



Use of periphyton cover to estimate chlorophyll *a* concentration

Performance of Canterbury conversion factors in Wellington Region rivers

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Executive summary

This report summarises the results of a study undertaken to assess when visual assessment of periphyton cover can be reliably used in place of measurement of chlorophyll *a* for monitoring compliance with Greater Wellington Regional Council's Proposed Natural Resources Plan periphyton outcomes (PNRP; GWRC 2015) and the National Policy Statement for Freshwater Management periphyton attribute (NPS-FM; MfE 2014). Use of visual estimates of periphyton cover in place of chlorophyll *a* measurement could reduce the staff time and funding needed for GWRC to meet its periphyton monitoring obligations under the PNRP and NPS-FM.

The study tested the performance of periphyton conversion factors developed from rivers in the Canterbury Region (Kilroy et al. 2013) in estimating periphyton chlorophyll *a* concentration from estimates of periphyton cover from rivers in the Wellington Region. Conversion factors were tested using data from monthly paired surveys of periphyton cover (using visual estimates) and quantitative sample collection for measurement of periphyton chlorophyll *a* at 13 river sites in the Wellington Region between August 2015 and July 2016. One-off paired samples were also collected at 17 sites during January/February 2016.

The report also presents a preliminary analysis of measured chlorophyll *a* results from 13 monthly monitoring sites against PNRP periphyton outcomes and the NPS-FM periphyton attribute bottom line. Results show that two sites, Mangaroa River at Te Marua and Kopuaranga River at Stuarts, do not meet periphyton outcomes identified in the PNRP. This is despite only 12 of the required 36 samples having been collected. These sites are also close to exceeding the NPS-FM periphyton attribute bottom line.

There was a moderately strong regression relationship between measured chlorophyll *a* and that derived from periphyton cover using conversion factors (R^2 of 0.79 and 0.77 for C1 and C2 Canterbury conversion factors respectively). However, only samples dominated by 'no algae' and 'film' cover categories could reliably be assigned to the correct NPS-FM band chlorophyll *a* range. There are likely to be several reasons why samples with even relatively low cover of chlorophyll *a* rich periphyton categories could not be confidently assigned to an NPS-FM band range. One key reason is a potential difference in chlorophyll *a* content of periphyton cover categories in Wellington Region rivers compared to those in Canterbury where conversion factors were developed. There is potential to improve conversion factors relating to periphyton cover categories for rivers in the Wellington Region. However, there may not be significant advantage in using chlorophyll *a* results derived from periphyton cover assessment for samples with moderate or high cover of chlorophyll *a* rich periphyton classes. For these samples there was only a small difference in the time taken to collect a sample for chlorophyll *a* analysis compared to that required to complete an assessment of periphyton cover.

It is recommended that the use of chlorophyll *a* derived using C2 conversion factors is trialled for samples with $\geq 95\%$ cover of 'no algae' and 'film' periphyton categories.

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1. Introduction

Greater Wellington Regional Council's Proposed Natural Resources Plan (PNRP; GWRC 2015) and the National Policy Statement for Freshwater Management (NPS-FM; MfE 2014) stipulate reporting of periphyton biomass (as represented by chlorophyll *a*) based on monthly measurements. Monthly sampling of periphyton chlorophyll *a* represents a significant increase in staff time and analytical costs compared to annual sampling that has been undertaken by GWRC in the past. This is likely to have significant resourcing implications as future monitoring of PNRP outcomes and objectives identified by GWRC's collaborative land and water management committees, known as Waitua, will likely require monitoring at numerous river and stream sites around the Region. However, Ministry for the Environment NPS-FM attribute guidance suggests that, where periphyton biomass is likely to meet freshwater objectives, a proportion of monitoring could be carried out using quicker and less costly visual assessment of periphyton cover (MfE 2015).

This report presents the results of a study undertaken to identify when visual assessment of periphyton cover can be used in place of chlorophyll *a* measurement when monitoring compliance with PNRP objectives and the NPS-FM bottom line in the Wellington Region. The study involved testing the performance of periphyton conversion factors developed from data collected from rivers in the Canterbury Region (Kilroy et al. 2013) to estimate periphyton chlorophyll *a* concentration from estimates of periphyton cover. Monthly paired surveys of periphyton cover (using visual estimates) and quantitative sample collection for measurement of periphyton chlorophyll *a* were undertaken at 13 river and stream sites in the Wellington Region. One-off paired samples were collected at an additional 17 sites.

1.1 Report purpose

This report has been prepared with the primary purpose of assessing when periphyton cover assessments can be used in place of chlorophyll *a* measurement when monitoring compliance with PNRP outcomes, Waitua objectives and the NPS-FM bottom line. It is intended that this report will help inform the design of future monitoring of periphyton in rivers and streams in the Wellington Region.

The report also aims to provide a preliminary analysis of measured chlorophyll *a* results from 13 river and stream sites against PNRP periphyton outcomes and the NPS-FM periphyton attribute bottom line.

1.2 Report outline

This report comprises six sections, including this Introduction:

- Section 2 provides a brief background on periphyton monitoring, the PNRP and NPS-FM.
- Section 3 describes the methods used to undertake the paired chlorophyll *a* and cover measurements as well as the methods used for analysis and reporting of results

- Section 4 presents the results of chlorophyll *a* measurements and assessments of periphyton cover as well as providing an analysis of the accuracy of chlorophyll *a* conversion factors.
- Section 5 discusses the key findings for section 4 and presents a method for using conversion factors
- Section 6 presents recommendations for future work

2. Background

Periphyton biomass is an important indicator of river and stream ecosystem health (Biggs, 2000). This is reflected in periphyton biomass outcomes (as measured by chlorophyll *a*) being included in GWRC's PNRP (GWRC 2015) and periphyton chlorophyll *a*, being included as a compulsory attribute in the NPS-FM (MfE 2014). In the past, periphyton biomass in the Wellington Region has been assessed once annually during summer/autumn as part of the Rivers State of the Environment monitoring programme. However, assessment of compliance with the NPS-FM periphyton attributes and PNRP outcomes requires monthly assessment for a minimum of three years at each site (Greenfield et al. 2015; MfE 2015). Periphyton biomass samples are time intensive to collect and require laboratory analysis. In comparison, visual assessment of periphyton cover is quick and no laboratory analysis is needed.

Conversion factors have recently been developed for estimating periphyton biomass from cover assessments in Canterbury rivers (Kilroy et al. 2013). These conversion factors were tested using a data set of paired periphyton cover and chlorophyll *a* measurements collected from the same three streams from which the conversion factors were developed. For these streams, the biomass equivalent derived from visual assessments using the conversion factors was strongly correlated with measured biomass ($R^2 = 0.89$) (Kilroy et al. 2013).

3. Methodology

Monthly estimates of periphyton cover and samples for measurement of periphyton chlorophyll *a* were taken from 13 river and stream sites (Figure 3.1; Appendix 1) between August 2015 and July 2016. One-off assessments were also made at 17 other sites during January and February 2016. Both periphyton cover and chlorophyll *a* measurements were taken from run habitat¹.

Sampler training was undertaken in August 2015 prior to the first sample round. Samplers also participated in a joint assessment in November 2015 to ensure that cover assessments and samples for chlorophyll *a* analysis were being collected in a consistent way.

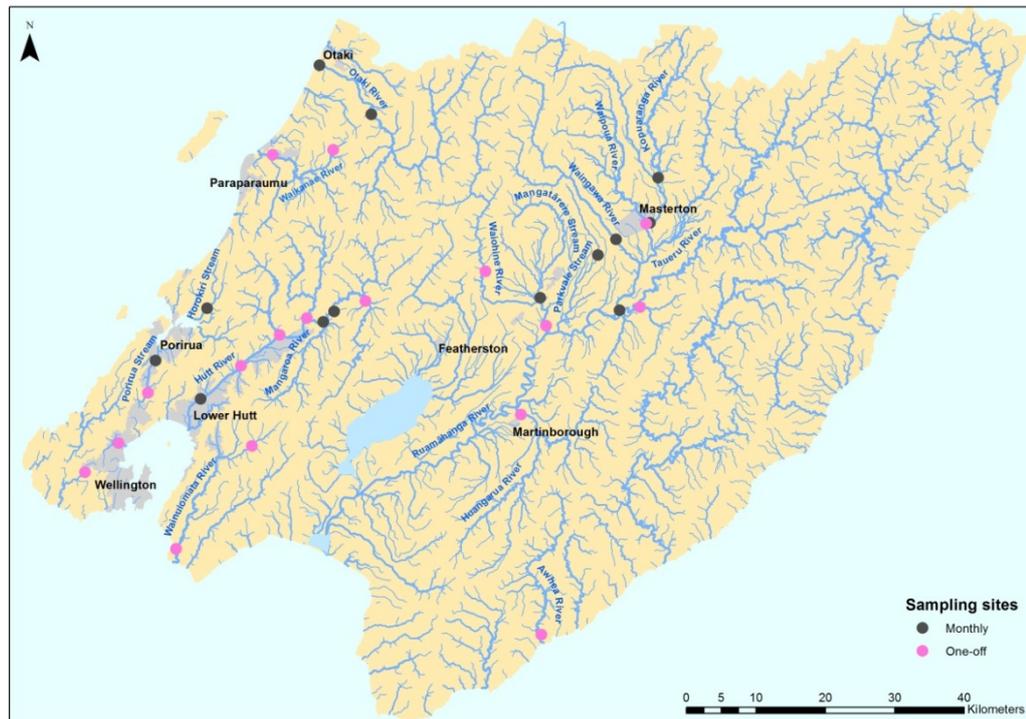


Figure 3.1: Map of river sites in the Wellington Region where paired periphyton cover and biomass (measured as chlorophyll *a*) were taken between August 2015 and July 2016

3.1 Sample collection

At monthly sampling sites, sampling was scheduled for the same time each month. However, if flow conditions did not allow safe access, sampling was delayed. At some sites, flow conditions meant that sampling could not be undertaken during some months.

On each sampling occasion, 20 points were marked out across four transects perpendicular to the direction of flow using flagging tape attached to fishing sinkers (Figure 3.2). Transects were spaced across a 30-50 m length of run habitat (or four or five times the width of the stream, whichever was smaller).

¹ A run is a stream segment where there is moderate water velocity that does not break the surface tension of the water and does not produce breaking wavelets that turn the surface water into white water.

On some occasions, the full width of the river/stream could not be accessed safely. In these cases samplers marked out sample points to the maximum safe depth (often around knee height). The minimum width of stream sampled was that at which five periphyton cover assessments could be undertaken without the viewing areas overlapping (approximately 1.8 m).



Figure 3.2: Fishing sinkers with coloured flagging tape used to mark periphyton viewing/biomass sampling points

Periphyton cover assessments were made at each of the 20 points. Assessments involved visually assessing the proportion of the river bed covered by each of nine periphyton or aquatic plant cover categories (Table 3.1). Periphyton cover categories were the same as those used by Kilroy et al. (2013). However, classes were added for macrophyte and bryophyte cover which can be considerable at some sites.

Table 3.1: Categories of periphyton/aquatic plants for cover assessments adapted from those in Kilroy et al. (2013)

Cover category	Description
No algae	No algae visible (no colour perceived); stone surface not slippery or slimy
Film	Fine, slightly slimy black, brown or greenish colouration, <0.5 mm thick (thin layer containing various algae including diatoms and Chlorophyta, but also bacteria and organic matter)
Mats	Definite consolidated layer of algae from 0.5 mm to >5 mm thick, variable colours; often dominated by diatoms but can be a mixture of diatoms, Cyanobacteria, Rhodophyta, Chlorophyta. Note that <i>Phormidium</i> -dominated mats are recorded as a separate category (Cyano)
Sludge	Loose, unconsolidated algae, easily dislodged; e.g. mucilage-rich accumulations of diatoms such as <i>Cymbella</i>
Cyanobacterial mats	Cyanobacterial mats, especially <i>Phormidium</i> ; distinctive black, brown or whitish flecked with black/brown, smooth surface
Green filamentous	Green filamentous algae, usually slimy and more than about 1 cm long; mainly Chlorophyta with some Xanthophyceae (Ochrophyta)
Other filamentous	Brown, reddish or other filaments, slimy or coarse, more than about 1 cm long. Includes Rhodophyta, filamentous diatoms and Chlorophyta covered with epiphytic diatoms
Bryophytes	Moss
Macrophytes	Aquatic plants such as oxygen weed, <i>Potamogeton</i> etc

Visual periphyton cover assessments were undertaken using an underwater viewer with a diameter of 350 mm (Nuova Rade, Genova, Italy). The bottom of the underwater viewer was divided into quarters to facilitate and standardise estimates of percentage cover. At each point the sinker was located just out of sight at the bottom of the viewed circle.

After periphyton cover assessments were made, a sample was collected for measurement of chlorophyll *a* from 10 of the marked points. Samplers randomly selected a single sample location at each of the 10 points by touching the river bed immediately upstream of the fishing sinker without looking and retrieving a single rock or a sample of smaller particles. Where rocks larger than 6 cm were collected Biggs and Kilroy (2000) method QM-1b (sampling from a defined area of rock) was used with a 5cm diameter lid. Where gravel or sand was collected Biggs and Kilroy (2000) method QM-3 (gravel/sand/silt sampling) was used with a 7 cm diameter lid. Samples from each of the 10 points were combined into a single composite sample for analysis.

Samples were frozen and sent to NIWA, Christchurch, for analysis for chlorophyll *a* using the Biggs and Kilroy (2000) spectrophotometric ethanol method.

3.2 Analysis of results

Chlorophyll *a* results received from the laboratory in units of mg/sample were converted to mg/m² using the sample area recorded. For five samples², periphyton chlorophyll *a* measurements are an estimate only as the area of the sample taken was not recorded. The area over which these samples were collected was estimated from previous samples.

For each sampling occasion, results from the 10 periphyton cover estimates that were paired with the 10 samples collected for chlorophyll *a* measurements were averaged.

Chlorophyll *a* results from samples which included macrophytes were excluded from the comparison with derived chlorophyll *a* estimates as no conversion factors exist for macrophytes and their high chlorophyll *a* content will invalidate the results.

Assessment of compliance with both PNRP outcomes (Table 3.2) and the NPS-FM bottom line (Table 3.3) is required to be based on three years of monthly samples (ie, 36 data points) (Greenfield et al. 2014; MfE 2015). However, an initial assessment of measured chlorophyll *a* from monthly sampling sites was made to assess whether any sites have already exceeded the maximum number of allowable exceedances. The exceedance frequency for each site was calculated as the number of exceedances of both PNRP outcome and the NPS-FM bottom line thresholds as a proportion of the 36 data points required.

² Ruamāhanga River at Gladstone Bridge and Mangatarere Stream at State Highway 2 in November 2015, Mangaroa River at Te Marua , Taueru River at Gladstone and Waiohine River at Gorge in February 2016.

Table 3.2: Proposed Natural Resources Plan (PNRP) periphyton outcomes and the National Policy Statement for Freshwater Management (NPS-FM) bottom line applying to each of the 13 monthly monitoring sites. See Appendix 1 for description of river classes

Site No.	Site name	PNRP river class	PNRP significant river	PNRP outcome (mg/m ²)	NPS-FM bottom line (mg/m ²)	Allowable exceedance frequency (%)
RS 05	Otaki River at Pukehinau	1	Y	≤50	≤200	8
RS 06	Otaki River at Mouth	4	Y	≤50	≤200	8
RS 13	Horokiri Stream at Snodgrass	2	N	≤120	≤200	8
RS 16	Porirua Stream at Wall Park	2	N	≤120	≤200	8
RS 20	Hutt River at Te Marua Intake	1	Y	≤50	≤200	8
RS 22	Hutt River at Boulcott	4	N	≤120	≤200	8
RS 24	Mangaroa River at Te Marua	1	N	≤50	≤200	8
RS 32	Ruamāhanga River at Te Ore Ore	4	N	≤120	≤200	8
RS 33	Ruamāhanga River at Gladstone	4	N	≤120	≤200	8
RS 38	Kopuaranga River at Stuarts	5	N	≤120	≤200	17
RS 41	Waingawa River at South Rd	4	N	≤120	≤200	8
RS 46	Parkvale Stream at Weir	5	N	≤120	≤200	17
RS 50	Mangatarere Stream at SH 2	4	N	≤120	≤200	8

Individual measured chlorophyll *a* results from all sampling sites were assigned to a chlorophyll *a* range corresponding to an NPS-FM band (Table 3.3). While individual samples can't be assigned to a band, this comparison was undertaken to assess the performance of conversion factors in the context of the NPS-FM periphyton attribute band thresholds.

Table 3.3: NPS-FM periphyton attribute band thresholds (MfE, 2014)

NPS-FM periphyton attribute band	Attribute state (mg chlorophyll <i>a</i> /m ²)
A	≤50
B	>50 - ≤120
C	>120 - ≤200
D (bottom line)	>200

For each sampling occasion, results from the 10 periphyton cover estimates that were paired with sample collection for chlorophyll *a* measurements were converted to a derived estimate of periphyton chlorophyll *a* using two sets of conversion factors³ (Table 3.4). The first set was that used in Kilroy et al. (2013) which was derived from samples from three streams in the Canterbury Region. The second set was derived from paired cover and chlorophyll *a* measurements from a larger set of rivers and streams in the Canterbury Region (Kilroy, pers comm).

³ An estimate of periphyton chlorophyll *a* is the sum of mean percent cover of each periphyton cover category multiplied by the conversion factor, divided by 100.

Table 3.4: Conversion factors developed for Canterbury rivers and streams to convert periphyton cover assessments to periphyton chlorophyll *a*

Periphyton cover category	Chlorophyll <i>a</i> equivalent (mg/m ²)	
	Conversion factors 1 (Kilroy et al. 2013)	Conversion factors 2 (C. Kilroy pers comm.)
No algae	1	0.3
Film	9	6.8
Sludge	72	27
Mats	118	147
Cyanobacterial mats	599	335
Green filamentous	404	340
Other filamentous	517	305

Estimates of periphyton chlorophyll *a* derived from cover estimates were then compared to measurements of periphyton chlorophyll *a* from samples taken at the same time. Comparisons were made using linear regression on log-transformed data.

Derived chlorophyll *a* results were compared to NPS-FM periphyton attribute band thresholds to assess the accuracy of predictions in relation to these.

4. Results

A total of 163 samples were taken including 146 monthly samples and 17 one-off samples. On eight occasions, samples could not be collected due to high flows making sampling unsafe⁴. On two occasions at Parkvale Stream at Weir samples could not be collected due to the site being dominated by macrophyte growth. Chlorophyll *a* results from five samples were excluded from analysis due to macrophyte being included in samples (two each from Kopuaranga River at Stuarts and Parkvale Stream at Weir and one from Taueru River at Gladstone).

On average periphyton cover assessments took 15-20 minutes to complete per site while collecting a sample for chlorophyll *a* analysis took 30-40 minutes. However, at sites with high coverage of a range of different periphyton categories, visual cover assessments would generally take longer. On these occasions visual assessment of periphyton cover could take almost as long as collecting a sample for chlorophyll *a* analysis.

4.1 Measured chlorophyll *a* and periphyton cover

Periphyton chlorophyll *a* measurements ranged from 0 mg/m² (measured in four samples from Otaki River at Pukehinau, Otaki River at Mouth, Ruamāhanga River at Te Ore Ore and Waingawa River at South Road) to 501 mg/m² recorded in a one-off sample taken at Huangarua River at Ponatahi Bridge in January 2016 (Figure 4.1).

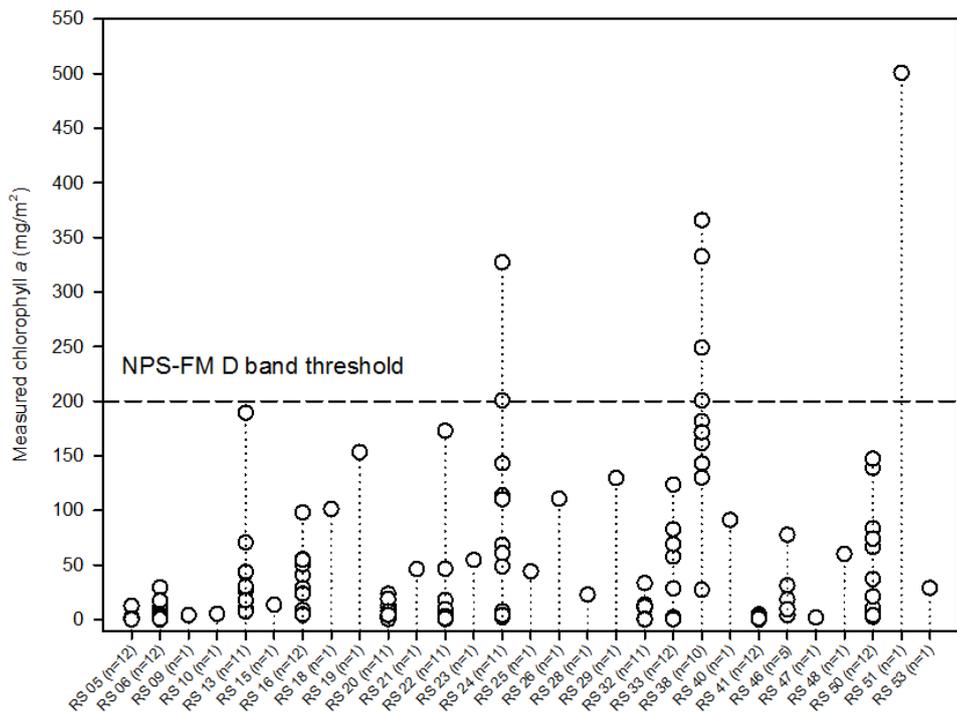


Figure 4.1: Measurements of periphyton chlorophyll *a* taken from river sites between August 2015 and July 2016. Monthly measurements were made at 13 sites and one-off measurements were made at 16 sites

⁴ One sample each at Horokiri Stream at Snodgrass, Hutt River at Te Marua, Hutt River at Boulcott and Mangaroa River at Te Marua and Ruamāhanga River at Te Ore Ore and three samples at Parkvale Stream at Weir

In most samples (117 or 71%) chlorophyll *a* was below the threshold defining the A band in the NPS-FM periphyton attribute (Table 3.3). Only 4% of samples had chlorophyll *a* that exceeded the threshold defining the D band.

Comparison of measured chlorophyll *a* results from monthly sampling sites to NPS-FM periphyton attribute band thresholds showed that, at five sites (RS05 Otaki River at Pukehinau, RS06 Otaki River at Mouth, RS20 Hutt River at Te Marua, RS32 Ruamāhanga at Te Ore Ore and RS41 Waingawa River at South Road), all results fell below the threshold defining the A band (Figure 4.2). In contrast, RS24 Mangaroa River at Te Marua (RS24) and Kopuaranga River at Stuarts (RS38) had two or more samples with chlorophyll *a* above the D band threshold.

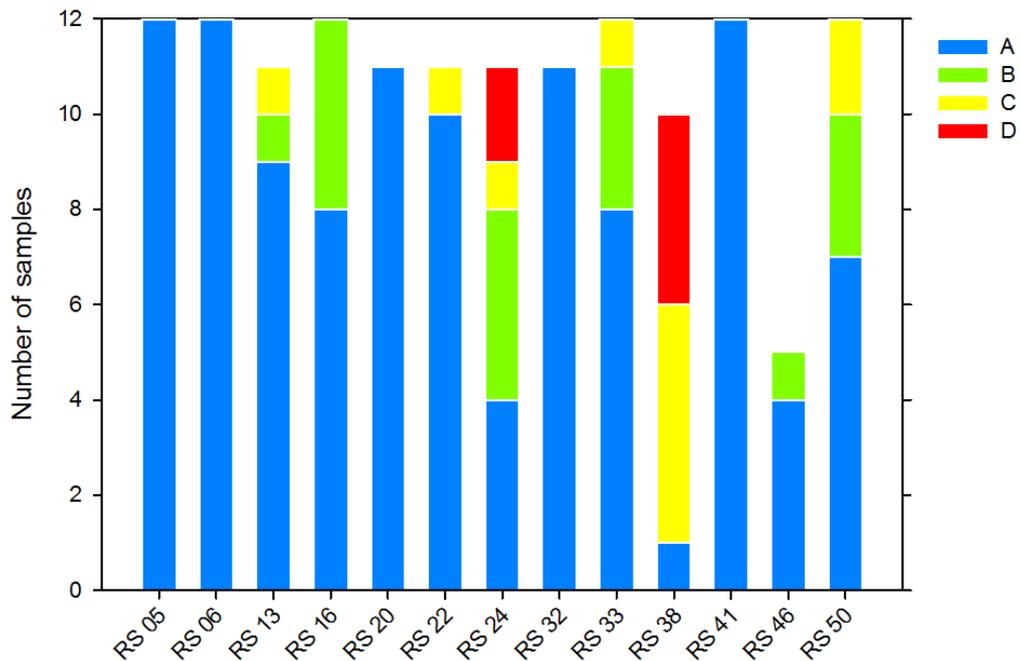


Figure 4.2: Number of samples at monthly monitoring sites with measured chlorophyll *a* in each of the NPS-FM band ranges

Comparison of measured chlorophyll *a* results with PNRP outcomes shows that Mangaroa River at Te Marua and Kopuaranga River at Stuarts have already exceeded the maximum number of samples allowed to exceed the threshold (50 mg/m² at Mangaroa River at Te Marua and 120 mg/m² at Kopuaranga River at Stuarts; Appendix 2). This is despite only 12 of the 36 required samples having been collected. Mangatarere Stream at SH 2 is close to exceeding the PNRP periphyton outcome.

Comparison of results with the NPS-FM D band threshold shows that Mangaroa River at Te Marua and Kopuaranga River at Stuarts have almost reached the maximum number of samples allowed to exceed this threshold based on 36 samples.

With regard to periphyton cover, Otaki River at Pukehinau, Hutt River at Te Marua and Waingawa River at South Road were generally dominated by cover categories ‘no algae’ and ‘film’ (Appendix 3). ‘Sludge’ was often the dominant

periphyton cover category recorded at Horokiri Stream at Snodgrass and Porirua Stream at Wall Park while the ‘other filamentous’ periphyton category often covered a high proportion of the river bed at Mangaroa River at Te Marua and Mangatarere Stream at SH 2 (Figure 4.3). A high proportion of cover of the ‘green filamentous’ category was recorded at Ruamāhanga River at Gladstone on several occasions. Periphyton cover at Kopuaranga River at Stuarts consisted of a range of chlorophyll *a* rich cover categories including ‘green filamentous’, ‘other filamentous’ and ‘cyanobacteria’ on virtually all sampling occasions. Macrophyte growth was significant on occasion at Kopuaranga River at Stuarts and Parkvale Stream at Weir.



Figure 4.3: Periphyton belonging to the cover category ‘other filamentous’ at Mangaroa River at Te Marua in February 2016. At this site, the periphyton in this category were generally filamentous diatoms

4.2 Accuracy of chlorophyll *a* conversion factors

Periphyton chlorophyll *a* estimates derived from periphyton cover assessments using conversion factors were moderately strongly correlated with measured periphyton chlorophyll *a*. Estimates derived using conversion factors 1 (C1) had a marginally stronger correlation with an R^2 of 0.79 compared to an R^2 value of 0.77 for conversion factors 2 (C2) (Figure 4.4). However, the linear regression relationship of estimates derived using conversion factors 2 to measured chlorophyll *a* was closer to the 1:1 line. Note that the relationships apply to log-transformed data.

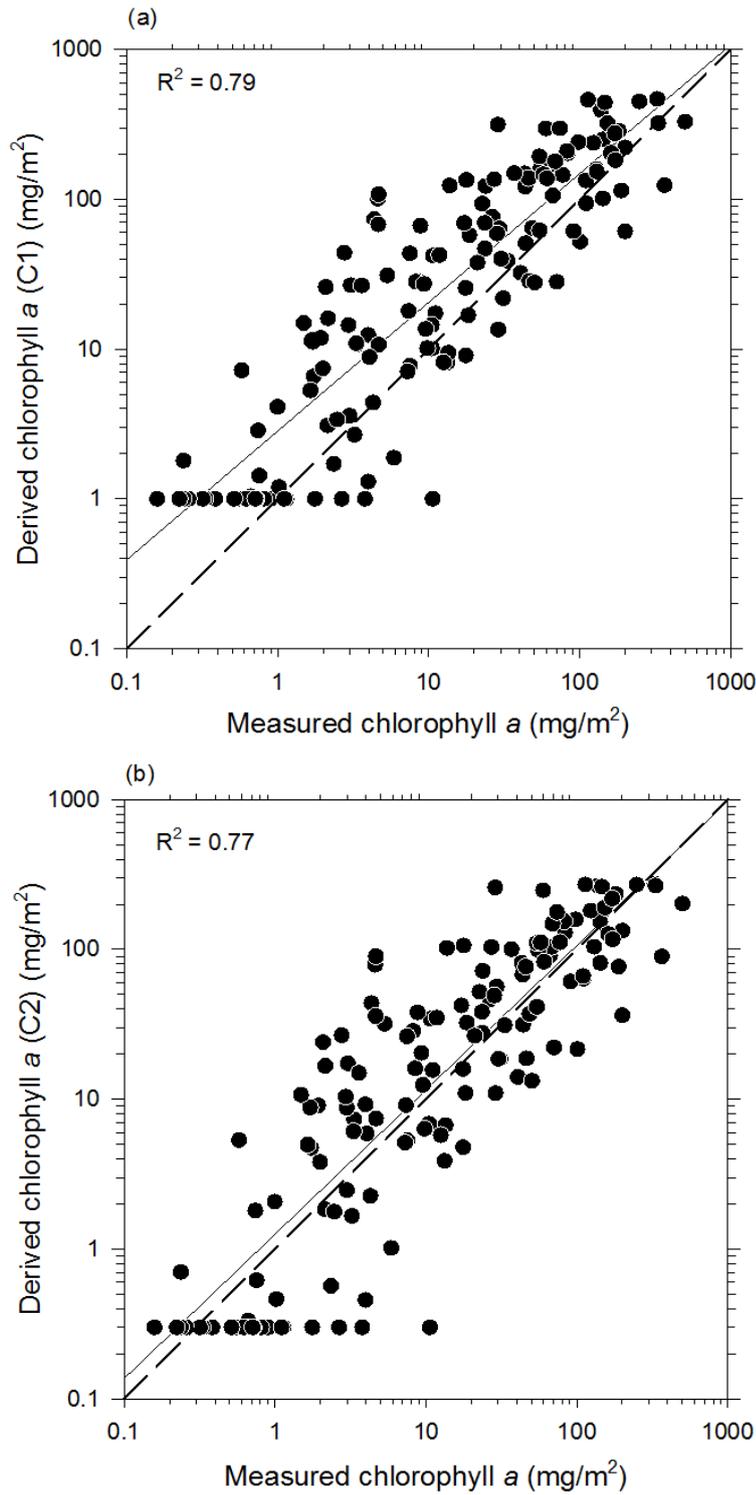


Figure 4.4: Periphyton chlorophyll *a* derived from visual estimates at 13 river sites monitored monthly and 16 sites sampled on a single occasion between August 2015 and July 2016, using (a) C1 and (b) C2 conversion factors plotted against measured periphyton chlorophyll *a*. The solid line represents the best fit regression ($P < 0.001$) while the dashed line is the 1:1 relationship

Using both C1 and C2 conversion factors the difference between measured and derived chlorophyll *a* was a less than 10mg/m² for 77 samples (49%). These samples generally had high coverage of ‘no algae’ and ‘film’ periphyton categories and very low coverage of chlorophyll *a* rich categories ‘cyanobacteria’, ‘green filamentous’ and ‘other filamentous’ (Figure 4.5).

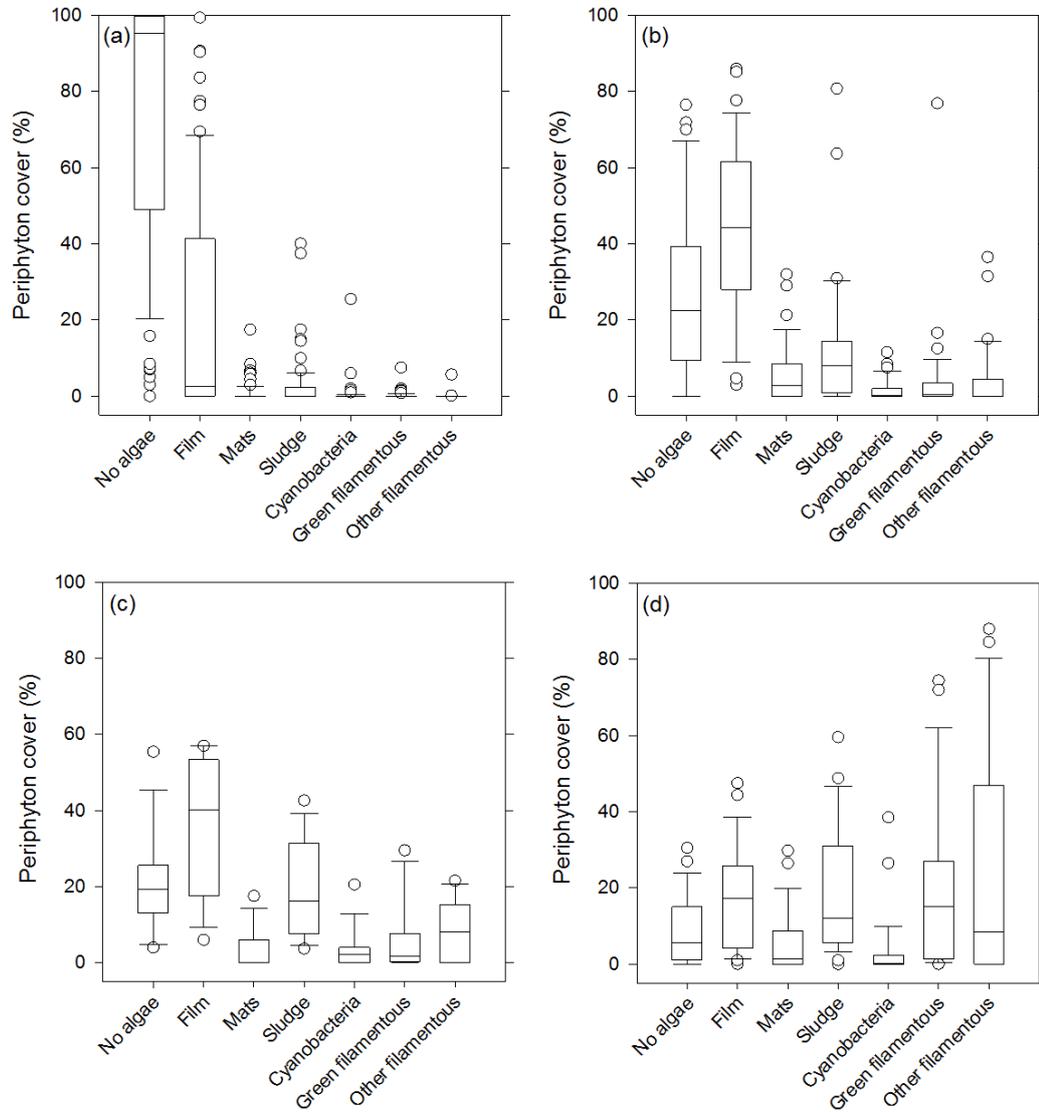


Figure 4.5: Box plot of cover of periphyton categories measured for samples with (a) $<10 \text{ mg/m}^2$ ($n=77$), (b) $10\text{-}50 \text{ mg/m}^2$ ($n=39$), (c) $51\text{-}100 \text{ mg/m}^2$ ($n=14$) and (d) $>100 \text{ mg/m}^2$ ($n=28$) difference between measured chlorophyll *a* and chlorophyll *a* derived using C1 conversion factors. A similar pattern was observed in results based on C2 conversion factors (not shown). The line inside the box is the median, the box edges are the 25th and 75th percentiles, whiskers are the 90th and 10th percentiles, and open circles are outliers

There was between 50 and 100 mg/m² difference between measured and derived chlorophyll *a* results for 14 samples using C1 conversion factors and 19 samples using C2 conversion factors. These samples included a wide range of periphyton cover categories and tended to have higher coverage of ‘sludge’, ‘green filamentous’ and ‘other filamentous’ categories (median coverage of

16%, 2% and 8% respectively for C1 conversions). There was more than 100 mg/m² difference between measured and derived chlorophyll *a* results for 28 samples (18%) using C1 conversion factors and 10 samples using C2 conversion factors. These samples had median cover of 12%, 15% and 8% for ‘sludge’, ‘green filamentous’ and ‘other filamentous’ categories respectively for C1 conversions.

For both sets of conversion factors, derived chlorophyll *a* estimates tended to be higher than measured chlorophyll *a*. Overall, C2 conversion factors resulted in derived chlorophyll *a* estimates closer to measured values (Figure 4.4).

A large proportion of the chlorophyll *a* estimates derived using conversion factors were correctly placed in the range of chlorophyll *a* used to define the NPS-FM A band (Table 3.3). Using C1 conversion factors, both estimated and measured chlorophyll *a* were < 50 mg/m² in 94 samples (80%). Using C2 conversion factors, 104 samples (89%) had both derived and measured chlorophyll *a* below 50 mg/m² (Figure 4.6). Using C1 conversion factors 14 samples were incorrectly assigned to the chlorophyll *a* range defining the B band and 8 to the C band. Using C2 conversion factors, 12 samples were incorrectly assigned to the B band range and no samples were incorrectly assigned to the C band range.

Both sets of conversion factors incorrectly placed one sample with measured chlorophyll *a* in the A band range to the D band range. This was a one-off sample collected from the Awhea River at Tora Road on 21 January 2016 which had a measured chlorophyll *a* of 29 mg/m². Coverage of 75% of the river bed by the ‘green filamentous’ periphyton category at this site meant that derived chlorophyll *a* values were 314 mg/m² and 258 mg/m² for conversion factors C1 and C2 respectively.

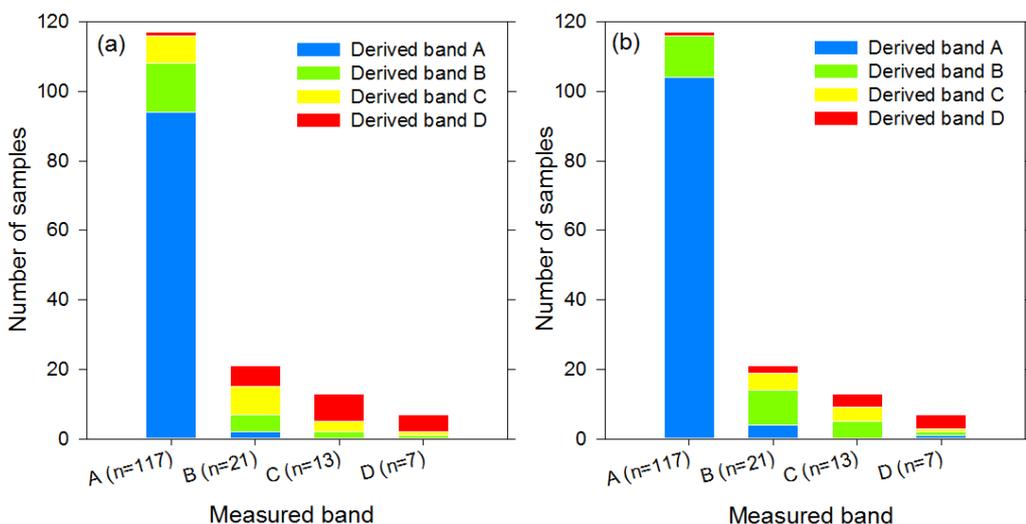


Figure 4.6: Number of measured chlorophyll *a* sample results in the range of each NPS-FM periphyton attribute band compared to number of derived chlorophyll *a* sample results assigned to each band range for (a) C1 conversion factors and (b) C2 conversion factors

The proportion of correctly assigned B and C band samples ranged between 23% for the C band range derived using C1 conversion factors, and 47% for B band using C2 conversion factors. In some cases there was a difference of up to two bands between measured results in the band B and C ranges and bands assigned based on derived chlorophyll *a*.

Of the small number of measured results that fell into the D band range 71% of results derived using C1 conversion factors were correctly assigned to the D band while 57% of results were correctly assigned using C2 conversion factors.

Overall, chlorophyll *a* derived using C2 conversion factors resulted in a higher proportion of samples being assigned the correct NPS-FM band range of chlorophyll *a* (77% compared to 68% for C1 conversion factors).

Due to the greater accuracy of chlorophyll *a* estimates derived using C2 conversion factors over those derived using C1 conversion factors, the following section refers to chlorophyll *a* results derived using C2 conversion factors only.

4.3 Performance of C2 conversion factors for NPS-FM band A samples

Of the samples correctly assigned to NPS-FM band A range ($\leq 50 \text{ mg/m}^2$) using C2 conversion factors in Figure 4.6, 90% had a measured biomass of less than 23 mg/m^2 while 75% had a measured biomass of less than 11 mg/m^2 (Figure 4.7).

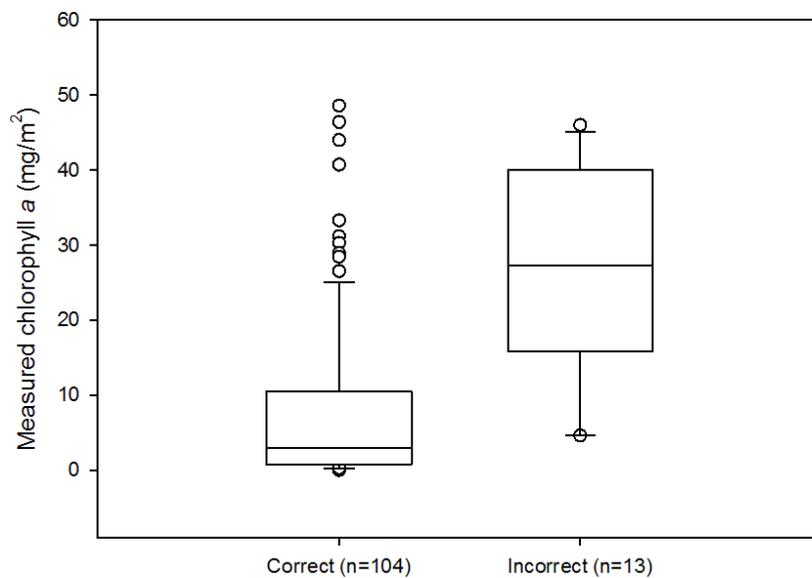


Figure 4.7: Box plot of measured chlorophyll *a* for samples falling into the NPS-FM A band range ($\leq 50 \text{ mg/m}^2$) and correctly or incorrectly assigned using C2 conversion factors. The line inside the box is the median, the box edges are the 25th and 75th percentiles, whiskers are the 90th and 10th percentiles, and open circles are outliers

Periphyton cover of samples correctly placed in the A band range was dominated by categories ‘no algae’ and ‘film’ (median cover of 72% and 19% respectively – data not shown). Correctly assigned samples had only very low cover of chlorophyll *a* rich categories such as ‘green filamentous’ and ‘other filamentous’ (90th percentiles < 2% for all).

Conversely, 90% of the samples incorrectly assigned to B, C or D band ranges had measured biomass greater than 6 mg/m² and 75% had measured biomass greater than 17 mg/m² (Figure 4.7). These samples had much lower median coverage of the ‘no algae’ cover category (21%) and higher median coverage of ‘sludge’ (13%) and ‘green filamentous’ (12%) categories.

In band A samples, the most reliable predictions were for samples with a high coverage of ‘no algae’ and ‘film’ cover categories. All samples with combined ‘no algae/film’ coverage of 80% or greater were correctly assigned to the A band. However, two of these samples had measured chlorophyll *a* results within 10 mg/m² of the 50 mg/m² A band threshold. In addition, while all samples with >80% ‘no algae’ and ‘film’ coverage were correctly assigned to the A band, the difference between measured and derived chlorophyll *a* across these samples was up to 39 mg/m² (Figure 4.8).

In contrast, for samples with combined ‘no algae’ and ‘film’ cover of 95-100% the maximum measured biomass was 14 mg/m² and the maximum difference between measured chlorophyll *a* and chlorophyll *a* derived using C2 conversion factors was 11 mg/m² (Figure 4.8).

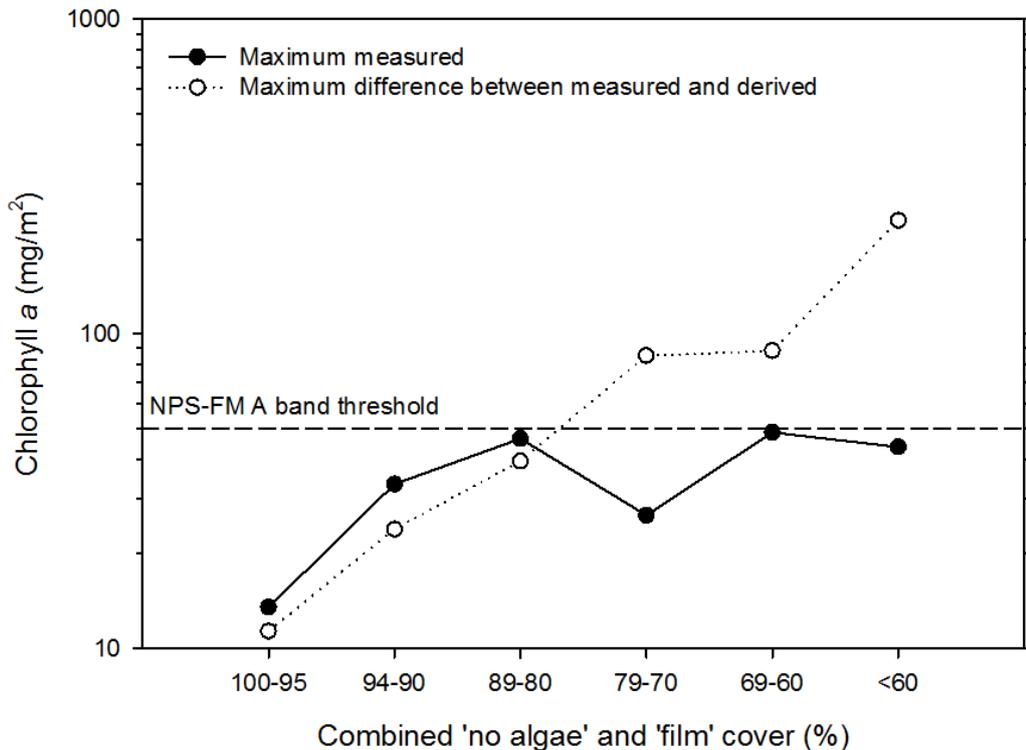


Figure 4.8: Maximum measured chlorophyll *a* and the maximum difference between measured and derived (C2) chlorophyll *a* for A band samples in six categories of combined ‘no algae’ and ‘film’ cover

5. Discussion

5.1 Measured chlorophyll *a* and cover results

Of the 13 sites sampled in this study, many in PNRP river classes 1 (steep, hard sedimentary) and 4 (lowland, large, draining ranges) frequently had chlorophyll *a* results $< 50 \text{ mg/m}^2$ throughout the year. However, it is concerning that two sites, Mangaroa River at Te Marua and Kopuaranga River at Stuarts, have already exceeded their PNRP periphyton outcome and are close to exceeding the NPS-FM periphyton attribute bottom line. This is despite only 12 of the 36 required data points having been collected.

The Mangaroa River at Te Marua has moderate levels of dissolved nutrients. Median concentrations of 0.41 mg/L Dissolved Inorganic Nitrogen (DIN) and 0.01 mg/L Dissolved Reactive Phosphorus (DRP) have been recorded in this river (Heath and Greenfield 2016). In addition, the Mangaroa River is often subject to long intervals between flows large enough to remove algal growth (average maximum accrual period of 89 days; Thompson and Gordon 2011). The Mangaroa River catchment is dominated by dry stock farmland and investigations have shown that nutrient inputs occur primarily in the lower part of the catchment (Heath and Greenfield 2016).

The Kopuaranga River at Stuarts has moderate to high dissolved nutrient levels (median DIN and DRP concentrations of 0.89 mg/L and 0.01 mg/L respectively; Keenan and Morar 2015) and is also subject to long periods between flushing flows (average maximum accrual period of 113 days; Thompson and Gordon 2011). The Kopuaranga River catchment is dominated by both high and low producing pastoral land uses (Perrie et al. 2012).

Water quality objectives and corresponding water and land management limits are currently being set for rivers and streams in the Ruamāhanga River catchment, including the Kopuaranga River, by the Ruamāhanga Whaitua. Objectives and limits will be set for the Mangaroa River by the Wellington/Hutt Valley Whaitua which is due to start in 2017.

Monthly monitoring of periphyton chlorophyll *a* needs to continue for two more years to be able to make a full assessment of compliance with PNRP outcomes and the NPS-FM bottom line. In addition, monthly monitoring of periphyton chlorophyll *a* will need to be expanded to other key river and stream sites in the Region to allow for adequate future reporting. Reporting against the NPS-FM bottom line and Whaitua objectives will be based on Freshwater Management Units (FMUs). As FMUs have yet to be set for the Wellington Region this poses some challenges in identifying appropriate monitoring sites. However, sites in the larger rivers and streams in each Whaitua are likely to be important in future NPS-FM and Whaitua monitoring.

5.2 Accuracy of chlorophyll *a* conversion factors

Chlorophyll *a* results derived using the two sets of conversion factors developed from Canterbury rivers had a moderate correlation with measured chlorophyll *a* (R^2 of 0.79 and 0.77 for C1 and C2 conversion factors respectively). The regression relationship was weaker than that identified between measured and derived chlorophyll *a* in Kilroy et al (2013; $R^2= 0.89$). In this study, derived chlorophyll *a* was generally higher than measured chlorophyll *a*. In contrast Kilroy et al. (2013) found that derived chlorophyll *a* was lower than measured chlorophyll *a* using C1 conversion factors.

In general, chlorophyll *a* derived using C2 conversion factors were closer to measured values than those derived using C1 conversion factors. This is likely to be due to C2 conversion factors being based on measurements from a wider range of rivers and streams in the Canterbury region and therefore capturing more periphyton types than C1 conversion factors. C1 conversion factors are based on results from three streams only (Kilroy et al. 2013).

Although there was a moderately strong regression relationship between measured and derived chlorophyll *a*, only results with low chlorophyll *a* dominated by ‘no algae’ and ‘films’ cover categories could reliably be assigned an NPS-FM band using either set of conversion factors. Derived chlorophyll *a* of samples with even relatively low coverage of chlorophyll *a* rich classes such as ‘green filamentous’ and ‘other filamentous’ could be 100 mg/m² or more than measured chlorophyll *a*. This means that samples with measured chlorophyll *a* in the range of the B, C or D NPS-FM bands could not be confidently assigned a NPS-FM band using derived chlorophyll *a*.

Some key likely reasons for differences between chlorophyll *a* derived using conversion factors and measured results are:

- Chlorophyll *a* content can vary between different periphyton taxa within the same cover category (Kasprzak et al. 2008) and within the same taxon at different times of year (Baulch et al. 2009). This makes it difficult to identify accurate conversion factors for periphyton types between different regions and even between different rivers within a region. For example, in this study chlorophyll *a* derived using C1 conversion factors from samples with a large amount of cover by the ‘other filamentous’ category was often considerably higher than measured chlorophyll *a*. It is likely that this overestimate is due to the type of algae that formed the basis of the ‘other filamentous’ cover category in Kilroy et al. (2013). In the Kilroy study the green algae *Cladophora* covered in epiphytic diatoms was the dominant type of alga in the ‘other filamentous’ category (Kilroy pers comm. 2016). This type of algae is likely to have a considerably higher chlorophyll *a* per unit area than the filamentous diatom taxa (e.g., *Melosira*) that generally dominated the ‘other filamentous’ category at sites sampled in this study (Figure 4.3).

- In paired studies such as this there is a mismatch between the area assessed for periphyton cover and the area from which samples for chlorophyll *a* analysis are taken. In this study, visual estimates of periphyton cover were taken over an area of approximately 0.96 m² of the river bed while samples for measurement of chlorophyll *a* were taken from an area of river bed of on average 0.03 m² (i.e., an area about 32 times less than that for visual assessment). This makes it difficult to directly compare measured and derived chlorophyll *a* results. In many cases, overestimates of derived chlorophyll *a* in samples with patchy cover of chlorophyll *a* rich classes are likely to be due to these classes simply not being captured in the area from which samples for chlorophyll *a* analysis were taken. For example, samples taken from Otaki River at Mouth on 3 February 2016 (Figure 5.1) and Waingawa River at South Road on 1 February 2016 both had patchy coverage of ‘green filamentous’ periphyton (18% and 26% cover respectively). This meant that based on chlorophyll *a* derived from cover assessments using C2 conversion factors these samples were assigned to the B band (derived biomass of 79 and 90 mg/m² respectively). However, measured chlorophyll *a* results for both these samples fell well within the A band (4.6 and 4.7 mg/m² respectively) suggesting that the ‘green filamentous’ algae had not been captured in the sample taken for chlorophyll *a* analysis.
- Some periphyton types such as filamentous diatoms, fine filamentous green algae and sludge are hard to capture in samples for chlorophyll *a* analysis. These types of algae can slip off the substrate as it is removed from the water for the sample to be taken. This was the case for the sample taken from the Awhea River at Tora Road in January 2016 mentioned in section 4.2. High coverage of green filamentous algae recorded at this site consisted of fine filaments which were largely unattached to the substrate. These filaments were difficult to capture in the sample for chlorophyll *a* analysis resulting in a large discrepancy between the measured and derived chlorophyll *a* results for this sample.
- As identified in Kilroy et al (2013) there can be bias in estimates of periphyton cover and one operator may consistently estimate higher or lower percentage cover for the more obvious cover categories such as ‘green filamentous’. There can also be difficulties in accurately assigning some cover categories. For example, it was often particularly difficult to assess coverage of periphyton in the ‘sludge’ category as algae in this category was often interspersed amongst other categories making it hard to decipher where one category stopped and the other started.



Figure 5.1: Green filamentous algae growing in the Otaki River at Mouth on 3 February 2016

It is possible that conversion factors could be developed further to provide more accurate estimates of chlorophyll *a* associated with the different periphyton cover categories in rivers in the Wellington Region. This development could include use of the data collected in this study to derive conversion factors using an iterative process. In addition, collection of samples from rock areas with 100% cover by a single cover category might result in more accurate estimates of mean chlorophyll *a* content for each category.

However, it is unclear whether chlorophyll *a* in samples with moderate or high periphyton cover could ever be predicted accurately enough to be used in PNRP and NPS-FM reporting using conversion factors. In addition, results from this study suggest that the time taken to complete a visual assessment of periphyton cover at sites where there is high coverage of a range of periphyton categories was often equivalent to the time taken to collect a sample for chlorophyll *a* analysis. This may mean that in these instances there is little benefit in using conversion factors to derive chlorophyll *a* from periphyton cover with regard to staff time.

5.3 Use of C2 conversion factors to derive chlorophyll *a* in samples with low periphyton cover

It is important that reporting against PNRP periphyton outcomes and the NPS-FM periphyton attribute bottom line is based on monitoring results that are as accurate as possible. This means that conversion factors to derive chlorophyll *a* from measures of periphyton cover should only be used when there is a high degree of certainty that samples will be assigned to the correct chlorophyll *a* range. Based on the paired data collected during this study, accurate

assignment to the correct chlorophyll *a* range occurred only for samples where either periphyton was not present across a large proportion of the river bed (as identified by high cover of the 'no algae' category) or where coverage was limited to 'film'. Even low cover of chlorophyll *a* rich classes such as 'green filamentous' and 'other filamentous' can result in samples being incorrectly assigned an NPS-FM band based on derived chlorophyll *a*.

Although all samples in this study with combined 'no algae' and 'film' cover of $\geq 80\%$ were correctly assigned to the A band range of chlorophyll *a*, several had measured and/or derived chlorophyll *a* results within 10 mg/m^2 of the A band threshold. This narrow margin represents a risk that a sample could be assigned an incorrect NPS-FM band based on derived chlorophyll *a* results.

Only samples with $\geq 95\%$ combined cover of 'no algae' and 'films' were considered to have a sufficiently low risk of being assigned to the incorrect chlorophyll *a* range used to define the NPS-FM bands. Based on results from this study the worst case scenario for a sample with $\geq 95\%$ combined cover of 'no algae' and 'films' is that a sample with a measured chlorophyll *a* result of 14 mg/m^2 would be identified as having a derived chlorophyll *a* result of 25 mg/m^2 . As both results are well within the A band range there is a high degree of confidence that the sample has been correctly assigned.

This suggests that conversion factors could be used with confidence in PNRP and NPS-FM reporting for samples with $\geq 95\%$ coverage of 'no algae' and 'film' categories.

Sixty six samples out of the 158 samples collected (42%) had combined 'no algae' and 'film' coverage of $\geq 95\%$. These samples were almost all collected from sites in PNRP river class 1 (steep, hard sedimentary) and river class 4 (lowland, large, draining ranges). There would be significant savings in both staff time and laboratory analysis costs if chlorophyll *a* can be confidently derived from periphyton cover for these samples.

In order to apply this method, field samplers need to be able to quickly assess in the field whether a sample for chlorophyll *a* measurement is required or whether a cover assessment will be sufficient. It is proposed that a quick assessment can be based on an initial instream cover assessment at five points across a single transect. If combined average cover of 'no algae' and 'film' categories is $\geq 95\%$ then the sampler continues to do a full instream cover assessment (i.e., 20 views across four transects). If combined average cover of 'no algae' and 'film' categories is $< 95\%$ then the sampler stops visual assessment and proceeds to take a sample for chlorophyll *a* measurement. Visual assessment of periphyton cover should be stopped at any time during a full assessment of average cover of 'no algae' and 'film' categories is $\geq 95\%$.

Visual assessment results for samples with $\geq 95\%$ combined cover of 'no algae' and 'film' categories are then used to derive chlorophyll *a* results using C2 conversion factors. These results can then be used in reporting along with measured chlorophyll *a*. It is recommended that derived results be identified in the data set for transparency of reporting.

6. Conclusion

Results from this study show that two sites, Mangaroa River at Te Marua and Kopuaranga River at Stuarts, do not meet periphyton outcomes identified in the PNRP. This is despite only 12 of the required 36 samples having been collected. These sites are also close to exceeding the NPS-FM periphyton attribute bottom line.

There was a moderately strong regression relationship between measured chlorophyll *a* and that derived from periphyton cover using conversion factors. However, only samples dominated by 'no algae' and 'films' cover categories could reliably be assigned an NPS-FM band. There are likely to be several reasons why samples with even relatively low cover of chlorophyll *a* rich periphyton categories could not be confidently assigned to an NPS-FM band. One reason is a potential difference in chlorophyll *a* content of periphyton cover categories in Wellington Region rivers compared to those in Canterbury where conversion factors were derived. Another is the mismatch in sampling areas and periphyton types captured between visual assessments of periphyton cover and collection of samples for chlorophyll *a* analysis.

There is potential to improve conversion factors relating to periphyton cover categories for rivers in the Wellington Region. However, for samples with moderate or high cover by chlorophyll *a* rich periphyton classes as there is often only a small difference in the time taken to complete an assessment of periphyton cover compared to that required to collect a sample for chlorophyll *a* analysis. Therefore, there may not be significant advantage in using chlorophyll *a* results derived from periphyton cover assessment for these types of samples.

In the meantime, there is likely to be benefit in trialling the use of derived chlorophyll *a* using C2 conversion factors for samples with $\geq 95\%$ cover of 'no algae' and 'film' periphyton categories.

6.1 Recommendations

Based on the results from this study it is recommended that:

- Monthly monitoring of periphyton chlorophyll *a* concentration continues at river and stream sites likely to be important for reporting against PNRP periphyton outcomes, Waitua objectives and the NPS-FM periphyton attribute bottom line as part of a long term periphyton monitoring programme. The sites monitored should be expanded as resources allow.
- In 2016/17 sampling at sites with consistently low chlorophyll *a* and therefore low risk of exceeding PNRP outcomes such as Otaki River at Pukehinau, Hutt River at Te Marua, Ruamāhanga River at Te Ore Ore and Waingawa River at South Road should cease. These sites should be replaced with other sites lower in the reaches of large rivers or key small stream sites. Monthly visual assessment of periphyton cover will continue to be undertaken at these sites as part of the River State of the Environment monitoring programme.

- Sites at Waikanae River at Greenaway Road, Kaiwharawhara Stream at Ngaio Gorge, Wainuiomata River downstream of White Bridge, Waiohine River at Bicknells, Huangarua River at Ponatahi and Ruamāhanga River at Waihenga are included in the monitoring programme in 2016/17.
- In 2016/17 use of C2 conversion factors to derive chlorophyll *a* from measures of periphyton cover is trialled for samples with $\geq 95\%$ combined cover of 'no algae' and 'film' categories, assessed using the method outlined in section 5.3. During the trial period, visual assessments of periphyton cover for these samples should be accompanied by measurement of chlorophyll *a* to further assess the accuracy of conversion factors at this level and whether cover thresholds require modification.
- Refinement of conversion factors for chlorophyll *a* rich periphyton categories such as 'green filamentous', 'other filamentous' and 'cyanobacteria' be considered. This refinement could be based on data in this study or collection of samples with 100% cover of these categories for measurement of chlorophyll *a* content.
- GWRC continue to support the development of methods and equipment that could allow for faster and most cost effective measurement of periphyton chlorophyll *a* in future. An example is the BenthosTorch, a fluorometric probe that provides *in situ* estimates of periphyton chlorophyll *a*.

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Appendix 1: Sampling site details

Table A1.1: Sampling site locations, river class descriptions and sampling frequencies

Site No.	Site name	Easting	Northing	PNRP river class	PNRP river class description	Sampling frequency
RS05	Otaki River at Pukehinau	1785426	5478749	1	Steep, hard sedimentary	Monthly
RS06	Otaki River at Mouth	1777983	5485886	4	Lowland, large, draining ranges	Monthly
RS13	Horokiri Stream at Snodgrass	1761804	5450653	2	Mid-gradient, coastal and hard sedimentary	Monthly
RS16	Porirua Stream at Wall Park	1754366	5443031	2	Mid-gradient, coastal and hard sedimentary	Monthly
RS20	Hutt River at Te Marua Intake Site	1780071	5450158	1	Steep, hard sedimentary	Monthly
RS22	Hutt River at Boulcott	1760858	5437486	4	Lowland, large, draining ranges	Monthly
RS24	Mangaroa River at Te Marua	1778543	5448643	1	Steep, hard sedimentary	Monthly
RS32	Ruamāhanga River at Te Ore Ore	1825574	5463019	4	Lowland, large, draining ranges	Monthly
RS33	Ruamāhanga River at Gladstone	1821208	5450327	4	Lowland, large, draining ranges	Monthly
RS38	Kopuaranga River at Stuarts	1826761	5469569	5	Lowland, large, draining plains and eastern Wairarapa	Monthly
RS41	Waingawa River at South Road	1820716	5460649	4	Lowland, large, draining ranges	Monthly
RS46	Parkvale Stream at weir	1818094	5458352	5	Lowland, large, draining plains and eastern Wairarapa	Monthly
RS50	Mangatarere Stream at State Highway Two	1809768	5452160	4	Lowland, large, draining ranges	Monthly
RS09	Waikanae River at Mangaone Walkway	1779974	5473638	2	Mid-gradient, coastal and hard sedimentary	One-off
RS10	Waikanae River at Greenaway Road	1771223	5472915	4	Lowland, large, draining ranges	One-off
RS15	Porirua Stream at Glenside	1753289	5438364	2	Mid-gradient, coastal and hard sedimentary	One-off
RS18	Karori Stream at Makara Peak Mountain Bike Park	1744213	5426874	2	Mid-gradient, coastal and hard sedimentary	One-off
RS19	Kaiwharawhara Stream at Ngaio Gorge	1749069	5431077	2	Mid-gradient, coastal and hard sedimentary	One-off
RS21	Hutt River Opposite Manor Park Golf Club	1766679	5442285	4	Lowland, large, draining ranges	One-off
RS23	Pakuratahi River 50m below Farm Creek	1784607	5451677	1	Steep, hard sedimentary	One-off
RS25	Akatarawa River at Hutt Confluence	1776183	5449184	1	Steep, hard sedimentary	One-off
RS26	Whakatikei River at Riverstone	1772256	5446748	4	Lowland, large, draining ranges	One-off

Site No.	Site name	Easting	Northing	PNRP river class	PNRP river class description	Sampling frequency
RS28	Wainuiomata River at Manuka Track	1768242	5430634	1	Steep, hard sedimentary	One-off
RS29	Wainuiomata River downstream of White Bridge	1757316	5415724	4	Lowland, large, draining ranges	One-off
RS37	Taueru River at Gladstone	1824148	5450815	3	Mid-gradient, soft sedimentary	One-off
RS40	Waipoua River at Colombo Road Bridge	1825018	5462890	4	Lowland, large, draining ranges	One-off
RS47	Waiohine River at Gorge	1801889	5455995	1	Steep, hard sedimentary	One-off
RS48	Waiohine River at Bicknells	1810615	5448099	4	Lowland, large, draining ranges	One-off
RS51	Huanga River at Ponatahi Bridge	1807009	5435213	4	Lowland, large, draining ranges	One-off
RS53	Awhea River at Tora Rd	1809951	5403289	5	Lowland, large, draining plains and eastern Wairarapa	One-off

Appendix 2: Preliminary PNRP and NPS-FM assessment

Table A2.1: Comparison of monthly sampling results with proposed Natural Resources Plan (PNRP) outcomes and the National Policy Statement for Freshwater Management (NPS-FM) bottom line

Site No.	Site name	PNRP river class	PNRP significant river	PNRP outcome (mg/m ²)	NPS-FM bottom line (mg/m ²)	Allowable exceedance frequency (%)	No. sample results	No. exceedances of PNRP threshold	Exceedances of PNRP outcome (%)	No. exceedances of NPS-FM bottom line	Exceedances of NPS-FM bottom line (%)
RS 05	Otaki River at Pukehinau	1	Y	≤50	≤200	8	12	0	0	0	0
RS 06	Otaki River at Mouth	4	Y	≤50	≤200	8	12	0	0	0	0
RS 13	Horokiri Stream at Snodgrass	2	N	≤120	≤200	8	11	1	3	0	0
RS 16	Porirua Stream at Wall Park	2	N	≤120	≤200	8	12	0	0	0	0
RS 20	Hutt River at Te Marua Intake Site	1	Y	≤50	≤200	8	11	0	0	0	0
RS 22	Hutt River at Boulcott	4	N	≤120	≤200	8	11	1	3	0	0
RS 24	Mangaroa River at Te Marua	1	N	≤50	≤200	8	11	7	19	2	6
RS 32	Ruamāhanga River at Te Ore Ore	4	N	≤120	≤200	8	11	0	0	0	0
RS 33	Ruamāhanga River at Gladstone	4	N	≤120	≤200	8	12	1	3	0	0
RS 38	Kopuaranga River at Stuarts	5	N	≤120	≤200	17	10	9	25	4	11
RS 41	Waingawa River at South Road	4	N	≤120	≤200	8	12	0	0	0	0
RS 46	Parkvale Stream at weir	5	N	≤120	≤200	17	5	0	0	0	0
RS 50	Mangatarere Stream at SH 2	4	N	≤120	≤200	8	12	2	6	0	0

Appendix 3: Periphyton cover results

Figure A3.1: Average cover ($n=10$) of nine periphyton/aquatic plant categories at each of 13 monthly and 17 one-off sites sampled between August 2015 and July 2016

