Freshwater ecology: periphyton taxonomic sampling and identification

Version 1.0

This specification was prepared by Duncan Gray in 2013.

Contents

Inventory and monitoring toolbox: freshwater ecology

DOCDM-784937

Department of Conservation
Te Papa Atawhai

Synopsis

This taxonomic sampling protocol is based on that presented by Biggs & Kilroy (2000). This protocol is designed to provide data suitable for statistical testing of differences amongst sites to detect impact effects, but also rapid assessment of the dominant taxa in a sample. However, the data produced is dependent on the laboratory protocols used, whilst field sampling does not vary. Sampling points are located along a single transect and 15 points sampled, with 5 samples being pooled into 3 replicates. The method described here is suitable for gravel or cobble substrate, but protocols for bedrock/boulder, sand/silt and artificial substrates are also described in Biggs & Kilroy (2000).

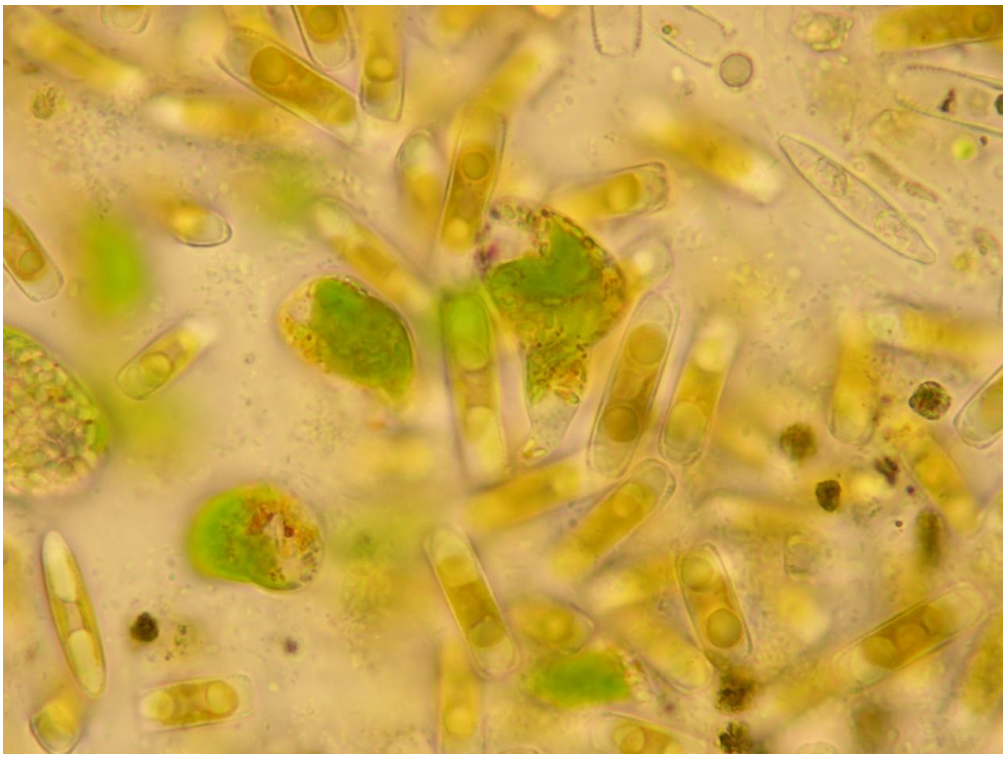

Figure 1. Typical compound microscope view of a periphyton community. Photo: Jon Bray.

Any quantitative sampling method must account for the level of variation within a site and the consequent degree of error associated with sampling. Greater heterogeneity in periphyton cover may require the number of replicates to be increased. However, in general it is recommended to collect at least 15 stones per site and pool these into 3 replicates. An in-depth discussion of methods for calculating error and estimating the required number of replicates may be found in Biggs & Kilroy (2000; section 3). Repeated surveys at regular intervals allow a comprehensive picture of periphyton community dynamics to be created. Results may be combined with physico-chemical data to assess the factors which influence periphyton biomass.

Taxonomy and identification of algal species groups is a highly specialised activity. Samples must be correctly stored and/or preserved before rapid transportation to a laboratory.

Assumptions

- Physical habitat conditions at each site are standardised as much as possible.
- Transects and points are positioned randomly within a transect at each site.
- The sample is representative of the periphyton community in the wider stream.

Advantages

- This method provides reliable high-resolution information about periphyton community composition in a stream reach.
- The method is robust against user bias provided the protocols are adhered to.

Disadvantages

- This method requires material to be transported from the site and the use of chilled storage or preservatives.
- The method incurs significant laboratory processing costs.
- Samples must be processed rapidly.

Suitability for inventory

This method is more or less suitable for inventory depending upon the laboratory protocol used; the rapid assessment protocol identifies the dominant taxa, but may miss rare taxa. The full count protocol provides information about community composition and optionally bio-volume, but data is resource-intensive to obtain, thus limited in spatial extent. If inventory is the primary objective of the study, novel laboratory protocols should be developed for producing taxa lists.

Suitability for monitoring

- This method is suitable for monitoring the effects of specific impacts to a stream ecosystem using either the quantitative or semi-quantitative protocol. Both methods provide high-resolution replicated data about periphyton community composition.
- Used in conjunction with qualitative methods to assess periphyton community composition and cover (RAM-1, RAM-2), and physico-chemical data, this method provides a powerful tool for understanding periphyton dynamics in a stream.
- This method is not suitable for assessing changes in periphyton community composition or cover.

Skills

Field observers will require:

- Basic training in stream periphyton and habitat sampling
- Basic outdoor and river-crossing skills
- A reasonable level of fitness

Study design and sample processing are specialised processes that require input from a TSO, Science Officer or external contractor.

Resources

Periphyton sampling of New Zealand streams may be carried out by a single field operative. However, in the interests of safety it is recommended that sampling is done by teams of at least two people.

Standard equipment includes:

- 20–30 m tape measure
- 2 pegs (> 20 cm long) and mallet
- Deep-sided laboratory tray or container (e.g. an ice-cream container)
- A range of scrubbing brushes (from toothbrush size to nail brush); size depends on the size of the stone
- Scalpel
- Squirt bottle with stream water
- Plastic callipers
- Labelled sample containers (pottles with a screw top lid are suitable for most stone scrubbing samples)
- Field data sheet or notebook (preferably made of waterproof paper) and pencil

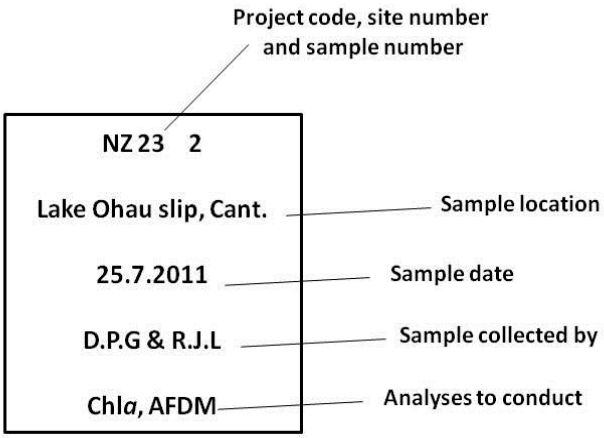

Figure 2. An example wet label which should be added to each sample collected.

Minimum attributes

Consistent measurement and recording of these attributes is critical for the implementation of the method. Other attributes may be optional depending on your objective. For more information refer to 'Full details of technique and best practice'.

DOC staff must complete a 'Standard inventory and monitoring project plan' (docdm-146272).

The more information that is collected at each site, the more thorough and complete will be any interpretation of the biological data collected. However, some basic information should be recorded with each sample collected:

- Substrate composition
- Riparian vegetation
- Stream width
- Stream depth
- Stream velocity

It is also commonplace to collect basic water chemistry information where possible. Temperature (°C), electrical conductivity (µS), pH and dissolved oxygen may all be measured by handheld meters to inform biological data. Some habitat and sites notes are also worthwhile, e.g. the occurrence of stock at the site or evidence of recent flooding. The 'Stream habitat assessment field sheet' (docdm-761873) is a good guide to the basic information that can be collected without recourse to specialised equipment or processing in a laboratory. Basic training in the use of this habitat sheet and/or a thorough perusal of Harding et al. (2009) is required before use.[1] As with all visual and qualitative assessments it is important to standardise collection protocols.

Data storage

During field sampling, data is conventionally recorded on a hardcopy data sheet prior to transfer to an electronic format. Hardcopy sheets should be clearly marked with the details of the project and identity/location of samples. Data should be entered into an electronic media in the same format to avoid confusion. Electronic data sheets should contain all the information required to identify each sample, and any habitat or water chemistry data that was collected simultaneously may be appended on a separate worksheet within the electronic file (usually Excel).

It is important that habitat and water chemistry data are entered in a comparable format to biological data, i.e. columns as sites, and this should be done as soon as possible by the field operative so that details are fresh. All hardcopies of habitat data and notes should be labelled and stored in a project file and retained.

[1] http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/stream-habitat-assessment-protocols.pdf

All electronic files should have a notes sheet which details any relevant information for future users. In particular, each user, beginning with the field operative who enters the data, should record details of any changes to the data, including when and why they were made. It is also recommended to retain a single version of the data which has undergone quality control and may not be altered. All analysis is performed on copies of this master sheet.

Forward copies of completed survey sheets to the survey administrator, or enter data into an appropriate spreadsheet as soon as possible. Collate, consolidate and store survey information securely, also as soon as possible, and preferably immediately on return from the field. The key steps here are data entry, storage and maintenance for later analysis, followed by copying and data backup for security.

Summarise the results in a spreadsheet or equivalent. Arrange data as 'column variables'—i.e. arrange data from each field on the data sheet (date, time, location, plot designation, number seen, identity, etc.) in columns, with each row representing the occasion on which a given survey plot was sampled.

If data storage is designed well at the outset, it will make the job of analysis and interpretation much easier. Before storing data, check for missing information and errors, and ensure metadata are recorded.

Storage tools can be either manual or electronic systems (or both, preferably). They will usually be summary sheets, other physical filing systems, or electronic spreadsheets and databases. Use appropriate file formats such as .xls, .txt, .dbf or specific analysis software formats. Copy and/or backup all data, whether electronic, data sheets, metadata or site access descriptions, preferably offline if the primary storage location is part of a networked system. Store the copy at a separate location for security purposes.

Analysis, interpretation and reporting

Seek statistical advice from a biometrician or suitably experienced person prior to undertaking any analysis.

Samples should be returned to the laboratory as soon as possible, particularly if taxa other than diatoms are of interest (Biggs & Kilroy 2000). 'Soft bodied' taxa lose shape and colour quickly after preservation or drying. Storage in a cool, dark space will keep samples fresh for 48 hours, while full freezing will keep samples indefinitely, although damage to organelles and cell structures is possible. Chemical preservatives, such as gluteraldahyde, are an option, but present a health risk and prevent the later analysis of chlorophyll *a*, which can be performed after freezing.

There are several options for enumeration of algal taxa. A basic technique appropriate for inventory or presence/absence analyses would be to list the taxa present in each sample. This would identify the occurrence of rare taxa, allow comparisons of taxa richness and basic community analyses such as ordinations (multidimensional scaling (MDS), principle components analysis (PCA), correspondence analysis (CA) and canonical ordinations such as CCA and RDA). A more

informative approach would be a semi-quantitative estimation of the relative abundance of taxa based on their contribution to sample bio-volume. This method does not permit an estimation of density or diversity, but allows a robust rapid assessment of the dominant taxa contributing to overall biomass. A third method involves a full count of the number of cells of the individual taxa. This quantitative technique allows an estimation of cell density, diversity and community composition expressed as the number of cells / mm^2 of stone surface sampled. This is the most detailed assessment of periphyton communities practicable and potential analyses are unlimited. A further level of detail can be added by estimating the mean biovolumes of specific taxa and multiplying by density. This can be particularly useful because different periphyton taxa cells vary considerably in dimension and estimates of relative biomass are only meaningful when density data is appropriately weighted by biovolume. See Biggs & Kilroy (2000) for more detail.

Quantitative analyses are, however, very time-consuming and an average analysis may require between 1–2 hours depending on the quantity of silt and taxa involved. Thus, the number of samples with adequate replicates required for even a basic study requires a considerable investment in time and resources. Biggs & Kilroy (2000) state that > 70% of the biomass in New Zealand stream communities often comprises only four or fewer taxa. Thus, the rapid assessment semi-quantitative method can be up to six times faster than the full count biovolume method. This allows greater within-site replication and across-sites spatial coverage, meaning the technique can be applied to impact assessment or state of the environment (SOE) monitoring without excessive expenditure.

Taxonomy and processing of periphyton samples is a specialised process which requires an accredited laboratory or qualified technician. As such, detailed descriptions of the laboratory methods are beyond the scope of this document. However, full details can be found in Biggs & Kilroy (2000). That document should be the first point of reference for anybody wishing to undertake taxonomic analyses of periphyton in New Zealand.

Case study A

Case study A: before and after control impact (BACI) assessment of mining impacts upon periphyton communities

Synopsis

JustCoal is a (fictional) opencast coal mining company based on the east coast of the North Island. They have excavated a new pit from the top of a hill while simultaneously infilling the headwaters of the adjacent Citrus Creek. Biomonitoring of fish, invertebrates and periphyton in lower Citrus Creek was performed before and after the mining operation in order to assess the impact of infill drainage on receiving environment water quality and biota. Citrus Creek contains one of the few nationally remaining populations of the (fictional) lemonfish, *Galaxias gordonsii* and so it was decided to invest in an intensive investigation of the effects of mining activities on the flora and fauna in Citrus Creek. Periphyton monitoring showed no difference in the density of algae as a result of mining activities. However, there was a dramatic decrease in the richness of taxa as a result of the extirpation of

clean water Chlorophyte taxa and the increased dominance of *Klebsormidium acidophilum,* an acidophilic species. Taxonomic analyses were required to detect the impacts of mining because both streams were dominated by similar quantities of long filamentous growths before and after mining. Qualitative cover (RAM) or quantitative biomass techniques would not have detected an impact.

Objectives

- To assess the effect of mining activities on the periphyton community in Citrus Creek.

Sampling design and methods

Biomonitoring of periphyton in lower Citrus Creek was performed before and after the mining operation in order to assess the impact of infill drainage on receiving environment water quality and biota. In order to control for effects on stream periphyton communities that were not due to the infill of Citrus Creek headwaters, a reference site was chosen on nearby Tonic Stream at a location of comparable discharge, riparian shading and in-stream habitat. Full count laboratory protocols were applied to 12 replicate quantitative periphyton samples along a 100 m stream reach before and after mining impact in both the Citrus Creek and the reference stream (Biggs & Kilroy 2000). Physico-chemical conditions at each site were assessed using handheld meters.

Results were analyses using nested ANOVA to account for the non-independence of samples taken in the same stream on different occasions. This is the standard way of analysing Before–After Control Impact (BACI) design studies (Quinn & Keough 2002).

Results

Citrus Creek and Tonic Stream are both (fictionally) 2nd order streams flowing through podocarp-*Nothofagus* forest. Both streams drain similar geological strata and exhibit relatively identical hydrographs. Substrate, shading and stream characteristics, and run-riffle-pool at the two study reaches were the same. Water chemistry in the reference stream (Tonic Stream) showed the stream was circum-neutral with low to moderate electrical conductivity, cool temperatures, and saturated with oxygen. Prior to infill of the headwaters, Citrus Creek had circum-neural pH, slightly higher conductivity than Tonic Stream, cooler temperatures and adequate dissolved oxygen levels for fish and invertebrates. After mining activities had taken place, pH decreased to a median value of 3.6, while electrical conductivity increased to a median of 1063. Temperatures and dissolved oxygen remained fairly similar.

Table 1. Physico-chemical conditions in Tonic Stream (reference) and Citrus Creek before and after the infilling of Citrus Creek headwaters. *N* = 12 for each site.

Variable median (and range)	**Reference *before***	**Reference *after***	**Citrus Creek *before***	**Citrus Creek *after***
pH	7.1 (6.9–7.6)	7.2 (7–7.6)	6.9 (6.4–7.3)	3.6 (3.1–4.3)
Conductivity (µS/cm^3)	103 (78–116)	101 (73–116)	145 (98–166)	1063 (678–1112)
Temperature (°C)	12 (9–15)	11 (8–16)	9 (7.5–11.2)	8.5 (7–11.2)
DO (%)	102 (97–106)	100 (94–102)	92 (90–101)	95 (91–104)

Density

Initial bank-side observations suggested that there were diverse and abundant periphyton communities in both waterways prior to mining; in particular, considerable growths of long green filamentous algae. Microscopic taxonomic analyses of the algal cell showed there was an average of 866 cells per ml from 6 cm^2 of stone surface. Nested ANOVA showed some significant differences between groups, but these were mostly due to stream effects rather than the effect of mining activities in the headwaters of Citrus Creek. Despite the drastic alteration in water chemistry, mining appears to have had no discernible effect on the density of algal cells.

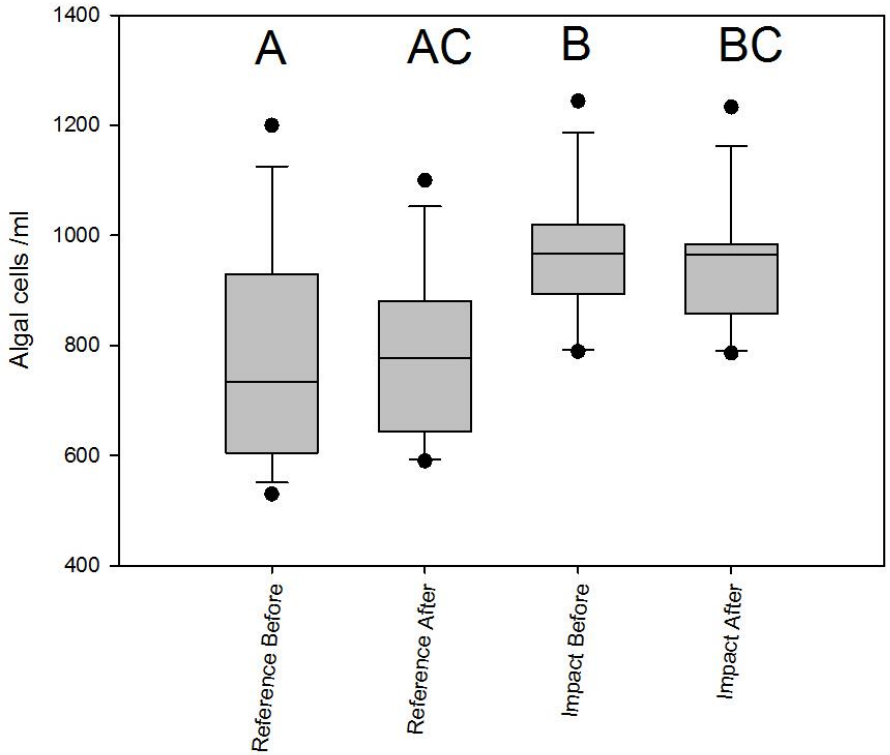

Figure 3. The abundance of algal cells / ml from 6 cm^2 of stone surface in both reference and impacted streams before and after mining activities. *N* = 12. Significant ($p < 0.05$) differences after nested ANOVA are shown.

Richness

The richness of algal taxa in the reference, Tonic Stream, did not vary significantly over the period of time covered by the monitoring (Fig. 4). However, richness in Citrus Creek decreases dramatically from a mean of 14.6 to 4.4 taxa. Despite their being no change in algal cell density, a large shift in richness was observed as a result of headwater infilling.

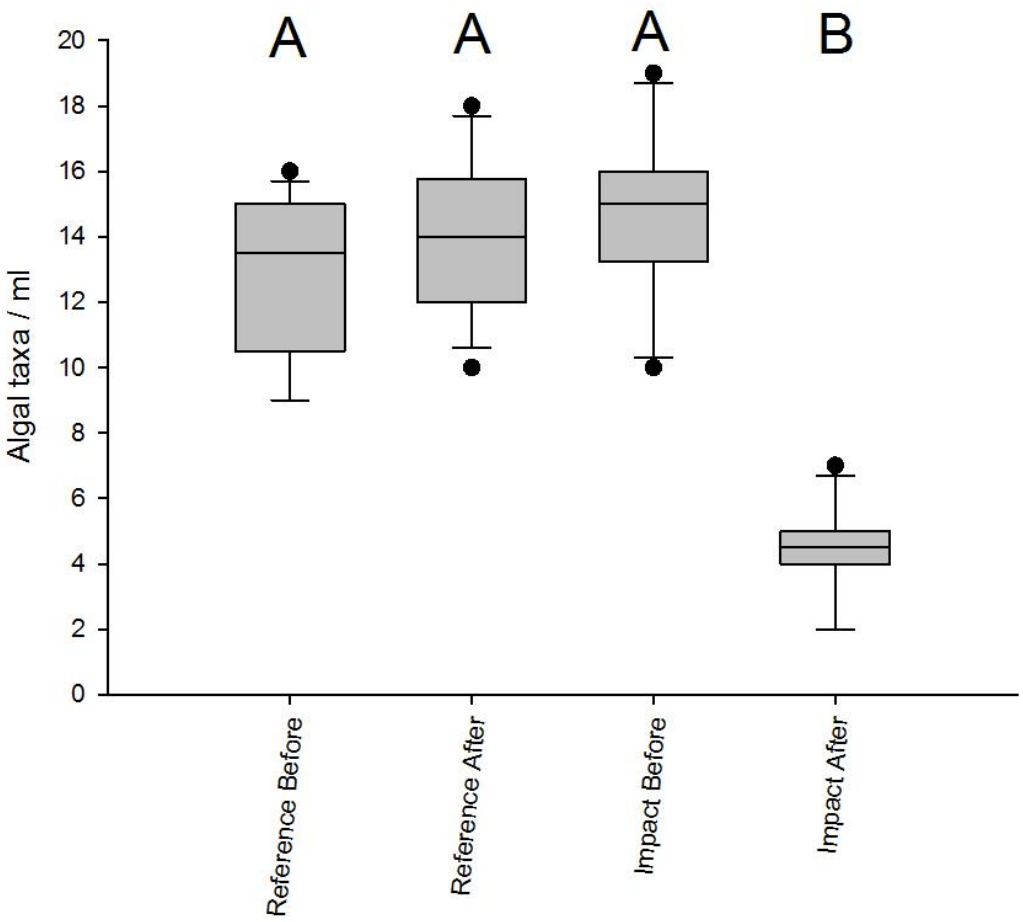

Figure 4. The richness of algal taxa / ml from 6 cm^2 of stone surface in both reference and impacted streams before and after mining activities. *N* = 12. Significant ($p < 0.05$) differences after nested ANOVA are shown.

Community composition

This apparent contradiction between abundance and richness is partially resolved by an inspection of the community composition of algae at the different sites and occasions (Fig. 5). In the reference stream and Citrus Creek prior to mining activities, the periphyton community was dominated by filamentous Chlorophyta, but there are portions of the community also composed of the other major algal taxa groups. However, in Citrus Creek following headwater infilling, Cyanophyta, Rhodophyta, Euglenophyta and Chrysophyta disappear and are replaced by a greater relative abundance of Chlorophyta, while Streptophyta and Bacilariophyta are relatively unchanged.

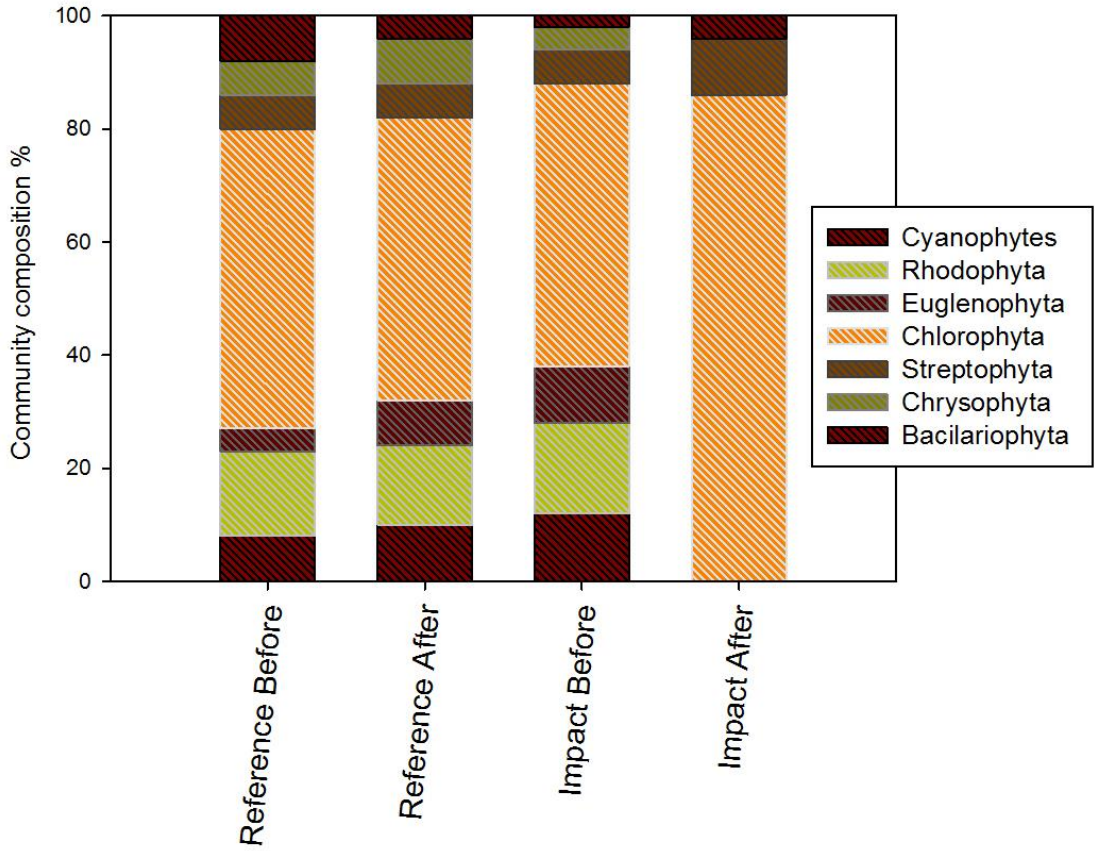

Figure 5. The community composition (%) of algal cells / ml from 6 cm^2 of stone surface in both reference and impacted streams before and after mining activities. *N* = 12.

The Chlorophyta are a diverse group of green algae with adaptation to a broad range of stream conditions. Prior to mining activities the Chlorophytes in both Tonic Stream and Citrus Creek were primarily dominated by *Ulothrix*, *Cladophora* and *Stigeoclonium* which all form distinctive green filamentous growths. Following infilling of the headwaters of Citrus Creek and the drastic changes in water chemistry these taxa were almost entirely replaced by *Klebsormidium acidophilum* (Fig. 6)*. K. acidophilum* also form long green filaments and can be dominant in acid mine drainage (AMD) streams due to a relaxation of competition with other algal taxa.

Figure 6. *Klebsormidium acidophilum*, a low pH loving filamentous chlorophyte algae found in an acid mine drainage (AMD) impacted stream. Photo: Jon Bray.

Limitations and points to consider

This illustrates the advantages of high-resolution taxonomic identification of periphyton communities. The application of periphyton cover protocols, such as RAM-1 or RAM-2, or the quantitative techniques would not have distinguished an effect of mining activities on the periphyton community. AMD impacts in this case did not result in changes to cover or likely biomass of periphyton, rather a complete shift in the community composition from taxa which prefer relatively clean water to those with a preference for low pH.

References for case study A

Biggs, B.J.F.; Kilroy, C. 2000: Stream periphyton monitoring manual. Prepared for the New Zealand Ministry for the Environment. National Institute of Water and Atmospheric Research, Christchurch.

Quinn, G.P.; Keough, M.J. 2002: Experimental design and analysis for biologists. Cambridge University Press. 537p.

Full details of technique and best practice

Field protocol for taxonomic analyses

The field protocol for taxonomic analysis is very similar to that described for 'Freshwater ecology: quantitative periphyton biomass sampling methods' (docdm-766000) and either method 1a or 1b would be appropriate depending on your objectives. Points of difference between sampling methods 1a/b and collections for taxonomic analysis are the number of replicates and subsequent pooling of samples.

The choice between methods 1a and 1b is dictated by the objectives of the study and pragmatism. Method 1a samples the entire stone periphyton community and assesses the entire diversity of microhabitat types around the stone. However, there will be little difference between communities on a particle when substrates are fine cobbles and gravel. Greater heterogeneity in biomass will be found around moderate-sized cobbles and larger substrates where whole stone sampling can provide more information about the overall community. Method 1a is generally used for assessments of general stream enrichment. Conversely, method 1b helps remove the effects of spatial differences in water velocity, erosion of communities along the edge of substrata, and the effects of grazing invertebrates that usually spend most of their time under or along the edges of particles. Accordingly, method 1b is very suitable for assessments of organic enrichment from a specific discharge. The available range of analytical techniques is the same for both techniques. The detail in this protocol can be applied to each method except where noted.

- Select a reference point in the middle of your site, then on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream. Anchor the far end with the second peg.
- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals.
- Move out to the first point across the transect (this will be near the water's edge on one side of the stream). Bend down and lightly touch the bed sediments without looking at what is there. Ideally, pick the first stone that you touch. If it is too big to retrieve, then take the nearest one that can be picked up. If you touch a small silty or sandy patch among the cobbles, then also take the nearest stone that can be picked up.
- Place the stone on the white tray with a small amount of stream water and return it to the stream bank.

Method 1a: whole stone surface sample:

- Use the scalpel to scrape off any filamentous algae and thick growths of brown algae from the stone. Wash onto the tray using minimal water from the squirt bottle.
- Then use the brush(es) to scrub the stone thoroughly. Periodically rinse off the stone and brush into the tray. Scrub all sides of the stone to remove as much periphyton as possible. A standard scrubbing time of 2 minutes is suggested for cobble-sized material.

- Transfer the contents of the white tray into your sample container (you may need to use a funnel if you have a narrow-necked bottle).
- Finally, rinse the tray into the sample container until no trace of periphyton remains.
- Measure the *x,y* and *z* dimensions of the stone with the plastic callipers.
- Proceed to the next sampling point and repeat the above procedures until five rocks have been cleaned. Pool the five samples into a single container and proceed to collect the next five samples which are also pooled. Three pooled samples is the bare minimum replication for this type of sampling.

Method 1b: known area sample from the upper surface of a stone (Fig. 7):

- Place the ring on top of the stone around the outside of the ring with the tip of a scalpel blade. Then, scrape away from the outside of the ring all the surrounding periphyton.
- Remove the ring and scrape off as much periphyton growth as possible from within the circle and rinse it off the scalpel into an appropriately labelled container.
- Scrub the defined area for 30 seconds with a toothbrush and remove the slurry from the circle using the small pipette.
- Rinse the area with a minimal amount of water. Remove any surplus water using the pipette and transfer into the sample container. Thoroughly rinse the brush into the container.
- Finally, rinse the tray on which the stone was resting into the sample container until no trace of periphyton remains. (Note: only use small amounts of wash water because you will quickly run out of space in the containers.)
- If the sampling point falls over a mat of filaments streaming in the current then a slightly different approach is required for sample collection. Slide your hand underneath the filaments and gently raise them to the surface taking care to not disturb their alignment. Take the ring used for defining a set area and press it down firmly on top of the filaments and into the palm of your flat hand. This action will cut a core out of the mat which then becomes your sample. If necessary, use fine nail scissors to cut the filaments from around the edge of the ring.
- Proceed to the next sampling point and repeat the above procedures until five rocks have been cleaned. Pool the five samples into a single container and proceed to collect the next five samples which are also pooled. Three pooled samples is the bare minimum replication for this type of sampling.

Figure 7. Quantitative periphyton sampling of a known area of the upper surface of stones, method 1b. Top left: a randomly selected stone is placed on the bank ready for sampling. Top right: a template of known area is used to protect the sample while all the unwanted periphyton around it is removed. Bottom left: periphyton sample after removal of surrounding material. Bottom right: the sample is carefully collected using a toothbrush. Photos: Duncan Gray.

Methods 1a and 1b:

- Add optional preservative and store the labelled container of periphyton sample on ice in a chilly-bin for transport to the laboratory.

For laboratory procedures see 'Analysis, interpretation and reporting'.

References and further reading

Biggs, B.J.F. 2000: New Zealand periphyton guideline: detecting, monitoring and managing enrichment in streams. Prepared for the Ministry for the Environment. National Institute of Water and Atmospheric Research, Christchurch.

Biggs, B.J.F.; Kilroy, C. 2000: Stream periphyton monitoring manual. Prepared for the New Zealand Ministry for the Environment. National Institute of Water and Atmospheric Research, Christchurch.

Biggs, B.J.F.; Kilroy, C.; Mulcock, C.M. 1998: New Zealand stream monitoring and assessment kit. Stream monitoring manual. Version 1. *NIWA Technical Report 40*. 150 p.

Harding, J.S.; Clapcott, J.; Quinn, J.; Hayes, J.; Joy, M.; Storey, R.; Greig, H.; Hay, J.; James, T.; Beech, M.; Ozane, R.; Meredith, A.; Boothroyd, I. 2009: Stream habitat assessment protocols for wadeable rivers and streams of New Zealand. University of Canterbury, Christchurch. http://www.cawthron.org.nz/coastal-freshwater-resources/downloads/stream-habitat-assessment-protocols.pdf

Weber, C.I. 1973: Biological field and laboratory methods for measuring the quality of surface waters and effluents. U.S. Environmental Protection Agency Report 670 / 4 / 73 / 001.

Appendix A

The following Department of Conservation documents are referred to in this method:

docdm-765928	Introduction to periphyton monitoring in freshwater ecosystems
docdm-766000	Freshwater ecology: quantitative periphyton biomass sampling methods
docdm-146272	Standard inventory and monitoring project plan
docdm-761873	Stream habitat assessment field sheet