Freshwater ecology: quantitative periphyton biomass sampling methods

Version 1.0

This specification was prepared by Duncan Gray in 2013.

Contents

Inventory and monitoring toolbox: freshwater ecology

DOCDM-766000

Department of Conservation
Te Papa Atawhai

Synopsis

Quantitative sampling methods 1a and 1b are based on that presented by Biggs & Kilroy (2000). These methods are designed to provide data suitable for statistical testing of differences amongst sites to detect impact effects. Sampling points are located along a single transect and 10 points sampled. The methods described here are suitable for gravel or cobble substrate, but protocols for bedrock/boulder or sand/silt substrates are also described in Biggs & Kilroy (2000). These methods estimate biomass for a known area of the entire exposed (1a) or upper (1b) surface of stones. Method 1a is suitable for general enrichment assessments and ecological studies of in-stream processes. Method 1b provides more precise information about the response of periphyton community biomass to specific impacts. Results are expressed in terms of surface area of exposed sediments (1a) or in terms of plane surface area of the stream bed (1b).

The choice between 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. 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 this method 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.

All quantitative sampling methods 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 10 replicate samples per site. These are most often collected as point samples along a transect across the river channel. 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 environmental data to assess the factors which influence periphyton biomass.

Figure 1. Examples of periphyton types commonly associated with nutrient enrichment and stable flows in streams. Top left: medium, possibly thick, dark brown mats. Top right: medium thickness green mat. Bottom left: long brown filaments adhere to rocks adjacent to a tracer stone used to measure stream bed movement. Bottom right: medium thickness dark brown mats and short green filamentous algae. Photos: Golder Associates.

Assumptions

- Methods (1a or 1b) assume that the physical habitat conditions at each site vary as little as possible.
- Probability based sampling (usually simple random or systematic designs) are applied to positioning of transects and points within a transect at each site.
- All biomass within the sample area is collected.
- The sample represents periphyton biomass in the wider stream area of interest.

Advantages

- Provides reliable, precise and detailed information about periphyton biomass in a stream reach.
- Robust against user bias provided the protocols are adhered to.

Disadvantages

- Material may be lost or degraded during sample collection and transport, negatively biasing estimates of biomass.

- These methods require material to be transported from the site and the use of chilled storage.
- The methods incur significant laboratory processing costs.
- Samples must be processed rapidly.
- Stream must be wadeable.

Suitability for inventory

These methods are not very suitable for inventory because it provides no information about community composition and data is resource-intensive to obtain, thus limited in spatial extent.

Suitability for monitoring

- These methods are suitable for monitoring the effects of general enrichment (1a), or specific impacts to a stream ecosystem (1b).
- Used in conjunction with qualitative methods to assess periphyton community composition and cover ('RAM-1'—docdm-769146, or 'RAM-2'—docdm-769150) and physico-chemical data, these methods provide a powerful tool for understanding periphyton dynamics in a stream.
- These methods in isolation are not suitable for assessing changes in periphyton community composition or cover by specific periphyton types.

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:

- One 20–30 m tape measure
- Two 6–10 mm diameter aluminium pegs (> 20 cm long) that are bent at one end to hold tapes in place (a mallet for securing pegs may be useful)

- Deep sided, white, laboratory tray container and ice container (e.g. chilly-bin)
- Scalpel
- Small scrubbing brushes (e.g. toothbrushes)
- Labelled containers (e.g. 120 ml specimen cups; 60 ml rigid clear plastic pottles) for each sample that will be collected.
- Pipettes (small eye-dropper is sufficient)
- Squirt bottle containing stream water
- A ring of an appropriate size (usually 4 to 8 cm diameter) which can be used to define a sampling circle on each stone (the top of the sample collection container can be used)
- Waterproof notebook
- GPS

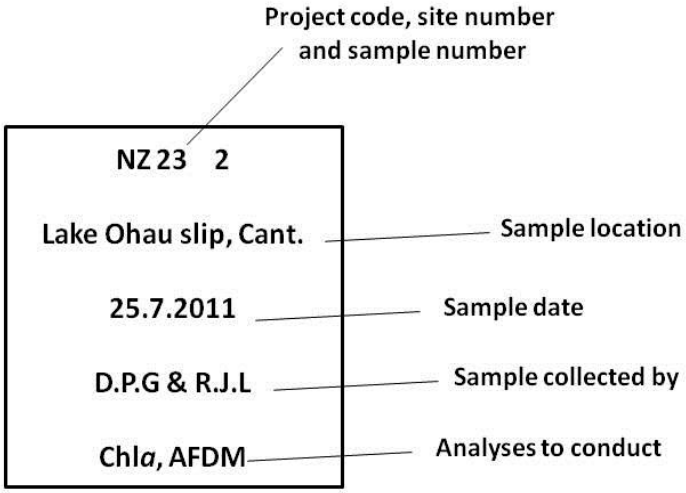

Figure 2. An example wet label which should be added to every sample collected.

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 contextual 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 common to collect basic water chemistry information. Temperature (°C), electrical conductivity (µS), pH and dissolved oxygen may all be measured by handheld meters and assist with the interpretation of biological data. Habitat and site notes are also useful, e.g. the presence of stock at the site or evidence of recent flooding. The 'Stream habitat assessment field sheet' (docdm-761873) specifies the minimum attributes that can be collected without recourse to specialised equipment or processing in a laboratory. Basic training in the use of this habitat sheet and 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 and ensure observations are calibrated. This is especially important if more than one observer is collecting data (e.g. if different teams will go to different sites or will repeat measurements in the future).

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. Forward copies of completed data sheets to the survey administrator, or enter data into an appropriate spreadsheet or database as soon as possible. Collate, consolidate and store survey information securely, also as soon as possible, 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.

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.

To avoid confusion, data should be entered into an electronic media in the same format as on the field data sheets. Electronic data files should contain all the information required to identify each sample, and any habitat or water chemistry data that was collected simultaneously should be captured in the same files. This is often achieved by entering habitat data on a separate worksheet within the same Excel workbook.

DOC best practice for Excel is to enter data in 'long format', i.e. with each field on the data sheet (date, time, location, transect designation, sample number, quantity, etc.) as a column heading and each row containing the values from a single sampling occasion. However, the convention for recording freshwater ecological data is to treat each site as a separate column. 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 contains basic metadata and 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, when and why they were made. It is also best practice 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.

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.

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.

Analysis, interpretation and reporting

Quantitative estimates of biomass are analysed using either or both the chlorophyll *a* (mg/m^2) or ash-free dry mass (AFDM) (g/m^2) methods. Laboratory analysis using specialist equipment is needed to calculate these data for each sample. Once data are obtained, seek statistical advice from a biometrician or suitably experienced person prior to undertaking any numeric analysis. Chlorophyll *a* gives an indication of the total amount of autotrophic (photosynthesising) organisms in the sample. AFDM estimates the total amount of organic material in the sample, and incorporates living autotrophic and heterotrophic micro-organisms, plus dead periphyton, micro-invertebrates and often some terrestrial leaf debris. It is best to analyse for both variables, which provide complementary information. The ratio of the two variables, AFDM / chlorophyll *a*, is called the autotrophic index or AI (Weber 1973). The AI indicates the proportions of the community composed of either heterotrophic or autotrophic material. Stream communities unaffected by organic pollution, and dominated by algae, usually contain 1–2% (by weight) of chlorophyll *a*. Therefore, AI values of 50–100 are characteristic of non-polluted streams with little organic detritus. Values greater than 400 indicate communities affected by organic pollution.

When periphyton growth is very sparse there may be insufficient material to perform an AFDM analysis. In this situation only chlorophyll *a* can be measured. Samples will usually be sent to external accredited laboratories for analysis because it requires experienced operators and considerable equipment. However, if the resources are available to perform the analyses in-house, detailed descriptions of the methods can be found in Biggs & Kilroy (2000).

The data collected using quantitative methods (1a and 1b) provides replicate estimates of periphyton biomass from an area of the surface of (usually) 10 stones. Conventionally, within-site data will be averaged and these averages (and the variance around the average) compared between sites or groups of sites. If samples are collected at multiple sites across an environmental gradient or on multiple occasions through time it will be possible to use correlation or regression analysis to compare biomass (indicated by chlorophyll *a*, AFDM or the AI) to physico-chemical data.

Alternatively, this biomass may be compared between groups or 'treatments' pre-defined by an experimental manipulation or land-use type using a method such as ANOVA.

Case study A

Case study A: monitoring change in periphyton communities as a result of riparian fencing

Synopsis

Puggings Creek is a (fictional) third order stream arising in bush-covered hill country and flowing for 13 km through low-lying, intensive dairying pastoral land. The stream is subject to nutrient enrichment and siltation due to stock access along much of its length. Local anglers and environmentalists noticed that the periphyton growths in the streams were impinging on the benthic biodiversity and trout fishing. Accordingly, they persuaded the farmers along the stream to fence stock out of the waterways and reduce discharges. Quantitative periphyton samples collected before and after fencing of the streams indicated a dramatic decline in the biomass of periphyton, but the Autotrophic Index (AI) indicated that the stream was still subject to organic pollution after 6 months.

Objectives

- Assess the effects of riparian fencing on the periphyton biomass in Puggings Creek.

Sampling design and methods

To maximise potential to detect the effect of fencing, periphyton biomass was monitored at the lowest reach of the stream on 24 occasions during 2 months before and after fencing was carried out with a 3-month gap after fencing to allow for changes to take place. Ten stone surface samples were collected and scrubbed to remove all periphyton according to method 1b (Fig. 3) (although method 1a would also have been appropriate: see 'Full details of technique and best practice') (Biggs & Kilroy 2000 and references therein). The samples were chilled and sent to a laboratory for chlorophyll *a* and AFDM analysis. Unfortunately, it was not possible to select a comparable reference stream in the area.

After laboratory analysis, results for before- and after-treatment were summarised graphically using boxplots, and the average of all before-treatment measurements was compared to the average of after-treatment measurements using ANOVA.

Figure 3. 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.

Results

Before fencing to exclude stock, and riparian planting along the stream banks, the average chlorophyll *a* concentration was 31 mg/m^2 of stone surface (Fig. 4). This quantity exceeds the nuisance guidelines for mean monthly chlorophyll *a* and confirms suspicions that benthic biodiversity was being degraded (Biggs 2000). Ash-free dry mass (AFDM) of periphyton had an average value of 40 g/m^2 which also exceeds the nuisance biomass guideline threshold for trout angling/habitat. This resulted in an AI with a mean value of 1334 which indicates a stream with severe organic pollution (Weber 1973). However, after fencing, both chlorophyll *a* and AFDM measures of periphyton biomass fell to 19 mg/m^2 and 12 g/m^2, respectively, below the nuisance threshold in the case of AFDM. The average AI declined to 806 which, although still indicative of an organically enriched stream, is a considerable improvement. Differences were shown to be statistically significant using ANOVA $p < 0.05$.

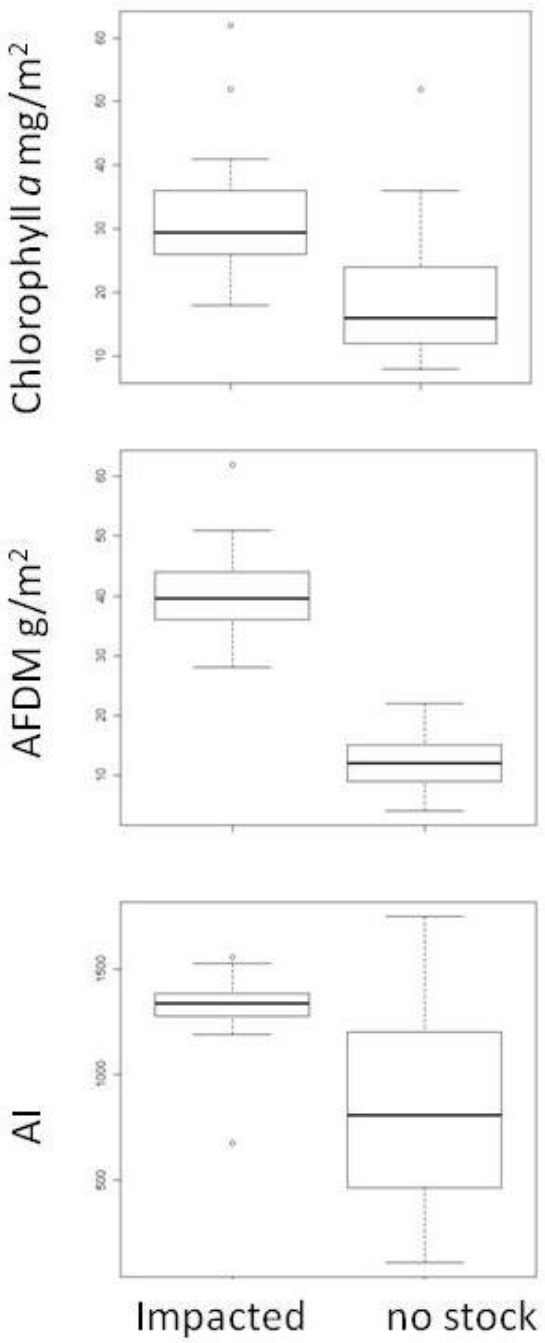

Figure 4. The average values of chlorophyll *a*, AFDM and AI before and after fencing prevented stock access to Puggings Creek.

The decrease in chlorophyll *a* was less dramatic than that seen for AFDM suggesting that the stream is still quite productive in terms of autotrophic production. The sharper decline in AFDM may be due to the reduction of direct additions of organic matter into the water by defecating stock, which would in itself contribute to AFDM and also encourage proliferation of heterotrophic organisms feeding on that matter. AI was consistently high prior to fencing, but after the removal of stock, variation in both chlorophyll *a* and AFDM created a large range in AI values.

Limitations and points to consider

This case study clearly demonstrates the response of periphyton communities to riparian fencing to prevent stock access to a stream. It would be informative to sample again after 1 year to allow the changes in periphyton biomass to settle after fencing. There are likely to be considerable stocks of organic matter and available nutrients contained within the stream reach which have to be depleted before a new periphyton community equilibrium can be achieved.

Sampling the whole stone (method 1a) rather than the upper surface may have been more sensitive at detecting whole stream changes, but it would likely take a longer period of time before the metric settled down compared to the stone surface method.

There is no measure of flow variability or nutrient concentrations in this study. This and any other basic stream habitat information is always very useful to make a full interpretation of periphyton data.

The lack of a control stream is unfortunate as it is not possible to discount seasonal effects on the periphyton community creating the observed changes independent of the impact of fencing and riparian planting.

One way to increase the reliability of the study in this situation would be to include multiple before and after measurements over a longer timeframe and/or at different distances up-stream and associate each measurement with reach-level habitat data, and data about seasonally variable factors such as water temperature and light intensity.

References for case study A

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.

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.

Full details of technique and best practice

Details of technique and best practice for this method are explained here. A full description of the method is given in Biggs & Kilroy (2000).

Monitoring project design

Each sample location consists of a single transect across the stream. Ten point samples are taken from each transect. If multiple transects will be sampled in a single day it is best to start downstream and work up.

Collecting habitat data and notes

- Use GPS to navigate to each pre-determined sample location.
- At each location, collect basic habitat assessment data (refer to 'Minimum attributes' section) and note relevant contextual information (e.g. presence of stock or indigenous species, flow conditions). Collect this information prior to sampling the stream.
- If required for a particular project, additional abiotic or habitat data may be collected.

Setting out transects

- Use GPS to find the point on the stream bank that is closest to the pre-determined sample location and on one bank drive a peg into the ground.
- Attach the tape measure to the peg and lay it out taut across the stream. The tape should be perpendicular to the stream (so that it crosses from bank to bank in the shortest possible distance). Anchor the far end with the second peg.
- Using the distances on the tape measure, divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals. Each of these will be one sampling location.

Collecting samples

- 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 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.
- 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.

Method 1b: known area sample from the upper surface of a stone:

- Place the ring on top of the stone and score/scrub around the outside of the ring with the tip of a scalpel blade or brush. Then, scrape away all the surrounding periphyton from the outside of the ring and discard into the stream.
- Remove the ring and then scrape off as much periphyton growth as possible from within the circle and rinse it off the scalpel into an appropriately labelled container. (Note: only use small amounts of wash water because you will quickly run out of space in the containers).
- Scrub the defined area for 30 seconds with a toothbrush and then 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.
- 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 lift 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.

Methods 1a and 1b:

- Store the labelled container of periphyton sample on ice in a chilly-bin for transport to the laboratory.
- Ideally samples are analysed immediately but if storage is required, freezing at < −10°C is recommended for periods of up to a few months but no longer. Chemical preservatives are also available but constitute a health risk (Biggs & Kilroy 2000).

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-769146	Freshwater ecology: periphyton rapid assessment monitoring in streams—method 1 (RAM-1)
docdm-769150	Freshwater ecology: periphyton rapid assessment monitoring in streams—method 2 (RAM-2)
docdm-146272	Standard inventory and monitoring project plan
docdm-761873	Stream habitat assessment field sheet