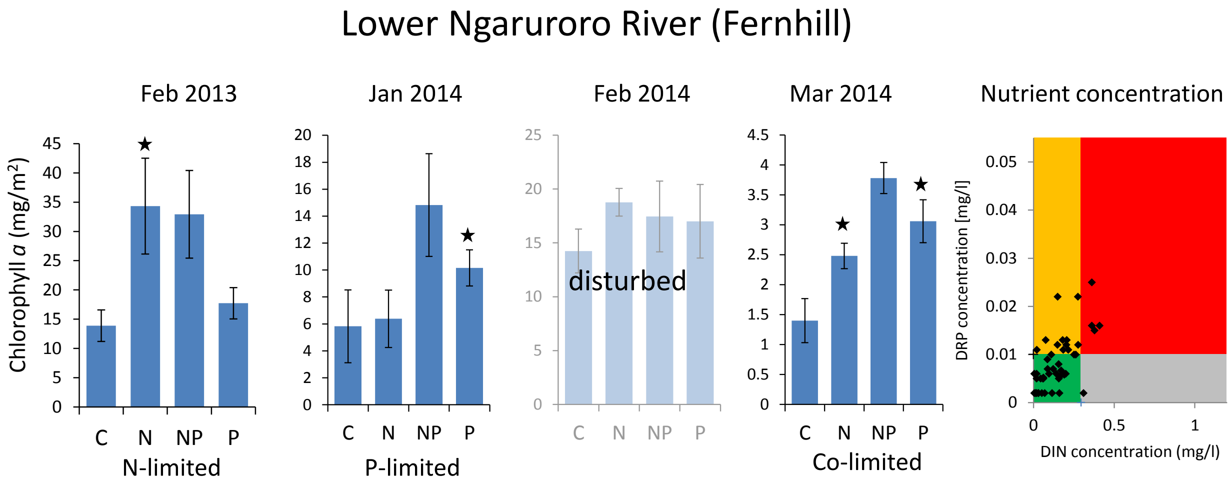

Figure 3-5: Nutrient Limitation Study results for the lower Ngaruroro River at Fernhill. Boxplots show NDS treatment results with standards errors (whiskers); C=Control, N=nitrogen addition, NP= nitrogen and phosphorus addition, P=phosphorus addition. Asterisks (*) above bars indicate a significant N effect, P effect, and (I) represents a significant interaction term as determined by 2-way ANOVA ($p < 0.05$) for positive responses to nutrient addition. The scatterplot displays long-term DIN and DRP concentrations (2003-2013, nutrient concentration at all flows). Box colours represent red= probably unlimited conditions, yellow=probably N-limited, grey=probably P-limited, green=probably co-limited; with box boundaries represent periphyton 20 day accrual guidelines for DIN and DRP (Biggs 2000).

The long-term nutrient concentrations (scatterplot in Figure 3-5) showed that most samples had DIN and DRP concentrations below threshold levels for periphyton accrual. In contrast to the site at Whanawhana, which had clearly co-limited conditions, some samples at Fernhill had elevated DRP-, and some (but fewer) samples had elevated DIN concentrations. This is consistent with the NDS studies, which show that nutrient limitation may vary over time between N-, P- and co-limitation.

The calculated DIN:DRP ratios over the NDS deployment period (Table 3-4) confirm switching nutrient conditions as observed in the NDS trials. However, many DRP values were below 0.004 mg/l (the limit of detection) which affects the accuracy of the calculated ratio. At this site DIN concentrations measured over the deployment period were significantly higher in January and February 2014 (whilst still well below the periphyton accrual threshold levels) than at other sites in the Tutaekuri and Ngaruroro, while DRP remained relatively low – which matches the NDS result of P-limitation in January 2014 at this site.

Table 3-4: Nutrient concentrations and calculated DIN:DRP ratios at the NDS study site in the lower Ngaruroro River at Fernhill. DIN:DRP ratios indicate N-limitation at <7:1 (N:P), P-limitation >15:1 (N:P) and co-limitation between 7 and 15 (McDowell et al. (2009). Bold italicised numbers: uncensored values below Level of Detection (LOD).

		Nutrient ratio during time of deployment			
	Date	DIN (mg/L)	DRP (mg/L)	DIN/DRP	Ratio indication
Fernhill					
Feb 2013	18/02/13	***0.007***	0.006	1.20	N-limited
	04/03/13	***0.003***	***0.004***	0.73	N-limited
	05/03/13	0.014	***0.004***	3.54	N-limited
Jan 2014	08/01/14	0.082	0.004	20.50	P-limited
	16/01/14	0.034	0.004	8.50	Co-limited
	20/01/14	0.064	***0.004***	16.84	P-limited
Feb 2014	29/01/14	0.014	0.007	2.00	N-limited
	04/02/14	0.062	0.006	10.33	Co-limited
	12/02/14	0.093	***0.003***	26.96	P-limited
Mar 2014	06/03/14	0.014	***0.002***	6.09	N-limited
	14/03/14	0.014	***0.002***	6.22	N-limited

3.3 Waitangi Estuary (estuary of the Ngaruroro and Tutaekuri rivers) NDS results summary

The Waitangi estuary receives water from the Tutaekuri and Ngaruroro rivers in its north-west quadrant, and water from the Karamu/Clive River in its south-west quadrant. The Waitangi estuary is the downstream receiving environment for the effects of land use on water in the Tutaekuri and Ngaruroro catchments.

Upstream catchment land uses affect estuaries through land use impacts on the levels of particulate and dissolved matter transported to the estuary by rivers. In estuaries this material is exposed to a complex suite of physical, biological and chemical processes. The material is either deposited in estuary sediments, used in plant and animal growth, transmitted to the atmosphere through gaseous exchange or flows directly to the oceanic environment. The waters of the estuary are also the mixing environment for water from these rivers and the ocean. Thus the water quality of the estuary is also influenced by water entering the estuary from the ocean.

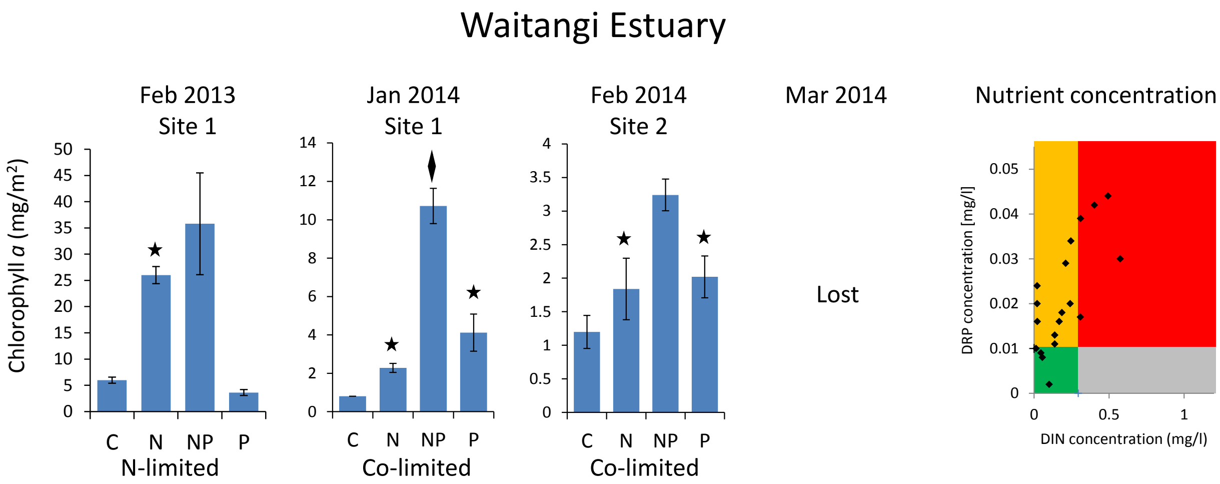

Figure 3-6: Nutrient Limitation Study results for Waitangi Estuary. Boxplots show NDS treatment results with standards errors (whiskers); C=Control, N=nitrogen addition, NP= nitrogen and phosphorus addition, P=phosphorus addition. Asterisks (*) above bars indicate a significant N effect, P effect, and (I) represents a significant interaction term as determined by 2-way ANOVA ($p < 0.05$) for positive responses to nutrient addition. The scatterplot displays long-term DIN and DRP concentrations (March 2013 – December 2014, nutrient concentration at all flows). Box colours represent red= probably unlimited conditions, yellow= probably N-limited, grey= probably P-limited, green= probably co-limited; with box boundaries represent periphyton 20 day accrual guidelines for DIN and DRP (Biggs 2000).

Estuary waters display a high degree of vertical stratification between denser salt water and lighter freshwater flowing over the top. In the Waitangi estuary there is also limited horizontal mixing between the waters of the three rivers until they reach the area of highest turbulence at the mouth of the estuary. The NDS tray sites were situated to try and distinguish the influence of the Tutaekuri and Ngaruroro rivers and the Karamu/Clive river.

Site 1, with NDS studies carried out in February 2013 and March 2014, is more influenced by the Tutaekuri and Ngaruroro rivers, and showed nitrogen limited conditions in 2013 and co-limitation in 2014. Site 2 which is closer to the Karamu/Clive river mouth showed co-limitation in March 2013.

The complexity of this environment may not be easily understood from a limited number of NDS results and discrete water samples. The fact that the NDS studies suggest nutrient co-limitation means that any change in nutrient concentrations entering the estuary from upstream will have an impact on this sensitive environment.

3.4 In-stream periphyton growth during the time of NDS studies

In-stream periphyton was assessed visually at all sites at the time of deployment, after one week and at retrieval of each of the NDS agar tray deployments between 8 January 2014 and 14 March 2014. During this period it was generally sunny, there was no significant rainfall, and flow was stable. These were ideal growing conditions for periphyton over the study period of 65 days. Figure 3-7 shows periphyton cover as ‘Periphyton Weighted Composite Cover’ (PeriWCC) which combines % filamentous algae cover and % mat algae cover into a single index giving a simplified integrated measure of proportional periphyton cover (Matheson et al. 2012).

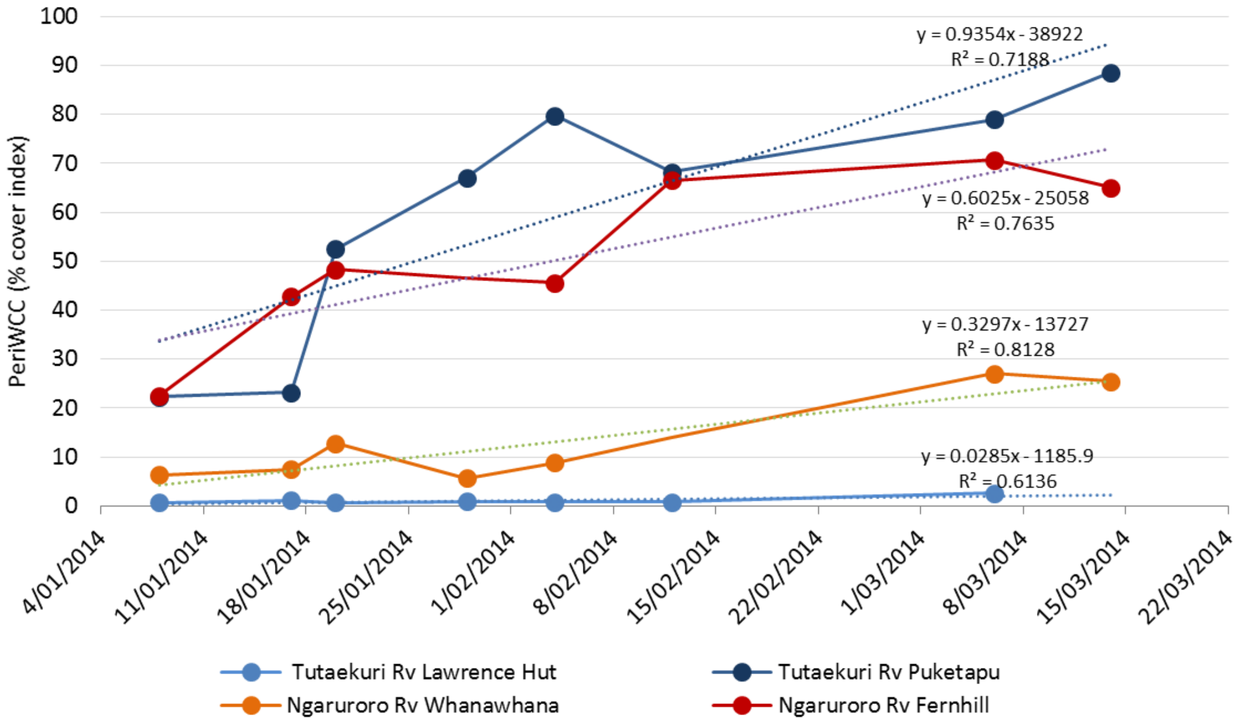

Figure 3-7: Periphyton cover as PeriWCC (% cover index) in the Tutaekuri and Ngaruroro. Monitored at the NDS study sites: upper Tutaekuri River at Lawrence Hut, lower Tutaekuri River at Puketapu upper Ngaruroro River at Whanawhana and lower Ngaruroro River at Fernhill over the time period of 8 January 2014 to 14 March 2014. Periphyton composite cover calculated after Matheson et al. (2012).

The gradient of the regression lines for each site in Figure 3-7 reflects the growth rate in terms of algal cover at each site. For example, the growth rate (as measured using the PeriWCC) for the Ngaruroro River at Whanawhana is approximately 0.33% PeriWCC/day. This contrasts with the Ngaruroro River at Fernhill, where the growth rate in periphyton cover is twice as high, with a rate of approximately 0.6% PeriWCC/day. The gradients of the regression lines may be used to estimate the accrual time required to reach a specific guideline value (in terms of PeriWCC) at each site, during conditions similar to those experienced in the 9-week study in 2014.

The availability of more nutrients in the lower reaches of the Tutaekuri River (at Puketapu) and Ngaruroro River (at Fernhill) is reflected in the higher growth rates of periphyton experienced here, compared to the upper catchment sites.

Growth rates appear to slow at these sites during the second half of the study period in February/March. After 9 weeks undisturbed accrual time, periphyton reached 70% PeriWCC stream bed cover in the lower Ngaruroro at Fernhill and 90% PeriWCC cover in the lower Tutaekuri at Puketapu (Figure 3-7).

The upper Tutaekuri site at Lawrence Hut experienced extremely low periphyton growth rates and had very low periphyton cover (maximum 2.6% PeriWCC cover) even after the study time period of 9 weeks, which shows that the availability of nutrients was clearly limiting periphyton growth, since flow and sunlight were ideal for periphyton growth.

The upper Ngaruroro site at Whanawhana had slightly higher growth rates but the guideline value of 30% PeriWCC was not reached over the 9 week study period. Periphyton growth rates in the Ngaruroro at

Whanawhana were approximately 10 times higher, (see gradients of the regression relationships in Figure 3-7) than in the Tutaekuri at Lawrence Hut, although nutrient concentrations measured during the study period were at a similar (very low) level at both sites. Under strongly co-limited conditions nutrients can be taken up rapidly by periphyton. Under those conditions it is possible to observe higher periphyton growth rates where more nutrients are available, but the nutrient concentration measured in the water column does not reflect higher nutrient availability because of its rapid uptake. This effect seems plausible as in the Ngaruroro 10% of the catchment is in dry stock farming upstream of Whanawhana (in the Taruarau tributary) and may contribute some dissolved nutrients, compared with an almost completely naturally vegetated catchment at Lawrence Hut. These nutrients may also be supplied via a groundwater pathway, which could be even more difficult to pick up during regular SOE monitoring.

Provisional guidelines for periphyton (as PeriWCC composite cover index) indicate that at a PeriWCC of below 20% the ecological condition is excellent, 20%–39% PeriWCC is good, 40%-55% PeriWCC fair and > 55% PeriWCC poor (Matheson et al. 2012). Both sites in the upper catchments achieve the most stringent 20% PeriWCC threshold for supporting ‘excellent ecological condition’. Less than 20% PeriWCC cover was observed at both sites for most of the time the NDS trays were deployed.

Algal cover estimates were not conducted in the Waitangi estuary since techniques used for assessing riverine visual algal cover are not applicable to estuarine environments, where the water is often deep and the sediment frequently soft. Increased nutrient concentrations can lead to some growth of benthic macroalgae, in particular *Ulva* species. However, increased nutrient concentrations in coastal environments are more likely to result in an increase in phytoplankton production. Phytoplankton is monitored in marine and estuarine environments by measuring the concentration of Chlorophyll *a* in the water column, and is reported elsewhere by HBRC.

4 Conclusions and implications for periphyton management

The long, hot, dry periods typical of a Hawke’s Bay summer result in lengthy periods of low flow, providing ideal conditions for algal blooms to occur in our rivers, by allowing prolonged undisturbed growth periods for periphyton. These catchments may accrue high volumes of algal biomass during long low flow periods, even if the nutrient supply is low. Although climatic conditions and extended periods of low flow are almost impossible to influence, river nutrient levels can be influenced by catchment management practices.

This study shows that the upper catchments of the Tutaekuri and Ngaruroro rivers contribute very low levels of nutrients to streams, with algal growth being strongly co-limited by both nitrogen and phosphorus availability. The risk of eutrophication of the rivers in these areas is low, because the upstream catchments are dominated by native vegetation protected in conservation land. These sites are pristine or near pristine, and can be considered as control sites.

Both the Tutaekuri and the Ngaruroro rivers become increasingly enriched in nutrients from upstream to downstream, largely because tributaries successively contribute water with high in-stream nutrient concentrations. Nevertheless, over the entire length of the main stem of the Ngaruroro, nutrient concentrations remain generally very low, and below recommended periphyton accrual thresholds. By contrast nutrient concentrations in the main stem of the Tutaekuri increase until they are above recommended periphyton accrual thresholds in the middle and lower main stem. The reason for the differences in nutrient concentrations between the Tutaekuri and Ngaruroro main stems is that there is a large volume of low-nutrient water from the upper Ngaruroro which dilutes nutrient-enriched tributary inflows lower in the catchment. This dilution effect does not occur as much in the Tutaekuri.

Periphyton growth was higher in the Tutaekuri compared to the Ngaruroro, and increased from upstream to downstream. This relationship parallels the increasing level of nutrients across the study sites.

The NDS studies have shown that algal blooms in both catchments are largely limited by both nitrogen and phosphorus. The Tutaekuri River showed co- and N-limitation over several trials, with some tendency towards secondary P-limitation in the lower Tutaekuri River. The upper Ngaruroro River was entirely co-limited. The lower Ngaruroro River at Fernhill potentially switches between different nutrient-limitation conditions. An increase in either N or P will result in increased algal growth rates and therefore a higher periphyton cover and frequency in algal blooms.

The NDS studies show that the Waitangi estuary is predominantly co-limited. This suggests that any increase in in-stream concentrations of either N or P could result in increased algal growth in the estuary. This increased algal growth would most likely take the form of increases in the frequency, extent and/or longevity of phytoplankton blooms. It is also possible there could be some increase in benthic micro- and macro-algae growth.

Low nutrient availability and growth limitation by availability of both N and P (co-limitation) does not necessarily mean that both nutrients are required together to increase algal growth rates. Plant communities can shift in composition to include algal species that prefer the new N:P ratios. In addition, individual algal species may vary their rates of uptake and loss of scarce nutrients, particularly P (Keck and Lepori 2012), (McDowell et al. 2009), (Klausmeier et al. 2004).

This means that any increase in N and/or P could result in higher growth rates of periphyton, which could lead to more frequent and extended algal blooms in the Tutaekuri and Ngaruroro rivers.

The occurrence of *Phormidium* is an example of how a change in the community composition of periphyton depends on nutrient availability. *Phormidium* is a cyanobacteria that grows on stable substrate amongst other periphyton; it produces compounds that taint water and can contain neurotoxins such as anatoxin-a, homoanatoxin-a and mycrocystins that are associated with dog poisonings. *Phormidium* is likely to occur naturally amongst benthic periphyton, but where it is found in greater abundance it may impair aesthetic and recreational use of water. There is also a risk that ingestion of *Phormidium* mats that have accumulated on the river bank could poison dogs (MfE/MoH 2009).

Phormidium is known to have a high tolerance of variation in flow velocity, river depth and substrate type. During times of stable flow *Phormidium* abundance has been positively correlated with increases in nitrogen concentrations, when phosphorus availability was low (Heath et al. (2011), Heath et al. (2013), Wood and Young (2011)).

In several North Island rivers *Phormidium* proliferated when TN:TP ratios were greater than 20 (Wood and Young 2011). This is consistent with the situation in the Ngaruroro and Tutaekuri catchments. At most of those sites where *Phormidium* mats were observed in the period 2003-2013 – such as the Ngaruroro downstream of Hawke’s Bay Dairies, the Ngaruroro at Motorway Bridge, and the Mangatutu Stream – DIN:DRP ratios were often greater than 20:1. An increase in in-stream nitrogen concentrations could potentially lead to increased *Phormidium* growth in both catchments.

The national cyanobacterial recreational guidelines suggest alert levels should be triggered when algal mats accumulate on the river banks. Interim cyanobacteria guidelines recommend two risk triggers for benthic cyanobacteria, which are 20% cover for surveillance, and 50% as an alert trigger (MfE/MoH 2009). These guidelines are presently rarely exceeded in the Tutaekuri and Ngaruroro catchments. However, *Phormidium* mats have been observed sporadically in summer since monthly visual algal assessments started in 2012 (further details will be provided in future HBRC reporting).

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Appendix A Sampling sites and deployment overview

Table A-1: Nutrient Diffusing Study (NDS) sites and deployment times in the Tuatekuri and Ngaruroro catchments and in the Waitangi Estuary.

NDS deployment site	NZTM E	NZTM N	Catchment	NDS Deployment	Mid deployment check	NDS Retrieval	comments
February 2013 deployment							
Tutaekuri Lawrence Hut	1896263	5636415	Tutaekuri	23.01.2013		04.02.2013	
Tutaekuri u/s Brookfields Bridge	1927490	5615183	Tutaekuri	23.01.2013		04.02.2013	
Mangaone at Rissington	1920082	5627751	Tutaekuri	05.03.2013		19.03.2013	
Ngaruroro at Whanawhana	1891976	5615740	Ngaruroro	18.02.2013		04.03.2013	
Ngaruroro d/s HB dairies	1907154	5610538	Ngaruroro	18.02.2013		04.03.2013	Tray disturbed by caught willow branch, some filter papers lost;
Ngaruroro at Fernhill	1922950	5611351	Ngaruroro	18.02.2013		04.03.2013	
Waitangi Estuary Site 1	1937173	5612748		23.01.2013		05.02.2013	Site 1 close to Tutaekuri/Ngaruroro river mouth.
January 2014 deployment							
Tutaekuri Lawrence Hut	1896263	5636415	Tutaekuri	08.01.2014	16.01.2014	20.01.2014	
Tutaekuri u/s Puketapu Bridge	1925600	5619590	Tutaekuri	08.01.2014	16.01.2014	20.01.2014	Some filamentous algae caught on tray, cleared 16.1.2014 at mid-check
Mangaone at Rissington	1920082	5627751	Tutaekuri	08.01.2014			vandalised
Ngaruroro at Whanawhana	1891976	5615740	Ngaruroro	08.01.2014	16.01.2014	20.01.2014	
Ngaruroro at Fernhill	1922950	5611351	Ngaruroro	08.01.2014	16.01.2014	20.01.2014	
February 2014 deployment							
Tutaekuri Lawrence Hut	1896263	5636415	Tutaekuri	20.01.2014	04.02.2014	12.2.2014	
Tutaekuri u/s Puketapu Bridge	1925600	5619590	Tutaekuri	20.01.2014	04.02.2014	12.2.2014	Some filamentous algae caught on tray first week, cleared at mid-check. May have affected first filterpaper out of 5 of each treatment
Ngaruroro at Whanawhana	1891976	5615740	Ngaruroro	20.01.2014	04.02.2014	12.2.2014	Lost filter papers C1,3;P1;N1
Ngaruroro at Fernhill	1922950	5611351	Ngaruroro	20.01.2014	04.02.2014	12.2.2014	Entire tray smothered by filamentous algae week 2; NP2 lost
March 2014 deployment							
Tutaekuri Lawrence Hut	1896263	5636415	Tutaekuri	06.03.2014			vandalised
Tutaekuri u/s Puketapu Bridge	1925600	5619590	Tutaekuri	06.03.2014	14.03.2014	14.03.2014	1 week only, cyclone Lusi
Ngaruroro at Whanawhana	1891976	5615740	Ngaruroro	06.03.2014	14.03.2014	14.03.2014	1 week only, cyclone Lusi
Ngaruroro at Fernhill	1922950	5611351	Ngaruroro	06.03.2014	14.03.2014	14.03.2014	1 week only, cyclone Lusi
Waitangi Estuary Site 1	1937173	5612748		06.03.2014		14.03.2014	Site 1 close to Tutaekuri/Ngaruroro river mouth.
Waitangi Estuary Site 2	1937462	5612300		06.03.2014		14.03.2014	Site 2 closest to Clive River mouth.

Appendix B Statistics for NDS treatment results

Table B-1: Two- factor ANOVA results for effects of nutrient treatments on periphyton biomass response (measured as Chlorophyll *a* concentration). N: nitrate; P: phosphate; n.s. no significant effect. Post-hoc least squares means (LSM) followed significant ANOVA. Relevant *p*-statistics are given only for positive responses to nutrient addition.

Site	Study period	N effect	P effect	Interaction N*P	Interpretation
Upper Tuatekuri					
Tutaekuri at Lawrence Hut	February 2013	<0.001	<0.001	0.012	N and P co-limited
Tutaekuri at Lawrence Hut	January 2014	0.001	-	n.s.	N-limited
Tutaekuri at Lawrence Hut	February 2014	0.022	0.025	n.s.	N and P co-limited
Tutaekuri at Lawrence Hut	lost				
Lower Tutaekuri					
Tutaekuri u/s Brookfields Br	February 2013	-	-	0.004	N and P co-limited
Tuatekuri at Puketapu	January 2014	<0.001	-	n.s.	N-limited
Tuatekuri at Puketapu	February 2014	<0.001	-	0.007	1°N-limited, 2°P-limited
Tuatekuri at Puketapu	March 2014	n.s.	n.s.	n.s.	Not limited by N or P
Mangaone at Rissington	February 2014	n.s.	-	-	Not limited by N or P
Upper Ngaruroro					
Ngaruroro at Whanawhana	February 2013	<0.001	<0.001	0.001	N and P co-limited
Ngaruroro at Whanawhana	January 2014	-	0.01	0.01	N and P co-limited
Ngaruroro at Whanawhana	February 2014	0.001	<0.001	0.001	N and P co-limited
Ngaruroro at Whanawhana	March 2014	0.031	0.01	n.s.	N and P co-limited
Lower Ngaruroro					
Ngaruroro at Fernhill	February 2013	0.008	n.s.	n.s.	N-limited
Ngaruroro at Fernhill	January 2014	n.s.	0.029	n.s.	P-limited
Ngaruroro at Fernhill	February 2014	n.s.	n.s.	n.s.	Not limited by N or P
Ngaruroro at Fernhill	March 2014	0.01	<0.001	n.s.	N and P co-limited
Estuary					
Waitangi Estuary Site 1	February 2013	<0.001	-	n.s.	N-limited
Waitangi Estuary Site 1	March 2014	<0.001	<0.001	0.002	N and P co-limited
Waitangi Estuary Site 2	March 2014	0.011	0.004	n.s.	N and P co-limited