A periphyton monitoring plan for the Manawatu-Wanganui region

NIWA Client Report: CHC2008-03
April 2008

NIWA Project: ELF08224

A periphyton monitoring plan for the Manawatu-Wanganui region

Cathy Kilroy
Barry Biggs
Russell Death*

Prepared for

Horizons Regional Council

* Massey University

NIWA Client Report: CHC2008-03
April 2008
NIWA Project: ELF08244

National Institute of Water & Atmospheric Research Ltd
10 Kyle Street, Riccarton, Christchurch
P O Box 8602, Christchurch, New Zealand
Phone +64-3-348 8987, Fax +64-3-348 5548
www.niwa.co.nz

Contents

Reviewed by: Approved for release by:

Dave Arscott Brian Sorrell

Executive Summary

Horizons Regional Council have identified a need to develop a new regional periphyton monitoring programme as part of the environmental management of waterways in the Manawatu – Wanganui region under the proposed One Plan. Horizons therefore used the Envirolink fund to hold a workshop on 6 November 2007, aimed at producing recommendations for an appropriate and cost-effective plan.

Any recommended programme needs to be suitable for long-term and spatially representative monitoring, to meet the following objectives:

(1) assess whether current periphyton and nutrient levels comply with the proposed One Plan nutrient and periphyton standards;

(2) develop a regional model that links nutrient levels and periphyton growth;

(3) determine the outcomes of management of nutrient inputs to water in terms of periphyton growth;

(4) over the longer term, separate local impacts of nutrient inputs from the broader impacts of flow variation resulting from, for example, climate change.

A suggested overall strategy for future periphyton monitoring is based on visual assessments of periphyton cover at sites associated with all current SOE hydrological monitoring sites (in all 117 river management zones with defined nutrient and periphyton standards).

To develop the regional model, the first step is to select sites from the 117 SOE sites, which are representative of the full range of nutrient conditions and flood frequency in the region. Additional sites to complete the coverage may be added from existing compliance sites, or may be selected as new sites. Monthly chlorophyll *a* and nutrient data are required from all the selected SOE and additional sites, for one complete year. Visual assessments of periphyton cover and type are also required for calibration against the model variable (chlorophyll *a*).

Periphyton monitoring at RMA compliance sites should include measurement of chlorophyll *a* and ash-free dry mass (AFDM). This enables calculation of the Autotrophic Index which can be used as an indicator of organic pollution.

The overall periphyton monitoring matrix therefore comprises sites used to fulfil the three demands for periphyton data: SOE monitoring, model development, and consent compliance. It is suggested that sites fulfilling all three demands form a core of sites used for ongoing calibration of the chlorophyll *a*, AFDM and visual assessment measures.

Periphyton monitoring specialists from NIWA and Massey University, along with Horizons staff, addressed nine pre-circulated questions.

1. Cost effective methods of sampling to measure periphyton biomass? The most cost-effective method of assessing periphyton cover is via visual assessments and this is recommended for ongoing long-term monitoring. The main advantage is that, for the same cost as periodic laboratory-based biomass measures, visual assessments can generate detailed time-series from which trends can be identified at multiple sites. Training and periodic QA checks are required to minimise the inter-observer variability that is inherent in these assessments.

2. Frequency of periphyton sampling? Monthly sampling allows calculation of annual and longer-term means and is highly likely to include times of peak biomass. Long term trends in biomass are therefore more likely to be captured.

3. Sampling locations at the water management zone scale, and at the reach / habitat scale? At the water management zone scale, it is recommended that the sampling framework is based on use of all 117 sub-catchments currently included in the SOE hydrological monitoring programme, with additional sites as necessary to ensure that all river types in the region are represented. These are defined by catchment land-use and geology, and flood frequency. At the reach/habitat scale it is recommended that periphyton collection is carried out in unshaded runs.

4. Methods for stone sample collection and number of stones collected? Quantitative sampling for chlorophyll *a* and AFDM determination should be based on a defined areas scraped from the upper surface of each of 10 stones collected along transect(s). For model development, the 10 samples can be pooled for analysis. In compliance monitoring, a statistical comparison of biomass between sites is needed and samples should be kept separate.

5. Rapid, robust visual assessment techniques? Based on experience in other regions, it is likely that a the method currently used for SOE periphyton monitoring by Horizons could easily be adapted for a routine monitoring programme and would yield reasonably reproducible results.

6. Analysis of visual assessment data? Once reduced to a mean value at a site, visual assessment data can be analysed in the same way as conventional biomass data. In both cases regular sampling leads to fixed sampling on random hydrological events. Data can be plotted and relationships to hydrological conditions examined using the appropriate statistical techniques. Long-term time series may be detrended to remove known regional climatic effects such as the Decadal Pacific Oscillation.

7. Best method for chlorophyll a *analysis (i.e., Acetone vs. Ethanol)?* We recommend the hot ethanol method as this was used for development of the current periphyton model.

8. Costs associated with changing analysis methods? Additional costs are negligible as the processing time is similar.

9. Ash-free dry mass (AFDM) analysis? AFDM analyses are generally relevant only for assessing the effects of wastewater discharges, through use of the Autotrophic Index (see above). Expected values for the Autotrophic Index under certain conditions were discussed. AFDM analysis is straightforward and the cost should be no more than that for chlorophyll *a*.

Issues discussed in addition to those covered by the nine questions included the use of the existing periphyton taxonomic and biomass data held by Horizons. It was recommended that this should be reviewed and analysed, and these data (both chlorophyll *a* and community composition) used to inform the future programme. This would be a separate project.

1. Introduction

Periphyton is the slime (mainly algae) that grows attached to rocks and other substrates on river beds. As well as forming a natural component of river ecosystems, periphyton is an important indicator of changes in water quality. In particular, periphyton may respond to increased nutrient levels (one of the major agents of anthropogenic water quality change) by increasing its biomass, usually with associated changes in species composition. At the same time, other environmental factors, especially flow variability (e.g., flood frequency), play important roles in determining the potential for periphyton biomass development in a river. Because excessive periphyton biomass has detrimental impacts on the recreational, aesthetic and ecological values of waterways, measuring periphyton biomass is one of the keys to determining the effects of water quality changes – specifically nutrient enrichment – on freshwater values.

Horizons Regional Council has identified a need to develop a new regional periphyton monitoring programme as part of the environmental management of waterways in the Manawatu – Wanganui region, under the recently completed "One Plan". As a means to achieve this objective, Horizons used the Envirolink fund to hold a workshop on 6 November 2007, aimed at producing recommendations for an appropriate and cost-effective periphyton monitoring plan. Attendees at the workshop were:

NIWA:	Barry Biggs, General Manager, Environmental Information
	Cathy Kilroy, Scientist, Freshwater Ecosystems
Massey University:	Russell Death, Senior Lecturer, Massey University
	Leonard Sandin, Visiting Scientist, Uppsala University, Sweden
Horizons RC:	Kate McArthur, Environmental Scientist – Water Quality
	Gareth Gray (absent), Senior Hydrology Technician
	Jon Roygard, Manager Science
	Maree Clark, Environmental Scientist – Water
	Carol Nicholson, Research Associate
	Jemma Callaghan, Group Secretary

Following an overview from Horizons of the issues faced by the Council with respect to periphyton monitoring, the workshop addressed pre-circulated questions covering aspects of monitoring design and sample collection and processing (Appendix 1). In

this report we present the outcome of the discussions, centred around a suggested general framework for monitoring, which was presented by Dr Barry Biggs at an early stage of the discussion. Recommended monitoring methods are outlined, with detailed protocols set out in Appendices. Answers from the discussion to each of the pre-circulated questions are presented, and further comments are included where appropriate.

2. Water quality management and periphyton monitoring in the Manawatu – Wanganui region

Horizons Regional Council's Proposed One Plan (POP) for environmental management in the Manawatu – Wanganui region focuses on four major issues that have been identified as requiring improvement in the region. One of these is water quality. The management framework proposed for water quality is based on identification of water management zones (catchment-based units) according to the values associated with those zones. These values include ecosystem, recreational, cultural, water use and social/economic values, and are set out in Schedule D of the Proposed One Plan (www.horizons.govt.nz/default.aspx?pageid=185#pub224). Forty-four management zones have been defined, which are further divided into 117 water management sub-zones.

Water quality standards have been developed and proposed for each water management zone, with the overall objective of maintenance of these standards where they are currently met, enhancement of water quality where they are not met, and management of activities where current water quality is unknown.

The water quality standards include measures of: maximum annual average concentrations for dissolved reactive phosphorus (DRP) and soluble inorganic nitrogen (SIN) (measured when the river flow is at or below 3 times the median flow); maximum ammonia concentrations; and maxima for chlorophyll *a* per square metre of stream bed (a measure of the amount of live algae in periphyton), and percentage cover of the visible stream bed by periphyton. The % cover standard is stated to apply only to the summer period (1 November to 30 April) and specifies filamentous algae more than 2 cm long.

Horizons acknowledge that, to be defensible, the defined standards for nutrients must be effects-based. For example, exceeding a nutrient standard might be expected to lead to a measurable increase in periphyton, which has some known impact on one or more waterway values. The first role of a periphyton monitoring programme should therefore involve defining these effects, especially adverse effects. In other words, the

monitoring should provide quantitative evidence of the effects on periphyton of changes in nutrient concentrations. To achieve this, data from a wide range of sites are needed to enable construction of regional "model" that will link periphyton growth with nutrient status. Ongoing monitoring will enable identification of local and regional trends, or departures from the model predictions.

Historically, Horizons have monitored periphyton biomass (as chlorophyll *a*) and community composition at approximately 30 sites, annually. Determination of chlorophyll *a* concentration in algal communities is an internationally accepted measure of the biomass of live algal cells, since all types of algae (including cyanobacteria) contain this photosynthetic pigment. Community composition can also provide clues about water quality because many algal taxa are characteristic of specific water chemistry conditions. The use of algal community composition as an indicator of the water quality status of streams and rivers is documented in, for example, Winter and Duthie (2000), Komulaynen (2002), Lavoie et al. (2004), Naymik et al. (2005). Horizons' existing data could similarly be used to determine the strength of such relationships in the Manawatu – Wanganui Region. In particular it may be possible to identify broad patterns in taxa – water quality linkages, which could be used to help interpret future visual assessments (see Section 5.1).

In general, annual data on periphyton can be informative especially if they are collected over a very long time. However, they are unsuitable for meaningful comparisons with nutrient concentrations using current models because such comparisons require quantification of mean biomass over seasonal or annual time scales, or identification of peak biomass, which is likely to be missed in a single annual sample (Biggs 2000a). Furthermore, there is always a time lag (e.g., up to 6 – 8 weeks under stable flows) between nutrient concentration changes and periphyton response. Only by monitoring both nutrients and biomass over time, or undertaking nutrient limitation experiments, can definitive links be identified.

In addition to annual periphyton monitoring at ~30 sites, Horizons currently undertakes invertebrate and fish monitoring and monthly SOE monitoring at hydrological sites. There are monitoring sites in every catchment with a standard applied, i.e., 117 sites. Impact water quality monitoring is also undertaken as part of resource consent compliance requirements, for example, to check for the effects of point-source and non-point source discharges.

The task for the 6 November 2007 workshop was to consider options for re-designing periphyton monitoring in the region so that it relates directly to the objectives set out the Proposed One Plan. This includes monitoring with respect to the standards defined for each water management zone, obtaining a comprehensive picture of periphyton –

nutrient concentration relationships in the region, and identifying trends and changes in the future.

To summarise, long-term and spatially representative monitoring of periphyton is required in the Manawatu – Wanganui region for the following reasons:

1. to develop and test a regional model that links nutrient levels and periphyton growth;

2. to assess the appropriateness of the Proposed One Plan standards, particularly with respect to the relationship between nutrient and periphyton standards;

3. to determine the outcomes of management of nutrient inputs to water in terms of periphyton growth;

4. over the longer term, to separate local impacts of nutrient inputs from the broader impacts of changes in river flows (e.g., resulting from climate change).

3. A periphyton monitoring framework to achieve the One Plan objectives

3.1. Data for a regional model

The goal of a regional model is to establish relationships between dissolved nutrient levels and periphyton standing crop / growth across a broad range of river types in the region. The relationship in any given river type can then be used to predict periphyton changes in response alterations in nutrient supply, or can be used to infer nutrient supply changes on the basis of changes in periphyton. Such predictions have multiple applications in river management because they enable the setting of limits to nutrient inputs (or periphyton biomass) according to river type.

The periphyton and nutrient standards currently listed in schedule D of the Proposed One Plan consist of three standards each for chlorophyll *a*, dissolved reactive phosphorus and soluble inorganic nitrogen, depending on the values of the water management zone they are set for. These standards were guided by the periphyton – nutrient – flood frequency model outlined in Biggs (2000b) (Figure 1), using the best available regional information at the time on periphyton cover, nutrient concentrations and flood frequency within each management zone. The proposed standard for % cover of periphyton (visual estimate) was set at 30% for all management zones,

applicable to the summer period and was based entirely on the nationally accepted aesthetic guidelines (Biggs, 2000b).

The regional model envisaged for the Manawatu – Wanganui region is also based on the Biggs (2000b) model, which was developed using data from 25 hill-country, cobble/gravel-bed rivers throughout New Zealand (Biggs 2000a). In the original model, conditions representing oligotrophic, mesotrophic and eutrophic conditions were predicted from dissolved N and P concentrations and the mean number of days of accrual for periphyton in a river. The three trophic conditions were defined by maximum periphyton biomass (Figure 1), based on an analysis of over 1000 streams, including a subset from New Zealand (Dodds et al. 1998). Mean number of days of accrual was calculated as the mean number of days between floods greater than 3 × median flow, i.e., 365/FRE3, where FRE3 is the annual frequency of floods > 3 × median. FRE3 has been shown to be one of the best hydrological predictors of mean periphyton biomass in a river (Clausen and Biggs 1997).

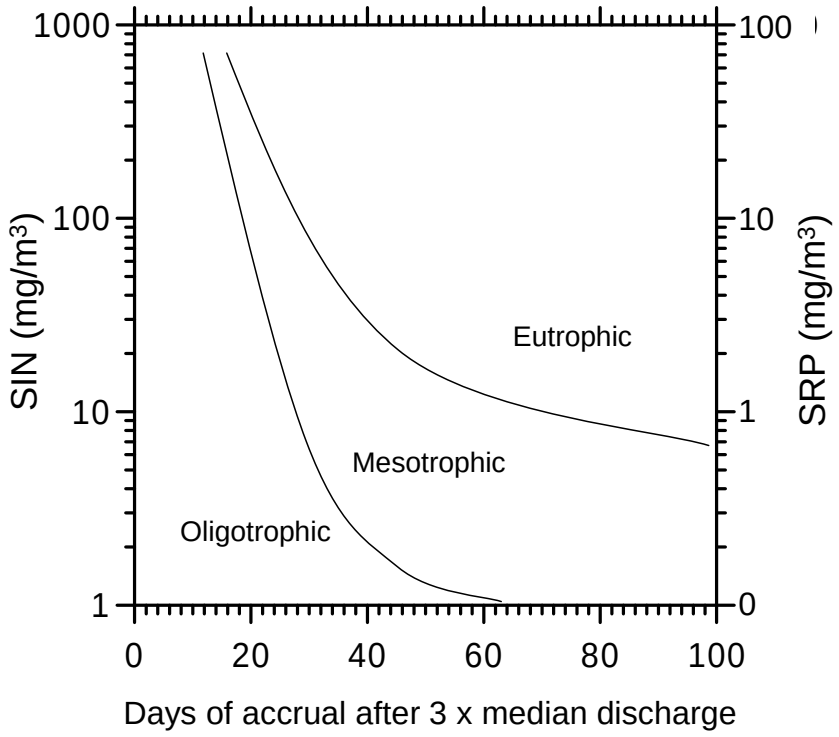


Figure 1. Diagram of periphyton chlorophyll *a* – nutrient concentration model developed using data from hill-country streams. The three areas in the graph show the range of nutrient concentration (primarily N) vs. days of accrual corresponding to oligotrophic (maximum chlorophyll *a* < 60 mg/m^2), mesotrophic (>60 < 200 mg/m^2) and eutrophic (> 200 mg/m^2) conditions. See Biggs (2000a) for full details.

To adapt this model to the region of interest, the first step is to establish current ranges of dissolved nutrient levels and flood frequency. This information should be readily available from existing hydrological and SOE water quality monitoring programmes. Following derivation of relationships with periphyton biomass, parameter values defining oligotrophic, mesotrophic and eutrophic conditions (or any other variations on these, as required) can be determined and plotted (e.g., Figure 1).

To develop the original model Biggs (2000a) obtained *time series* of both nutrient concentrations and periphyton chlorophyll *a*. The rationale was that, because of seasonal and flow-related variability in both periphyton (e.g., Biggs et al 1999) and nutrients (e.g., Scarsbrook et al. 2003; NRWQN data, NIWA), robust river-scale relationships are most likely to be found using long-term averages or maxima of data that are collected at the same time. The nutrient data were reduced to monthly means, and maximum values for chlorophyll *a* were used.

A similar approach needs to be taken in the current programme. Thus, although Horizons have historical nutrient data from multiple sites, ideally, the model must be developed using nutrient concentration and periphyton biomass (as chlorophyll *a*) collected *together*. At least one complete year of data, collected monthly, is required for model development. Because the long-term monitoring plan suggested is based on visual assessments of periphyton cover (see Section 4, answer to question 1) a further objective of the first year of data collection is to undertake visual assessments at all sites on each monitoring occasion, so that biomass (chlorophyll *a*) – visual assessment relationships can be developed. Chlorophyll *a* data enable the definition of the trophic status of rivers in terms of the parameters used in the initial model. Because we are proposing long-term monitoring using visual assessments, we need to establish the equivalent in terms of cover, which will be used in subsequent years to discriminate between different trophic states. Precise relationships are not essential. We simply need to know the range of levels of percentage cover of different algal types that correspond to the boundaries between oligotrophic and mesotropic (maximum of 60 mg/m^2 chlorophyll *a*[1]) and mesotrophic and eutrophic (maximum of 200 mg/m^2 chlorophyll *a*).

Because different algal communities yield different amounts of chlorophyll *a*, it will be important to incorporate recording of different algal types into the visual assessments. This is covered in more detail in Section 5. Development of an empirical relationship between chlorophyll *a* and visual cover will involve applying weightings to each type of recorded algal cover. For example, green filaments yield more chlorophyll *a* than diatoms/cyanobacteria and this is reflected in the different

[1] Chlorophyll *a* measured using the hot 95% ethanol extraction method (Biggs and Kilroy 2000).

chlorophyll *a* guideline applied to the two communities (120 and 200 mg/m^2 respectively) for maintenance of trout habitat and angling values (Biggs 2000). Very much higher chlorophyll *a* associated with filamentous green algal communities compared with diatom-dominated communities has been noted in field studies (e.g., Suren et al. 2003).

A suggested procedure for developing and testing an empirical relationship between visual estimates and chlorophyll *a* is as follows:

1. Calculate a range of indices from the visual assessment data by weighting the mean proportional cover of each visual category by an appropriate factor, e.g., green filaments x 2; cyanobacterial mats x 1; diatom mats x 1.2, then summing the weighted proportions.

2. Regress each index against the chlorophyll *a* value for that sampling occasion (these data may need to be log or square-root transformed).

3. Test the predictive power of the best relationships using an independent dataset (a subset of the original dataset that was not used in the regression.

3.2. Sampling sites

Site locations should be based on the current network of 117 SOE monitoring sites. For advice on details of site selection at a local scale, refer to Section 4, answer to question 3.

The overall requirement for site selection is to have sites representative of the full range of nutrient conditions and frequency of flood events (FRE3). The current 117 hydrological sites should be reviewed in this context to determine how many and what type of additional sites are needed to enable sampling of as many nutrient x flow regime combinations as possible. For example, sites could be assigned to classes of enrichment and FRE3 within a matrix (Figure 2). Some combinations shown in Figure 2 are probably rare (e.g., eutrophic streams with high flood frequency). However, for modelling purposes, attempts should be made to include at least 3 representatives of all combinations.

Classes included in the matrix would be defined from the ranges found in existing water quality data. Without prior knowledge of those ranges it is difficult to suggest the boundaries between categories. A suggested approach for nutrients is to first plot mean SIN versus mean DRP from all available sites, using log-transformed data

(which would be expected to show a significant positive correlation), then mark on concentric zones corresponding to low medium and high nutrient concentrations. An example is shown in Figure 3, using data from the National River Water Quality Monitoring Network. In this case, each of the three concentric zones (defined by solid lines) encompass roughly equal numbers of sites, and the values of maximum DRP are equivalent to published average values separating oligotrophic, mesotrophic and eutrophic lakes on the basis of total P (5, 10 and 30 mg/m^3, respectively) (Wetzel 2001). A fourth, ultra-oligotrophic, category can be defined from this dataset (enclosed by the dashed line). For flood frequency classes, we suggest use of the approach taken by Clausen and Biggs (1997), in which rivers were grouped into equal-length FRE3 bands of >5, 5 –<10, 10–<15, 15–<20 , and so on. Other flood frequency metrics could also be used.

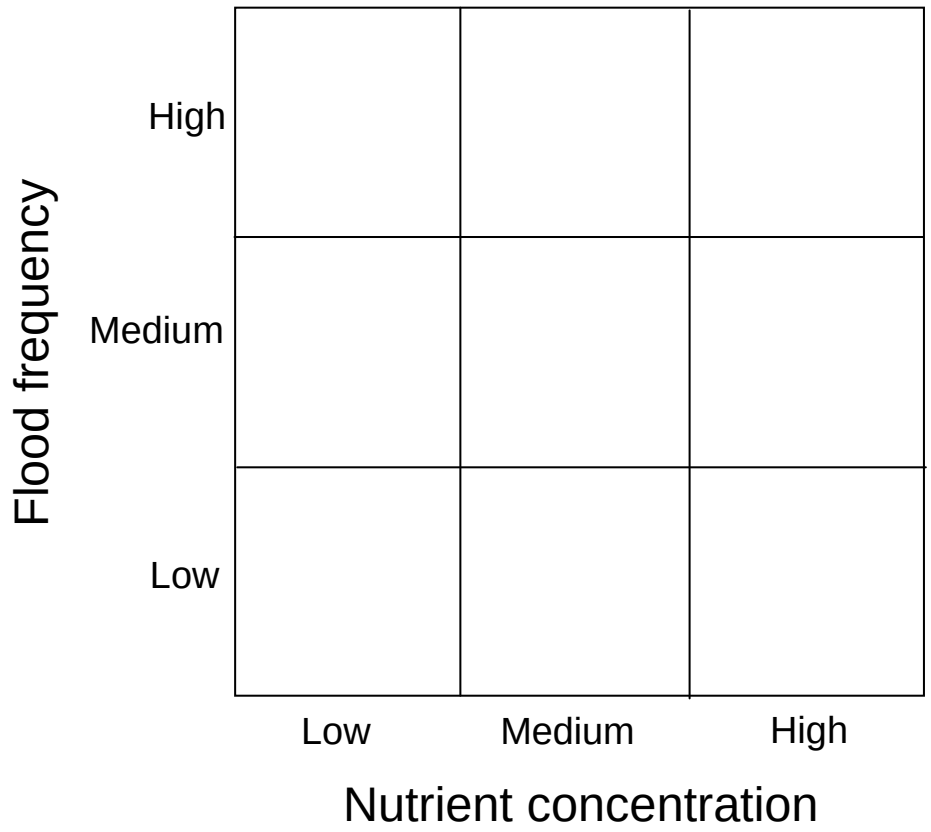


Figure 2: Example of a matrix set up to check for coverage of sites in the dataset used for development of the regional model. Both nutrient concentration and flood frequency ranges should be set according to the complete range known in the region. More nutrient and flood frequency categories can be added as necessary, with numbers of classes guided by the range of the existing data.

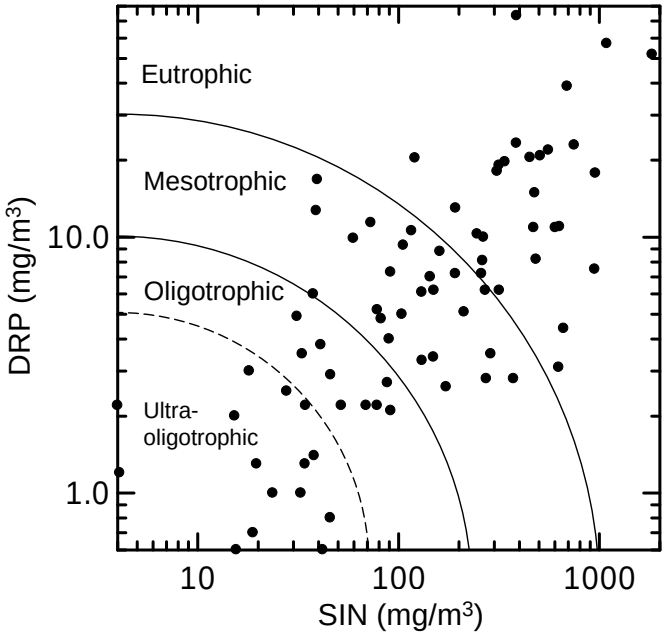


Figure 3: Example of division of river sites into low, medium and high nutrient concentrations on the basis of the range in the data of both dissolved reactive phosphorus (DRP) and soluble inorganic nitrogen (SIN). Units are mg/m^3. The dashed line encloses a fourth category of very low nutrients. (Data from >70 rivers in the national rivers water quality monitoring network.)

3.3. Model development and transition to long-term monitoring

Priority needs to be given to prompt evaluation and analysis of the first year's data. A priority should be to work on the chlorophyll *a* – visual indices relationships (section 3.1). One potential outcome of this is that no satisfactory relationship is found, suggesting that nutrient – flood frequency models need to be developed for both chlorophyll *a* and visual estimates, which may require a reassessment of data collection at some sites (e.g., maintenance of chlorophyll *a* sampling at more sites). The aim will be to determine the need for any site changes or changes to protocols as soon as possible as the long-term monitoring gets underway. It is assumed that practical issues with the sampling and sampling protocols will also be addressed as the sampling programme proceeds during the first year.

3.4. Overview of periphyton monitoring framework

The following places the data collection discussed above (for model development and ensuing long-term monitoring) in the context of the current network of monitoring sites maintained by Horizons.

The three main demands for periphyton data by Horizons are for: A. SOE monitoring; B. development of a regional model; C. consent compliance purposes. Each requires different information, but there is some overlap. To obtain data appropriate for these different purposes *and* to achieve sufficient spatial and temporal frequency to provide data for the regional periphyton model (predicting nutrient loadings from periphyton) the following site framework is suggested (Figure 4):

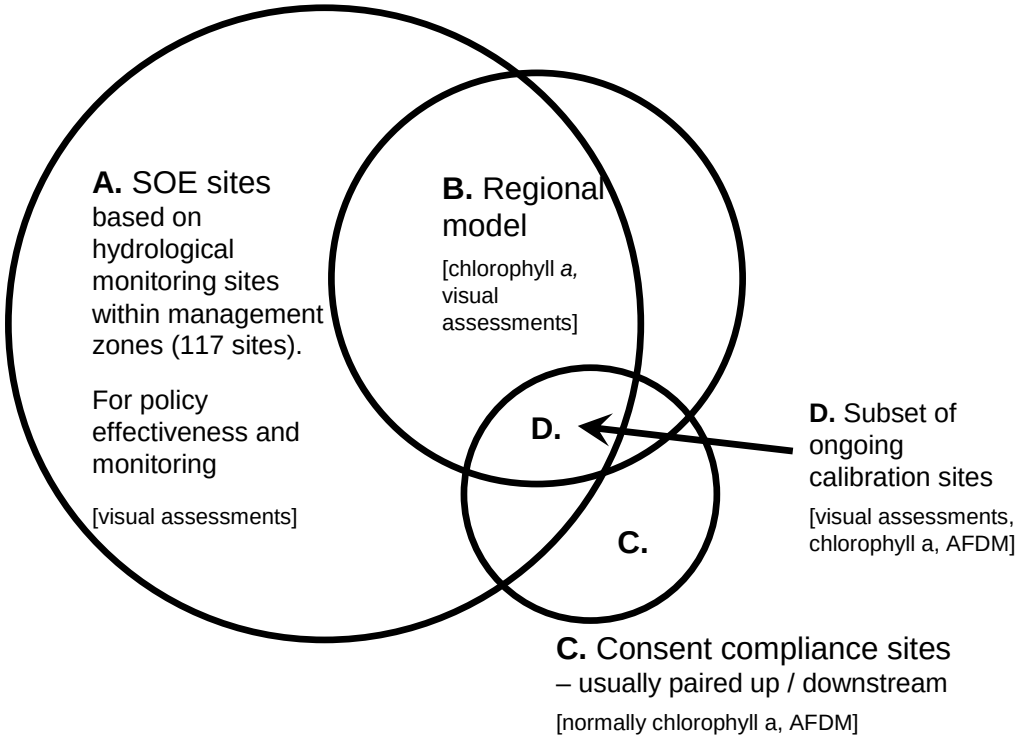


Figure 4: Conceptual diagram of periphyton monitoring recommended for Horizons Regional Council. The three circles each represent numbers of monitoring sites set up for different purposes, with circle size roughly proportional to the number of sites in that category. Sites for ongoing calibration (set D) are selected from any of groups A, B and C, ensuring that all broad river types (especially in terms of nutrient loading and hydrology) are represented.

A. Plan to undertake visual cover assessments (see Section 4, answers to questions 1 and 5, and Appendix 2) in *all* the current SOE (hydrological) sites, on a monthly basis, in the long term. In other words, the visual monitoring should become a standard component of regular site visits to obtain SOE information. A fixed sampling programme is ideal because this represents randomisation of hydrological conditions. Refer to discussion on visual monitoring in section 4.1 below, and Appendix 2.

B. For the first year only, quantitative samples for subsequent chlorophyll *a* analysis, are required monthly from at least a subset of the SOE sites, for use in development of the regional periphyton model. The number of sites included should be as large as resources allow (all, if possible) to maximise the number of datapoints available for developing chlorophyll *a* – visual estimate relationships. The exercise described in Section 3.2 should be used to select sites (if necessary) and also to identify any gaps in the regional range of nutrient concentrations and flood frequency, which are poorly, or not represented in the current SOE sites. These are additional sites in the non-overlapping part of circle B. in Figure 4.[2] For example, these may include upstream reference sites not currently included in the SOE network.

C. Monitoring programmes designed for resource consent compliance monitoring need to include quantitative measurements of both chlorophyll *a* and ash-free dry mass (AFDM). AFDM is a measure of the total organic matter in a sample, and therefore includes non-living material and also heterotrophic (non-photosynthetic) organisms. The ratio of AFDM to chlorophyll *a* is called the Autotrophic index which is useful in assessing the effects of discharges to waterways (see Section 4, answer to question 9).

D. Some sites in categories A, B and C will overlap. It is suggested that these sites be considered for ongoing monitoring of all three periphyton measures: chlorophyll *a*, AFDM *and* visual cover, which will permit ongoing intercalibration of the three biomass measures. Sites included in this subset will need to be selected carefully to ensure that they represent a stratified subsample of the rivers throughout the region, covering about 10% of all sites.

In summary, it is recommended that future periphyton monitoring is largely based on visual assessments of periphyton cover at sites associated with all current hydrological monitoring sites. This will enable Horizons to keep track of both peak and mean annual periphyton cover and to relate this to nutrient data and the nutrient standard at each defined river management zone. Initially, a full year of chlorophyll *a* data at these sites (or at least a stratified subset based on past nutrient concentration and flood frequency data) will allow:

a) development of regional relationships between nutrients, flood frequency and periphyton chlorophyll *a*;

b) calibration of the traditional laboratory measure of biomass (chlorophyll *a*) against the new visual methods.

[2] Note that the figure is diagrammatic only and not to scale.

In parallel Horizons will also maintain resource consent compliance monitoring sites (normally measuring chlorophyll *a* and AFDM at paired sites, for example above and below a discharge), and some of these may be appropriate for inclusion in the dataset for development of a regional model linking periphyton biomass and nutrient status. For example, a site may fall into one of the matrix squares (Figure 2) which is under-represented in the SOE dataset. Additional sites are likely to be required to achieve complete coverage of river types for model development. It is beyond the scope of this exercise to recommend protocols for compliance monitoring. However, if compliance sites need to be used to obtain complete coverage of nutrient – flood frequency combinations for development of the model, then monthly visual assessments and sample collection for chlorophyll *a* will need to be taken as at all other sites used for model development. We recommend the methods suggested in the answer to question 4 (Section 4).

Ongoing calibration of the visual assessments and biomass measures can be undertaken at a proportion (~10%) of sites in the current SOE, consent compliance and new regional model sites, selected to represent the whole range of river types.

4. Responses to pre-circulated questions

At the workshop, the monitoring structure described above was introduced and discussed in answer to the first of the pre-circulated questions. Subsequent questions were addressed in the context of structure. Below we provide a summary response to each question, based on the workshop discussion, followed by further discussion around the topic, derived from both the workshop and from consideration of the topic during the preparation of this report.

1. What are the best and most cost-effective methods of sampling to measure periphyton biomass?

- *Answer:*[3] The most cost-effective method of assessing periphyton cover is via visual assessments and this is recommended for ongoing long-term monitoring (see Section 3 above). Visual assessments do not require any sample collection and do not strictly measure biomass, but can be calibrated with biomass measured (as chlorophyll *a* and/or AFDM) from samples scraped from defined areas of substrate. Some knowledge of community composition is also informative regardless of the biomass measure used, because different communities have different biomass responses to changes in nutrient concentrations. It was suggested that a basic visual assessment of

[3] Note that much of Section 3 was discussed in response to this question. The response here is confined to the discussion of the field methods.

major algal growth forms could be useful in this respect (see Section 5 for further discussion). Whatever the final form of the visual assessment, a move away from laboratory-based, quantitative biomass and community composition measures should enable many more sites to be included in the programme, at closer time intervals, for the same cost. The main advantage of visual assessments is that this can generate detailed time-series from which trends can be identified at multiple sites.

Discussion

Visual assessments are not as widely used internationally as the traditional laboratory-based biomass measures. To our knowledge the methods suggested here have been largely developed in New Zealand. For example, visual assessments of cover of green filaments and brown algal mats have been included in the New Zealand River Water Quality Network sampling protocol since 1989 (Quinn & Meleason 2002). An expanded version of the NZRWQN methodology was developed for the New Zealand Stream Health Monitoring and Assessment Kit (SHMAK) (Biggs et al. 2002). Two protocols are set out in detail in the Stream Periphyton Monitoring Manual (Biggs and Kilroy 2000). These methods have been recently adapted for use in a range of monitoring programmes, including research on *Didymosphenia geminata*. There are examples in the international literature of long-term monitoring of benthic biotic using different visual techniques (e.g., the point-transect method, Arscott et al. 1998, Bott et al. 2006, Benstead et al 2007), and these may be appropriate alternatives in some cases to the methods currently used in New Zealand.

Despite their limited use compared to traditional biomass assessments, visual assessments are promising for acquisition of maximum information for minimum cost. In a recent survey of invertebrate and periphyton monitoring by New Zealand Regional Council, six out of 14 councils reported using visual assessment methods in periphyton surveys (data from 2008 survey, K. McArthur, K. Collier, C. Nicholson). Thus, the technique is already becoming accepted. In our experience, after training, use of a standardised method yields reasonably reproducible results. We have also found that careful visual assessments incorporating both % cover and mat thickness correlate well with quantitative biomass measures, when applied to communities dominated by a single species (especially *D. geminata*) (Kilroy et al. 2005). QA checks can be incorporated to ensure acceptable consistency of assessments. For example, parallel assessments could be undertaken by all monitoring personnel at selected sites (e.g., 10% of all sites, picked at random) on an annual basis to identify the cause of any inter-observer discrepancies, and to resolve these as necessary. If required, calibration against laboratory biomass measures can be incorporated into a monitoring programme (as suggested above).

Details of visual assessment methodology were discussed at the workshop, and a summary of the issues follows under question 5. Suggestions and recommendations have been incorporated into a detailed protocol for visual assessments (Appendix 2).

2. How often should periphyton sampling be undertaken in order to gain an 'average' picture of algal biomass in the region's rivers and streams?

- *Answer:* Monthly is the ideal. This allows calculation of annual and longer-term means and is highly likely to include times of peak biomass. Long term trends in biomass are therefore more likely to be captured.

Discussion

Monthly periphyton monitoring on a large scale is economically feasible only if rapid visual assessment methods can be used. It is envisaged that these can be incorporated into routine monthly hydrological monitoring. Once sites are established and some experience has been gained in the technique, each visual assessment should take no more than 20-30 minutes. This is the only cost (apart from the time for data entry) as there are no subsequent laboratory analyses. Refer to Appendix 2 for a detailed suggested protocol, and see further comments of visual assessments under Questions 1 and 5.

3. Where should sampling be undertaken (both at the water management zone and reach/habitat scale)?

- *Answer 1:* At the water management zone scale, it is recommended that the sampling framework is based on use of all 117 sub-catchments currently included in the SOE hydrological monitoring programme, with additional sites as necessary to ensure that all types of river in the region are represented. This applies to representation in all combinations of nutrient concentrations and flood frequencies (see Section 3.2). In addition a complete review of the SOE sites in terms of catchment features is recommended. This includes identification of catchment land use and geology (REC classes). A matrix similar to that in Figure 2 could be used to ensure that the maximum number of combinations is represented.

- *Answer 2:* At the reach/habitat scale it is recommended that periphyton collection is carried out in **runs** (rather than riffles – even though riffles often support richer and more prolific periphyton). The reason is that runs are always available in rivers, therefore it is possible to standardise all sampling

to areas with similar flow characteristics. Water velocity measurements could confirm this, but are generally not necessary as most river runs would fall within the range of 0.1 to 0.3 m/s, which represents a homogeneous habitat in terms of periphyton. It is also important that all sites have wadeable portions that are **unshaded**, since shade changes the rate of development of periphyton biomass, adding another confounding variable to any relationships. This criterion will exclude many heavily shaded smaller streams in forested areas. However, any significant nutrient inputs into smaller streams will have downstream effects, which may be covered by locating some sampling sites just downstream of forested areas. Note that the existing periphyton model was developed using data from unshaded sites only (Biggs 2000a). Note also that existing hydrological monitoring sites will not necessarily conform to these requirements because flow recorders are purposely located to obtain a consistent stage – flow relationship (e.g., at the outlet of a gorge or similar). In these cases, we suggest first checking downstream, then upstream, for a suitable sampling site (and unshaded, wadeable run) that also has sufficiently easy access.

Discussion

In the past Horizons' hydrological programme has focused on larger rivers. Sites on some of these may present difficulties for sampling, either temporarily (e.g., when in flood) or permanently (e.g., large, steep-sided rivers that are difficult to access regardless of flow). For comments about dealing with rivers in flood, see comments following question 6. Large, steep-sided rivers may have to be excluded because visual assessments of periphyton can only be undertaken in wadeable areas. In these cases, sites in tributaries may need to be found. Because the tributaries feed into larger rivers, extrapolation of any biomass – nutrient relationships can be justified provided the sites meet the criteria above, i.e., unshaded, wadeable runs, and are still sizable streams (i.e., not so small that their temperature regime differs markedly from that of the main river).

Checking the management zone sites for representativeness for use in the model should include stratification on the basis of geology and land use. We recommend use of the broad categories defined by the River Environment Classification. Taking all categories into consideration ensures that expectations for periphyton growth can be managed realistically. For example, soft-rock geologies tend to be naturally rich in nutrients and therefore will tend to have higher periphyton than expected in undeveloped catchments.

4 If appropriate, what methods should be used for stone sample collection and how many stones should be collected?

- *Answer:* As indicated above, most of the monitoring will be based on visual assessments (see Appendix 2 for recommended protocol). We recommend that quantitative sampling for chlorophyll *a* and AFDM determination should be based on 10 stones collected along a transect (or transects) (Biggs and Kilroy 2000). This method has been used to characterise periphyton biomass in several research and monitoring programmes. Examples include most of the data used in Biggs (2000a), and recent monitoring on the lower Waiau River, Southland, and the Opuha River, Canterbury (data in NIWA Client Reports). For monthly monitoring for model development, samples scraped from a defined area on the upper surface of each stone can be pooled into a single container for analysis. Note that for compliance monitoring where a statistical comparison of biomass between sites is needed, samples should be kept separate.

Discussion

Standard methods for field collection of periphyton samples are already documented (e.g., in Biggs and Kilroy 2000). Methods for cobble-bedded rivers include: (1) scrubbing whole stones; (2) sampling from a defined area on the top of the stone; (3) processing whole stones. We suggest adoption of (2) on the grounds that (a) it is consistent with the method used previously, and (b) it can be adapted for use in rivers with finer substrates. Refer to Appendix 3 for a detailed protocol, including an option for dealing with small sized substrates (including sand/silt).

5. Are there visual assessment techniques that yield robust results and are able to be rapidly assessed by field staff?

- *Answer:* Yes, based on our experience in other regions, it is likely that a variation on the method currently used for SOE periphyton monitoring by Horizons could easily be incorporated into a routine monitoring programme and would yield reasonably reproducible results. For example, visual assessments based on 20 areas defined by an underwater viewer (see below) are being used for routine periphyton monitoring in the lower Waiau River. In a recent QA check at a single site, average percentage cover of algal types was within 5%, and overall scores (incorporating mat thickness) were 340 and 480 (from a possible range of 0 – >2000). See further comments under Question 1 above.

Discussion

Until 2 years ago, visual assessments of percentage cover by green filaments and mats were undertaken in the existing periphyton monitoring programme (~30 sites), using quadrats placed along three transects. In the past two years, this has been replaced by the full SHMAK visual assessment (i.e., incorporating colour and thickness categories), again on quadrats viewed with an underwater viewer. The recommended method combines some colour categories that may be difficult to distinguish so that the observations are based on a combination of colour, texture and thickness. A suggested method that might be adopted by Horizons is shown in Appendix 2. Some aspects of this method were discussed at the workshop. For example, a detail is whether to define areas for visual assessments using a quadrat placed directly on the riverbed, or to use the circular area seen through the underwater viewer. Pros and cons of each are as follows:

- Pros for quadrat: defines exactly the same area for each sample.
- Cons for quadrat: can be difficult to scan the quadrat area using the viewer; hard to keep in place in moderately flowing water; difficult for a single person to organise both the viewer and quadrat, and also make recordings.
- Pros for viewer area: ease of use by a single operator; easy to scan area.
- Cons for viewer area: area varies with depth; more open to "selection" of stream bed areas by operator rather than truly random survey locations.

Because of funding constraints, it is likely that regular visual periphyton surveys will be undertaken by a single person. On this basis, we recommend use of the viewer area rather than the quadrat. The difference in area at different depths is not likely to be great as all surveys will be undertaken at wadeable depths. The detailed protocol includes a procedure to ensure random survey areas. Both methods become difficult to use in deep water because of visibility limitations.

6. How are visual assessment data best analysed?

- *Answer:* Visual assessments can easily be reduced to a single figure. For example, the data can be used to calculate mean percentage cover by filamentous algae or thick mats, or an index calculated from mean mat thickness and percentage cover. The calculation used will depend on the objective of the study. As an example, in studies on *Didymosphenia geminata*, we found that proportional cover × measured mat thickness (mm) was

significantly correlated with both chlorophyll *a* (R^2 = 0.564) and AFDM (R^2 = 0.842) (Kilroy et al. 2005). For the present case, we suggested (Section 3.1) that an index should be derived in order to establish a relationship between chlorophyll *a* and visual cover. This would be the index calculated in ongoing monitoring.

Once reduced to a mean value at a site, visual assessment data can be analysed in the same way as conventional biomass data. In all cases regular sampling leads to fixed sampling on random hydrological events, so that samples are random and independent. A variety of analytical techniques is available. For example, trends over time can be plotted. Long-term time series can be detrended to remove known regional climatic effects such as the Decadal Pacific Oscillation. Relationships with flow metrics such as FRE3 can be examined. Relationships between nutrient concentrations (or daily loads of nutrients) and visual indices may be examined, preferably using annual averages for both. Partitioning data from individual site – times according to percentile flows – e.g., flow levels exceeded, say, 20% of the time – may show flow-dependent differentiation in the nutrient–biomass relationship.

Discussion

During the discussion of data analysis the question of how to deal with "difficult" sites was raised, including any sites during times of floods. During floods, it will be possible to collect water samples for nutrient analyses, but periphyton sampling / visual assessments will not be possible. In these circumstances, biomass should be assumed to be 0, and this can be checked by returning to the sites immediately after the flood has receded.

7. What is the best method for the analysis of chlorophyll *a* that will allow for consistent results with the periphyton model (i.e., Acetone vs. Ethanol)?

- *Answer:* We recommend the hot ethanol method. This was the method used for analyses in development of the current periphyton model (Biggs 2000a).

Discussion

Ethanol is also generally cheaper and safer to work with than acetone. In addition, hot ethanol extraction has been shown in several studies to be more efficient than acetone extraction (e.g., Sartory and Grobelaar 1984; Wasmund et al. 2006). It should be possible to compare future chlorophyll data (using the ethanol method) with data from the previous monitoring programme (analysed using the acetone method) by running

parallel analyses on a subset of samples. Samples should be blended prior to splitting to ensure that the parallel samples are homogeneous (see Biggs 1987). Samples from about 20 different sites should be sufficient to develop a relationship between the two sets of results. Extraction efficiencies for different periphyton groups (e.g., cyanobacteria and diatoms) can differ with solvent type (for references see Thompson et al. 1999), therefore some variability is expected in any relationship.

8. What are the potential additional costs associated with changing analysis methods?

- *Answer:* Additional costs are negligible as the processing time is similar.

Discussion

It was noted that ethanol was only a minor expense relative to the other expenses incurred for the analysis.

9. Should we be investigating AFDM analysis as well? What are the additional costs associated with AFDM analysis?

- *Answer:* AFDM analyses are generally relevant only for assessing the effects of wastewater discharges. Normally, monitoring for resource consent compliance would include both chlorophyll *a* and AFDM so that the autotrophic index[4] can be calculated. AFDM analysis is relatively straightforward and the cost should be no more than that for chlorophyll *a*.

Discussion

Two further questions were raised with respect to the use of SOE sites in compliance monitoring.

1. How close should an SOE site be to a discharge for it to serve as a compliance monitoring site?

Answer: The distance depends on the size of the river and the size of the discharge. For downstream SOE sites, the discharge needs to be fully mixed, but the site should not be too far downstream (e.g., > 1 km). One rule of thumb is to place the site immediately downstream of the first riffle below the discharge (i.e., assume that the riffle aids mixing). The sampling location should be kept at a consistent distance below the discharge from occasion to occasion. However small variations (e.g., up to

[4] The autotrophic index is calculated from the ratio of AFDM : chlorophyll *a* (expressed in the same units).

30 metres if, say 1 km downstream from the discharge) are less important than ensuring that the habitat sampled is similar in terms of water velocity and depth. Sampling locations will also need to account for reasonable mixing zones as determined by the consent conditions and Proposed One Plan requirements.

2. What levels of autotrophic index indicate desirable / undesirable conditions?

Answer: Some "rules-of-thumb" for use of the autotrophic index should be noted. First, biomass levels should be reasonable (e.g., at least 2 g/m^2) (Biggs 2000). At lower levels, measurement error in both chlorophyll *a* and AFDM can be a large proportion of the actual measurement, therefore the ratio of the two measures may be biased. Second, community composition can influence AI (see Biggs 2000b, reproduced in Appendix 4) so it is difficult to specify general levels.

However, as a general guide (with AFDM > 2 g/m^2), healthy communities in unpolluted streams generally have AI of 100 – 200. Values over 400 indicate that the system is dominated by non-autotrophic matter, and 600 – 1000+ indicate an abundance of non-autotrophic organic matter (Collins and Weber 1978). When calculating AI, ensure that both chlorophyll *a* and AFDM are expressed in the same units. The AI has proved to be a useful indicator of pollution in studies in New Zealand and overseas (e.g., Biggs 1989, Hill et al. 2000).

It is preferable to tailor the monitoring and compliance requirements to the discharge (for an example, see Biggs, 1989) and for this reason, it is usually preferable to keep such monitoring separate from SOE monitoring. Another consideration is that use of artificial substrates may provide the best information, and periphyton growth on artificial substrates cannot be directly compared with that on natural substrates (see Biggs and Kilroy 2000).

5. Related issues

5.1. Periphyton community composition

Community composition assessments require identification skills and are time-consuming, and therefore costly. These are not recommended as a routine part of a long-term monitoring programme, but are probably necessary for the development of a regional model. The reason is that different algal taxa tend to be associated with different environmental conditions, and respond differently to changes in nutrients. Communities also respond differently to floods – some are more scour-resistant than others. Differences in community composition will account for some of the variability

in biomass seen at similar levels of enrichment. Species composition differences therefore highlight the importance of stratifying sites in a large monitoring programme according to land-use, geology, and flood frequency. Awareness of the different communities will assist in understanding biomass responses to nutrients at different sites.

During the workshop discussion it was suggested that existing periphyton data (chlorophyll *a* and community composition over 7 years at approximately 30 sites per year, with associated data on nutrient concentrations) could be analysed to look for patterns relating species composition (especially dominant species) to environmental factors. The results of such an analysis could contribute to the design of the new monitoring programme. For example, we would expect to find patterns in community composition that can be related to nutrient availability. It was suggested that discrimination between some broad visual groups of algae could be readily included in the visual assessments of cover. For example, with a small amount of training it should be possible to distinguish between, say, five groups of algae:

- fine films that give rocks a green, brown or brown/black colour, and a slimy texture, but that produce barely any material when scraped with a fingernail (e.g. < 0.5 mm thick).
- slimy / sludgy coatings that easily fall apart, light to dark brown-coloured (various diatoms)
- cohesive mats; greenish, brown, or dark-coloured (usually Cyanobacteria, but could include red algae and some diatoms)
- fine, slimy green filaments (short or long, e.g., *Stigeoclonium*, *Spirogyra*, *Oedogonium*)
- long, coarse tough filaments (*Rhizoclonium*, *Cladophora*, red algae such as *Compsopogon*)

Photographs showing examples of these categories are provided in Appendix 5. Note that these categories (and the monitoring form and photographs) may need to be adjusted following training, if other identifiable categories recur in rivers in the Manawatu – Wanganui region

5.2. Turning a monitoring plan into practise

Horizons have emphasized that budget constraints will play a large part in determining what the final monitoring progamme looks like. A programme based on visual assessments should maximise use of the budget, but only if:

a) the staff undertaking the monitoring can incorporate it relatively easily into their current monthly hydrological programme;

b) the suggested methods are feasible and produce reproducible results.

This clearly requires that thorough training is undertaken in the methods, and that trial runs are undertaken at the outset to ensure that the protocol is practical and achievable. It was emphasised that this step is essential if the programme is to successfully become long-term. An important part of the protocol will be ongoing quality control, in particular cross-checking of inter-observer variability, and maintenance of records of all such checks.

The same applies with respect to development of the regional periphyton model: in other words it will be important to spend significant time on the site selection process to ensure the data provide complete coverage. At the time of writing, further work on the site selection process was being investigated.

6. Acknowledgements

We thank Kate McArthur and Jon Roygard of Horizons Regional Council for initiating this project, and for their reviews during preparation of this report. We also appreciate review comments from Dave Arscott. Funding for the workshop and report was provided by an Envirolink grant from the Foundation for Research, Science and Technology.

7. References

Arscott, D.B.; Bowden, W.B.; Finlay, J.C. (1998). Comparison of epilithic algal and bryophyte metabolism in an arctic tundra stream. Journal of the North American Benthological Society 17: 210-227.

Benstead, J.P.; Green, A.C.; Deegan, L.A.; Peterson, B.J.; Slavik, K.; Bowden, W.B.; Hershey, A.E. (2007). Recovery of three arctic stream reaches from experimental nutrient enrichment. Freshwater Ecology 52: 1077-1089.

Biggs, B.J.F. (1987). Effects of sample storage and mechanical blending on the quantitative analysis of river periphyton. Freshwater Biology 18: 197-203.

Biggs, B.J.F. (1989). Biomonitoring of organic pollution using periphyton, South Branch, Canterbury, *New Zealand. New Zealand Journal of Marine & Freshwater Research 23*: 263-274.

Biggs, B.J.F. (1995). The contribution of flood disturbance, catchment geology and land use to the habitat template of periphyton in stream ecosystems. *Freshwater Biology 33*: 419-438.

Biggs, B.J.F. (2000a). Eutrophication of streams and rivers: dissolved nutrient–chlorophyll relationships for benthic algae. *Journal of the North American Benthological Society 19*: 17-31.

Biggs, B.J.F. (2000b). New Zealand Periphyton Guideline: detecting, monitoring and managing enrichment of streams. Ministry for the Environment, Wellington.

Biggs, B.J.F., Stevenson, R.J.; Lowe, R.L. (1998). A habitat matrix conceptual model for stream periphyton. *Archiv fur Hydrobiologie 143*(1): 21 - 56.

Biggs, B.J.F.; Kilroy, C. (2000). Stream Periphyton Monitoring Manual. Published by NIWA for MfE. 226 p.

Biggs, B.J.F.; Kilroy, C.; Mulcock, C.; Scarsbrook, M. (2002). SHMAK Stream Monitoring Manual. Version 2. *NIWA Technical Report No. 111*. 190 p.

Biggs, B.J.F.; Smith, R.; Duncan, M.J. (1999). Velocity and sediment disturbance of periphyton in headwater streams: biomass and metabolism. *Journal of the North American Benthological Society 18*: 222-241.

Bott, T.L.; Montgomery, D.S.; Newbold, J.D.; Arscott, D.B.; Dow, C.L.; Aufdenkampe, A.K.; Jackson, J.K.; Kaplan, L.A. (2006). Ecosystem metabolism in streams of the Catskill Mountains (Delaware and Hudson River watersheds) and Lower Hudson Valley. *Journal of the North American Benthological Society 25*: 1018-1044.

Clausen, B.; Biggs, B.J.F. (1997). Relationships between benthic biota and hydrological indices in New Zealand streams. *Freshwater Biology 38*: 327 - 342.

Collins, G.B.; Weber, C.T. (1978). Phycoperiphyton (algae) as indicators of water quality. *Transactions of the American Microscopical Society 97*: 36-43.

Dodds, W.K.; Jones, J.R.; Welch, E.B. (1998). Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen and phosphorus. *Water Research 32*: 1455-1462.

Hill, B.H.; Willingham, W.T.; Parrish, L.P.; McFarland, B.H. (2000). Periphyton community responses to elevated metal concentrations in a Rocky Mountain stream. *Hydrobiologia 428*: 161-169.

Kilroy, C.; Biggs, B.J.F. et al. (2005). Ecological studies on *Didymosphenia geminata*. NIWA Client Report CHC2005-128. For Biosecurity New Zealand. 77 p.

Komulaynen, S. 2002. Use of periphyton to assess water quality in north-western Russian rivers. *Journal of Applied Phycology 14*: 57-62.

Lavoie, I.; Vincent, W.F.; Pienitz, R.; Painchaud, J. (2004). Benthic algae as bioindicators of agricultural pollution in the streams and rivers of southern Québec (Canada). *Aquatic Ecosystem Health and Management 7*: 43-58.

Naymik, J.; Pan, Y.; Ford, J. (2005). Diatom assemblages as indicators of timber harvest effects in coastal Oregon streams. *Journal of the North American Benthological Society 24*: 569-584.

Quinn, J.M.; Meleason, M. (2002). Periphyton in our rivers: too much of a good thing? *Water & Atmosphere 10(3):* 20-21.

Scarsbrook, M.R.; McBride, C.G.;, McBride, G.B.;, Bryers, G.G. (2003). Effects of climate variability on rivers: Consequences for long term water quality analysis. Journal of the American Water Resources Association, 39: 1435-1447.Sartory, D.P., Grobbelaar, J.U. (1984). Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia 114*: 177-187.

Suren, A.M., Biggs, B.J.F.; Kilroy, C.; Bergey, E.A. (2003). Benthic community dynamics during summer low-flows in two rivers of contrasting enrichment 1. Periphyton. *New Zealand Journal of Marine and Freshwater Research 37*: 53-70.

Thompson, R.C.; Tobin, M.L.; Hawkins, S.J.; Norton, T.A. (1999). Problems in extraction and spectrophotometric determination of chlorophyll *a* from epilithic

microbial biofilms: towards a standard method. *Journal of the Marine Biological Association, U.K. 79*: 551-558.

Wasmund, N., Topp, I., Schories, D. (2006). Optimising the storage and extraction of chlorophyll samples. *Oceanologia 48*: 125-144.

Wetzel, R.G. (2001). Limnology. Lake and river ecosystems. Academic Press.

Winter, J.; Duthie, H. (2000). Epilithic diatoms as indicators of stream total N and total P concentration. *Journal of the North American Benthological Society 19*: 32-49.

Appendix 1. Text of document circulated to workshop participants prior to the meeting

Key questions for periphyton sampling and analysis workshop

Background:

Since 1999 Horizons has contracted Russell Death from Massey University to undertake periphyton and invertebrate biomonitoring as part of the Council's State of the Environment (SOE) monitoring programme. Five stone samples were collected for periphyton analysis at the time of invertebrate sampling either once annually (annual sites) or once every three years (rolling sites). Some visual assessment was also undertaken in the field; in 2006 the SHMAK method was implemented for this.

Chlorophyll *a* analysis was then undertaken using Acetone extraction at 5 degrees for 24 hours before measurement using spectrophotometer. Chlorophyll *a* was corrected for stone surface area, calculation methods for this correction changed in 2004. Prior to 2005 stone scrapings were also collected for periphyton community composition analysis.

Council staff now undertake the sample collection of invertebrates, increasing the Council's capacity in biomonitoring of invertebrates and fish (which have never been formally monitored for SOE purposes). Additionally, the Proposed One Plan contains water quality standards for all water management zones in the Region which refer to algal biomass (chlorophyll *a*) and invertebrate community 'health' (QMCI). These Plan standards increase the monitoring required by Council to assess the objectives of the Plan in meeting these standards.

Also, Horizons has been interested in using local periphyton data to run a predictive model to examine the potential relationship between nutrient concentration and periphyton biomass. The methods used previously have made running such a predictive model (developed for the NZ periphyton guidelines (Biggs, 2000) difficult and unreliable. Horizons are still interested in compiling a periphyton data set for this specific use in the future.

Questions:

Horizons is seeking expert advice, through this workshop, on the best sampling and analysis methods to collect periphyton biomass data in order to assess this data against the Plan standards. Specifically Horizons wish to know:

- What are the best and most cost effective methods of sampling to measure periphyton biomass?
- How often should periphyton sampling be undertaken in order to gain an 'average' picture of algal biomass in the region's rivers and streams?
- Where should sampling be undertaken (both at the water management zone and reach/habitat scale)?
- If appropriate, what methods should be used for stone sample collection and how many stones should be collected?
- Are there visual assessment techniques that yield robust results and are able to be rapidly assessed by field staff?
- How is visual assessment data best analysed?
- What is the best method for the analysis of chlorophyll *a* that will allow for consistent results with the periphyton model (i.e. Acetone vs. Ethanol)?
- What are the potential additional costs associated with changing analysis methods?
- Should we be investigating AFDW analysis as well? What are the additional costs associated with AFDW analysis?

PREPARED BY
Kate McArthur
Environmental Scientist - Water Quality
Horizons Regional Council

Date: 10/10/07

Appendix 2: A protocol for routine visual assessments of periphyton cover in rivers

Background

The following protocol is based on the RAM-I and RAM-II methods in Biggs and Kilroy (2000). It has been adapted for potential use as part of Horizons Regional Council periphyton monitoring programme, following a workshop of periphyton monitoring on 6 November 2007. Highlights of the following method are:

- it is designed to be undertaken by a single person (though it can be undertaken more rapidly by two people when appropriate);
- it is based on visual estimates of periphyton cover using the view through a clear-bottomed, circular underwater viewer;
- periphyton categories have been simplified to five classes, based on the quantity and texture of growth (these classes can be modified / expanded following field testing of the method in the region). "No algae" makes up a sixth class.

In an earlier survey using a method similar to the following the number of sites completed per day by a team of two ranged from 8 to 16. Access and site selection time was often more time-consuming than the survey itself. At established sites it should be possible to complete the following procedure in 15 – 20 minutes (two people) or 30 mins (one person).

Equipment

- Underwater viewer: e.g., Nuova Rade viewer (available from, for example, http://www.marisafe.com/store/viewItem.asp?ID=506050907, price approx. US$40.00). These viewers allow a clear view of the stream bed with no interference from surface turbulence. They also enable definition of a more-or-less standard area of the stream bed at each survey point (i.e., equivalent to a quadrat in terrestrial ecology).
- Clipboard
- Datasheets (see below), preferably printed on waterproof paper, or adapted to fit on a smaller sheet for easier use when one person is monitoring alone.

- Pencils
- GPS unit and 1:50 000 maps
- If working alone, you will need a means of attaching the clipboard to the underwater viewer or to your person, so that you always have one hand free to hold the viewer upright. Alternatively, the viewer could be tethered to your waist or arm.

Sampling team

This protocol has been designed for a single person, but the work can be split efficiently between two people, one person making the observations in the river and the other person recording.

Site selection

Site locations will usually be determined by access. Often they will be the same sites as used for other aspects of river monitoring. Site requirements are:

1. Must be wadeable for at least part of the river width (e.g., 10 metres);
2. Must be in a run typical of the river (i.e., smooth, unbroken water);
3. Must be unshaded.

Decide on a 40 – 50 m reach where the survey will be undertaken on a regular basis. Place a marker (e.g., a post or cairn) on the bank at the downstream end of the site so that others can easily find the location.

Timing of monitoring runs

SOE monitoring should be random in relation to river flows. This can be achieved by monitoring at regular intervals. An issue with this is that in high flows, it is sometimes not possible to access the permanently wetted part of the river. This is also a safety issue: in higher flows you will need to view deeper areas to avoid areas that have only recently been inundated. Therefore in very high flows, assume that all periphyton has been scoured and record cover = 0. This can be checked as soon as flows have receded sufficiently.

Monitoring procedure

1. Mark out four transects in the selected reach by placing marker rocks along the water's edge, 10 – 15 m apart.

2. Complete the first section of the monitoring form: site, date, time, etc., and note any unusual features such as flood debris, etc.

3. Record GPS coordinates at the downstream end of the site. As a backup in case of any fault in the GPS unit, or no satellite connection, we recommend also locating the site on a 1:50 000 map as accurately as possible and noting the map reference.

4. With the underwater viewer and attached datasheets (if working alone), wade into the stream at right angles to the water's edge, about 1 m downstream of the marker stone. Go out to a depth of approximately 0.6 m, if the river is not wadeable all the way across. The five recording points should be equally spaced along this transect or part-transect. An easy way to measure the distance is to count strides as you wade into the stream.

5. Record the maximum water depth.[5] This information isn't used directly in the survey results, but can be useful if you need to compare different sites, or different monitoring occasions. The easiest strategy is to stick to a similar depth (e.g., 0.6 m) at all sites, where possible.

6. Turn to face upstream and hold the underwater viewer about 20 cm under the water on the transect line. The area you are viewing should not be one you have just walked over. Holding the viewer steady and as vertical as possible, estimate the percentage cover by periphyton in the following categories. Typical examples of each are shown in the photographic guide (see Appendix 5). Note that you may need to retrieve stones to confirm categories.

   no cover (clean stones)

   thin film (green or brown colour, slimy texture)

   loose "sludge" (usually brown)

   cohesive mats (usually brown/black, don't fall apart when handled)

[5] If working alone, an easy way to do this while you are carrying the viewer and clipboard, etc., is to mark a scale (e.g., 5 cm intervals) on the outside of your waders.

slimy, fragile filaments (usually bright green but can be brown or dark coloured)

tough, coarse filaments (usually green or brown)

7. Record these percentages in the appropriate boxes for transect 1. Normally they would add up to 100. However, if algal types obviously overlap (e.g., green filaments overlying brown mats) then the total may be >100.

8. When you have finished the first assessment take the appropriate number of strides towards the water's edge (see step 4 above). Lower the viewer into the water and repeat the assessment. Pre-selecting the viewing point means that they are random points in a variable river bed.

9. Repeat for the remaining three points on transect 1. The fifth point (nearest to the water's edge) should ideally be at a depth of 0.1 – 0.15 m, though this depth will vary according to the type of river. For example, if the river bank is incised (channelised) the closest survey point will be deeper.

10. Move upstream to transects 2, 3 and 4, and repeat steps 4 to 9.

11. Alternatively, if the stream is shallow and wadeable all the way across, you can work on two complete transects, with 10 points on each transect. Estimate the distance between viewing points using stride counts, as in step 4. Indicate on the datasheet that there were two transects (e.g., by bracketing transects 1&2 and 3&4 on the right hand side) and note the maximum depth on each transect.

12. From your observations of the stream bed as you carried out the survey, estimate the % cover of the stream bed by seven categories of stream bed substrates (bedrock, boulders, large cobbles, small cobbles, gravels, sand, silt). Refer to monitoring form for definitions. Substrate size is important in determining maximum periphyton biomass potential.

It is a good idea to measure water temperature and conductivity on each sampling occasion. It is assumed that water samples for nutrient analysis will be taken on the same day.

Visual assessment of periphyton: monitoring form

Sampling team: …….. ……..……...

River: ……...… **Site:** ……..

Date: ……..…… **Time:** ……..…. **GPS:** E ……..……...…..… N: ……

Photos taken? yes / no ref. (if yes) …….……... **Water temp.** …… **Conductivity** . ……

Site/weather observations ..……...…...

At each 40–50 m site view the bed **5 equally spaced points** along **4 transects** to a max. depth of ~0.6 m. Start downstream. Estimate % coverage of each view by periphyton in 6 categories.

View	Clean	Film	Sludge	Mats	Fils_slimy	Fils_coarse
Transect 1 – maximum depth:						
1						
2						
3						
4						
5						
Transect 2 – maximum depth:						
1						
2						
3						
4						
5						
Transect 3 – maximum depth:						
1						
2						
3						
4						
5						
Transect 4 – maximum depth:						
1						
2						
3						
4						
5						

Estimate of bed substrate composition (%)

Bedrock	Boulders (25 cm across)	Large cobbles (12 – 25 cm)	Small cobbles (6 – 12 cm)	Gravel (0.2 – 6 cm)	Sand (< 0.2 cm)	Silt (fine, not gritty)

Appendix 3. Field method for routine collection of periphyton samples for chlorophyll *a* analysis.

Background

The following method is based on Quantitative Method 1b (QM-1b) in Biggs and Kilroy (2000). The principle of QM-1b is that at each site periphyton is collected from a known area at 10 predetermined points (usually individual cobbles) across a transect (i.e., equivalent to random points in a heterogeneous river bed). The main difference is that a procedure is added for sample collection from very fine substrates. Athough fine substrates generally do not support much periphyton growth, in periods of stable, low flows, significant biomass can accumulate.

Directions are given for varying the method according to the purpose of the sample collection. In general, for SOE purposes, periphyton biomass is determined along with dissolved nutrient data. Since the latter are single values for each site – time combination, it is only necessary to obtain a single value for chlorophyll *a*. In other words, the samples collected from the 10 sampling points can be pooled into a single container and analysed as one sample. For compliance purposes, it is usually necessary to compare biomass a different sites, e.g., above and below a discharge. In this case, there should be at least three replicate samples to enable statistical testing. Ideally, all 10 samples should be kept separate. Alternatively, samples could be pooled in groups of two or three. Note that the use of artificial substrates is appropriate for compliance monitoring as this provides eliminates variability in substrate size and stability that might also influence biomass accrual.

Equipment

- Sample containers (e.g., 120 ml stackable “Elkays”)
- Tape measure – long enough to span the wadeable part of the river (the entire width in some cases)
- Two marker post and mallet
- Deep-sided laboratory trays (2-litre ice cream containers are suitable); it is useful to have two or three.

- Knife, scalpel and scissors for scraping/cutting off thick algae
- Small scrubbing brushes for scrubbing thin, tightly attached algae (e.g., nail and toothbrushes)
- Squirt bottle containing stream water
- Small disposable pipetters
- A ring to define the sampling area. Normally an Elkay lid works well, but in case the substrate size is small, also carry lids of smaller sizes. For sampling fine substrates, it may help to drill a hole in the lid.
- A thin sheet of stiff plastic large enough to completely cover the lid (e.g., a sheet cut out of an ice-cream container lid.
- A chilly bin with ice for storing samples.

Method

1. First label the sampling containers. At the minimum, include site name, date and sample number.

2. Select a sampling location at your site and drive a marker post into the ground near the water's edge.

3. If the river is wadeable across the entire width, run the tape measure out from the marker post to the opposite bank, and secure to the second post.

4. If the river is too deep/swift after 10 m or less perpendicular from the bank, drive in the second post 8-10 m upstream of the first. In this case you will be working on two short part-transects and will take the tape out to the deepest point sampled, and work back to the bank.

5. From the width of the river, or the distance to the deepest point, calculate the distance between 10 equally spaced points (or 5, if you are working on two part-transects <10 m).

6. Move to the first point on the transect line (either near the water's edge, if sampling the entire width, or at the deepest point, if sampling part-transects).

Taking care not to sample an area you have trodden over, bend down and lightly touch the bed sediments without looking. Ideally, pick up the first stone you touch. If it is too big, shift to the next one that can be picked up.

7. If the substrate at the sampling location is too small (e.g., all particles no larger than ~2 cm diameter), follow the procedure in 14. to 17. below.

8. Without disturbing its periphyton cover, place the stone in a tray and return to the bank.

9. Place the sampling ring or lid on the upper, central surface of the stone. Holding the lid firmly, use a knife or brush to scrape/brush away all the algae on the rest of the stone. Rinse away the surplus using the squirt bottle, leaving the circular sample.

10. If periphyton cover consists of streaming filaments, you may need to use scissors to cut around the lid to obtain a sample.

11. Scrape/brush the sample into the appropriately labelled container. Rinse off using the squirt bottle. Use a transfer pipetter to suck up any sample in crevices or depressions in the rock. It may be easier to first scrape/brush/rinse the sample into a 2-litre ice cream tray, then transfer to the smaller sample container.

12. Rinse the knife and brush in a small amount of stream water and also transfer to the sample container. Finally rinse the tray until no periphyton remains stuck to the sides or bottom.

13. Use minimal water to ensure that the sample size is no more than the container capacity.

14. To collect a sample from fine substrates, place the lid directly on the substrate and push in until level with the substrate surface. Slide the stiff plastic under the lid full of sediment and lift out. Smooth off the sample surface so that it just fills the lid.

15. Transfer the sample to an ice cream tray with a small amount of stream water and mix together thoroughly. Individually scrub any larger particles using a toothbrush, rinse and discard.

16. Drain the supernatant (which may be a brown or greenish colour) into an appropriately labelled sample container.

17. Add a little more stream water to the sample in the ice cream tray, mix again and transfer the supernatant to the sample container. Repeat one more time.

18. Repeat the stone/sample collection and sampling procedure for all 10 points on the transect(s). Always make sure that you are not sampling from areas that have been trodden on.

19. Transfer all samples to the chilly bin as soon as they are collected. It is important to keep the samples in a dark, cool environment until they are ready for processing in the laboratory. If processing will commence more than 24 hours after collection, then freeze as soon as possible.

Appendix 4. Use of the Autotrophic index (from page 74, Biggs 2000a)

Autotrophic index: A measure of the degree of organic enrichment

Dissolved organic wastes (particularly sugars and low molecular weight organic compounds) tend to favour the growth of heterotrophic periphyton taxa such as the filamentous bacterium *Sphaerotilus natans* (sewage fungus). These communities can eventually outcompete autotrophic taxa (algae and cyanobacteria) and dominate biomass at high concentrations of dissolved organics, creating nuisance slime growths that are unsightly and smother the streambed rendering it unsuitable for many other organisms (particularly some groups of invertebrates such as mayflies and stoneflies).

Historically, nuisance conditions have occurred downstream of discharges from some dairy factories, meat works, food processing industries and domestic sewage treatment plant outfalls. However, with increase treatment of wastes in New Zealand (to remove the labile organic content), there has been a major decrease in the incidence of periphyton proliferations dominated by heterotrophic organisms. Nevertheless, some organic rich discharges do occur and can become quite concentrated in receiving waters during summer low flows.

A good measure to forewarn of an impending shift from a periphyton communities dominated by autotrophic organisms to one dominated by heterotrophs is the autotrophic index (AI) (Collins and Weber 1978). The index is simply determined as the ratio of AFDM:chlorophyll *a* (ensuring that both measures are in the same units). The greater the degree of organic contamination, the higher the value of AI. Biggs (1989) used intensive monitoring over an accrual period to determine that the AI of communities on artificial substrates was highly correlated with the biochemical oxygen demand (BOD) in the water (r = 0.962, P. <0.001). Collins and Weber (1978) have suggested that once AI values exceed 400 then waters are starting to become impaired by pollution.

Several precautions need to be taken when employing the AI for monitoring of organic enrichment. First, there are considerable errors when measuring AFDM at low levels. Usually, much higher values of AFDM are recorded than actually occur because of a lack of sensitivity in the method. This can then result in a very high bias in AI values. Thus, AI should only be determined on samples with a reasonable biomass (e.g., >2 g/m^2 AFDM). Second, some mucilaginous diatom and cyanobacterial communities can have naturally high AI values which could be misleading in data interpretation (particularly for control sites). For example, Biggs and Hickey (1994) recorded AI values of >2000 (documented as percent chlorophyll *a*

in Biggs and Hickey) for a large number of samples from a thick *Gomphoneis*/*Cymbella*/*Synedra*-dominated mucilaginous diatom community in the regulated Ohau River, South Canterbury, where no organic waste discharges occurred. Thus, it is important to ensure that plenty of biomass is collected for this analysis and that the community is not dominated by slime-forming diatoms (or cyanobacteria).

References

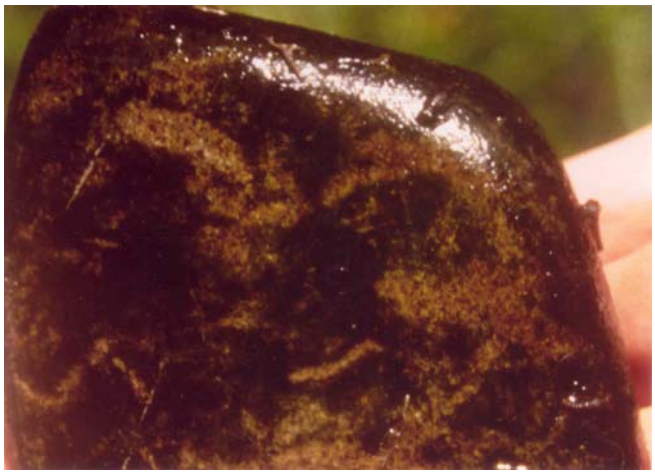
Biggs, B.J.F. (1989). Biomonitoring of organic pollution using periphyton, South Branch, Canterbury, New Zealand. New Zealand Journal of Marine & Freshwater Research 23: 263-274.

Biggs, B.J.F.; Hickey, C.W. (1994). Periphyton responses to a hydraulic gradient in a regulated river in New Zealand. Freshwater Biology 32: 49 - 59.

Collins, G.B.; Weber, C.T. (1978). Phycoperiphyton (algae) as indicators of water quality. Transactions of the American Microscopical Society 97: 36-43.

Appendix 5. Photographic guide to the different visual categories of periphyton

1. Fine films



Fine films can comprise diatoms, cyanobacteria, and/or green algae. They give rocks brown, brown/black or green colour, and a slimy texture, but produce barely any material when scraped with a fingernail (e.g. < 0.5 mm thick).

2. Slimy / sludgy coatings

Slimy / sludgy coatings. These are usually diatoms forming loose slimy masses that easily fall apart and/or have no particular structure (e.g., individual colonies are not distinguishable). They may appear fluffy when viewed underwater and are light to dark brown-coloured .

3. Cohesive mats

These mats are firmer and retain their structure when lifted from the substrate. They range in colour from greenish to brown, reddish or dark-coloured and are usually Cyanobacteria, but could include red algae and some diatoms.

4. Fine, slimy green filaments

These have a definite slimy texture, and individual filaments are fine and fragile, bright green to brownish, often appearing like greenish clouds under the water. They may be short or long, e.g., *Stigeoclonium*, *Spirogyra*, *Oedogonium.*

5. Long, coarse tough filaments

These green or brown filaments have a coarse texture (barely slimy), with tough, easily distinguishable individual filaments, sometimes very thick (> 0.5 mm in diameter). Includes the green algae *Rhizoclonium* and *Cladophora*, and red algae such as *Compsopogon*. A brown colour can be due to heavy colonisation of the filaments by epiphytic diatoms.