Stream Periphyton Monitoring Manual

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Prepared for

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by

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1   Introduction

1.1   Background

Water management in New Zealand is principally controlled by the Resource Management Act (1991) (RMA). The RMA is holistic in its approach and encourages the inclusion of a wide range of values in assessing the potential impacts of any water resources development. A fundamental part of river ecosystems, and the aesthetic, amenity, biodiversity and fishing values that they sustain, is the periphyton community. This community is the slimy coating found on rocks and other stable substrates in streams and rivers. It consists mainly of algae, but the term also includes fungal and bacterial matter, and varies greatly in appearance from a thin brownish or greenish film, to thick, dark-coloured "mats", to masses of streaming green or brown filaments. More specifically, periphyton:

- can provide much of the food, and therefore energy, to maintain higher levels of the food chain such as insects and the fish that feed on these insects;

- are an important agent to help purify waters. The periphyton absorb many impurities such as nitrogen and phosphorus (nutrients), heavy metals and are sites for the breakdown of bacterial and other organic matter contamination;

- are very responsive to degradation of water quality, often changing in both taxonomic composition and biomass where even slight contamination occurs;

- can proliferate when high concentrations of nutrients occur in the water, velocities are low, and there hasn't been a flood for a while.

1.2   Purpose and scope

The *New Zealand Periphyton Guideline: Detecting, Monitoring and Managing Enrichment of Streams* (Biggs 2000a) reviews periphyton in the context of their importance to water resources management. In particular, a background overview is given of factors controlling periphyton growth in streams, communities normally found in different habitats, the use of periphyton as environmental indicators, and guidelines to prevent their proliferation. In this stream periphyton monitoring manual we build on this by describing a standard set of methods to enable Regional Councils, consultants and researchers to collect and analyse data on periphyton for resource surveys, impact assessments/monitoring, State-of-the-Environment (SOE) reporting and research studies. *We stress the need for comprehensive study planning and a clear definition of what the data are required to resolve before a study is commenced.*

Periphyton monitoring is suitable for use in streams and rivers with any type of substrate (silt through to boulders and bedrock). However, for practical and safety reasons, it is best to apply the sampling methods described in this manual in streams and rivers that can be waded, with water velocities of no more than about 1.0 m/s and a depth of < 0.75 m. Throughout this manual we use the term "*stream*" because it more closely reflects the type of environment where the methods are likely to be applied.

Internationally, many different methods are used for periphyton research and monitoring depending on the habitats and study objectives. It is not the intention to review all these methods in the present manual. Instead, the objective is to prescribe a set of protocols that will be applicable to most of the common stream habitats in New Zealand. We cover two general levels of data collection that are likely to be needed in resource management investigations: rapid assessments, and comprehensive (quantitative) assessments. The methods

have all been used extensively. We describe methods for:

- non-destructive sampling, surveying and data collection in the field;

- collection of periphyton samples from natural and artificial substrates for later analysis in the laboratory;

- in-stream nutrient-diffusing substrate assays for assessing the degree and type of nutrient limitation of periphyton growth;

- laboratory analyses for two measures of biomass (ash-free dry mass and chlorophyll *a*);

- laboratory analysis of taxonomic composition (including a guide to common taxa).

The manual also includes:

- guidance on design of sampling programmes;

- statistical definition of variability and numbers of sample replicates;

- procedures for sample identification and tracking;

- monitoring and cross-checking procedures;

- some approaches to data analysis.

We use "*sampling programme*" as a general term to mean a data collection exercise of no fixed frequency or duration. For example, this might be an experiment to define the nutrient that limits periphyton growth in a stream. Alternatively it might entail the detailed assessment of the effects of a discharge from a dairy shed using artificial substrate sampling above and below the discharge point on several occasions. A more specific sub-set of this is a "*monitoring programme*". This involves defining a set location, sampling interval and duration in relation to establishing some longer-term condition or potential effect on the resource. For example, you might sample a site monthly for a year to establish the average state of the community in relation to the degree of nutrient enrichment. Alternatively, a set of sites might be sampled quarterly over a long period (e.g., 10 years) to detect trends as part of SOE monitoring.

While this manual is geared toward agencies involved in water resources management issues, it should also be useful for researchers as a guide to some robust methods for use in New Zealand stream and river environments. It is hoped that the adoption of standardised approaches will greatly assist in data interchange throughout New Zealand.

2   Design of sampling programmes: "begin with the end in mind"

2.1   Introduction

In developing any sampling programme, whether it be for bio-monitoring of waste discharges, SOE monitoring or a research study, the quality of the information generated will largely hinge on good study planning. Good study planning, particularly the setting of clear objectives and hypotheses, can save time later in the study, and also greatly simplify data analysis and reporting. In other words, it really helps to "*begin with the end in mind*". Time-consuming, and usually quite complicated data analysis is often needed where study objectives have not been explicitly developed and used to thoroughly plan a sampling programme. Such analyses can be very frustrating, and lead to imprecise results with weak conclusions. Indeed, your study is likely to be much more useful if you clearly set out what you want to define. We therefore discuss programme planning in some detail below. Two examples of planning and executing studies are used to illustrate the concepts and types of decisions that need to be made.

2.2   Types of sampling programmes

Three basic types of periphyton sampling programmes are likely to be needed in New Zealand.

1. *Resource surveys* establish general patterns of periphyton biomass and composition in time and/or space. Such data can then be used for desk-top assessments in discussions of possible changes to water resources/landuse management regimes, classification of waterways according to degree or type of human impact, etc.

2. *Impact assessments* define effects on periphyton of a change in management regime. These are best treated in terms of BACI designs (Before vs. After in time, Control vs. Impact sites in space) (e.g., Green 1979). However, this is rarely possible in practice so such assessments generally have to rely on Control vs. Impact site analysis.

3. *State-of-the-Environment monitoring* tests the effectiveness of regional water resources management policies. Sites are selected to represent some pre-defined condition within a region, and are monitored over an extended period so that any changes in these conditions that may relate to policy decisions will be unambiguously defined.

These study designs will be discussed in more detail below.

Before embarking on a sampling programme, it is essential that you clearly define the issues or questions being asked. As Green (1979, p.25) suggests: "Be able to state concisely to some-one else what question you are asking. Your results will be as coherent and as comprehen-sible as your initial conception of the problem". It is particularly important that you study Figure 1 if embarking on resource surveys and impact assessments. This figure summarises a general approach for planning, setting consent criteria and verifying appropriateness of the chosen criteria for managing instream values in relation to periphyton. You should turn your questions into instream management objectives and then, if possible, develop testable hypotheses.

2.3   Defining your objectives

The most fundamental aspect of establishing a sampling programme and fulfilling the over-all goals of your study is adequate definition of objectives. These objectives might be formal instream management objectives (ISMOs), as described in Biggs (2000a). However, if the
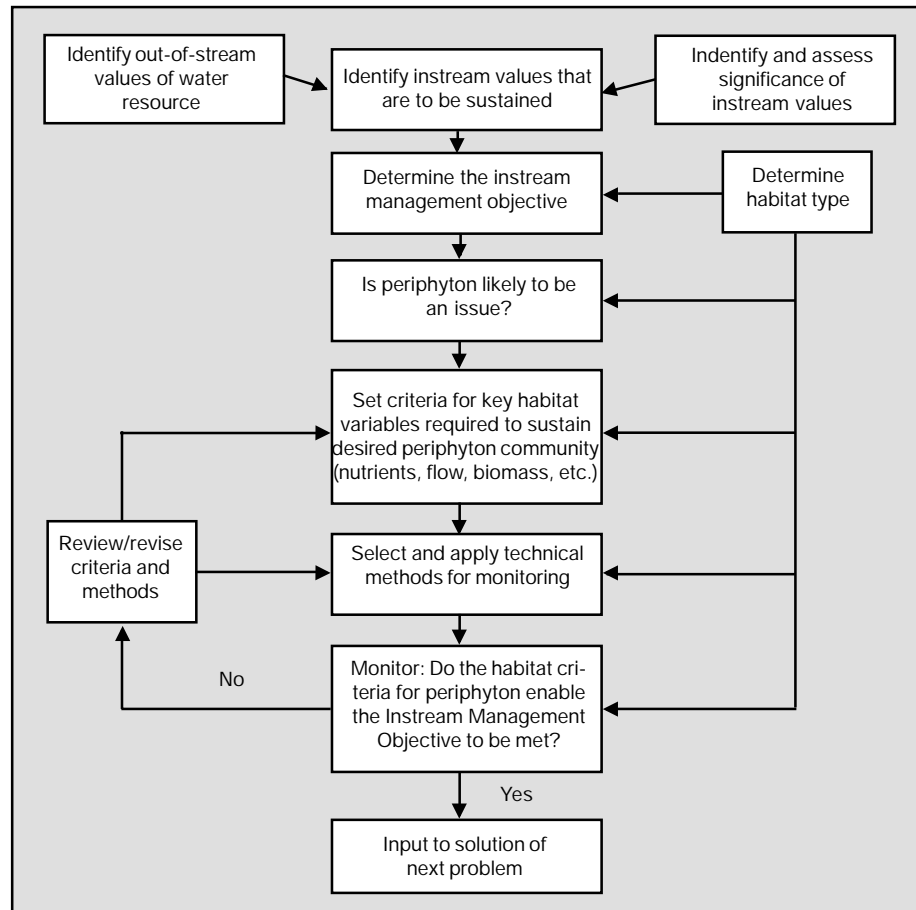
Figure 1: General procedures for planning, setting consent criteria and verifying appropriateness of consent criteria for managing instream values in relation to periphyton (from Biggs 2000a).

study relates more to general resource analysis, or testing for the effects of an existing water management practice, the objectives might be specific to your particular study. Well-defined objectives will help in resolving:

- where to sample;
- how often to sample;
- variables to consider;
- sampling methods and replication;
- study budget;
- approaches to data analysis;
- reporting milestones and formats.

Planning for many types of studies can be sharpened even further by translating the objective into a hypothesis testing framework. This might seem rather too scientific for many resource management studies or general resource surveys. However, the planning for even a simple study can benefit from hypothesis testing. One of the principal benefits of doing this is that it forces you to think about what important effects or differences you might need to be able to confidently detect and the processes that might be operating (i.e., "*start with the end in mind*"). For most questions you are confronted with, it should be possible to develop a reasonable study approach using your accumulated knowledge, literature, and some sensible reasoning (based on sound scientific principles) (see Section 2.5). A literature search is always a powerful entry point to a new study area or for solving a new problem. Electronic

searches should be possible through most libraries. NIWA holds databases on all material published in New Zealand. It might be possible to access overseas databases (e.g., "Aquatic Sciences and Fisheries Abstracts") through some University libraries.

The means of assessing sample numbers and performing calculations is given in Section 3. But first we need to consider some more general issues, such as scale. We then give some examples of planning and carrying out periphyton monitoring studies to illustrate the concepts.

2.4 Issues of scale

A component of clearly defining your objectives and setting hypotheses is developing a sense of the scale of your problem. This will enable you to better assess what data might be required to resolve your issue. Scale is a somewhat nebulous concept to most people, but underlies many discrepancies that arise in concepts and differences in data interpretation. It is also vital to understand scale when attempting to rationalise data collection to obtain the most cost-effective solution.

Set the right scale and you will establish the right reference point to analyse your problem. Scale is mainly about the size of the "window" that you use to see the biological and physical variability in nature. For example, our perception of the world as we walk down the street is different from that we obtain from an aircraft, which is different again from what we would obtain from a spacecraft. *In essence*, the patterns we perceive are strongly influenced by how big our window is in relation to spatial and temporal variability in our environment. So, a motile diatom that is <10 μm long sees the surface of a stone like we might see the Sahara Desert. The diatom might then perceive undulations on the surface of the stone as we would see a large sand dune. Conversely, a mayfly would see variability in terms of the shape of individual stones and their orientation to the flow and a trout would see variability in terms of the extent of pools, riffles and runs. Trout wouldn't be influenced by the surface texture of individual stones and a diatom wouldn't even know about pools, riffles and runs.

Humans have the ability, particularly through modern technology, to view things over a very broad range of scales. Often it is very tempting to become swamped by detail in an effort to understand variability in stream periphyton communities. At some point it becomes important to stand back and ask "what level of detail and quantification of variability do I really need to answer the question being posed?" Should I be looking at the spatial arrangement and type of community patches on individual stones (which might be controlled most by local variations in water velocity)? Should I be integrating everything over individual stones and looking at the variability within the reach (which might be controlled most by spatial variations in sediment type such as cobbles vs. sands)? Or, should I be looking at variability among reaches (which might be controlled most by variations in riparian vegetation)? Or perhaps it is more important for the study objectives to pool all the samples together within each stream, but sample many streams along an environmental gradient to determine the effects of, say, catchment development among streams?

In other words, clearly define the potential factor(s) whose effects you want to examine, and the scale of biotic response that could occur. While strongly influencing the nature and success of your study, these questions also have a major bearing on the cost of carrying out the investigation (see below).

Scale has important implications for environmental predictions (Levin 1994). In complex systems it is usually possible by carrying out time and/or space averaging, to reduce vari-

ability to such an extent that general trends or responses become clear and can be simulated. This might be the appropriate scale to base management on. Alternatively, so much detail may be lost that the predicted shifts in biological response lie within the error of the model. For example, periphyton biomass in streams is typically highly variable over time (e.g., Fig. 16, Biggs 2000a). Indeed, this variability has defied efforts to develop realistic time-series models of biomass as functions of controlling variables such as nutrients, floods, light, temperature and invertebrate grazing. However, considerable progress has been made in developing models by moving up to the next scale by averaging out the temporal variability over a year, and then comparing biomass from many streams that differ widely in their landuse and flow characteristics (e.g., Biggs 2000b).

Thus, while we cannot predict exact periphyton biomass in a given reach of a river at a particular time, we can get estimates of likely average and worst case biomass as functions of flood disturbance frequency and nutrient concentrations. The question then arises: are these estimates accurate enough to enable the required decision to be made, or are they within the range of error of the model? Such questions of scale need to be addressed at the planning stage of a programme. Obtaining sensible answers requires careful thought about the processes you wish to investigate and their effects.

2.5   Examples of setting objectives and carrying out a sampling programme

Two examples of setting objectives and how one might develop associated study strategies are summarised below. For completeness, we have also included a brief summary of the results. The first example is an investigation of the effects on periphyton of land use in the stream catchment. The second is an investigation of the effects of a change in point source pollution discharge from a meat works.

2.5.1   Example 1: Enrichment of the Kakanui River, North Otago

The Otago Regional Council (ORC) were confronted with a perceived issue of possible over-allocation of waters in the Kakanui River in North Otago. It was suggested by community groups that abstraction in summer was reducing flows to such an extent that it was compromising the "life-supporting capacity" of the river. In particular, there was a perception that trout fishing opportunities had decreased greatly in recent years in the lower river and this was possibly because low flows were causing periphyton proliferations. This provided a good research opportunity to develop a greater understanding of the interactions between flow regimes and stream enrichment. The study was carried out as a collaboration between NIWA and the Otago Regional Council and the results have been published in Biggs *et al.* (1998b). After considering the problem we established a monitoring objective within the context of an hypothesis testing framework as defined below. The primary questions were:

1. Is there a downstream increase in *mean* and *maximum* periphyton biomass associated with intensification of landuse and the accompanying reduced flows caused by abstractions?

2. If so, does biomass breach MfE guidelines for the maintenance of life supporting capacity in streams (i.e., are periphyton proliferations really to blame for a perceived degradation in the fishery)?

To sharpen the scientific and conceptual basis for the investigation we then formulated a simple hypothesis:

"We predict that the headwater reaches of the river will be less enriched, and have lower periphyton biomass, than the lowland reaches."

The testable null hypothesis then became:

"There will be no statistically significant difference in periphyton biomass among sites at the 95% confidence level".

The monitoring objective then became:

"To define the effects of downstream intensification of landuse on enrichment of the Kakanui River with particular concern for defining the timing and magnitude of possible periphyton proliferations."

From this monitoring objective we could start to address the specific issues of study planning, as noted above (Section 2.3):

• **Where do we sample?**

Sites were needed in reaches that were all morphologically similar (i.e., similar slope, substrate type, water velocity, degree of shading, etc.) in an undeveloped headwater reach, a moderately developed mid-catchment reach and a highly developed lowland reach. Ideally, to give robust and conclusive answers, it would be best to replicate sites within each of these reaches (this would allow assessment of within reach variability). However, because of budgetary constraints, this was not possible. In each reach we sampled in a riffle and a run in order to identify whether enrichment influenced these habitats differently.

• **How often should we sample?**

We were most interested in summer periods of low flows resulting from abstractions. However, many high country streams in the South Island have periphyton biomass maxima at other times of the year - particularly mid-autumn (Biggs and Close 1989, Biggs *et al.* 1999). Therefore we chose to sample monthly over 2 years. This would allow us to quantify temporal variability, the timing of maximum biomass, and the magnitude of these growths to see how well they correlated with periods of abstraction.

• **What variables should we consider?**

Enrichment effects will be manifested as high amounts of biomass. Samples could be analysed for either ash-free dry mass (AFDM – which measures total organic matter) or chlorophyll *a* (which measures the amount of live algae). Chlorophyll *a* is cheapest, and easiest to relate to existing guidelines. However, AFDM gives a better measure of overall mass and, with an extra step in the analysis, can generate data on siltation of the bed. We chose to carry out both analyses. We also chose to determine the type of periphyton dominating the mats. This is because the species (or taxa) dominating a community provides a good additional indicator of relative levels of enrichment and may become an important qualifier if, for example, invertebrate grazing is quite high (in which case biomass becomes a less useful indicator of enrichment). Habitat variables such as near-bed water velocities, dissolved nutrients in the water, and nutrient concentrations in the periphyton mat were also measured.

• **What sampling method and replication is needed?**

The objective was "To define the effects of downstream intensification of landuse ... on ... possible periphyton proliferations". If restrictions on land development and/or use of the water are to be contemplated, then we need to be sure that changes in biological response down the catchment really are significant and that biomass criteria for proliferations are being consistently broken. Therefore, we chose sampling method that allowed biomass to be

measured as weight per unit area (see Section 6.5). Further, it was also necessary to carry out a statistical comparison of biomass among the sites to test the null hypothesis (that there was no statistically significant difference in biomass among sites at the 95% level), so some degree of replication was required within the sites. The amount of replication needed to reflect the spatial variability of growths. After considering the degree of variability from a preliminary survey, the degree of difference in mean biomass that might be ecologically important among sites (> 50%), and the budget we ended up choosing five samples per transect for each sampling occasion (see Section 3.4 for more guidance on determining required numbers of replicates for different levels of precision).

• **How should we analyse the data?**

We needed to define:

1. changes in biomass as a function of position in the catchment;

2. the timing and magnitude of peak biomass events.

A graph summarising data from one of the sites is given in Fig. 16 of Biggs (2000a). Because the sampling programme had been planned around a testable hypothesis, it was possible to use analysis of variance (ANOVA) to infer whether biomass differed significantly (at the

Table 1: Summary of periphyton statistics from runs and riffles at three sites in the Kakanui River, Otago. Head = headwater reach, Mid = mid-catchment reach, Low = lowland reach. ANOVA - *P* is the probability statistic of a one-way ANOVA to determine whether the mean monthly chlorophyll *a* or AFDM at the sites is significantly different at the 95% confidence interval (*) or the 99% significance level (**). Samples collected at monthly intervals were considered to be independent of one another. N.S. indicates that the difference is not statistically significant at the 95% level. Statistics with the same superscript for a given variable are not significantly different from each other at the 95 % confidence level as determined from a post-hoc Tukey test. %C.V. is the percentage coefficient of variation (i.e., the standard deviation/mean x 100). The analysis was carried out after transforming the raw data to natural logarithms to correct for a non-normal distribution in the data as required by the ANOVA test. Periphyton mean values were calculated as geometric means (from Biggs *et al.* 1998a).

Variable	Head	Mid	Low	ANOVA - *P*
1. Runs				
Mean chlorophyll *a* (mg/m^2)	10.1	17.0	8.6	N.S.
% C.V.	88.7	48.3	83.0	
Maximum chlorophyll *a* (mg/m^2)	143 (May)	64 (Apr.)	104 (Sep.)	
Mean AFDM (g/m^2)	6.2	15.1	5.6	N.S.
% C. V.	84.9	50.5	56.8	
Maximum AFDM (g/m^2)	75 (May)	56 (Apr.)	33 (Sep.)	
2. Riffles				
Mean chlorophyll *a* (mg/m^2)	18.0[1]	13.7[1]	59[2]	**
% C.V.	76.6	31.8	16.6	
Maximum chlorophyll *a* (mg/m^2)	220 (Aug.)	60 (Dec.)	159 (Jan.)	
Mean AFDM (g/m^2)	10.7	14.5	21.6	N.S.
% C. V.	84.9	27.6	17.9	
Maximum AFDM (g/m^2)	67 (Aug.)	33 (Mar.)	47 (Jan.)	

nominated confidence level) down the valley. If sites are replicated for a given landuse, then it is important to carry out a nested ANOVA to enable the among replicate site variability to be assessed against the variability among reaches (landuse treatments). However, this was not applicable in this example because only one site in each headwater, mid-catchment and lowland reach was sampled. A summary of the results is given in Table 1, together with the ANOVA results.

In brief, the results indicated the following.

• There was not a statistically significant difference in mean chlorophyll *a* nor AFDM biomass in the runs moving downstream, however there was a statistically significant difference in the riffles (for an explanation of this see below) which partially supported the hypothesis of increased levels of enrichment down the catchment;

• Maximum chlorophyll *a* concentrations in the runs did not show a clear difference down the catchment, while maximum AFDM did differ statistically down the catchment. Chlorophyll *a* was not greater than levels deemed to be a proliferation at any site and which could lead to the degradation of higher communities such as trout (= 200 mg/m² chlorophyll *a* – see guideline in Biggs 2000a). However, AFDM levels were greater than the recommended criteria (= 35 g/m² AFDM) at the headwater and mid-catchment sites suggesting a high accumulation of non-photosynthetic organic matter on the bed at these sites. These accumulations occurred in autumn (April and May) and not in summer, as expected.

• Maximum chlorophyll *a* concentrations in the riffles also did not increase downstream. Values at the headwater site and not the lowland site exceeded the proliferation criteria for chlorophyll *a*. This occurred in winter. AFDM in the riffles exceed the biomass criteria at both the headwater and lowland sites, in winter and summer respectively. The variation in timing of maximum chlorophyll *a* and AFDM was unexpected and did not support the contention that artificially reduced summer low flows were enhancing periphyton proliferations.

When interpreting the data for management decisions it is important to take into account the areal extent of the habitat that is being sampled. In the lowland section of the river, whilst high biomass of periphyton occurred in the riffles, they occupied only a small part (~ < 20 %) of the reach. The remaining area was dominated by runs which we found did not have a high biomass of periphyton. The reason for this was high grazing activity by snails. Experiments were carried out with nutrient-diffusing substrates to isolate nutrient and grazing effects (Biggs and Lowe 1994). These experiments clearly indicated that under the low water velocities of the runs the snails were able to build up high densities and graze most of the accumulating biomass production from the stones. However, higher velocities in the riffles prevented snails from accessing the stone surfaces so that they were unable to control periphyton biomass.

2.5.2 Example 2: Removal of a meatworks discharge to the South Branch of the Waimakariri: what were the benefits?

In 1985, Canterbury Frozen Meats introduced a new waste-water management policy in an effort to reduce the impact of their activities on the environment. This policy involved: commissioning an upgraded waste-water treatment facility to remove most of the solids from their effluent; removal of much of the oxidisable organic matter; and a reduction in phosphorus outputs. It was also planned to progressively reduce the amount of waste being discharged to the South Branch of the Waimakariri and eventually divert the entire, more highly treated, waste stream to the much larger main stem of the Waimakariri River.

The South Branch is a spring-fed stream with a mean flow of about 1 m³/s, few floods, clear waters and a diverse, productive benthic community dominated by macrophytes and periphyton. In contrast the main Waimakariri is a flood prone gravel-bed river with a mean flow of ~50 m³/s and a relatively depauperate benthic community. The waste was organic in nature and previous field observations had identified proliferations of sewage fungus in the reaches of the South Branch downstream of the discharge. The heterotrophic growths are undesirable because they suffocate the stream bed and can cause deoxygenation of the near-bed waters where the invertebrates live. A progressive reduction in waste volume started in May 1986 with complete diversion from the South Branch by September 1986. The question we had was "what benefits would an improvement in waste quality have on the ecology of the South Branch and what would be the benefits of complete diversion?". We carried this study out as a government-funded research exercise because it provided a good opportunity to test periphyton monitoring methods and the time required for communities to recover. As it turned out, no other studies were commissioned so it provided the only quantitative biological data to determine whether the very large expenditure on improved waste management had any benefits.

After viewing the discharge area, the following hypothesis was developed:

> *"The waste discharge is degrading the benthic communities in the South Branch by causing sewage fungus proliferations. As a result the periphyton communities are changing from being dominated by autotrophic to heterotrophic organisms".*

The monitoring objective then became:

> *"To determine the benefits of removing a treated meat works effluent discharge to the ecology of the South Branch of the Waimakariri River."*

The testable null hypothesis then became:

> *"There is no statistically significant difference at the 95% confidence level in the proportions of autotrophs and heterotrophs in periphyton between sites upstream and downstream of the discharge."*

Because we were forewarned, we had the opportunity to collect data prior to a change in discharge. Thus, we were able to develop a BACI type study design (i.e., sample before any change in management, after the changes, and with upstream control sites and downstream impact sites; see Section 4.2 for more information on this type of monitoring design).

- **Where do we sample?**

Sites needed to be carefully selected so that the physical constraints were identical at both the control and impact sites (i.e., any observed differences in periphyton would be due to water quality, not physical differences between sites). Also, it was essential that the impact site be "after full mixing" of the effluent since most consent conditions will specify this. It would have been preferable to replicate sites but this was not possible because of a major increase in encroachment of riparian willows on the channel immediately upstream of the control reach. Sampling locations were chosen to avoid effects of changes in local hydraulic conditions (velocity and turbulence) and of the effects of patches of macrophytes.

- **How often should we sample?**

At least two samplings before, and two samplings after, the change in management were nominated. Greater numbers of samplings would have been desirable but were not possible because of the timing of the planned waste reduction programme. We were also able to carry out four samplings during the period when the effluent quantity was being reduced.

- **What variables should we consider?**

We needed fully quantitative variables to assess the effects on heterotrophic (sewage fungus) growths vs autotrophic (algal) growths. We also needed quantitative analysis of the relative abundance of different algal taxa and sewage fungus. Therefore, we chose to analyse samples for ash-free dry mass, chlorophyll *a* and the density of cells in different taxonomic groups. We also calculated the autotrophic index (AI). This is the ratio of ash-free dry mass to chlorophyll *a* where high values indicate large amounts of non-photosynthetic organic material compared to live plant material (see Section 7.1).

- **What sampling method and replication is needed?**

Sampling needed to be quantitative. However, inspection of the reaches above and below the discharge point revealed that the local substrate conditions differed between the two reaches. It was therefore necessary to employ artificial substrate samplers in order to remove the influence of differences in bed habitat (see Section 6.6). Three sets of samplers, each with five
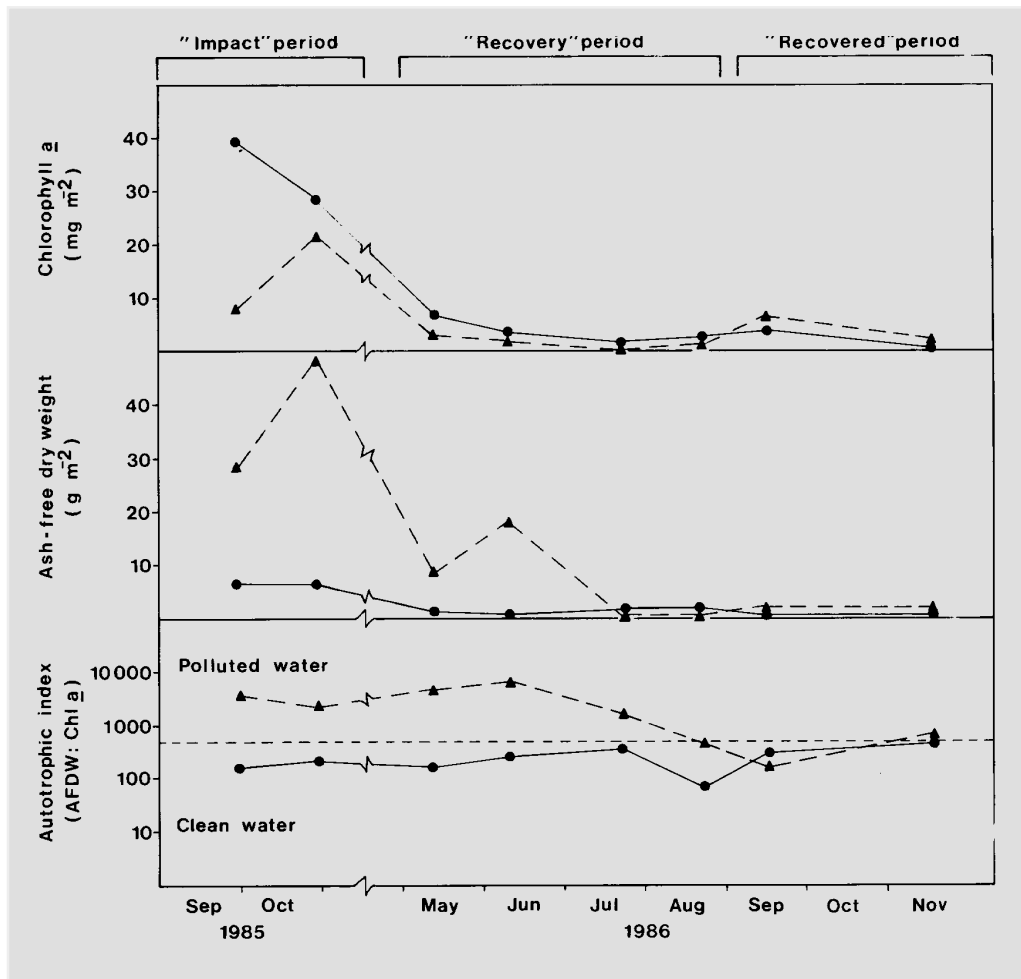
Figure 2: Changes in chlorophyll *a*, AFDM, and the autotrophic index (AI) over time in the South Branch of the Waimakariri River. Periods of full effluent discharge ("Impact" period), reducing discharge ('Recovery period'), and no effluent discharge ("Recovered period") are shown. The fine dashed line on the AI graph denotes a value of 400 which has been suggested by Collins and Weber (1978) as the upper limit for clean waters (•, control site; ▲, impact site) (from Biggs 1989, reproduced with permission of the *New Zealand Journal of Marine and Freshwater Research*).

replicate substrates, were used in each of the reaches above and below the discharge point. The sampling plates were loaded vertically in the sampler rack to avoid siltation. Previous analysis (Biggs 1988a) had identified that 28 replicates per sampler would be needed to estimate mean biomass to within ±20%. However, this was not possible because of budgetary constraints. So five replicates were collected from each of three samplers, giving quite a low level of precision per sampler, but improving to around 25 % with a pooling of the results within a site once the blocking effect of the different samplers was accounted for in the ANOVA (see Section 3.4 for more guidance on determining required numbers of replicates for different levels of precision in impact testing).

- **How should we analyse the data?**

Two main approaches were used:

1. ANOVA of differences between sites;

2. analysis of trends over time.

Prior to the improved waste treatment, the most abundant taxa at the control site were unicellular diatoms that indicated reasonably clean or slightly enriched waters whereas at the downstream impact site the communities were dominated with "sewage fungus". This resulted in statistically significantly different AI between the control and impact sites. These differences can be clearly seen in Figure 2.

With a reduction in effluent volume over a four-month period there was a decrease in quantity of sewage fungus in the mat at the impact site and an associated reduction in the AI. Within a month of complete diversion of the effluent, ANOVA indicated that there were no statistically significant differences in the AI between control and impact sites.

2.6 Reference sites

Reference sites are sites located in areas that are not clearly impacted by human activity. Possible locations for these can be identified through the process of physical habitat classification or local knowledge. Ideally, reference sites as well as impacted sites should be included in all long-term monitoring programmes (e.g., State of the Environment). Reference sites are essential if the objective is to determine the effects of environmental changes brought about by a specific activity that could introduce non-point source contaminants, such as forestry or horticultural developments. This is because broad-scale changes may also be occurring as a result of, say, climate change. It is then necessary to separate the effects of changes in management from such broad-scale influences.

Reference sites should not be confused with "control" sites. Control sites are sites upstream of a specific perturbation that are paired with a downstream "impact" site and are used to define the effects of that perturbation regardless of what the upstream conditions are. While reference sites might serve the needs of a control site (depending on where in the catchment the analysis is located), the reverse need not be true.

In choosing reference sites, it is important to define the habitat type being represented. This will usually only need to be done at quite a high level of the hierarchy of controlling variables in a region, and for controllers that are largely unaffected by human activity, plus the local native vegetation type. So for example, in Hawke's Bay we might define a reference site for hill country streams with predominantly greywacke geology at a location near the head of the catchment where the land cover is predominantly beech and broadleaf forest. We may identify another reference site in a hill country stream with predominantly soft, Tertiary mudstone and a native bush upper catchment to represent the natural background setting∕

conditions in Tertiary hill-country streams of the region. These sites are then used as the reference points against which data from downstream, impacted, reaches are compared. These sites may also serve as reference points for other streams/catchments in a region that share the same base classification of source of flow and geology. Ideally, one should attempt to sample at least three reference sites on several occasions for a given habitat class to gauge the variability among them before settling on using one for long-term monitoring.

It should be recognised that it may not be possible to obtain reference sites for some major habitat types in many regions. For example, finding a lowland stream with a predominantly forest catchment upstream of a given point is now very difficult. A regional river environment classification will assist in defining the availability/rarity of such habitats (see Snelder *et al.* 1998).

2.7 Maximising information for the available budget

In developing a sampling programme, there will always be a trade-off between the detail required and the resources available. This problem arises because of the amount of variability in nature and the scale of the changes or impacts that you usually need to define for management of streams. These considerations impinge heavily on replication and the degree of statistical precision (i.e., degrees of freedom) required for the analysis (this is discussed more in Section 3). There are always budget limits, and there will always be compromises. The compromises should be based on prior knowledge and the requirements of each study. The limits to compromise also need to be set from this prior knowledge. The limit is the point at which the data collected are unable to satisfactorily resolve the issue(s) under consideration.

In the past, we have often compromised detail in our investigations in the following order (i.e., first to last):

1. analytical detail such as the number and type of parameters (this should not be confused with analytical precision which relates to how well an analysis is performed);

2. analytical replication;

3. sampling replication in space;

4. sampling replication in time.

These reflect a hierarchy of increasing sources of variability in periphyton data. In other words, periphyton communities are most variable in time (periods of weeks to months), followed by being quite variable across the bed of a stream within a given reach or even valley segment, followed by being less variable among analytical replicates. Finally, the type and detail of the analysis can add a huge amount to the cost of a study (perhaps doubling it), but for only a marginal increase in quality of information. Unless that increase in quality of information could become vital at a later stage, then save your resources and only analyse for the variables that are absolutely necessary. There are a number of ways to optimise the quality of the data, whilst reducing the cost of a programme. A real example of such a series of compromises to fit a relatively small budget is as follows. This was to enable the study in Example 1 (Section 2.5.1) to be carried out (from Biggs *et al.* 1998a):

Study objective:

To define the effects of downstream intensification of landuse on periphyton communities in the Kakanui River catchment, North Otago, with particular concern for defining the timing and magnitude of possible proliferations.

What we most needed to know (arranged in decreasing order of priority):

- average monthly periphyton biomass over a year;

- maximum biomass (to see if it breached proliferation criteria);

- what taxa dominated peak biomass;

- variability/gradients in community biomass/composition within the catchment;

- overriding effects of other perturbations/disturbances (e.g., invertebrate grazing and floods).

Sampling regime:

In order to satisfy the above priorities, while staying within the budget, we decided on a periphyton sampling and analysis programme as follows.

- Monthly sampling over two years at three sites representing the three main valley segments in the catchment (headwaters segment, mid-catchment confined segment, lowland segment) with sites located near water-level recorder sites;

- Five replicate samples scraped from a set area on the tops of stones along a transect across a riffle (see Section 6.5.5). These replicates were then pooled into one sample per sampling date rather than being kept separate for individual analysis. Another five replicates were then collected in the same way from runs. This protocol was repeated at each site on every visit.

- In the laboratory, each pooled sample was thoroughly blended and then three aliquots (sub-samples) of suspension were removed and pooled for analysis of one sub-sample for biomass. A further pooled sub-sample was retained and frozen. Once the sampling programme and all biomass analyses were complete, then sampling occasions when peak biomass occurred were analysed for taxonomic composition using a rapid assessment method to define relative abundance (see Section 8.3).

Sampling regime and budgetary compromises and efficiencies:

- We sampled for two years because the catchment was known to be prone to major inter-annual variations in flow regime. A single-year sampling programme would not enable the average conditions of the river to be characterised, nor the effects of meso-scale variations in climatic regimes. A longer time period would have been preferred to establish year-to-year variability; two years is the minimum time required to start to define inter-annual variability.

- Monthly sampling was carried out as part of the standard hydrological/water-level recorder site visits to the river by the Otago Regional Council. This meant that specific field visits by our team (including the down-time of travel from Christchurch to the Kakanui River, 4 hours each way) was not required. Minimal new resources were therefore necessary to carry out the sampling. It would have been good to have extra sites in order to determine variability within each section of the river, but these could not be accommodated within the budget. A once-off, more detailed synoptic survey of 8 sites down the catchment was carried out during summer low flows to help define downstream variations in biomass and composition.

- Only five samples were collected which would have give means with quite high variability, but we expected to be able to confidently detect differences of > 50% with 95% confidence (using formulae as defined in Section 3.4). A much lower error

would have been achieved had we collected 10 samples per habitat (a standard error of ~ 10–20% of the mean, depending on how patchy the distribution of the periphyton was). However, we needed to sample both riffles and runs (as opposed to just runs, which is our normal sampling habitat) because proliferations had been observed in the runs in headwater reaches and riffles in downstream reaches. The time required to collect 20 samples per site (10 from the riffle and 10 from the run) would have meant that the field team could not complete the sampling and all their other tasks within daylight hours in winter. Another consideration in the decision was that the time-scale of the programme was long (2 years) and so we expected that the size of the temporal fluctuations in biomass would have been much greater than the errors associated with individual measurements (2–20 times greater).

- The decision to pool the five replicate samples was to enable analytical costs to be greatly reduced (to about 30% of what they would have been). We lost the ability to test for significance of difference between habitats and sites on any given sampling occasion, or to test the effects of specific perturbations (e.g., the effect of a given flood event). However, the primary objective was to identify differences among sites over at least a year and the magnitude and timing of biomass peaks. This was the primary scale of our objective and thus we didn't believe that pooling of replicates would compromise this objective greatly. For example, the single values for each month resulted in 12 values for a given habitat over a year. This provided the replication required to test for differences among sites in mean monthly biomass (i.e., a comparison of the average of the monthly measurements at each site). These data were also suitable for testing differences among years for a given site.

- Analysis of individual aliquots would have enabled the error in sub-sampling + analysis to be assessed. However, previous studies have shown that the standard deviation was in the order of only 12–15% of the mean (Biggs 1987), so pooling sub-samples reduced analytical costs greatly. It was not anticipated that the data from analytical replication would be used. The samples were thoroughly blended before sub-sampling because periphyton tend to aggregate into clumps, which can result in very large sub-sampling error and the necessity for analysing many more replicates (see Section 7.1). Every taxonomic analysis is quite time consuming. By only analysing samples from specific times of interest (i.e., times of high biomass), it was possible to also greatly reduce the expense of this part of the study.

3 Determining data precision and number of sampling replicates required

3.1 Background

In any quantification of the environment, we are attempting to take a representative "snapshot" of the conditions present. In other words, we analyse a sub-set of the overall conditions or populations and make inferences about the way the whole population or community works from this sample. The degree to which our "sample" represents the "whole" of what we are interested in is a function of the number of samples we take in relation to the degree of variability (or patchiness) of communities or populations.

To illustrate ways to deal with variability we can use a simple hypothetical example based on a well-known terrestrial "habitat": a golf course.

Suppose we are presented with the question: "what is the average biomass of plant communities on the putting green around the 7th hole?" To determine this we might retrieve three replicate samples from different parts of the putting green (e.g., as a core down through the grass to a depth of ~5 cm). Because putting greens are generally created and maintained to be very uniform, the three samples might give us data on the mean biomass of vegetation (grass) that is very close to the true average for the whole putting green (e.g., the sample mean might be within, say, 10% of the true mean).

However, the question might become: "what is the average biomass of vegetation on all 18 putting greens of the golf course?" In this situation, the results from the 7th green would probably be a poor representation of all the greens. It would then be necessary to randomly select (i.e., using a set of random number tables) several of the greens to sample. If the greens were all being maintained in a similar way and had very similar vegetation cover, then a set of three samples from each of three greens might represent the full population of greens very well. However, if for some reason (e.g., soil type, irrigation regime etc) there was considerable variability in grass growth among greens then a sample of three greens would give a poor representation of average green vegetation biomass.

At an even higher spatial scale, the question might become: "what is the average biomass of vegetation for the golf course?" Clearly, if we were to take three randomly located samples of vegetation from the whole golf course, we would obtain a mean that is likely to be very different from the true mean because of an increase in the degree of variability scaling up from the case of a single green to the whole course. One sample might be located in a bunker, another on a green and yet another in the rough. What about the stands of trees that line some areas and the extensive areas in fairways? In essence, how many random samples would we need to represent the mean biomass of vegetation for a high level of precision in such a heterogeneous habitat? This is the sort of problem we are continually confronted with when sampling stream periphyton.

In the following sections we discuss several measures of variability, illustrate some important concepts with regard to variability and the effects of sample number, and give guidance on how to estimate the number of samples needed for determining the mean of a variable for any pre-determined level of precision. Different approaches are required for estimating sample numbers in different types of studies and tests. Here we cover estimating sample numbers for: (1) general resource surveys; (2) testing for differences in the means of variables between two sites/populations; and (3) testing for differences between the mean level of a variable and a guideline or standard. Finally, we describe how to stratify sampling according to

physically distinct habitat units (e.g., greens vs. bunkers vs. patches of trees, etc.) in order to make more effective use of resources and increase information content about the habitats.

3.2   Measures of variability

The standard error of the mean is generally used as a measure of how reliable the sample mean may be when it is used to estimate the true population mean (note that the standard error is not a measure of variability – the standard deviation does this). Indeed, when presenting summary plots of the data, it is standard practice to include standard error bars on mean values to show the precision of our estimate of that mean (i.e., an indication of how precisely we have quantified the population mean). The standard error of the mean (*SEMx*) is calculated as the standard deviation of the data (*s*), divided by square root of the number of samples taken (*n*). In symbols, this is:

$$SEM_{\bar{x}} = \frac{s}{\sqrt{n}} \qquad\qquad (1)$$

For most periphyton assessments we wish to be able to state that:

"the mean abundance or biomass is $\bar{x} \pm y$ numbers of cells or biomass/m² ".

In practice, we have to accept that the data we use to estimate the mean are "normally" distributed about the true (and unknown) value so that no matter how large *y* is, there is a chance that our estimates could be further away from the true mean than we have stated. It is common to accept a 5% chance of being wrong and this is achieved if *y* is approximately twice the standard error of our estimate of the mean. In other words, there is a 95% chance that the true mean will be within two standard errors of our sample mean. The standard error of our estimate of the true mean can be made as small as we like by taking more replicate samples. That is, the more we sample from an area, the better we average out the variability. We discuss this more below.

It is the 95% confidence intervals (C.I.) around the sample mean that are the most useful statistics for interpreting whether sets of samples from different populations are statistically significantly different. These are easily calculated from the standard error of the mean using Student's *t*-statistics as follows:

$$\bar{x} \pm (t_{\alpha(2),\ n-1}) \times SEM_{\bar{x}} \qquad\qquad (2)$$

where the *t*-statistic is obtained from tables of Student's *t*-values by selecting the required % chance of being wrong ($\alpha = 0.05$, in this case), whether the test is one-tailed or two-tailed (indicated by the subscript (1) or (2) – see page 23), and also the number of degrees of freedom (indicated in the tables by ν, where $\nu = n - 1$ degrees of freedom). (See, for example, Appendix Table B.3 of Zar 1996). If the 95% C.I. for two sets of samples do not overlap (e.g., when the means and C.I.s of the two samples are represented together on a bar graph), then the samples are considered to be statistically significantly different with a 95% probability that this conclusion is correct (i.e., $P < 0.05$ for the test).

3.3   Estimating the number of replicate samples needed for general resource surveys

For general surveys, we can use the standard deviation of the data to tell us how many samples (*n*) we might need to collect to achieve a pre-selected precision (after Zar 1996, p. 107). We would use preliminary survey data to calculate the initial standard deviation (as a measure of the variability), or data from a similar site collected previously. We then enter this data into the following equation:

$$n = \frac{s^2 \, t^2}{d^2} \tag{3}$$

where s is the standard deviation of the preliminary data, $t = t_{a(2)}, \, n_{-1}$ the two-tailed critical value of the Student's t distribution with $v = n - 1$ degrees of freedom and $\alpha = 0.05$ (found in Appendix Table B.3 of Zar 1996), and d is a pre-selected half-width of the desired confidence interval of the sample mean. [Note: this formula corrects that given in Zar (1984) which is incorrect (G.B. McBride, NIWA pers. comm.)].

The degrees of freedom for t are not known because n is unknown, so it is necessary to iterate Equation (3) to obtain a solution. The half-width of the pre-selected precision, d, is set based on the objective of the study and practical or resource limitations (e.g., we may wish the confidence interval around a mean to be ±30% of the mean so $d = 0.3/2$ x the mean = 0.15 x the mean). As we discuss further below, as the level of precision increases more samples need to be collected and processed. This is because with a doubling of precision, the required number of samples increases not 2-fold, but 4-fold (i.e., to the power of two). The balance that needs to be struck between being over-precise and under-precise, will be discussed further below.

The above assessment assumes that the preliminary data for assessing variance are drawn from a population with a statistical distribution that is approximately "normal". If the data are very skewed to the right (as is often the case for periphyton measurements) then a logarithmic transformation can be used to normalise the data and the calculations done using the standard deviation of the logs. However, for very skewed data, the mean is a very poor representation of central tendency and it may, therefore, be better to use the median. Procedures for assessing the number of samples required for calculating medians with a given level of precision are given in texts such as Gilbert (1987, p. 174, eq. 13.23).

Let us briefly work through an example. Suppose we want to know the mean chlorophyll a concentration of periphyton in a run of a foothills stream (we could be interested in the extent of enrichment from agricultural activities in a catchment). We might want to estimate this mean biomass with a precision that enables the sample mean to lie within a 95% C.I. that is ±20% of the mean (i.e., the sample mean is to be within ± 20% of the population mean at $p \oplus 0.05$). How many samples would be required? Preliminary survey data of chlorophyll a from one reach were tested and found to be approximately normally distributed. The mean of these data was 267.5 mg/m^2 chlorophyll a ($n = 10$), with a standard deviation of 86.4, and $d = 0.2$ x $267.5 = 53.5$. We then need to start the process of iteration by guessing the number of samples that might be required and then using this as a basis to select a critical value for the t distribution. We start by guessing that 15 samples would be required (for the iterations it is better to initially overestimate the number required). Therefore, the critical value for the t distribution is $t_{0.05, 15-1} = 2.145$. Inserting our values for s, t and d in equation (3) and completing the calculation we therefore have:

$$n = s^2 \, t^2 / d^2 = 86.4^2 \text{ x } 2.145^2 / 53.5^2 = 12.00 \text{ samples}$$

We then iterate the equation again to see if we can get close to 12 by using a smaller starting value than 15. If we use 12 samples as the starting point we insert a critical value for the t distribution for $n = 12 - 1$ degrees of freedom (= 2.201). This iteration then gives an estimate of 12.6 samples required. This is close enough to the first estimate of 12 to conclude that we probably need 12–13 samples to enable us to be 95% confident that our sample mean will be within ± 20% of the population mean.

Figure 3: Effect of increasing number of sample replicates on the standard error of the mean (*SEM*) (normalised to its respective means) for chlorophyll *a* of diatom dominated communities on cobbles at three sites. +, mean chlorophyll *a* with 10 samples = 268 mg/m^2; X, mean chlorophyll *a* = 64 mg/m^2; •, mean chlorophyll *a* = 4 mg/m^2.
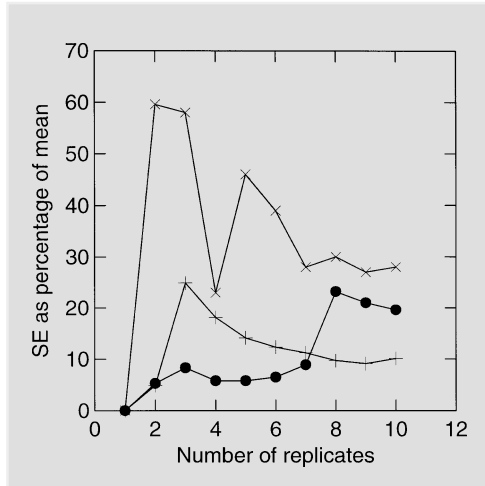
Such assessments can be easily carried out for any parameters. However, in pre-selecting precision there is a trade-off between the precision of estimating the population mean (which influences the chance of incorrect conclusions) and resource availability for the study.

Let's take a closer look at the effect of the number of replicate samples on how precisely we can estimate the mean. We will consider biomass and then species richness (i.e., total number of species encountered) because analysis of variability for these requires different approaches.

In Figure 3 we plot the *SEM*s (as a percentage of each mean) for chlorophyll *a* as a function of increasing replication. The samples are from low, medium, and high biomass diatom dominated communities. These data show several important patterns. First, with few samples for the medium and high biomass communities the variability in the data tends to be high, and thus the standard error of our estimate of the mean is high relative to the mean. However, with increasing numbers of replicates the error decreases, then stabilises (at ~ 7–8 replicates).

Second, precision is lowest (i.e., the *SEM* is highest relative to the mean), and more variable, for the medium biomass community than for both high and low biomass. With medium biomass, periphyton cover is usually more patchy and we often see stones with large tufts of green filaments in reaches that otherwise only have mats of diatoms (see Figure 6 and Figure 28 in Biggs 2000a). Once a tuft of filamentous green algae is encountered, variability suddenly increases (e.g., at 5 replicates for the medium biomass community, Figure 3). This variability in the *SEM* does not tend to occur as much with high biomass communities (particularly of diatoms), because the distribution of organic matter at such times has generally become more uniform across the stream bed. Thus, variability peaks at 3 replicates and then decays with increasing sample replication.

Third, with low biomass communities, variability tends to be very low because cover is also usually quite uniform. However, as with the medium biomass community, there is an increased chance of encountering higher biomass tufts with more sampling such as occurred for replicate 8. Such tufts may have developed from relict communities that survived the last flood disturbance, or they may be near some localised enrichment (e.g., from groundwater sources). The analysis of changes in standard errors as a function of numbers of replicates can also be carried out on cell count data.

A different approach is normally used for determining "species richness" in a population (NB: we use the term "species" liberally here because many of the identifications can only be made to the generic level with periphyton, but "species richness" is the accepted terminology in other fields of ecology). When species richness is an important consideration, we are

Figure 4: Effect of increasing number of samples on total species richness of periphyton samples at three sites in a cobble bed river. +, mean chlorophyll *a* with 10 samples = 69.3 mg/m^2; x, mean chlorophyll *a* = 45.0 mg/m^2; •, mean chlorophyll *a* = 2.1 mg/m^2.
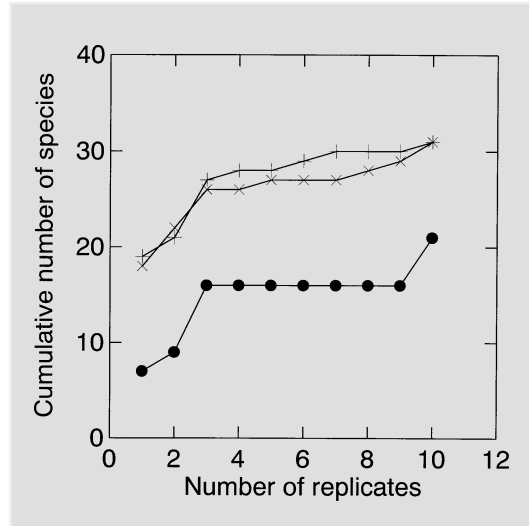
generally interested in knowing what the total species pool might be (e.g., for assessing biodiversity values or the effect of, say, landuse on community composition). We therefore assess cumulative richness as a function of increasing number of replicate samples in our power analysis. As an example, we have summarised the cumulative number of species as a function of cumulative replicates for medium and low biomass communities in Figure 4. These illustrate two common features. First, low biomass communities tend to have fewer species because succession has not usually proceeded far enough for a more complex community to develop. Second, low biomass communities tend to be more stable in their species richness unless, as occurred in the example for replicate 10, a much higher biomass "patch" is encountered. With medium (and high) biomass communities, there is a rapid increase in recorded richness to 3 replicate samples, after which the increase slows. However, it does still trend upward and generally levels off at about 7–10 replicates.

It is often worthwhile to check the variability of sample values for resource surveys using the approaches described above. Consistent redundancy in the information collected can justify reducing sampling effort. Conversely, with high variability, more sampling effort may be required.

3.4 Estimating the number of replicate samples required for testing hypotheses of differences in two population means for periphyton biomonitoring and consent evaluation

There are situations where we may be required to test differences between two population means. An example is looking for the relative differences in some variable between two sites (e.g., impact vs. control or reference sites) as part of a biological monitoring programme. For this, we might need to set a level of difference between the two sites that should not be breached (e.g., as part of a consent condition). The study will then set out to establish whether a breach of the specific condition is occurring. In this case we need to determine how many samples are required from both sites at the nominated level of difference to ensure a low probability of being wrong. Assessing differences where there are gross disturbances or differences is a trivial procedure. However, if we are to distinguish more subtle differences then we need to be sure that we have collected enough samples so that our error does not swamp the differences we are trying to detect. For example, it might be that the biomass of the periphyton mat needs to change by >30% (measured as chlorophyll *a*) compared with the

control site before the consent has been breached (i.e., any change of less than 30% might be considered ecologically unimportant because of natural spatial or temporal variability). The formula for such estimates (from Zar 1996, p.133) is:

$$n \geq \frac{2s_p^2}{\delta^2} \ (t_{\alpha(2),v} + t_{\beta(1),v})^2 \tag{4}$$

where n is the size of the sample from each site (or at one site over time), δ is the minimum difference in populations that we wish to detect ($\delta = \mu_1 - \mu_2$ for the two-sample t-test of the hypothesis, where μ indicates a population mean), sp is the pooled within-population standard deviation determined from previous data, $t_{\alpha(2),v}$ is the critical value for the t-distribution for v = $2(n-1)$ degrees of freedom and with α, the nominated significance level, usually set at 0.05 (or perhaps 0.01 or 0.001 for really stringent tests). We can set the significance level of the test, α, to anything we want. However, $p = 0.05$ is a generally accepted limit and tells us that we have less than a 5% chance of making a Type I error (i.e., falsely rejecting the null hypothesis that there is no difference between the two population means). β is the probability of committing a Type II error (i.e., erroneously concluding that there is no difference between our populations) and $1 - \beta$ is the power of the test to detect a difference between population means. Values for $1 - \beta$ may be set at 0.8, 0.9, 0.95, etc. depending on what are considered to be ecologically important differences in population means, and budgetary constraints. High values require larger sample sizes. The value of $t_{\beta(1), v}$ is related to the probability that the desired exceedence will be detected (see Table B.3, Appendix B of Zar 1996 for the t values).

Because n is on both sides of the equation (the degrees of freedom depend on n), the solution can be found only by trial and error. However, this is usually done in only 2 or 3 iterations. Note that this is a "two-tailed" statistical analysis. In other words no assumption is being made of whether the change is up or down, or that a specific criterion is going to be exceeded. If the latter circumstances prevail, then a "one-tailed" test is required (see below). Also, the analysis assumes that the samples are from two populations with approximately normal distributions. If the data are strongly skewed to the right, transformation of the preliminary data (e.g., to logarithms) will be required before calculating the variance for the analysis .

Let us work through an example. The data are from the patchy "intermediate" biomass community used in Figure 3. Suppose that these data are representative of an upstream control site and there is a discharge of treated dairy shed effluent that is moderately enriched with phosphorus just downstream of the site. We set up a physically identical "impact" site in a reach after full mixing of the effluent (say 0.5 km downstream of the discharge). We need to assess, using a once-off survey during worst-case conditions during summer low flows, whether the conditions for the discharge developed as part of the consent procedures under the Resource Management Act have been broken. The consent is defined as:

> "there shall not be more than a 30% change in chlorophyll *a* between upstream and downstream sites (after full mixing) at any time over the summer months as a result of the discharge".

While we would expect that a discharge of treated dairy shed effluent would result in nutrient enrichment, and therefore an increase in periphyton biomass, we can never be sure of this. For example, in some situations higher levels of suspended solids may restrict periphyton production. We therefore want to test for *any* change in chlorophyll *a* (a "two-tailed" test) rather than testing for a specific level of increase in biomass (a "one-tailed" test). To do this we need to sample the impact and control sites with sufficient precision that we could detect a difference of ±30%. We would normally nominate a level of significance (α) of 0.05 for the

test, with a power $(1 - \beta)$ of say 0.90 (i.e., $\beta = 0.1$). Our preliminary study determined that the *arithmetic* mean biomass at the control site was 63.9 and the within-population variance (i.e., s^2) was 2865. However, the test assumes that the samples are drawn from two approximately normally distributed populations and a quick plot of the density distribution of the raw data identified that they were right skewed. We therefore require a logarithmic transformation of the data before determining sample size (indeed such a transformation would also be required before carrying out any parametric statistical tests such as *t*-tests or analysis of variance (ANOVA)). The mean of the logged data (using natural logs) was then 3.8898 and s^2 of the logged data was 1.7122. The antilog of the mean of the logarithms is the *geometric* mean, and this is $\exp(3.8898) = 48.90$. (Note that the geometric mean is close to the median of the untransformed data.) Thus, the change to be detected (δ, best called the "minimum detectable difference") with the desired power is $\delta = 0.3 \times 48.9 = 14.67$, and in terms of logarithms, the difference is $\ln(\delta) = 2.686$. We need to guess what the sample size, *n*, might be so we will start with $n = 10$. Therefore, the critical value for significance level of 0.05 on the *t* distribution is calculated based on $2(10 - 1) = 18$ degrees of freedom, so for a two-sided test $t_{0.05(2),18} = 2.101$. The critical value of β, the probability of committing a Type II error, is determined for a one-sided test as $t_{0.1(1), 18} = 1.330$. We then insert these numbers in equation (4) above:

$$n \geq \frac{2\,(1.7122)}{(2.686)^2}\,(2.101 + 1.330)^2 = 5.60$$

We now iterate the calculation again using a number near the result of the first iteration to see how close the next result is to the first. Say we try with $n = 6$:

$$n \geq \frac{2\,(1.7122)}{(2.686)^2}\,(2.225 + 1.372)^2 = 6.14$$

We therefore conclude that we need at least 6 samples per site to be able to detect a 30% change in chlorophyll *a* between the two sites with a power of 90%.

A few general, perhaps obvious, comments are warranted here. First, as noted in Section 3.3, the smaller the difference that we want to detect, the larger the number of samples we will need. Thus, in the above example, if we wanted to detect a difference of just 10% instead of 30% then we would need 16 samples per site. Second, the number of samples also increases greatly with increased variability, or patchiness, of the community. In the example there was a relatively low variability once the data had been log transformed. Many sites will have communities with considerably more variation. Third, if we desire a high probability of detecting a difference between population means (i.e., having a low probability of committing a Type II error), the power of the test, $1 - \beta$, needs to be relatively large which will, in turn, result in a larger sample size.

3.5 Estimating number of replicate samples required for testing hypotheses regarding compliance with periphyton cover or biomass guidelines

Another common situation to arise is monitoring to determine whether a breach of a specific guideline or standard on a consent might have occurred (e.g., for cover of filamentous algae or biomass). In such situations it is advisable to try to estimate the mean with as much precision as possible because the consequences of an incorrect assessment could be major for the Resource Consent holder or the environment. In other words, one could easily accept that there is a difference between the sample mean and the guideline value when there really isn't

(a Type I error in statistical terms), or that there isn't a difference between the sample mean and the guideline value when there really is (a Type II error). Both these scenarios can be problematical, the first might result in unnecessary restrictions for a water user (or prosecution) and the second might result in under-protection for the environment. This analysis is very similar to that described in the preceding section. However, the equation is slightly different because our interest lies in whether a given level for a variable is exceeded and not the simpler case of whether a significant change in any direction had occurred. Thus, we must use a "one-tailed" or ("one-sided") test. Zar (1996, p. 108) defines the following equation:

$$n \geq \frac{s_p^2}{\delta^2} \; (t_{\alpha(1),\,v} + t_{\beta(1),v})^2 \qquad (5)$$

where n is the size of the sample for the site, δ is the minimum difference in population that we wish to detect, s is the within-population standard deviation determined from previous data, $t_{\alpha(1),v}$ is the critical t-value at the nominated significance level for the test (α) at $v = n - 1$ degrees of freedom (the significance level is usually set at 0.05, but could be 0.01 or 0.001 for really stringent tests). β is the probability of committing a Type II error (i.e., erroneously concluding that there is no difference between our populations) and $1 - \beta$ is the power of the test to detect a difference between population means. Values for $1 - \beta$ may be set at 0.8, 0.9, 0.95, etc. depending on what is considered to be an ecologically important difference in population from the guideline value and budgetary constraints. High values require larger sample sizes. The value of $t_{\beta(1),\,v}$ is related to the probability that the desired exceedence will be detected (see Table B.3, Appendix B of Zar 1996 for the t values).

As with Equation (4), n is on both sides of the equation (the degrees of freedom depend on n), so the solution can be found only by trial and error. However, this is usually done in only 2 or 3 iterations. As noted above, this is a "one-tailed" statistical analysis. In other words we are testing whether our population is *greater* than a hypothesised (or guideline) value. Also, the analysis assumes that the samples are from a population with approximately normal distributions. If the data are strongly skewed to the right a transformation of the preliminary data (e.g., to logarithms) will be required before calculating the variance for the analysis.

We will now work through an example, again using the data from Figure 3. Suppose we want to detect an exceedance of the 50 mg/m² chlorophyll *a* biomass guideline for the protection of natural biodiversity values in streams (Biggs 2000a) and the minimum ecologically important exceedance of this value that we are interested in is >30%. The mean of the logged data was then 3.8898 and s^2 of the logged data was 1.7122. Thus, a change in biomass of ±~30% of the mean at the control site would equate to needing to detect a difference in the geometric mean of the data of $\delta = 48.9 \times 0.3 = 14.67$, and $\ln(\delta) = 2.686$, which replaces δ in Equation (5). We need to guess what the sample size, n, might be so we will start with $n = 10$. The critical value for significance level of 0.05 on the t distribution is calculated based on $n = 10 - 1 = 9$ degrees of freedom, so for a one-sided test $t_{0.05(1),9} = 1.833$. Also, with $\beta = 0.1$ and $v = 9$, the critical value of $t_{\beta(1),\,v} = 1.383$. We then insert these numbers in Equation (5) above:

$$n \geq \frac{(1.7122)}{(2.686)^2} \; (1.833 + 1.383)^2 = 2.45$$

We then iterate the equation again by starting with $n = 3$ and determining new critical values on the t distribution for α and β based on $3 - 1$ degrees of freedom for the one-sided test. This gave an estimated number of samples of 5.5, and repetitions lead to alternative values of 3

and 6. We would therefore settle on a sample size of 6 as the minimum required to fulfil the design criterion of being able to detect a 15 mg/m² (i.e., 30%) exceedance of the 50 mg/m² chlorophyll *a* guideline at the given site.

3.6   Stratifying sampling effort to increase precision and reduce effort

In highly heterogeneous environments, or with very patchy communities, there are simple ways to focus sampling effort to give more cost-effective results (i.e., maximising precision for a given cost). This is carried out by dividing an area or reach into several more-or-less homogeneous strata. This is also useful because the assumption that is the basis of random sampling – that features vary randomly in time and/or space – is not always true. If we partition our sampling effort by strata, it allows us to increase the precision of population estimates and to "partition variance". Let us return to the hypothetical example of assessing the mean biomass of the golf course, introduced in Section 3.1 above. Rather than take many samples randomly over the golf course, we would be better off to identify physically distinct sub-units (or strata), characterise these, and then determine their areal extent. These sub-units might comprise the greens, fairways, bunkers and "the rough". The parallel for stream periphyton sampling might be to divide a reach of interest into areas of pools, riffles and runs or based on substrate type such as bedrock vs. sands and silts vs cobbles. At a larger scale, dividing streams up according to source of flow and then local geology and landuse, as done using stream classification, is another form of stratifying stream systems. This type of stratified sampling is usually done when carrying out general resource assessments rather than testing specific hypotheses.

First, we need to define the areal extent of the habitats and decide how many samples are required meet our monitoring requirements. We then allocate the sampling effort in proportion to the contribution of each area to the overall study area or catchment. The actual areas to be sampled within each stratum are then located randomly. However, if the variance of the variable is known within each stratum, then the optimum allocation of sampling effort is to allocate samples in relation to variance within strata (Snedecor and Cochran 1980):

$$n_i = \frac{N_i\, s_i}{\sum_{i=1}^{k} N_i\, s_i} \tag{6}$$

where n_i is the number of samples in stratum i, N_i is the total number of sampling units available in the ith stratum, s_i is the standard deviation of the ith stratum.

Stratified samples have an overall mean calculated as:

$$\overline{x} = \frac{\sum_{i=1}^{k} n_i\, \overline{x}_i}{\sum_{i=1}^{k} n_i} = \frac{n_i \overline{x}_i + n_2 \overline{x}_2 \dots + n_k \overline{x}_k}{n} \tag{7}$$

where $\overline{x}$ is the overall sample mean, n is the total number of samples and the other samples are as defined above.

The sample variance of the overall mean is calculated as follows:

$$s_{\overline{x}}^2 = \sum_{i=1}^{k} \left[\frac{N_i}{N}\right]^2 \left[\frac{s_i^{\,2}}{n_i^2}\right] \left[1 - \frac{n_i}{N_i}\right] \tag{8}$$

where s_i^2 is the sample variance for ith stratum for a random sample, N_i, n_i and N are the total number of sampling units available in the ith stratum, the number of sampling units

sampled in the *i*th stratum, and the total number of sampling units in all strata. The standard deviation of the overall mean is simply calculated as the square root of overall sample variance.

3.7 Estimating the number of replicate samples required and level of differences in testing effects: a cautionary note

There are several issues relating to application of the above equations for estimating sample size and required precision that require some cautions. First, the equations give only a rough approximation of the number of samples needed to achieve prescribed levels of precision. Periphyton have high spatial and temporal variability, so a preliminary estimate of the population variance at one point in time may not result in a very accurate estimate of number of samples in other locations or at other points in time. In particular, if there has been flood disturbance since a preliminary sample was obtained, then the degree of variability is likely to be quite different. In many situations, it may not be possible to collect preliminary samples or there may be doubt about the transferability of data from another site. In such situations, our recommendation is to collect at least 10 replicate samples which will normally give quite a high level of precision. Indeed, this has become our standard number of replicates per site because we are rarely afforded the luxury of preliminary sampling.

Second, as shown in the preceding section and Figure 3, it is possible to narrow down the error of estimates by taking more replicate samples. In doing this, it might then be possible to discriminate statistically significant, but very small (and possibly trivial) differences in periphyton communities among sites (e.g., control vs. possible impact sites). Very small differences if placed in the context of natural variability of the communities in both space and time may have little meaning for the ecosystem as a whole. Thus, we need to ensure that we give adequate thought to the fundamental question: "what differences are likely to be ecologically important?" There is further discussion on this by G.B. McBride in Appendix 1.

There are no set rules for determining what the most ecologically important differences in space or time might be for periphyton communities, which may then be used as a basis for assessing impacts or breaches of guidelines. It will vary with variables and habitats in relation to how sensitive a particular set of wider ecosystem processes might be to the variable in question.

For example, a 20% breach of the 200 mg/m² chlorophyll *a* guideline for the protection of trout and fishing values could be unacceptable during summer low flows because of the low capacity of the waters at high temperature to hold oxygen, periphyton respiration rates are high (respiration rates tends to double with every 10°C increase in temperature), and lower volumes of water to buffer the system from periphyton mediated diurnal fluctuations in dissolved oxygen. However, such a breach may be of little or no consequence in winter when water temperatures are much lower, periphyton respiration activity is lower, and river flows are higher (unless the area of concern is a prime spawning habitat as well as fish rearing location).

Thus, determining whether a difference in a particular variable is of much management consequence must be done with some common sense, consultation with river managers and other end-users, knowledge of the interdependence of different communities or levels of the food chain, and knowledge of the natural variability of communities.

4 Specific considerations in planning studies

4.1 Resource surveys

4.1.1 General

Resource surveys are probably the least complicated sampling programme. However, they still require good planning to generate useful data. The aim of a resource survey is to describe habitats, and associated communities, and are generally only carried out on a "once-off" basis, or very irregularly. They might be required in the following circumstances:

- classification of the enrichment status of different valley segments in a catchment (along with some water quality indicators);

- definition of the location, extent and timing of periphyton proliferations;

- identification of unique, rare or endangered populations or communities;

- a baseline to enable assessments of the effects of a planned developments (e.g., a dam or discharge of pollutants);

- to characterise habitats that might be important for specific values (e.g., conservation status, salmon spawning, aesthetics, etc.);

- testing or validating the biological significance of catchment/valley segment habitat classifications based on physical variables.

It is also possible, with care, to use resource survey data as a basis for predicting possible communities and biomass in similar habitats elsewhere in the region. A table of commonly found community types is given in Appendix 2, together with a description of the habitats in which they are normally found. Resources surveys could also be used to test these community/habitat classifications and then modify or add to them as necessary for a particular region.

When using data from such resource surveys that have been collected more than, say, a year previously it is important to consider whether the data are still valid. Factors to review might include:

- whether there have been significant changes in landuse upstream of the site since the original survey;

- how representative weather conditions were during the year of the survey (e.g., was it a drought year?);

- whether the extent of abstraction and/or baseflows has changed markedly since the original survey,

- whether the bed sediment composition or channel configuration has changed.

All these factors have an important bearing on transferability of data from the past to the present.

After setting your objectives, the following main steps are required for a once-off resource survey.

- Carry out a river environment classification of the catchment to define the main combinations of climate, sources of flow, geology, landuse and frequency of flood events (Snelder *et al.* 1998). If there are major differences in valley morphology, you may also need to carry out valley segment classifications.

- Select a sample of the most common habitat types where access is good and the data

are likely to be most relevant to management issues. If possible, make sure that there is at least one "reference" site. This will be a site with minimal human impacts, which will provide a reference against which to compare conditions elsewhere in the catchment (see Section 2.6).

- Try to sample at a time of year that is most relevant to the questions being asked and that represents the usual state of periphyton growth for the types of habitats being sampled. For example, a common objective might be to define when and where in a catchment proliferations might occur. In hill-country stream (where highest biomass often occurs in spring and autumn), surveys in spring and autumn would be appropriate. However, for surveys on lowland-fed streams, or low altitude parts of hill-country fed rivers, then it might be best to sample in mid to late summer.

- Regardless of season, always try to sample at least 4 weeks after last flood event that moved the coarse parts of the stream bed, so that communities have developed to a reasonably mature state which will reflect their local environment. If you are in a catchment that is prone to frequent spates or floods, then sample as long after the last flood as is sensibly possible.

- Select sampling and analytical methods. These should reflect the detail with which you need to characterise the communities. In many circumstances, a rapid assessment survey using the SHMAK protocol (Section 6.4.3) will be sufficient. However, if it is likely that the data might be used to assess values for a contentious development, then quantitative sampling with semi- or fully quantitative analyses will be needed.

- Carry out the survey. Attempt to complete the sampling in the shortest time possible so that there are no major changes in flow or weather conditions which may influence the periphyton while carrying out the survey.

- Analyse your samples in the laboratory (if a quantitative survey).

- Analyse and report your data. In particular plot up your results in relation to, say, habitat type or variations in some environmental gradient such as percentage of the catchment developed for agriculture. *Use your original objectives to focus the analysis on specific issues.*

- Store your data in a form that is safe and accessible to others. Historical data collections are becoming increasing valuable as benchmarks against which longer-term changes in the environment can be assessed. At present there is no national database for stream benthic communities such as exists for hydrological and freshwater fish data.

4.1.2   How many sites should we sample?

This is a perennial question that defies a simple recommendation. If we were to be completely objective, the number of sites should reflect the heterogeneity of the environment and the scale at which it was necessary to characterise habitats and communities. The more variable the environment the more sites we generally need. If possible, we recommend statistically based approaches to assess the number of sites, based on variability in data from preliminary surveys such as described in Section 3.3 for sample replication, but treating sites as sampling replicates for the statistical analysis. However, in many situations the resources don't permit such an approach and some reasoned pragmatism is called for. If you are in this situation use your habitat classifications to guide you. This process partitions much of the broadscale variance and allows you to focus on habitats of greatest relevance. A general rule is:

> *Aim for at least three sites in the main habitat types of interest so that within-habitat variability can be quantified.*

Formal habitat classification (Snelder *et al.* 1998) allows division of the environment and allocation of the location and number of sampling units to be carried out objectively/reproducibly. Basing sample sites and locations on habitat classifications generally results in fewer sites being required than if you were to sample a region randomly. Such sampling is known as "stratified random sampling" (see Section 3.6).

4.1.3   Where should sites be located: riffles, runs, pools or backwaters?

Once again, the answer to this question depends on the objectives of the survey and the habitats being considered. Runs are by far the dominant meso-scale habitat feature in most New Zealand streams and should be the first priority for sampling to gain the best representation of the general nature of the resource. This is the area where periphyton proliferations will usually develop because lower velocities allow higher biomass to accumulate under enriched conditions. In streams with moderate to low nutrient levels, riffles will usually have the highest biomass because of greater nutrient mass transfer (e.g., see Biggs and Hickey 1994, Biggs *et al.* 1998b). However, while biomass in riffles can be high, it usually does not exceed proliferation guidelines because higher velocities in this habitat slough accumulations from the bed. If a comprehensive study is required on a large river system, such as to assess the effects of future river diversion or damming, then stratify your sampling by mapping the extent of meso-habitats (as noted above and described in general terms in Section 3.6). Among other things, this will help identify unusual or rare communities and habitats. An example of such an approach is given in Biggs and Shand (1987).

4.1.4   How many replicate samples should be taken at each site?

For valid statistical comparisons of stream communities in different habitats, resource surveys must include replicate samples at each site. Each replicate is collected independently of the others, but must be taken in exactly the same way from each sampling point. The more replicates taken at a site, the more statisically robust the analysis will be (see Section 3). However, as noted earlier, there is a trade-off between increasing the accuracy of the survey and the extra cost of more sampling replicates. The level of discrimination is very much dependent on local variability or "patchiness": the more patchy, the less precision there will be for a given number of samples (e.g., see Section 3.3).

4.2   Impact assessments

4.2.1   General

The aim of periphyton biomonitoring for impact assessment is often to compare biomass and taxonomic composition of communities:

- that have been impacted by a disturbance plus at least one paired control site; *or*
- at a site before and after a disturbance; *or*
- at impacted and paired control sites, both before and after the disturbance.

Impact assessments are probably the most complicated sampling programmes because the effects of a particular pollutant need to be isolated from the effects of the local physical habitat. They require good planning to generate data that will be defensible. Unlike resource surveys, impact assessments do not usually enable a full characterisation of the aquatic habitats and associated communities. They normally focus on testing for a specific effect/impact. Com-

monly they might be used in the following situations:

- definition of the extent and cause of enrichment from a point source discharge such as an oxidation pond or a zone of possible diffuse source enrichment along a stream;

- monitoring for compliance of conditions on a consent;

- identifying possible impacts from an existing discharge where there is little historical information or if there has been a change in effluent quantity/quality;

- testing or validating conditions on a resource consent.

After setting your objectives, the following main steps are required for implementing an impact assessment.

- Carry out a reach-scale river environment classification in the vicinity of the impact point to help establish where control and impact sites might be located.

- Select at least two sites above (control sites) and two sites below (impact sites) the point/zone under investigation. Attempt to have all physical habitat variables the same among the sites (e.g., shading, depth, velocities, temperature, etc.) so that the periphyton results reflect only the effects of the impact being investigated.

- Attempt to carry out sampling at a time of year that is most relevant to the questions being asked and the usual state of periphyton growth for the types of habitats being sampled.

- Regardless of season, always try to have about >4 weeks of stable flow prior to sampling, and during the incubation period (if artificial substrate samplers are being used). This is to ensure that communities have developed to a reasonably mature state that will reflect their local environment and any effects of the potential problem being investigated. If you are in a catchment that is prone to frequent spates or floods, then sample as long after the last flood as is sensibly possible.

- Select sampling and analytical methods. These will need to reflect the detail with which you need to characterise the communities. In most circumstances, a quantitative approach will be required.

- Carry out the assessment. Attempt to complete the sampling in the shortest time possible so that there are no major changes in flow or weather conditions which may influence the periphyton during sampling.

- Analyse your samples.

- Analyse and report your data. In particular, plot up your results in relation to the disturbance being investigated. Commonly, histograms are used for such analyses followed by analysis of variance. *Use your original objectives to focus the analysis on specific issues.*

- Store your data in a form that is safe and accessible to others.

4.2.2   How many sites should be sampled?

Ideally there should be multiple impact and control sites. With two or more control sites we can better assess any longitudinal variation in periphyton along the stream between apparently similar sites which cannot be attributed to the disturbance. With two or more impact sites it might be possible to identify downstream recovery. We are then looking for differences between the impact and control sites which are greater than any which exist within each set of sites. An example of this is shown in Figure 5.

Figure 5: Chlorophyll *a* concentrations from 10 replicate samples across the bed at two sites above and three sites below a nutrient discharge. All sites were in runs. The data show a minor increase in periphyton chlorophyll *a* moving downstream before the discharge point (Sites 1 and 2). This increase continued downstream of the discharge (Sites 3–5). A nested analysis of variance showed that the chlorophyll difference was not statistically different among the upstream sites and among the downstream sites. However, the difference between upstream and downstream was highly significant.
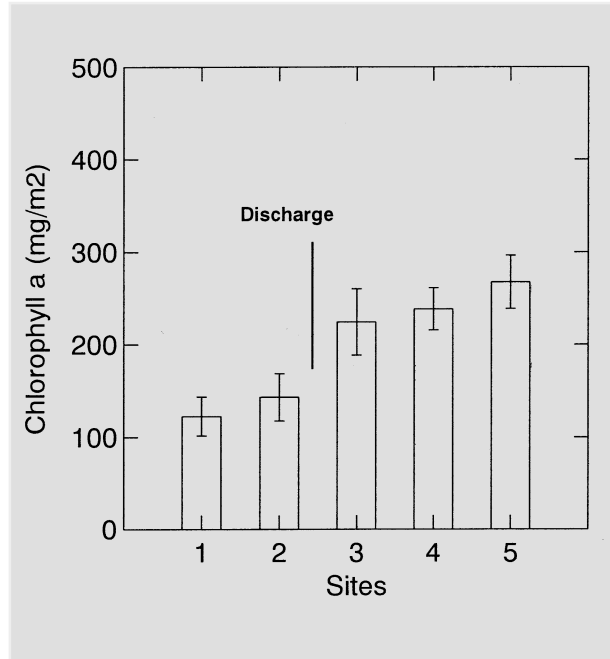
Again, it is important to ensure that all impact and control sites are matched in all conditions except those resulting from the disturbance. Where monitoring is requested in an area which has highly variable physical characteristics, then it may be difficult to isolate the effects of the human disturbance on the periphyton from other habitat influences. The number of replicate sites may also be limited by the size of the stream. A further limit may be set by the available budget, as mentioned previously. Single impact and control sites will provide some information, but the statistical comparison is not nearly as robust as that provided by multiple sites. In cases where there have been limitations on placement of sites, then be sure to make other data users aware of the problems.

4.2.3   Where should sites be located?

The locations of impact and control sites must be selected on a case-by-case basis. It may be possible to select general locations for sites from a 1:50,000 map of the area. The exact location is determined in the field after a reach-scale habitat assessment.

For impact assessments, choose sites in river runs wherever possible. It is understood that in some streams it may be difficult to find areas of runs large enough for sampling. As noted earlier, the most important consideration in site selection is to ensure that impact and control sites are similar in all respects *except* in location relative to the disturbance and in relation to habitat/water quality features being influenced by the disturbance. The main local habitat features to consider in site selection are:

- bed slope
- water velocity
- water depth
- bed sediment composition
- shade
- other disturbances (e.g., stock crossings).

If depths and velocities are highly variable and difficult to standardise, or if the bed is silty/sandy, then consider using artificial substrates (see Section 6.6). These enable a much more accurate isolation of effects of specific water quality disturbances.

4.2.4 How many replicate samples should be taken at each site?

As discussed in earlier sections (e.g., see Section 3.5), it is necessary to collect replicate samples at each site for valid statistical comparisons of stream communities at different locations on a stream or river to accurately determine the effects of some perturbation. Each replicate is collected independently of the others and must be taken in exactly the same way from a spot within the site which is similar to that for the other replicates. Replicates are not sub-samples from a single sample obtained from a site. The more replicates taken at a site, the more statistically precise the analysis will be and the smaller the differences that can be detected among sites. A guide to calculating the likely numbers of replicates for a given level of precision (which needs to reflect the degree of difference among sites that you think is ecologically important; see Section 3.7) is given in Section 3.5.

4.3 State of the Environment monitoring

State of the Environment (SOE) monitoring is a relatively new concept that was initiated in New Zealand following a Cabinet directive in April 1993 with the overall purpose of measuring and reporting how well we are looking after the environment (MfE 1997). The specific objectives are to:

- systematically measure the performance of environmental policies and legislation;
- better prioritise policy and improve decision making;
- systematically report on the State of New Zealand's environmental assets.

Section 35 of the Resource Management Act requires Regional Councils to monitor the effectiveness of policies at the regional level.

At present there are no frameworks or guidelines for setting up and reporting SOE monitoring. These will be needed to ensure that data will be usable for assessing the effects of policy, and making connections between the indicator data and environmental issues. The indicators used will need to be sensitive to the types of pressures expected to influence the environment and have a clear link back to mechanisms causing changes in these variables (e.g., percentage silt in the periphyton matrix in rivers used for gravel extraction). Any framework will also need to incorporate an environmental classification system for identifying reasonably homogeneous parts to the environment and to allow stratifying of monitoring effort. Such classification also allows the identification of "reference" sites, enables physically similar environments to be compared over time, and enables data and conclusions from individual sampling sites to be extrapolated objectively to larger areas or a whole region.

Percentage cover of the stream bed by different types of periphyton has been included as part of a proposed suite of environmental indicators for SOE monitoring of New Zealand's freshwaters (MfE 1997). This inclusion recognises the importance of periphyton as an indicator of environmental quality.

To date, regional and national SOE reports have generally just collated monitoring data. These data are either summarised as means for different sites/geographic areas (to define "snap-shots") of current conditions and/or are summarised as time series for indicators in an attempt to define trends over time (e.g., Waikato State of the Environment Report 1998). However, a few simple steps can be included in these reports that would greatly add to their

value and information content. In the following we suggest some general steps to be considered if using periphyton for SOE reporting. A number of these principles are common to those required for resource surveys and impact assessments.

- Set clear objectives that explicitly focus on measuring the success of Regional Policy Statements and Regional Plans so that changes that might ensue (to the most affected habitats) can be measured with the pre-selected level of precision.

- Carry out a habitat classification of the catchment to define the main combinations of climate, sources of flow, geology, landuse and frequency of flood events. If there are major differences in valley morphology, you may also need to carry out valley segment classifications.

- Select a sample of the most common habitat types where access is good and the data are likely to be most relevant to management issues. If possible, make sure there are at least three "reference" sites (i.e., where no human induced changes occur; see Section 2.6) for each of the main habitat types. Data from reference sites are essential if you want to effectively determine the long-term benefits of policies designed to improve stream health (such as planting riparian buffer strips in pastoral lands).

- Select indicator variables that are most relevant to the questions being asked and that will be sensitive to expected changes in environment. Also, ensure that there is a clear link back to mechanisms of change and management policies.

- Set a sampling frequency that targets your information needs while taking into account the natural temporal variability of the variable. For example, you may wish to know if a new policy of increasing the extent of riparian buffer strips in pastoral lands has resulted in a reduction in frequency of periphyton proliferations. Thus, we might nominate to carry out monthly surveys of percentage cover of filamentous algae and after 10 years of monitoring assess the trends in terms of the number of months per year when long filamentous algae exceeds 40% cover.

- Unlike resource assessment surveys, which can be carried out a certain times of the year to target specific conditions, SOE monitoring involving periphyton will generally need to be carried out on a routine basis with frequent sampling during the year to establish "average" conditions. See Section 3 for statistical methods for assessing number of replicate samples that may be required for testing differences in space or time at different levels of precision.

- Plot up your results according to broad habitat types to obtain an overall "snap-shot" characterisation. Then carry-out any statistical tests required to compare results from sites where you expect a change because of a particular policy decision with results from your reference site. Trends in indicator variables over time can also be plotted with test- and reference- site data overlayed on single graphs so that departures in trends over time can be easily detected. *Use your original objectives to focus the analysis on specific issues.*

- As for resource surveys, ensure that you store your data in a form that is safe, and accessible to others.

Discussion of other details applicable to SOE monitoring including number of sampling sites, where sites should be located, and how many replicates to collect is contained in Sections 4.1.2, 4.1.3, and 4.1.4. Discussion on sampling statistics is in Section 3.

4.4   Some practical considerations before you start your study

Before starting a monitoring programme, do some homework on the area and sites for which periphyton monitoring is proposed. This preliminary work should be done before designing the specifics of the programme. Consider the following.

- Examine maps of the area. Identify exactly where human disturbances might occur or are proposed. Identify other possible disturbances and select catchments in which control sites can be located.

- If possible, obtain a flow record for the stream in question, or for the nearest stream with a similar type of catchment in the area. This, combined with the GIS habitat classification information will give you a good idea of the type of stream that you are dealing with.

- Obtain any previous data that might be available (e.g., from previous monitoring programmes). Also, obtain information about any resource consents issued on the stream. This information might be a good lead on what communities to expect, how variable communities might be in space and/or time, the possible confounding effects of disturbances, etc.

- Visit the area and familiarise yourself with the different stream/river reaches before developing any specific monitoring plans.

5 Common New Zealand stream habitats and their periphyton communities

In this section we summarise the types of periphyton communities that might be found in different habitats around New Zealand. These habitats are defined in the first instance by differences in source of flow, because this dictates the general hydrological and sediment transport regime of streams and rivers. Sources of flow have an overriding influence on local habitat conditions and, through the associated disturbance regimes (which includes flood events), constrain the potential effects of the lower-order habitat variables of geology and landuse, which most commonly control the nutrient supply. These habitat features form the basis of the hierarchical river environment classification that is currently being developed for New Zealand (Snelder *et al.* 1998) and referred to earlier. General periphyton community types have been discussed in Biggs (2000a) and are further summarised in Appendix 2. The following sections discuss habitat characteristics of the main sources of flow of most New Zealand streams and rivers, and the periphyton communities most commonly found there.

5.1 Spring-fed streams

The overriding habitat characteristic of spring-fed streams is the high degree of flow stability, followed by a generally low bed gradient, fine bed sediments (usually silts, sands and fine gravels) and stable water temperatures. Thus, these habitats are often colonised by aquatic macrophytes. Indeed, gravel/cobble substrata for periphyton to adhere to may be rare. If the bed gradient and associated water velocities are sufficiently high, then areas of coarse gravels and cobbles may become more prevalent. Spring-fed streams are often quite enriched, depending on the geology of the catchment or whether the seepage waters are derived from gravels in an unconfined aquifer. Thus, a broad range of community types can be expected in such habitats. *Vaucheria* can often be found in cold, spring-fed streams forming very large mats over the silts and sands.

Of all the different stream types, spring-fed streams are most likely to have seasonal variations in biomass because spates don't occur regularly or often enough to "re-set" community development to early successional stages (Biggs 1996). For example, in spring-fed streams of Canterbury, the highest biomass (usually caused by blooms of *Ulothrix zonata* or *Oedogonium* depending on the degree of enrichment) occurs in late summer/autumn and late winter. Between these times, a moderate to low biomass of periphyton usually persists on the rocks. Intense invertebrate grazing during these periods probably keeps biomass from accruing. The reason for the two seasonal peaks in biomass of filamentous green algae is unclear. An intense period of sporulation may be triggered in these taxa by a change in light intensity or photoperiod. Water temperatures do not change much between winter and summer in spring-fed streams (except in downstream reaches, far from source) and always appear sufficient for high invertebrate activity.

5.2 Lake-fed streams

Lake-fed streams have some physical elements in common with spring-fed streams, in particular they generally have low flow variability. Both stream types usually have high densities of benthic invertebrate grazers. However, there is a great range in bed gradients and flows among lake-fed streams and, therefore, in water velocities and substrate particle size.

There is also a considerable range in the trophic state of lake outlet streams, reflecting the varying trophic states of the feeder lakes.

A wide range of periphyton communities is expected to dominate such lake-fed streams, however, few data have been collected in such habitats. Most sampling has been carried out in regulated lake-fed rivers in relation to hydroelectric power development, and it is unlikely that this information is totally applicable to unregulated lake-fed systems. Some lake-fed streams near their outlet are dominated by diatoms such as *Synedra*, *Gomphoneis* and *Diatoma*, whereas others are dominated by green algae such as *Ulothrix*, *Oedogonium* and *Bulbochaete* (S. Moore unpublished data; B.J.F. Biggs unpublished data).

5.3 Wetland-fed streams

Wetland-fed streams are distinctive in generally being low gradient with associated moderate or low water velocities and fine bed sediment particles. These streams are also often low in nutrients, and the waters often have a low pH and are dark through tannin staining. This represents a distinctive environment for periphyton. Much of the periphyton community is expected to be episammic (associated with sands) and epiphytic with abundant desmids and Zygnemataceae (*Spirogyra, Zygnema, Mougeotia*) and *Microspora* and filamentous diatoms such as *Eunotia, Fragilaria* and *Tabellaria*.

Few wetland-fed streams have been surveyed in New Zealand, so little definitive information exists to confirm these predictions. Considerably more basic research is needed on these habitat types, particularly given that such ecosystems have greatly diminished over the last 100 years, and are still under considerable threat. It is quite possible that they could harbour some unique communities and even new taxa. Several new diatom species have been discovered recently in brown water streams on the West Coast and on Stewart Island (R. L. Lowe and C. Kilroy, pers. comm.).

5.4 Low-altitude streams

With an average catchment elevation below about 400 metres, most of these streams are low gradient (with associated long reaches with low velocities and fine silty/sand bed sediments) located near the coast. Most of these streams are at least partially spring-fed and thus tend to have a low variability in flow (although some may be ephemeral) and tend to have prolonged periods of low flow in summer. They also tend to be very enriched, being at the most downhill end of farming activities. Few of these streams have natural, intact, riparian and/or catchment vegetation and we have no knowledge of what periphyton communities would naturally exist in such habitats. Some basic research is required on the communities inhabiting the remaining, natural lowland streams around New Zealand (mainly Fiordland, parts of Southland and the West Coast).

In the agriculturally developed areas such streams often don't have significant periphyton communities because of a lack of suitable substrate. Where there are extensive beds of macrophytes, filaments may grow as entangled masses over the larger plants. Where bed gradients are steeper and cobbles and gravels predominate, mesotrophic and eutrophic communities tend to occur, often forming a very high biomass. In particular, *Oedogonium* and *Microspora* can form very extensive cover in enriched South Island lowland streams, and *Cladophora glomerata* in North Island lowland streams.

5.5   Hill country-fed streams

This is by far the most common stream type in New Zealand and has the greatest range in physical conditions. In particular, most of these streams are flood prone with a frequency commonly being between 10 and 30 events per year. These streams are generally relatively steep so often have strongly developed reach-scale morphological features (e.g., pools, runs and riffles), the bed sediments are generally gravels and cobbles (except in mudstone catchments), and there is a very wide range of water velocities. They also encompass the full range of enrichment regimes expected for streams as a result of differences in land use and catchment geology. On stable substrates during summer low flows, the specific dominant community is usually determined by (in decreasing order of importance): shading, enrichment, water velocity and invertebrate grazing. Since most New Zealand streams and rivers do not have riparian shade except in their very headwater reaches, the most important controllers during low flows are usually enrichment, velocity and invertebrate grazing.

Diatom communities (e.g., *Fragilaria* and *Gomphoneis* groups) that rapidly colonise the stream bed most commonly dominate such streams over the year. However, if sediments are reasonably stable (armoured or imbricated) then a range of different communities will develop during low flows reflecting the local conditions of nutrient and light supply, and reach velocities. In oligotrophic streams draining bush or tussock catchments during such summer low flows, cyanobacterial communities dominated by *Schizothrix/Calothrix/Lyngbya* are often abundant as small black patches on the stones. *Coleodesmium* can be abundant in hill-country streams of Otago (S. Moore, Otago Regional Council pers. comm.). The communities appear to be very resistant to grazing by invertebrates. The cold water streams in the upper catchments of South Island hill country streams are often dominated by the green filamentous alga *Ulothrix zonata*. Communities dominated by the red alga *Audouinella* tend to be restricted to stream reaches with very stable boulder or bed rock substrata. Mats dominated by this taxon are often light purple in colour.

Where low–moderate enrichment occurs, the low-flow communities in swift flowing reaches and riffles of such New Zealand streams are most often dominated by thick mucilaginous
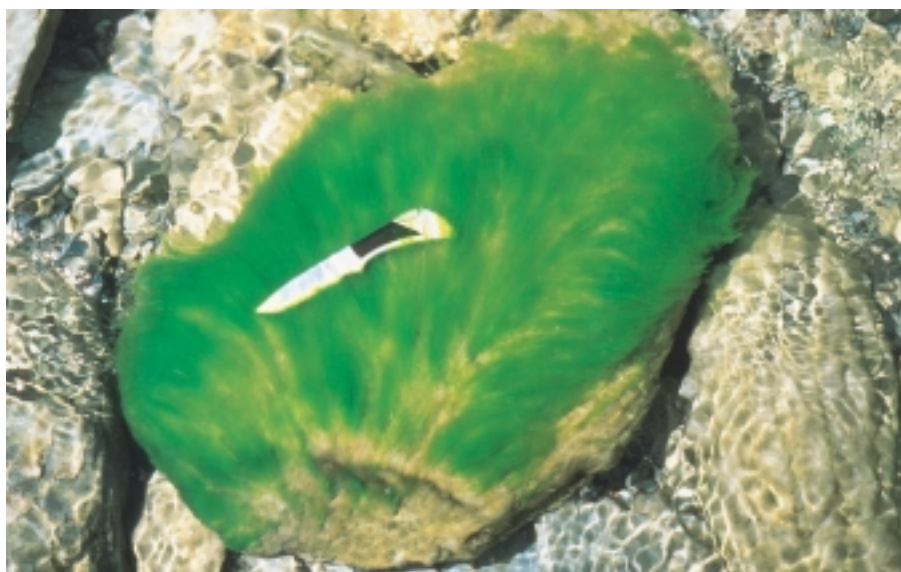
Figure 6:   Distinctive, bright green, tufts of the branched filamentous alga *Stigeoclonium lubricum* growing through an understorey of *Gomphoneis minuta* var. *cassieae* in a moderately enriched foothills-fed river.

mats of *Gomphoneis/Cymbella*. These can be overgrown by dark filamentous cyanobacterial (*Phormidium*) growths late in the summer (or late in the successional cycle if prolonged low flows occur at other times of the year). Large tufts of the branched filamentous alga *Stigeoclonium lubricum* often develop among the diatom community in moderately enriched streams and are a very conspicuous bright green colour (Figure 6). It is rare for a whole stream reach to be dominated by *Stigeoclonium*.

Similarly, *Spirogyra* is probably the most common filamentous alga throughout New Zealand. However, it is rarely the dominant community when a whole stream reach, or a transect across a stream, is surveyed. This taxon is often common among communities dominated by *Oedogonium* and diatoms, or as tufts of dark green filaments on stable stones in mesotrophic streams. *Spirogyra* can also form clouds of green filaments in pools during summer low flows.

With increasing enrichment, it is common to find extensive areas of hill country-fed gravel-bed streams dominated by *Oedogonium/Microspora/Zygnema*. These taxa can form very high biomass in the lower velocity runs of such streams (so long as there are adequate stable substrata).

5.6   Non-glacial mountain streams

Non-glacial mountain streams and rivers are usually characterised by harsh hydrological conditions of frequent flood events, steep, high-energy channels with generally unstable bed sediments, are rarely shaded and are most commonly unenriched. The windows of stable conditions that enable communities to proceed through to maturity are rare.

The overall species pool in the upper reaches of such rivers is probably limited by the frequency of flood disturbance events and thus even during infrequent periods of low flows the periphyton communities will usually be dominated by rapidly colonising diatoms (e.g., *Gomphoneis/Cymbella* communities and communities of *Fragilaria/Diatoma* and associated taxa) with *Ulothrix zonata* being the principal filamentous green alga (because this taxon is particularly disturbance resistant). Steep mountain headwater streams are often dominated by the filamentous diatom *Diatoma hiemale*. Grazing mayflies probably exert considerable "top-down" control on such communities because these invertebrates can be very common and the rate of periphyton production is usually quite slow because of low levels of nutrients. As a result of these joint processes, mean and peak biomass are usually relatively low.

5.7   Glacial mountain streams

These habitats are strongly dominated by high levels of sediment supply and associated bed sediment movement, and also have low levels of nutrient supply. Flood disturbance events are usually more seasonal than in non-glacial mountain and foothills-fed rivers, with the lowest flows usually occurring in late summer/early autumn when lower precipitation falls and then in winter when most precipitation falls as snow. Because of the high suspended and bedload sediment supply in these systems only minor increases in flow can mobilise sediments creating harsh abrasive conditions for benthic biota. These sediment supply processes are so prevalent that river communities may be strongly controlled by the glacial origin of flows even though glaciers may only constitute a very small proportion of the catchment. For example, at its mouth to the Pacific Ocean, only 2% of the catchment of the Rakaia River is of glacial origin, but the physical regime (flow variability and sediment movement) and benthic communities are strongly influenced by this source of flow.

It is important to recognise different valley segments because physical conditions change strongly down the river continuum in these glacial rivers. For example, conditions are very harsh in the single-thread, down cutting phase of these rivers, but where they braid in the less confined outwash plains, many sections of the braids usually have considerably less hydraulic power with very stable (often armoured) bed sediments. Periphyton are almost always very sparse in the main channels with the only cells being diatoms washed in from the more stable braids. However, extensive mats of diatoms (particularly *Gomphoneis/Cymbella* dominated) and mats of the green filamentous alga *Ulothrix zonata* can occur during periods of stable flow in the braids with stable sediment. Mats dominated by the red alga *Audouinella hermanii* can also occur in these stable braids.

In Canterbury, many braided reaches of such rivers receive groundwater inflows enriched with nitrate. These can result in locally high biomass (and sometimes proliferations) of taxa such as *Ulothrix* along the edges. High quantities of silt usually accumulate in such mats.

5.8   Streams regulated by dams

As already discussed in Biggs (2000a), rivers regulated by mainstream dams have unusual hydrological disturbance regimes which tend to encourage the development of high biomass communities of diatoms and filamentous green algae. These conditions include a reduction in bed sediment supply, which results in armouring, of the river bed (see Figure 19 of Biggs 2000a); a reduction in the frequency of small and medium sized flood disturbance events; and enrichment of waters from groundwater upwelling during periods of very low flows (Figure 7). The dominant periphyton cover during low flows in such systems is often filamentous and, depending on the degree of enrichment, can be either indicative of oligotrophic (e.g., *Ulothrix zonata*) or mesotrophic habitats (e.g., *Oedogonium*, *Phormidium*). Thick mucilaginous diatom slimes composed of *Gomphoneis*, *Cymbella* and *Synedra* populations occur in some South Island regulated rivers during summer low flows (Biggs and Hickey, 1994).

Figure 7:   Boulder bed, typical of a regulated river downstream of dams, with luxuriant growths of periphyton dominated by the mucilage forming taxa *Cymbella kappii*, with *Gomphoneis minuta* var. *cassiae* as an understory and overgrowth of patches of the green filamentous alga *Spirogyra*.

6 Field procedures

6.1 General

Floods, particularly those which cause the bed material to move, can scour away much of the periphyton from the stream bed. It may take much more than four weeks of stable flows for periphyton communities to regain the biomass and species diversity present prior to such floods (see Biggs 2000a). Therefore, to obtain a representative sample of periphyton, at least four weeks should elapse between floods that have caused major scouring of the coarse bed sediments and sample collection. Such floods can generally be taken as at least 5-6 times the average flow for the previous week (if the flow was constant). Note that for oligotrophic streams such a change may not re-set the periphyton communities significantly because they are generally lower in growth form and tightly attached. In these cases a scouring flood can be taken as one capable of moving at least the 84th percentile size fraction of the bed, which usually takes a much higher increase in flow. If you are in a catchment that is prone to frequent spates or floods, then sample as long after the last flood as is sensibly possible. Conversely, long periods of low flow may lead to the natural growth of large accumulations of algae, particularly filamentous types. However, after peak biomass for the accrual cycle has been reached, extensive natural sloughing often occurs. If sampling takes place while sloughing is in progress you are likely to get highly variable biomass amongst replicate samples.

In the following sections, we describe methods for a rapid, semi-quantitative, assessment of periphyton as a function of cover of the streambed by different groups of algae. For this method, all information is collected in the field. The rapid assessment method does not provide information on community composition, nor biomass. However, the thickness of the different types of mat is assessed. It is also possible with some experience to identify many of the macro-algal taxa in the field and express the results in terms of percentage cover of a reach by certain community dominants. Such methods are very useful for broadscale surveys, for example, to understand the effects of variations in landuse on low flow periphyton growth, or monitoring adherence with consent conditions for the prevention of proliferations. We then describe fully quantitative methods of sampling, where the samples then need to be returned to the laboratory for later analysis. These methods include natural and artificial substrate sampling. Natural substrate sampling is recommended where ever possible, but artificial substrates are valuable to isolate most clearly the effects of water quality/pollution from other habitat constraints which might complicate interpretation of results. Quantitative methods need to be used if attempting to provide clear and comprehensive information on the possible effects of any perturbation to water quality or physical habitat.

6.2 Location of sites

Always select sites that are physically representative (i.e., depths, velocities, bed sediments, shading, etc.) of the reach in the stream that you need to characterise or monitor. As noted in Section 4, this will most commonly be in a "run". These are places (or habitat units) where the water velocities are uniformly moderate–low (e.g., 0.2–0.4 m/s), the water depths are 0.2–0.6 m, and the bed sediments are uniformly similar in size and without significant variations in bed level. The water surface is also unbroken, or rarely covered by ripples.

For more details on requirements for study planning, site selection and determining the number of replicate samples required, refer to Sections 2, 3, and 4.

6.3   Site records

It is important to record all background information at a site. This might include:

- site name (the same as you put on sampling containers);
- map reference;
- vegetation on left bank; on right bank;
- degree of shade;
- water surface slope;
- catchment vegetation;
- water depth & velocity (measured at each sampling point on each sampling occasion);
- substrate composition (by visual assessment or Wolman particle size count (Wolman 1959)).

Also, take a photograph of the site on each monitoring occasion from a fixed location. A polarising filter improves the view through the water to the bed of the stream.

This record of the site is used both to confirm the exact location of the site and to establish the degree of similarity of conditions amongst sites, or at a site over time, during a monitoring programme. Site information is usually important in the interpretation of the results.

6.4   Rapid assessment protocols

6.4.1   Introduction

Many studies will require only rapid assessment of the cover of the stream bed by different general types of periphyton. We define two line transect methods below. The first is the simplest and focuses on measuring the cover of a site by filamentous algae using quadrats. This is specifically designed for assessing compliance with the periphyton guidelines for cover to protect aesthetic, recreational and fishing values (see Table 14; Biggs 2000a). The second method, while still a rapid assessment protocol, is more detailed focussing on the cover of a sample of individual rocks and enables the general evaluation of the degree of enrichment and water quality at a site. This method was developed for the Stream Health Monitoring and Assessment Kit (SHMAK) (Biggs *et al.* 1998c) and recognises 12 main types of periphyton based on colour and thickness. Repeated surveys at regular intervals over a year (e.g., monthly) using this method will provide a comprehensive picture of the temporal dynamics of communities and state of the environment. Necchi *et al.* (1995) compared line transect, point intercept and quadrat methods for rapid assessment of cover of macroscopic growth forms of periphyton and found little difference in overall results among the methods, but did show some differences in how depiction of spatial distributions. See Necchi *et al.* (1995) for more details.

Recordings from both rapid assessment methods can be supplemented with more detailed analyses of, say, biomass and taxonomic composition at certain times of the year. This might be during summer low flows when maximum biomass for the year has developed. The data that these methods generate are quantitative and thus can be analysed statistically. As mentioned above, an experienced person can also usually identify the predominant taxa forming the most conspicuous catagories of periphyton rather than just relying on the colour descriptors (a field microscope really helps such evaluations). There is some potential for inter-operator differences in assessments through different perceptions of colour, estimates of thickness and estimates of percent cover of the substrates. However, during testing of the methods, we have found that such variation is usually much smaller than differences in the community

over time or among sites covering a broad range of conditions. Nevertheless, there are advantages in standardising the person who carries out the assessment at a given site for routine surveys, or for multiple sites across a region where variations among sites/streams are to be assessed for a given time of the year.

Prior to using the following methods, ensure that your study objectives are clearly defined (preferably in writing) and that you have chosen a suitable site in the stream reach of interest. Refer to Sections 2, 3 and 4 for more details on design of sampling programmes sampling replication.

6.4.2 Rapid Assessment Method 1 (RAM-1): Quadrat method for percentage cover of a site by filamentous green/brown algae for assessing compliance with aesthetic/recreational guidelines for proliferations

This method involves setting up transects across a site and recording the percentage cover of filamentous algae > 3 cm in length for a given number of quadrat points. Percentage cover values for the individual points are then averaged to obtain an estimate of the average cover of the site by filamentous green/brown algae. These individual records can also be used to later construct a map of the distribution of filamentous algae and, if repeated sampling is performed, then changes in the distribution of mats or patches of these algae can be traced over time. Such analyses, if combined with some physical measurements (e.g., shading, water velocities, depths and/or substrate composition), can provide useful insights into the primary factors controlling the local development of proliferations.

Equipment:

1. 2 tape measures (50 m and 20 m long).

2. 4 pegs (> 20 cm long) and mallet.

3. Sampling quadrat consisting of an open steel/alloy ring or square 15 - 20 cm in diameter (size is not critical, but try to use the same size consistently for a given stream/site).

4. Glass or clear plastic bowl ~ 20 cm in diameter, with a flat bottom for looking into the water.

5. Field data sheet (preferably of waterproof paper).

Procedure:

•   Select a reference point at the downstream end of your site and on one bank drive a peg into the ground.

•   Attach the 50 m tape measure to the peg and lay it out taut for its full distance (or 5 x the stream width, whichever is the smaller). Attach the upstream end of the tape to a second peg.

•   Calculate 10 equally spaced intervals along the tape measure (e.g., 5-m intervals for a 50 m distance).

•   Attach the end of the 20 m tape measure to a third peg at the location of the reference peg and unwind the tape at right angles to the main tape. Anchor the far end with the fourth peg on the far bank.

•   Divide the width of the stream (water's edge to water's edge) into 10 equally spaced points.

•   Place the sampling quadrat on the stream bed centred on the first point.

•   Hold the glass bowl on the surface of the water to obtain a clear view of the stream bed

(this may not be necessary in shallow, clear streams).

- Estimate the percentage cover within the quadrat of filamentous green/brown algae which have filaments > 3 cm long. Record these on your field sheet then move across to the next point.

- Complete the transect then move the transect upstream for the pre-selected interval and repeat the recordings.

- When complete, enter the data onto a spreadsheet and calculate the mean percentage cover of the site by filamentous algae > 3 cm long.

Notes:

- If the cover of the site is clearly very homogeneous (e.g., broad mats just along the periphery of the channel) then it is acceptable to reduce the number of points across each transect from 10 to 5.

- If the stream is so narrow that the area of the quadrat from one sampling interval overlaps with adjacent points then it is also acceptable to reduce the number of points across each transect or to employ a small quadrat.

- If the stream is > 20 m in width or too deep to wade safely, then sub-divide your site in a stream-wise fashion. This involves setting the limits of your transects out into the river based on a fixed depth or distance from shore. Using depth contours is most preferred. You then need to express your results in terms of percentage cover of filamentous algae for, say, the < 0.6 m deep section of the reach.

- There may be some difficulty in determining what constitutes a green/brown filamentous algal community with filaments > 3 cm long. In such cases, familiarise yourself with the communities at the site before commencing the survey by picking up stones from the stream bed and examining them carefully just below the surface of the water (so that the filaments stream in the current), then lift them above the water for closer examination. Note that the mats can look quite different under the water vs. out of the water.

- Taxa which form such growths include: *Spirogyra, Oedogonium, Stigeoclonium, Microspora, Mougeotia, Cladophora, Rhizoclonium* and *Zygnema.*

6.4.3 Rapid Assessment Method 2 (RAM-2): Line transect – point method for percentage cover of substrates by different categories of periphyton for general resource surveys and assessing broadscale effects of perturbations

As with RAM-1, this method involves setting up transects across the site of interest and recording the percentage cover of algae at fixed points. However, much more information is obtained on the types of algae present and many fewer points are examined. Four transects are used with five points across each transect. The number of points and/or number of transects can be increased for greater levels of precision. This method does not allow an estimate to be obtained of the percentage cover of the site by different algal groups. However, the percentage cover values for various types of algae are estimated, then multiplied (i.e., weighted) by a pollution score to give a general assessment of water quality conditions. Such analyses, if combined with some physical measurements (e.g., shading, water velocities, depths and/or substrate composition), can provide useful insights into the primary factors controlling the local development of different periphyton communities and assessment of causes of stream habitat degradation.

Equipment:

1. 2 tape measures (10 m and 20 m long).
2. 4 pegs (> 20 cm long) and mallet.
3. Small tea strainer (approximately 8 cm in diameter).
4. Field data sheet (preferably of waterproof paper).
5. Periphyton field identification chart (Appendix 3).

Procedure:

- Select a reference point at the downstream end of your site and on one bank drive a peg into the ground.

- Attach the tape measure to the peg and lay it out taut for a distance of 10 metres (or 5 x the stream width, whichever is the smaller). Attach the upstream end of the tape to a second peg.

- Divide the distance along the tape into thirds and mark the tape (i.e., 3.3-m intervals for a 10 m site distance).

- Attach the 20-m tape measure to a third peg at the location of the reference peg and unwind the tape across the stream at right angles to the main tape. Anchor the tape on the far bank with the fourth peg.

- Divide the width of the stream (water's edge to water's edge) into 5 equally spaced points.

- Working from the downstream end of the site, move out to the first point across the first transect to be sampled (this will be near the water's edge on one side of the stream). Bend down to lightly touch the bed sediments without looking at what is there. Ideally, pick up the first stone that you touch. Because the stones need to be a reasonable size to provide useful information sometimes you will have to disregard the initial piece of sediment that you touch if it is very small (e.g., gravel or sand) and pick up a stone no more than 10 cm away which is bigger than about 4 cm across.

- If the stream bottom is gravel, sand or silt, take a scooped sample at the sampling point with the tea strainer.

- Examine each stone carefully and identify the categories of periphyton present according to their colour and thickness using the periphyton field identification chart in Appendix 3. Estimate the percentage cover of the stone in each category (+ 5%) and enter this on the field data sheet reproduced on the next page.

- Complete the transect then move the tape upstream for the second transect at one-third interval and repeat the recordings.

- When complete, calculate the mean percentage cover of sampling points for each category of periphyton. You can also use the table shown below the sampling form to calculate an overall score for the sampling.

Periphyton (on exposed surfaces)		Peri.	Transect 1					Transect 2				
		score	1	2	3	4	5	6	7	8	9	10
Thin mat/film:	green	7										
(under 0.5 mm thick)	light brown	10										
	black/dark brown	10										
Medium mat:	green	5										
(0.5-3 mm thick)	light brown	7										
	black/dark brown	9										
Thick mat:	green/ light brown	4										
(over 3 mm thick)	black/dark brown	7										
Filaments, short	green	5										
(under 2 cm long)	brown/reddish	5										
Filaments, long	green	1										
(over 2 cm long)	brown/reddish	4										
		Peri.	Transect 3					Transect 4				
		score	1	2	3	4	5	6	7	8	9	10
Thin mat/film:	green	7										
(under 0.5 mm thick)	light brown	10										
	black/dark brown	10										
Medium mat:	green	5										
(0.5-3 mm thick)	light brown	7										
	black/dark brown	9										
Thick mat:	green/ light brown	4										
(over 3 mm thick)	black/dark brown	7										
Filaments, short	green	5										
(under 2 cm long)	brown/reddish	5										
Filaments, long	green	1										
(over 2 cm long)	brown/reddish	4										

Stone number:

	Transect 1					Transect 2				
Stone/sample no.	1	2	3	4	5	6	7	8	9	10
(a) Enter total % of stone surface covered by all types of periphyton										
(b) Enter list of: percentage cover x score for that type of periphyton										
(c) Sum of all multiplied % scores										
(d) Average score per stone/ sample [(c) / (a)]										
(e) Total of all average scores in line (d)										
(f) Total average periphyton scores (d) /no. samples with periphyton (= overall avge.)										

Notes:

- In the present procedure we are recommending the use of 4 transects per site with 5 points/stones being examined per transect giving a total of 20 assessments/replicates per site. This is equivalent to the most detailed level of the SHMAK protocols (Level 2+) and will allow a good level of precision for activities such as State of the Environment reporting and detailed regional water quality assessments.

- Table 2 summarises the main taxa that typically dominate the different periphyton categories. Note that some taxa are found in several categories and it is not just their presence, but the thickness of the mat (i.e., degree of biomass development) which is important for the evaluation of water quality

- The indicator scores in Table 2 are preliminary at this stage and are derived from Biggs *et al.* (1998c) and unpublished data of the authors. These will be refined in future years with greater experience in their application in various habitats.

Table 2: Summary of periphyton categories for field assessment of community type in the SHMAK rapid assessment protocol. Also given are the enrichment indicator scores and taxa which could be expected to be dominating the biomass of the samples (*, diatom epiphytes give the green filaments a brown colouring).

Periphyton category		Indicator score	Typical taxa
Thin mat/film: (<0.5 mm thick)	Green	7	*Cymbella, Achnanthidium, Cocconeis, Ulothrix, Stigeoclonium* (basal cells), young *Spirogyra*
	Light brown	10	Assorted diatoms and cyanobacteria (e.g., *Cocconeis, Fragilaria, Synedra, Cymbella, Lyngbya, Amphithrix*
	Black/dark brown	10	Assorted cyanobacteria (*Schizothrix, Calothrix, Lyngbya*)
Medium mat: (0.5-3 mm thick)	Green	5	*Stigeoclonium, Bulbochaete, Chaetophora, Oedogonium, Spirogyra, Ulothrix*
	Light brown ±dark green/black bobbles	7	*Gomphonema, Gomphoneis, Synedra, Cymbella, Diatoma, Fragilaria, Navicula, Nostoc*
	Black/dark brown	9	*Tolypothrix, Schizothrix, Phormidium, Lyngbya, Rivularia*
Thick mat: (< 3mm thick)	Green/light bown	4	*Navicula, Gomphoneis, Synedra, Rhoicosphenia, Ulothrix, Oedogonium, Microspora, Spirogyra, Vaucheria*
	Black/dark brown	7	*Phormidium, Schizothrix, Audouinella, Batrachospermum, Nostoc*
Filaments, short (<2 cm long)	Green	5	*Ulothrix, Oedogonium, Microspora, Spirogyra, Cladophora*
	Brown/reddish	5	*Cladophora*, Oedogonium*, Rhoicosphenia, Navicula, Batrachospermum, Diatoma*
Filaments, long (>2 cm long)	Green	1	*Ulothrix, Oedogonium, Microspora, Zygnema, Spirogyra, Cladophora, Rhizoclonium*
	Brown/reddiish	4	*Melosira, Cladophora*, Rhizoclonium**

6.5   Quantitative protocols

6.5.1   Introduction

For many resource surveys and most impact assessments, sampling and analyses must be quantitative to enable testing for statistical significance of differences amongst sites and for diagnosis of impacts. There are two main parts to such sampling:

- locating the sampling points along a transect, and
- collecting the periphyton from the substrata.

Sampling points are located using transects as described for the Rapid Assessment Method 1 (Section 6.4.2) except that only one transect with 10 equally spaced points is used instead of 10 transects. Each point is defined by dropping an imaginary vertical line from the transect at a set interval; then picking up the nearest stone.

Transects have an important drawback for quantitative sampling in impact assessments. This is that it may not be possible to match physical characteristics of the sites at both control and impact sites. This may result in differences in periphyton community composition and biomass that are unrelated to the effects of the effect being monitored. In such situations it may be possible to just sample a sub-set of conditions that are common to each site (e.g., depths of 0.2–0.4 m, velocities of 0.3–0.5 m/s, only sample cobble sized substrates, etc).

When collecting the periphyton sample, the aim is to take samples in a consistent way from a known area. Different methods are used depending on whether the stream bed is:

- Gravel/cobble (2 methods, Method 1a, 1b),
- Bedrock/boulders (Method 2), or
- Sand/silt (Method 3).

The two most common methods for collection of periphyton samples from gravels and cobbles are whole stone sampling (Method 1a) and scraping and/or brushing a sample from a defined area on the top of the stones (Method 1b). With Method 1a, the samples provide representative data on the whole community inhabiting the stone, whether it is located on the tops or sides of the stone. There will be little difference in communities among such micro-habitats in fine cobble and gravel dominated habitats. However, high heterogeneity in biomass and taxonomic composition can occur over the surface of individual substrates where they are moderate sized cobbles and larger. This method is preferred for overall resource assessments and studies of instream ecological processes where it might be important to assess the total biomass and composition of the community across micro-habitats. Data generated from this method of sampling are usually expressed in terms of surface area of exposed sediments.

If you are only interested in the chlorophyll *a* concentration, then it is also possible to collect the rocks and return them with their communities intact to be directly extracted for chlorophyll in the laboratory.

With Method 1b, samples are only collected from the tops of the stones (i.e., the area of the substrata exposed to the direct flow of the water). This helps to remove the effects of spatial differences in water velocity, erosion of communities along the edge of the substrata, and effects of grazing invertebrates that usually spend most of their time under or along the edges of the substrata. Thus, this method is preferred for assessments of pollution effects, enrichment, etc. It is also the best method to use if large mats of long filamentous algae are expected because such mats can be "cored" with this method for an aerial estimate of biomass. Data generated from this method are expressed in terms of plane surface area of the stream

bed (which is, of course, considerably less than the total surface area of substrates exposed to the water).

With any quantitative method it is important to have an appreciation of the degree of error associated with your sampling. This reflects the natural heterogeneity of the periphyton, size of your sampling unit and the number of replicates. It is generally most easy to vary the number of replicates. Methods for calculating error are described in Section 3. In general we recommend at least 10 replicate samples per site, because periphyton are particularly hetero-geneous. However, more may be required in some situations.

Before describing the sampling methods in more detail, we will outline requirements for sampling containers and labelling.

6.5.2   Sample containers

Different kinds of sample collection containers will be needed for different projects according to the volume of the samples being collected. For many purposes, Elkay-type containers are ideal. These are stackable flexible polyethylene specimen cups with screw lids and a volume of about 125 ml. Smaller (60 ml) plastic containers are also suitable for small samples.

For whole stone sampling, strong plastic bags (especially the snap-lock type) make the most convenient collection containers. Alternatively, 500 ml plastic bottles are also suitable.

6.5.3   Sample labelling

Prepare suitable containers for samples prior to field collection. Ensure that all containers are labelled with the following information:

• Date of sample collection.

• Name of collector (with organisation name if applicable).

• Job number (or job name).

• Site name or number.

• Sample number.

• Analyses to be done.

An example of label follows:

```
Date:          16 Feb 2000
Collected by:  A. Helper (ORC)
Job:           Kakanui monitoring
Site:          A
Sample:            1
Analyses:      Chla, DM, AFDM, Taxon.
```

Use pre-prepared paper labels, or write directly on the containers using a permanent marker pen. Remember that permanent marker ink is soluble in ethanol and do not label the lid (these can get mixed among samples very easily).

Correct labelling of sample collection containers at the time of sampling is crucial for the validity of the entire sampling programme.

6.5.4   Quantitative method 1a (QM-1a): Whole cobble/gravel sampling

Equipment:

1. 20–30 m tape measure.

2. 2 pegs (> 20 cm long) and mallet.

3. Deep sided laboratory tray or container (e.g., an ice-cream container).

4. A range of scrubbing brushes (from toothbrush size to nail brush – the size depends on the size of stone).

5. Scalpel.

6. Squirt bottle filled with stream water.

7. Labelled sample containers (500 ml bottles or large zip-lock plastic bags).

8. Plastic calipers.

Procedure:

• Select a reference point in the middle of your site 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. 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 to lightly touch the bed sediments without looking at what is there. Ideally, pick up 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.

• 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.

• Store the labelled container on-ice in a chilli-bin (cooler) for transport to the laboratory.

• Measure the x, y and z dimensions of the stone with the plastic calipers.

• Proceed to the next sampling point and repeat the above procedures.

6.5.5 Quantitative method 1b (QM-1b): Scraping or brushing a sample from a defined area on the top of a stone

Equipment:

1. 20–30 m tape measure.

2. 2 pegs (> 20 cm long) and mallet.

3. Deep sided laboratory tray or container (e.g., an ice-cream container).

4. Scalpel.

5. Small scrubbing brushes.

6. Labelled containers (e.g., 120 ml specimen cups; 60 ml rigid clear plastic pottles).

7. Pipettes (small "eye-dropper" is sufficient).

8. Squirt bottle containing stream water.

9. A ring of an appropriate size which can be used to define a sampling circle on each stone (e.g., the top of the sample collection container can be used).

Procedure:

• Select a reference point in the middle of your site 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. 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 to lightly touch the bed sediments without looking at what is there. Ideally, pick up 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.

• Place the ring on top of the stone to define a circle in the centre, or as near as possible to the centre, of the stone.

• Scratch a mark on 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 then 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 then remove the slurry from within the circle using the small pipette (Figure 8).

Figure 8: Sampling periphyton from a defined area on the surface of a stone. *Left:* scraping the diatom film from within circumscribed area to show the difference in colour of the sampled area within the circle and the diatom film (additional scrubbing with a coarse toothbrush will complete the sampling). *Right:* a defined circle of periphyton mat which will be scraped/brushed off into a container as the sample.

- Rinse the area with a minimal amount of water. Remove any surplus water using a 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).

- Store the labelled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.

- 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.

6.5.6   Quantitative method 2 (QM2): Underwater bedrock/boulder sampling

It will be possible to use QM1a,b for most sites in New Zealand streams. However, sites with bed sediments of predominantly large cobbles, boulders or bedrock that are too large to retrieve for sampling (e.g., Figure 7), the samples must be collected in-situ with an underwater sampler. This sampler is made up from 2 x 60 ml wide-bore syringes with one of the syringes having a soft, thick rubber flange on the bottom to seal against the rock. The head of a flat toothbrush is attached to the plunger and this is used to brush the periphyton off the rock within an area defined by the base of the syringe (Figure 9). A second syringe is used to suck the slurry containing the abraded periphyton from within the base of the first syringe. This slurry is retained as the periphyton sample. The method was developed by Loeb (1981).

Figure 9:   Double syringe periphyton sampler. *Top:* the apparatus, showing the components, and an assembled set. *Bottom:* the device in position in a stream ready for use.

Equipment:

1. 20–30 m tape measure.

2. 2 pegs (> 20 cm long) and mallet.

3. Double-syringe underwater sampler.

4. Squirt bottle filled with stream water.

5. Labelled containers (e.g., 120 ml specimen cups; 60 ml rigid clear plastic pottles).

Procedure:

*   Select a reference point in the middle of your site 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. 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). Press the sampler firmly against the boulder/bed rock at this selected point, ensuring that there is a good seal around the base. The horizontal syringe should be inserted in the base of the main syringe as illustrated in Figure 9.

*   Push the plunger down in the first syringe so that the brush is in contact with the substrate. Turn the plunger three full rotations to the right, then three full rotations to the left

*   Draw some water into the vertical syringe by raising the plunger about 1 cm. Then draw this water/periphyton mixture into the horizontal syringe (this takes some dexterity, but is reasonably easy with practice).

*   Carefully remove the apparatus from the water and discharge the sample into its appropriate container. Rinse off the brush into the container.

*   Store the labeled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.

*   The recommended number of rotations of the plunger may be insufficient to remove tightly attached periphyton. If possible test the method in a shallow area, or on a stone that can be retrieved at your site, so that you can check periphyton removal efficiency before using it for proper sample collection. If necessary increase the number of rotations, but then ensure that you use this number consistently for all samples.

    Note: Cattaneo and Roberge (1991) have tested the efficiency of this sampler for removal of periphyton in streams and lakes. They found that it depends largely on the relative proportions of loosely and tightly attached community. In stream, the syringe sampler tended to underestimate biomass, and the severity of this underestimation increased with water velocity under which the communities had grown. Thus, if it is necessary to use this sampler in streams, we recommend that for consistency within a study, it is used to collect all samples in that study.

6.5.7 Quantitative method 3 (QM-3): Sand/silt sampling

Equipment:

1. 20–30 m tape measure.

2. 2 pegs (> 20 cm long) and mallet.

3. Deep sided laboratory tray or container (e.g., an ice-cream container).

4. Labelled containers (e.g., 120 ml Elkays; 60 ml rigid clear plastic pottles).

5. Pipettes (small "eye-dropper" is sufficient).

6. Squirt bottle containing stream water.

7. Top half of a 47 mm diameter plastic petri dish.

8. Large stainless-steel spatula.

Procedure:

• Select a reference point in the middle of your site 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. 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 gently press the petri dish lid into the top layer of sand/silt (to a depth of 5–7 mm).

• Slide the spatula blade under the petri dish to isolate the sediment in the dish.

• Gently bring to the surface, ensuring that as little material/water is lost from inside the petri dish as possible.

• Empty the petri dish into a tray/container, rinsing any residue from the petri dish, and then transfer into a labelled container.

• Finally rinse the tray into the sample container until no trace of periphyton remains.

• Store the labelled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.

6.6 Methods for sampling periphyton on artificial substrates

Artificial substrate sampling is used to measure net biomass production rates where the precise effects of water quality need to be assessed but where the natural substrates cannot be sampled. For example, the water may be too deep or the bed sediments may differ greatly among sites. Artificial substrate sampling is a type of in-stream "bioassay" of water quality conditions. This is because such substrata allow sampling surfaces and other habitat factors such as water velocities to be standardised, thereby allowing a more precise focus on differences in water chemistry. Factoring out confounding effects of habitat can be particularly important when evaluations cover broad geographic areas. Artificial substrata also allow easier and more reproducible sampling because they reduce heterogeneity in microhabitats. Artificial substrata are widely used for periphyton sampling in water quality surveillance, particularly in North America. However, artificial substrata sampling is not recommended if the study objective is to characterise local community composition, the effects of natural physical perturbations, the interaction between physical and chemical perturbations, or the effects of water quality perturbations on natural periphyton communities.

Various materials, sampler frames and sampling surface configurations have been used for periphyton artificial substrate samplers. Sampling surface materials commonly include etched acrylic and glass, but have also included slate, brick, glass microscope cover slips, clay tiles, filter papers, acrylic rods, and wooden dowels (Aloi 1990). The sampler frames also vary greatly, from bed-mounted samplers to fully floating devices. The samplers' frames should

reflect the type of environment to be monitored. For example, frames holding racks of glass microscope slides that float just below the water surface and that are tethered to the bed (like the Wildco periphyton sampler – see below) are commonly used in deep, sluggish rivers. However, a much more robust sampler comprising a rack of acrylic slides mounted on their edges in a steel caged that prevents debris damage (Figure 10), has been recommended for use in swift and shallow streams and rivers (Biggs 1988a).

While there are some advantages in using artificial substrate samplers, there are also several disadvantages. These have been summarised by Porter *et al.* (1993) as follows.

- Artificial sustrata require at least two visits to each sampling site (installation and retrieval), separated by a colonization period.

- The samplers can be susceptible to vandalism or washout during floods.

- The taxa found growing on the samplers can be biased toward those actively immigrating at the time of and in the location of the incubation.

- Artificial substrates may provide a less sensitive indicator than natural substrate communities of changes or differences in water quality associated with land disturbance, and changes in more general habitat conditions.

Also, biomass on artificial substrata is generally less than on natural rocks (Biggs 1988a). In relation to the taxonomic composition of communities, natural diatom assemblages are usually well simulated by those on artificial substrates, whereas green algae and cyanobacteria at high biomass can often be misrepresented (Cattaneo and Amireault 1992). Thus, data collected using artificial substrata should generally not be compared with data from natural substrata in assessments of possible effects of pollution. If a study is being carried out over a broad area (within a catchment or among streams) and artificial substrates are necessary at some times, then we recommend that such substrata be used at all sites so that valid comparisons of water quality can be made among the sites.

Changes in community composition can also occur during incubation. Indeed, duration of substratum exposure is critical to data interpretation and should be standardised among all sites within any one survey (Biggs 1988b). Rate of colonisation and succession varies as a function of time since the last major flood disturbance in the catchment (which affects density of available immigrants); degree of enrichment, water temperature and light (which affects growth rates), and local water velocities and invertebrate grazing (which affects delivery and losses of immigrants) (Biggs 1996). Suitable incubation periods can be from 1 to 2 weeks for maximum diatom diversity (Cattaneo *et al.* 1975) and for comparative studies of enrichment effects on growth rates across a gradient of nutrient concentrations, to 4–6 weeks for maximum biomass in mesotrophic and eutrophic habitats (Biggs 1988b). It is therefore important to be clear about your monitoring objectives and understand that artificial substrate sampling enables measures of relative differences; rarely do they enable assessments of absolute effects on natural communities.

Although using artificial substrates has advantages in relation to isolating water quality effects, there is a risk that data loss will occur through:

- vandalism;

- flooding;

- flotsam;

- stock crossing streams;

- recreational four-wheel-drive vehicles.

Careful sampler placement can help minimise such effects. For example, it is often possible to position samplers out of view (e.g., behind a willow tree) and away from "traditional" stock or vehicle crossings (preferably upstream!). Even taking such precautions, Biggs (1988a) reported a loss of 17% of samples due to vandalism over a 12 month study of nine rivers in Canterbury. Loss of data through vandalism can be reduced through replication of samplers at sites. Loss of samplers through flooding is always difficult to mitigate if sampling in foot-hills or mountain-fed streams or rivers. For example, Biggs (1988a) lost 19% of data due to flooding in the study of Canterbury rivers noted above. Conversely, flotsam from detached macrophytes is a problem in many low-gradient and spring-fed streams, particularly if a floating sampler is to be used.

The following are some general points to considered when designing an assessment programme using artificial substrate samplers.

- Local habitat conditions should be standardised among all samplers.

- The sampling surfaces should be moderately textured/etched to allow good colonisation and adhesion of the mat.

- The materials used for the sampling surfaces and holding frame should be inert so that no materials leach from the substrata which may influence community composition.

Standardising local habitat conditions among sampling sites can be difficult, but is essential to avoid erroneous conclusions about variations in water quality. For example, the data in Table 3 were obtained from three samplers exposed to habitat conditions that were as similar as could be obtained at a single site. There were no water quality differences between samplers, but significant differences were evident in the quantities of periphyton among the samplers. Had these samplers been placed individually at three different sites, as might have been done for a surveillance programme, erroneous 5-fold differences in productivity (and thus degrees of enrichment) might have been deduced. The sampling method is thus very sensitive to even minor differences in non-water quality habitat variables between samplers.

Table 3: Periphyton biomass on three artificial substrate samplers incubated at a single site in the Halswell River, near Christchurch. Eight replicates were collected per sampler and all samplers had biomass significantly different from each other ($P < 0.05$) (from Biggs 1985).

Sampler	Depth of sampler (m)	Velocity over sampler (m/s)	Mean biomass (mg chlorophyll *a*/m^2 + 95% C.I)
1	0.78	0.253	5.3 + 0.83
2	0.90	0.233	2.1 + 1.55
3	0.80	0.200	10.2 + 2.79

The type of frame used to mount the sampling surfaces is relatively unimportant provided that there is a good supply of light and water is free to flow across the surfaces without a high degree of turbulence or high velocities. If the samplers described below are considered inappropriate for your particular task, then three main factors should be evaluated when considering alternative designs (Biggs 1988a).

- The design must be appropriate to the physical conditions of the river being monitored.

- The sampler must hold substrates that will harbour a community as similar as possible to that normally found in the river so that major biases will not occur.

• The sampler should give reproducible results.

In the following sections we describe two types of artificial substrate samplers and one set of protocols for their deployment.

6.6.1   Sampler designs

Bed-mounted artificial substrate sampler

The bed-mounted sampler (Figure 10) has a oval acrylic (perspex) or marine-ply base-plate, fixed to a large concrete paving block (~ 10 kg) by masonry bolts which have been inserted into the block. This bolting system enables the sampler to be removed for sampling without disturbing the anchoring system. The block is fixed to the riverbed by wire and a stake which is driven in flush with the sediment to avoid catching debris.

A stainless steel wire cage is fitted to the front of the sampler base-plate to deflect debris. Two blocks of wooden or (preferably) acrylic slats are fixed to the base-plate under the cage. These slats hold vertical acrylic slide sampling plates, with the plates being held in place by wire rods which extend through a line of holes in the slats and base of the sampling plates. The leading edge of the plates is angled to avoid, or reduce, the collection of debris being washed downstream. The plates are first roughened by lightly sanding them with coarse sandpaper. The sampler can hold up to 28 plates, depending on the number of slats used.
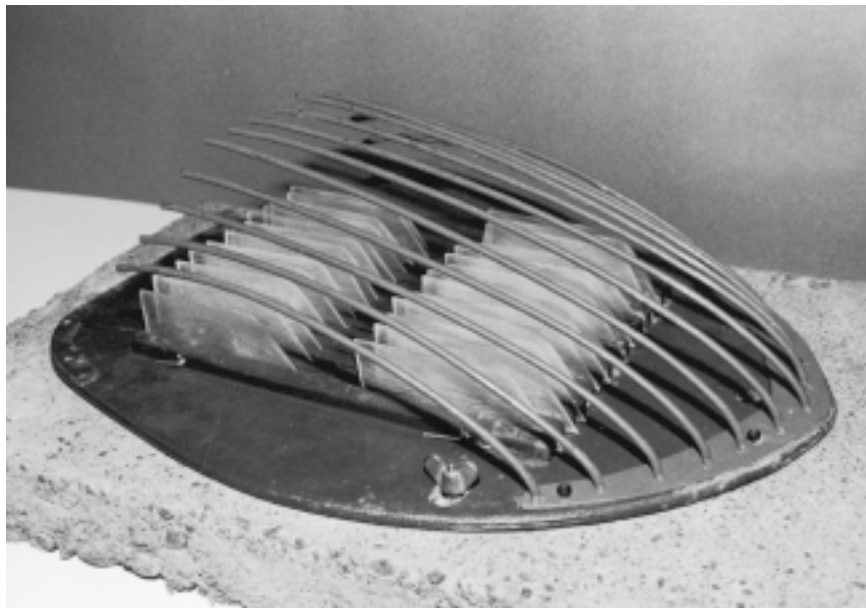
Figure 10: An artificial substrate sampler comprising a stainless-steel wire cage and edge mounted acrylic sampling plates. The sampler is attached to a large paving block to hold it in place on the stream bed (via bolts glued into the block and wing-nuts). The block can also be secured to the bed using long tent pegs and wire attached to the bolts on the block. The wing nuts allow easy removal of the sampler for periodic sampling and cleaning.

Floating sampler

Floating samplers have limited use in New Zealand because of our generally shallow, swift rivers and in deeper, low gradient rivers they tend to become clogged with macrophytes. There are a range of designs for floating samplers. These samplers can be purchased in North America (e.g., Wildlife Supply, Saginaw, Michigan – www.wildco.com/flowing.html#periph), but it might be cheaper to have them made (see diagram on p. 10-31 in APHA 1995). If you do

decide to construct your own, then the following points should be considered.

- The sampler frame should be constructed from perspex to avoid shadows on the sampling surfaces.
- Metal components should be corrosion-resistant (e.g., stainless steel).
- Ensure that there is a debris deflection guard.
- Position the floats away from the sampling surfaces.
- Rack the sampling surfaces vertically to minimise silt deposition.
- Use roughened acrylic or roughened glass microscope slides as sampling surfaces.

6.6.2 Sampler deployment

As noted in Sections 2.5.2, 4.2.3 and 6.6, placement of samplers is critical for:

- obtaining representative results;
- minimising variations among sites due to differences in physical habitat so that water quality effects can be isolated.

The second point is critical for valid comparisons of water quality among sites or habitats. As shown in Table 3, even minor differences in water velocity can result in highly significant differences in a number of periphyton metrics which are unrelated to water quality. Critical aspects of the physical habitat to standardise among sites are:

- degree of shading;
- water velocity;
- depth of sampling surfaces;
- proximity to the thalweg;
- influence of secondary currents.

It is usually necessary to carry out a preliminary physical survey of all sites where samplers are to be deployed before selecting exact positions for the samplers. Such a survey will help identify the constraints that might limit possible locations for deployment across the full range of sites. For example, water velocities might only cover a range of 0.1–0.3 m/s at one site, but range from 0.1 to 0.8 m/s at all other sites. In such a situation, you would need to standardise the placement of samplers at all sites at a velocity within the range of the first site (i.e., 0.1–0.3 m/s). Standardising for degree of riparian shade is usually quite difficult when monitoring in smaller streams.

When deploying bed mounted samplers, it is also important to ensure that the sampler is oriented so that the sampling surfaces are exactly parallel to the water flow. Otherwise, turbulence will be created around the slides which will cause a high degree of patchiness in the communities and siltation on the downstream side of the slides.

While it is very important to standardise for water velocity among samplers, depth is also an important factor. Variations in depth might influence growth particularly in more turbid streams and rivers where light attenuation is rapid. Therefore, where possible, attempt to also standardise depth among samplers.

6.6.3   Sampler retrieval

Samplers should be checked regularly to:

- clear any debris;
- check growth so that sloughing doesn't commence before retrieval. If growth at any one site appears about to slough from the substrates (i.e., it is loosely attached with small pieces

starting to break off), then the substrates should be removed at all sites.

It is important that incubation time be standardised among sites for any given sampling run so that differences in productivity and successional state can be adequately compared. Large variations, particularly in taxonomic composition, can occur with different rates of growth and leaving samplers at some sites to incubate longer than other sites for a particular sampling run will usually complicate data interpretation.

When retrieving the samplers:

- Approach them from downstream (i.e., move upstream to the sampler). It is often good to re-measure the depth and velocity of water immediately in front of the sampler to determine whether conditions have changed markedly since deployment.

- Detach the sampler from its "holdfast" (concrete block or attachment line).

- Gently lift the sampler from the water moving the sampler downstream with the current as you draw it through the water.

- When the sampler starts to clear the water, take a note of anything that might influence the periphyton communities and thus effect interpretation of the results. Write these down once on the river bank. Things to observe might include the amount of silt accumulated on the slides or substrates, any debris such as leaves, twigs or macrophyte stems that have caught on the sampler and might have brushed against the sampling surfaces or influenced local flow paths, numbers and types of invertebrates. A quick count of animals to the lowest taxonomic level that you can clearly recognise is often very useful in data interpretation and might provide another useful assessment of water quality (see the SHMAK rapid bioassessment protocol for a general grouping of invertebrates based on their pollution tolerances and ease of identification: Biggs *et al.* 1998c).

- On the river bank, carefully remove the sampling surfaces (e.g., perspex slides) and place them in individual labelled containers of stream water.

- Chill the samples to ~ 5°C, then return to the laboratory for analysis. Make sure that you keep the samples chilled and in the dark.

  *Notes:*The number of replicate substrates that you retrieve will depend on study objectives and the degree of precision. If you are assessing rates of production and need to construct a growth rate curve, then you may only retrieve three replicates at, say, 3-day intervals over a 2-week period (you will need to determine that you have started the incubation with enough replicate sampling surfaces to allow this). However, if you are assaying the effects of a perturbation on community composition (e.g., a discharge from a treatment plant), then one set of, say, five replicates might be sufficient. See Section 3 for statistical methods for determining sample replication.

6.7 Nutrient limitation assays

It is important to determine what nutrient might be limiting production of periphyton in streams, and the degree of limitation. Traditionally, this has been inferred from nitrogen and phosphorus concentrations and/or nutrient ratios in the water. More recently, nutrient limitation has been assessed by cellular nutrient quotas. However, these approaches have their drawbacks (Biggs 2000b), and the least ambiguous approach for establishing the nutrient limiting growth is the instream nutrient assay (Francoeur *et al.* 1999a). The simplest of these assays is the nutrient-diffusing substrate. These are artificial substrates of a porous material

placed over a container of agar impregnated with nutrients. The nutrients slowly diffuses out to the substrate providing a localised nutrient source. Periphyton grows on the substrate. After a defined time, the substrates are processed for chlorophyll *a* to compare periphyton growth in response to the different nutrient treatments. The treatment with the highest chlorophyll *a* indicates the most limiting nutrient for periphyton growth. Such assays have been used to determine the degree of enrichment caused by different landuses (e.g., Chessman *et al.* 1992, Corkum 1996), the effects of forest harvesting (e.g., Lowe *et al.* 1986), the effects of enrichment from point source discharges (e.g., Dube *et al.* 1997, Scrimgeour and Chambers 1997), and trophic interactions between nutrients, periphyton and invertebrates (e.g., Winterbourn 1990, Biggs and Lowe 1994).

Several types of nutrient-diffusing substrate have been used in periphyton monitoring and research. One type designed for use in New Zealand gravel-bed rivers comprises a large galvanised steel tray which holds plastic jars containing the nutrient enriched agar (Biggs and Lowe, 1994) (Figure 11). This steel-tray sampler is robust to floods, quick to set up and deploy, and has samples that are easily retrieved and analysed. Two incubations of approximately 2 weeks duration each can be obtained from each set of agar jars. Its disadvantages are that only chlorophyll *a* can be assessed from the substrates, large amounts of agar are required, the trays are difficult to fabricate by non-specialist engineers (they can be purchased or hired from NIWA, Christchurch), and the filter paper sampling surfaces sometimes rip. This design of substrata has been used extensively in New Zealand for both biomonitoring and research purposes (e.g., Francoeur *et al.* 1999a).

Other types of samplers are made from unglazed flower pots, through which the nutrients leak (e.g., Fairchild *et al.* 1985, Scrimgeour and Chambers 1997) and petri dishes containing the nutrient agar mix with a coating of sand set into the agar (or Nitex netting over the top) as collection surfaces (e.g., Pringle and Bowers 1984, Winterbourn 1990, Corkum 1996). This latter method uses much less agar and nutrients, but is also much less robust for use in flood prone and swift streams (e.g., Scrimgeour and Chambers 1997). Tate (1990) modified the flower pot and petri dish methods by using an inverted clay flowerpot saucer. This is more robust, does not protrude significantly into the flowing water where it might be damaged, and uses a substrate that is more analogous to natural hard rock surfaces. It also has the advantage over the steel-tray method that the samples can be analysed for a full suite of variables and not just chlorophyll *a.* Both the steel-tray and clay flowerpot saucer methods are described below.

Two problems occur with nutrient-diffusing substrate assays which may confound the interpretation of results. First, nutrients are released from the reservoirs at a negative exponential rate (Corkum 1996, Scrimgeour and Chambers 1997). This means that the supply concentration of nutrients that seeps from the agar and through the porous substrata reduces rapidly over time. Therefore, the volume of the agar reservoir is an important influence on how long samplers can be left out to incubate for. Corkum (1996) found that nutrients were depleted from 80-ml agar reservoirs in 6 days, with phosphate depleting at a much faster rate than nitrate. Similarly, Scrimgeour and Chambers (1997) found that nutrient release declined to relatively low levels after 32 days incubation of 325 ml nutrient-agar reservoirs.

Second, grazing by benthic invertebrates can have a major effect, and this may vary among sites. Such grazing influences are particularly acute in lowland or spring-fed streams where densities of snails may be high. The snails tend to gather on the patches of higher periphyton production and remove large amounts of the newly produced biomass. In such cases, the nutrient enhancement may not result in a significant difference in chlorophyll *a* compared

with controls, but an increase in consumer biomass may occur (e.g., Biggs and Lowe 1994). It is possible to reduce this effect by placing the substrates in relatively high velocities (e.g., > 0.5 m/s) where it is too harsh for snails to migrate onto the periphyton colonisation surfaces. Biggs and Lowe (1994) placed some of their substrates in riffles to dissuade snail colonisation. Another approach that has been used to control grazing is the use of the insecticide malathion in the agar which leaches out with the nutrients (e.g., Gibeau and Miller 1989, Winterbourn 1990, Peterson and Grimm 1992). However, recently, malathion has been found to inhibit algal growth and the optimal dose of malathion that will inhibit grazing without inhibiting periphyton growth has yet to be found (Francoeur *et al.* 1999b).

6.7.1 Steel-tray nutrient-diffusing substrate method (suitable for large streams and flood prone rivers)

Overview

The nutrient-diffusing substrates are galvanised steel trays (dimensions 600 mm x 400 mm x 200 mm), with a handle at each end and a drainage aperture at one end. An internal thick plastic frame holds 20 nutrient-diffusing reservoirs (Figure 11). Each reservoir is a 400 ml square plastic jar filled with nutrient agar.

The periphyton colonisation surfaces are hardened ashless filter papers placed over the top of the reservoir jars and held in place by push-fit plastic rings. A thick plastic lid fits over the entire box. Twenty holes cut in the lid fit exactly over the colonisation papers so that the stream current flows over a flat, unbroken surface. The lid is secured by tightening nuts onto four long bolts welded to the base of the box. On the surface of the lid, five 20 mm high longitudinal partitions maintain parallel flow and prevent diffusion of different treatments onto neighbouring rows. The trays and internal fittings are not easy to fabricate and detailed design notes are not given here. Samplers can be purchased or hired from NIWA Instrument Systems, P.O. Box 8602, Christchurch (phone 03 348 8987; e-mail g.elley@niwa.cri.nz).

For deployment on the stream bed, ideally the tray is placed in an excavated depression so that the surface is no more than 100 mm above the bed. Often this is not practical. Where possible, it is a good idea to secure the tray by wiring it to metal stakes (one at each end) driven into the bed, or by running anchor lines from a tree or post on the stream bank.

To assess periphyton growth, the entire hardened ashless filter papers are analysed for chlorophyll *a.*

The standard assay uses five replicates of each of four nutrient treatments:

- Phosphorus (0.05 molar solution of trisodium orthophosphate in agar)
- Nitrogen (0.5 molar solution of sodium nitrate in agar)
- Phosphorus + nitrogen (both the above in agar)
- Control (no nutrients added to agar).

Preparation of nutrient-agar solutions

Materials:

Standard chemicals used, with quantities, are:

- Granulated agar: 20 g per litre
- Sodium nitrate ($NaNO_3$) 42.5 g per litre
- Trisodium orthophosphate ($Na_3PO_4.12H_2O$) 19 g per litre

Each tray holds 20 jars, five each of:

Figure 11: Steel-tray nutrient-diffusing substrate sampler. *Top:* sampler without the lid attached showing the internal frame of the sampler. The four agar-filled jars at the rear show stages in securing the filter-paper colonisation surfaces to the jars. The phosphorus-enriched agar jars are dark in colour. *Bottom:* nutrient-diffusing substrate sampler after incubation with the lid attached (the upstream end is marked with red paint on the left). Treatments from the far side are: control, nitrate + phosphate, phosphate, nitrate .

- Plain agar
- Agar + nitrate
- Agar + phosphate
- Agar + phosphate + nitrate

The trays are designed to hold 400 ml Labserv jars with square bottoms and a round opening (Catalogue no. LBS 33030, 400 ml containers, natural cap, unlabelled). Allow a little extra agar solution for spillage.

Equipment:

1. Scales.
2. Measuring cylinder/jug.
3. Hotplate.

4. Large pot for boiling agar.

5. Mixing tools (glass rods).

6. Plastic jars for enriched agar.

Procedure:

- Measure out the required volume of water into the container.

- Sprinkle onto the water the required amount of granulated agar (20 g per litre).

- Heat the water and stir until the agar is completely dissolved.

- Weigh out the appropriate amounts of phosphate and/or nitrate and add to the warm agar solution. Heat and stir until completely dissolved.

- Bring the mixture to boiling point then allow it to cool down before pouring into the jars. If it's too hot the jars will buckle and collapse.

- Fill the jars to the brim. The growing surfaces for periphyton (filter papers) need to be in contact with the agar.

- Store the filled jars with their lids on in the refrigerator.

- Use coloured stickers or similar to distinguish jars of different media. (Those containing phosphate turn dark brown; nitrate and plain media are uncoloured.) We use the following colour codes:

- N (nitrate) only   ORANGE or RED stickers
- P (phosphate) only   YELLOW stickers
- N and P together   PINK or WHITE stickers
- Control (agar only   GREEN stickers

Preparation of trays

Equipment:

1. NDS trays, including 20 rigid plastic rings for each.

2. Appropriate numbers of jars of nutrient agar.

3. Rubber bands (10 x 76 mm, no. 73).

4. Filter papers (Whatman no. 542, hardened, ashless, 9 cm diameter).

The jars of agar are always placed in the trays in the same configuration, i.e. four rows of five:
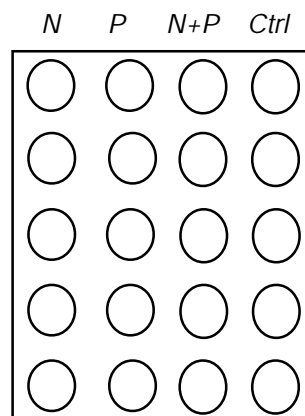
Position the jars in the tray, then:

- Place a rubber band around neck of each jar.

- Place a filter paper over the jar opening.

- Gently push one of the plastic rings supplied with the tray onto the jar over the top of the filter paper to fold the paper down over the neck and rubber band. The rubber band ensures a tight fit.

- Ensure the paper is stretched smoothly over the agar surface, with no creases or tears.

Notes:

- The lid of the tray fits over the top of the jars so that only the tops of the filter papers are exposed.

- Screw the lid down tightly.

- Each tray has a numbered tag attached to one of the handles. This number should match the one etched on the tray lid. Lids and trays are generally not interchangeable.

Deployment

Equipment:

1. Spanner for tightening nuts.

2. Metal stakes (warratahs).

3. Wire and wire cutters.

4. Mallet.

5. Spade (may be needed to dig a cavity in the stream bed for the tray).

Procedure:

- Prepare the trays with agar jars and filter papers shortly before deployment. This could be the night before, but don't do it much further in advance as the agar shrinks on exposure to air.

- Rinse off the lids and keep them handy. You'll need them if the media is to be re-used.

- Select a suitable location in the river. If monitoring at multiple sites, ensure that depth and velocities are the same at all deployment points (see Section 6.6.2).

- Once your limitations for water depth, velocity, shade, etc. have been set, then choose more-or-less stable locations on the stream bed.

- Make a depression in the bed (if bed material is suitable for this) and secure the trays, tags upstream, using a warratah wired onto the upstream handle. If in doubt, secure the downstream handle as well.

Notes:

- If you are deploying trays in pairs place them side by side, around 30–50 cm apart (or as convenient).

- Always deploy the trays with the numbered tag at the upstream end. For ease of recognition (and to allow for correct orientation of trays from photographs) the upstream end is also marked with brightly-coloured paint. As a further check, mark the nutrient rows at the upstream end of the inner plastic frame (N, P, N+P, C) with an indelible marker pen.

- Numbers 1–20 are systematically assigned to the jars. Always use these numbers as part of your labelling system for the containers into which you collect the filter papers, for example, site no. (or letter) + jar number.

Incubation time:

Normally, 14–17 days is enough to achieve good growth on the substrates before significant sloughing takes place. Of course this varies from river to river, and with season. After your first experiment you may need to adjust the length of any subsequent incubations. Keep the periods as consistent as is practical. It is best to avoid leaving the samplers to develop thick mats as sloughing may occur, and this would distort the results. Also large volumes of ethanol are required for chlorophyll extraction from thick mats.

We have found that the substrates give good growth responses when deployed for a second incubation with new filter papers. Beyond this time, results are doubtful.

Storage:

Freshly made media keep well for extended periods under refrigeration if the jar lids are kept tightly sealed. The media have limited "shelf life" between incubations, especially if not kept in a cool place.

Collection

Materials:

1. Collection containers for the filter papers (e.g., 60 ml. plastic containers, small whirl-pack or zip-lock plastic bags).
2. Permanent markers for labelling (can be done in advance).
3. Spanner to undo nuts.
4. Lids of agar jars for resealing (prior to re-use).
5. Paper towels.
6. Plastic bags.
7. Camera (possibly – see below).

Procedure:

- Gently remove the tray from the stream bed moving it downstream with the current as you lift it up. *Warning: the trays are very heavy when full of water.*
- Keep the tray close to horizontal while draining, especially if there is any chance of material becoming detatched from the filter papers.
- When the sampler starts to clear the water, take a note of anything that might influence the periphyton communities and thus effect interpretation of the results. Write these down once on the river bank. Things to observe might include the amount of silt accumulated on the slides or substrates, any debris such as leaves, twigs or macrophyte stems that have caught on the sampler and might have brushed against the sampling surfaces or influenced local flow paths.
- Count any invertebrates on each filter paper to the lowest taxonomic level that you can clearly recognise. These data are often very useful for interpreting the enrichment results and might provide another useful assessment of water quality (see the SHMAK rapid bioassessment protocol for a general grouping of invertebrates based on their pollution tolerances and ease of identification: Biggs *et al.* 1998c).
- Remove the lid and carefully slide off the plastic rings.
- Starting at the upstream left hand corner (the first N treatment), remove the whole filter paper from the agar substrate.
- Drain off as much water as possible.

- Fold the filter paper and sample in half (upper sides together).

- Clean off any periphyton growing on the underside of the filter paper with a soft toothbrush and discard.

- Fold the paper again, or roll up loosely, and place the whole paper in an appropriately labelled container.

- Put the 20 containers for each tray into a separate plastic bag – this greatly simplifies sorting out back in the lab.

- Transport the bag of samples back to the lab for analysis as quickly as possible. In the meantime, keep them frozen, if at all possible; otherwise keep them in a cool dark place.

Notes:

- Occasionally the papers become very fragile or are destroyed by invertebrate grazing.

- The most important part of the collection procedure is getting the filter papers into correctly-labelled containers.

- It is often very helpful for interpretation of the results to photograph each tray in the stream, and on the bank straight after removal. Make sure that the coloured paint is visible on the photo, as this means that you know for certain which treatment is which.

Post-collection analysis:

Chlorophyll *a* is analysed directly from the filter papers from the NDS trays using the method described in Section 7.3, but with slight variations. These are as follows:

- 60 ml containers are used for extraction of the chlorophyll because the NDS filter papers are so large.

- The 60 ml containers do not fit in standard tube racks so need to be placed in the water bath on a tray, while being held down with another tray on their lids.

- After boiling and leaving for 12 hours in the refrigerator, the containers with extractant are shaken and a 5 ml aliquot is removed which is then placed in a centrifuge tube for the balance of the analysis as described in Section 7.3.

- Blending is not required.

- Calculate chlorophyll *a* per square metre, assuming that the collecting surface is 65 mm in diameter (i.e. 0.003318 m^2).

6.7.2   Clay flowerpot-saucer nutrient-diffusing substrates (suitable for small streams and non-flood prone rivers)

Overview

The clay flowerpot-saucer nutrient-diffusing substrate technique is cheaper to construct than the steel tray method. However, more effort is required in the field for deployment and these substrates a more prone to disturbance during freshes and floods. The substrates comprise an inverted, unglazed, clay flowerpot saucer attached to a perspex base. Instructions for fabricating these samplers are given below. The saucer is filled with nutrient-agar solution through a hole in the top which is then sealed (e.g., with silicon caulk or a bung). Replicates of each treatment are then placed randomly on the streambed.

The nutrients diffuse out through the clay saucer and are absorbed by the adhering periphyton. The periphyton are removed at the end of the incubation period using a toothbrush. The sample is then analysed for chlorophyll *a.*

The standard assay uses five replicates of each of four nutrient treatments:

- Nitrogen (0.5 molar solution of sodium nitrate in agar)
- Phosphorus (0.05 molar solution of trisodium orthophosphate in agar)
- Nitrogen + phosphorus (both the above in agar)
- Control (no nutrients added to agar).

Fabrication of the sampler

Materials:

1. Unglazed clay flowerpot saucers (10–15 cm diameter) (purchase from garden suppliers).

2. Perspex (plexiglass) baseplates, cut in squares to be at least 1 cm larger than the diameter of the saucer (purchased as polycarbonate sheet; check Yellow Pages under "Plastics").

3. Silicon glue.

Procedure:

- Drill a small hole (e.g., 5–8 mm diameter) in the base of each saucer using a masonry bit and slow speed drill. Take care not to crack the saucer.
- Cut the perspex sheet to the appropriate size and drill holes in the corners for 10–15 cm galvanized nails (with flat heads) or ties to secure the samplers to the stream bed.
- Invert the saucer and glue it to the base plate using silicon fish-tank glue. Leave for several days to cure.
- Soak each sampler in water (preferably distilled) for a week to leach any mobile chemicals from the saucers. The samplers are now ready for filling with agar.

Preparation of nutrient diffusion solutions

Standard chemicals used, with quantities, are:

- Granulated agar: 20 g per litre
- Sodium nitrate ($NaNO_3$)   42.5 g per litre
- Trisodium orthophosphate ($Na_3PO_4.12H_2O$) 19 g per litre

For each site, prepare five substrates each of:

- Plain agar
- Agar + nitrate
- Agar + phosphate
- Agar + phosphate + nitrate

Allow a little extra agar solution for spillage.

Equipment:

1. Scales.
2. Measuring cylinder/jug.
3. Hotplate.
4. Large pot for boiling agar.
5. Mixing tools (glass rods).
6. Pre-prepared saucers.

Procedure:

- Measure out the required volume of water into the container.

- Sprinkle onto the water the required amount of granulated agar (20 g per litre).

- Heat the water and stir until the agar is completely dissolved.

- Weigh out the appropriate amounts of phosphate and/or nitrate and add to the warm agar solution. Heat and stir until completely dissolved.

- Bring the mixture to boiling point then allow it to cool down before pouring into the saucers.

- Fill the saucers to the brim. Any air trapped in the saucer will increase its buoyancy making it easier to dislodge.

- Store the filled saucers in the refrigerator.

- Use coloured stickers or similar to distinguish saucers of different media (those containing phosphate turn dark brown; nitrate and plain media are uncoloured.). We use the following colour codes:

- N (nitrate) only   ORANGE or RED stickers
- P (phosphate) only   YELLOW stickers
- N and P together   PINK or WHITE stickers
- Control (agar only   GREEN stickers

Deployment

- Select a suitable location in the river. If monitoring at multiple sites, ensure that depth and velocities are the same at all deployment points (see Section 6.6.2).

- Once your limitations for water depth, velocity, shade, etc. have been set, then choose more-or-less stable locations on the stream bed.

- The replicate substrates can be deployed in 5 groups of 4 treatments (N, P, N+P and Control) or haphazardly around the site. Given the constraints of depth, velocity shading and the main flow path, it is usually very difficult to deploy these substrates in a truly random arrangement.

- Secure the samplers using a several large galvanised nails driven into the gravels with a hammer. Alternatively a long tent peg (or short warratah) may be driven into the bed upstream and the substrate wired to the peg. Try to ensure that the pegs are driven down far enough so that the head is flush with the surrounding sediment to avoid catching debris which may then brush against the substrates. If in doubt about how well secured the substrates may be then secure the downstream end as well.

- Take care that the substrates do not influence one another (i.e., nutrients diffusing from an N treatment does not diffuse directly onto a P treatment). If substrates must be downstream of another because, say, the stream is very narrow, then attempt to have at least a 1.5 m gap between substrates.

Notes:

- Always deploy the saucers with a number on the upstream end (this helps for photo identification and later analysis).

- Numbers 1–20 are assigned to the saucers. Always use these numbers as part of your labelling system for the containers into which you collect the periphyton.

Incubation time

Normally, 14–17 days are enough to achieve good growth on the substrates before significant sloughing takes place. Of course this varies from river to river, and with season. After your first experiment you may need to adjust the length of any subsequent incubations. Keep the periods as consistent as is practical. It is best to avoid leaving the saucers to grow very thick mats as sloughing may commence which will distort the results.

Collection

Materials:

1. Collection containers for the periphyton removed from the samplers (e.g., 60 ml plastic pottles).
2. Toothbrush (flathead, medium hardness).
3. Scalpel.
4. Small pippette.
5. Permanent markers for labelling (this can be done in advance).
6. Paper towels.
7. Laboratory tray.
8. Plastic bags.
9. Camera.

Procedure:

- Detach the securing pins or wires first.
- Gently lift the substrates from the streambed moving then in a downstream direction with the current.
- When the sampler starts to clear the water, take a note of anything that might influence the periphyton communities and thus effect interpretation of the results. Write these down once on the river bank. Things to observe might include the amount of silt accumulated on the saucers, any debris such as leaves, twigs or macrophyte stems that have caught on the sampler and might have brushed against the sampling surfaces or influenced local flow paths.
- Pace the sampler on a laboratory tray.
- Count any invertebrates on each saucer to the lowest taxonomic level that you can clearly recognise. These data are often very useful for interpreting the enrichment results and might provide another useful assessment of water quality (see the SHMAK rapid bioassessment protocol for a general grouping of invertebrates based on their pollution tolerances and ease of identification: Biggs *et al.* 1998c).
- Scrape off as much periphyton growth as possible with the scalpel into an appropriately labelled container.
- Scrub the saucer with a toothbrush transferring the slurry into the sample container using the small pipette.
- Rinse the saucer with a minimal amount of water. Remove any surplus water using a pipette and transfer into the sample container. Thoroughly rinse the brush into the container.
- Store the labelled container of periphyton sample on-ice in a chilli-bin for transport to the laboratory.

- Group the samples into separate bags for each site – this greatly simplifies organisation back in the laboratory.

Notes:

- The most important part of the collection procedure is getting the periphyton thoroughly removed from the saucer and placing it in the correctly labelled container of water.

- It is often very helpful for interpretation of the results to photograph groups of saucers in the stream, and then on the bank straight after removal. Make sure that label is visible in the photo.

- You should probably count invertebrates before removing them from the samplers.

- Make notes on any unusual features such as the spatial distribution of periphyton, parts that may have been abraded etc.

Post-collection analysis

Analyse the slurry collected from each replicate sampler for biomass and/or taxonomic composition as required. See Section 7.

6.8 Community metabolism (maximum primary productivity and community respiration) using benthic respirometry

Primary productivity and respiration are two important process variables of periphyton communities. These measures are based on the oxygen evolved during photosynthesis and oxygen consumed by algae and heterotrophic organisms during respiration.

The method described below enables maximum primary productivity and community respiration to be measured; it does not allow net nor gross primary production to be measured (these latter variables require use of the diurnal oxygen curve method– see Bott 1996). The method described here can be used to indicate rates of carbon assimilation (a surrogate for growth rates), oxygen uptake rates/heterotrophic activity, and the relative balance between autotrophic and heterotrophic activity in the periphyton community. Such variables can be important for understanding the more detailed mechanisms resulting in response of periphyton to natural and artificial perturbations. Respirometers can also be used for nutrient limitation/uptake experiments, assessments of responses to different light levels (i.e., developing photosynthesis vs. irradiance curves), and toxicity assays.

Briefly, the method involves placing a sample of substrates (stones, gravel or artificial substrates) into a container with a perspex/plexiglass lid (the respirometer). The respirometer is then submerged in a shallow pool in the stream/river, the system sealed, and then stream water is pumped across the surface of the substrates and then recirculated back into the chamber through a short return hose with a 12-V DC submersible pump. Oxygen concentrations in the water within the respirometer are monitored over time (10–15 min.). The output from the dissolved oxygen (DO) meter recorder port is routed via a cable through a scale expander to amplify the DO signal within its working range and then this signal is routed to a field chart recorder. A black rubber sheet is placed over the respirometer for measuring respiration. The rate of change in oxygen during each treatment is used to estimate maximum photosynthetic rates (P_{max}) and community respiration (CR).

A significant trend in DO is difficult to obtain with low biomass and when the stream is near DO saturation. This leads to spontaneous degassing with the result that there is accumulation of air bubbles under the lid of the respirometer. Such degassing results in a major bias in

recordings and very noisy data. Ways to avoid this are explained below including purging the water in the respirometer with nitrogen gas. Respirometers are also prone to resource (CO_2 and/or nutrient) limitation. However, the method described below uses only short incubation times which greatly reduces the potential for this problem. Non-uniform velocity fields within the chambers are another potential problem. Water flow is often centered in the middle of the chamber. Baffles are inserted in the entrance region in an effort to create more uniform flow across all the substrate. Regardless of this, flow is always quite turbulent, which is probably more realistic if test substrates are from shallow, cobble bed streams. Dodds and Brock (1998) have recently designed a new chamber configuration in an attempt to reduce the problem of non-uniform velocity fields.

If biomass is low, there is little that can be done except attempt to fill the chamber with as much substrate as possible. Sometimes there can be difficulties detecting trends in oxygen evolution or consumption. It is important that each analysis is monitored carefully as it progresses so that problems or lack of trends are quickly identified and remedied. These measurements can be quite problematical at times.

P_{max} is usually influenced by both biomass and temperature (Boston and Hill 1992, Hill 1996, Biggs *et al.* 1999). It is therefore essential that both these variables are measured. Temperature should be recorded at the beginning and end of each respiration and photosynthesis run. If the respirometer is fully submerged in water with a moderate current (e.g., 0.2–0.4 m/s) then changes in temperature should be minimal during each incubation. At the end the substrates brushed clean (using sampling Method 1a, Section 6.5.4) to remove the periphyton and the slurry is analysed for both chlorophyll *a* and ash-free dry mass.

The following chamber design and methodology is based on that developed and tested by Hickey (1988).

6.8.1   Equipment

1. Benthic respirometer (Figure 12), consisting of:

    -   A 25 cm x 40 cm internal dimensions stainless steel base tray,

    -   A perspex/plexiglass chamber that clips over the top of the tray and seals around the edges with rubber flanges. The chamber has baffles at the inlet end to dissipate the flow.

    -   A re-circulation hose with an in-line 12-V DC submersible pump and a housing for the dissolved oxygen probe.

    - 12 V DC deep cycle truck battery to power the submersible pump.

2. Precision dissolved oxygen meter with a recorder output port and a 0–10 V range for the output signal.

3.   A scale expander to amplify the DO meter signal within a specific range.

4. Thin rubber sheet with a weighting chain attached to the perimeter.

5. A 1000–1500 watt quartz halogen lamp mounted on a stand with adjustable legs.

6. Portable generator capable of > 1500 watts and isolating transformer to power the lamp.

7. Large rectangular plastic bin (~ 0.8 m x 0.5 m x 0.3 m).

8. Large finely graduated plastic measuring jug (2–3 litres).

9. Small bottle of nitrogen gas (optional).

Figure 12: Benthic respirometer on adjustable legs. The submersible pump is mounted on the left end and dissolved oxygen probe is insert through a port on the lid of the main chamber. An in-line electromagnetic flow meter (not shown) can be inserted in the return flow line (foreground). Behind the respirometer are the (from left): 12-V DC marine grade battery to power the pump, voltage controller, chart recorder, scale expander and dissolved oxygen meter. The overhead lighting system is not shown (see Figure 13).

Figure 13: Undertaking P_{max} measurements with the benthic respirometer. A 1500 Watt lamp is mounted over the respirometer to provide standardised, high light for incubations. The light is power by a generator on the bank (out of photo).

Notes:

- Respirometers and scale expanders can be purchased from NIWA through the authors. These would be made up on demand. Customised designs could also be considered, or alternative designs such as that proposed by Dodds and Brock (1998).

- Most perspex/plexiglass filter out UVB wavelength light and thus creates a modified light environment for the periphyton. If it is important that these wavelengths are not altered, then special UV transparent perspex/plexiglass needs to be used for the chamber lid.

- The return hose length and depth in the chambers needs to be minimised to reduce the volume of water being re-circulated. This increases the sensitivity of the method.

- The volume of the respirometer needs to be known exactly to enable the photosynthesis/respiration rate calculations to be completed.

- We have designed a variation on the above respirometer that is quite small to accomodate unglazed ceramic tiles (up to 5 tiles) for experimental studies. This respirometer is used in the laboratory under broad wavelength artificial light (~ 1000 $\mu E\ s^{-1}\ m^{-2}$).

6.8.2   Procedure

- Locate a suitable swift pool or run (> 0.3 m deep, velocity 0.2–0.4 m/s) at your study site and set-up the respirometer in the water. It may be necessary to excavate a small pit to place the respirometer if the stream is quite shallow. Ensure that the chamber is level.

- Start the motor and check that it is pumping water through. If it is not then the pump probably has air trapped inside it. This can usually be removed by raising one of the respirometers. Then ensure that all air bubbles are purged from the system then disconnect the pump.

- Connect the DO probe to the meter, connect the DO recorder output line to the scale expander and then connect the expander line to the chart recorder. Check that all are working.

- Check the calibration of the DO probe (ensure that it is properly warmed up first – this can take longer than 10 minutes). Then connect the probe to the housing in the chamber re-circulation hose.

- Remove the stainless steel base tray of the respirometer and collect stones from the stream bed according to the requirements of the study (this will normally be from a transect across a specific riffle or run).

- Place the stones in the base tray so that their upstream–downstream orientation is maintained.

- Reconnect the battery to start the pump and check that the water is flowing through the respirometer and that there are no further air bubbles. Adjust taps on the inflow lines to get a reasonably even inflow of water. Then close the lid (you may need to wipe the underside of the lid to remove any attached bubbles. Run for pump for 2–5 minutes before starting DO measurements.

- Carry out the respiration measurement first by placing the black blanket over the chamber. Ensure that there are no gaps around the side where light could penetrate.

- Turn on the chart recorder (set it to run at ~ 1 cm/min and 1 mm = 1 mg/l scale). Set the scale expander to the appropriate range. Note the temperature, start time, begin-

ning oxygen concentration from the meter readout, and chart recorder speed on the chart.

- Track the change in DO. The line should trend down after a few minutes. Note the oxygen concentration on the chart recorder chart at several different concentrations to ensure a correct calibration of the chart divisions with DO.

- Run the respirometer until a monotonic linear trend has been recorded for at least 8 minutes. There should be only minor high frequency fluctuations in DO (1–2 mm maximum on the chart).

- Once a clear trend has been established, note the temperature again, then remove the rubber blanket to record photosynthetic rates.

- Place the light over the respirometer and start the generator to power it. The downward trend in DO should be reversed within a few minutes. Note the DO concentration regularly during the upward trend.

- Run the respirometer again until a clear upward linear trend occurs for at least 8 minutes.

- When a satisfactory record has been obtained, record the temperature, remove the light and carefully remove the tray of rocks.

- Collect all the periphyton from the surfaces of the rocks using collection Quantitative method 1A (Section 6.5.4).

- Measure the *x*, *y* and *z* axes dimensions of the cobbles/stones.

- Determine the volume of the rocks using water displaced in a finely graduated measuring jug.

Notes:

- In some streams the water may be near or at saturation with oxygen. This can result in spontaneous de-gassing of the water going through the pump with the result that large quantities of oxygen bubbles accumulate in the chamber. It helps to run the sealed chamber for about 5 minutes then clear the bubbles before doing a measurement. Doing the respiration measurement first also helps. However, in super-saturated water it is necessary to purge any excess oxygen by bubbling a small amount of N_2 gas into the system (through the bung hole in the chamber lid). Take the O_2 down to 8–9 mg/l. Release the bubbles through the bung hole on the lid at the downstream end. If you overdo it, then exchange a little stream water with chamber water through the lid bung holes.

- As the recording proceeds, write any notes on the chart paper that may be relevant in interpreting the results or calculating rates of change in oxygen.

- Carry out a blank run (i.e., no rocks in the chamber) for both respiration and productivity at the beginning of each day of measurements to ensure that the system is running well and there is no drift in the DO meter. If there is drift, and this cannot be fixed, then the results of the real measurements need to be corrected for the rate of drift. If you suspect problems, then re-run blanks at regular intervals.

- It pays to switch the chart recorder off for a few seconds when recording temperature from the DO meter because there is a major change in strength of signal and the recorder immediately goes off scale.

- Regularly check the membrane on the DO probe. It should be replaced every few days if doing many measurements.

- Wipe the underside of the lid after each run to remove the very small oxygen bubbles that sometimes accumulate there.

- To avoid electrical shocks always wear rubber gumboots in the water, and avoid putting your hand in the water while running the overhead light. It is a good policy to not enter the water while the generator is running in case the light falls over. Also, use a restricted current device (RCD isolating transformer) between the light and the generator.

6.8.3   Calculations

- Determine the rate of uptake and evolution of oxygen for the respiration and photosynthesis measurements, respectively, from the chart record by converting the graph scale into DO units (mg/l) and the linear distance into time in minutes, then calculating the amount of change in DO divided by time. The respiration rate will be a negative number, but the sign is dropped as it is inherent in the definition of community respiration (CR) that it is an oxygen uptake (i.e., loss) rate. Now convert the rate to mg O_2/l/hr by multiplying by 60.

- Subtract the volume of rocks used in the chamber from the gross chamber volume (chamber plus hose-line volumes) to give net chamber volume for the analysis. Multiply the rate of oxygen change by the net chamber volume (in litres). This gives rates for CR and net maximum productivity (Pn_{max}) in mg/l/hr.

- To normalise the rate measurements to surface area of the substrate, use:

$$\text{Stone surface area (cm}^2) = 1.59 + 0.811 \, (xy + yz + xz)$$

where x, y and z are the lengths of the three main axes of the stones in centimetres. This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area). This gives the results in mg/min/m².

- Calculate gross maximum productivity (P_{max}) as:

$$P_{max} = CR + Pn_{max}$$

Notes:

- When determining the rate of change in DO off the chart paper, adopt a systematic procedure in relation to how you draw a line through the readout. If there is some noise in the signal, then attempt to average this out by drawing a line up through the mean of the short-term fluctuations.

- At the beginning of the CR section of the incubations, there is often a non-linear reduction in oxygen. Ensure that you only use the linear portion of the output for your calculations.

6.8.4   QA/QC for metabolism measurements

This method does not lend itself particularly well to the development of formal QA/QC procedures. However, the following points should always be borne in mind when carrying out these measurements:

- The largest source of interference is the accumulation of gas bubbles under the chamber lid. This can cause high frequency noise with large magnitude variations in DO. Ensure that the system is as free of bubbles as possible at the beginning of a run. If possible avoid opening the system up between CR and productivity sections of the run. If you

find that this is needed, then you may need to use N_2 gas to lower the background concentration of dissolved oxygen in the water.

- Probe drift and temperature variations within runs is a common source of error in results. As noted above, always run a blank for both productivity and respiration at the beginning of a set of measurements. The blank is just stream water without any substrates. Both lines of DO output should be flat without any upward or downward trend. If temperature increases by more than 3˚C over both the CR and productivity sections of the incubation, then the results are probably subject to unacceptable error.

- Ensure that all calculations are checked.

6.9   Sample transport, storage and registration

6.9.1   Sample transport

Ideally all types of samples should be transferred to the laboratory as soon as possible after collection. Pack samples into chilly bins. Use crushed ice or slicka pads to keep the bins cool. Pack any spaces with crumpled newspaper or equivalent. It is important to keep samples chilled and in darkness.

If transport cannot be arranged within 18 hours of sample collection, the samples may be frozen and dispatched later at a convenient time (see below). Ensure that samples sent chilled are not in transit for more than 6 hours and those sent frozen are not in transit for more than 18 hours.

6.9.2   Sample storage

Three methods can be used to store periphyton samples:

- Freezing, for samples that are to be analysed for biomass, diatom composition and semi-quantitative abundance of soft-bodied taxa such as green algae and cyanobacteria. This is the only method that is practical for large biomass samples.

- Gluteraldahyde, for small samples (or sub-samples) to be analysed quantitatively for taxonomic composition. This method of preservation should not be used if biomass is to be analysed.

- Lugol's iodine/acetic acid, for small samples (or small sub-samples) to be analysed quantitatively for taxonomic composition. This method of preservation should not be used if biomass is to be analysed.

Freezing is widely used in New Zealand. Periphyton samples will remain intact for several months if kept at <–10˚C, but probably should not be kept for more than 3 months if chlorophyll *a* is to be analysed. We find that freezing periphyton samples does not significantly affect ash-free dry mass measures (Biggs 1987). While diatom form is not affected by freezing, the contents of filamentous green algae may distort after freezing. However, the common types retain a characteristic appearance.

Gluteraldehyde and Lugol's iodine enables the soft-bodied algae to be preserved. Glutaraldehyde is now most commonly used for preservation of algae for detailed examination of soft-bodied forms because it generally does not degrade the structure of the organelles or colouration. This preservative is active indefinitely. Lugol's solution facilitates sedimentation (particularly of very small diatoms), stains starch bodies, maintains fragile cell structures. However, this preservative is generally only active for 1–2 years and samples must be stored in the dark. If detailed taxonomic and biomass analyses are planned, then the samples should be blended and a small sub-sample removed (see Section 7.2) for preservation in

gluteraldehyde or Lugol's iodine.

Check the sample containers before freezing. If you are using rigid plastic pottles, ensure that there is some air space in the container (1 cm at the top is safe). Otherwise the containers will crack on freezing. This costs valuable time when you have to decant the sample on thawing and re-label another container. It also increases the chances of mis-labelling. Elkays are flexible and will rarely crack on freezing.

Method:

- Glutaraldahyde (this is available as 50% stock solutions from Biolab Scientific).
  - Assess the volume of sample to be preserved.
  - Under a fume hood, add sufficient gluteraldehyde to give a 3–5% final concentration. **NB: glutaraldehyde is a toxic solution and you should avoid skin contact or breathing the fumes.**
- Lugol's iodine:
  - Under a fume hood, dissolve 25 g of iodine and 50 g of potassium iodide in 50 ml of glacial acetic acid.
  - Add distilled water to bring the volume to 500 ml.
  - Store in an amber glass bottle.
  - Assess the volume of sample to be preserved.
  - Under a fume hood, add sufficient Lugol's solution to give 3–5% final concentration. **NB. Lugol's solution can irritate your eyes and nose. Avoid breathing fumes and minimise skin contact.**

6.9.3   Sample labelling and registration

- Often it is most convenient to pre-label sample containers. Store sets of containers for a site separate from those for other sites. For example, hold each set in a labelled plastic bag. Before you begin sampling, satisfy yourself that the containers are the correct ones for this site.
- Labelling containers on-site is OK, but again double check that you are using the correct name/number for the site.
- Enter the samples onto a sample registration system as soon as they arrive in the laboratory. This will ensure that a record exists that they are on the premises and await analysis by a certain date.

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7 Biomass analyses

7.1 Overview of biomass analyses

Two standard measures of biomass are used:

Chlorophyll a*:* this gives an indication of the total amount of (predominantly) autotrophic organisms in the sample.

Ash-free dry mass (AFDM): this is a measure of the total amount of organic material in the sample, and includes living autotrophic and heterotrophic microorganisms, plus dead periphyton, micro-invertebrates, and usually some terrestrial leaf debris.

It is preferable to analyse for both of these variables because: (a) they provide complementary information; (b) they can be combined to form a ratio (i.e., AFDM in mg/m²:chlorophyll a in mg/m²) called the "autotrophic index – AI" (Weber 1973), which itself is an informative measure. The AI is indicative of the proportions of the community composed of heterotrophic and autotrophic organisms. Stream periphyton communities unaffected by labile 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 conditions with little organic detritus (where autotrophy dominates) (Biggs 1989). Values greater than 400 are taken to indicate communities affected by organic pollution (Collins and Weber 1978) (but see caution in Section 7.4.6, bullet #8).

In cases where periphyton growth is extremely sparse, the material collected may be insufficient to carry out an analysis of ash-free dry mass (errors in sample measurement are larger than the actual measurement). In this case analyse for chlorophyll *a* only. If available resources only allow one measure of biomass then chlorophyll *a* is recommended (and is the most commonly used measure) because it is cheaper to perform and more sensitive at low levels. Also, it is less prone to error associated with allochthonous organic matter.

7.2 Sample preparation

Most periphyton samples will contain clumps of filamentous algae or diatoms. This creates difficulties in sub-sampling to obtain representative portions for analysis. Representative sub-sampling is essential for accurate assessment of periphyton communities. Homogenising the sample with a simple, hand-held laboratory or kitchen blender can significantly reduce variability in sub-sample analyses, thus increasing the precision of the results. Biggs (1987) found that the assessed density of cells in a periphyton sample could increase by up to 500% and coefficients of variation decrease by nearly 90% following blending compared with just shaking the sample to achieve mixing. The highest increase in precision can be expected with samples that are dominated by filamentous algae. The blending process generally does not greatly damage cells, although some colonial species tend to break apart and long, lightly silicified diatoms and chrysophytes can be damaged. Most filamentous taxa break apart into much smaller fragments at inter-cellular connection points. Certain green filamentous algae do not break apart easily (e.g., *Cladophora*) during blending and tend to get wrapped around the cutting blades. The use of sharp blades on the blender, only a small volume of sample, and a slightly longer blending time usually overcomes this problem (Biggs 1987).

7.2.1 Equipment

1. Glass beaker.

2. Stoppered bottles with narrow necks (marked at 50 ml intervals).

3. Kitchen or laboratory tissue blender.

4. Squirt bottle with distilled water.

7.2.2 Procedure

- Tip the contents of the sample container into a glass beaker (the width of the beaker should be only slightly greater than the width of the housing holding the cutter blades of the blender). Rinse out any sample residue from the container and lid into the beaker. Distilled water is preferred for rinsing and making up the sample volume. However, tap water can be used providing the water isn't heavily chlorinated.

- Pick out any invertebrates, pieces of gravel, leaves, moss, etc. from the sample.

- Ensure that there is enough water to fully cover the blender-blade housing.

- Blend for about 30 seconds or until the mixture is free of obvious clumps of material. If the sample contains much filamentous algae, break the strands up by repeated cutting with a pair of sharp scissors. Every 10 seconds, or so, stop the blender and free any filaments that may have become caught on the blades or blender housing using a squirt of water. You need to end up with a homogeneous solution suitable for sub-sampling.

- Transfer the blended liquid to a stoppered bottle. Rinse out the beaker and make up the solution to a known volume (usually 50, 100 or 150 ml). Do a quick calculation of how much solution is required for all your sub-samples (variables to be analysed x aliquots x replicates) and ensure that you will have at least 50% more volume than this to allow for re-analysis of variables where there may have been some analytical errors or problems – see Sections 7.3.6, 7.4.6, and 8.7. Enter this volume onto your lab analysis sheet.

- If the sample is frozen between blending and different sets of analyses, then it should be re-blended briefly before sub-sampling for the next analysis because freezing tends to coagulate some filamentous and mucilaginous periphyton.

7.3 Chlorophyll *a*

While different taxonomic groups have distinctive assemblages of pigments, it has become standard procedure to assay the quantity of chlorophyll *a* as a measure of live biomass because this pigment is abundant in most periphytic algae including cyanobacteria. However, there are several important sources of bias with this parameter. First, the amount of chlorophyll *a* does vary among taxonomic groups and this can result in apparent biomass differences among communities when quantities of organic matter vary only a little. Second, the chlorophyll degrades naturally as communities age and cells die. This results in the formation of degradation products called phaeopigments which occurs through the loss of a magnesium ion from the chlorophyll molecule. Indeed the periphyton mat is also a sink for dead algal cells from in-situ growth, upstream deposition and also terrestrial plant debris. These phaeopigments interfere with the analysis of "live" chlorophyll *a* because they have an absorption peak the same as for chlorophyll *a*. It is therefore essential to carry out an acidification step as a second part to the analysis analysis to correct the measurements for phaeopigments. Acidification removes the magnesium ion from the chlorophyll molecules converting the whole sample to phaeopigment.

Three main methods have been developed for the determination of chlorophyll *a* in algae:

• Spectrophotometry

• Fluorometry, and

• High performance liquid chromotography (HPLC).

Fluorometry and HPLC are more precise than spectrophotometry. For example, spectrophotometry has been found to overestimate pigment concentrations in natural phytoplankton by 13–46% (Sartory 1985). This overestimation has been attributed to interference by carotenoids in the assessment of phaeopigment concentrations (Riemann 1978). However, a number of studies have also shown that the differences between spectrophotometry, fluorometry, and HPLC are not systematic (e.g., Jacobsen and Rai 1990). Fluorometry and HPLC techniques have a major advantage with very low concentrations of algae in that they are far more sensitive (Lorenzen 1966, Sartory 1985). However, this is rarely a problem with stream periphyton analyses. We describe the spectrophotometric technique here, because of the much wider availability of spectrophotometers.

A second issue that has varied widely among researchers internationally, is what method to use to extract the chlorophyll from the algae for analysis. Four solvents have been commonly used:

• Acetone

• Methanol

• Ethanol

• Dimethyl sulfoxide (DMSO).

Acetone has been the most popular method, but many studies have shown that alcohol solvents (particularly ethanol) are more efficient than either acetone or DMSO at extracting pigment from many algae (e.g., Holm-Hansen and Reimann 1978, Riemann and Ernst 1982, Sartory 1982). For example, Jacobsen and Rai (1990) reported that methanol and ethanol extractions were almost complete after 4 h, but were still incomplete after 24 h with acetone from a culture of *Scenedesmus* (a colonial green alga). The final acetone extraction efficiency was only 50% of the ethanol extraction. Maceration of the sample using a tissue grinder can greatly assist extraction (e.g., Marker 1972), but may not totally compensate for acetone's poor extraction efficiency. Grinding also results in an additional step in the analysis that is not required for ethanol extraction (Sartory and Grobbelaar 1984). The use of ethanol over methanol has now been recommended by a number of researchers (e.g., Nusch 1980, Sartory 1982, Sartory and Grobbelaar 1984, Webb *et al.* 1992). Because ethanol is a very effective solvent, there is only a minor shift in the absorbance peak with acidification, interference from chlorophyll *b* is less, ethanol presents a much lower health and safety risk, and it is cheaper. We have carried out a number of our own tests on extraction efficiency of various solvents with periphyton communities from New Zealand streams and these support the above results. Thus, since 1984, we have adopted the spectrophotometric ethanol method for analysis of chlorophyll *a* for general use in New Zealand.

The procedure detailed below is based on Sartory (1982) and Sartory and Grobbellar (1984), where absorption coefficients and acid ratios were re-defined based on the work of Nusch (1980). The method involves the following steps:

• Concentrate a sub-sample of homogenised periphyton on a glass-fibre filter.

• Extract the chlorophyll in a solution of 90% boiling ethanol (i.e., 78 °C. Boiling increases extraction efficiency and helps to "fix" the chlorophyll by destroying enzymes thus

making the chlorophyll relatively stable when in storage).

- Leave to complete extraction in a refrigerator overnight.

- Centrifuge to remove particulates from the solution.

- Read background turbidity and pigment concentration on a spectrophotometer.

- Acidify sample to convert the chlorophyll *a* to phaeopigments and re-read absorbances

- Correct for the presence of turbidity and phaeopigments

- Calculate chlorophyll *a* concentrations by multiplying by an absorbance coefficient.

If you have collected whole rocks for direct extraction of chlorophyll *a*, then a larger quantity of the ethanol extractant is put into the zip-lock bags with the rocks. The bags and rocks are then placed in the hot water bath for extraction.

Notes:

- The absorbance peak for chlorophyll *a* is very narrow and varies slightly among spectrophotometers, so it is important that you locate the wave length of the peak exactly by scanning absorbance of a standard solution. This is done as follows:

  - Make up a dilute standard solution of chlorophyll *a* in 90% ethanol (concentrated chlorophyll is available from some laboratory supply companies) and bring to the boil at 78°C for 5 minutes

  - Cool the standard to room temperature and then measure the absorbance of the standard at wavelengths ranging from 660 to 668 nm. This will enable you to determine where the peak in absorbance is for your spectrophotometer. This will normally lie between 663 and 665 nm. Use this waveband for all future measurements on your spectrophotometer.

7.3.1 Equipment

1. Data sheets.

2. Ethanol, 95 or 96% ex-stock, diluted to 90% with distilled water.

3. Glass fibre filters, 47 mm Whatman GFC.

4. Polythene centrifuge tubes, stoppered, numbered and stored in order in racks.

5. Forceps/tweezers.

6. Vacuum filtration apparatus.

7. Pipettes set to 5 ml and 0.1 ml (the 5 ml pipette needs to have a wide aperture ~ 2 mm diameter).

8. Squirt bottle with distilled water.

9. 0.30 molar HCl.

10. Water bath.

11. Spectrophotometer.

7.3.2   Sub-sample filtering

- Prepare tubes by placing 5 ml of 90% ethanol in each and re-capping the tube firmly.

- Set up filtering apparatus.

- Place fresh filters in each filtering chamber.

- Apply suction pressure (be careful not to have a high pressure as this will rupture cells releasing the chloroplasts; <10 mm Hg vacuum is recommended).

- The filtered sub-sample comprises three smaller aliquots of the blended sample which are pooled. To take these aliquots, shake the bottle of blended sample and withdraw 5 ml with an automatic pipette from half-way down the solution while the liquid is still agitated. Release this solution into the filtering chamber. Repeat this twice more to give the full sub-sample (i.e., the total sub-sample volume = 15 ml) (see QA/QC recommendations).

- If there is not an obvious colouring from periphyton on the filter, then you should filter more aliquots. However, if you intend to do other analyses as well, ensure that you have enough sample left (AFDM analyses aren't as sensitive as chlorophyll *a* and require larger sub-samples where there is a low concentration of periphyton).

- Check for any fragments of leaves, mosses, invertebrates etc. on the filter paper and remove these with forceps.

- Record the volume of sub-sample (i.e., number of 5 ml aliquots used).

- Rinse the pipette by sucking up and discarding some clean water.

- If you are sub-sampling for both ash-free dry mass and chlorophyll *a*, get into a routine of always doing them in the same order. We take sub-samples for chlorophyll *a* first, then for AFDM.

- Remove the filter from the filtering apparatus, fold in half, loosely roll up and place in its numbered tube. Make sure it is completely covered with the ethanol and the tube is firmly sealed.

Notes:

- Always check that clumps of algae or a leaf fragment, etc., hasn't blocked the intake to the pipette.

- If the aliquots are taking a very long time to filter for each sub-sample, you probably need to dilute your sample or take a smaller volume aliquot (e.g., 2 ml). Ensure that you record the degree of dilution and aliquot volume so that this can be used in the calculations of AFDM concentration.

- Remember to have sufficient sample solution for multiple analyses as required (N and P content, species analysis, repeat analyses, etc.).

- Throughout the filtering process, periodically check that the centrifuge tube numbers you use correspond to the numbers and the sample identifiers marked on the data sheets.

7.3.3   Analysis

- Pre-heat the water bath to 78 °C (boiling point of ethanol). Immerse the racks of tubes in the bath for exactly five minutes.

  NB. Make sure the tubes are held firmly in the racks. Loosen the tops to prevent them popping off as the ethanol boils. But leave the tops ON, otherwise you will lose ethanol through evaporation.

- Place the racks in the refrigerator overnight.

Notes:

- At this stage, if any of the chlorophyll seems to be really concentrated (i.e., a very deep green), then dilute the extractant with 90% ethanol. The aim is to keep the absorbance readings to less than 1.000. Readings higher than this indicate concentrations which may no longer be linearly proportional to absorbance.

- To dilute, first add an extra 5 ml of 90% ethanol to the tube. Push down the stopper firmly and shake well. Note on the data sheet that the volume of extractant is 10 ml (instead of the usual 5 ml). If the solution still looks very concentrated, centrifuge the solution (as described above), then withdraw 5 ml from the tube, add another 5 ml of 90% ethanol and shake well. Note on the data sheet that the (effective) volume of extractant is now 20 ml.

7.3.4   Absorbance readings

Equipment:

1. Spectrophotometer including cuvettes, printout paper.

2. 90 % ethanol for washing out cuvettes and for blank solutions.

3. Pipettes set to 4 ml and 0.1 ml.

4. Tissues.

5. 0.30 molar hydrochloric acid (6.25 ml concentrated HCl made up to 200 ml with distilled water).

Procedure:

- Switch on the spectrophotometer, wait for initialisation and for the machine to completely warm up (warm-up time will vary among brands).

- Set the wavelengths at the wavelength peak for chlorophyll *a* for your spectrophotometer and 750 nm.

- Ensure that the outside surfaces of your cuvette are clean and free of drips and dirt. Take blank readings using 90 % ethanol to check this.

- Using a glass rod, or metal forceps, push the filter papers as far as possible to the bottom of the centrifuge tubes, and re-close firmly.

- Centrifuge at a speed of 6000 rpm for 10 minutes.

- Take the first centrifuged sample and pipette out 4 ml into the cuvette.

- Read absorbances at 665 and 750 nm.

- Remove the cuvette, inject 0.1 ml of 0.3 M HCl in the cuvette, place a cap on the cuvette and invert to mix. Wipe the side of the cuvette again with a clean tissue.

- Place the cuvette back into the spectrophotometer and re-read absorbances at 665 and 750 nm, leaving a 30 second delay.

- Remove the cuvette, discard the acidified sample and rinse it thoroughly with 90% ethanol. Invert and tap dry on a clean tissue.

Notes:

- Always make sure that the spectrophotometer is adequately warmed up and has stabilised before analyses. This may take up to an hour for some machines.

- Always use the same cuvette for each sample batch, and always use it facing the same way (e.g. always place it in the compartment with the "L" facing to the left.). Make sure that the outside of the cuvette is clean and dry before each reading.

- Always hold the cuvette by the frosted side panels.

- Acidification occurs within a few seconds, but ensure that there has been a delay of at least 30 seconds before re-reading to ensure that acidification is complete before taking the next reading.

- Make sure you clean the cuvette thoroughly after each sample has been acidified to

avoid contaminating the next sample with an acidic solution.

- Be careful not to over-acidify the extract because this may cause the development of products which interfere with the phaeopigment absorbance peak.

- Always close the sliding door over the cuvette compartment before taking a reading.

- The optimal band pass width for the spectrophotometer is 1–2 nm, with a slit width of ~ 0.2 nm.

7.3.5 Calculations

$$\text{Chlorophyll } a \text{ (mg per sample)} =$$
$$\frac{[(\text{absorbance}_{665 \text{ before}} - \text{absorbance}_{665 \text{ after}}) \times 28.66 \times \text{sample vol.} \times \text{extractant vol.}]}{\text{(filtered sub-sample volume)}}$$

where:

- absorbance$_{665 \text{ before}}$ and absorbance$_{665 \text{ after}}$ are the absorbance readings at a wavelength of 665 nm before and after acidification (having already subtracted the respective turbidity blanks read at 750 nm);

- 28.66 is the absorption coefficient for chlorophyll *a* as defined by Sartory and Grobbelaar (1984) (based on the specific absorption coefficient for chlorophyll in ethanol of 83.4 g/l/cm and an acid ratio for chlorophyll *a* in ethanol of 1.72 (Sartory 1982));

- all volumes are in litres.

Phaeopigment concentrations may be calculated as follows:

$$\text{Phaeopigments (mg per sample)} =$$
$$\frac{[(1.72 \times \text{absorb.}_{665 \text{ after}}) - \text{absorb.}_{665 \text{ before}}] \times 28.66 \times \text{sample vol.} \times \text{extractant vol.}]}{\text{(filtered sub-sample volume)}}$$

where all volumes are in litres.

It is then necessary to normalise the chlorophyll or phaeopigment concentration for the area from which the sample was collected. If whole stone sampling was used (i.e., from sampling Method 1a; Section 6.5.4), you need to calculate the surface area of stone from which the sample was obtained:

$$\text{Stone surface area (cm}^2) = 1.59 + 0.811 \, (xy + yz + xz)$$

where *x*, *y*, and *z* are the lengths of the three main axes of the stones in centimetres. This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area). Otherwise, calculate the area based on the diameter of the sampling circle used (i.e., from sampling Method 1b).

If samples have been collected from a set area on top of the stones (Quantitative Methods 1b, 2 and 3: Sections 6.5.5, 6.5.6, 6.5.7), or from artificial substrates (Section 6.6), then calculate the surface area of the individual replicate and multiply by the number of replicates collected.

Finally, calculate the concentration of chlorophyll *a* or (phaeopigments) in mg/m² as follows:

$$\text{Chl. } a \text{ (mg/m}^2) = \text{Chlorophyll } a \text{ (mg/sample)/area of sample (m}^2)$$

(Ensure that you have converted your sampling area from cm² to m² for this final calculation.)

7.3.6   QA/QC for chlorophyll *a*

Sources of error

Two main areas in the chlorophyll *a* analysis can introduce errors. These are:

- sub-sampling;

- analysis.

Variation associated with sub-sampling is generally <10% (as % coefficient of variation), but may be as high as 25% for samples of communities that are difficult to break up during blending (Biggs 1987). Higher levels of sub-sampling precision are expected with diatom dominated communities than with filamentous communities (particularly mat forming cyanophytes). It is common to only remove and analyse one sub-sample. If you only wish to analyse one sub-sample then we strongly recommend that this sub-sample be composed of at least 3 ↔ 5-ml aliquots. This considerably reduces sub-sampling variability.

When sub-sampling and filtering a large batch of samples it is easy to loose track of how many aliquots you have put through the filters. Also, it can be easy to get centrifuge tube numbers and sample numbers interchanged when working between the tubes and laboratory sheets. Establish a clear, systematic, routine for the filtering process. Don't attempt to carry out the sub-sampling/filtering procedure too fast. Ensure that the sample bottle labels are clearly readable, the bottles are arranged in order on the lab bench, and the samples are listed in the same order on the laboratory bench sheet. Never label the sample bottles on their lids only (these can be swapped too easily!). Mislabeling or accidental swapping of samples and or sample labels is one of the easiest and most significant sources of error in these analyses.

Analytical error should, in most cases, be small relative to variability associated with the patchy distribution of mats on the stream bed (i.e., sampling error). For chlorophyll *a* analyses, several sources of analytical error are possible:

- Incorrectly measuring the 5 ml aliquot of 90 % ethanol. The viscosity of the ethanol is relatively low, so sometimes you don't withdraw the exact 5 ml from the working solution. Also, the ethanol often drips from the automatic pipette as the solvent is being transferred to the centrifuge tubes. These problems can introduce large errors.

- Not shaking the sample thoroughly to ensure a homogeneous mixture when sub-sampling. Be very careful to ensure that the blended mixture is homogeneous. Do not swirl the container as this distributes the heavier cells/filaments to the perimeter of the container. Shake the container and take the sub-sample from approximately the middle of the container.

- Incorrectly measuring the 5 ml aliquots that are pooled for the sub-sample. When processing large numbers of sub-samples it is easy to become a little sloppy in the sub-sampling technique. This usually occurs through incorrectly releasing the pressure on the automatic pipette so that the sub-sample gets sucked up too fast. This results in incomplete withdrawal of the aliquot. Always check that clumps of algae or a leaf fragments haven't blocked the intake to the pipette.

- Not allowing the spectrophotometer to warm up properly.

- Not correctly locating the peak in absorbance for chlorophyll *a* on your spectrophotometer. This can introduce very large errors because the peak is very narrow.

- Not allowing sufficient time for acidification.

- Errors in calculations for dilutions. These calculations must be done very carefully and

checked because you can double, quadruple, half, or quarter the apparent concentrations very easily without knowing it.

For overall errors associated with sub-sampling and analyses, Biggs (1987) reported mean co-efficients of variation of 10.6% for chlorophyll a based on 258 sets of analyses of three sub-samples. The samples covered a wide range of biomass and community types including diatoms, filamentous green algae and Cyanobacteria.

Ethanol dissolves ink from marker pens, so be sure that any drips created by pouring the chlorophyll extract from the centrifuge tube to the cuvette don't wash the identifying mark off the outside of the tube. If this does happen, then ensure that the drip with the associated ink doesn't land in the cuvette. Wipe the outside of the tube immediately and re-apply the identifier.

Formal quality control procedures

The following steps should be included in a formal QA/QC system for chlorophyll *a* analysis.

- Check the location of the chlorophyll absorbance peak on the spectrophotometer using a pure chlorophyll a in 90% ethanol solution at least twice per year (see page 79).

- Check the calibration, and volume setting, on the automatic pipette used for sub-sampling before each batch of analyses by weighing sub-samples of distilled water on a precision balance.

- All field sample labels should be checked and signed off as being clearly readable.

- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.

- Check the transcribing of sample numbers/labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet. Prepare data sheets before you begin the analyses. Enter each site/sample number in a logical order (e.g., sites in upstream to downstream sequence).

- Prepare racks of numbered centrifuge tubes for extraction of chlorophyll *a* and enter these numbers onto the data sheets. Start numbering from 1 for each sampling run and ensure that the tubes are arranged in their racks in the correct order.

- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.

- For analyses where the results are likely to be used for a resource consent hearing or the Environment Court, then 3 sub-samples should be analysed from each sample and the results averaged. If the value for any one sub-sample is >2 X the mean of the two nearest sub-samples, then the outlier is considered to be a sub-sampling or analytical error and the result of the outlier should be discarded.

- The scientist responsible for analysing the data and reporting must check the results and sign them off as being acceptable. The balance of all samples should be retained (frozen) until the data has been approved in case re-analysis is required.

Notes:

- It is difficult to develop Quality Control charts to allow "benchmarking" of batch analyses for chlorophyll *a* as has been suggested for AFDM (see below). This is because we are, as yet, uncertain of the stability of frozen chlorophyll samples or ethanol extracts if the sub-samples are kept for more than a few months. Preserving samples for later analysis and benchmarking is an essential component of a QC chart.

- A good quality assurance system in a laboratory is dependent on feedback. Feedback between:

  - laboratory staff and laboratory manager, regarding problems with samples, techniques etc.;

  - laboratory staff and scientists regarding expected capabilities, variability, and expected levels for the results, and required levels of precision, etc.;

- Feedback helps to ensure:

  - the objectives of the project are being met;

  - variability of results is reduced and thus confidence in the results is maintained;

  - all participants in the analyses know what is expected of them in the QA system.

7.4   Ash-free dry mass (AFDM)

In brief, the method involves drying a sub-sample to drive off all water, determining the dry weight, ashing the sample and then re-weighing. The difference between these two weights gives the ash-free dry mass value (sometimes called loss-on-ignition or ash-free dry weight).

As with analyses for chlorophyll *a*, many methods have been used and no one standard method has been adopted internationally. Variations in technique have arisen mainly in the temperature and time used for ashing. Ridley-Thomas *et al.* (1989) assessed the effects of different sample sizes, ignition time and ignition temperature on AFDM results for a set of stream periphyton samples. They found significant variation as a function of differences in all these aspects, but did not develop a recommended procedure.

Most commonly it has been recommended to ash samples at 500–550˚C for 1–2 hr (e.g., APHA 1995). However, at such high temperatures there is a risk of dehydroxylation of the clay minerals (Mook and Hoskin 1982) which tend to accumulate in periphyton mats (sometimes to high concentrations; Davies-Colley *et al.* 1992). If biomass is low, this can potentially result in a very high biasing of results. An ignition temperature of 400˚C for 4 hr has been most commonly used for analysis of stream periphyton in New Zealand in an effort to reduce such problems (e.g., Biggs 1987, Biggs and Close 1989, Biggs 1990, Davies-Colley *et al.* 1992, Biggs 1995). This method is described in detail below.

7.4.1 Equipment and materials

1. Data sheets.

2. Glass-fibre filters, 47 mm Whatman GFC.

3. Crucibles, with number/ID mark, on metal trays.

4. Forceps/tweezers.

5. Vacuum filtration apparatus.

6. Glass beakers, stoppered bottles (marked at 50 ml intervals).

7. Blender.

8. Automatic pipettes set to 5 ml (with a wide aperture ~ 2mm diameter).

9. Squirt bottle with distilled water.

10. Desiccator.

11. Muffle furnace, drying oven, and balance capable of measuring to 0.1 mg.

7.4.2 Procedure

• Place one filter in each crucible. Do not fold or tear.

• Pre-ash in the muffle furnace at 400°C for two hours – this is necessary in case any traces of combustible material in the GFC filters interfere with the ash-free dry weight measurements.

• If dry weight of the samples is required, then transfer the crucibles and filters to a desiccator to cool. Allow expanded hot air to escape for a few seconds through the desiccator lid valve, then close the valve to create a vacuum seal. About 30 minutes is usually enough for cooling.

• Record the weight of each crucible and filter paper after they have cooled (if sample dry weight is to be determined).

Notes:

• Always handle the crucibles with forceps/tweezers to avoid contamination with grease, etc., from your hands.

• If dry weight is not needed, then allow the crucibles to cool on trays.

• Replace the lid on the desiccator between each weighing. Once out in the open, the filter papers start to absorb moisture, which increases their "dry" weight.

• Allow the crucibles to cool fully. Weigh the crucibles at room temperature. If they are warm or hot, you get spuriously low values.

7.4.3 Sub-sample filtering

• Set up the filtering apparatus.

• The filtered sub-sample comprises at least three smaller aliquots of the blended sample which are pooled. To take these aliquots, shake the bottle of blended sample and withdraw 5 ml with an automatic pipette from half-way down the solution while the liquid is still moving. Release this solution into the filtering chamber. Repeat this twice more to give the full sub-sample (i.e., the total sub-sample volume = 15 ml).

• If there isn't a significant accretion of periphyton on the filter then you should filter more aliquots.

• Check for any fragments of leaves, mosses, invertebrates etc. on the filter and remove these.

• Apply suction pressure to the filtering apparatus.

• Record the volume of sub-sample (i.e., number of 5 ml aliquots used).

• Rinse the pipette by sucking up and discarding some distilled water.

• If you are sub-sampling for both ash-free dry mass and chlorophyll *a*, get into a routine of always doing them in the same order.

• Replace the filter in its crucible when filtering is complete.

Notes:

- If the aliquots are taking a very long time to filter for each sub-sample, you will probably need to dilute your sample or take a smaller volume aliquot (e.g., 2 ml). Ensure that you record the degree of dilution and/or smaller aliquot volume so that this can be used in the calculations of AFDM concentration.

- Pipette tips can be widened slightly to overcome persistent blockage.

- Remember to have sufficient sample solution for multiple analyses as required (N and P content, species analysis, repeat analyses etc.).

- Throughout the filtering process, periodically check that the crucible numbers you use correspond to the numbers and the sample identifiers marked on the data sheets.

7.4.4   Ashing of samples

- Dry the sub-samples (crucibles + filters with filtered material) for 24 hours at 105 °C.

- Weigh each crucible after cooling in a desiccator, as before.

- Ash for 4 hours at 400°C, cool in the desiccator, and weigh for the final time.

7.4.5   Calculations

Ash-free dry mass (g per sample) =

[{(weight of crucible + filter + sample after drying) – (weight of crucible + filter + sample after ashing)} x sample volume]

/ [volume of filtered sub-sample]

Dry mass (g per sample) =

[{(weight of crucible + filter + sample after drying) – (weight of crucible + filter after drying)} x sample volume]

/ [volume of filtered sub-sample]

All volumes are in litres.

If whole stone sampling was used (i.e., sampling Method 1a; Section 6.5.4), you need to calculate the surface area of stone from which the samples were obtained:

Stone surface area (cm^2) = 1.59 + 0.811 ($xy + yz + xz$)

where x, y, and z are the lengths of the three main axes of the stones in centimetres. This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area).

If samples have been collected from a set area on top of the stones (Quantitative Methods 1b, 2 and 3: Sections 6.5.5, 6.5.6, 6.5.7), or from artificial substrates (Section 6.6), then calculate the surface area of the individual replicate and multiply by the number of replicates collected.

Finally, calculate the AFDM or dry mass in g/m^2 as follows:

AFDM or DM (g/sample) = AFDM or DM (g)/area of sample (m^2)

Ensure that you have converted your sampling area from cm^2 to m^2 for this final calculation (x cm^2/10,000 = y m^2).

Additional variables may be calculated as follows:

$$\text{\% organic matter} = (\text{ash-free dry mass} \times 100) \, / \, (\text{dry mass})$$

$$\text{Autotrophic index (AI)} = \text{AFDM (in mg/m}^2) \, / \, \text{chlorophyll } a \text{ (mg/m}^2)$$

7.4.6   QA/QC procedures for ash-free dry mass

Sources of error

There are two main areas in the ash-free dry mass analysis that can introduce errors. These are:

- sub-sampling;

- analysis.

Variation associated with sub-sampling is generally < 10% (as % coefficient of variation), but may be as high as 25% for samples of communities that are difficult to break up during blending (Biggs 1987). Higher levels of sub-sampling precision are expected with diatom dominated communities than with filamentous communities (particularly mat forming cyanophytes). It is common to only remove and analyse one sub-sample. If you wish to analyse only one sub-sample then we strongly recommended that this sub-sample be composed of at least 3 × 5-ml aliquots. This considerably reduces sub-sampling variability.

When sub-sampling and filtering a large batch of samples it is easy to loose track of how many aliquots you have put through the filters. Also, it can be easy to get crucible numbers and sample numbers interchanged when working between the sample containers and laboratory sheets. Establish a clear systematic, routine for the filtering process. Don't attempt to carry out the sub-sampling/filtering procedure too fast. Ensure that the sample bottle labels are still clearly readable, the bottles are arranged in order on the lab bench, and the samples are listed in the same order on the laboratory bench sheet. Never label the sample bottles only on their lids (these can be swapped too easily!). Mislabelling or accidental swapping of samples and or sample labels is one of the easiest and most significant sources of error in these analyses.

Analytical error should, in most cases, be small relative to variability associated with the patchy distribution of mats on the stream bed (i.e., sampling error). For AFDM analyses, several sources of analytical error are possible:

- Not shaking the sample thoroughly to ensure a homogeneous mixture when sub-sampling. Be very careful to ensure that the blended mixture is homogeneous. Do not swirl the container as this distributes the heavier cells/filaments to the perimeter of the container. Shake the container and take the sub-sample from approximately the middle of the container.

- Incorrectly measuring the 5 ml aliquots that are pooled for the sub-sample. When processing large numbers of sub-samples it is easy to become a little sloppy in the sub-sampling technique. This usually occurs through incorrectly releasing the pressure on the automatic pipette so that the sub-sample gets sucked up too fast. This results in incomplete withdrawal of the aliquot. Always check that clumps of algae or a leaf fragment, etc., hasn't blocked the intake to the pipette.

- Errors in calculations for dilutions. These calculations must be done very carefully and checked because you can double or half the apparent concentrations very easily without knowing it.

- Non-uniform heat distribution within ovens and the muffle furnace. Ovens should have an internal fan to mix the heated air.

- Dehydration of the inorganic fraction. The ashing temperature has been set at a relatively low level of 400°C to help reduce the effect of dehydration of the clay fraction in the sample. However, if clays are a large fraction of the sample and precise results are required, then it is recommended that the mineral fraction is re-hydrated after ashing (but before final weighing). This is done by cooling the sample after ashing, adding a few ml of water to the sample, allowing it to stand overnight, drying again at 105°C for 4 h, cooling in a desiccator and then taking the final weight.

- Re-hydration of the organic component can occur after the initial drying step if the sample is not cooled in a desiccator. This can result in positive biases to the results. Ensure that the silica gel in the desiccator is fully dry.

- Weighing errors can occur. This is a particular problem when biomass is very low. Indeed, AFDM is not nearly as accurate as chlorophyll *a* as a measure of biomass at low levels. The magnitude of error introduced may be illustrated by taking the ratio of AFDM:chlorophyll *a* (both in mg/m^2, which gives the Autotrophic Index [AI] – see above), and making the assumption that the chlorophyll measurement is a more sensitive and accurate analysis. Healthy communities in unpolluted streams normally have an AI of 100–200. However, for healthy low-biomass communities dominated by, say, diatoms the error associated with weighing will usually result in spurious AI values of 0.1–2,000. Occasionally there appear to be net gains in biomass during ashing. Therefore, if you are attempting to discriminate differences among sites or treatments in an experiment where biomass levels are all low, then use chlorophyll *a* as the measure of biomass. Autotrophic indices should not be calculated for samples where AFDM < 2 g/m^2.

For overall errors associated with sub-sampling and analyses, Biggs (1987) reported mean coefficients of variation of 8.9% for AFDM based on 258 sets of analyses of three sub-samples for each variable. The samples covered a wide range of biomass and community types including diatoms, filamentous green algae and cyanobacteria.

Formal quality control procedures

The following steps should be included in formal QA/QC procedures for AFDM:

- Balances and temperatures for the ovens and muffle furnace should be calibrated at least annually.

- The calibration and volume settings on the automatic pipette used for sub-sampling should be checked before each batch of analyses by weighing sub-samples of distilled water on a precision balance.

- All sample labels should be checked and signed off as being clearly readable.

- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.

- Check the transcribing of sample numbers/labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet. This checking should be done by another analyst and signed off.

- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of

errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.

- For analyses where the results are likely to be used for a resource consent hearing or the Environment Court, three sub-samples should be analysed from each sample and the results averaged. If the value for any one sub-sample is > 2 x the mean of the 2 nearest sub-samples, then the outlier is considered to be a sub-sampling or analytical error and the result of the outlier should be discarded. For such analyses, you should also carry out the re-hydration step after ashing.

- Use QC charts to benchmark the analyses. With every new batch of analyses three sub-samples are included from low, medium and high biomass samples with known means and error. The sub-samples must fall within two standard deviations of the known mean for the batch results to be accepted. The following gives detailed instructions:

  - Collect three large samples (from the field from areas 2–3 times bigger than usual) covering low (e.g., < 10 g/m² AFDM), medium (20–40 g/m² AFDM) and high biomass (> 40 g/m² AFDM) communities.

  - Transport the samples to the laboratory and analyse while still fresh.

  - Blend the samples thoroughly as described in Section 7.2. Ensure that there is sufficient blended solution for removal of 30 sub-samples, each 15 ml (i.e., at least 450 ml of blended solution for each sample).

  - From each sample, remove and filter 30 sub-samples through pre-ashed filters as described in Section 7.4.3.

  - Randomly select five of these sub-samples and place them in labeled crucibles. Fold the filter papers for the remaining 25 sub-samples, place in individual small zip-lock plastic bags, and label each with a unique identifier consisting of the QC batch, biomass group, and sub-sample number. Store in a freezer at < –20°C.

  - Analyse the five QC sub-samples for AFDM as described above and calculate the results. Because the results of these analyses are then used to benchmark the QC checks in subsequent routine analyses, it is very important that they be carried out very carefully. It is recommended that two analysts work on carrying out the QC benchmark analyses, to review each other's manual operations (if possible, one analyst should have considerable experience), and fully check the calculations.

  - Prepare a QC chart for each of the low, medium and high biomass samples by calculating the mean and standard deviation of the five sub-samples from each sample and then plotting the mean and 2 x the standard deviation of the mean as lines across the chart (Figure 14).

  - Every time a batch of analyses is run, remove one of the QC sub-samples from storage for each of the levels of biomass and analyse these three sub-samples with the batch.

  - Plot the results of the QC sub-samples on the QC chart. The value should lie within the two standard deviation boundaries around the mean of the original five QC sub-samples. If one of the three QC results falls outside these limits you should re-check all your calculations and methodology. If there are no errors in the calculations flag the results for the whole batch of samples and check with the person using the results whether there is a requirement for high precision (in which case the batch should be re-analysed). If two or more of the QC sub-samples fall outside the 2 standard deviation limits and there are no errors in the calculations, then you
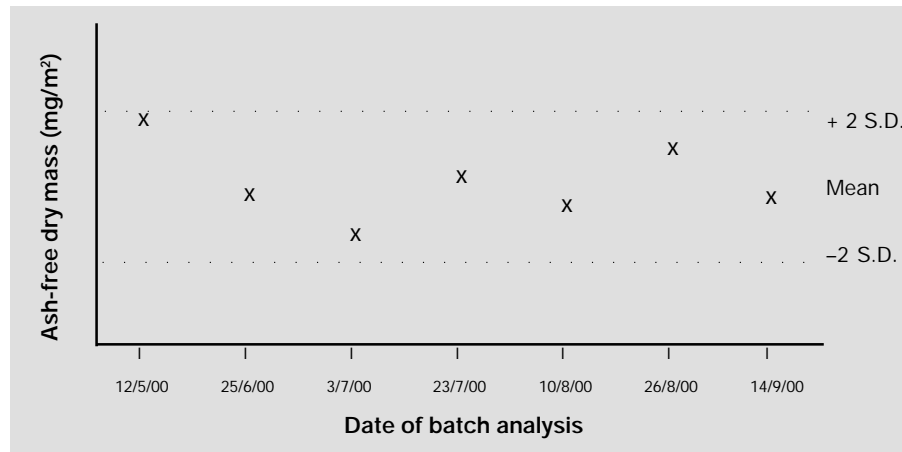
Figure 14: Example of a quality control chart for ash-free dry mass analysis. Preserved QC sub-samples from a given QC batch are analysed with each batch of project samples and the values are plotted and compared with the mean and 2 x the standard deviation (2 S.D.) for the initial analysis of the QC group of sub-samples.

should repeat the whole set of analyses for that batch as a matter of course.

• The scientist responsible for analysing the data and reporting must check the results and sign them off as acceptable. The balance of all samples should be retained (frozen) until the data has been approved, in-case re-analysis is required.

Notes:

• The frozen AFDM QC sub-samples should not degrade appreciably over time. However, look for any trends when you plot up individual results over time. If you detect a trend (expected to be downward) then a new set of QC sub-samples should be prepared. As a general rule, QC sub-samples should not be kept for more than a year.

• A good quality assurance system in a laboratory is dependent on feedback. Feedback between:

   - laboratory staff and laboratory manager, regarding problems with samples, techniques, etc.

   - laboratory staff and scientists regarding expected capabilities, variability, and expected levels for the results, and required levels of precision, etc.

• Feedback helps to ensure that:

   - the objectives of the project are being met;

   - variability of results is reduced and thus confidence in the results is maintained;

   - all participants in the analyses know what is expected of them in the QA system.

7.5   Biomass accrual and growth rate estimates

7.5.1   Net biomass accrual rates

It is often useful to determine the rates per unit area at which the periphyton community accumulates on substrates. This provides an approximation of growth rates in situations where losses due to factors such as detachment and invertebrate grazing are low. However, the term "accrual rate" is preferred over "growth rate" because the variable being calculated is area specific biomass change (not biomass specific or cell specific biomass change) and

reproduction rates are not accurately estimated. Net accrual rate measures the rate of accumulation of community biomass on the substrate per day (Biggs 1990, Stevenson 1996). Net accrual rates are particularly useful for assessing differences in the degree of enrichment of sites (e.g., to assess the effects of a waste discharge; see Biggs 1990) and to assess the effects of differences in habitat variables within sites (e.g., Biggs and Stokseth 1996).

Net accrual rates are most easily determined using artificial substrates, whereby a large number of replicate plates (say 20) are loaded onto a sampler at the beginning of the assay, then subsets (3–5 replicates) are randomly selected and removed at progressively greater time intervals (e.g., see Biggs 1990). Natural substrates can also be sampled if you can commence sampling immediately after a flood which has reduced stream bed biomass to near zero (e.g., see Biggs and Stokseth 1996). It is recommended that you sample at least four times during the exponential phase of growth to accurately define the rate of change of biomass (Figure 15). With artificial substrates in a stable flowing river, the time interval between sampling during the exponential phase may need to be as little as 3 - 4 days. However, the time to the first sampling can be much greater if colonisation is slow (e.g., soon after a flood disturbance when the pool of colonists is low). If possible, sampling should continue until peak biomass. This may only take several weeks in some spring-fed streams, but several months in some foothills streams if they have been recently disturbed by high flows (e.g., Biggs and Stokseth 1996).

Net accrual rate is calculated according to the following model:

$$B = a \exp(kT) \tag{9}$$

where B is the biomass measure per square metre at day T, a is the initial biomass concentration and k is the net accrual rate during the exponential growth phase. The way we calculate k is to perform a $\log_e (B + 1)$ transformation on the data which changes the exponential part of the accrual curve into a straight line (Figure 15, plot B). We then use these points on the curve in a regression of B against T (using a statistical calculator or statistical software). The resulting coefficient for T is the value we use for k. Low values for k are < ~ 0.10 and high values for k are >~ 0.35. Since k is the natural log exponent for the rate of change, it has no units except day^{-1}.

An example of accrual rate results used to determine the enriching effects of a discharge from a domestic sewage oxidation pond is given in Figure 16. Artificial substrate samplers were placed upstream and downstream of the discharge point in the South Brook near Rangiora, North Canterbury. The samplers were incubated for up to 15 days on three occasions. No significant differences were found between upstream and downstream accrual rates even though phosphorus concentrations were a factor of 10 higher in the downstream reaches (nitrogen was available in excess quantities). The results indicated that the waters had sufficient P to not be limiting accrual rates at both upstream or downstream sites and therefore that the discharge was unlikely to be stimulating periphyton growth. For more details see Biggs (1990).

While we usually use chlorophyll *a* as a measure of biomass accrual, it is also possible to use AFDM and cell densities. If cell densities are used, and immigration, emigration, death and consumption losses can be quantified (or estimated), then the approach outlined above can be used to calculate growth rates for specific taxa (see Stevenson 1996 for more discussion on this topic).

7.5.2   Specific and relative specific growth rates

If losses by emigration, death and invertebrate grazing appear to be minimal (as usually

Figure 15: Idealised periphyton biomass (*B*) accrual curve (as chlorophyll *a*) on artificial substrates for (A) untransformed chlorophyll data, and (B) log$_e$ transformed data. Each data point represents the mean of 5 replicates collected for different lengths of accrual time (T). PB = peak biomass for the accrual cycle, T$_{PB}$ = time to peak biomass.
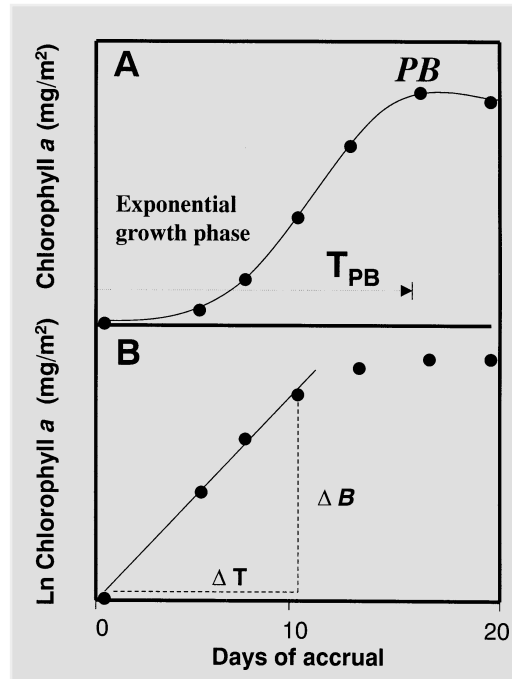
Figure 16: Periphyton chlorophyll *a* accrual curves (and 95% confidence limits) for three trials to test the effects of an oxidation pond discharge on periphyton accrual in the South Brook, North Canterbury (•, above the discharge; ▲, below the discharge). Trial 1: Upstream *k* = 0.180, Downstream *k* = 0.338; Trial 2: Upstream *k* = 0.351, Downstream *k* = 0.310; Trial 3: Upstream *k* = 0.411, Downstream *k* = 0.385 (reproduced from Biggs 1990 with permission of the *New Zealand Journal of Marine and Freshwater Research*).
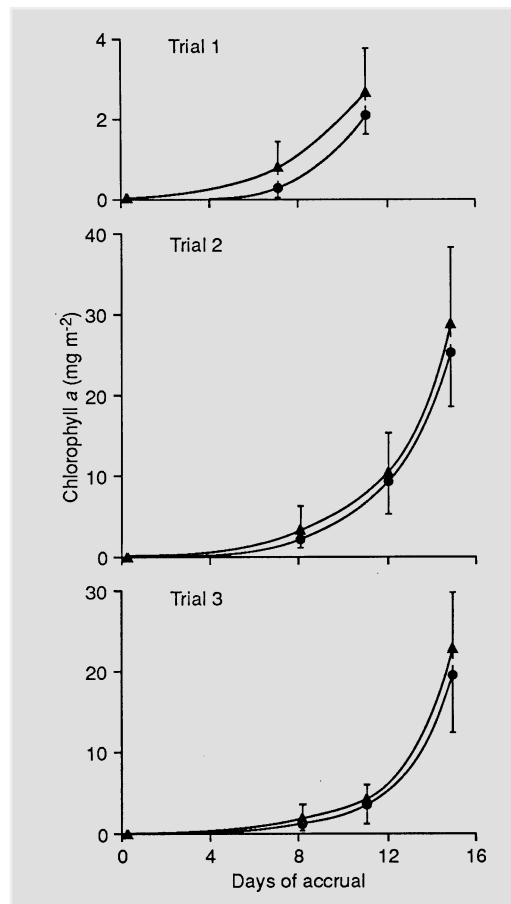
occurs on artificial substrates raised off the stream bed in slow moving water), then specific growth rates (μ) can be calculated. To do this we use the value for *k* derived from analysis of chlorophyll *a* accrual in the preceding section and apply a correction to convert the value to $\log_2$:

$$\mu = k/0.693 \tag{10}$$

where μ is expressed as cell divisions per day (Bothwell 1988). This is the measure for growth more commonly used in phytoplankton studies.

The advantage of calculating μ is that it is then possible to use μ as a nutrient availability indicator by normalising μ to μ_{max} – the nutrient-saturated, maximum specific growth rate. Goldman (1980) promoted the use of this concept as a means of removing the effects of temperature on algal growth rates and thus to isolate growth as a function of limiting nutrient concentration (this is because growth is mainly a function of temperature if nutrients and light are not limiting). We can estimate the P-saturated maximum specific growth rate as a function of temperature using the model of Bothwell (1988):

$$\mu_{max\text{-}P} = 0.189 + 0.0278 \leftrightarrow \text{temperature (°C)} \tag{11}$$

The "P" subscript with $\mu_{max\text{-}P}$ denotes the communities used to calculate the relationship were P-unlimited, but some slight limitation by other nutrients could not be ruled out by Bothwell (1988). If one assumes that growth is unlimited by any other nutrients, then the degree of nutrient limitation is simply calculated as the relative specific growth rate:

$$\mu{:}\mu_{max\text{-}P}. \tag{12}$$

Bothwell (1985, fig. 3) provides data which indicate that P-deplete relative specific growth rates occur when $\mu{:}\mu_{max\text{-}P}$ is < 0.3, and P-replete growth rates occur when $\mu{:}\mu_{max\text{-}P}$ is > 0.8. Biggs (1990) used a series of other nutrient limitation assays such as alkaline phosphatase activity, cellular nutrient quotas and phosphorus uptake rates to test the applicability of this method for enrichment monitoring in New Zealand streams. While highly nutrient-limited conditions did not occur in the test communities, the results for moderately P-limited and P-saturated periphyton communities conformed well with the criteria of Bothwell (1985).

Note that in making these calculations we assume that nutrient limitation is the only factor affecting biomass reduction. However, other factors – such as light, grazing and emigration – could potentially reduce biomass independently. Therefore the conversion of biomass accrual to relative specific growth rates and the use of these as indicators of nutrient limitation should be used with caution.

8 Taxonomic analyses

8.1 Overview of taxonomic analyses

Finding out what taxa comprise a periphyton community is fundamental in most periphyton studies. It sets the scope for interpretation and evaluation in any resource or pollution monitoring investigation. For some investigations this can be adequately carried out in the field using small portable microscopes (particularly where communities are dominated by distinctive macro-algae). However, for most investigations it will be necessary to bring samples back to the laboratory for analysis. Methods for sampling will vary depending on the objective of the work as discussed in Sections 2 and 6.

Where possible, samples should be returned to the laboratory and analysed fresh if any taxa except diatoms are of interest. This is because the colouring and cell shape in many "soft-bodied" taxa is lost with preservation. Where analysis is delayed for less than 48 h it is usually adequate to store samples in a cool, dark place. Most commonly, though, it will be necessary to preserve samples for later analysis. Longer preservation is easily achieved with preservatives or through freezing. Freezing can result in the contraction of some organelles such as chloroplasts in *Spirogyra*, but this is generally easier and less of a health risk than many other methods based on toxic preservatives such as gluteraldahyde. Freezing also enables later analysis of chlorophyll *a*; this is not possible if chemical preservatives are used. For diatoms, sub-samples may need to be cleaned in acid or peroxide to remove organic coatings and clearly reveal the morphology of the silica frustule, from which detailed identifications are made. As with biomass analyses, sub-sampling is usually carried out after the samples have been homogenised to break up large clumps and very long filaments (see Section 7.2). Sub-samples of some green algae may need to be stained to assist with identification of important bodies within the cells such as starch granules.

There are several possible levels of enumeration. One is a semi-quantitative determination of the relative abundance of taxa based on their contribution to sample biovolume. A rapid scan technique is used for this. This method does not allow for the calculation of densities of the different taxa, or diversity indices, but it does allow a robust assessment of what is mainly contributing to overall community biomass (which is usually also measured using chlorophyll a or ash-free dry mass). The method is based on the Braun-Blanquet approach that is widely used for terrestrial vegetation analysis (e.g., see Kershaw 1973).

A second method is fully quantitative and involves a detailed count of the number of individuals of each taxa. This enables the calculation of cell densities (usually expressed as number of cells/mm²), diversity indices and the proportion of individuals of different taxa in a given sample. For this method, sub-samples are systematically scanned and all cells counted in a series of randomly located fields until 300 cells have been enumerated. A calculation involving the area of the sample, the proportion of the sample used as a sub-sample, and the number of fields analysed then enables densities to be determined. It is often necessary to move to a further level of detail by calculating the size/biovolume of each of the common taxa and multiplying the densities of individual taxa by their mean biovolumes so that the data are appropriately weighted by the proportion of the biomass made up by each taxon. This step is usually necessary because of the great range in sizes of the different taxa.

Quantitative analyses, however, are very time consuming. An average analysis, for example, will generally take 1–2 hours depending on how much silt is in the sample and how familiar you are with the taxa. Because of eye strain while working on the microscope, and the high

potential for developing repetitive strain injuries, it is unwise to spend more than six hours a day doing analyses. Thus, it can take a considerable time (and therefore expense) to analyse a group of 50–100 samples. In practice, we are often only interested in the most abundant taxa and somewhat less than four taxa (often only one or two) usually comprise > 70 % of biomass in samples from most New Zealand gravel bed streams. Therefore, we most frequently use the semi-quantitative method. This method is up to six times faster than the cell count - biovolume method which then allows many more samples to be analysed and a better range of information to be generated than detailed enumerations for a given budget. The semi-quantitative method is appropriate for impact assessment and state of the environment reporting, where major differences in periphyton are expected between sites or sampling dates. However, there can be inter-operator variations in evaluations.

It is highly recommended (and an essential part of a formal quality assurance system: see Section 8.7) that every laboratory develops a photographic register of the taxa that have been encountered and the taxonomic designations given. Attach the photographs to filing cards and note on the cards the literature source used to obtain the designation. This will enable you to be consistent with your designations and to have them checked by an expert. Indeed, in taxonomic analyses it is essential that identification of taxa is done based on consistent criteria. It is also good practice to have a voucher collection of preserved sample from each study so that identifications can be verified in the future.

We recommend that you do a preliminary scan of samples if they have been collected from a site not previously sampled, or you are expecting some changes in community composition. During this scan it is a good policy to attempt to identify every cell to the lowest taxonomic level you can. If you distinguish two taxa which you later find are the same, then it is easy to combine them. However, the reverse is not possible. Always photograph any new taxa that you encounter and add them to your photographic library so that there is a record of your designation. If you discriminate one taxon as being clearly different, but cannot determine a species or genus name, then identify the specimen to the lowest level you can and give it a unique number (e.g., *Phormidium* sp. 1).

8.2   Sample preparation

Most periphyton samples will contain clumps of filamentous algae or diatoms. This creates difficulties in sub-sampling to obtain representative portions for analysis. Representative sub-sampling is essential for accurate assessment of periphyton communities. Homogenising the sample with a simple, hand-held laboratory or kitchen blender can significantly reduce variability in sub-sample analyses, thus increasing the precision of the results. Biggs (1987) found that the assessed density of cells in a periphyton sample could increase by up to 500 % and coefficients of variation decrease by nearly 90 % following blending compared with just shaking the sample to achieve mixing. The highest increase in precision can be expected with samples that are dominated by filamentous algae. The blending process generally does not greatly damage cells, although some colonial species tend to break apart and long, and lightly silicified diatoms and chrysophytes can be damaged. Most filamentous taxa break apart into much smaller fragments at inter-cellular connection points. Certain green filamentous algae do not break apart easily (e.g., *Cladophora*) during blending and tend to get wrapped around the cutting blades. The use of sharp blades on the blender, only a small volume of sample, and a slightly longer blending time usually overcomes this problem (Biggs 1987).

8.2.1   Equipment

1. Glass beaker.

2. Stoppered bottles with narrow necks (marked at 50 ml intervals).

3. Kitchen or laboratory tissue blender.

4. Squirt bottle with distilled water.

8.2.2   Procedure

- Tip the contents of the sample container into a glass beaker (the width of the beaker should be only slightly greater than the width of the housing holding the cutter blades of the blender). Rinse out any sample residue from the container and lid into the beaker. Distilled water is preferred for rinsing and making up the sample volume. However, tap water can be used providing the water isn't heavily chlorinated.

- Pick out any invertebrates, pieces of gravel, leaves, moss, etc. from the sample.

- Ensure that there is enough water to fully cover the blender-blade housing.

- Blend for about 30 seconds or until the mixture is free of obvious clumps of material. If the sample contains much filamentous algae, break the strands up by repeated cutting with a pair of sharp scissors. Every 10 seconds, or so, stop the blender and free any filaments that may have become caught on the blades or blender housing using a squirt of water. You need to end up with a homogeneous solution suitable for sub-sampling.

- Transfer the blended liquid to a stoppered bottle. Rinse out the beaker and make up the solution to a known volume (usually 50, 100 or 150 ml). Do a quick calculation of how much solution is required for all your sub-samples (variables to be analysed X aliquots ↔ replicates) and ensure that you will have at least 50% more volume than this to allow for re-analysis of variables where there may have been some analytical errors or problems – see Sections 7.3.6, 7.4.6 and 8.7. Enter this volume onto your lab analysis sheet.

- If the sample is frozen between blending and different sets of analyses, then it should be re-blended briefly before sub-sampling for the next analysis (freezing tends to coagulate some filamentous and mucilaginous periphyton).

8.3   Relative abundance assessments using an inverted light microscope

In the following, we describe a relative abundance method based on scores ranging from 8 ("Dominant") to 1 ("Rare"). This method is used for a rapid evaluation of community composition and the main taxa comprising biomass.

8.3.1   Equipment

1. Inverted microscope with a 12.5X eyepiece and 10X, 40X and 63X objectives.

2. Ocular and stage micrometers for cell measurements.

3. 0.5–2 ml automatic pipette with a ~ 2 mm wide bore at the tip to allow the free passage of cells.

8.3.2   Procedure

- Blend your sample to get a homogenous solution as described in Section 7.2. If the sample has already been blended and then stored frozen, we recommend that you re-blend it. Freezing can result in coagulation of the cells (particularly filaments).

- Transfer the freshly blended sample to a narrow-necked bottle.

- The sub-sample for analysis comprises at least three smaller aliquots of the sample which are pooled. To take these aliquots, shake the bottle well and withdraw 2 ml with an automatic pipette from half-way down the solution while the liquid is still moving. Check that the liquid is not swirling around the perimeter of the bottle - this will cause density separation of the cells and prevent the removal of a representative sub-sample. Release the sub-sampled solution into the settling chamber. Repeat this procedure twice more to give the full sub-sample (i.e., the total sub-sample volume = 6 ml).

- Attempt to obtain a thin covering of cells on the bottom of the settling chamber – not so thick that they overlap frequently.

- Ensure that the cells are evenly distributed across the entire bottom of the settling chamber. This requires some practice and can be done by gently shaking the chamber from side to side. Don't swirl the sample as it will just result in the cells being redistributed to the perimeter of the chamber.

- If your first sub-sample is too dense, then dilute the sample by 2x or 4x, or whatever is appropriate. Rinse out the chamber with water then sub-sample again.

- If the first sub-sample is too dilute, then leave the sample to settle for several hours (most periphyton cells are relatively heavy and settle quickly) then decant off the supernatant to reduce the original volume by half or quarter, or whatever is appropriate. The amount of dilution or concentration of the cells is unimportant for this type of analysis.

- Allow the sample to settle in the microscope chamber for 2–5 minutes.

- First, scan the whole sub-sample at low power (about 100x) and write down the names of taxa you can see. You may get an idea at this stage of which contribute most to the volume of cells. Only consider those cells containing chlorophyll (i.e., live cells), but the diatoms are often easier to identify to species level by viewing dead cells of a similar size and shape from which the organic matter has decayed.

- Scan again at dry high-power (400–700x), check your original identifications made at low power and note down the small taxa present.

- Rank the main taxa in decreasing order of importance of their contribution to the volume of cells in the chamber relative to the most common taxon. The DOMINANT taxon contributes the most volume to the sample. Note that you can make 2 or even 3 taxa CO-DOMINANT if you find it impossible to decide on a single one being dominant.

- Rank all the other taxa you have listed in relation to the dominant taxon (taxa) as follows:

8	Dominant
7	Abundant
6	Common – abundant
5	Common
4	Occasional – common
3	Occasional
2	Rare – occasional
1	Rare

Notes:

- You don't have to include all ranking categories in every assessment. For example, if a sample is overwhelmingly dominated by a single taxon, and the next most common one is much less common, then you should rank the second one as "common", for example, rather than abundant.

- The number of ranks we recommend is quite high (i.e., 8). We have found that with practice it is quite easy to use an 8-rank scale and gives much more information (it is also more satisfying as an analyst). High numbers of ranks are also advocated, and used, for modified Braun-Blanquet assessments of terrestrial vegetation cover (e.g., see Kershaw 1973, Table 1.5). However, some analysts for varying reasons, may prefer to use fewer ranks (e.g., 3; rare, common, abundant). This is quite acceptable. In general terms the equivalence with our scale would be as follows:

Recommended ranks		**Alternative (reduced) ranks**	
8	Dominant	3	Abundant
7	Abundant		
6	Common – abundant		
5	Common	2	Common
4	Occasional – common		
3	Occasional		
2	Rare – occasional	1	Rare
1	Rare		

- If adopting a reduced ranks system, then it is important that you still use the enumeration protocol defined in the preceding method (i.e., the taxa/taxon that dominate the biovolume of the sample are given a ranking of 3 – Abundant, and everything else is ranked in relation to this score). The use of a reduced, but semi-quantitative, ranking system has been advocated by some workers. In such an approach, the above ranks are intuitively given boundaries in terms of absolute cell densities (i.e., a few cells = rare, dense cells = common, too dense to count = abundant). However, we strongly discourage this approach because it is almost impossible to keep a mental benchmark of absolute densities among samples, sampling occasions and analysts. It also doesn't take into account different sample sizes. Such an approach would only be possible if a fixed area of substrate is always sampled; the sample and sub-sample volumes are always identical, and a system is setup where by taxa are ranked according to a frequency of occurrence for a given number of microscope fields. If you are going to this trouble then you are better off doing a quantitative assessment, but with a reduced count (say, 100 cells).

- When using size as a diagnostic feature, refer to identification texts for typical sizes. The microscope should have an eyepiece micrometer and you should keep handy a conversion table which lists units on the scale and their equivalent actual lengths for the magnifications.

- Refer to Section 10 (an identification guide to common periphyton taxa in streams and rivers) to assist with identifications. See Section 10.5 for a selection of other published texts.

- If resources allow, you should complement relative abundance assessments with some quantitative measure of biomass such as chlorophyll *a.*

- If you are a novice, repeat your analysis on the same sub-sample and then different sub-samples until you are confident that you are getting consistent results (see QA/QC procedures).

- The most efficient way to record the scores is to use a standard taxa list. These can be made up for a range of monitoring programmes and you should select a list made up from samples in the region being monitored. Leave spaces on the sheets to add new taxa as required. With such recording sheets you end up with a matrix of sites vs. periphyton taxa, with blank cells wherever a listed taxon did not occur in a sample.

- It is preferable to use fresh material for microscope assessments of periphyton. However, this may be impractical due to the need to process blocks of samples that have been collected elsewhere or over time. Such samples usually require storage (freezing or preservatives) to prevent decay.

- Sub-samples may be preserved in gluteraldahyde, which retains the cellular form of both green algae and diatoms (see Section 6.9.2).

8.4   Quantitative analysis (cell counts) using an inverted or compound microscope

The quantitative microscope method involves counts of cells in a known number of microscope fields in a sample and sub-sample of known volumes. It provides the most accurate assessments of the taxonomic composition of a periphyton sample, providing enough cells are counted and that sub-sampling is representative. Its drawback is that it is very time-consuming (therefore expensive). In addition, the level of detail obtained from a full count is often superfluous.

This quantitative method may be employed using an inverted microscope or a normal compound microscope. The difference is in how the samples are prepared for examination. In general, if there are large quantities of filamentous taxa (e.g., *Cladophora*, *Vaucheria*, *Batrachospermum*) it is recommended that an inverted microscope be used because many of the filamentous cells are so large that it is difficult to mount them properly for examination with a compound microscope.

In brief, an aliquot of the homogenised sample is placed into a settling chamber (inverted microscope) or Palmer-Maloney chamber (compound microscope). The cells settle to a glass plate which forms the bottom of the chambers. This base plates are the thickness of a cover-slip. For the inverted microscope, up to 5 ml of sub-sample is placed in the counting chamber. The objective lens is oriented to capture the image from below the chamber and therefore permits the examination of cells and filaments that have settled on the glass base-plate without disrupting them. For the compound microscope, the objective looks down through the sample in the Palmer-Maloney chamber. This chamber is much smaller than for an inverted microscope (it is only as deep as a microscope slide) so a much smaller sub-sample is used (e.g., 0.1 ml). With both techniques it is possible to examine the samples at up to 790x magnification using dry lenses which is sufficient to identify all soft-bodied algae and most diatoms. However, for samples with large quantities of very small diatoms, >1000x magnification is required with oil immersion lenses. This is possible with the inverted microscope, however, it is recommended that such enumerations be carried out after samples have been cleaned and mounted on a slide (see Section 8.5), so that oil immersion analysis using a high power compound microscope can be used. This will give much greater optical resolution and,

therefore, greater certainty with identifications.

Cells and 10 μm long units of filamentous taxa with very small cells (mainly cyanobacteria) are counted in fields that are located according to a set of random numbers and a stage grid. Counting in strips across the microscope chamber has often been carried out, but is not recommended here because random fields are less prone to error due to non-random settling of cells in chambers (Sandgren and Robinson 1984). We are recommending that a 300 cell/ unit count be carried out as the standard for quantitative enumeration. This number of cells/ units and random fields gives good reproducibility/precision, is in line with the standard U.S. Geological Survey protocol for their National Water-Quality Assessment programme (S.D. Porter, US Geological Survey, pers. comm.), and has been recommended in most recent methods descriptions in the international literature (e.g., Lowe and LaLiberte 1996).

8.4.1   Concentrating and diluting samples

Ideally, you want about 30–50 cells per field at 500x magnification for efficient and/or accurate quantitative enumeration. Often the cells in the samples will be too dilute, or too concentrated. In such cases it is necessary to alter the volume of water that they are suspended in after the sample has been homogenised to give a "workable" concentration of cells. It is usually best (but not critical) to perform dilution or concentration of the samples before any sub-sampling (e.g., for analysis such as chlorophyll *a*).

Dilution is simply achieved by adding more water to the sample, while concentration is achieved by siphoning off the supernatant after the sample has been left to settle for at suitable time in a measuring cylinder (usually allow about 1 hour per centimetre of settling depth). When transferring the sample to the measuring cylinder, make sure that you thoroughly rinse the original container to remove cells adhering to the walls. It is useful to add or dilute water in multiples of the original sample volume (e.g., doubling or halving the volume). This makes it much easier to perform calculations for cell density and to calibrate your eye on the microscope when determining how much dilution/concentration a sample might need. Always record the final sample volume.

If you are already working with a sub-sample that has been previously removed from a larger sample (e.g., used previously for biomass analyses), then we treat the sub-sample as "the sample" in terms of volumes and calculations. This is because the concentration of cells in the taxonomic "sample" should be the same as the original, larger, sample (assuming that it was properly homogenised before sub-sampling).

8.4.2   Materials

1. An inverted microscope or compound microscope with 10x, 40x, 63x and 100x objectives and 12.5x oculars.

2. An ocular micrometer and calibration slide for measuring cell size.

3. Settling chambers (generally 5 ml is adequate for inverted microscopes) or a Palmer-Maloney chamber for a compound microscope.

4. Settling board or leveled table. A settling board can be made easily by inserting a leveling screw in each corner of a 20 cm x 20 cm flat board. The screws are adjusted until the board is level. Check with a small spirit level.

5. Pipettes: 1 ml serological, or accurate automatic pipettes with a ~ 1 mm wide bore at the tip to allow the free passage of cells.

6. Measuring cylinder (~ 1 litre) (required only if a sample needs to be concentrated).

Notes:

- The inverted microscope settling chamber is a plastic or glass cylinder of variable height (the height determines the volume of sub-sample that can be settled) and that screws into a metal ring that fits into a movable stage of the microscope. The metal ring has a lip on which a thin glass base-plate sits (equivalent to the cover slip of a compound microscope). The cylinder is then screwed into the base-plate glass to make the settling chamber. Silicone grease or vaseline may be required to make a water-tight seal between the cylinder and base-plate. The base-plates are easily broken when the chambers are being washed, so ensure that you always have a good supply of plates from the microscope supplier.

- Palmer-Maloney counting chambers can be purchased from specialist biological instrument suppliers (check the Web for your most convenient source or try the "Biology Shop" in Australia; fax: +61 247 586 806). They can also be fabricated easily by making a clean hole in the middle of a plastic microscope slide using a cork borer, then gluing a cover slip to the underside. A small cut is made on the edge of the well across the lip to allow excess water to escape when sealing off the top of the sample with a second cover slip. Calculate the volume of the chamber from its dimensions.
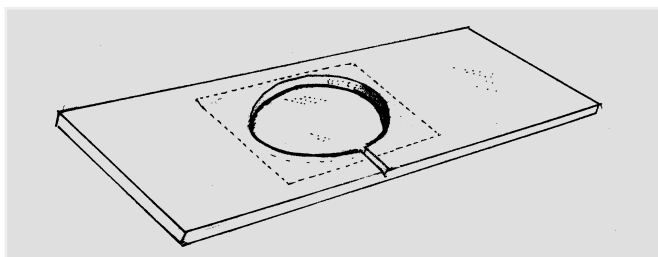
Figure 17: Palmer-Maloney counting chamber.

8.4.3   Preparation procedure

- Blend the sample to get a homogenous solution (see Section 7.2).

- Transfer the sample to a narrow-necked bottle that is graduated for volume measurements. Rinse any residue from the original container into the bottle.

- Record the volume of the blended sample. This is essential for calculating cell densities.

- For the inverted microscope, shake the sample and using a precision pipette remove a 0.1 – 0.5 ml aliquot from half-way down the sample and place it into the centre of a clean settling chamber. Repeat this step twice more (shaking the sample between each) to give a total sub-sample of three pooled aliquots in the chamber. You may need to add a few extra drops of water to the chamber after the sub-sample to give enough liquid for the cells to be evenly distributed (Note: this extra liquid doesn't affect the volume calculations for density estimates). Shake the chamber from side to side to distribute the cells. Be careful not to swirl the chamber because this will re-distribute the cells to the perimeter of the chamber. Record the total sub-sample volume.

- For the compound microscope, shake the sample and using a precision pipette remove a 0.1 ml aliquot from half-way down the sample and gently place it into the Palmer-Maloney chamber. Then slip a cover slip over the chamber ensuring that there are no air bubbles trapped under the cover slip. Record the sub-sample volume.

- Allow the sample to settle for 2 – 5 minutes in the chambers.

Notes:

- Try to obtain a *thin* covering of cells on the bottom of the chambers – not so thick that they overlap frequently. Check the density of cells. If your first sub-sample is too dense, then dilute the sample as described above (ensure that you record the new sample volume). Rinse out the chamber with water then add a sub-sample from the diluted sample. Don't forget to shake the sample between the withdrawal of each aliquot. Re-record the sub-sample volume.

8.4.4 Examination procedure

- First scan the whole sub-sample at low power (about 100 x). Check the distribution of cells to ensure that they are reasonably evenly spread across the base plate. If they aren't, then gently shake the chamber from side to side to re-suspend the material then allow to settle. Avoid swirling.

- If the cells are evenly distributed, then continue with the analysis.

- Change to a magnification of 400–500x. This is adequate for most enumerations.

- Locate your first field for the cell counts using a random number table: one number to give the horizontal setting and one number to give the vertical setting on the movable stage. (These numbers can be generated from most scientific calculators or obtained from statistics texts. Compile a table that covers the range of numbers and degree of movement on the stage rack so that all positions are within the area of the base-plate. If there is a low density of cells then you will need many pairs of random numbers. This table can be part of your laboratory bench data sheet as shown in Table 4).

- Count all cells for each taxon within the first field. Only count those cells with chloroplasts (however, the diatoms are often easier to identify to species level by viewing empty frustules of a similar size and shape from which the organic matter has decayed). Only include cells which are > 50% within view. For filaments with very fine cells that are difficult to discern (mainly filamentous cyanobacteria), count the number of 10 µm long units.

- When all cells/units in the field have been enumerated, use another pair of random numbers to locate the next field. Count all cells as for the first field. Continue this procedure until at least 300 cells/units have been counted.

- Record the number of fields examined.

Notes:

- Set out your lab bench sheet so that it is easy to keep a running check on the total number of cells/units that have been counted (e.g., groups of five ticks).

- If you reach 300 cells/units before a full field has been completed then carry on to finish the field. This is necessary so that the densities can be accurately determined. Counting more cells/units than the recommended number just improves the precision of the density estimates.

- It can be useful to also count the number of empty (dead) frustules. However, these should not be included as part of the 300 cells/units for the primary analysis.

- Instead of manually logging your counts, there are several software programs that run on laptops to allow you to register counts as you examine the samples (e.g., see www.bgsu.edu/department/biology/algae/count/count.html). These programs give a range of running statistics such as the total number of cells/units, diversity indices and confidence limits on the counts. However, it is relatively easy to develop your own

Table 4: Example of a laboratory sheet for quantitative taxonomic analysis of periphyton samples. The following are entered: sample ID, sample and sub-sample volumes; sub-sample number. Each taxon is entered as it is encountered and the number of cells or 10-μm long units of fine-celled filaments in each field is registered as ticks. These ticks are sorted into groups of five for ease of summation. Each set of random numbers for the stage settings is crossed out as it is used. This enables the total number of fields that have been counted to be determined. This number is entered at the end of the analysis.

Sample/ Lab. ID	Sample (ml.) = Sub-sample no. = Sub-sample (ml) = No. of fields =	Comments:					
Stage setting (x, y)	Taxa	No. cells/units					
		5	10	15	20	25	30
39, 113							
41, 113							
39, 108							
29, 112							
43, 113							
39, 109							
40, 114							
31, 110							
35, 121							
29, 109							

Etc.

customised entry systems using Microsoft Excel and Access if you are familiar with these programmes. Direct entry onto spreadsheets or databases is a significant time saving and reduces the potential for transposition errors.

• Permanent mounts of samples dominated by soft bodied taxa can also be made and used for quantitative analysis using Taft's media (see Lowe and LaLiberte 1996 for instructions on preparing Taft mounts). It is not recommended that quantitative analyses be carried out under a compound microscope by just placing the coverslip directly over the sub-sample on a slide. This usually results in the material being forced out the sides of the coverslip, thus changing the volume of the sub-sample to an unknown degree which can introduce large errors in the analysis.

8.4.5 Calculations

The density of cells for given taxa or total cell density is calculated as follows (all volumes in ml):

Cells per sample =

(total number of individuals x sample volume x area of the counting chamber or base or coverslip)

/ (area of each field x number of fields counted x volume of sub-sample)

If whole stone sampling was used (i.e., sampling Method 1a; Section 6.5.4), you need to calculate the surface area of stone from which the samples were obtained:

$$\text{Stone surface area (mm}^2) = [1.59 + 0.811\ (xy + yz + xz)] \times 100$$

Where x, y, and z are the lengths of the 3 main axes of the stones in centimetres. The "× 100" is to convert the area from cm² to mm². This formula has been adjusted to only include the area of the stone normally protruding into the water on which the periphyton can colonise (~ 65 % of the total surface area).

If samples have been collected from a set area on top of the stones (Quantitative Methods 1b, 2 and 3: Sections 6.5.5, 6.5.6, 6.5.7), or from artificial substrates (Section 6.6), then calculate the surface area of the individual replicate in mm, then multiply by the number of replicates collected.

Finally, calculate the density of cells follows:

$$\text{Cell density/mm}^2 = \text{Cells per sample/area of sample (mm}^2).$$

8.5 Procedures for cleaning diatoms and making permanent mounts

Some diatoms (particularly very small taxa) will be difficult to identify with confidence from fresh or frozen material, even under high power dry objectives. In particular some genera such as *Nitzschia* sp., *Synedra rumpens*, and some *Gomphonema* spp. can be difficult to distinguish. For these cases it is advisable to clean and mount a sub-sample of the algal suspension for examination under an oil immersion objective at ≥1000× magnification.

8.5.1   Cleaning methods

Ashing

The ashing method is relatively straightforward and suitable for samples that comprise mostly diatoms and little other organic material. It is also good for preparing fine or delicate diatom frustules (particularly plankton samples).

Equipment:

1. Muffle furnace.
2. Pipettes (disposable glass droppers are fine).
3. Forceps.
4. Coverslips (No. 1 thickness).

Procedure:

- Pipette a few drops of the well-mixed suspension onto a clean coverslip.
- Sit the coverslip on a slide (with the solution facing up) and view under 40× magnification to determine whether the dilution or concentration of the sample is necessary to easily observe the cells for the enumeration.
- Once the appropriate density has been determined, allow the sub-sample to air-dry on the coverslip overnight.
- Using fine forceps, transfer the coverslip to a crucible and ash at 400°C for about an hour. This burns off the organic matter leaving behind only the silica frustules ("skeletons") of the diatoms. Allow to cool. Alternatively, heat the coverslip on a hotplate at about 600°C for up to 10 minutes.

Acid cleaning

Many samples will contain all sorts of material as well as diatoms – other algae, detritus, plant fragments. In these cases, cleaning with acid (or some other strong oxidising agent) is recommended because it is more effective at removing the larger amounts of organic matter without leaving an ash residue. There are numerous methods involving the use of various types of acid, and hydrogen peroxide, with and without heating. We have found the following to be effective for most periphyton samples.

Equipment:

1. Concentrated sulphuric acid.

2. Hydrogen peroxide.

3. Clean glassware (tall beakers or conical flasks, 600 to 800 ml).

4. Fume hood.

5. Distilled water.

6. No. 1 cover slips.

7. pH papers.

8. Glass transfer pipettes.

Procedure:

- Allow the sample to settle for a few hours, then drain off as much water as possible.

- Place a small amount of sample – say a quarter of a teaspoonful – in a labelled container. You could allow the sample to dry out further at this stage.

- Working under a fume hood, add a small amount of concentrated sulphuric acid to the container. The amount you use depends on how much material you have. Usually 10–20 ml should be sufficient.

- Add a little ~25% hydrogen peroxide. Both additions will set off a reaction, with generation of heat and sulphur dioxide. Keep adding a few drops of hydrogen peroxide until there is no more fizzing.

- When the reaction has completely subsided and the container has cooled down, top up the container with distilled water and leave to settle.

- Rinse the residue of diatom valves until the supernatant is neutral. This normally takes up to eight changes of distilled water. After each addition, allow the residue to settle for at least 4 hr, then carefully pour off as much supernatant as possible without losing any of the settled sample. Top up the container with the next rinse of distilled water and repeat until the pH is 7.

- Using a forceps, pick out any remaining large fragments.

- The rinsing process can be speeded up by centrifuging the sample between each rinse. However, this could possibly damage some diatom valves.

- When rinsing is complete, drain off as much supernatant as possible. Transfer the sample to a smaller container and continue to draw off water until the sample is at a suitable concentration. Check this by examining a small drop (on a slide) under the microscope.

- Pipette the samples onto coverslips ready for drying and mounting (see Section 8.5.2). Pipette on enough sample to cover each slip but not so much that the surface tension is broken.

Notes:

- Always take safety precautions when handling sulphuric acid and hydrogen peroxide. Use protective clothing including gloves and facemask. Always carry out the cleaning under a fume hood.

- The heat generated in the reaction causes most diatom frustules to dissociate into their two valves.

- It is advisable to use a new transfer pipette for each diferent sample, to prevent cross-contamination.

- The slips can be arranged on trays for drying and should be carefully labelled.

- If you wish to keep the cleaned sample for any length of time, add 95% ethanol to the storage container – about equal in volume to the sample. This will prevent fungal growths.

8.5.2   Mounting

Equipment:

1. Slide mounting medium (Naphrax – this is available from only a few outlets world-wide. One is: Northern Biological Supplies, U.K.; fax +44 1473 612 148). Instructions for preparation of the Naphrax with toluene come with the product.

2. Clean slides.

3. An electric hotplate, preferably with no ridge around the perimeter.

4. Glass dropper (for dispensing Naphrax)

4. Forceps.

5. Slide labels and/or permanent marker (e.g., a diamond-tip glasscribe).

Procedure:

- Drop a small amount of Naphrax (in toluene) fixative onto the centre of a clean glass slide.

- Place the slide onto a hotplate at ~ 120ΥC and heat until the Naphrax–toluene solution begins to bubble gently.

- Place the coverslip (with ashed or cleaned diatoms facing down) onto the Naphrax–toluene solution and ease down very gently.

- Continue heating until bubbling subsides then remove the slide from the hotplate.

- Gently press down the coverslip with forceps until all air bubbles have been squeezed out.

- Allow to cool then label the slide.

- Examine the slide under oil immersion as described in Section 8.4.

Notes:

- Always ensure that all glassware is kept clean. Dried diatoms can easily stick to slides, beakers, etc. and then contaminate other samples. Also, keep laboratory benches and preparation areas clean and free of dust. This dust could also include diatom frustules.

- *Always* use No. 1 coverslips (the thinnest available). This is especially important if you are intending to use a 100X objective because of the very small working distance between the sample and the lens.

- Heating the Naphrax drives off the toluene solvent after which the mountant hardens.

Some practice will be required to judge the right amount of heating necessary. Too little, and the Naphrax stays sticky; too much and the mountant becomes yellow and brittle.

- Toluene is toxic (carcinogenic). When mounting slides, always have an extractor fan above the hotplate or use a facemask.

- When processing multiple samples, ensure that slips, crucibles and slides are correctly labeled throughout.

- Slides prepared in this way can form the basis of a reference collection of samples from different sites and at different times.

8.6   Biovolume estimates

The most meaningful quantification of periphyton communities is given by converting density information into biovolumes. This enables a more accurate analysis of the biomass dominance of the different taxa. For example, a filamentous green alga may dominate a site in terms of the biomass (and what you see), but have fewer cells than taxa such as very small diatoms in the sub-canopy. By calculating representative biovolumes for a sample of each of the main taxa, the data can be corrected for the contribution of each taxon to the total amount of organic matter at the site.

Biovolumes are usually determined using the calibrated eyepiece micrometer by measuring the length (A), width (B) and depth (C) dimensions of 10–15 randomly selected cells of all the common taxa and then inserting the data in the geometric formula that best represents the shape of the taxa. Note that the volume of each specimen should be calculated individually and then the volumes averaged for each taxa. Calculating the mean dimensions first and then calculating the volume gives a biased estimate of the mean volumes.

Alternatively, Dr D. Kirchtel (University of Vermont, USA) has written software that allows an even more accurate assessment of biovolume. This is available free from: www.uvm.edu/ ~kirschtel/biovol/

8.7   QA/QC procedure for taxonomic enumerations

8.7.1 Sources of interference and error

Quantitative analysis of periphyton is prone to several sources of interference:

- Periphyton often accumulates silt within their matrix. This may result in many of the smaller taxa being obscured.

- Previously used sample bottles, settling chambers and slides need to be thoroughly cleaned before re-use. Many taxa (particularly diatoms) tend to stick very well to glass surfaces.

- Convection currents and air bubbles in the settling chambers can interfere with sedimentation. The distribution of material needs to be checked before any analysis to ensure that cells are randomly distributed across the bottom of the settling chamber.

There are three main areas of the analysis that can introduce error. These are:

- Sub-sampling,
- Counting (a lower-scale than sub-sampling), and
- Identifications.

Variation in cell density estimates with sub-sampling is generally <15% (as % coefficient of

variation), but may be as high as 50% (Biggs 1987). Less precision generally occurs with filamentous communities (particularly mat-forming cyanophytes) because fine clumps still often exist after blending. Generally only one sub-sample is withdrawn from the sample for analysis. However, we have recommended that 3×0.1–0.4 ml aliquots be removed and pooled for each sub-sample analysed (if using the inverted microscope) to reduce this sub-sampling variability. However, this isn't possible using the Palmer-Maloney chambers because of their small volume. Thus, analysis of multiple sub-samples will probably be required if using a Palmer-Maloney chamber to obtain the levels of precision commonly required for quantitative analyses. Assessments of error and sub-sampling precision can be carried out as has been detailed for sampling error and precision (see Section 3).

There should be minimal chance of mislabelling results for taxonomic analyses as the person carrying out the analyses will normally work from the original sample container. Double check at the end of each taxonomic assessment that you have entered the correct site number on the data sheet.

As mentioned in Section 8.1, it is recommended that every laboratory sets up a photographic library of the taxa encountered and the designations given. Identifications should be regularly checked against this library to ensure that there is consistency with designations. If you later find a mis-classification, it will be necessary to return to the earlier databases and correct them. To assist tracing the location of re-classified taxa in older datasets, it is valuable to develop a spreadsheet of taxa encountered at the lab and the studies in which they were found (this is an essential part of a formal quality assurance system). It is also recommended that a collection of preserved or mounted samples from each study be set up. This enables identifications, relative abundance assessments, and density estimates to be checked later (as with a photographic library, this is an essential part of a formal quality assurance system).

Errors can also arise from failure to discriminate distinct taxa, microscope calibration errors, errors in calculating densities and transcription errors.

8.7.2   Formal quality control procedures

The following steps should be carried out if formal QA/QC procedures are required for taxonomic enumerations:

- The calibration on the automatic pipette used for sub-sampling should be checked before each batch of analyses.

- All sample labels should be checked and signed off as being clearly readable.

- All samples should be registered in a properly prepared and maintained electronic or hard copy register as they arrive in the laboratory. Information should include unique sample identifiers (this would normally be a site + replicate sample number), date of collection, date of receipt in laboratory, method of preservation, analysis required, person who collected sample, person responsible for the job, and job number.

- Check the transcribing of sample numbers/labels onto the laboratory bench sheet and ensure that the samples on the bench are in the same order as on the laboratory bench sheet.

- Results, calculations, and transposition of data should all be checked and signed off by someone other than the analyst. All errors should be logged in a laboratory registry of errors. Apart from helping ensure the quality of the analysis, this registry also helps isolate problem areas in the analytical process.

- For analyses where the results are likely to be used for a resource consent hearing or the Environment Court, 3 sub-samples should be analysed from each sample and the results averaged. If the value for any one sub-sample is > 2x the mean of the 2 nearest sub-samples for a given taxon or total density, then the outlier is considered to be a sub-sampling or analytical error and the result of the outlier should be discarded.

- A comprehensive photographic library to record all designations used by the laboratory must be kept. The photographs need to be high quality colour images using a fine grained film. Attach the photographs to filing cards and note on the cards the literature source used to obtain the taxonomic designation. Where possible, include examples of fresh and preserved material, and images covering the range of sizes/shapes for each taxon. For diatoms, there should also be examples of cleaned material in valve and girdle view. Include dimension data with each image. A register of when new designations are added to the library should also be prepared and kept up to date.

- A voucher collection of preserved, representative sub-samples from each study should be maintained. To do this, pool a sub-sample from every sample collected for a particular site or habitat on each sampling occasion. This composite sample should be preserved in gluteraldehyde, labelled with the site and study details, date of collection, and the number of samples the sub-sample was derived from. On a reference sheet, record the volume of the samples and sub-samples, and the surface area of substrate from where the samples were collected so that mean densities can also be checked.

- When doing batches of routine analyses, it is standard practice to only analyse one sub-sample per sample unless pre-determined requirements for precision dictate more sub-sample replication. If only one sub-sample per sample is being analysed then three replicates from one sample in every 10 should be analysed as an estimate of variability for the batch. This QC sample should be selected randomly from the batch and the data sheet for the extra two replicates labelled with the addition of a QC identifier. The data from the three replicates (either as total density or abundance/relative abundance of specific taxa) is then used to calculate a measure of variability such as the standard error (see Section 3). This measure is reported to the client or project leader who will then decide whether the sub-sampling variability is acceptable in relation to study objectives or the batch needs to be re-analysed.

- No samples are to be disposed of until the data have been checked and signed off by the client/project leader.

Notes:

- The voucher collection sub-samples should not degrade appreciably over time. However, ensure that the containers are air tight so that the preservative doesn't evaporate off.

- A good quality assurance system in a laboratory is dependent on feedback between:
  - laboratory staff and laboratory manager, regarding problems with samples, techniques, etc.
  - laboratory staff and scientists regarding expected capabilities, variability, and expected levels for the results, and required levels of precision, etc.

- Feedback helps to ensure:

- the objectives of the project are being met;
- variability of results is reduced and thus confidence in the results is maintained;
- all participants in the analyses know what is expected of them in the QA system.

9 Data analysis and reporting

9.1 Introduction

In the following section we briefly summarise some approaches and precautionary notes that might assist in analysis of periphyton monitoring data. The idea is not to try to give a comprehensive guide on how to do the analyses, but to give pointers on good practice. This will reduce the range of possibilities for data analysis and help to standardise approaches while improving analytical quality. We assume that you have access to suitable spreadsheet software such as EXCEL and specialised statistical software such as SYSTAT, GENSTAT, SPSS, STATISTICA, etc. (note that some caution needs to be used with non-standard data or experimental designs if using the statistical procedures in EXCEL). We also assume that you have access to several of the many excellent books which give statistical details for analyses such as Elliott (1977), Green (1979), Sokal and Rohlf (1981), Zar (1996) and Underwood (1997). There are a number of journal papers/book chapters that should also be consulted as they give reviews of approaches and specific recommendations for analysing biological monitoring data from streams (e.g., Norris and Georges 1993, Lowe and Pan 1996).

9.2 A general philosophy for data analysis

Any biological data analysis is potentially a minefield of difficulties and errors. This is particularly true for periphyton data because it is usually high in:

- temporal variability (community dynamics and processes are strongly controlled by stochastic processes as well as rapid colonisation potential and fast species turnover);

- spatial variability (community structure, biomass and function are strongly controlled by spatial variations in local controlling variables such as velocity and turbulence, substrate particle size and texture, history of substrate stability, nutrient and light resource supply, and herbivore activity) (for further details see chapters in Stevenson *et al.* 1996 and Biggs 2000a).

In carrying out data analysis, it is necessary to distill the data to a form that is understandable and useful. This is often not a simple process. It is also necessary to reconcile your data with the issues which spawned the study and this is often not as easy at it might seem. It is also important to be able to determine and understand the causes and mechanisms for changes/differences that may have been recorded.

We have three suggestions that should assist in data analysis with the goal of obtaining "information from data". The first point has already been discussed in some detail (see Section 2) and relates to study planning; the second cuts across all processes of data analysis.

1. **"Begin with the end in mind"** – thorough study planning will almost always facilitate speedy and meaningful data analysis. Ultimately, we usually want to test for some difference or impact…in effect we are hypothesis testing. So, set up your hypothesis and sampling programme with the test in mind (preferably written down as part of your planning documentation). Then ensure that you do everything correctly to enable that test to be valid and meaningful. Enough is known about the ecology of periphyton communities that it would be rare that even a basic survey of "what's there" would need to be carried out without first guessing (based on the literature and your experience) "what might be there" before you start, and then "why might it be there". Once you do guess, it then becomes a simple and logical step to set up a hypothesis. This

hypothesis then drives your whole sampling, data analysis and reporting procedures. Embedded in the data is the answer to the first basic question "what's there" (you just need to extract this information specifically). However, you learn so much more about the system and controlling processes by having thought about the potential results and processes of causation, and then testing for something specific.

2. **"Use simple and meaningful metrics"** – this applies particularly to taxonomic results. There is a strong tendency in ecological sciences (and particularly in biomonitoring) to want to distill community data down to indicator metrics and multivariate component scores. The intent is to facilitate understanding. However, in the effort to provide simplification, the biology is often lost and we are (unwittingly) starting to provide ammunition for "single number environmental management". For example, single number invertebrate community indicators are widely used for assessing pollution effects in streams both in New Zealand and internationally. While they do have their place, we have sometimes witnessed discussions with water managers in which such data have been summarised and then the presenters criticised for using such approaches because the non-specialist has more difficulty seeing the issues or effects. The following dialogue is an example:

Water manager or tribunal member – *"Well Dr Bugs, what does this all mean?"*

Biologist – *"Quite simple really ... all the diatoms and other algae that like clean water to live, and are found abundantly upstream of the discharge, are being killed off in downstream areas and replaced by sewage fungus and green filamentous algae that thrive in polluted waters."*

Water manager or tribunal member – *"Then, why didn't you say that in the first place? So what are the implications of this change for us?"*

Biologist – *"These slime communities downstream of the discharge are not good habitat for large insects such as mayflies and stoneflies so these insects will also be displaced from downstream reaches. Trout like to feed on such insects so we expect that this could also reduce trout growth and numbers in the impact reach".*

Such simple, and informative, depiction of results isn't possible with complex metrics and many pollution indices. A far more powerful story is conveyed using a simple biologically based description of the system and effects. As Hynes (1974, p.163) has stated:

> *"If numerical data are collected and tabulated, or drawn as histograms, the effects of pollution are clearly shown even when it is very slight. There is neither need of, nor advantage in, a formal classification into zones, which in any event are not clearly defined, nor is anything to be gained by elaborate graphical methods."*

We believe that Hynes' advice is as salient today … particularly when attempting to convey information to the public and water managers.

As a result of the above scenario, we have been very hesitant to develop periphyton pollution and eutrophication indices for New Zealand. Indeed, we don't believe that such indices would facilitate a better incorporation of periphyton data into the decision making processes. We have, however, developed a stream-health indicator score system based on percentage cover of the bed by different periphyton growth forms (Biggs *et al.* 1998c; see also Section 6.4.3). This has been to assist untrained biologists to get some information from their data as part of community monitoring programmes. Once identifications to generic or species level are made, then the corresponding ecological data (e.g., see Table 9 of Biggs 2000a) should be used to understand and interpret

results in relation to specific components of the community.

A further problem with employing multi-component metrics is that it becomes much more difficult to discern "what is an ecologically important difference?". The multi-metric may change significantly (say from 120–100), but how does this relate to the important taxa and wider ecological processes? We seldom know the answer. However, if we clearly depicted data showing that clean water diatom taxa dominated at a control site and filamentous green algae such as *Cladophora* (that require high nutrient concentrations) dominated at the impact site just downstream, then we would have a good basis for interpreting the possible wider ecological effects of the perturbation (as illustrated in the earlier example). This issue of "ecological importance" biomonitoring results is discussed further in Section 3.7 in relation to constraints on sampling replication.

3. **"First, plot your data"** – a visual representation of your results in relation to the hypothesis being tested is an essential first step to analysis and reporting of any study. Use the plots as the initial assessment of your hypothesis. For many of us, this is one of the most exciting and interesting parts of the whole scientific process: are we right or wrong with our predictions and associated hypotheses? These plots then usually form the basis for statistical testing of trends and differences to determine if what you see in the plots is statistically significant. While it is good to look for overall patterns in your data, avoid going on "fishing expeditions" to create hypotheses based on what you see plotted up. Be careful about jumping to early conclusions about causes for observed patterns. This is particularly important if doing correlation plots.

9.3 Some common data analysis approaches

In the following sections we will briefly discuss three families of data analysis procedures that we would commonly use in relation to periphyton monitoring or experimentation in streams. Full statistical background for most procedures is given in texts such as Zar (1996). Here, we present examples illustrating two methods for assessing differences among treatments, in order to clarify the principles behind the methods. The third procedure is for assessing trends over tim. An worked example is presented because the method is not yet available in standard software programs.

9.3.1 Assessing differences among "treatments"

Treatments might refer to sites in streams with different landuses, sites above and below waste discharges, or experimental application of different nutrients such as described for the nutrient-diffusing substrate assays (Section 6.7). The most widely adopted method for discriminating treatment differences is the use of bar plots (with standard error bars) or "box and whisker" plots (e.g., Figure 5). Such plots are used to show a periphyton response variable on the vertical axis for various category states of a treatment on the horizontal axis. For many analyses a simple analysis of variance (ANOVA) will assist to determine the magnitude of differences among the treatments relative to the sampling variability. This may be carried out using parametric analyses (after first checking for assumptions of normality in the distribution of data – and correcting if necessary – and approximately equal variances among the treatments) or using the non-parametric Kruskal-Wallis procedure which requires fewer assumptions; in particular it does not require normality in the sample data. In relation to assumptions required for tests such as ANOVA, it should be noted that they are robust to departures from assumptions if the experiment/data sets are "relatively large". This is par-

ticularly true where data are balanced (i.e., sizes of samples for the different populations are the same). For ANOVA, Underwood (1997) suggests that "relatively large" means having more than about 5 treatments, with sample numbers exceeding about 6 per treatment.

A number of situations can occur when a more complex form of the ANOVA is required. This is when there is some "structure" to the way the treatments have been applied. The most common of these is nesting of secondary effects within the overall variations being assessed among treatments (a "nested" ANOVA) and secondly when the effects of two treatments and their interaction are being assessed simultaneously (a "factorial experiment" ANOVA). An example of a nested design might commonly be when attempting to discriminate the effects of a point source perturbation such as a nutrient-rich discharge from a waste water treatment plant using multiple sites above and below the discharge point (e.g., Figure 5). The structure of this data is summarised in Table 5. This table also represents how the data need to be arranged and coded in the data file to allow the categorical treatment effects to be tested in the ANOVA model.

For such an analysis, the ANOVA model statement for this would need to be:

$$\text{Chlorophyll } a = \text{Constant} + \text{Location} + \text{Site\{Location\}}$$

The results of this analysis are summarised in Table 6. They show that chlorophyll *a* biomass

Table 5: Summary of data structure for an analysis of the effects of a point source discharge to a river using multiple sites above and below the discharge point. This is a "nested" ANOVA structure. The actual data are depicted in Figure 5. In reality each site had 10 replicate samples.

"Treatments"		Response
Site (and replicate samples within each)	**Location** (relative to discharge point and replication within)	**Chlorophyll *a*** (mg/m^2)
1_1	Above$_1$	52.3
1_2	Above$_1$	167.6
1_3	Above$_1$	110.0
etc.	etc.	etc.
2_1	Above$_2$	153.2
2_2	Above$_2$	112.7
2_3	Above$_2$	80.2
etc.	etc.	etc.
3_1	Below$_1$	187.5
3_2	Below$_1$	378.5
3_3	Below$_1$	232.5
etc.	etc.	etc.
4_1	Below$_2$	317.2
4_2	Below$_2$	164.0
4_3	Below$_2$	266.0
etc.	etc.	etc.
5_1	Below$_3$	292.0
5_2	Below$_3$	265.0
5_3	Below$_3$	113.5
etc.	etc.	etc.

did differ significantly as a function of location above and below the discharge, but within each location there weren't any inter-site differences.

Table 6: Summary ANOVA results for data in Table 5. Data were $\log_e$ transformed prior to analysis.

Source	Sums-of-squares	Degrees of freedom	Mean-squares	F-ratio	*P* - value
Location	5.939	1	5.939	19.727	< 0.001
Site{Location}	0.507	3	0.169	0.562	0.643
Error	13.548	45	0.301		

An example of a 2-way ANOVA data structure is summarised in Table 7. In this analysis, we were interested in determining whether the effect of higher nutrient enrichment of periphyton chlorophyll *a* biomass was dependent on degree of stream shading. The nutrient treatments were 19 vs. 188 ppb nitrate-N, and light treatments were 400 vs. 1,200 μE m^{-2} s^{-1}.

In reality, this study was done as an experiment so that all conditions except those of the treatments were held constant among the artificial stream channels. Thus, we can specify an interaction term as well as "main-effects" terms in the ANOVA:

$$\text{Chlorophyll } a = \text{Constant} + \text{Nutrients} + \text{Light} + \text{Nutrients} \leftrightarrow \text{Light}$$

Table 7: Summary of data structure for an analysis of the effects of two nutrient concentrations (low and high) and light levels (low and high) on periphyton chlorophyll *a* in experimental streams. This is a two treatment "factorial" ANOVA structure.

"Treatments"		Response
Nutrients (and replicate samples within each)	**Light** (and replicates within each sample)	**Chlorophyll *a*** (mg/m^2)
Low_1	Low_1	1.16
Low_2	Low_2	0.33
Low_3	Low_3	0.26
Low_4	Low_4	1.36
Low_5	$High_1$	0.76
Low_6	$High_2$	1.59
Low_7	$High_3$	1.50
Low_8	$High_4$	0.37
$High_1$	Low_5	56.9
$High_2$	Low_6	38.3
$High_3$	Low_7	94.0
$High_4$	Low_8	64.2
$High_5$	$High_5$	135.0
$High_6$	$High_6$	77.2
$High_7$	$High_7$	55.2
$High_8$	$High_8$	76.0

The output table will define whether nutrients and light are statistically significant effects on their own. If the Nutrients x Light interaction is also statistically significant, it would mean that the effects of nutrients is not uniform over the range of light intensities tested. Table 8 summarises the results. It identifies that the periphyton responded only to the nutrient treatment, and that the interaction term with light was not statistically significant, meaning that the nutrient effect was not modified by light within the tested levels.

Table 8: Summary ANOVA results for data in Table 7. Data were $\log_e$ transformed for the analysis.

Source	Sums-of-squares	Degrees of freedom	Mean-squares	F-ratio	P - value
Nutrients	82.708	1	82.708	226.268	< 0.001
Light	0.489	1	0.489	1.338	0.270
Nutrients x Light	0.010	1	0.010	0.027	0.873
Error	4.386	12	0.366		

One more important aspect of ANOVA can be illustrated here. This is the presence of another environmental variable which may also be changing among the treatments, but which we cannot control. This is called "co-variance". Such phenomena can be accommodated in the ANOVA using most modern statistical software so long as the state of the co-varying variable has been measured. In the above experiment on the effects of nutrient enrichment under different light conditions, it is plausible that the high light treatments were warmer than the low light treatments which could confound interpretation of the results (i.e., temperature differences alone could explain the results). By including temperature as a co-variate (this is defined in different ways in the model statement for the various statistical packages) the analysis will first remove the effect of temperature by accounting for the temperature correlation with chlorophyll and then assess the relationship between the residual variance in chlorophyll with light. This process isolates "main effects" from secondary effects and provides a much more robust analysis and set of conclusions.

In carrying out any of the above assessments it is also important to remember to try and recognise what might be ecologically important (see Section 3.7). Periphyton communities at most sites or patches on the streambed can be shown to be statistically significantly different just by collecting more samples. But … so what? Think about differences and what they might mean for wider issues such as ecosystem functioning, trophic interactions etc., before concluding whether treatment differences are important.

9.3.2   Assessing trends over time

There are many situations, such as routine monitoring for SOE reporting, where we may need to determine whether the periphyton communities have changed over time as a result of, say, a change in landuse or other management decision. When using the term "trend" we are adopting a reasonably strict definition in that we are referring to multiple points collected over a time series. There are a number of situations where a site might be sampled only on two occasions. Analysis of such data can be easily done as bar or box and whisker plots followed by a Student's *t*-test or Mann-Whitney U-test. There are several methods by which longer time series of data can be analysed.

As already emphasised, the data must first be plotted. These plots just consist of the response variable being plotted as a function of date of collection. An example is given in
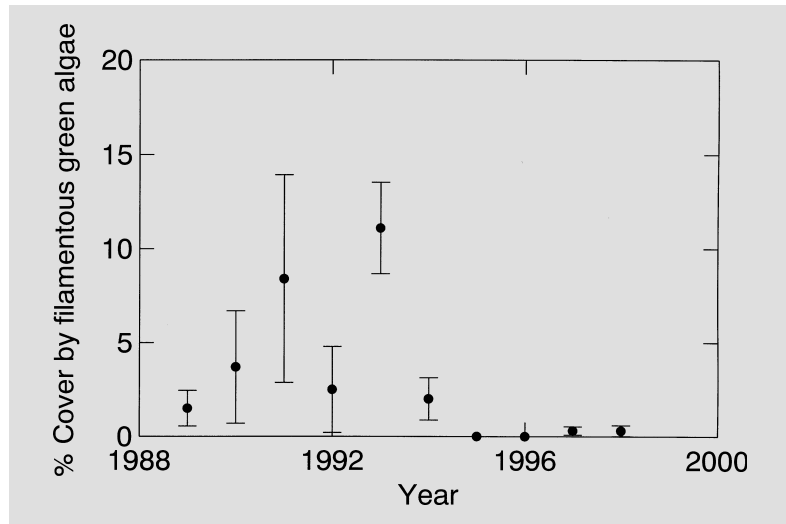
Figure 18 Time series of percentage cover (± 1 SEM) of filamentous green algae during summer low flows in the Manawatu River (data from the NIWA National Water Quality Monitoring Network courtesy of Dr J.M. Quinn).

Figure 18. This depicts 10 years of summer percent cover of the river bed by filamentous green algae. It depicts what appears to be a reduction in algal cover over the duration of the monitoring. The plot also illustrates a difficulty we are often confronted with when analysing such time series. This is that simple, linear trends rarely occur. Nonetheless we will use the standard approach to determining whether a trend is occurring in periphyton cover over time. This involves assessing the significance of the slope on a linear regression of the dependent variable as a function of time:

$$Y = c + b(t) \qquad\qquad (13)$$

Where Y is the dependent variable (say percentage cover of filamentous green algae), c = a constant (the intercept on the Y axis), and b = coefficient giving the average rate in Y for a given increment in time (t).

This approach assumes that the change is monotonic (i.e., only proceeds up or down) and that each observation is independent of the previous. The second assumption is generally easy to fulfill with periphyton data if they are sampled at intervals of > 4 weeks because of the rapid turnover time for the community. The results of this analysis do not indicate a clear monotonic trend. Indeed, the slope of the regression line between percentage cover and time is not significant ($P = 0.194$). Instead percentage cover appears to increase from 1989–1993, then is suddenly very low again through to 1998.

This plot also illustrates a common difficulty in such trend analysis in which periphyton data are collected very infrequently: variability over time is so great that it is difficult to distinguish clear trends. An alternative approach to analysing this data for trends in slope over time is to divide the time periods up according to any clear discontinuities. In this example we might want to compare cover pre-1994 vs. 1994 and after using a Student's *t*-test or Mann-Whitney U-test. This indicated that there was a significant difference in cover ($p = 0.016$ for a Mann-Whitney test) between the two time periods suggesting that there has been a reduction in cover of filamentous green algae in the Manawatu River during late summer since 1994.

Several other approaches are available such as the Mann-Kendall test for trend (Gilbert 1987) which is a non-parametric test for monotonic trend that does not assume linearity, but does

assume independence of samples. This approach has been developed further to account for seasonality, with the option of removing flow dependence, and is widely recommended for trend analysis in water quality monitoring (Ward *et al.* 1990, Loftis and Ward 1994, Manly 1999). A software package (WQStat-Plus) is now available to enable such calculations (contact NIWA Instrument Systems: g.elley@NIWA.cri.nz).

9.3.3   Correlation and regression analyses

These are probably the most familiar type of statistical analyses to most stream ecologists. They are the "bread and butter" tools of our trade. While ANOVA focuses on defining the statistical significance of differences in responses to set levels in a controlling variable (often just as binary treatments), correlation and regression analysis have their strengths in establishing graduated responses to gradients in the independent variables. Such "gradient analysis" is often much more useful to management. Also, biotic responses to changes in the environment are seldom linear and it is usually important to define the shape of this response. For example, the biotic responses may be asymptotic, Gaussian (i.e., bell-shaped), or display critical thresholds whereby the biota can resist the effects of changes in an environmental variable and then there is a sudden response. Correlation and regression analysis can be used to analyse and model such non-linear effects providing the appropriate equations are adopted for curve fitting. Thus, these analyses provide core tools for developing simple predictive models that can be useful to water managers.

Correlation and regression are closely related techniques and have similar roots. With correlation analyses we are generally only interested in whether one variable changes in harmony with another variable (i.e., there is no implied causative relationship between one variable and the other). There are no statistical assumptions to be satisfied in order to compute the correlation coefficient, however there are assumptions underlying testing of hypotheses about, and the determination of confidence intervals for, correlation coefficients (Zar 1996). Parametric (Pearson's correlation) and non-parametric (Spearman's rank order) methods can be used. The parametric method assumes linearity, whereas the non-parametric method does not. The non-parametric test is particularly appropriate to use when the distribution of the data for each variable is far from normal. It is important to remember that a non-significant correlation using the parametric test does not necessarily mean that there isn't a relationship between your variables. It may just mean that the relationship isn't linear. Thus, to reiterate our earlier advice … always plot your data first and inspect the relationship before designing an appropriate data analysis approach.

Regression analysis is used to define the dependence of one variable (a response, or "dependent" variable) on another variable (the variable causing that response – usually environmental – called the "independent" variable). The general form of the linear model is summarised in Equation 14. As with correlation analysis, most statistical software will offer a range of approaches for regression analysis from simple bivariate regression to complex stepwise procedures for use when attempting to isolate the best set of predictors for the dependent variable. The statistical engine of these analysis is defined in texts such as Zar (1996) and Underwood (1997). General procedures for fitting complex curves are given in Snedecor and Cochran (1980). We will not repeat any of this background information here, but it is useful to reiterate the main assumptions of regression analysis. These are:

- Independence of data,
- Homogeneity of variables,

- The independent variable is measured without error, and

- The errors in the dependent variable for any given value of the independent variable are normally distributed (see Underwood 1997, p. 424 for discussion of these assumptions).

One of the critical issues in using regression analyses in studies of stream ecology (particularly periphyton), is that some thought be put into the processes or mechanisms that might be expected to result in a given relationship. If the study has been well planned (see Section 2), then possible causal mechanisms will have already been isolated and the investigator will find it much easier to draw sound conclusions from a significant relationship. However, if regression (or correlation) analysis is used to search for patterns in large datasets, then it is often difficult to interpret relationships in any meaningful way and, indeed, many absurd linkages can be defined. Use some thought and common sense before embarking on such analyses.

One of the difficulties in regression analysis that we are often confronted with is finding an appropriate model to fit non-linear relationships. In practice, what we would normally do is adopt a general equation that best simulates the shape of our relationship, apply the transformations given by the models and then use least-squares regression to estimate the parameters. A summary of general types of curves and their equations is given in some statistical texts (e.g., Steel and Torrie 1960).

10 Identification guide to common periphyton in New Zealand streams and rivers

10.1 Introduction

This identification guide covers many of the simple photosynthetic organisms (algae) that occur in the periphyton of streams and rivers in New Zealand. It is intended to provide rapid and practical assistance for non-specialist biologists and stream ecologists wishing to identify the most common taxa in samples of periphyton. For completeness we also include some less common taxa because they can be abundant regionally.

The guide is organised into broad groups based on easily distinguishable features. These groups do not necessarily correspond to those used in taxonomic classification.

10.2 Nomenclature and classification

The term "algae" has traditionally been used to encompass all simple organisms that are capable of photosynthesis and that are not included in the bryophytes (mosses and liverworts) or vascular plants. Therefore algae do not form a single evolutionary group. For example, the prokaryotic "blue-green algae" (see page 124) are only distantly related to the rest of the algae, which are eukaryotic organisms (see page 122) and it is now usual to refer to the blue-green algae as cyanobacteria. Nevertheless, the term "algae" remains a useful ecological grouping of chlorophyll *a*-containing organisms that occur in mixed communities in aquatic habitats.

The classification of algae is based on a wide range of characteristics, the more obvious of which are listed in Table 9. Using various combinations of these features it is possible to distinguish the main groups, as shown in the key (Table 10). The groups normally encountered in stream and river periphyton are shown highlighted in colours, with their common names in **BOLD CAPITALS**. Sections 10.2.1 to 10.2.6 give more information about each of the groups.

Each description in this manual is based on a genus and includes basic taxonomic information (e.g., Division, Order, Genus). The colours highlighting each group in Table 10 are repeated throughout the guide as a quick indication of the algal group to which each genus belongs. Note that, at the level of Order, you will find a slightly different classification scheme in almost every phycology guide. For the eukaryotic algae we follow the orders used by Prescott (1978) and Round *at al.* (1990) (for the diatoms). For cyanobacteria, the groups used are those proposed by Anagnostidis and Komàrek (1985) (see Section 10.2.1). For an up-to-date account of algal classification see Graham and Wilcox 2000.

Table 9: Characteristics of algae used in classification and identification

Cellular organisation:	The simple photosynthetic organisms found in periphyton are either: • "prokaryotes", in which the cell inclusions are not bound by membranes and pigments are distributed throughout the cell, giving a diffuse appearance (these are the "blue-greens" (Cyanobacteria)); or • "eukaryotes", in which cell inclusions such as nucleus, mitochondria and chloroplasts are distinguishable and are enclosed by membranes.
Cell pigments	All possess the photosynthetic pigment chlorophyll *a*, but chlorophylls *b* and *c*, and phycobilins also occur in certain groups.
Flagella	Flagella (singular: flagellum) are long, thin flexible appendages that allow many algal cells to move around. Some algae have no flagella; sometimes they occur only at the reproductive stages; in some cases they are always present and there may be one, two or four. Where there are two or more flagella they may be the same or different lengths. Flagellated species are mainly planktonic and are rarely encountered in stream periphyton.
Colour	Cell colour ranges from red to brown through yellow and green to blue-green and even blue. The major taxonomic groups are named on the basis of colour but, because many taxa contain a mixture of pigments, colour is not always a reliable guide to the groups. Colour may vary with environmental conditions and may also vary according to the type and duration of preservation.
Food reserves	Some of these organisms have food reserves of starch and therefore a positive reaction to the iodine starch test. Others store food as leucosin or oil and do not react to the starch test.
Cell wall type	Cyanobacteria often possess a muco-polysaccharide sheath; green algae have cellulose cell walls laid down in a criss-cross pattern; diatoms have rigid walls made of silica.
Reproduction	Some algae and cyanobacteria reproduce by cell division, each new cell being independent of all others. Most algae also produce specialised reproductive bodies, or spores, and in some groups the form of these is the main character that distinguishes species. Sexual reproduction is also common.
Gross structure	This is the group of characteristics that is of most practical use in *identifying* algae. For example, is the alga single-celled, filamentous, colonial or sheet-like? Does the alga have different types of cells – e.g., heterocysts? Many of the same structural characteristics occur in different groups, and thus do not reflect natural relationships.
Ecology	Aquatic algae including cyanobacteria are capable of inhabiting almost all damp to wet habitats in existence, from sea water through to hot springs. However, many types occur only within a certain range of conditions.

Table 10:   Key to the major groups of common freshwater periphyton

1a	Cells without chloroplasts (though granules in the cells could be mistaken for chloroplasts), diffuse blue-green, olive or red-brown. Cells often very small.
	Division: **Cyanobacteria (BLUE-GREENS) [see page 124]**
1b	Cells with chloroplasts (discrete structure in which the cell pigment is concentrated). **2**
2a	Chloroplasts pale to deep grass green. **3**
2b	Chloroplasts some other colour. **4**
3a	Single-celled, filamentous or colonial form, normally with one or two chloroplasts arranged as sheets inside the cell wall or along the length of the cell. Sometimes with many chloroplasts. Where flagella are present there are two or four and their lengths are equal. Positive reaction to starch test.
	Division: **Chlorophyta**; Class: Chlorophyceae **(GREEN ALGAE) [see page 124]**
3b	Large, erect plants, typically 4-50 cm long, with regular branches. Rooted by rhizoids in soft sediment. Cells large with numerous chloroplasts.
	Division: **Chlorophyta**; Class: Charophyceae
3c	Single-celled, colonial or filamentous, normally with two or more small, discoid chloroplasts per cell. Where flagella are present there are two, of unequal length. Negative reaction to starch test.
	Division: **Xanthophyta (YELLOW-GREEN ALGAE) [see page 125]**
3d	Single cells with one long, thick flagellum emerging from a depression at the end of the cell. Two-to-many discoid chloroplasts. Cell wall can be elastic and striated.
	Division: **Euglenophyta**
4a	Cells with a rigid, ornamented silica wall composed of two halves, sometimes forming filaments. Often motile, but no flagella. One to many brownish chloroplasts.
	Division: **Bacillariophyta (DIATOMS) [see page 126]**
4b	Cells not as above. **5**
5a	Cells with two flagella, partly within two furrows on the cell surface, one around the cell, the other at right angles. Cell walls of smooth or angular plates, flat or with projecting horns. Positive reaction to starch test.
	Division: **Dinophyta (= Pyrrophyta {dinoflagellates})**
5b	Cells without two deep furrows **6**
6a	Chloroplasts pale yellow to brown, usually 1 or 2 per cell. Cells single, colonial or (rarely) filamentous. If flagella are present they are either one long one, or one long and one short.
	Division: **Chrysophyta (GOLDEN-BROWN ALGAE) [see page 128]**
6b	Chloroplasts some other colour, no cysts, flagella (if present) not strongly unequal. **7**
7a	Single-celled, bean-shaped with two slightly unequal flagella arising from a small depression of furrow. One or two chloroplasts, coloured olive, red or blue.
	Division: **Cryptophyta**
7b	Plants filamentous and frequently with complex structure, or flattened and encrusting, or (rarely) single-celled. One or more chloroplasts per cell, coloured olive, red or blue. Usually attached to rocks and mosses in streams. No flagella.
	Division: **Rhodophyta (RED ALGAE) [see page 128]**

Throughout the idenification guide, the above colours are used to indicate the major algal group to which each genus belongs.

10.2.1   Cyanobacteria

Cyanobacteria are easily distinguished from other algae by the absence of chloroplasts – the discrete organelles that contain the photosynthetic pigments in eukaryotes. Instead, these pigments are diffused throughout the cell protoplasm. Additional pigments found in this group are responsible for the range of colours they exhibit, most notably the blue-green appearance. The orders of cyanobacteria represented in this guide are listed below. These groups are those used by Anagnostidis and Komàrek (1985), with details in Anagnostidis and Komàrek (1988a, b) and Komàrek and Anagnostidis (1986, 1989). This classification system follows the traditional botanical approach, being based on "Geitler's" system (see Geitler 1925, 1932, 1942) but now incorporating morphological, cytological and ecophysiological characters. Note that several other classification schemes have been proposed based on the bacteriological approach (Stanier 1977 and Rippka *et al.* 1979) or on "Drouet's" system (Drouet and Daily 1956 and Drouet 1981), where the number of taxa was reduced considerably.

Chroococcales: Unicells, colonies, pseudoparenchymatous colonies or pseudofilamentous colonies. Trichomes, heterocysts and akinetes are lacking. Cell division in one, two or more perpendicular planes. Examples include *Gloeothece* and *Chamaesiphon*.

Oscillatoriales: Cells forming trichomes (a single row of cells connected by cross walls); false branching, gas vesicles and sheaths lacking or facultatively present. Heterocysts, akinetes and true branching absent. Reproduction occurs by "hormogonia" formation through trichome fragmentation. Examples are *Phormidium*, *Oscillatoria* and *Lyngbya*.

Nostocales: Cells forming trichomes with a wide or narrow mucilaginous sheath. Trichomes unbranched or falsely branched (initiated at a heterocyst or between two vegetative cells). Specialised nitrogen-fixing cells (heterocysts) and spore cells (akinetes) may be present. Reproduction mainly by hormogonia or hormocysts. Examples are *Nostoc*, *Tolypothrix*, *Calothrix* and *Rivularia*.

Stigonematales: Cells forming true trichomes, sometimes combined with pseudotrichomes. True branching always present while false branching may occur. Akinetes rarely present while heterocysts occur facultatively in several genera. Reproduction mainly by hormogonia and hormocysts. Best known example is *Stigonema*.

10.2.2   The green algae (Division: Chlorophyta)

The orders listed below belong to the class Chlorophyceae and are represented in the periphyton of New Zealand streams and rivers. These are traditional orders (largely as used in Prescott 1973). See Graham and Wilcox (2000) for recent classification schemes.

Tetrasporales: These occur in a non-motile vegetative form, usually in colonies held together by mucilage (e.g., *Gloeocystis, Palmella*). The cells can reproduce by simple cell division. The chloroplasts are usually described as cup-like – that is, they are curved so that they line part of the inside of the cell.

Chlorococcales: These can look similar to the Tetrasporales (e.g., *Oocystis*). The main difference is that the cells do not reproduce by simple cell division in the vegetative state, though they do divide to form spores. Single-

celled or colonial. Examples found in periphyton are *Pediastrum, Ankistrodesmus, Scenedesmus.*

Ulotrichales:	Unbranched simple filaments with mostly cylindrical cells containing a single band-like chloroplast similar to that in the Chlorococcales. Most species are attached when young, but become free-floating later. The best known filamentous alga in this order is *Ulothrix*. Others include *Geminella.*
Ulvales	Many cells in a sheet-like arrangement, e.g., *Enteromorpha,* in which the sheets form hollow filaments.
Microsporales:	Unbranched filaments of cylindrical cells with walls in sections with a cell wall at the centre – so that broken-up filaments comprise H-shaped pieces. The chloroplast covers the whole wall of the cell and may be thin or dense. The only genus in this order is *Microspora.*
Cylindrocapsales:	Another order with only one genus – *Cylindrocapsa.* Usually filamentous with dense, large chloroplasts.
Chaetophorales:	Branched filaments that arise from a holdfast. Cells forming branches often smaller than those nearer the base. Chloroplasts are parietal, sometimes completely covering the cell wall. Examples are *Chaetophora, Draparnaldia* and *Stigeoclonium.*
Cladophorales:	(also known as Siphoncladales) Repeatedly branched filaments, cylindrical cells, thick walls. Chloroplast parietal and net-like in young, healthy specimens, but sometimes appearing as many small disks. Often the cell walls are very thick and the filaments frequently carry many diatom epiphytes, e.g., *Cladophora, Rhizoclonium.*
Oedogoniales:	There are both unbranched and branched forms in this order, which is characterised by occasional ring-like scars at the front end of cells, caused by cell division. Genera described in this guide are *Oedogonium* (unbranched) and *Bulbochaete* (branched).
Zygnematales:	Unbranched filaments of long or short cylindrical cells with a cellulose cell wall; end walls are separated by a middle lamella. The chloroplasts are large, with 1–2 per cell usually. Species in this order have no motile reproductive cells. Instead, cells transform into amoeboid gametes, two of which fuse to form the zygospore (via "conjugation" of cells). Genera represented in this guide are *Mougeotia, Spirogyra* and *Zygnema.*
Desmidiales:	The desmids are single-celled forms (occasionally in filaments) related to the Zygnematales through having a similar mode of reproduction. Most desmids are divided into two equal halves. The chloroplasts are variable and can be complex. Examples found in periphyton are *Cosmarium, Closterium* and *Staurastrum.*

10.2.3   The yellow-green algae (Division: Xanthophyta)

The yellow-green algae can be difficult to distinguish from the green algae. The main features that separate the two divisions are a predominance of yellowish pigments (e.g., carotenoids) in the yellow-greens, and leucosin or oils as food reserves, rather than starch. Just two orders are included in this manual.

| Tribonematales | Branched or unbranched filaments, e.g., *Tribonema.* |
| Vaucheriales | Branched, cylindrical filaments, no cross walls, e.g., *Vaucheria.* |

10.2.4   The diatoms (Division: Bacillariophyta)

The structure of diatoms means that it is relatively easy to identify many of them to species or even variety. In this guide, we include descriptions of many *common* species – particularly those that are useful indicators of environmental conditions. This should enable you to distinguish them from other species that are similar in appearance, but that you won't necessarily be able to identify beyond genus level. Some identifications should be possible using fresh or frozen material.

For detailed studies on diatoms it is necessary to examine acid-cleaned specimens at magnifications of up to ×1000, using an oil-immersion objective on a light microscope in order to see the ornamentation of the cell walls. In addition, you need an understanding of their structure and a comprehensive collection of specialised diatom taxonomy texts because, as yet, there is no complete guide to diatoms in New Zealand.

Briefly, the diatom cell wall is made of silica and the basic construction is of two halves (valves) that fit together with one half overlapping the other so that the whole structure resembles a chocolate box. The two valves together are called the frustule. Classification is based on the arrangement of various surface features and etchings on the frustule. A major feature is the raphe, a pair of slits in the valve face. When present, the raphe may be on both valves or on one valve only. The two valves may be connected by a

Structure of a generalised diatom frustule

raphe

stigma

striae, made up of alveolae (or puncta)

valves

girdle, made up of girdle bands

(drawing: Liz Bergey)

girdle or a series of girdle bands. The valve faces may carry rows (called striae) of openings or depressions (known as puncta or areolae). Larger holes are called "stigmata" (singular: stigma). Most descriptions of diatoms are based on the view from the top ("valve view"). The view from the side ("girdle view") can look quite different.
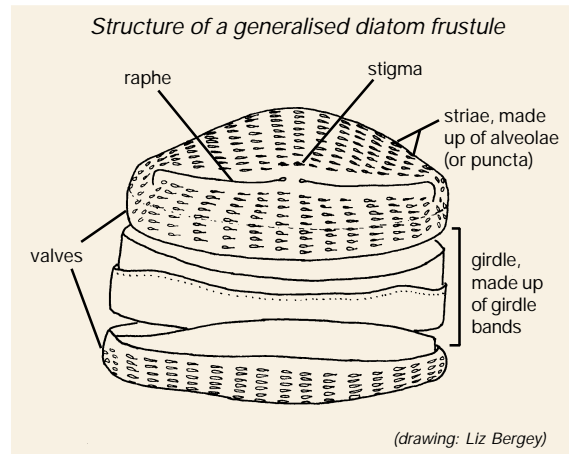
The structure of the chloroplasts and other cell inclusions may also be considered in descriptions. For more details about the fine structure of diatoms refer to Barber and Haworth (1981), Round, Crawford and Mann (1990) and Cox (1996).

As for the algae in general, various classification systems have been proposed for diatoms (see Bourelly 1981, Krammer and Lange-Bertalot 1987–1997, Round *et al.* 1990) and we follow the system of orders of Round *et al.* 1990. For descriptive purposes, these orders can be placed in seven groups (as used by E. Stoermer, pers. comm.) that are convenient though not necessarily natural (i.e., made up of related organisms). The illustrations below show representative valve and girdle views in each group.

Centrics: Radially symmetric valves. No raphe system.
Thalassiosirales: *Cyclotella;*
Melosirales: *Melosira;* Aulacosirales: *Aulacoseira.*
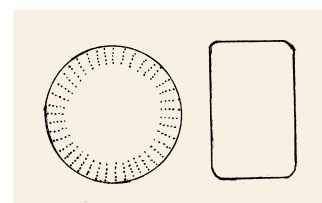
Araphid pennates: Usually symmetrical along the all axes of the valve (exceptions: *Meridion, Asterionella*). No raphe system on either valve, but usually a "pseudo-raphe" present (i.e., a break in the striae, as if a raphe were present).
Fragilariales: *Fragilaria, Fragilariaforma, Staurosira, Diatoma, Meridion, Synedra;*
Tabellariales: *Tabellaria.*
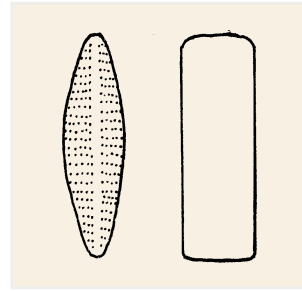
Monoraphid pennates: Usually symmetrical along at least two axes. A raphe system present on one valve only. The second valve may or many not have a pseudoraphe. The ornamentation on the two valves can be quite different. (Illustration shows raphe valve, girdle view and pseudoraphe valve.)
Achnanthales: *Achnanthes, Achnanthidium, Cocconeis.*
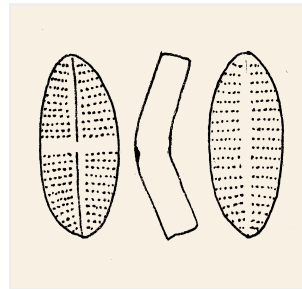
Eunotioids: A rudimentary raphe system on both valves, with the two valves usually similar. There may be asymmetry along all three axes.
Eunotiales: *Eunotia, Eunophora, Actinella.*
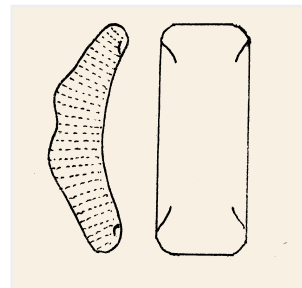
Biraphid pennates: Symmetric naviculoids: These are usually more or less symmetric along both the long and cross-valve axes (exception: some *Pinnularia* species). A fully developed raphe system present on both valves. The two valves are usually identical.
Mastogloiales: *Mastogloia;*
Naviculales: *Brachysira, Frustulia, Navicula, Neidium, Pinnularia, Stauroneis.*
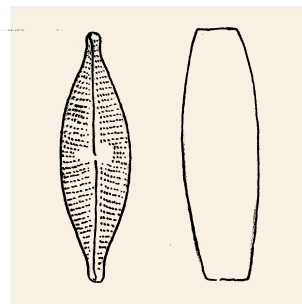
Biraphid pennates: Asymmetric: Lacking symmetry along at least one axis. A raphe system present on both valves. The two valves are usually identical (an exception is *Rhoicosphenia*).
Cymbellales: *Cymbella, Rhoicosphenia, Encyonema, Gomphonema, Gomphoneis, Reimeria;*
Thalassiophysales: *Amphora.*
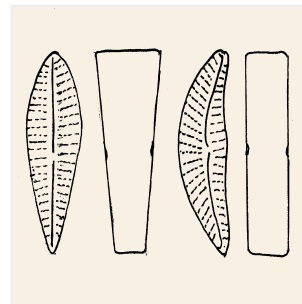
Biraphid pennates: "External" raphe: The raphe lies partly or wholly along the edge of the valve, sometimes within a raised canal. The structure can be quite complex. Some diatoms in this group are nitrogen-fixing because of a symbiotic relationship with cyano-phytes, and can therefore tolerate nitrogen-limited situations. Bacillariales: *Nitzschia;*
Rhopalodales: *Epithemia, Rhopalodia;*
Surirellales: *Surirella, Stenopterobia.*
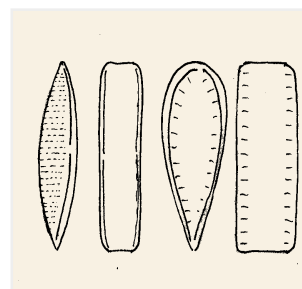
10.2.5   The golden-brown algae (Division: Chrysophyta)

Mostly motile cells, though the division includes some filamentous and sheet-like forms. This Division is more commonly found in lake habitats than in flowing waters. Chrysophytes are characterised by possessing a very large chloroplast, and by their particular combination of pigments. The one example described is *Dinobryon* (Order: Chrysomonadales).

10.2.6   The red algae (Division: Rhodophyta)

The red algae are distinguished from the other algal divisions by their methods of reproduction and life histories. The "red" comes from the predominance of the red pigment phycoerythrin in many species. Most red algae are marine. The examples in this manual represent the two subclasses of the Rhodophyta. See Bourelly (1985) for a version of classification finer than this. See Entwistle and Kraft (1984) for an account of freshwater red algae in south-east Australia, including notes on the New Zealand flora.

Bangiophycidae   Freshwater forms have a branched, unbranched or plate-like thallus, with thick cell walls. Asexual reproduction is by non-motile spores. Sexual reproduction rare. Represented by *Chroodactylon, Compsopogon.*

Florideophycidae   The more "advanced" group, with a wide range of morphologiesfrom slightly branched filaments to more complex growths. The female sex organs (carpogonia) are characteristic. Representatives are: *Audouinella, Batrachospermum, Bostrychia.*

10.3 How to use this guide

To aid identification, each taxon described in this guide is assigned to a group on the basis of cell type and growth form. These groups do not necessarily correspond to the traditional classification outlined above.

Photosynthetic periphyton may be subdivided into two **cell types**:

- Containing "organelles" (distinguishable structures like chloroplasts and a nucleus), but may not have cell walls. These are the green, yellow-green and red algae, and the diatoms.
- Cell contents diffuse, granular, with no distinguishable organelles (but take care not to interpret granules as chloroplasts). These are the cyanobacteria.

These groups can then each be divided into four broad **growth forms** that are easily distinguished:

- flamentous, unbranched;
- filamentous, branched;
- single-celled;
- colonial or multi-celled (e.g., sheets).

Since diatoms are readily recognised from their silica frustules they are placed in groups on their own.

A further obvious feature of algae is colour. In periphyton this can be:

- green (various shades);
- brown – golden-brown;
- pale bluish green, olive-green or pinkish.

However, even though colour is used to name the major taxonomic groups, the colour of a particular algal genus or species can vary quite a lot depending on, for example, the environmental conditions of the habitat, the health of the cells, preservatives used, and the light source and filters on your microscope. Therefore, you should be cautious in using colour as a diagnostic feature. Just remember that it is often helpful, but sometimes can be misleading.

The groups used in this guide are as follows.

(a) Group C: cells containing chloroplasts (eukaryotic algae)

1. **C: unbranched filaments**. All of these are green algae, except one representative from the yellow-greens. 11 genera

2. **C: branched filaments**. This is a more diverse group, including genera from the green, yellow-green and red algae. 10 genera

3. **C: unbranched filaments, or as single cells (diatoms)**. This group includes diatoms that grow in filaments. They are grouped separately from the unbranched filaments above because all of them are also frequently seen in periphyton as single cells. 7 genera, some with individual species described.

4. **C: single cells (diatoms)**. Because diatoms are relatively easy to recognise from their solid "skeletons", and because many species are found in periphyton, they are all grouped together. 35 genera, with individual species described for most.

5. **C: single cells (non-diatom)**. This much smaller group includes single-celled green algae. 3 genera.

6. **C: colonial**. Included here are cells that are always found growing in colonies of 4 cells or more. 6 genera (5 green, 1 golden-brown).

7. **C: colonial (sheet-like)**. This includes just one distinctive green alga.

(b) Group BG: cells with diffuse cell contents (prokaryotic algae: Cyanobacteria)

8. **BG: unbranched filaments**. Filaments growing in masses or singly. mixed with other algae. 7 genera.

9. **BG: filaments in gelatinous masses**. These are filamentous forms that always grow in cohesive gelatinous clumps. 2 genera.

10. **BG: branched filaments**. Filaments growing in masses or singly, mixed with other algae. 5 genera.

11. **BG: single cells**. One example of a single-celled blue-green alga in periphyton.

12. **BG: colonies**. Two examples of colonial cyanobacteria occasionally found in periphyton.

To use the guide, decide on the category to which your specimen belongs, then look for possible matches in the relevant section. All genera included are listed on page 131.

The coloured bars alongside the descriptions refer to the algal group (e.g., Division) to which the genus belongs (see Table 11 on page 123 for a key to the groups).

As noted above, each description refers initially to a *genus.* In each section the genera are usually in alphabetical order. Most green, yellow-green and red algae require detailed studies – including examination of the reproductive bodies – in order to get past genus level. Many diatoms, on the other hand, can be identified to *species* relatively easily. Common species within many of the genera are described (in words and/or photographs), with the more

frequently encountered species first – though these may not be the most common in all samples. In environmental studies species identification can be important because some diatom species are known to have particular environmental preferences that are not shared by other species in the same genus. Their identification can add considerable value to the interpretation of taxonomic data.

Notes on *macroscopic appearance* and *microscopic details* are included. The former is included only where a taxon has some recognisable feature, rather than being part of a mixed periphyton community. For microscopic examination we assume the use of a good quality light microscope capable of magnifications of at least 400x. An oil-immersion objective allowing magnifications of >1000↔ is also desirable for examining the smaller algal taxa. Ideally you will have an eyepiece micrometer on your microscope so that you can measure the dimensions of your specimens. This should be calibrated using a stage micrometer.

A typical *size range* is given for most taxa.

For a few genera we include notes on the *appearance of algae following freezing*, with illustrations where the changes are particularly noticeable. Many types of algae – particularly diatoms – are not greatly altered by freezing other than some shrinkage of the chloroplasts.

Although examination of frozen material is not ideal, it is often impractical to examine fresh material in routine environmental monitoring programmes. Normally part of the sample needs to be analysed for chlorophyll *a* (a measure of total live biomass). Unless this can be done immediately, the samples must be temporarily preserved. Freezing is the most effective way of doing this. Preservatives such as glutaraldeyde retain the cellular structure of algae, but interfere with the chlorophyll content (see Section 8).

For each taxon there are notes on typical habitat and environmental preferences (if known), as well as observations on abundance and distribution. These comments are based on published notes on algal distributions in New Zealand (Cassie 1984a,b, Biggs and Price 1987), on information in the literature on habitat preferences (Cassie 1989, Cox 1996, Winter and Duthie 2000), and on personal observations.

We also note taxa that might be confused with the one being described. In addition, for some of the diatoms we include notes on recent name changes.

All the photographs in this guide are of periphyton from New Zealand rivers and streams. Magnifications are approximate.

Following the illustrated guide, Sections 10.5 to 10.8 comprise:

- A glossary explaining the technical terms used in the descriptions.

- A table summarising recent new diatom genera that are becoming generally accepted. These genera do not appear in older identification texts, though many of the species can be found under their traditional genus name.

- A list of general references used for identification of algae. (See Section 11 for a full list of literature cited.)

- A list of the diatom species described, with authorities, and reference(s) to one or more source(s) from which the identification may be made.

Definitions

Conductivity:	high, >200 μS/cm		LM = light microscope
	medium, 80–200		SEM = scanning electron microscope
	low < 80		μm = micrometre (1 mm = 1000 μm)
			cf. = "compare with" (i.e., looks like)

10.4   Guide to periphyton genera

Contents (names in parentheses indicate a partial description only)

Cylindrocapsa

How to recognise the genus:

Macroscopic: Cylindrocapsa grows as dark green-brown masses.

Microscopic: Thick cell wall with a many-layered clear gelatinous sheath between cells. Each cell contains a single large dense chloroplast. In the example illustrated the cells have a distinctive purple colouration, which occurs during reproduction. Normally cells are green. The filaments are about 25 µm across

Habitat and distribution: The genus *Cylindrocapsa* is found mostly in boggy areas but also in slow-flowing shallow streams downstream of wetlands. It has been reported rarely in New Zealand and we have found it only in high altitude, pristine areas. The specimen photographed was collected in the Arthur's Pass region, South Island.

Possible confusion: Distinctive, therefore unlikely to be confused.
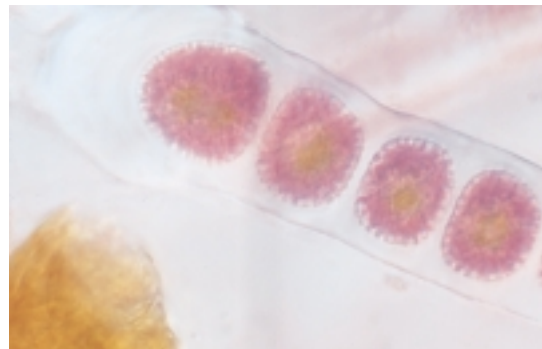
Cylindrocapsa *filaments. top, x 200, bottom, x 800.*

Geminella

How to recognise the genus:

Macroscopic: Geminella has no distinctive macroscopic features.

Microscopic: Small, separate cells, each with a single folded chloroplast, embedded in a thick mucilaginous sheath to form filaments. Individual cells are about 8 µm across and 10 µm long. The entire sheath is around 30 µm in diameter.

Habitat and distribution: *Geminella* is typically a lake-dwelling genus but has been found in the periphyton of lake-fed rivers, usually close to the lake outlet.

Possible confusion: Distinctive, therefore unlikely to be confused.
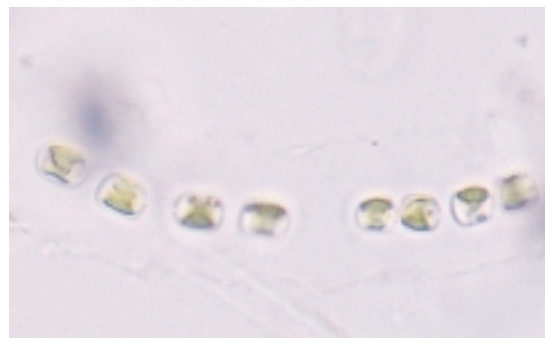
Geminella. *Note the sheath, just visible. x 800*

Klebsormidium

C: unbranched filaments

Division: Chlorophyta **Class:** Charophyceae

How to recognise the genus:

Microscopic: Narrow filaments (less than 10 µm across). The curved chloroplast covers a relatively small proportion of the cell wall.

Habitat and distribution: Not certain.

Possible confusion: The chloroplasts in *Ulothrix* (page 138) are similarly curved but occupy much more of the cell wall.

Note: *Klebsormidium* was recently placed in the Charophyceae (see Margulis *et al.* 1990)
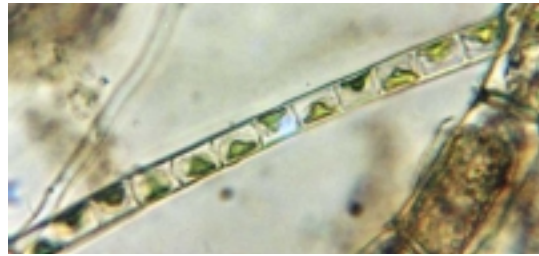
Klebsormidium. *x 550 (photo: Stephen Moore, Otago Regional Council)*

Microspora

C: unbranched filaments

Division: Chlorophyta **Order:** Microsporales

How to recognise the genus:

Macroscopic: Microspora forms long, deep-green filaments, often trailing in water and entangled around other algae or vascular water plants.

Microscopic: Unbranched filaments of regular, oblong or, usually, squarish cells; the chloroplast is reticulate (net-like) and parietal (arranged against the cell walls). Through the microscope the chloroplast appears as a dense layer around the inside of the cell wall.

The clearest distinguishing feature of this genus is the H-shaped join between cells, although this is not always obvious. Try to find the end of a filament; the cells normally break at the end of the H rather than at the dividing wall. Under high power (1000x) it should be possible to see the where successive sections of cell wall overlap. Filaments range from about 10 to 40 µm across.

Habitat and distribution: *Microspora* is found in a range of conditions, but often in clean, gently flowing streams. The genus is common and widespread. It may proliferate in enriched cold streams.

Possible confusion: *Tribonema* (see page 137). This also has H-joins. However, the two genera are easy to distinguish as *Tribonema* has several discoid chloroplasts rather than a dense net.

from top:

Microspora *growing amongst macrophytes in a spring-fed stream.*

Microspora *filaments (with diatoms). x 150*

Fragment of Microspora *in which the cells are dividing. Note the new H-shapes alternating with the older (outer) pieces. x 350*

Microspora *sp. x 680*

Mougeotia

How to recognise the genus:

Macroscopic: Mougeotia grows in light green cottony-looking masses that feel slimy and are often mixed with other types of algae.

Microscopic: Unbranched filaments of oblong cells, usually much longer than they are wide. The chloroplast is a ribbon-like and runs along the cell. It may be twisted in places. Pyrenoids often visible at regular intervals along the chloroplast. In many cases, between each cell is a well-defined lens-shaped (or "rice-grain" shaped) area.

Typically filaments are 25–40 μm across, with cells up to 150 μm long.

Habitat and distribution: *Mougeotia* is typically found in moderately enriched to highly enriched slow flowing streams. It has been observed as the dominant taxon during low flows in an enriched lowland river in Canterbury.

Possible confusion: After freezing, *Mougeotia* and *Spirogyra* (see page 136) can be confused, though the lens-shape between cells (if present) reamins clear. The two genera are easily distinguishable when fresh.

C: unbranched filaments
Division: Chlorophyta, **Order:** Zygnematales
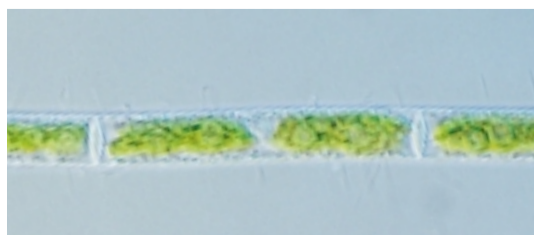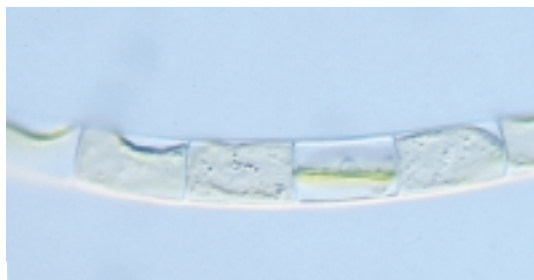
from top:

Mougeotia *from the Waiau River. Note the sheet-like chloroplasts, in some cases folded over. x 500*

Mougeotia *filament from the Waipara River, Canterbury. x 650*

Filament following freezing. x 650

Rhizoclonium

How to recognise the genus:

Macroscopic: Long, coarse, tough filaments, dull-green coloured.

Microscopic: Cells from 10 to over 50 μm wide, usually much longer than wide. Chloroplast is a dense or loose network.

Habitat and distribution: *Rhizoclonium* is found only in high conductivity, warm rivers and streams, Encountered most often in Hawkes Bay (usually very large species). Also found in tidal rivers in Otago (Stephen Moore, pers. comm.)

Possible confusion: Possibly with *Cladophora,* which is branched, though sometimes *Rhizoclonium* puts out rhizoids.

C: unbranched filaments
Division: Chlorophyta, **Order:** Cladophorales
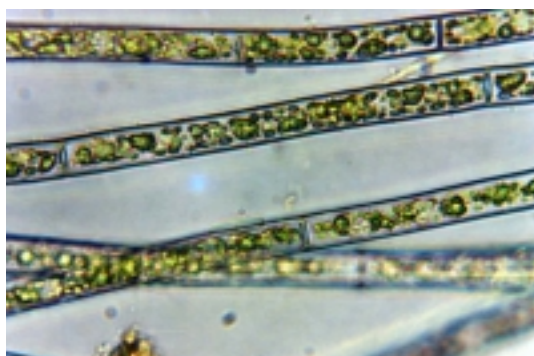
Rhizoclonium *sp. x 450 (photo: Stephen Moore, Otago Regional Council)*

Oedogonium

How to recognise the genus:

Macroscopic: Oedogonium sp. grows in dull green masses and can form massive blooms under warm, stable, low-flow conditions.

Microscopic: Unbranched filaments attached to the substrate at one end. The cells are fairly regular and square to oblong and sometimes slightly barrel-shaped. The chloroplast is a light green parietal network with a lacy appearance. Pyrenoids are scattered throughout the network.Look for the scarring that occurs as a result of cell division – one or more rings encircling the outer cell wall at the end of some cells. This, with the non-branching form, is diagnostic for the genus.

In frozen material, the chloroplast shrinks away from the cell wall but remains attached at the perimeter at each end of the cell. Although it looks quite different from the fresh cells, it is fairly distinctive.

There are many species of *Oedogonium*. To distinguish them it is necessary to consider a combination of size of the vegetative cells, and the size and form of the reproductive bodies. Filaments range from about 10 to 40 µm across.

Habitat and distribution: *Oedogonium* is normally associated with fairly enriched conditions but may also occur in streams with stable, low flows, sometimes with little apparent enrichment. It is extremely common and widespread and occasionally forms blooms.

Possible confusion: Usually there is no difficulty in identifying this genus, although the branched genus *Bulbochaete* (closely related) has similar cells (see page 142).
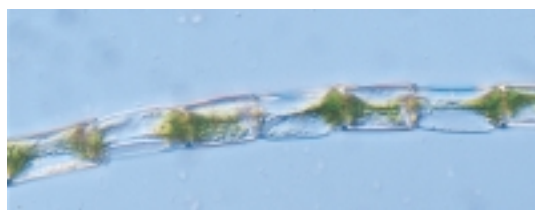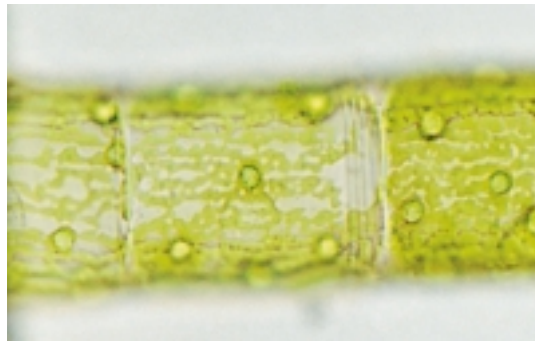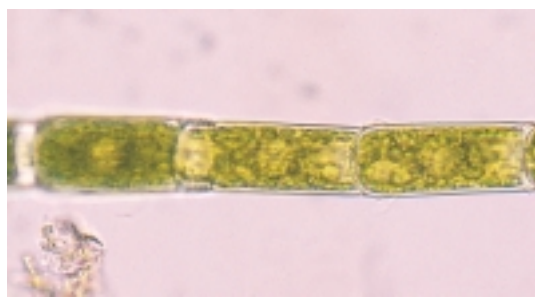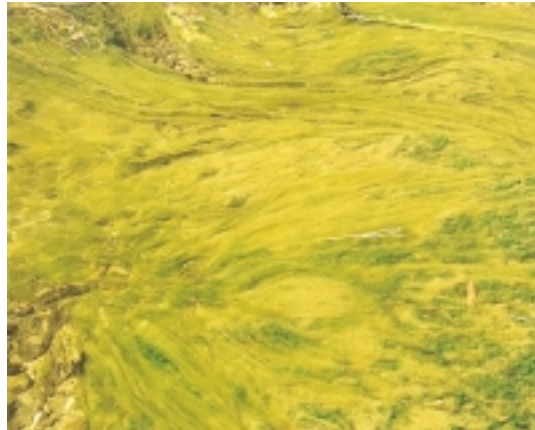
from top:

Oedogonium *bloom in the Hakataramea River, North Otago, 1996.*

Fresh Oedogonium *filament. Nore the scarring on the left hand cell. Specimen from clean, foothillls stream, Canterbury. x 400*

A different Oedogonium *species, from an enriched lowland river. Note the lacy chloroplasts and cell-scarring. x 600*

Filament following freezing. This is the typical appearance: cell walls collapse and the chloroplast shrinks to the middle of the cell. x 300

Spirogyra

How to recognise the genus:

Macroscopic: Clouds of fine, bright green filaments often with a foamy look (bubbles at the water surface). Very slimy to touch.

Microscopic: Smooth-sided filaments with cells varying from almost square to very much longer than they are wide. Cells up to 70 μm wide and 100 μm long, or more. Within the cells, one or more long ribbon-like chloroplasts are arranged in a spiral or series of overlapping spirals. Pyrenoids are dotted along the chloroplasts.

The walls between successive cells are usually more or less flat; sometimes somewhat convex (into the cell). Some species have "replicate" end walls that are evident under the microscope as an H-shape on the dividing wall.

In frozen material the spiral chloroplasts shrink together to form a dense mass at the centre of each cell.

Habitat and distribution: *Spirogyra* is found most often in slow-flowing backwaters in open (unshaded) situations in a range of environments from pristine mountain rivers to lowland streams and often appears in response to point sources of nutrients such as from groundwater inputs. The genus is extremely common and widespread. Species with replicate ends seem to occur mostly in lakes.

Possible confusion: With *Mougeotia* (when frozen) (see page 134). In both cases the chloroplast shrinks to the centre of the cell.

from top:

Spirogyra *growing along the margins of the Ashley River, Canterbury.*

Dense Spirogyra *from Siverstream, Canterbury. x135*

Three species with different spiral patterns. Note the pyrenoids (first specimen). nuclei (second and third specimens) and replicate ends (third specimen) x 270

Spirogyra *filaments following freezing.*
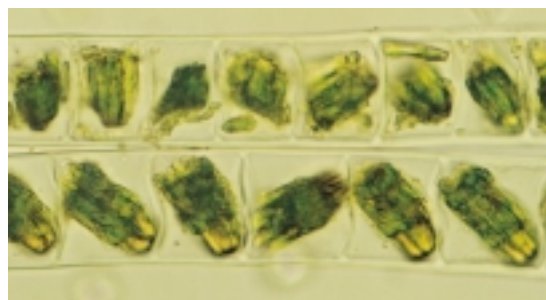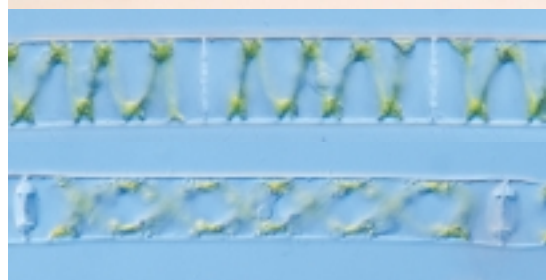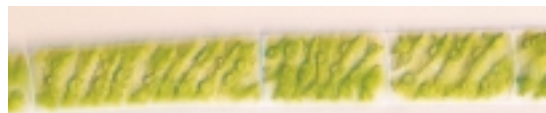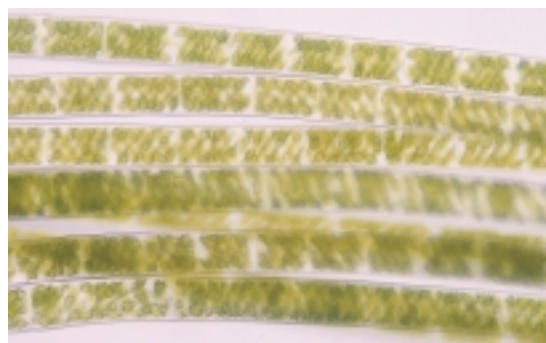
Tribonema

How to recognise the genus:

> *Macroscopic:* Light green masses of filaments.
>
> *Microscopic:* Cells longer than wide, usually slightly inflated at the central part of the cell (barrel-shaped). The cell walls are thin and successive cells are joined at the central area (rather than at the cell dividing walls) where cell walls overlap. Thus when the cells dissociate, they break into H-shaped pieces – halves of adjacent cells. There are several disc-shaped pale green chloroplasts dotted through each cell.
>
> *Tribonema* filaments are usually quite fine, ranging in size from 3 to 10 μm in diameter and 15 to 50 μm long.

Habitat and distribution: This genus occurs in open situations in a range of conditions, from clean headwater streams (mixed with filamentous diatoms, during low-flow conditions), to lowland high-conductivity streams.

Possible confusion: *Microspora* also has H-shaped cell walls. However, in *Microspora* the chloroplasts are much denser and are single sheets rather than several separate bodies (see page 133).
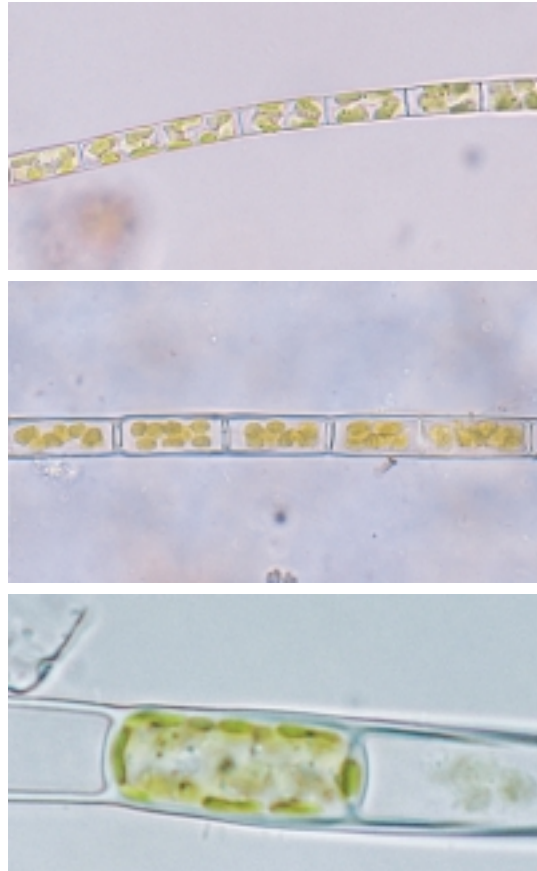
from top:

A fine filament of Tribonema, *showing the disc-like chloroplasts. Cell joins not obvious. x 500.*

Tribonema *filament clearly showing H-joins between calls, and also the yellow-green colour of the disk-like chloroplasts. x 500.*

Higher power photo of a Tribonema *cell separating at the H-join. x 1400*

Ulothrix

How to recognise the genus:

Macroscopic: Vivid green skeins of fine filaments.

Microscopic: Unbranched filaments of cells ranging in length from noticeably shorter than they are wide to (occasionally) longer. Each cell has a single chloroplast that forms either a complete or partial ring around the inside of the cell wall. The width of the ring varies from almost as long as the cell to very narrow. The filaments may be attached to the stream substrate with a holdfast. Cells of *Ulothrix zonata* – a very common species in New Zealand – may be up to 45 μm in diameter. Other species are smaller – up to 20 μm in diameter.

Habitat and distribution: *Ulothrix* sp. (especially *U. zonata*) is very widespread. Much growth occurs in spring and, under suitable flow conditions (i.e., low, stable flows) blooms of *Ulothrix* are common in many lowland rivers. It also dominates the periphyton communities of many mountain streams in spring and late summer.

Possible confusion: Perhaps could be confused with sparsely branched types of *Stigeoclonium* (see page 145), in which the chloroplasts are similar.

from top:

Spring growth of Ulothrix *sp. in a steep headwater stream (Avoca catchment, Canterbury).*

Three examples of Ulothrix *(probably* U. zonata*). Note the air bubbles in the cells in the top two photographs. x 270*

Two examples of Ulothrix *at high power showing the different extent of the chloroplast. x 680*
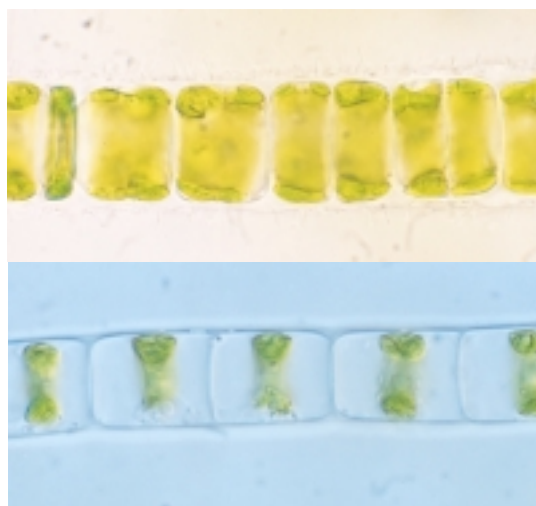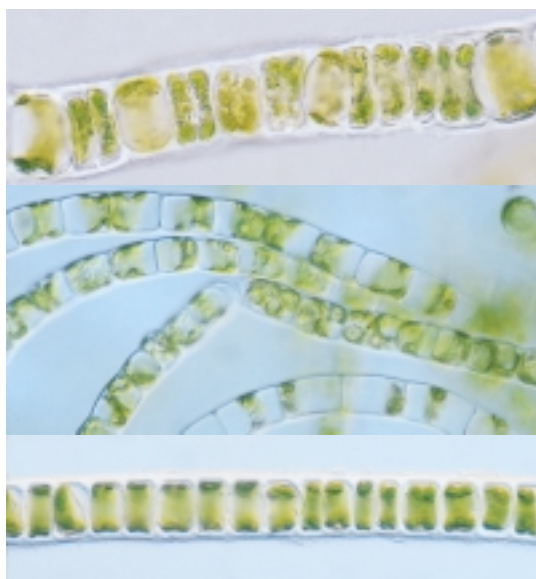
Zygnema

How to recognise the genus:

Macroscopic: Light green slimy filaments.

Microscopic: Unbranched filaments of cylindrical cells with straight or rounded end walls. The cells vary in length from almost square to oblong. There are two star-shaped chloroplasts per cell, arranged side-by side. Sometimes this arrangement is extremely clear. Where the chloroplasts more or less fill the cell, the arrangement is not quite as obvious. In fresh material, often the cell nucleus can be seen between the chloroplasts, as a greyish, more-or-less circular body.

In frozen material the chloroplasts shrink and lose their star-like appearance but it is usually still possible to discern the double side-by-side arrangement.

Filaments vary from about 20 to 50 μm wide.

Habitat and distribution: *Zygnema* is usually found in relatively still waters in lakes, rather than rivers. However it has been collected from slow-moving backwater areas in rivers in a range of environmental conditions: pristine to lowland. This genus is widespread but is not commonly found in periphyton.

Possible confusion: *Zygnema* is usually easy to recognise though there may be confusion with *Spirogyra* in frozen material (see page 136) as the chloroplasts clump together in both genera.
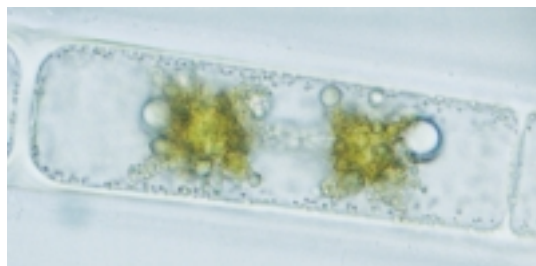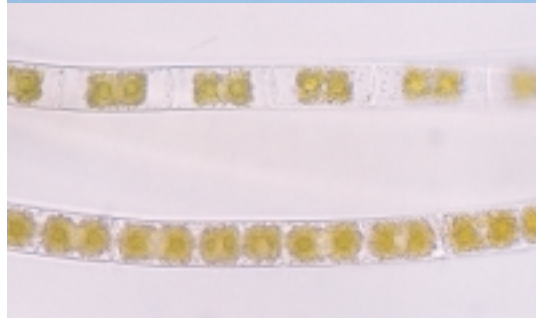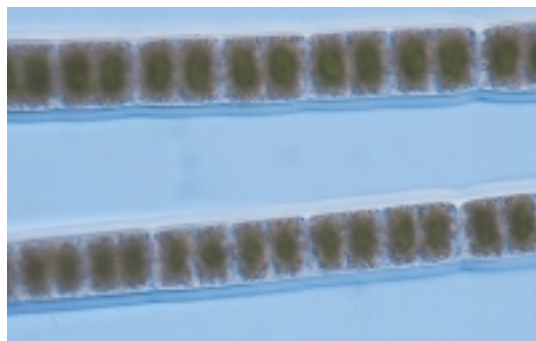
from top:

Zygnema *growing in the Waipawa River, Hawkes Bay*

Filaments with various densities of cell contents: note the muclaginous covering to the filaments in the top two filaments. x 270

Lower photo: x 600.

Audouinella

Division: Rhodophyta, **Subclass:** Florideophycidae

How to recognise the genus:

Macroscopic: Pink–brown patches, like felt, sometimes forming a continuous, firmly attached mat, on rocky substrates.

Microscopic: Tufts of branched filaments growing from a basal holdfast. Thick cell walls and square to oblong cells, sometimes with constrictions at the cell walls. The filaments branch repeatedly with often no clear "main" filament; tips usually rounded or slightly tapering. The chloroplasts are a dull greyish green sometimes with a pink or purple tinge. There are no pyrenoids.

The most common species in New Zealand is *Audouinella hermanii.* Cells are 10–25 μm in diameter.

Habitat and distribution: *Audouinella* can be very common in clean foothills and lowland rivers, where the reddish brown mats may be visible particularly on very stable substrates like bedrock and large stones. Also found in shady forest streams. Widespread.

Possible confusion: Usually none, though note that *Batrachospermum* (page 141) goes through a young stage that resembles *Audouinella.*
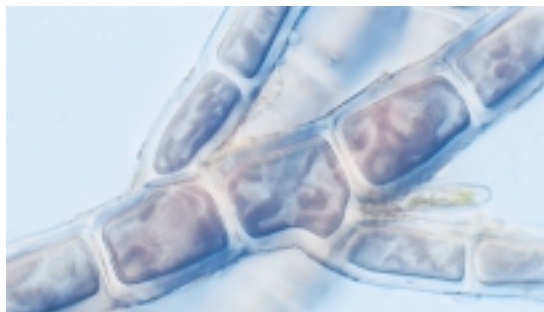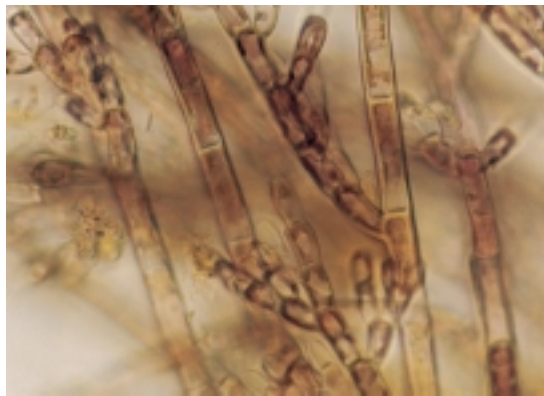
from top:

Audouinella *growing amongst moss and green algae, recently dried out.*

Audouinella *from the Cass River, Canterbury. x 220 (photo: Nelson Boustead)*

Audouinella*. x 680*

Batrachospermum

Division: Rhodophyta, **Subclass:** Florideophycidae

How to recognise the genus:

Macroscopic: Red–brown to green–grey fronds growing in streaming brown jelly-like masses up to about 15 cm long. The main branches are clearly visible to the naked eye and have a beaded appearance.

Microscopic: Batrachospermum comprises a thick central filament bearing closely spaced whorls of much branched filaments. Individual cells are bulbous with rounded cells at the branch tips. The central filament is also branched.

Habitat and distribution: Clean, cool streams, often spring fed, or shady forest streams.

Possible confusion: Unlikely, though note that *Batrachospermum* goes through a young stage that resembles *Audouinella* (page 140). If you see both genera in a sample, there is a good chance that it is all *Batrachospermum.* However, without culturing the sample you cannot be certain.
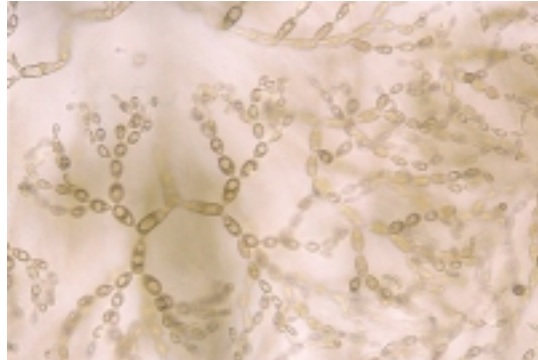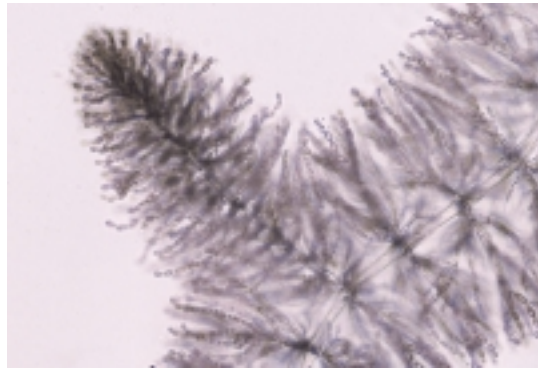
from top:

Batrachospermum *sp. from a spring-fed creek, Canterbury. x 70*

A different species of Batrachospermum *sp. from the same spring-fed creek. x 135*

As above. x 270

Bulbochaete

How to recognise the genus:

Macroscopic: Green filaments with no particular distinguishing features.

Microscopic: Bulbochaete has branched filaments of somewhat bulbous cells, most bearing a long hair – a "seta" (thugh these can detach). Cells are 20–30 µm across. *Bulbochaete* is closely related to *Oedogonium* (see page 135) and has a similar chloroplast – a lacy network lining the cell.

Habitat and distribution: *Bulbochaete* is encountered most often in lakes and pools, however it does occur in the periphyton of outlet streams and rivers.

Possible confusion: Usually none; a very distinctive genus.

from top:

Bulbochaete, *from a lake outlet, Canterbury. x 135*

Bulbochaete *with setae missing Note the resemblance of the cells to* Oedogonium. *x 270.*

Chaetophora

How to recognise the genus:

Macroscopic: Bright green firm jelly-like blobs up to 10 mm across, or elongated branching fronds in mucilage.

Microscopic: Chaetophora is closely related to *Stigeoclonium* and is distinguished by being enclosed in firm mucilage (hence its macroscopic appearance) and by its much denser branching. Each tuft arises from a flattened mass of cells. The chloroplast is a parietal band covering most of the cell wall.

Habitat and distribution: *Chaetophora* seems to prefer cool, clean and moderate- to fast-flowing streams. It is not often found in periphyton but may possibly be more common than recorded because of confusion with *Stigeoclonium* (see below).

Possible confusion: Maybe with *Stigeoclonium*, though growth form differs: *Chaetophora* grows in compact masses, *Stigeoclonium* in streaming tufts. The growth form of *Chaetophora* can be preserved quite well even after samples have been blended and frozen.

from top:

Chaetophora *cf.* incrassata *(branching fronds). x 70*

Chaetophora, *a type that forms firm globules. x 80*

Chroodactylon

Division: Rhodophyta; **Subclass:** Bangiophycidae

How to recognise the genus:

Microscopic: Single rows of vivid blue-green, oval cells, usually isolated from each other, within a thick, clear sheath. Branching is irregular. The chloroplast is star-shaped, though this is not always evident. .

Habitat and distribution: *Chroodactylon* is found occasionally in periphyton, and can be common within individual samples. Distribution not known.

Possible confusion: Distinctive. The genus could be mistaken for a cyanobacteria because of its colour. However, discrete chloroplasts are usually obvious.
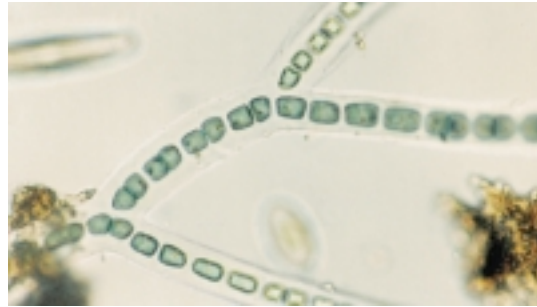
Chroodactylon, *showing branching (top, x250) and the striking blue-green colour (bottom, x 450). (Photos: Nelson Boustead)*

Compsopogon

Division: Rhodophyta; **Subclass:** Bangiophycidae

How to recognise the genus:

Macroscopic: Forms coarse, stringy red–brown or bleached filaments, sometimes very long ("like fishing line").

Microscopic: Main filaments can be 400 μm wide or more and comprise a "cortex" (layer) of angular cells overlying a central axis of large, clear cells. Branches reduce to a single row of disc-like cells at the tips.

Habitat and distribution: Usually reported from tropical areas. In New Zealand, most records of *Compsopogon* have been from lowland rivers in the North Island.

Possible confusion: Distinctive, though it could possibly be confused with another red alga, *Bostrychia* (Subclass: Florideophycidae) which also has multi-cellular branched filaments (see photo). In *Bostrychia* the cells are arranged in longitudinal lines (i.e., a bundle of several filaments with the outer layer forming a cortex of flattened cells), clearest in young filaments. The branch tips tend to curl. In *Compsopogon*, the cells are not clearly in rows. *Bostrychia* is mainly a marine genus but one species occurs in fresh water.
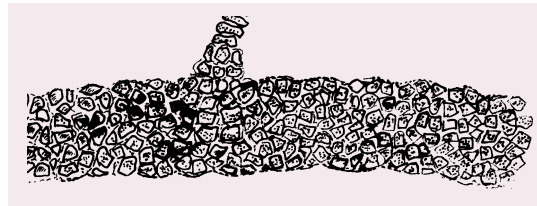
Compsopogon. *Top: SEM of main stem showing the cortex of flattened, angular cells overlying rounded axial cells. x 120. Bottom: drawing showing main stem and branch.*
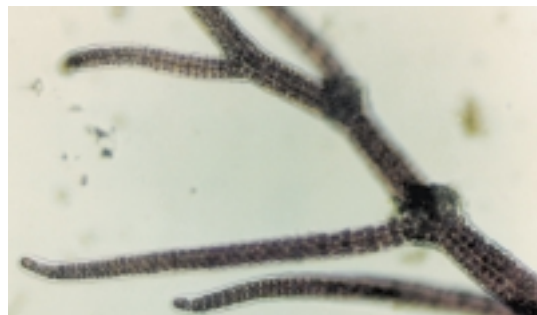
Bostrychia. *x 70 (photo: Nelson Boustead)*

Cladophora

How to recognise the genus:

Macroscopic: Deep or dull green to brown, tough filaments in mats or masses; individual filaments sometimes thick, stringy and khaki brown because of epiphytes.

Microscopic: Large branched filaments with very long cells. Branches originate from cell cross walls and are usually smaller than the main stem. Filaments taper slightly towards rounded ends. The cell contents appear granular – a combination of the lace-like chloroplast lining the inside of the cell walls (often appearing as many small chloroplasts) and numerous pyrenoids.

There is no mucilaginous layer on the outside of the cell walls, accounting for the coarse feel and appearance of *Cladophora*, and the ease of colonisation of the filaments by epiphytes – mainly diatoms.

In frozen material the cell walls may collapse and become distorted.

In New Zealand, *Cladophora glomerata* has been identified as the most common species. Cells on the main axes may be up to 75 – 100 µm across, on branches they are about half that size and also shorter.

Habitat and distribution: *Cladophora* is often found in high-conductivity streams and rivers and can form large blooms in summer and autumn under stable flow conditions. The taxon is widespread in soft-rock areas of North Island (naturally high conductivity) and occurs elsewhere in response to other sources of enrichment.

Possible confusion: *Rhizoclonium* (see page 134). *Rhizoclonium* has large, long, cylindrical cells, very occasionally branched, but not with the tree-like pattern in *Cladophora.* There is a slight chance that *Cladophora* filaments could be confused with *Vaucheria,* which lacks cross walls (see page 147).
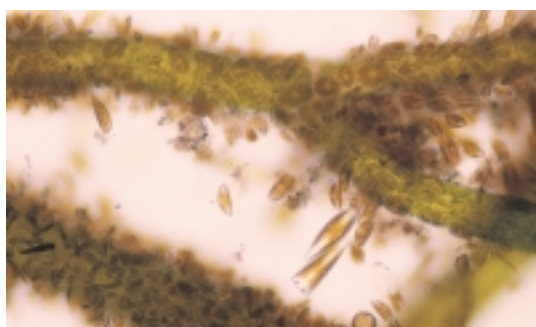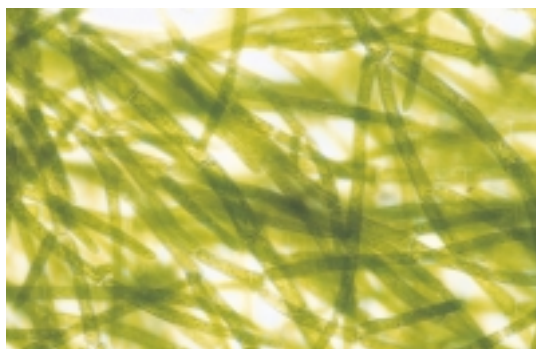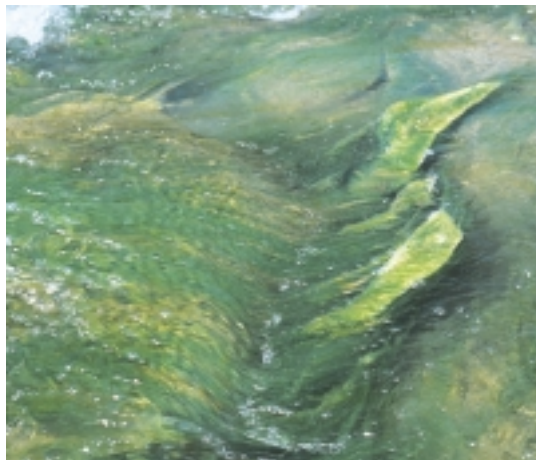
from top:

Cladophora, *Wairoa River, Hawkes Bay.*

Cladophora *sp.: top: x 70; bottom: x 270. Note the pattern of branching.*

Cladophora glomerata, *with epiphytes (diatoms). x 270*

Draparnaldia

Division: Chlorophyta, **Order:** Chaetophorales

How to recognise the genus:

Macroscopic: Light green, soft masses, up to about 5 mm across, slimy because of copious mucilage.

Microscopic: Draparnaldia is closely related to *Stigeoclonium* and *Chaetophora*. It is distinguished by the thick central filament that carries densely branching tufts of filaments whose ends taper to fine hairs. The large central cells have a single parietal chloroplast that extends part way around the cell and is much narrower than the cell. The central stem is up to 100 µm across. Branch cells are up to 10 µm across, and longer than they are wide.

Habitat and distribution: *Draparnaldia* occurs mostly in clean, cool streams, often spring-fed. Distribution is uncertain.

Possible confusion: Usually no problem identifying this genus.

from top:

Draparnaldia *from a spring-fed stream, Canterbury. x 80*

Note the very wide main branches. x 200

Stigeoclonium

How to recognise the genus:

Macroscopic: Bright green tufts growing on stony substrates, often dotted amongst growths of brown algae (diatoms).

Microscopic: Branched alga with small, squarish to elongated cells, sometimes inflated at the centre and constricted at the cross walls. The cross-walls are straight. Within the cells, the chloroplast partly lines the inside of the cell wall. Each tuft is attached to the stream substrate by a holdfast structure of similar cells in a sheet-like arrangement. Branching varies among species and can be quite sparse. The filaments taper either to a point or to a more blunt end. Cell diameter is typically up to about 15 μm for main axes and less for the branches.

Stigeoclonium does not change greatly in overall appearance following freezing. The branching habit and tapering filaments of this genus make it easy to recognise, fresh or frozen.

Habitat and distribution:

Stigeocloneum seems to prefer moderate- to fast-flowing streams in a range of environments, from clean to enriched waters. The genus is common and widespread and may dominate the periphyton.

Possible confusion:

In very sparsely branched specimens the filaments may be mistaken for *Ulothrix*, because of the similar arrangement of the chloroplast. If you identify a doubtful *Ulothrix*, check along the filaments to see if there is any branching. Also watch out for confusion with *Chaetophora*, which forms firm mucilaginous clumps (see page 143).
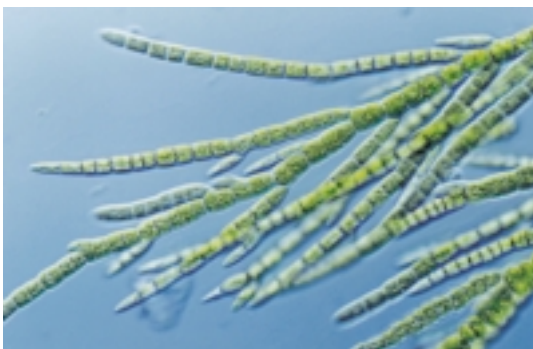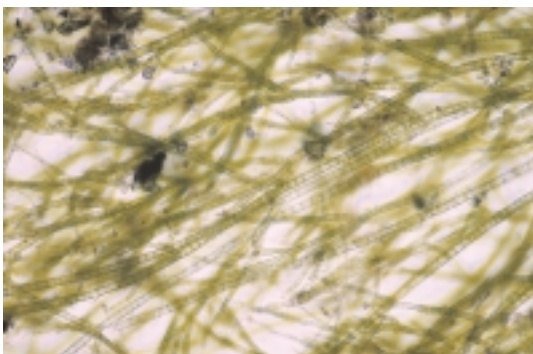
from top:

Tuft of Stigeoclonium *with diatoms.*

Stigeoclonium *from the Hokitika River, West Coast. x 70.*

Stigeoclonium *from the Cust River, Canterbury. x 270*

Vaucheria

How to recognise the genus:

Macroscopic: Dark green round or oval "cushions" – up to about 20 cm across – attached to stones or other stable substrate. When squeezed free of water the cushion has a tough "woolly" feel.

Microscopic: Large branched filaments with rounded tips and no cell cross walls. Multiple small, disc-like chloroplasts line the cell wall. Diatom epiphytes are often present.

In frozen material, the cell walls collapse and become distorted and the chloroplasts clump together.

The filaments are up to 150 µm in diameter and decrease in size with branching. Note that branching can be infrequent and the clearest diagnostic feature is the lack of cross walls.

Habitat and distribution: Found in a wide range of conditions, from clean, cool headwater and spring-fed streams, to more enriched lowland locations.

Possible confusion: The macroscopic appearance of *Vaucheria* is very characteristic. It is possible that isolated filaments under the microscope could be mistaken for *Cladophora*. Therefore in cells with apparently multiple small chloroplasts, you should always check for the presence of cross walls.
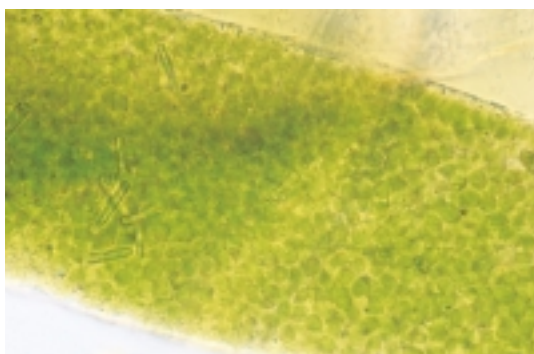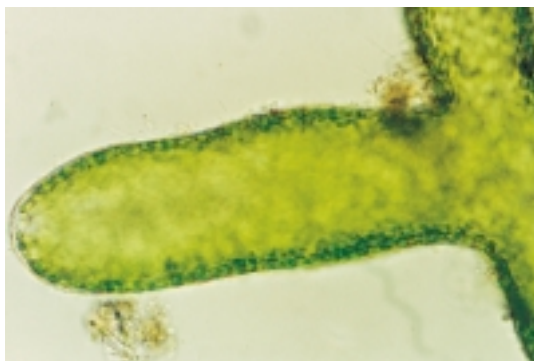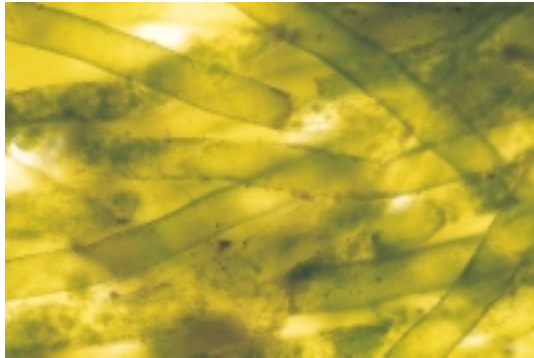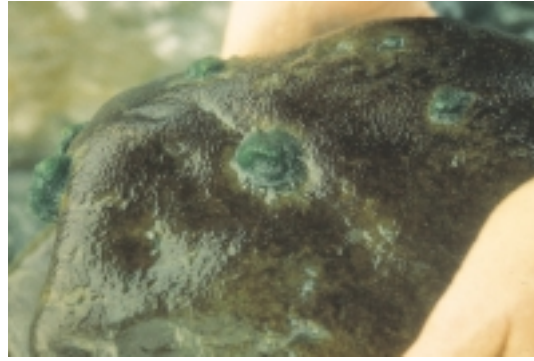
from top:

Vaucheria *growing in tufts.*

Vaucheria *from the Avon River, Christchurch. x 70*

Showing branching, x 135

Showing many small chloroplasts. x 270

Aulacoseira   C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta **Order:** Centrales

How to recognise the genus:

Microscopic: Aulacoseira forms distinctive short filaments in which the ornamentation of the valves is often visible even under relatively low power (e.g., x 400). Several irregular chloroplasts.

Possible confusion: Usually none.

Aulacoseira species

The most common species of *Aulacoseira* in New Zealand is probably *A. granulata* (and its varieties). The species is is characterised by widely spaced long spines linking successive valves. These cannot be seen easily in live material. The valves range from 5 to 25 μm wide and can be short and fat or very elongated (*A. granulata* var. *angustissima*). The specimens illustrated are tentatively identified as this species.

Habitat and distribution: *Aulacoseira* species are most often found in lakes as part of the phytoplankton. However they may appear occasionally in the periphyton of lake-fed streams and rivers.
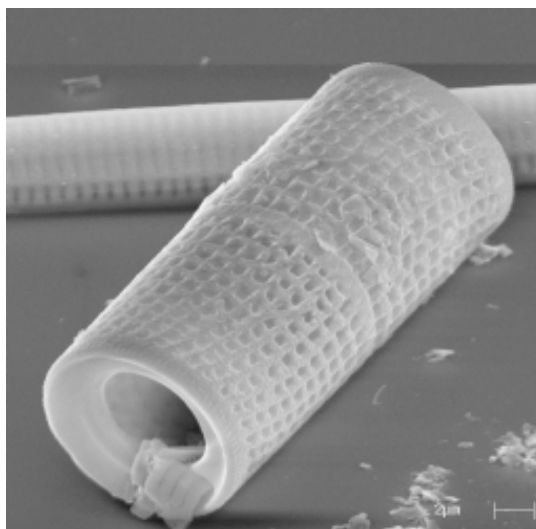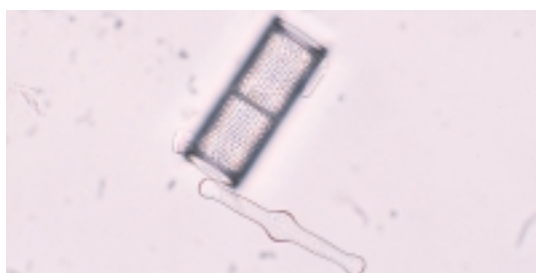
Note: The old genus name *Aulacoseira* has recently been resurrected to include several species previously placed in *Melosira*, but separated because of their very different cell wall structure (see Round *et al.* 1990).

from top:

Aulacoseira *filament, Tarawera River. x 270*

Cleaned specimen, Waiau River. x 680

Aulacoseira *sp. SEM. Cells separated at the girdle.*

Diatoma   C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Macroscopic: Brown shiny, slimy covering on rocks, though often *Diatoma* species are mixed with other algae – both diatoms and green algae.

Microscopic: Diatoma species form ribbon-like filaments or zig-zag or star-like colonies. They are also seen as solitary cells. Each cell contains several chloroplasts. In cleaned material *Diatoma* cells lie in either valve or girdle view. Thick ribs of silica across the valve face ("costae") are visible as dark lines (valve view) or knobs at the sides (girdle view).

Possible confusion: The two *Diatoma* species described below are fairly distinctive.

Diatoma hiemale var. *mesodon*

Short ribbon-like filaments of square cells each containing several irregular, brown-coloured chloroplasts; sometimes seen from the top. Often seen as single cells. Two or 3 ribs across the valve face. Cells 15–20 µm long and 5–12 µm across (valve view).

Habitat and distribution: Common and widespread; "occasional" or less in many periphyton samples. This species often dominates the periphyton in cold, clean headwater streams.

Diatoma tenuis

Solitary or in zig-zag colonies; cells elongated. In valve view, the two ends may bulge out slightly (sometimes in different directions). In girdle view you see a rectangle with dark dots down each long edge. Cells are typically 15–30 µm long and up to 4 µm wide.

Habitat and distribution: *D. tenuis* has been identified mainly from the periphyton of southern South Island rivers, from the lowlands to the headwaters. This species has been recorded as dominant in the periphyton, e.g., in some locations on the Waiau River, Southland, in summer 2000.

from top:

Filaments of D. hiemale *growing in a backwater of a pristine mountain stream. (The green is* Spirogyra.*)*

Live Diatoma hiemale *chains. x 450*

Diatoma hiemale *valve and girdle views. x 1000*

Joined cells of Diatoma tenuis, *girdle view, plus valve view (right). x 1200*
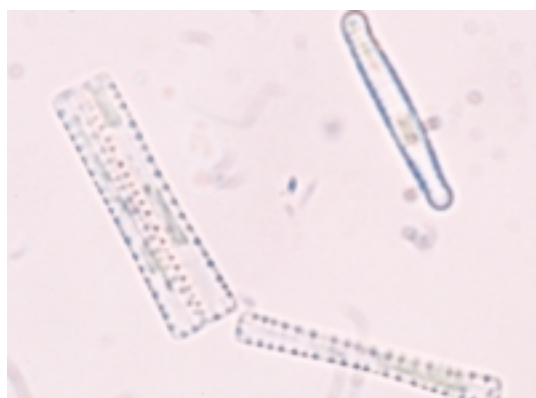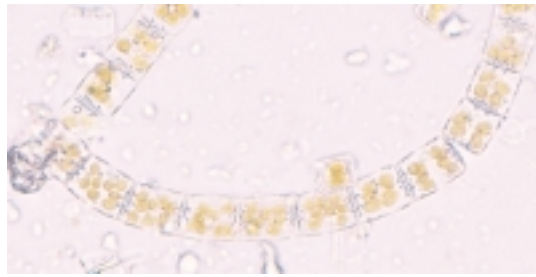
Eunotia

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Eunotiales

How to recognise the genus:

Microscopic: In valve view *Eunotia* species are mostly variations on a crescent shape; the girdle view is rectangular, sometimes rhomboidal. Each part of the raphe system is very short. Often all four raphe ends may be seen in girdle view. Some species of *Eunotia* form ribbon-like filaments in which they lie in girdle view. There are two sheet-like chloroplasts in each cell. Species range in size from 10 μm up to 150 μm long.

Possible confusion: Single cells of *Eunotia* are usually recognisable, especially if lying in valve view. In filaments, there could be confusion with *Fragilaria.* Look for single cells in valve view that are likely to be the same species as the filaments (from their size, etc). In wet samples you can agitate the sample to get cells to turn over. Check the chloroplasts: *Fragilaria* has irregular plates; *Eunotia* usually has two larger sheets per cell. Under high power LM, focus through to find the raphe system in *Eunotia.*

Eunotia species

Identification of many *Eunotia* species is difficult without access to a good microscope and to cleaning and mounting facilities. Therefore we do not provide any individual descriptions. For characterising periphyton it is more important to be able to distinguish between filaments of *Fragilaria* and *Eunotia.*

Habitat and distribution: *Eunotia* species tend to be very common in low-conductivity, acid still waters, e.g., in lakes and upland bogs and tarns. The genus can also be an important part of stream periphyton, especially as filaments in relatively clean, spring-fed lowland streams, or mountain-fed streams, e.g. *Eunotia* cf. *serpentina* (illustrated).

from top:

Eunotia *filaments from a spring-fed stream, Canterbury. Note the chloroplasts. This is probably* Eunotia serpentina, *which has undulating valve margins when seen in valve view, discernible here as faint shadows running along the filament. x 375*

Girdle view of a single valve of Eunotia *cf.* serpentina*. x 800*

Valve view, Eunotia serpentina*. x 800*

Valve view of Eunotia cf. incisa *(from a small stream, Stewart Island). x 2000*

SEM showing structure of valve of Eunotia*. Note short raphes extending over the edge of the valve face.*
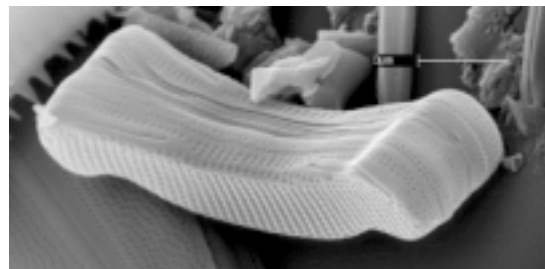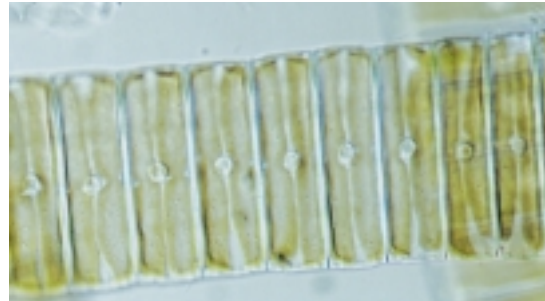
Fragilaria

C: unbranched filaments/single cells (diatoms)

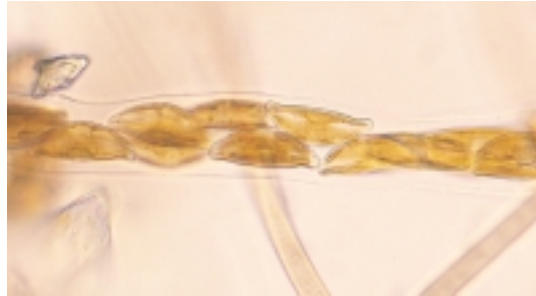**Division:** Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Microscopic: Fragilaria species form ribbon-like colonies in which cells lie in girdle view. Some species are commonly seen as single cells. An elongated chloroplasts lies against the face of each valve. Currently, the genus *Fragilaria* is reserved for species that have certain structural features that cannot usually be seen under LM (including, in many species, spines around the margins of the valve face, which allow them to form chains).

Possible confusion: Single cells of some *Fragilaria* species cannot be distinguished from *Synedra* under LM. Some *Synedra* species also have spines around the valve edge and form chains (page 187). *Fragilaria* filaments could be confused with *Eunotia* (see page 150).

Fragilaria vaucheriae

Small spindle-shaped cells, sometimes with capitate ends, rectangular in girdle view. Often seen as single cells but also in filaments. The species is distinguished by the presence of a slightly swollen area, without striae, to one side of the valve centre. There are two narrow chloroplasts. Up to 30 µm long and 6 µm wide.

Habitat and distribution: Very common; seems to occur in a wide range of conditions.

Fragilaria capucina

Pencil-shaped cells generally longer than *F. vaucheriae* (up to over 100 µm long, but often only 25 µm). Central area spans the whole width of the valve, clear axial area. Fine striae usually, though some varieties have a coarser arrangement with striae clearly alternating on either side of the valve (see photo).

Habitat and distribution: Probably widespread. When not in chains, some varieties of this species may be identified as *Synedra rumpens* (see page 188).
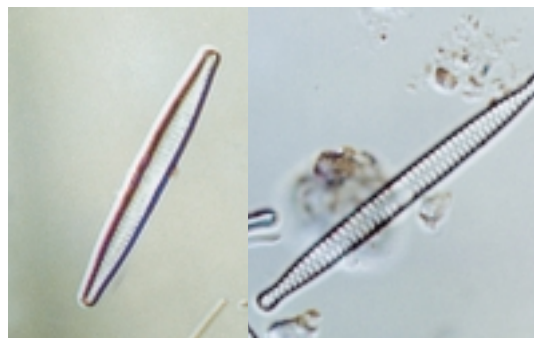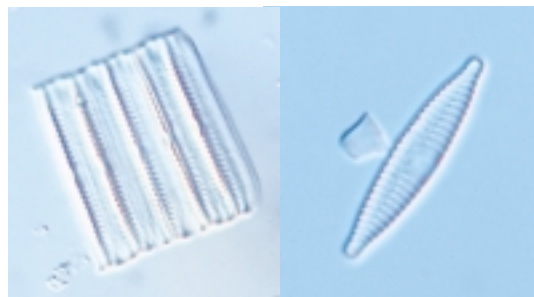
from top:

Fragilaria filaments growing over a thick diatom mat.

Filament of Fragilaria vaucheriae*. Fresh material. x 500.*

Fragilaria vaucheriae, *cleaned frustules, valve view (left), girdle view (right). x 2000*

Fragilaria capucina (left); Fragilaria capucina *var.* distans (right – *note the widely-spaced striae). x 2000*

SEM , Fragilaria capucina. *Note the small spines at the valve margin.*
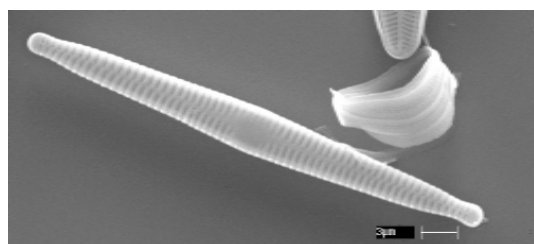
Fragilariforma   C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Microscopic: Fragilariforma is a new genus based on *Fragilaria viriscens*. It is distinguished from *Fragilaria* by the type of puncta and its very narrow pseudoraphe, i.e., striae almost span the whole valve width. Other diagnostic features are visible only under SEM. *Fragilariforma* species form ribbon-like colonies in which they lie in girdle view.

Possible confusion: With other *Fragilaria* species and also with *Eunotia* species in chains (see page 150). Always try to find a valve view in the sample.

Fragilariforma viriscens

Small elongated cells (10–20 µm long by about 10 µm wide) usually seen as filaments. The cells have fine striae, a very narrow axial area and no central area.

Habitat and distribution: Occasionally found in South Island periphyton samples. Reported to prefer low-conductivity spring and stream waters (Cox 1996).
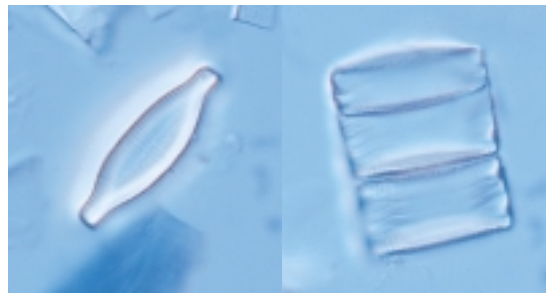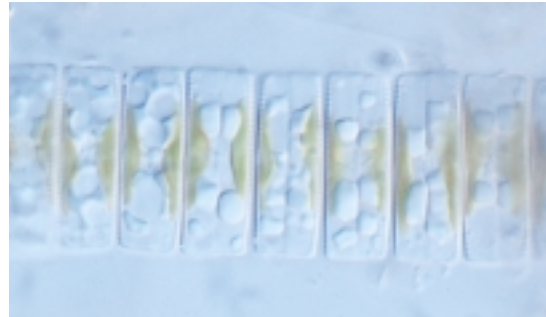
from top:

Fragilariforma cf. viriscens*: live filament. x 1000*

Cleaned material, valve and girdle views; from a South Island West Coast stream. x 850.

Note on the re-classification of *Fragilaria*

The genus *Fragilaria* has recently been downsized by splitting off newly established genera including *Fragilariforma.* The other new genera represented in this guide include species that are most often seen as single cells, though they may also form short filaments. They are covered on pages 184 (*Staurosira*, based on *Fragilaria construens*) and 185 (*Staurosirella*, based on *Fragilaria lapponica*).

Pseudostaurosira (based on *Fragilaria brevistriata*) is also seen occasionally (see photo, right)

See Williams and Round (1987, 1988) for a full account of the new genera.

Pseudostaurosira brevistriata *valve view and short chain, Okuku River, Canterbury. x 1300*

Melosira　　　C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Centrales

How to recognise the genus:

Macroscopic: Medium brown fluffy masses, though often *Melosira* is mixed with other algae – both diatoms and green algae. Can sometimes form whitish to iridescent green mats as a monoculture.

Microscopic: Cylindrical filaments with cells approximately as long as they are wide. There is no ornamentation on the cell walls either in valve or girdle view. Several to many irregular, brown-coloured, chloroplasts line the cell walls. Sometimes the chloroplasts fit together in a neat jigsaw pattern. Cells are loosely joined and the filaments break up easily into single cells, especially following blending. It is common to see *Melosira* only as single cells unless the sample is dominated by the genus.

Possible confusion: *Aulacoseira* (page 148) forms similar chains but the valves have noticeable ornamentation. If Melosira is not recognised as a diatom filament, the species may be confused with a range of non-diatom filaments.

Melosira varians

This is the only *Melosira* species known in New Zealand. Cleaned material viewed under high power shows barrel-shaped frustules with little ornamentation. Easy to identify. *M. varians* ranges from 10 to 40 μm in diameter.

Habitat and distribution: *Melosira varians* is found throughout the country in slow to medium flowing open lowland streams. It can dominate the periphyton community in moderately enriched situations. It is reported as both a "cleanwater species" and "moderately polluted water species" in Cassie (1989).

Note: Other species of *Melosira* listed in some texts have been transferred to the genus *Aulacoseira*, notably *A. granulata* (see page 148).

from top:

Masses of Melosira varians *growing on a stream bed.*

Melosira varians *-dominated periphyton community, photographed following storage (freezing). x 400*

Healthy Melosira varians *filament showing chloroplasts in two planes of focus. x 480*
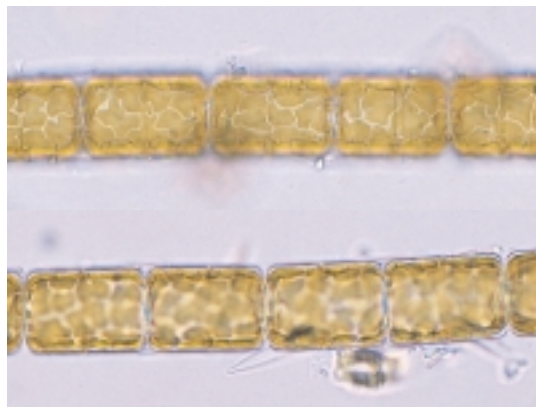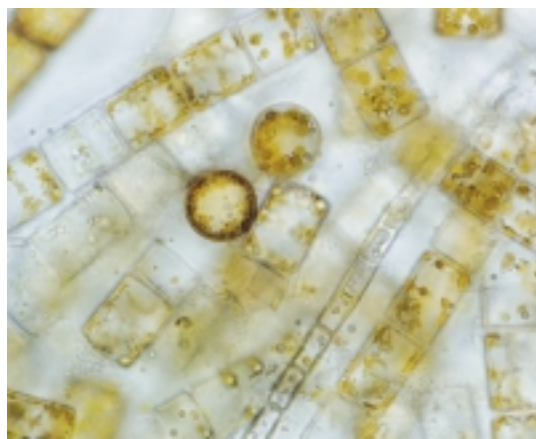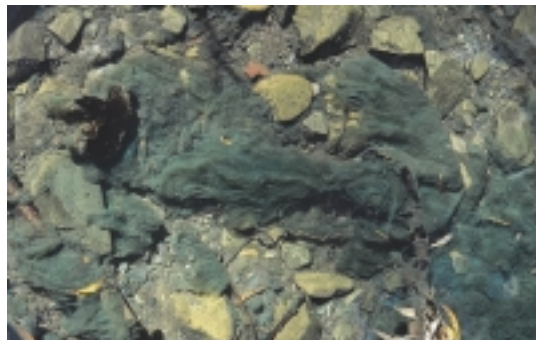
Tabellaria

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Tabellariales

How to recognise the genus:

Microscopic: *Tabellaria* species form zig-zag or straight filaments and are also often seen as single cells. The cells usually fall in girdle view – oblong to square with prominent dark projections running towards the centre of the cell ("septa" – sheets of silica partly filling in the girdle bands). Valve views – narrow with swollen ends and centre – are usually seen in cleaned preparations where frustules have broken apart into the two valves and several girdle bands. This is an araphid genus. There are several small chloroplasts throughout the cell.

Possible confusion: Unlikely.

Tabellaria flocculosa

Tabellaria flocculosa forms zig-zag chains of valves in girdle view. Each valve is square to oblong in girdle view and usually about 10–40 μm wide by 30–100 μm long. There are several (up to 7 or 8) irregular looking septa at each side, usually with short rudimentary septa opposite. In valve view there is a prominent central bulge and expanded ends.

Habitat and distribution: This is the only *Tabellaria* species found widely in New Zealand periphyton. Although widespread, it is not often common in stream periphyton communities. It seems to do best in low-conductivity, clean waters. (see Cassie (1989), p. 18). We have found the elongated form illustrated mostly in clean, high-altitude outlets from tarns.
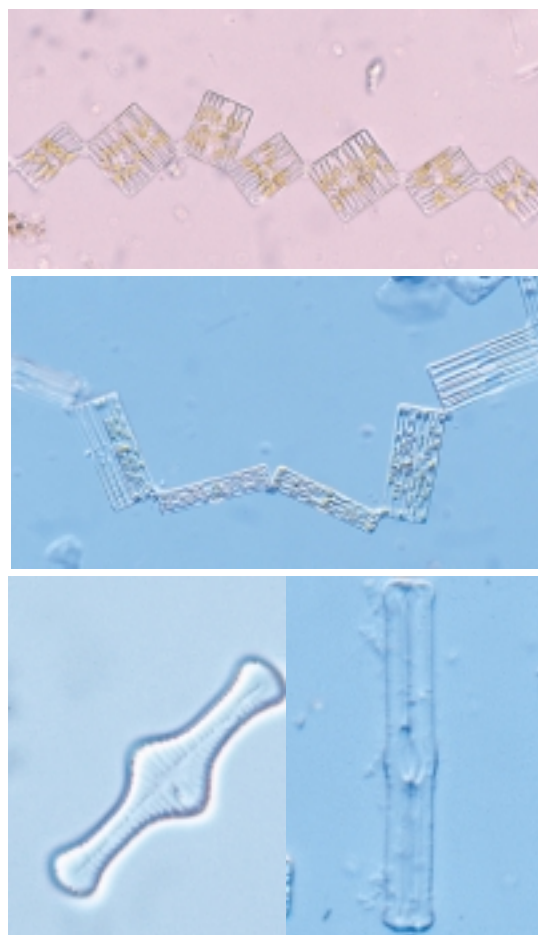
Note: The elongated *Tabellaria* species illustrated is most likely a form of *Tabellaria flocculosa*. A widespread species that has the same shape in girdle view is *Tabellaria fenestrata*. However, in *T. fenestrata* there are usually four septa only and no rudimentary septa. Some the the cells shown here appear to have both rudimentary septa and sometimes more than four full-sized septa.

from top:

Chains of Tabellaria flocculosa, *typical cells, more or less square (top), and elongated oblong cells (bottom). x 350*

Tabellaria flocculosa, *valve views. Left: typical form. x 1500. Right: elongated form. x 700.*

The following two genera form "filaments" in mucilage tubes in which the cells are not joined into true filaments. Both genera are frequently seen in single-celled form, and are described in more detail under C: single cells (diatoms)

Encyonema

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Microscopic: See page 166 for a full description. Some species of *Encyonema* typically form long "filaments" of somewhat irregularly arranged frustules in mucilage tubes.

Possible confusion: Single cells of *Encyonema* are easily confused with *Cymbella*, though the latter tend to grow on stalks (like *Gomphonema* or *Gomphoneis*) and do not occur in mucilage tubes.

Encyonema caespitosa *packed into a mucilage tube, forming a filament. x 400*

Frustulia

C: unbranched filaments/single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: See page 169 for a full description. *Frustulia* may occur scattered in long mucilage tubes, but is normally encountered as single cells.

Possible confusion: Single cells of *Frustulia* may be confused with other naviculoid diatoms but this is the only naviculoid genus you will find in mucilage tubes.

Frustulia *cells in a mucilage tube. x400*

Achnanthes

How to recognise the genus:

Microscopic: This is a monoraphid genus. In girdle view the valve is slightly bent into a V-shape with the raphe valve on the inside of the V (i.e., concave). The raphe and pseudoraphe are somewhat off-centre and the areolae are large and clearly seen at 1000 x.

Possible confusion: Unlikely. Most representatives of this genus are marine, and the freshwater species are large and distinctive.

Achnanthes inflata

Microscopic: The only common representative of *Achnanthes* in periphyton in New Zealand is *A. inflata*, which is recognised from its large size (up to 80 µm long) and distinctive shape.

Habitat and distribution: Normally found mainly in clean, cool, shaded streams and never in great numbers. This species seems to be able to withstand some salt-water intrusion (Patrick and Reimer 1966) and has been reported as common in an Otago estuary (Steven Moore, Otago Regional Council, pers. comm.)
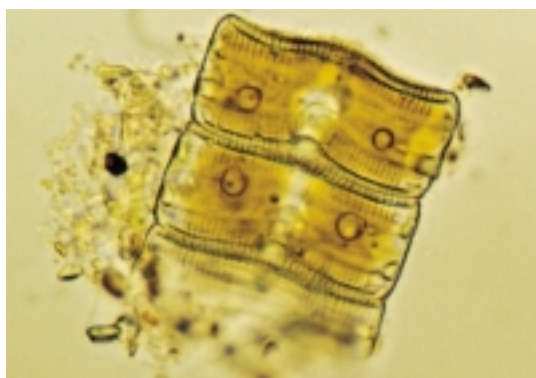
from top:

Achnanthes *cf.* inflata, *valve view (raphless valve). x 1000*

Achnanthes *cf.* inflata, *live cells in girdle view. x 1000*

A note on the re-classification of *Achnanthes*

Until recently many monraphid diatom species with a "bent" valve were included in *Achnanthes*, but many freshwater species have now been transferred to the genus *Achnanthidium* [and subsequently to other genera, see Ross *et al.* 1990] (see page 157). The genus *Achnanthes* is currently reserved for species with a particular type of areola and raphe structure. The chloroplast is also different.

Achnanthidium

How to recognise the genus:

Macroscopic: Achnanthidium is usually part of a mixed community of periphyton. When dominant these diatoms form a dense, dark brown slimy layer.

Microscopic: A monoraphid genus. Most *Achnanthidium* species are very small and live attached by mucilage to substrates or aquatic plants. The valve is bent so that in girdle view they are slightly V-shaped. In valve view they are oval, elongated or captitate (i.e., with a "head" at the ends). In many species the raphe and pseudoraphe valves differ in their ornamentation. There is one chloroplast.

Possible confusion: Other small diatoms. Look for the V-shape in girdle view.

Achnanthidium minutissimum

A very small species (5–25 μm long), narrow with slightly pinched ends. In girdle view the V is flattened out a little at each end.

Habitat and distribution: Common and widespread in a range of ecological conditions but does best in clean, low-conductivity streams. The species often dominates the periphyton following flooding, growing in dense masses attached to substrates. In more stable conditions other species take over.

Achnanthidium lanceolatum (now transferred to *Planothidium lanceolatum*, see note page 158)

Planothidium lanceolatum has an obvious "empty area" on the pseudoraphe valve on one side of the central area, formed by a flap of silica. The cells can be relatively large (up to 35 μm long) compared with other species in this genus. Each stria is made up of more than one row of puncta (visible only under SEM).

Habitat and distribution: *P. lanceolatum* is widespread and occasionally abundant. It occurs in the periphyton of a wide range of river and stream types, is tolerant of moderate levels of organic pollution and is reported to favour alkaline waters (e.g., see Round and Bukhtiyarova 1996).
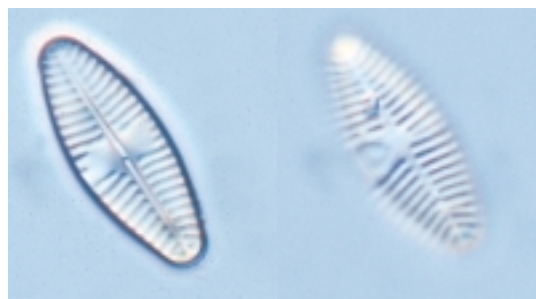
from top:

Dense brown coating of diatoms on a river stone. Achnanthidium minutissimum *dominated this community from the Selwyn River, Canterbury.*

Achnanthidium minutissimum, *valve and girdle views (g.v. in centre). x 1400*

Planothidium lanceolatum, *showing the raphe valve (left) and rapheless valve (right). Note the silica "flap". x 1100*

Achnanthidium linearis (now transferred to *Rossithidium linearis*, see note below)

A very small diatom (10–20 µm long), elongated oval in shape and often seen in girdle view as a straight-sided shallow "V". Striae are quite close together and usually parallel, with no break at the centre except sometimes a slight gap on the raphe valve.

Habitat and distribution: Common, wide-spread and seems to tolerate a wide range of conditions.

The following related species have yet to be formally transferred to an appropriate genus.

Achnanthes oblongella (= *A. saxonica* in Foged 1979)

Another small species, 10–20 µm long. The raphless valve has quite prominent and slightly irregular striae.

Habitat and distribution: Widespread but not usually common.

Achnanthes exigua

Small, 10–20 µm long, with noticeably pinched ends.

Habitat and distribution: Not very common. Habitat unknown.

from top:

Rossithidium linearis, *valve view (left) and girdle view (right). x 1400*

Achnanthes oblongella, *raphe valve (left) and raphless valve (right). x 1400*

Achnanthes exigua, *rapheless valve. x 1400*

A note on the re-classification of
Achnanthidium

Some species included in *Achnanthidium* have been re-assigned to new genera, proposed by Round and Bukhtiyarova (1996) on the basis that the original genus lacked any unifying diagnostic characters except for the "bent" frustule and for being monoraphid. The genera commonly found in the New Zealand periphyton are represented by the first three species described above:

Achnanthidium (e.g., *A. minutissima*)

Planothidium (e.g., *A. lanceolata*)

Rossithidium (e.g., *A. linearis*)

Actinella

Division: Bacillariophyta, **Order:** Eunotiales

How to recognise the genus

Microscopic: Elongated cells expanded at one end, and living attached to plants or substrate by the other (narrow) end. Some species are wedge-shaped in girdle view (like *Gomphonema).* Closely related to *Eunotia, Actinella* species also usually have an inconspicuous raphe system that lies to one side of the valve.

Possible confusion: *Actinella* species are often very distinctive, however small forms that are wedge-shaped in girdle view could easily be mistaken for *Gomphonema* under low power LM. Check for raphe endings at the centre of the valve in *Gomphonema* (visible as a slightly thickened area in the middle of each long side in girdle view, see page 171).

Actinella species

Until recently *Actinella* had been rarely reported in New Zealand. The genus has now been found in several locations and these finds include new species that are still being described.

Habitat and distribution: *Actinella* seems to be confined to clean, acid habitats. It can be extremely common locally. Distribution not certain. Most recent findings have been in the South Island and Stewart Island.

Actinella *sp. valve and girdle views. From the outlet stream of a high altitude tarn, South Island. x 1400*

Asterionella

Division: Bacillariophyta, **Order:** Eunotiales

How to recognise the genus

Microscopic: An araphid genus with long thin cells inflated at one or both ends, often forming star-shaped colonies.

Possible confusion: Should be no problem recognising this genus.

Asterionella formosa

Long cells, inflated at both ends, more at one end than the other. Often seen joined at one end to make a star-shape.

Habitat and distribution: *Asterionella formosa* is most common in lake plankton, but can be common in lake-fed rivers and streams.
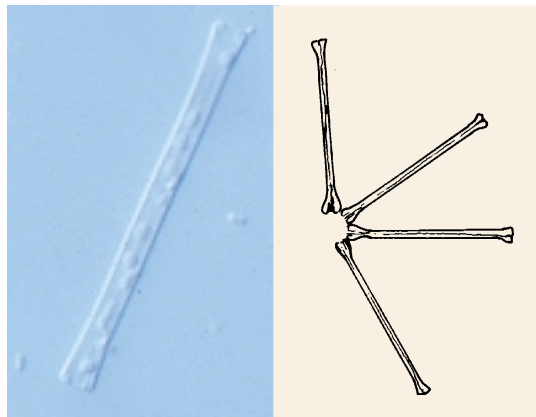
Asterionella formosa. *Left: single cell from periphyton (Waiau River). x 600. Right: drawing of part of star-shaped colony.*

Amphora

Division: Bacillariophyta, **Order:** Thalassiophysales

How to recognise the genus:

Microscopic: An asymmetrical biraphid diatom. *Amphora* species in valve view are roughly crescent-moon-shaped, like *Cymbella.* However, because the girdle band is much wider on the convex side of the valve than on the concave side, the two valves are usually visible side-by-side, i.e., the girdle and valve views are the same. Single valves are seen when the frustule has broken apart. There is an H-shaped chloroplast. In many *Amphora* species, the ornamentations on the valve are too fine to be seen except under very high power.

Possible confusion: The valve views of *Amphora* and *Cymbella/Encyonema* are similar in shape: *Amphora* tends to be finer. In *Cymbella* and *Encyonema* the girdle may be also wider at the top (curved) side of the valve than at the bottom so that in girdle view, both valve faces are visible obliquely. You'll need to look for cells in valve view to be sure of your identification. Also note that *Eunophora* (see photo below) looks superficially like *Amphora,* but is actually closely related to *Eunotia. Eunophora* (a recently described genus, see Vyverman *et al.* 1998) is common in pristine, high-altitude lakes but is rarely found in streams and therefore is not described separately in this manual.

Amphora veneta var. *capitata*

A very small, fine diatom in which the valve markings are difficult to see. Size: 20–40 μm long and up to 15 μm wide (whole valve).

Habitat and distribution: *A. veneta* has been reported as common in a moderately enriched North Island stream and has occurred occasionally elsewhere. It is reported (Cox 1996) to occur in enriched waters and to be pollution tolerant.

Amphora ovalis

Much larger, 30–100 μm long and up to 50 μm wide.

Habitat and distribution: Not seen very often to date. We have found some in the Waiau River. This species is reported to be widespread in medium conductivity waters overseas.

Compare with *Eunophora*:

from top:

Amphora veneta *var.* capitata, *single valve (left) and whole frustule. Cleaned material, from the Waitekauri River, Coromandel. x 1000*

Amphora ovalis, *from the Waiau River, Southland. x 1000*

Eunophora *sp. (Order: Eunotiales). As in* Amphora *the valves lie side by side; however the genus has four to many chloroplasts and features in common with* Eunotia. *Left: live cell , x 700 ; right: cleaned frustule x 1000*
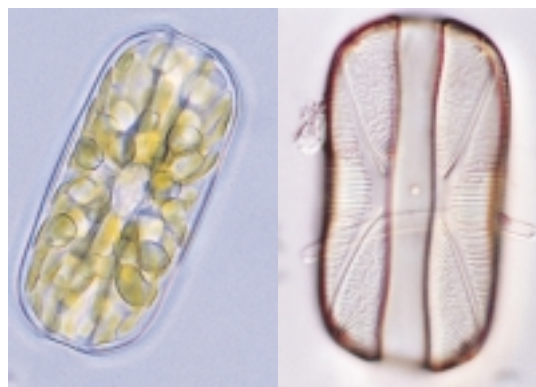
Brachysira

How to recognise the genus:

Microscopic: A symmetrical biraphid diatom. *Brachysira* species are naviculoid in valve view and more or less rectangular in girdle view. Instead of the regular radial striae seen in *Navicula* species, the valve decorations are more irregular, generally appearing as dashes that line up to form wavy lines down the valve face. There is single, lobed chloroplast.

Possible confusion: The shape is similar to that of *Navicula* (see page 174). However, in the latter, the paired chloroplasts should be distinctive. Under medium to high power (over 400x) it should be possible to see clearly the irregular markings on cleaned frustules of *Brachysira*.

Brachysira vitrea (= Anomoeoneis vitrea)

Slender naviculoid shape with pinched ends, 15-40 μm long and about 5 μm wide. Striations not clear on the valve face, even at high power.

Habitat and distribution: Widespread mostly in low to medium conductivity waters. Most commonly found in lakes and tarns, but has has been found in periphyton samples from lake-fed streams and rivers, e.g., it is quite common in the Waiau River in Southland.

Brachysira serians (= Anomoeoneis serians)

Cells larger: 50–100 μm long and 10–20 μm wide. Pronounced naviculoid shape. Variety *acuta* is smaller and has drawn out ends. Identification not certain.

Habitat and distribution: Less often seen in periphyton. More likely to occur in the bottom sediments of clean, acid-water lakes and tarns.
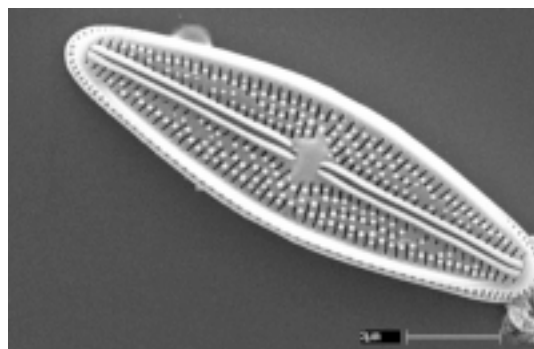
Note: Until recently, *Brachysira* species were included in the genus *Anomoeoneis.* Species remaining in the latter genus are typically found in high conductivity waters. *A. sphaerophora* has been recorded in New Zealand but has not been noted in any of our periphyton samples.

from top:

Brachysira vitrea, *from the Waiau River. x 1400*

Brachysira *cf* serians *var. acuta, live specimen showing the form of the chloroplast (compare with* Navicula*); also note the large oil droplets (food storage). x 1000*

Brachysira *sp. (unknown), Waiau River. x 1400*

*SEM (*Brachysira *sp.) showing markings on the valve face.*

Cocconeis

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Achnanthales

How to recognise the genus:

Microscopic: A monoraphid diatom. *Cocconeis* species are oval in shape and virtually always seen in valve view. Commonly epiphytic. There is a single flattened or lobed chloroplast, and the puncta – especially around the edge of the frustule – are often clearly visible. Examining cleaned material under high power (x 1000) should allow you to see both the raphe and rapheless valves, which can be quite different.

Possible confusion: Usually none

Cocconeis placentula

A oval-shaped diatom that varies considerably in size – from 10 to 90 µm long and 8 to 40 µm wide. Look for the distinctive ridge around the perimeter of the raphe valve. The ornamentation of the raphless valve varies in different varieties.
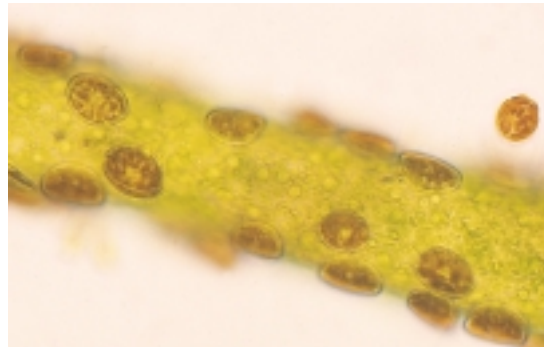
Habitat and distribution: *C. placentula* is common and widespread, occurring in a range of conditions from clean to moderately enriched to very enriched waters. This species sometimes dominates the periphyton following high flows because its flattened shape allows it to withstand turbulent conditions. Found on stones or growing as an epiphyte on wood, filamentous algae or vascular plants.
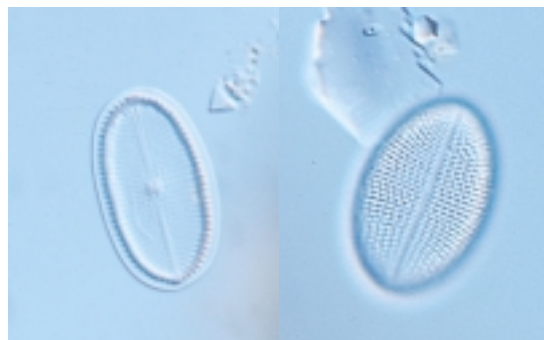
Cocconeis pediculus

C. pediculus differs from *C. placentula* in that it is distinctly curved (so that it is like a bowl) and lacks an extra ring around the outer edge of the raphe valve. Its size range is smaller: 10 to about 30 µm long and up to 20 µm wide.

Habitat and distribution: Grows as an epiphyte on filamentous algae (e.g., *Cladophora*) or on vascular plants. Tends to occur in high conductivity waters. Not as widespread or as common as *C. placentula.* All of our examples have come from periphyton from North Island rivers.

from top:

A filament of the green alga Cladophora *colonised by* Cocconeis *as an epiphyte. x 300*

Cocconeis placentula, *raphe valve (left), rapheless valve (right). x 1200*

Cocconeis *cf.* pediculus*. x 1200*

Cyclotella

How to recognise the genus:

Microscopic: This is a centric diatom. In valve view it is round with radial striae around the edge that differ from the pattern in the middle. The girdle view is rectangular (with rounded corners). There are numerous small chloroplasts.

Possible confusion: Many small centric diatoms are difficult to identify. However, the common *Cyclotella* species (below) is one of just a few that turn up regularly in periphyton samples, and it is fairly easy to recognise.

Cyclotella cf. *stelligera*

Microscopic: Cyclotella stelligera is typically 5–40 µm in diameter and circular in valve view, with radial striae around the margin and a few raised bumps in the centre – the overall effect a bit like a daisy. In girdle view the cells are rectangular with rounded edges.

Habitat and distribution: *Cyclotella* is normally planktonic, in lakes. *C. stelligera* is seen occasionally in the periphyton of lake-fed streams and rivers.

from top:

Cyclotella stelligera *in two planes of focus. x 1200*

Cyclotella *cf.* meneghiniana, *another species found in habitats similar to those of* C. stelligera*. x 1400*
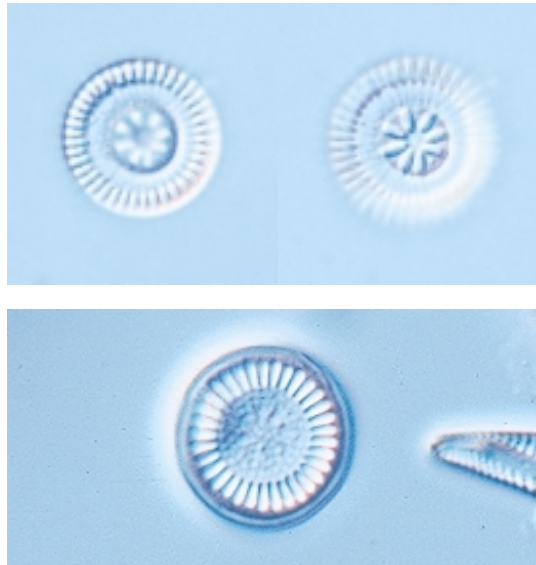
Cyclostephanos

How to recognise the genus:

Microscopic: Another centric diatom. It differs from *Cyclotella* (above) in having a pattern of radiating markings on the valve face that is continuous between the edge and centre, though you'll need to view cleaned specimens in different planes of focus. Other ultra-structural details separate the two genera including the presence of spines in *Cyclostephanos.*

Possible confusion: Other centric diatoms (see above); however this genus is clearly distinguishable from *Cyclotella* (above).
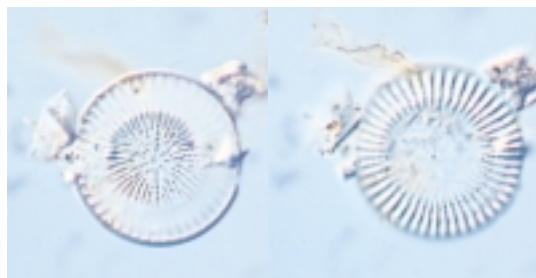
Cyclostephanos *sp. in two planes of focus; note that the striae are continuous from the edge to the centre of the valve but the valve is not flat. Common in the Moawhango River, North Island. x 1200*

Cymbella

How to recognise the genus:

Macroscopic: Cymbella kappii can accumulate as mats of light brown-green streaky slime.

Microscopic: An biraphid diatom that is asymmetric across the valve. Most species are more or less half-moon shaped in valve view. Some grow on mucilage stalks.

Possible confusion: *Cymbella* resembles *Encyonema* (page 166), and the differences are obvious only under high power (1000 x). In *Cymbella* the raphe ends turn upwards at their outer ends and are deflected downwards in the middle of the valve. In *Encyonema*, it is the other way around. Features of the chloroplast are also reversed in the two genera.

Other half-moon shaped genera include *Epithemia* (page 168) and *Amphora* (page 160). These are fairly easy to separate from *Cymbella*.

Cymbella kappii

Cymbella kappii is typically 25–35 μm long and about 10 μm wide. Because this species is so common in New Zealand periphyton, you can be reasonably confident from the shape alone that the identification is correct. If in doubt, check that there are two stigmata (isolated holes) in the central area, below the raphe endings (see photo).

Habitat and distribution: Very widespread in New Zealand and has been recorded as dominant or abundant in several lowland streams and rivers.
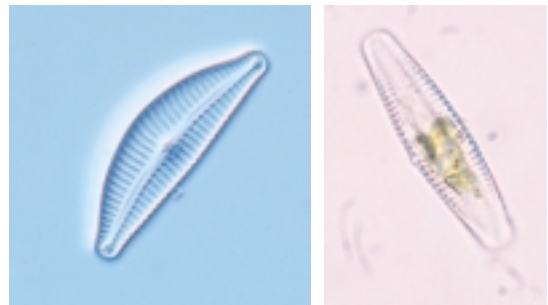
Cymbella aspera

C. aspera is a large, easily recognised diatom, typically 70 to 150 μm long and 20 to 45 μm wide. The striae and puncta are large enough to distinguish and count easily. There are no stigmata in the central area of the valve.

Habitat and distribution: This species is seen in the periphyton fairly often, and can be abundant in low-conductivity streams. Widespread.

from top:

Cymbella *slime in the Wangapeka River, near Nelson*

left: Cymbella kappii. *x 1000; right:* Cymbella *sp., showing girdle view. x 800*

Cymbella aspera: *live specimen showing chloroplast.Note the "fold" at the dorsal (top) side of the cell. In* Encyonema, *this is at the bottom. x 350*

Cymbella aspera, *cleaned specimen. Note lack of stigmata. x 350*

Cymbella tumida

A larger, chunkier diatom than *C. kappii,* typically 40–80 μm long and 15–20 μm wide. Slightly bulbous ends; striae radiating from a round central area with a single stigma.

Habitat and distribution: *Cymbella tumida* seems to occur in rivers with moderate to high conductivity. It has been noted as common in some North Island samples.

Other *Cymbella* species

Stream periphyton may contain various other species of *Cymbella*. To distinguish these from the three common species described, examine cleaned material under high power and look for: overall shape and size, density and direction of the striae, shape and size of the central area and number of stigmata present.

from top:

Cymbella tumida, *from the Maowhango River, North Island. x 1200*

Cymbella cistula *var* gracilis. *Note 4 stigmata. x 700*

Cymbella *sp. (unknown). x 1000*

Diatomella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales
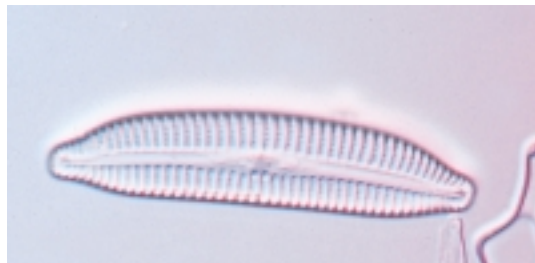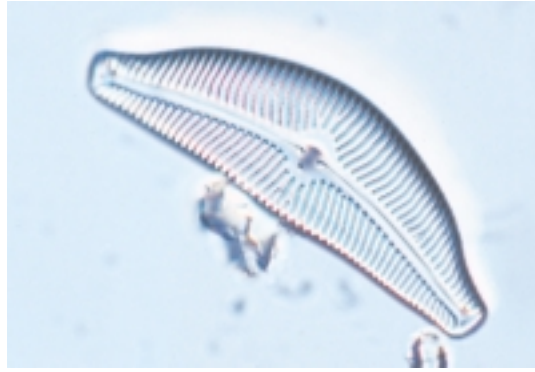
How to recognise the genus:

Microscopic: A symmetric biraphid genus with noticeable "septa" present, each a plate with three large holes. In girdle view the solid sept show up as short thick lines.

Possible confusion: A distinctive genus.

Diatomella balfouriana
Diatomella parva

Diatomella balfouriana is up to 30 μm long; *D. parva* around 10 μm.

Habitat and distribution: Possibly confined to clean mountain-fed streams. Distribution not certain. Specimens found in a stream in Abel Tasman National Park (*D. balfouriana*) and in the Waiau River, Southland (*D. parva*).

Diatomella balfouriana, *valve view focussed to show the raphe (lop) and the septum (bottom). x 1400*

Diatomella parva, *girdle view. x 1400*
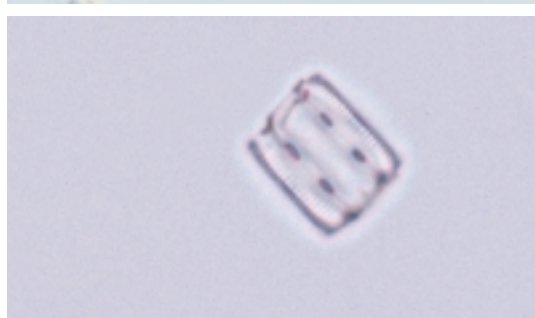
Diploneis

How to recognise the genus:

Microscopic: A symmetric biraphid diatom. Usually oval in shape, but sometimes with a constriction in the middle of the valve. The raphe lies in a thick rib of silica. Obvious rows of spots (holes) run parallel to the outer edges of this rib. The striae are easy to see even under low power. Two chloroplasts.

Possible confusion: A fairly distinctive diatom genus.

Diploneis elliptica

Diploneis elliptica is basically oval, usually between 20 and 80 μm long. It is distinguished from other species by its shape, rounded central area and the coarse striae and puncta. *Diploneis ovalis* is similar in shape but has finer striae and puncta.*

Habitat and distribution: Usually inhabits lakes, though we have found this diatom in the periphyton of lake-fed rivers; can be occasional to common.

Diploneis *cf.* elliptica. *x 1800*

Encyonema

How to recognise the genus:

Macroscopic: Some species of *Encyonema* grow in mucilage tubes (see page 155) and can accumulate as extensive mats of light brown slime.

Microscopic: A biraphid diatom, asymmetric across the valve with most species roughly half-moon shaped. Look for upward-pointing central raphe endings and downward-pointing terminal endings. The chloroplast in valve view is more or less H-shaped.

Possible confusion: *Cymbella* (see page 164).

Encyonema minutum (= Cymbella minuta = Cymbella ventricosa)

Small (15–30 μm long, 5–7 μm wide) with a more or less flat ventral (bottom) side and a strongly rounded dorsal side. The striae can be seen clearly at 400↔ and the raphe runs close to the ventral (bottom) margin. There are no stigmata.

Habitat and distribution: *E. minutum* is common and widespread throughout New Zealand. It has been recorded as dominant or abundant in periphyton from streams draining moderately developed catchments.

Encyonema minutum, *cleaned specimens showing range of size. x 1500*

Encyonema gracile (= Cymbella gracilis = Cymbella lunata)

Narrow cells, slightly rounded on the dorsal (upper) side and more or less flat beneath, from 30 to 50 µm long and 5 to 7 µm wide. The rounded ends sometimes seem to point slightly downwards. Fine striae.

Habitat and distribution: This species seems to occur mostly in the periphyton of clean-water lake-fed rivers. It has also been recorded in the periphyton of high altitude lakes and tarns (acid conditions?). Most examples seen in South Island samples.
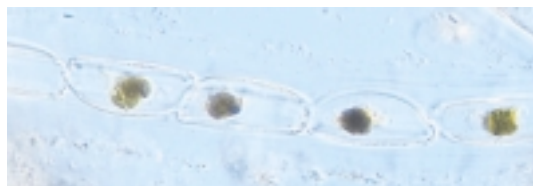
Encyonema prostratum (= Cymbella prostrata)

Chunky cells with prominent markings, often seen as filaments in mucilage tubes. Cells are 40–80 µm long and 20–30 µm wide. The striae contine right around the ends of the valve.

Habitat and distribution: Seems to occur usually in relatively high conductivity streams. This species does not appear to be widespread but it has been reported in abundance in the Shotover River (S. Moore, pers. comm.) and in a stream near Murchison.

Encyonema caespitosum (= Cymbella caespitosa)

Microscopic: Smaller than *E. prostratum*, with more pointed ends; 20–40 µm long and 10–15 µm wide. The cells often have a somewhat asymmetrical appearance. Again the striae are quite prominent. Occurs as filaments in mucilage tubes (see illustration page 155).

Habitat and distribution: Found in the periphyton of large rivers. Distribution unknown.
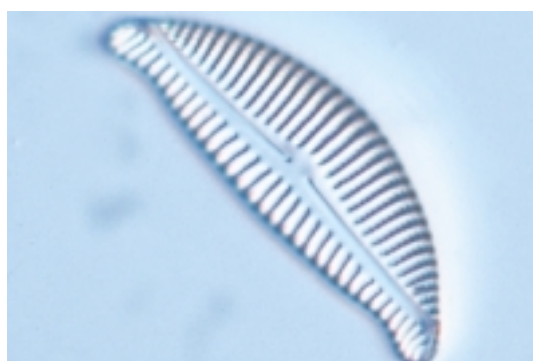
Note: The old genus *Encyonema* has now been expanded to include many species placed in *Cymbella*, including the examples above. (See Round *et al.* 1990.)

from top:

Encyonema gracile. *x 1400*

Encyonema prostratum *cells in a mucilage tube. x 350*

Encyonema prostratum. *x 1400*

Encyonema caespitosum. *x 1400.*

Epithemia

How to recognise the genus:

Microscopic: Epithemia has an "external" raphe. In valve view the cells are typically shaped like an orange segment (side view) or half-moon; the girdle view is roughly rectangular. Thickened areas (costae) on the valve wall show up as obvious dark lines under LM. The raphe runs in a "canal" along the ventral (i.e., the bottom, concave) edge of the valve, curving up at the centre. A single deeply lobed sheet-like chloroplast lies at the ventral side of the valve. All cells contain symbiotic cyanobacteria (visible as small spheres mainly in the girdle view of live specimens).

Possible confusion: Possibly *Cymbella* and *Encyonema* (see pages 164, 167). However, the structure of *Epithemia* is quite different from either of these genera.

Epithemia sorex

Epithemia sorex is 20–60 μm long and 6–16 μm wide. In valve view it looks like a "Napolean's Hat". In girdle view it is oval with squared-off ends (because of the way the valve bulges out at the sides). In valve view the entire raphe canal is visible.

Habitat and distribution: Common, found in lowland (enriched) streams and rivers throughout New Zealand, often growing attached to plants and other algae. It occasionally dominates periphyton, usually in moderately enriched streams after prolonged low flows.
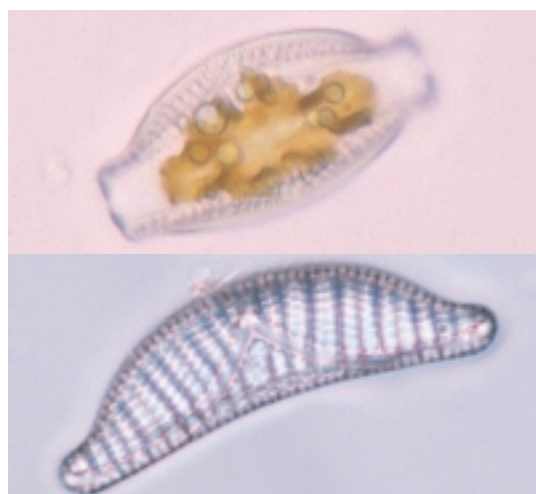
Epithemia cf. *adnata* (= *Epithemia zebra*)

Larger than *Epithemia sorex*, up to 150 μm, with rounded ends, coarser markings and costae usually more widely and less regularly spaced. Rectangular in girdle view.

Habitat and distribution: Seems to be widespread, though not usually common in periphyton. Often occurs with *Epithemia sorex.* Reported to prefer moderate to high conductivity streams, possibly extending to brackish water (Cox 1996).
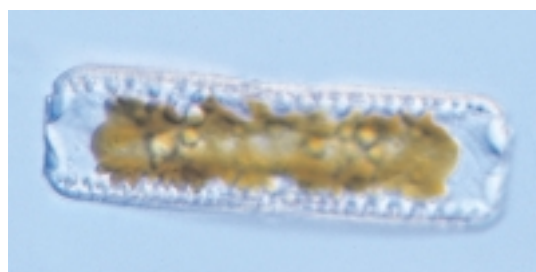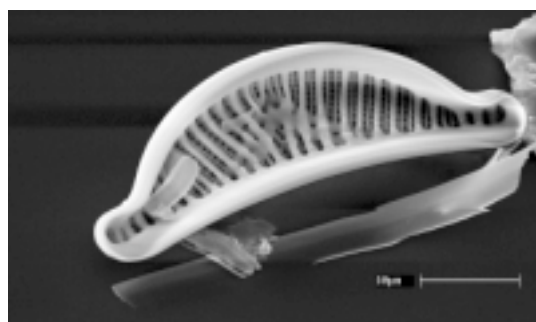
from top:

Epithemia sorex, *girdle view (live cell). x 850, valve view (cleaned specimen). x 1400*

SEM showing internal "costae" and raphe canal.

Epithemia *cf.* adnata, *girdle view (live). x 500; valve view (cleaned specimen). x 1000*

Frustulia

How to recognise the genus:

Microscopic: Frustulia is a symmetrical biraphid naviculoid genus, rectangular in girdle view. The raphe is distinctive: instead of a simple slit (or line), there is a ridge at either side (actually on the inside of the valve) so that the raphe appears to lie in a channel. The striae are very fine, barely visible under LM. Two chloroplasts, as in *Navicula*, but usually extending futher across the valve face.

Possible confusion: Other naviculoid genera. Look for the form of the raphe, especially the outer ends, and the extent of the chloroplasts. In *Navicula* (see page 174) the two lateral chloroplasts do not extend the width of the valve.

Frustulia rhomboides

A fairly large species, somewhat angular in shape, from 70 to 150 µm long and to 30 µm wide (at the widest point).

Habitat and distribution: Usually occurs only occasionally in stream periphyton, but this species can be abundant. It can also be very common in clean-water lake sediments. Widespread.
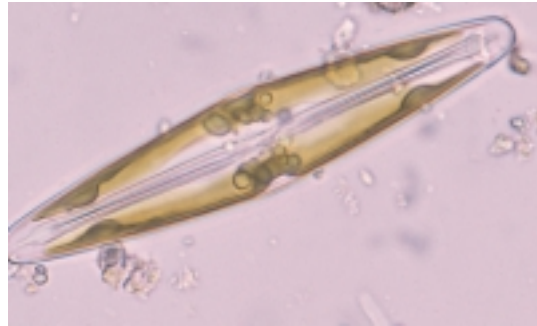
Frustulia rhomboides var. *crassinerva*

Smaller than *Frustulia rhomboides,* 30–50 µm long. This variety has capitate (pinched) ends and very slightly undulating margins.

Habitat and distribution: Not certain. Again may be abundant in streams in some areas (e.g., West Coast). May be more common in cleaner (acid) waters.

Note: Other varieties of *Frustulia rhomboides* have more rounded sides, or are longer and more slender.

Frustulia vulgaris

Up to 60 µm long, 12 µm wide. The cell has barely capitate ends and slightly rounded sides. The valve face sometimes looks slightly asymmetrical, with the raphe tending to one side.

Habitat and distribution: This species occurs quite often in periphyton samples over a range of conditions; can be common.

Note: Some species of *Frustulia* form "fila-ments" of cells in mucilage tubes. See page 155.

from top:

Frustulia rhomboides, *live specimen. x 450*

Frustulia *cf.* rhomboides *var.* crassinerva. *x 1300*

Frustulia *cf.* vulgaris. x 1300

Gomphoneis

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Macroscopic: Gomphoneis can form thick, glistening, light-brownish mats on river substrates.

Microscopic: An asymmetric biraphid diatom, *Gomphoneis* is club-shaped in valve view, with the raphe lying along the centre of the long axis. Under high power (in cleaned specimens) there seems to be a discontinuity in the density of the striae about half way between the valve margin and the raphe. This occurs where the puncta go right through to the inside (see SEM below). Double rows of striae may just be visible. In girdle view the genus is wedge-shaped. *Gomphoneis* grows attached to the substrate by long branching mucilage stalks. The chloroplast seems to be H-shaped, with sheets flattened against each valve face and joined at the centre.

Possible confusion: *Gomphonema* spp. (page 171). Look for the larger size, double striae (barely visible under LM) and long stalks in *Gomphoneis.*

Gomphoneis minuta var. cassieae

Gomphoneis minuta var. *cassieae* ranges in size from 60 to 100 μm long and about 20 μm wide (at the widest point).

Habitat and distribution: This species occurs all over New Zealand and often dominates periphyton communities in moderately enriched to enriched waters.

Note: This species has formerly been identified as *Gomphoneis herculeana* and was re-assigned by Kociolek and Stoermer 1988. We assume here that the New Zealand form of *Gomphoneis* is *G. minuta* var. *cassieae.* However, the difference between the two species is subtle.
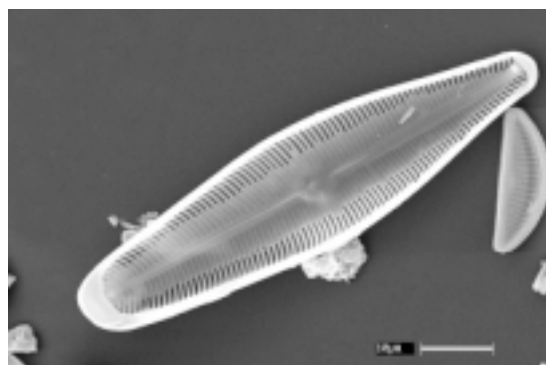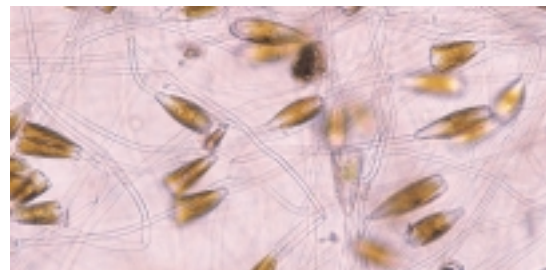
from top:

Thick Gomphoneis *growth on a stone, with* Fragilaria *(the darker strands in the middle).*

Mass of live Gomphoneis, *showing stalks. x 125*

Gomphoneis minuta *var.* cassieae, *cleaned specimen, x 820*

The interior structure of Gomphoneis*. SEM.*

Gomphonema

How to recognise the genus:

Microscopic: There are many species of *Gomphonema* in New Zealand, some of which still await description. Obvious features of this asymmetric biraphid genus are longitudinal asymmetry and a wedge shape in girdle view. Many live attached to the substrate – either rocks or water plants, for example, and sometimes growing in masses – by long or short mucilage stalks that enable them to withstand water currents.

Possible confusion: *Gomphoneis* (see page 170).

Gomphonema parvulum

Distinguished mainly by size and shape: 10–25 µm long; narrow leaf-like with pinched ends (slightly pinched or markedly so). Under high power, cleaned specimens are identified by the more-or-less parallel and relatively densely spaced striae (though the latter is very variable), a space on one side of the central area, and a single "stigma" opposite the space.

Habitat and distribution: *G. parvulum* is generally considered to be pollution tolerant, and if common to dominant in a sample, this often indicates that there is some enrichment (particularly organic) to the water.

Note: Most *Gomphonem*a specimens of the shape and size illustrated are likely to be *G. parvulum.* However, another species, *Gomphonema angustatum* , is similar and it can be difficult to decide which one you have, even under high power. Many specimens seem to have spaced out central striae characteristic of *G. angustatum*, but also have striae that are close to parallel rather than radiate. The former is characteristic of *G. parvulum*). *G. parvulum* and *G. angustatum* are clearly distinguished in, for example, Foged (1979, p. 57/59, pl 38). However, illustrations in other publications (e.g., Krammer and Lange-Bertalot 1997) are not as clear cut.

Note: Don't confuse the similarly named species *Gomphonema angustatum* and *G. angustum* (see page 172)
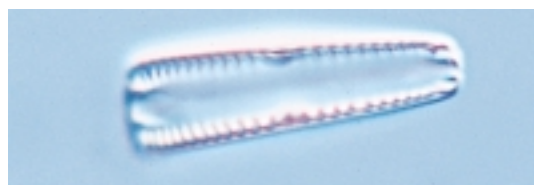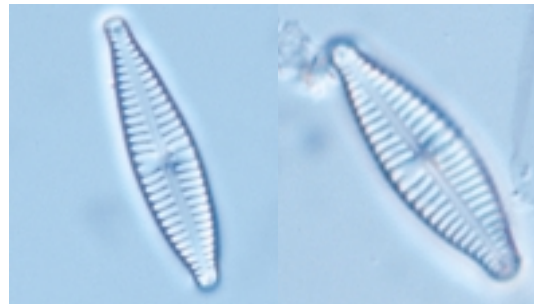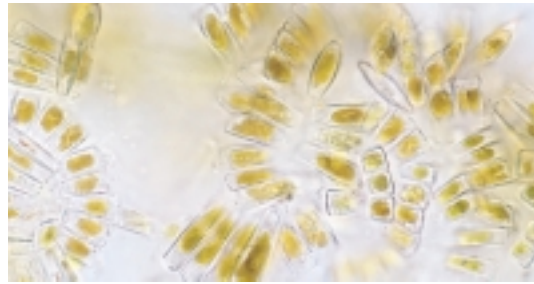
from top:

Small Gomphonema *sp., growing massed together attached to substrate, mostly in girdle view.*

Two specimens of G. *cf.* parvulum. x 1600

Left: Uncertain species, possibly a variety of G. *cf.* parvulum, *wider, with more densely spaced striae than we see normally. Right:* G. parvulum *tending towards* G. angustatum? *x 1600*

Gomphonema *sp., girdle view. Note the thickened areas on each side, indicating the raphe endings.*

Gomphonema truncatum (= G. constrictum)

"Jelly-baby"-shaped with a rounded end. 20–70 μm long, striae radiate at the centre with a space at centre on both sides of valve and a single stigma.

Habitat and distribution: A fairly common epiphyte, usually in moderate to high conductivity streams.
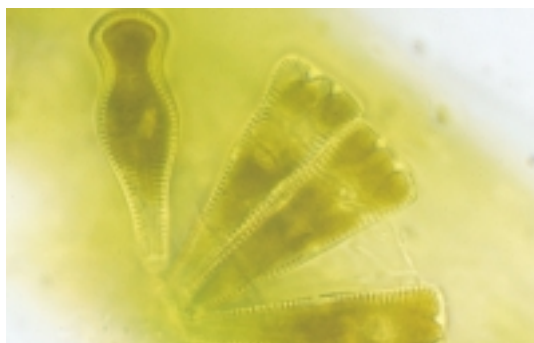
Gomphonema acuminatum

A very distinctive shape, like *Gomphonema truncatum* but with an extra projection at the end. 30–100 μm long.

Habitat and distribution: Sensitive to moderate levels of pollution (Cox 1996). Widespread and can be common.
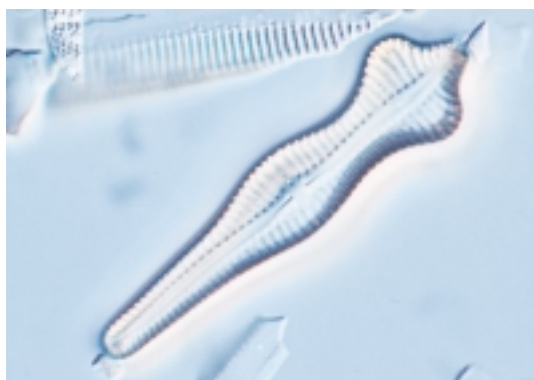
Gomphonema cf. minutum (= G.tenellum)

Small, narrow species, up to 25 μm long and about 5 μm wide, with quite widely spaced striae.

Habitat and distribution: This small species seems to be widespread and can be very common. Often in fairly clean streams. Identification is uncertain.

Gomphonema cf. angustum (= G.intricatum)

Narrow, elongated cell, up to 50 μm long and 8 μm wide, with a wide axial area.

Habitat and distribution: Reported to prefer clean streams.Seems to be widespread and can be common.

Gomphonema clavatum (= G. longiceps)

Club-shaped, blunt ends, 25–100 μm long. narrow axial area with central stria shorter, wider spaced, one stigma.

Habitat and distribution: Encountered occasionally. Distribution not certain.

Note: The above are some of the more obvious species of *Gomphonema* found in stream periphyton. There are many others. In processing periphyton samples, the best way to deal with them is to note the different forms in a sample, assign any to the above species, then simply assign numbers to the others (see Section 8.1).

from top:

Gomphonema truncatum, *live cells. x 1000*

Gomphonema acuminatum. *x 1250*

Gomphonema *cf.* minutum. *x 1250*

Gomphonema *cf.* angustum. *x 1250*

Gomphonema clavatum. *x 1250*

Gyrosigma

How to recognise the genus:

Microscopic: Gyrosigma has a sigmoid shape, with fine striae. It is biraphid, like *Navicula*. There are two chloroplasts, one along each side of the girdle bands.

Possible confusion: Another genus, *Pleurosigma*, has a similar shape, but is rarely seen in fresh water in New Zealand. In *Pleurosigma* the puncta often appear to lie in diagonal rows, while in *Gyrosigma*, if visible, they lie parallel to the raphe.

Gyrosigma cf. *scalproides*

Parallel sides, with the ends turning in opposite directions to form an S-shape. 60–100 µm long and about 15 µm wide. Central raphe endings are T-shaped (but this can be difficult to make out).

Habitat and distribution: This species has been recorded as abundant in lowland streams in North Island, but does not seem to be widespread.

Note: *Gyrosigma spencerii* (not illustrated) is similar in size but tapers more gradually towards the ends. It seems to be quite widespread, but not common. Reported to occur in high-conductivity streams.

Gyrosima *cf.* scalproides *from the lower Waitekauri River, Coromandel. live specimen, x 700; cleaned valve, x 700*

Mastogloia

How to recognise the genus:

Microscopic: Mastogloia is biraphid and fairly "chunky" (i.e., heavily silicified). Naviculoid in valve view with a distinctive wavy raphe. Focussing through the specimen reveals a series of chambers on each side of the valve, which is part of the girdle. In valve view the frustule is rectangular, with the chambers visible. Two chloroplasts, one at each end.

Possible confusion: Normally very distinctive, but when separate from the girdle, the valve faces could be mistaken for *Navicula* (see page 174). Look for the wavy raphe.

Mastogloia elliptica

Microscopic: Roughly oval, with slightly pinched ends, 30–70 µm long and 10–20 µm wide.

Habitat and distribution: *M. elliptica* can be common in the periphyton of lake-fed rivers in both North and South Islands.
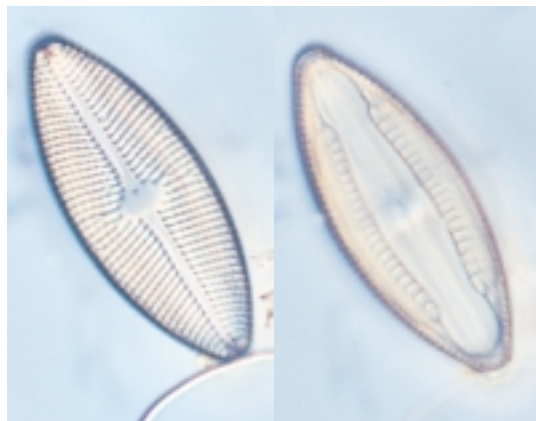
Mastogloia elliptica, *focussing on the valve face (left) and the internal chambers (locules) (right). x 1200*

Meridion

How to recognise the genus:

Microscopic: An araphid genus, *Meridion* forms semi-circular or radial colonies of valves joined face to face. In girdle view, the valves are wedge shaped. Like *Diatoma*, thick ribs ("costae") cross the valve face and these are visible in girdle view as small knobs down each long edge. There are several small chloroplasts dotted around the cell. This is a small genus with only one main species.

Possible confusion: Unlikely.

Meridion circulare

From 20 to about 80 μm long.

Habitat and distribution: *Meridion circulare* seems to prefer clean, cool streams, in which cells attach themselves to the substrate with a mucilage pad. It is usually not very common. A "cleanwater species" in Cassie (1989).
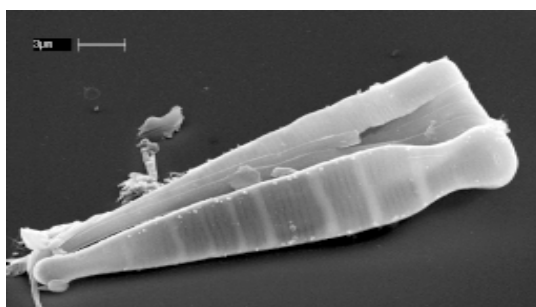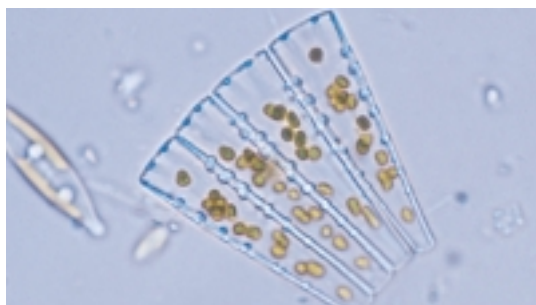
from top:

Meridion circulare *colony. x 900*

SEM of a single frustule of M. circulare.

Navicula

How to recognise the genus:

Microscopic: Navicula occurs in almost all periphyton samples containing diatoms. The genus is biraphid, with basically lens-shaped ("naviculoid") frustules. Two narrow chloroplasts are located laterally in valve view. Striae usually radiate from the centre. In fresh samples *Navicula* species tend to be noticeably motile.

Possible confusion: Other naviculoid diatoms include *Frustulia, Mastogloia, Neidium, Brachysira, Stauroneis.* Look for the narrow paired chloroplasts in healthy *Navicula.*

Navicula lanceolata (= *N. avenacea*)

Up to about 70 μm long, with noticeable striae radiating from a round central area, becoming convergent at the ends. which are blunt and barely capitate.

Habitat and distribution: *N. lanceolata* is widespread, common and can dominate the periphyton in streams of low to moderate conductivity. Often called *N. avenacea.*
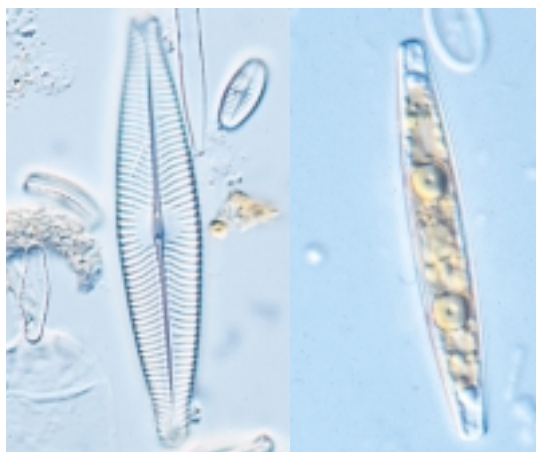
left. Navicula lanceolata *x 900. right, Girdle view of a live specimen. x 900*

Navicula cryptocephala

Small, slender, with capitate ends; 20–40 µm long and 5–7 µm, wide. Narrow axial area expanding to a rounded area at the centre of the valve. In live material look for fine striae, and the arrangement of the two narrow chloroplasts exactly opposite each other.

Habitat and distribution: Widespread and common; occasionally dominates the periphyton in relatively clean streams.

Navicula capitoradiata

Similar in size to *N. cryptocephala*, but a little fatter with a smaller central area. The chloroplasts are slightly offset from each other (see Cox 1996).

Habitat and distribution: Cox (1996) reports that *N. capitoradiata* prefers high-conductivity streams and is pollution tolerant. Thus it may be important to separate it from *N. cryptocephala.* Distribution in NZ uncertain.

Navicula rhynchocephala

Resembles a bigger (35–80 µm long) version of *N. cryptocephala.* The striae are coarser and the central area rounded (like *N. avenacea*). The chloroplasts are wider and rather ragged looking.

Habitat and distribution: Widespread in low to moderately enriched streams draining foothills, but not usually common. Reported as a "grossly polluted water species" (Cassie 1989).

Navicula radiosa

Microscopic: Longer and narrower than either *N. avenacea* or *N. rhynchocephala*, up to 100 µm long. Ends definitely not capitate. Striae radiate out from the centre. Central area smaller than *N. avenacea.* Long thin chloroplasts lie opposite each other.

Habitat and distribution: Found in cool, clean streams. Widespread but not usually common. Reported to be sensitive to moderate pollution (Cox 1996).
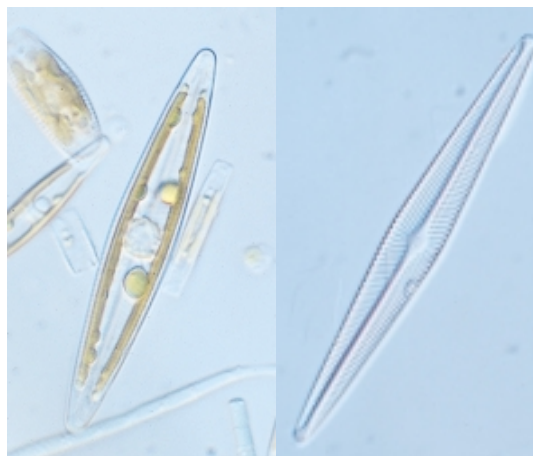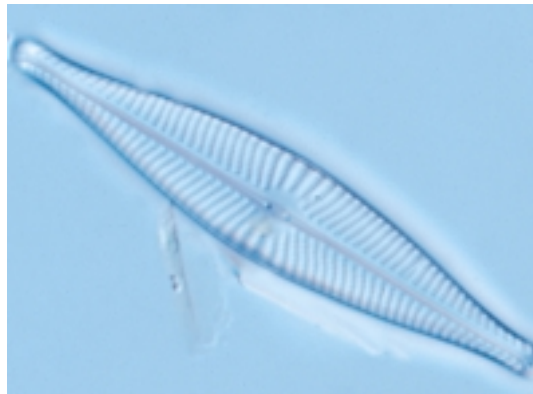
from top:

Navicula cryptocephala, *x 1500*

Navicula capitoradiata, *x 1500*

Navicula rhynchocephala, *x 1500*

Navicula radiosa*; (left) live specimen x 600; (right) cleaned specimen, x 600*

Navicula cf. *margalithi*

Blunt cigar-shaped typically 40–50 μm long and 10 μm wide. Distinctive in its almost parallel striae.

Habitat and distribution: This species has been recorded as common in some North Island locations. Not yet found in South Island yet. (Tentative identification.)

Note: Many other species of *Navicula* occur in stream periphyton usually as "occasional" or less, e.g., :

N. gregaria: about 15 μm long, pinched ends, striae parallel, chloroplasts markedly offset.

N. cincta: 15–25 μm long, smooth-sided (like a rugby ball), chloroplasts opposite.

(Both species reported to occur in high-conductivity streams. *N. cincta* may be pollution-tolerant.)

Navicula *cf.* margalithi, *Moawhango River, North Island. left, live cells x 700; right, cleaned specimen x 1400.*

NB. Navicula *cf.* gregaria *appears on the photo of* Meridion, *page 174.*

Neidium

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: Biraphid, symmetric naviculoid cells, rectangular in girdle view. Very fine striae that lie more or less parallel across the valve. In most species, at the centre of the valve the raphe endings curve in opposite directions. There are four chloroplasts per cell.

Possible confusion: Usually easy to recognise by its size and shape, the distinctive central raphe endings (if present) and the four chloroplasts.

Neidium affine

Usually 40–80 μm long and about 20 μm wide, with rounded capitate ends and slightly curved margins, not quite parallel.

Habitat and distribution: Fairly widespread but not very common. Usually found in clean streams.

Neidium iridis

A larger species, 50–90 μm long. Slightly rounded sides curving smoothly to the ends.

Habitat and distribution: Reported usually from very slow moving streams or ponds. Widespread but not common.

left: Neidium affine, *from the Waiau River, Southland. x 1100*

right: Neidium iridis*: live specimen from a pond, Wairau Valley, Canterbury. x 700*

Nitzschia

Division: Bacillariophyta, **Order:** Bacillariales

How to recognise the genus:

Microscopic: A large genus with an "external" raphe system. Most *Nitzschia* species are narrow needle-like cells. Some are slightly sigmoid (S-shaped). The raphe lies on a ridge (the "keel") near or at one edge of each valve. A series of internal ribs across the keel appear as a row of dots (the "keel puncta") under the microscope. This is very obvious in some species. There are two chloroplasts, arranged along the valve – a good indicator of this genus.

Possible confusion: Small *Nitzschia* species may be confused with some small *Synedra* species, especially where the chloroplasts are not clearly visible (see page 187). Look for the more delicate structure (less silicon) of many *Nitzschia*. If possible, examine specimens at 1000x and look for the keel puncta. The genus *Hantzschia* (not in this manual) is closely related; the main *visible* difference from *Nitzschia* is strong asymmetry across the valve. *Nitzschia* has many species that are difficult to identify. All identifications below are tentative.

Nitzschia cf. *palea*

Usually 15–60 μm long and up to 5 μm wide. Delicate-looking sometimes with a slightly asymmetric appearance. The keel edge is rounded and the opposite edge is slightly rounded, flattened or even slightly concave. The striae are very fine – virtually invisible except at very high power. The keel puncta can also be difficult to make out under low power (400 x and less).

Habitat and distribution: Widespread, common and well-known as a pollution tolerant species.

Nitzschia cf. *amphibia*

From about 10 to 40 μm and 4–6 μm wide. The striae and keel puncta are conspicuous compared with those of *N. palea.*

Habitat and distribution: Widespread and common in a wide range of conditions; may not as pollution-tolerant as *N. palea*, but possibly does best in high-conductivity streams.

Nitzschia cf. *inconspicua*

A minute diatom, 3–15 μm long, with rounded ends. Valve markings not visible except under high power.

Habitat and distribution: Common in high-conductivity streams. Tolerates moderate pollution levels.

from top:

Nitzschia *cf.* palea, *x 1800*

Nitzschia *cf.* amphibia, *x 1800.*

Nitszchia *cf.* inconspicua, *several specimens. x 1800*

Scanning electron micrograph of Nizschia *sp. to illustrate structure. (NB. valve broken at centre.)*

Nitzschia cf. *dissipata*

Usually small but can be up to 70 μm long and 3 to 6 μm wide, with tapering ends. The elongated keel puncta are set in from the valve edge. Very fine striae (not visible under LM).

Habitat and distribution: Seems to be widespread, but not common in stream periphyton samples. Usually in higher conductivity waters.

Nitzschia cf. *linearis*

Typically large (over 90 μm long), with obvious, elongated keel puncta and fine striae.There is a notch at the centre of the valve where the two raphe ends meet (see photo). Long chloroplasts. In fresh samples usually lies in girdle view.

Habitat and distribution: Reported overseas as being tolerant of polluted conditions (Cox 1996). Seems to be widespread in New Zealand. In fresh or frozen material could be confused with *N. intermedia* (below).
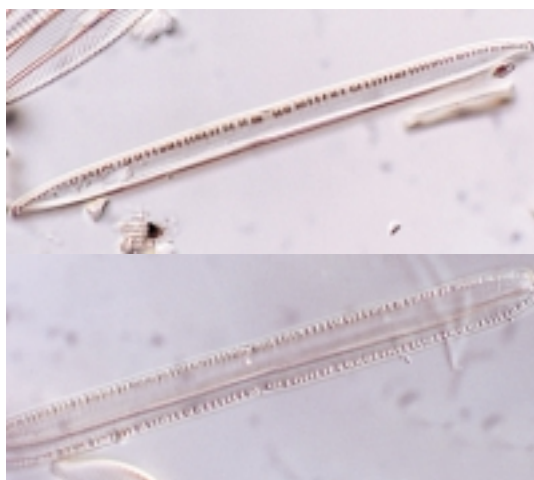
Nitzschia cf. *intermedia*

Another large species, up to 150 μm long. The keel puncta are dot-like, more widely spaced than in *N. linearis*, and there is no obvious central notch. Long chloroplasts.

Habitat and distribution: Found widely in periphyton from lowland rivers, and can be common. This species is reported to prefer moderate to high conductivity, but is not particularly tolerant of organic pollution (Cox 1996).

Nitzschia cf. *acicularis*

A delicate species, 20–60 μm long, including fine, drawn-out ends. The keel puncta and striae not visible under the light microscope.

Habitat and distribution: Encountered widely in stream periphyton, but usually not very abundant. This species is reported overseas as being tolerant of moderately polluted conditions (Cox 1996).

from top:

Nitzschia *cf.* dissipata. *Small specimen from the Avon River, Christchurch. x 1400*

Nitzschia *cf.* linearis, *cleaned material. Top: valve view – note notch; lower: girdle view. x 800*

Nitzschia *cf.* intermedia, *x 350, live specimen (girdle view) from the Ashley River, Canterbury, showing chloroplasts and continuous keel puncta.*

Nitzschia *cf.* acicularis. *Live specimen. x 900.*

Nitzschia cf. *gracilis*

Fine, elongated, up to 100 µm long. Ends drawn out and rounded at the tips. Keel puncta and striae visible only under high power.

Habitat and distribution: Not known.

Note: *Nitzschia sigmoidea* (not illustrated) is a large species (150 –500 µm long and up to 15 µm wide) which is S-shaped in girdle view. In other smaller species the valve is have a sigmoid shape in valve view, e.g., *Nitzschia nana* (= *N. ignorata*, about 50 µm long and 4 µm wide). None of these are common in stream periphyton.
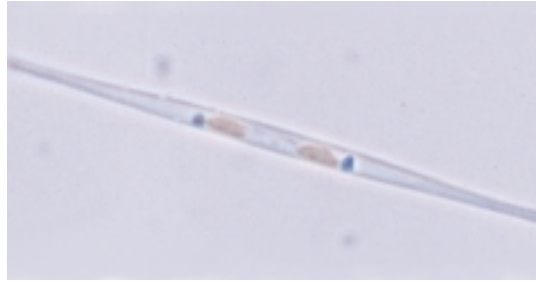
Nitzschia *cf.* gracilis. *live specimen x 1100. Note the two small chloroplasts.*

Pinnularia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A biraphid symmetric naviculoid genus. Each stria is made up of many tiny holes opening into a chamber, so that the striae appear wide and continuous rather than made up of a series of puncta. This is especially visible in large species. The raphe endings curve strongly in the same direction and some species seem almost asymmetrical across the valve (like *Cymbella*). The two chloroplasts lie one each side of the frustule. In valve view you see both; in girdle view you see a single sheet. *Pinnularia* species are fairly motile.

Possible confusion: Can sometimes look like *Navicula* sp. (see page 74). Note that there are many species of *Pinnularia*, species can be variable and identification can be difficult. Most of the following identifications are tentative.

Pinnularia viridis

A large diatom (up to 150 µm long and 25 µm wide), cigar-shaped with prominent striae that are slightly radiate in the middle of the valve changing to parallel and then convergent at the ends.

Habitat and distribution: Occurs occasionally in stream and river periphyton. Along with many other *Pinnularia* species, the preferred habitat is clean, acid lakes and ponds. Widespread.
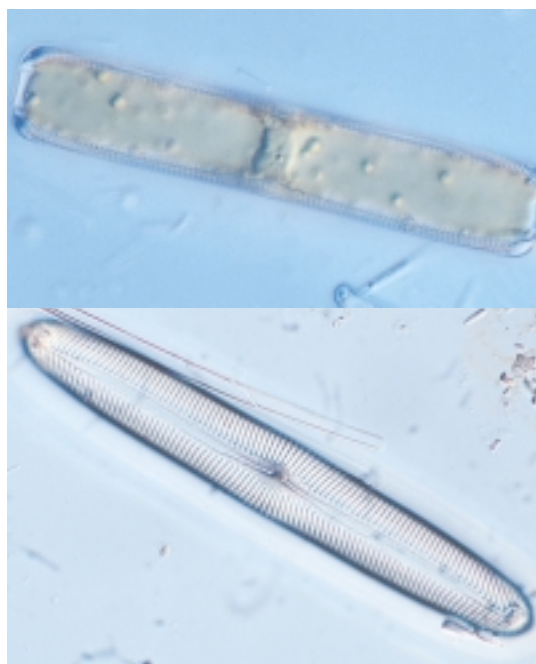
from top:

Live Pinnularia. *cf.* viridis *in girdle view, showing the large sheet-like chloroplast. x 500*

Pinnularia viridis, *cleaned specimen. x 700*

Pinnularia cf. *gibba*

Large (80–120 μm long and about 15 μm wide), with very slightly capitate ends. Quite short striae, sometimes with a gap at the centre of the valve; wide axial and central areas.

Habitat and distribution: Habitat uncertain. Not usually common in stream periphyton. Widespread.

Pinnularia cf. *microstauron*

Smaller – up to 70 μm long and 15 μm wide with slightly undulating margins and blunt rounded ends. A gap in the striae at the central area, but much less empty space in the central and axial areas than *P. gibba*.

Habitat and distribution: Probably widespread, usually in low conductivity.

Pinnularia cf. *mesolepta*

Margins definitely undulating, with rounded ends, from 25 to 80 μm long and 15 μm wide.

Habitat and distribution: Probably widespread and found in a range of stream types.

Pinnularia cf. *subcapitata*

Usually small (25 to 60 μm long and are 6–7 μm wide); slightly undulating sides, and rounded slightly capitate. There is a gap in the striae each side of the central area. The axial area can be quite wide.

Habitat and distribution: Occasionally found in stream and river periphyton. Most findings from South Island samples, especially high country streams.

Note: *Pinnularia* species are typically abundant in acid wetland areas. Note also that there are many small species of *Pinnularia*. To distinguish between species look at the cell shape and arrangment of the striae. Is there a gap at the central area? Do the striae radiate from this area? Do they change direction part way along the valve (as in the specimen in the bottom photograph)?

from top:

Pinnularia *cf.* gibba, *Waiau River. x 1200*

Pinnularia *cf.* microstauron. *Tentative identification. x 1200*

Pinnularia *cf.* interrupta, *live cell from a lowland stream, Banks Peninsula, Canterbury. x 1200*

Pinnularia *cf.* subcapitata *variety?. This example has a very wide axial area so the identification is tentative only. x 1200*

Pinnularia *sp. found in a stream on Stewart Island. x 1200*

Placoneis

Division: Bacillariophyta, **Order:** Mastogloiales

How to recognise the genus:

Microscopic: Placoneis species are biraphid symmetric naviculoids, usually with fat, rounded sides and capitate ends. The main diagnostic feature is the single lobed chloroplast. Cleaned specimens show strongly radiating striae; individual puncta may be visible under high power.

Possible confusion: *Navicula*: check the chloroplast structure if possible. There are other differences but these are clearly visible only under SEM. *P. placentula* is distinctive.

Placoneis placentula (= Navicula placentula)

Usually quite large, up to 60 μm long and noticeably wider than most naviculoid diatoms (up to 25 μm wide).

Habitat and distribution:

Reported to be a clean-water species, but usually in moderate to high conductivity streams (Cox 1996). Widespread but usually not common.

Placoneis placentula *from the Moawhanga River, central North Island. x 1300*

Note: *Placoneis* was formerly included in *Navicula*, but is now reinstated as a genus in its own right (see Cox 1987).

Reimeria

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Microscopic: Reimeria is a biraphid asymmetric genus. Features are a small, slightly curved valve view (like *Cymbella*) with a bulge on the concave side, widely spaced striae and small size. Rectangular in girdle view and quite motile.

Possible confusion: With other small diatoms such as *Fragilaria* especially in girdle view.

Reimeria sinuata (= Cymbella sinuata)

This is the only species known in New Zealand. *R. sinuata* varies in size from 10 to 25 μm long.

Habitat and distribution: Quite common and widespread in New Zealand stream periphyton. It has been recorded as abundant in lowland rivers, e.g., in Westland, during summer low flows.

Reimeria sinuata, *with girdle view above. x 1900.*

Note: The genus *Reimeria* was established in 1987, based on *Cymbella sinuata* (see Kociolek and Stoermer 1987).

Rhoicosphenia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Cymbellales

How to recognise the genus:

Microscopic: Rhoicosphenia is a small biraphid genus, asymmetric in three planes. In valve view it is club-shaped (like *Gomphonema*); in girdle view it is bent (like *Achnanthidium*) and also wider at one end. Under high power (x 1000) the valve view shows a "thickening" at each pole where an internal layer of silica extends into the cell. The concave valve face has a more highly developed raphe than the convex face. *Rhoicosphehia* grows on short stalks often as an epiphyte on other algae.

Possible confusion: *Gomphonema* (see page 171) – though the girdle view is distinctive.

Rhoicosphenia abbreviata (= R. curvata)

Only one species of *Rhoicosphenia* is found commonly in streams. *R. abbreviata* varies in size from 20 to 60 μm long and up to 8 μm wide.

Habitat and distribution: Very common and widespread in New Zealand periphyton. It has been recorded as dominant (growing massed together on substrates) in small lowland streams with moderate to high water velocities. It seems to be pollution tolerant.
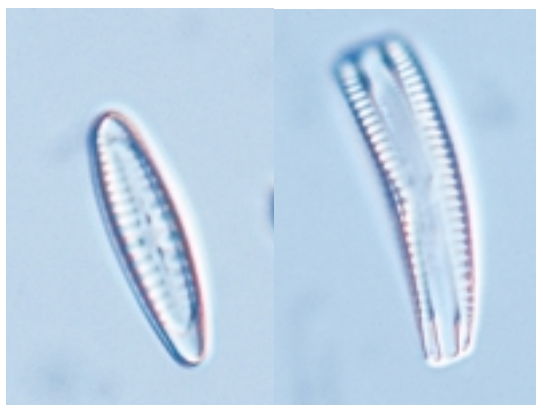
Rhoicosphenia curvata *from the Tarawera River; left: valve view; right: girdle view. x 1800*

Rhopalodia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Rhopalodiales

How to recognise the genus:

Microscopic: In this genus the raphe system lies close to the valve edges, and the valve faces are rotated so that they lie roughly in the same plane (as in *Amphora*, for example). so you normally see both valve faces at the same time. Thick ribs of silica (costae) may run across the inside of the valves, visible as heavy lines or stripes under LM. There is a single large chloroplast. Some *Rhopalodia* species contain symbiotic bacteria which enable them to fix inorganic nitrogen.

Possible confusion: Unlikely.

Rhopalodia novae-zealandiae

A large diatom up to 200 μm long and 25 μm wide (both valves). Cells have rounded ends and a bulge at the middle of each side, with a slight notch where the raphe ends meet. Striae should be visible at 400x in fresh material,There are no thicker ribs (costae).

Habitat and distribution: Very abundant in lakes but can also be common in clean lake-fed rivers and streams.
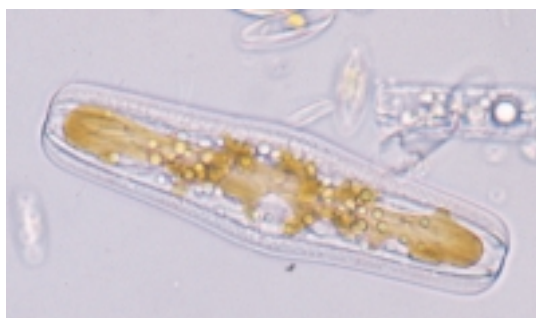
Rhopalodia novae-zealandiae, *live specimen showing chloroplast. x 500*

Rhopalodia operculata

Smaller than *R. novae-zealandiae* (20–40 μm long and about 20 μm wide). Valves are rounded on their outer margins and have thick costae running across them.

Habitat and distribution: This species is encountered occasionally in river periphyton. Distribution and habitat preferences uncertain.

Note: May be the same as *R. musculus* in Foged 1979.

Rhopalodia operculata. *x 1200*

Sellaphora

Division: Bacillariophyta, **Order:** Naviculales

How to recognise the genus:

Microscopic: A biraphid genus with rounded ends (valve view). The raphe lies in a sort of raised plate running along the centre of the valve. There is often a clear area at each end of the valve. A single chloroplast, with lobes lying against each side of the valve and joined in the middle to form an "H" shape.

Possible confusion: With other naviculoids, though the shape is fairly distinctive, and the chloroplast different.

Sellaphora pupula

S. pupula is 30–70 μm long and 5–20 μm wide. Elongated shape with rounded, slightly capitate ends.

Habitat and distribution: *Sellaphora pupula* seems to be quite widespread in stream periphyton in New Zealand, though never abundant. Overseas, it is reported as being tolerant of a wide range of ecological conditions (Cox 1996).

Sellaphora bacillum

*S*imilar in size to *S. pupula* but has parallel sides.

Habitat and distribution: *Sellaphora bacillum* has been recorded throughout New Zealand (Cassie 1984). It is reported overseas as being tolerant of some pollution.

Note: Species in *Sellaphora* were formerly placed in *Navicula* but have now been transferred back to their original genus (see Mann 1989).
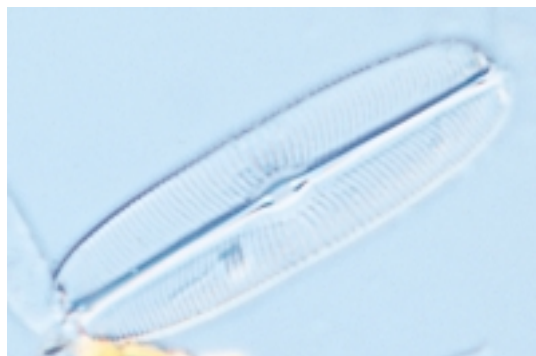
from top:

Sellaphora pupula, *cleaned specimen from the Tarawera River. x 2000*

Sellaphora bacillum, *cleaned specimen from a stream, West Coast, South Island . x 2000*

Stauroneis

<div style="text-align: right">

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Naviculales

</div>

How to recognise the genus:

Microscopic: A symmetric biraphid naviculoid. Some species can be quite large (over 100 µm long). The main diagnostic feature is the thickened band of silica across the middle of the valve (this may be difficult to see). There may also be internal flaps of silica at the ends of the valve (cf. *Rhoicosphenia*). Fine striae in which the puncta may be visible. Two chloroplasts lie against the girdle (one each side) and extend under the valve face.

Possible confusion: *Navicula (see page 174)*, other naviculoids.

Stauroneis cf. anceps

Up to 75 µm long and 15 µm wide. Rouded margins terminating in rounded captitate ends. Striae radiate throughout the valve.

Habitat and distribution: Widespread, mostly in ponds and lakes or slow-moving stream outlets. Reported to occur over a wide ecological range (Cox 1996).
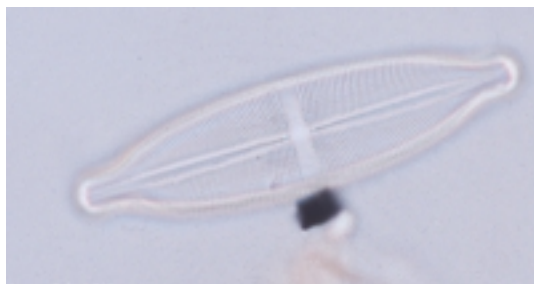
from top:

Stauroneis *sp., live specimen showing chloroplasts. x 450*

Stauroneis anceps, *cleaned specimen from the Waiau River. x 1100*

Staurosira

<div style="text-align: right">

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

</div>

How to recognise the genus:

Microscopic: A small araphid genus formerly included in *Fragilaria*. Small cells often cruciform (shaped like a cross), with a prominent striae and a pseudoraphe that usually widens at the centre of the cell. Rectangular in girdle view.

Possible confusion: Other small araphid genera formerly placed in *Fragilaria*, especially *Staurosirella*, which has a very different stria structure (see page 185). In *Staurosira* the striae comprise single rows of small round or oval puncta.

Staurosira construens (= *Fragilaria construens*)

Cruciform to oval cells 8–25µm long, up to 12 µm wide, rectangular in girdle view, sometimes forming short chains.

Habitat and distribution: Has been recorded as common in clean, spring-fed streams. Widespread.
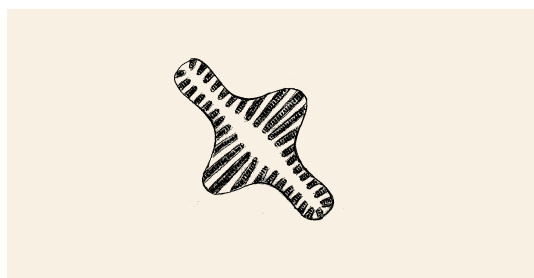
Drawing of Staur osira construens *showing features visible under LM. x 2000.*

Staurosirella

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Fragilariales

How to recognise the genus:

Microscopic: An small araphid genus formerly included in *Fragilaria*. Small cells cruciform or oval with wide striae; pseudoraphe may be wider at the centre. Rectangular in girdle view.

Possible confusion: Cruciform cells of *Staurosira* (page 184). Difficult to distinguish except under high-power LM, although *Staurosira* is usually smaller. In *Staurosirella* the coarse striae are spaced farther apart than the finer striae of *Staurosira*.

Staurosirella leptostauron (= *Fragilaria leptostauron)*

Cruciform cells 15–35μm long and up to 20 μm across, s ometimes forming short chains. Coarse striae widely spaced (less than 9 in 10 μm). Under high power fine lines across the striae may be visible.

Habitat and distribution: Usually occurs in clean rivers and streams, and can be locally common. Distribution not certain.
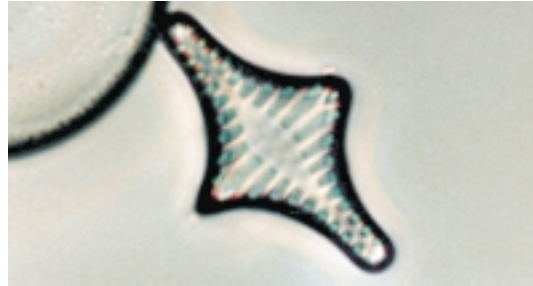
Staurosirella leptostauron *from the Okuku River, Canterbury. x 2000*

Stenopterobia

C: single cells (diatoms)

Division: Bacillariophyta, **Order:** Surirellales

How to recognise the genus:

Microscopic: Elongated cells, sometimes S-shaped, with an "external" raphe system. As in *Surirella* (page 196), the raphe runs around the entire margin of the valve so that the two"distal" raphe ends are adjacent to each other, and the central join is at the opposite end of the cell. There are small ribs around the whole cell margin. A single chloroplast has a large lobe lying against each valve face.

Possible confusion: The S-shaped species could be confused with some *Nitzschia* species that have a similar shape and also *Gyrosigma*. Check the chloroplast.

Stenopterobia species

Stenopterobia delicatissima is spindle shaped with noticeable ribs around the margin. Size: from 30 to 100 μm long. *Stenopterobia curvula* is longer, slender, sigmoid with somewhat angular ends (rather than rounded). Marginal ribs faint. Size: up to about 250 μm long.

Habitat and distribution: Both these species have been found in pristine streams draining small lakes and wetlands. Distribution uncertain.

left: Stenopterobia delicatissima*. x 1100*

right: Stenopterobia curvula *with chloroplast partly intact. x 350*

Surirella

Division: Bacillariophyta, **Order**: Surirellales

How to recognise the genus:

Microscopic: Surirella species have an "external" raphe. They can be oval to rounded, sometimes with a "waist" at the middle. The "keel" (containing the raphe) encircles the entire valve. Prominent ribs lead onto it. Valves are generally rectangular in girdle view with the ribs visible at each side. There is a single large lobed chloroplast arranged as a plate against each valve face, joined by a narrow "bridge".

Possible confusion: The valve views of *Surirella* and *Epithemia* (page 168) species are occasionally confused.

Surirella linearis

Elongated oval, more or less symmetrical, sometimes with a slight "waist" in the middle. From 40 to 100 μm long and 10 to 20 μm wide. Ribs round the edge quite large and widely spaced.

Habitat and distribution: Seems to occur in a range of conditions, from low-conductivity to enriched. Widespread, can be quite common.
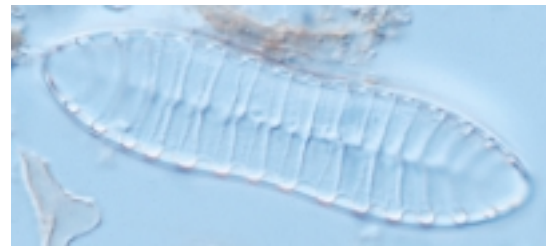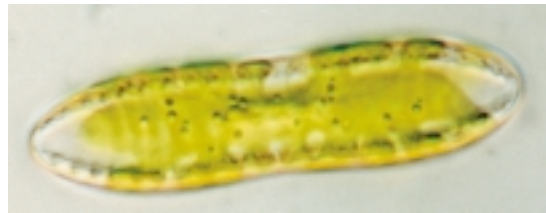
Surirella angusta

Smaller with parallel (or slightly rounded) sides, conical ends and closely spaced ribs. Typical size 20-30 μm long and about 10 μm wide.

Habitat and distribution: Widely found in nutrient-rich lowland rivers and streams.
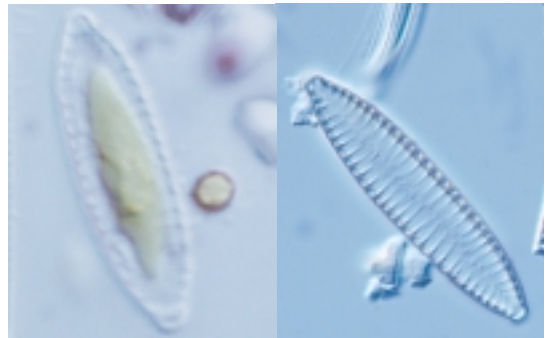
Surirella brebissonii var. *kuetzingii*

Small and rounded, slightly pointed at one end. Usually 10-20 μm wide.

Habitat and distribution: Not certain, but has been recorded as common in a stream in farmland in the Catlins region.
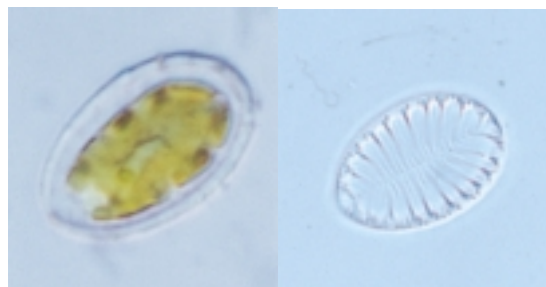
Note: *S. minuta (*not illustrated) also occurs in enriched streams. It is elongated egg-shaped with parallel ribs, 10-45 long and 10 μm wide.

S. tenera (not illustrated) occurs occasionally in stream periphyton. This species is much larger (up to 200 μm long), an elongated oval shape, rounded at one end and much sharper at the other. The ribs are noticeably closer together at the ends of the valve than in the middle. (See Foged 1979 for illustrations.)

Many species of *Surirella* are extremely abundant in freshwater wetland areas.

from top:

Surirella linearis, *live cell showing large chloroplast. x 900*

Surirella *cf.* linearis, *showing ribs. x 900*

Surirella angusta, *live cell and cleaned frustule. x 1200*

Surirella cf. brebissonii var. kuetzingii, *live cell and cleaned frustule. x 1200*

Synedra

How to recognise the genus:

Macroscopic: Sometimes *Synedra* species can dominate the periphyton and may have the appearance of masses of short, thick, brown "filaments".

Microscopic: An araphid genus. *Synedra* are thin pencil-like cells, solitary or occasionally in fan-shaped colonies where the frustules attach to the substrate by one end. A couple of species form filaments like *Fragilaria*. A "pseudoraphe" runs the length of the cell on both valves often with a clear central area in the middle. In the larger species under high power LM, a single "rimoportula" (a small hole) is visible at each end of the valve. This is diagnostic for the genus. Two long chloroplasts, one lying against each valve face.

Possible confusion: The small species of *Synedra* can sometimes be confused with *Nitzschia* (page 177): check the chloroplasts, or examine under higher power. Also some types of *Synedra* and *Fragilaria* (page 151) may be impossible to distinguish without the use of an SEM.

Note: A range of different schemes has been published for classifying *Synedra* species. The following are suggested to cover the common forms found in New Zealand periphyton.

Synedra ulna

Very variable length, up to 200 μm long. Regular parallel striae and parallel margins tapering or rounded at the apices. (Some varieties differ in shape.) There is usually a clear central area (absent in some varieties).

Habitat and distribution: *Synedra ulna* seems to grow well in response to nutrients. It can dominate the periphyton in enriched lowland streams. The species is wide-spread and extremely common.

Synedra ulna *var.* contracta

As for *S. ulna*, except that the centre of the valve is slightly concave. Size, up to about 120 μm long.

Habitat and distribution: Frequently found in the periphyton of large lowland rivers. Widespread.

from top:

Synedra ulna *cf. var.* biceps, *growing in a colony joined face-to-face. x 270*

Synedra *cf.* acus, *from the Avon River, Christchurch, growing in star-shaped colonies, each cell attached at one end. x 270*

Synedra ulna, *x 900*

Synedra ulna *var.* contracta. *x 900*
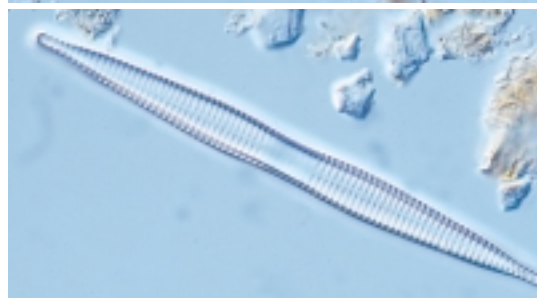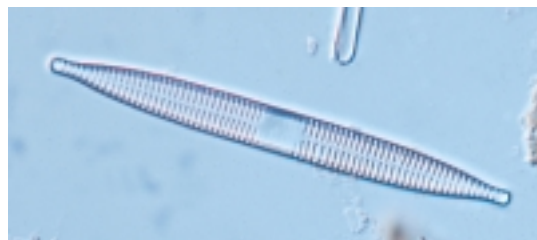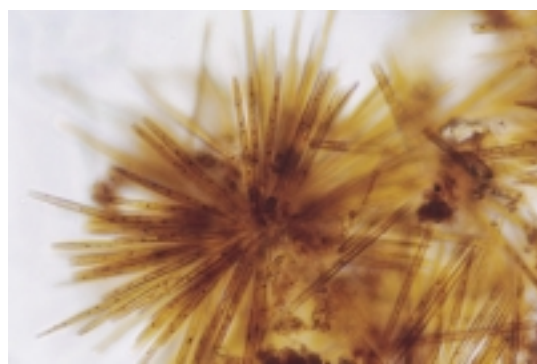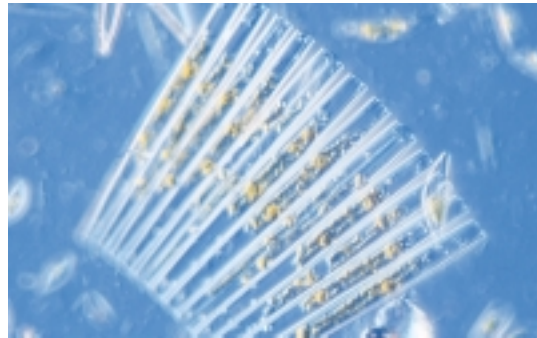
Synedra ulna var. *biceps*

Very long cells (up to as much as 500 μm). No central area. Small spines along the valve margins allow the formation of colonies joined face-to-face (cf. *Fragilaria*).

Habitat and distribution: Found dominating enriched streams in summer. Probably widespread.

Synedra cf. *ulna* var. *ramesi*

Short, stubby with pinched ends and a narrowing at the centre of the valve. Striae fine and slightly irregular-looking. Size: up to 60 μm long.

Habitat and distribution: Seems to occur mostly in clean-water streams and rivers. It is can be quite common.

Synedra acus

The valve definitely tapers from the central area to the ends and there is no narrowing at the centre. 90–180 μm long.

Habitat and distribution: Common. Seems to be mostly in moderate conductivity streams.

Synedra rumpens

Small fine cells, 25–50 μm long and 2–4 μm wide. Central area apears to be very slightly swollen.

Habitat and distribution: Can be very abundant locally, but its small size and lack of detail make it difficult to discern. There may be confusion with *Fragilaria capucina* (see page 151). Indeed Williams and Round (1987) consider *S. rumpens* and *F. capucina* to be synonomous.
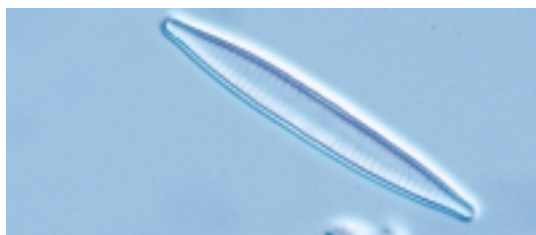
Synedra delicatissima

Very fine, needle-like cells tapering to the apices; up to about 200 μm long and 3–5 μm wide

Habitat and distribution: Not certain.

from top:

Synedra ulna *var.* biceps. *Half of valve. Note spines around the valve margin. Also note the rimoportula (islolated hole) near the tip of the valve. One at each end is characteristic of* Synedra *species. x 700*

Synedra ulna *var* ramesi. *x 1000*

Synedra acus, *live specimen. x 800*

Synedra rumpens. *x 1400*

Synedra delicatissima. *x 400*

Cosmarium

How to recognise the genus:

Microscopic: Like other desmids, *Cosmarium* is clearly divided into two parts, visible in "face view" (see photographs). From the top, the shape is usually rounded or oval. There are many species. Those encountered in periphyton range from relatively small (around 25 μm across) to larger species such as *C. margaritatum* (illustrated).

Habitat and distribution: *Cosmarium* is most abundant in lakes and wetland areas. However some species regularly occur in the periphyton of streams and rivers and can be common.

Possible confusion: Potential confusion with some species of *Staurastrum* (see page 190) which have a triangular top view but may resemble *Cosmarium* in face view.
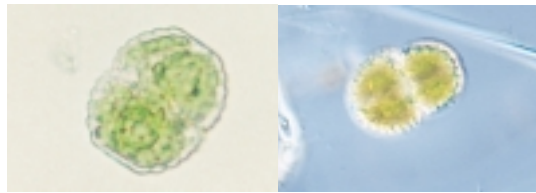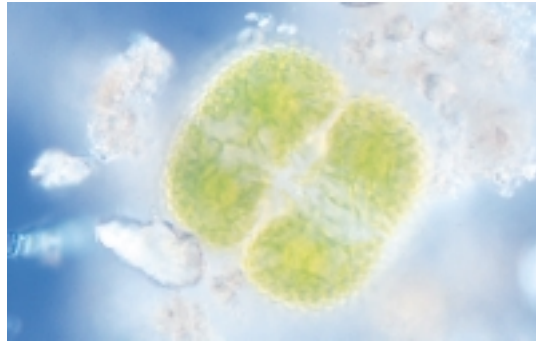
from top:

Cosmarium *cf.* margaritatum *from the Waitekauri River , Coromandel. x 400*

left: Cosmarium *cf.* impressulum *from the Waitekauri River , Coromandel. x 400; right: Cosmarium sp. x*

Closterium

How to recognise the genus:

Microscopic: Crescent-shaped cells with an obvious division of chloroplasts at the centre (but no constriction to the outside of the cell). In live material you should be able to make out vacuoles at each end containing moving granules. Usually large – up to 500 μm long and 40 μm wide.

Habitat and distribution: The genus as a whole is more typical of lake than river habitats, but a large form often occurs in the periphyton. It can be quite common in moderately enriched (e.g., lowland agricultural) streams, in a mixed periphyton community.

Possible confusion: Unlikely.

Note: For a full account of Desmids in New Zealand, see Croasdale and Flint (1986, 1988) and Croasdale, Flint and Raine (1994).
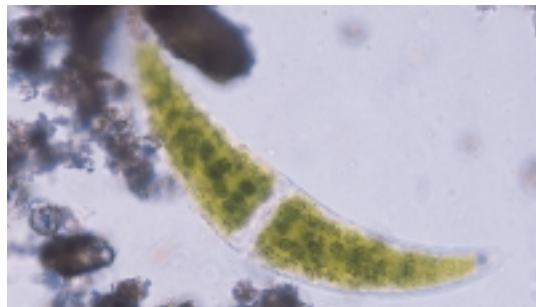
from top:

Closterium *sp. from the Wairau River, North Canterbury. x 270*

A smaller species of Closterium*. x 270*

Staurastrum

Division: Chlorophyta, **Order:** Desmidales

How to recognise the genus:

Microscopic: Similar to *Cosmarium*, but the top view is three-sided rather than rounded. The form found most often in periphyton resembles a solid triangle rather than the more delicate three-pronged planktonic form. Up to about 40 μm across.

Habitat and distribution: Very common planktonic genus in enriched ponds and lakes. The solid triangular forms can be common in the periphyton of lowland sreams and rivers.

Possible confusion: Possibly with *Cosmarium* (see page 189).

from top:

Side view of Staurastrum *sp., a "solid" type often encountered in periphyton. The top view is triangular. x 400*

The more delicate type of Staurastrum *normally seen as part of the plankton communitiy in ponds and lakes. x 400*

Ankistrodesmus

Division: Chlorophyta, **Order:** Chlorococcales

How to recognise the genus:

Microscopic: Small colonies that look like loose bundles of spindle-shaped cells, though the bundles seem to break apart easily and you often seen individual cells scattered among other taxa. Cells may be up to 100 μm long but are often much smaller. The chloroplast covers most of the cell wall and there is usually a pyrenoid.

Habitat and distribution: *Ankistrodesmus* can be common in the periphyton of low- to moderately enriched streams.

Possible confusion: There are other fine, spindle-shaped green algae, but these generally are not a common component of periptyon communities.
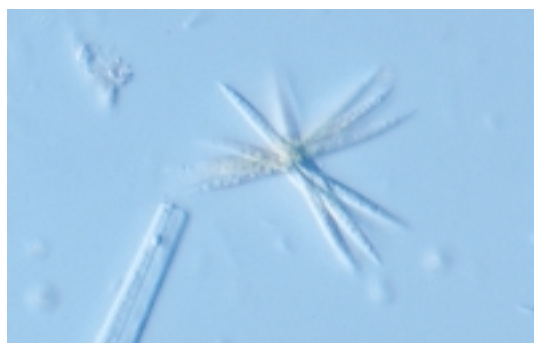
Ankistrodesmus *sp. x 650*

Dinobryon

Division: Chrysophyta, **Order:** Chrysomonadales

How to recognise the genus:

Microscopic: colonies of cup-like individuals, each containing an oval cell with a light yellow-green chloroplast. This is one of the golden-brown algae.

Habitat and distribution: Usually part of lake phytoplankon, but has occurred in periphyton of lake outflows.

Possible confusion: Unlikely.

Note: Another representative of the golden-brown algae sometimes found in periphyton is *Chrysocapsa* (Order Chrysocapsales), which forms colonies of round cells embedded in mucilage.

from top:

Dinobryon *sp. x 500*

A brown gelatinous coating of a colonial alga identified as Chrysocapsa, *found as dominant in summer/autumn low flows in a foothills Canterbury river with moderate conductivity.*
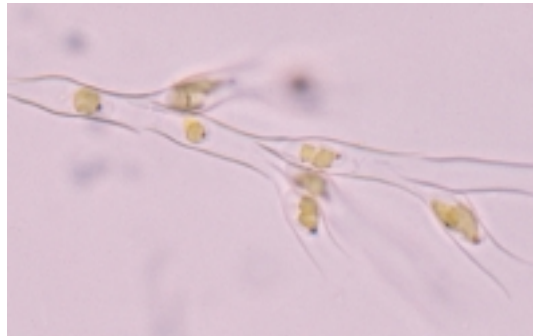
Gloeocystis

Division: Chlorophyta, **Order:** Tetrasporales

How to recognise the genus:

Microscopic: Small colonies of 4 or 8 green, spherical cells around 10 μm in diameter, each enclosed in a mucilage "envelope". The cells may separate during blending. The single chloroplast can cover the entire cell wall.

Habitat and distribution: Not certain. Has been found in abundance in a stream on the Coromandel Peninsula.

Possible confusion: There are many kinds of green colonial cells similar to *Gloeocystis* and all are difficult to identify. For example, the lower photograph is possibly *Palmella* (Order: Tetrasporales) in which many spherical cells (up to 10 μm across) are arranged in an amorphous mass with cell sheaths not always visible. It is often only possible to identify such green cells as "unknown colonial greens" or "unknown green unicells".
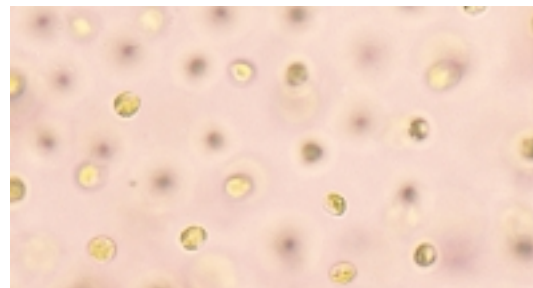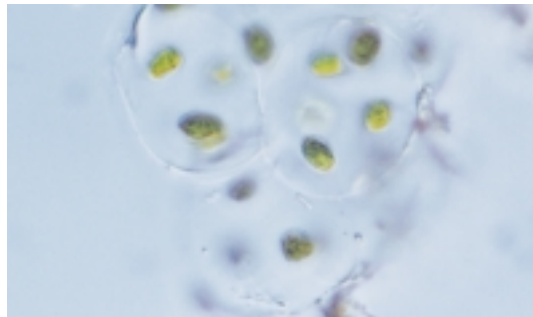
from top:

Gloeocystis. *Note the definite sheaths around groups of 4 cells. x 500*

Possibly Palmella: *smaller cells scattered in a mass of mucilage. x 500*

Pediastrum

C: colonial

Division: Chlorophyta, **Order:** Chlorococcales

How to recognise the genus:

Microscopic: More or less circular colonies of lobed cells, up to about 100 μm across.

Habitat and distribution:

Mostly found in slow-moving streams. Not usually common.

Possible confusion: Unlikely.
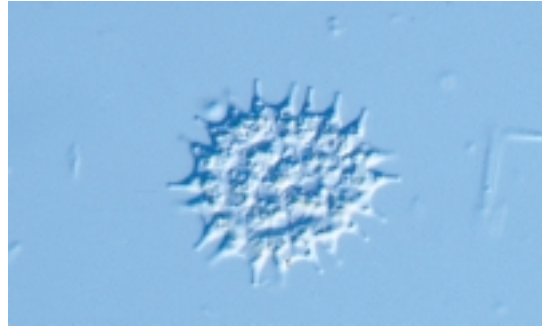
Pediastrum*: a relatively small colony (about 40 μm across). x 600*

Scenedesmus

C: colonial

Division: Chlorophyta, **Order:** Chlorococcales

How to recognise the genus:

Microscopic: This genus grows as small colonies that look like stacks of oblong cells. Sometimes there are two stacks side-by-side and there are usually 4, 8 or 16 cells. The many species in this genus are distinguished on the basis of size and shape of the cells and the presence and form of spines. Cells may be up to 35 μm long. The chloroplast covers most of the cell wall and each cell has one pyrenoid.

Habitat and distribution: *Scenedesmus* can be extremely common in the periphyton of low- to moderately enriched streams, especially in the absence of other green algae. It occurs as "rare" or "occasional" in many periphyton samples.

Possible confusion: Unlikely.
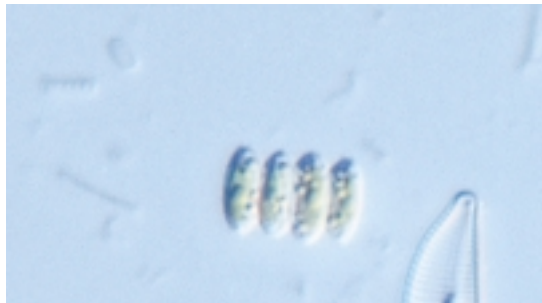
Two different species of Scenedesmus*. The upper one is often common in periphyton. x 700*

Tetrastrum

Division: Chlorophyta, **Order:** Chlorococcales

How to recognise the genus:

Microscopic: Colonies of four cells arranged in the shape of a cross. About 30 µm across.

Habitat and distribution: Not often found in periphyton, but has been recorded as common in a moderately enriched stream (Waitekauri River)

Possible confusion: Similar to *Crucigenia*. *Tetrastrum* has "spines" protruding from the cells, *Crucigenia* does not. Also *Tetrastrum* does not tend to form multiple colonies. In blended material it may be difficult to decide between them.
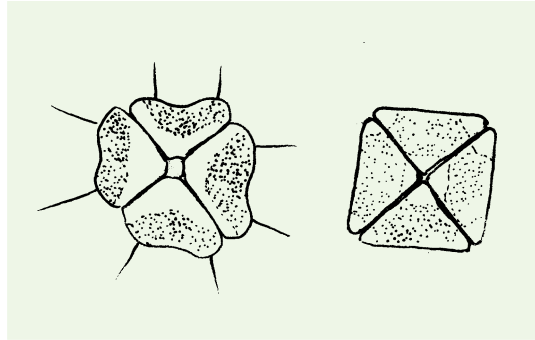
Diagrams of Tetrastrum *(left) and* Crucigenia *(right). Note that* Crucigenia *is often found growing in multiple groups.*

Enteromorpha

Division: Chlorophyta, **Order:** Ulvales

How to recognise the genus:

Macroscopic: Thin, bright-green fronds, like a tiny seaweed.

Microscopic: Sheets of cells organised into hollow tubes a single cell thick, often with branches.

Habitat and distribution: Most species of *Enteromorpha* inhabit brackish or marine habitats. We have found the genus in high conductivity streams, for example in the Waipara River, Canterbury during summer low flows (measured conductivity about 180 µS/cm).

Possible confusion: Unlikely.

from top:

Enteromorpha, *from the Waipara River, Canterbury.*

Enteromorpha. *x 90*

Enteromorpha. *x 300*

Anabaena

BG: unbranched filaments

Division: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Macroscopic: Usually no distinguishing macroscopic features but in lakes, some species of *Anabaena* can form severe toxic blooms.

Microscopic: Unbranched trichomes (filaments) may be straight or curved to spiral. Individual cells are rounded or barrel-shaped, and there are frequent intercalary heterocysts (appearing between vegetative cells), approximately the same size as the vegetative cells. Akinetes (reproductive cells), where present, are often much larger ellipsoidal to cylindrical, highly granular cells.

Habitat and distribution: We have found *Anabaena* species in slow-flowing high-country streams, mixed with green filamentous algae, diatoms and other cyanobacteria.

Possible confusion: *Anabaena* looks very similar to *Aphanizomenon* (not in this manual). However, the end cells of *Aphanizemenon* are elongated and sometimes narrowed.

from top:

Anabaena *sp., from an inland Canterbury stream, curved species. x 270*

Anabaena *sp. x 680*

Calothrix

BG: unbranched filaments

Divison: Cyanobacteria, **Order:** Nostocales

How to recognise the genus:

Microscopic: Relatively short trichomes growing from a heterocyst at the base of the filament and usually tapering to a fine point. The trichome is enclosed in a firm sheath. *Calothrix* may grow in star-like tufts or as solitary trichomes.

Habitat and distribution: *Calothrix* often grows amongst other algae and can be epi-phytic. It is not seen often in periphyton.

Possible confusion: *Rivularia* (page 200) also has trichomes tapering from a basal heterocyst, though these are smaller than *Calothrix*, and always arranged side-by-side in a macroscopic colony.

Note: *Calothrix* can have limited false branching, and merges into *Dichothrix* (see page 200).

Calothrix. *x 700 (photo: Donna Sutherland)*

Cylindrospermum

How to recognise the genus:

Microscopic: Light blue-green symmetrical trichomes with terminal heterocysts. Akinetes develop just beside the terminal heterocyst at both ends of the trichome in most species.

Habitat and distribution: Mostly in still waters but has been found in slow-flowing pool outlets.

Possible confusion: Could be confused with *Cylindrospermopsis.* However, the position of akinetes in *Cylindrospermum* is adjacent to the terminal heterocyst while in *Cylindrospermopsis* they are always slightly distant from the heterocyst.
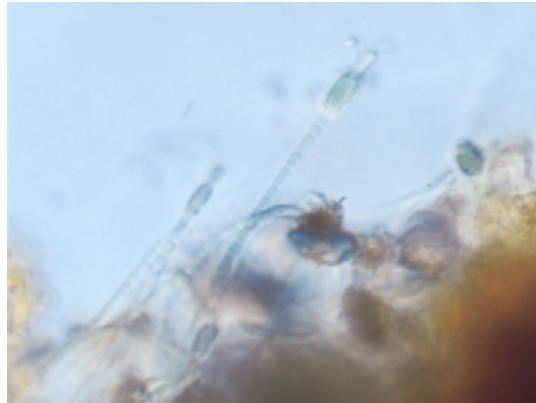
Cylindrospermum. *x 400*

Leptolyngbya

How to recognise the genus:

Microscopic: This genus includes most of the very narrow species (0.5–3 μm wide) previously included in *Phormidium* or *Lyngbya.* Little detail will be visible except under very high magnification. The filaments do not taper and are enclosed within a fine but distinct sheath The colour varies from pale blue-green to brown. The cylindrical cells are often shorter than they are wide, but can be longer. There are no specialised cells. The thallus comprises a free cluster of tangled trichomes.

Habitat and distribution: Clean-water upland streams and rivers. Probably widespread in New Zealand.

Possible confusion: *Leptolyngbya, Leibleinia,* and *Jaaginema* are all very similar in appearance. However, *Leibleinia* differs by its special epiphytic mode of life (see photo) and *Jaaginema* (not in this manual) has no sheath.

from top:

Masses of Leptolyngbya. *Outlet from tarn, Richmond Range, Nelson/Marlborough. x270*

Leibleinia *growing as an epiphyte on* Tolypothrix. *x 700 (photo: Donna Sutherland)*

Lyngbya

How to recognise the genus:

Microscopic: Straight filaments that do not taper, usually blue-green, and with a definite sheath which is often evident beyond the tip of the trichome. The cells are usually disc-like, i.e., wider than they are long, and there are no heterocysts. Cell division is rapid, and often new cell walls can be seen forming even before the walls in the previous division are completed. Size varies from 5 μm to 25 μm across.

Habitat and distribution: Clean-water upland streams and rivers. *Lyngbya* is widespread in New Zealand.

Possible confusion: Some *Lyngbya* species resemble *Oscillatoria* (see page 197) and *Phormidium* (see page 198). *Oscillatoria* seldom has a sheath and the cells are disc-like; *Phormidium* usually has a sheath and the cells appear square.

Note also the appearance of *Heteroleibleinia* (formerly included in *Lyngbya*).
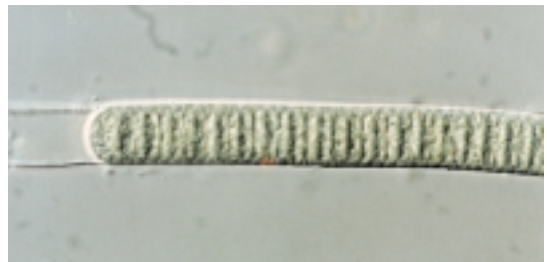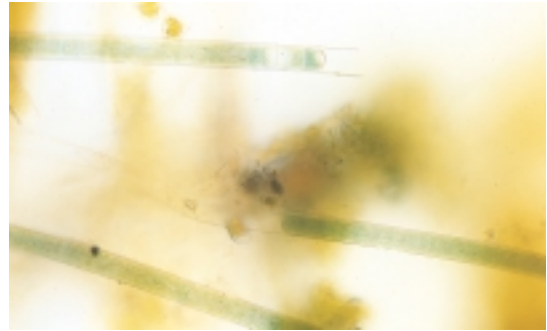
from top:

Lyngbya *from a clean mountain stream (Arthurs's Pass). x 270.*

Lyngbya. *x 600 (photo: Donna Sutherland)*
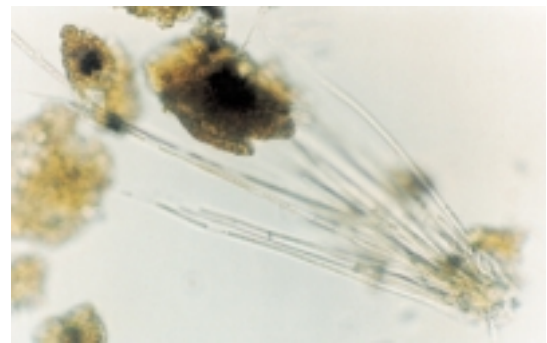
Possibly Heteroleibleinia, *previously included in* Lyngbya. *This can be quite common in clean streams. x 250 (photo: Nelson Boustead)*

Oscillatoria

How to recognise the genus:

Macroscopic: Slimy, dark-coloured layers, or undistinguishable, mixed with other periphytic algae.

Microscopic: The trichomes are cylindrical, straight or slightly wavy. Akinetes and heterocysts are always absent. Sheath absent for most part, but some species will rarely produce sheaths under sub-optimal conditions. Trichomes can move by a waving (oscillation) action. The cells are characteristically discoid (i.e., cells wider than long) and are between 6–23 μm wide. A thickened cap (calyptra) sometimes forms on the end of older trichomes (this feature is age-related). Cell division occurs rapidly and often new cell walls can be seen forming before the walls in the previous division are completed.

Habitat and distribution: Widespread in New Zealand, and found in a wide range of conditions.

Possible confusion: *Lyngbya* (see page 196), *Phormidium* (see page 198) and *Oscillatoria* all look somewhat similar. *Phormidium* and *Lyngbya* typically have sheath (most easily seen extending beyond the end of the trichome).
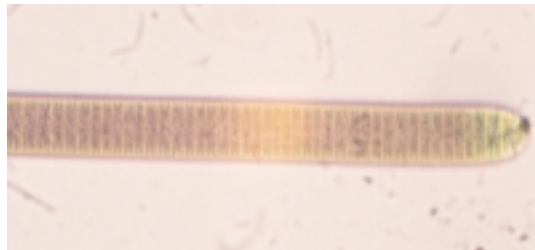
from top:

Oscillatoria, *showing the thickened cap (calyptra) at the end (found in older trichomes). x 500*

Note the new cell walls forming within each narrow cell. x 270 (photo: Donna Sutherland)

As above. x 540 (photo: Donna Sutherland)

Phormidium

How to recognise the genus:

Macroscopic: Forms expanded, gelatinous, dark green to black mats growing on stable substrates – stones, other alga, plants. These mats can be fine and thin to compact and leather like. Very rarely, single trichomes are scattered throughout a mixed periphyton community.

Microscopic: The cylindrical trichomes are slightly to intensely waved, and unconstricted or slightly constricted at the cross-walls. The sheaths when present, are firm and adherent to the trichome, never lamellated. Trichomes move with a gliding, trembling or waving action. Cells are typically box-like, that is as long as they are wide. Ther are no heterocysts.

Habitat and distribution: The genus is widespread in New Zealand, often very abundant in high-conductivity streams and rivers.

Possible confusion: *Phormidium*, *Lyngbya* (see page 196) and *Oscillatoria* (see page 197) all look somewhat similar. *Phormidium* has square, box-like cells, rather than the disk-like cells seen in the other two genera. Specimens that can not be distinguished between these three genera are typically provisionally placed in *Phormidium*.
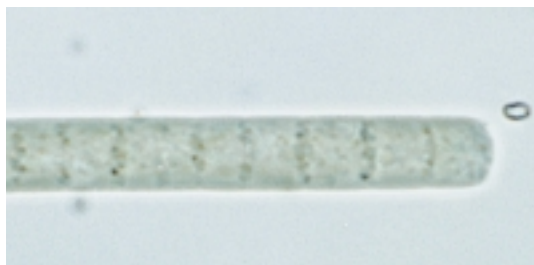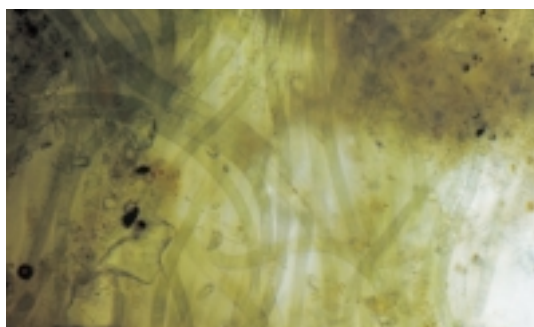
from top:

Phormidium *mat. Waipapa River, Hawkes Bay.*

Phormidium *filaments (from the Waipara River, Canterbury). x 270*

Phormidium. *x 400*

Phormidium. *x 1400*

Nostoc

How to recognise the genus:

Macroscopic: Tough, irregularly shaped or rounded, gelatinous nodules, clear to yellow-brown to dark brown in colour; usually growing on stone substrates.

Microscopic: Under low power (up to 200x) *Nostoc* appears as a compact, cohesive mass in which numerous looped and twisted filaments are embedded. These may be denser around the outsides of the colony. Under higher power (up to 1000x) you can see that individual filaments are unbranched and comprise chains of bead-like rounded cells. (Don't mistake overlapping trichomes for branches.) The cells can be up to 8 μm in diameter, while the heterocycts are often slightly larger. Akinetes, where present, develop between adjacent heterocysts.

Habitat and distribution: *Nostoc* is usually found in fast-flowing relatively clean-water upland streams and rivers, growing attached to rocky substrates. Some species also grow on damp soil. The alga can become abundant and quite noticeable. It is widespread in New Zealand.

Possible confusion: Macroscopically, *Nostoc* is quite characteristic, though some types of green algae grow as tough nodules in similar environmental conditions. These are usually much darker than typical *Nostoc*. Under the microscope *Anabaena* (see page 194) and *Wollea* (not in this manual) look very similar to *Notsoc*. These three genera are distinguished on the basis of growth form – *Anabaena* never forms gelatinous colonies while the colonies of *Wollea* are long and tube-like with parallel trichomes.

from top:

Nostoc *growing on a stone taken from a fast-flowing hill-country stream in Otago.*

Nostoc colonies on Cladophora.

Nostoc *sp., showing edge of nodule. x 135*

Nostoc *trichomes. x 270.*

Rivularia

How to recognise the genus:

> *Macroscopic:* Small dark raised hemi-spherical colonies attached firmly to substrates, up to 3 mm across.

> *Microscopic:* Masses (colonies) of short tapering trichomes arranged parallel to each other, sometimes with false branching. Each trichome is enclosed within a sheath and a heterocyst is located at the base of each.

Habitat and distribution: *Rivularia* is usually found in fast-flowing relatively clean-water upland streams and rivers, growing attached to rocky substrates. Some species also grow on damp soil.

Possible confusion: *Gloeotrichia* (not included in this manual) is similar to *Rivularia* except that it has an akinete next to each heterocyst. *Gloeotrichia* colonies tend to be less firm than *Rivularia* and are usually found floating in lakes rather than in streams.

BG: filaments in gelatinous masses
Division: Cyanobacteria, **Order:** Nostocales
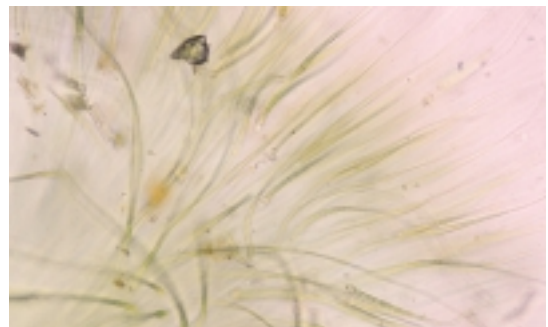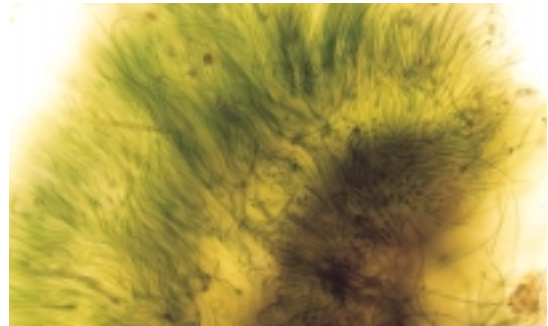
Rivularia, *Waiau River. x 70 (top) and x 135 (bottom). Note tapering filaments with a heterocyst at the base.*

Dichothrix

How to recognise the genus:

> *Macroscopic:* Brown gelatinous layer.

> *Microscopic:* Brown to blue-green branching trichomes. The heavily pigmented sheaths are either thin and firm or lamellated and several times wider than the trichome. Each trichome originates with a heterocyst and gradually tapers towards the end forming a hair-like structure. False branching always originates at a heterocyst (compare with *Scytonema*).

Habitat and distribution: Found mostly in lakes but also in clean-water streams. Probably widespread.

Possible confusion: *Tolypothrix* (see page 203) has a similar type of false branching, but the new branches part immediately from the original trichome. The hair-like tapered ends are absent in *Tolypothrix*.

BG: branched filaments
Class: Cyanobacteria, **Order:** Nostocales
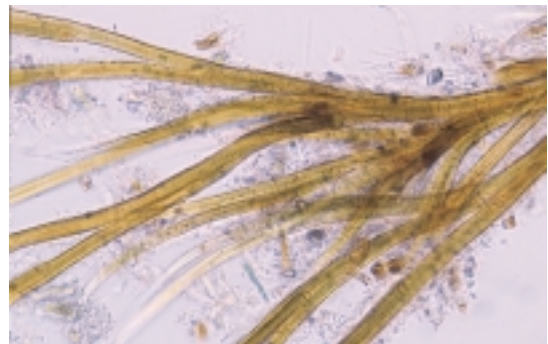
from top:

Dichothrix. *Note false branching with trichomes running parallel before branching off. x 135*

Note thick sheath and tapering ends. x 270

Coleodesmium

How to recognise the genus:

*Macroscopic:* Tiny blue-green tufts up to 3 mm across, atttached to substrates in streams.

Microscopic: Bundles of trichomes that lie side by side and entwined around each other within a common sheath, then gradually branch off (with additional false branching) to form an expanded fan-like thallus. At the ends of the branches, each trichome lies within an individual, finer sheath and often can be seen breaking up to form rounded fragments. Heterocysts seem to be both intercalary and at the origins of false branches. The cells are slightly constricted at the cross walls. Trichomes typically 10 to 15 µm in diameter.

Habitat and distribution: *Coleodesmium* was first found in New Zealand in 1996 by Steven Moore of Otago Regional Council, and identified by Dr Paul Broady (University of Canterbury). It is apparently common in Otago, and has been found in Taranaki. We have recently (May 2000) found the genus in clean foothills streams in both Canterbury and Nelson/Marlborough. The genus may well be widespread.

Possible confusion: *Schizothrix* (not illustrated in this manual) also has multiple filaments within a sheath, with progressive branching. However, there are no heterocysts and spores do not form. (See Bourelly 1985 for a description of both genera).
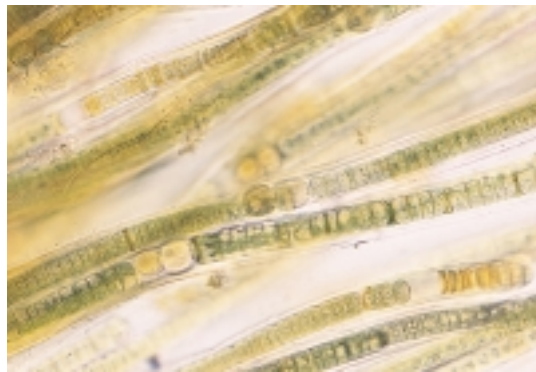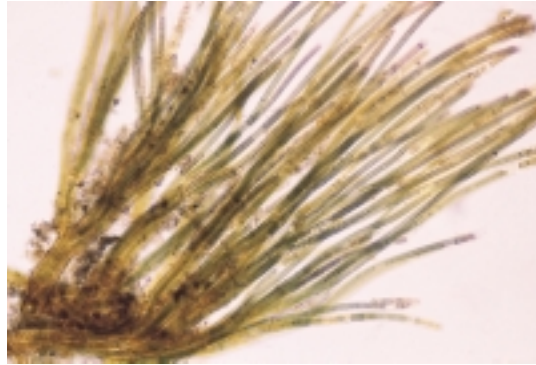
from top:

Coleodesmium. *Note typical branching fan-like growth. x 70*

Note the heterocysts. x 270

Filaments showing sheath and individual cells. x 270

Scytonema

How to recognise the genus:

Microscopic: Long trichomes with a definite base and tip, always enclosed in a usually heavily pigmented sheath. False branches always arise between two vegetative cells. Usually two branches arise beside one another, rarely only one. Heterocysts are always intercalary.

Habitat and distribution: Most often found in still waters, but can occur in outlet streams and rivers.

Possible confusion: *Tolypothrix* and *Scytonema* are habitually very similar. However, the false branches of *Tolypothrix* arise from heterocysts.

from top:

Scytonema *amongst other periphyton, Waiau River, Southland. x 90*

Branching in Scytonema*. Note wide sheath. x 680*
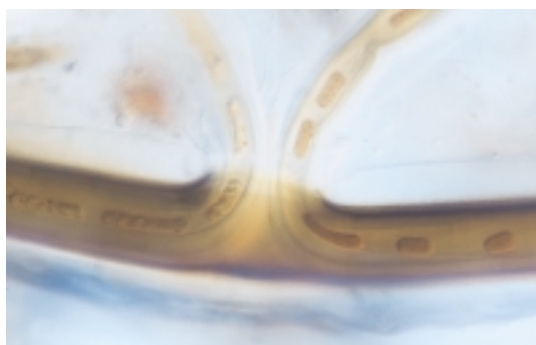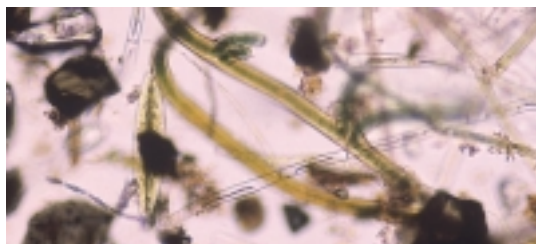
Stigonema

How to recognise the genus:

Macroscopic: Tough, irregularly shaped, gelatinous nodules, yellow-brown to dark brown; usually on stone substrates.

Microscopic: Trichomes divided into main filament and branches, with the rounded cells generally in multiple rows. Heterocysts are either intercalary or absent. True branching occurs, i.e., a new branch grows directly from cells in the main stem (compare with false branching in *Dichothrix* and *Tolypothrix*).

Habitat and distribution: *Stigonema* is often found in damp habitats rather than in flowing water. The specimen photographed was from a shallow, clean, upland stream near Motueka, South Island, growing with other cyanobacteria on marble bedrock. Distribution not known.

Possible confusion: *Stigonema* is unlikely to be confused with any other taxon in this manual. A similar taxon, *Fischerella* (see photo) has cells in single rows.

from top:

Stigonema: *note the true branching, and multiple rows of cells. x 250*

Probably Fisherella, *from a spring-fed stream, Canterbury. There is true branching but the cells are mainly in single rows. x 70*

Tolypothrix

How to recognise the genus:

> *Macroscopic:* Forms tangled, brown to yellow-brown clumps/tufts often mixed with other periphyton, or attached to rocks or aquatic plants.

> *Microscopic:* Long, straight (not tapered) blue-green trichomes in a thin, firm sheath. Cells typically barrel-shaped and rounded at the edges forming a slight constriction between successive cells. *Tolypothrix* has false branches that always arise just below a heterocyst.

Habitat and distribution: *Tolypothrix* is more often encountered in lakes than rivers but we have found it in a range of rivers and streams. The genus seems to be associated with clean conditions and can be very abundant in hard-water streams.

Possible confusion: A distinctive genus. Possible confusion with *Dichothrix* (page 200). However, the latter has a thick brown sheath and tapering trichomes.

from top:

Tolypothrix *in a mixed periphyton community, Waiau River. x 270*

Tolypothrix, *the beginnings of a new "branch". x 400*

Chamaesiphon

How to recognise the genus:

> *Microscopic:* Single cells forming polarized "pseudofilaments". The cells, encased in an open-ended sheath, are attached to the substrate at the narrow end. Division of cells occurs rapidly at the terminal end. Often you will see the new young cells separating off from the mother cell as round "exospores".

Habitat and distribution:

> *Chamaesiphon* grows as an epiphyte on other periphyton, attached to larger substrates, e.g., wood. It is fairly widespread in fast-flowing waters throughout New Zealand, but not common. It seems more likely to occur in cleaner waters.

Possible confusion:

> A distinctive genus, unlikely to be confused with any others.

from top:

Chamaesiphon *colonising* Tolypothrix, *from a foothills stream, Canterbury. x 350*

Long Chamaesiphon *cells on* Cladophora, *Moawhango River, central North Island. x 600*

Aphanocapsa
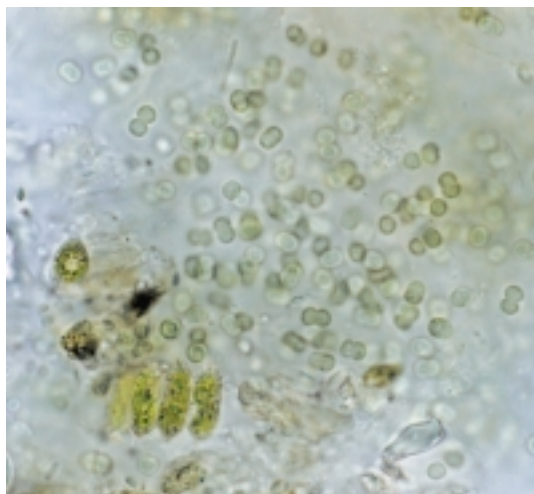
Division: Cyanobacteria, **Order:** Chroococcales

How to recognise the genus:

Macroscopic: Gelatinous masses.

Microscopic: Usually pale blue-green globular cells, around 2 to 4 µm across, loosely distributed through a gelatinous mass.

Habitat and distribution: Most often in tarns and lakes, but does occur in outflow streams.

Possible confusion: *Aphanothece* and *Gloeothece* (not in this manual) also form colonies of cells embedded in mucilage. In both of these the cells are more oval or elongated than round and in *Gloeothece* there are sheaths around individual cells.

Aphanocapsa. *x 1400*

Merismopedia

Division: Cyanobacteria, **Order:** Chroococcales

How to recognise the genus:

Microscopic: Flat colonies of round or oval blue-green cells arranged in mucilage (not always visible) in a regular right-angled criss-cross pattern. Species are determined from the number of cells in each colony (from 4 to hundreds) and the shape and size of individual cells. Cells are typically 3 to 6 µm across.

Habitat and distribution: Although usually found in lakes, *Merismopedia* occasionally appears in periphyton samples from a range of river types. The genus is normally rare.

Possible confusion: None. A distinctive genus.
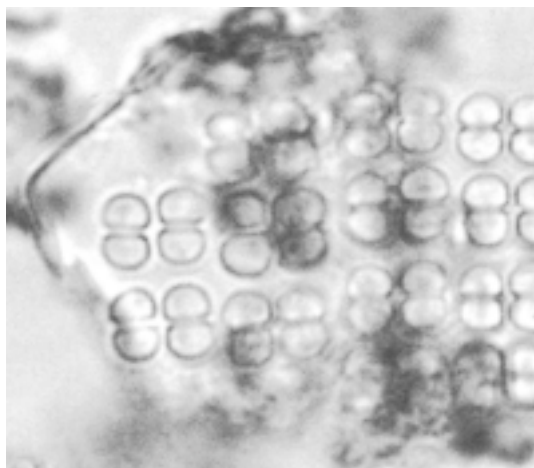
Merismopedia. *x 700 (photo: Phillippe Gerbeaux)*

10.5   Glossary of terms used in taxonomic descriptions

akinete	in cyanobacteria, specialised reproductive cell within the trichome.
araphid	term for diatoms that have no raphe.
areolae	in diatoms, holes or depressions on the valve surface that make up the striae (see puncta, which often means small holes).
axial area	in diatoms, a break in the striae that runs along the middle of the valve; may contain the raphe system.
biraphid	term for diatoms that have a raphe system on both valves.
capitate	ending in a knob or head.
central area	in diatoms, the part of the axial area at the centre of the valve.
chloroplast	structure within a cell which holds the photosynthetic pigments.
convergent	in diatoms, striae that are inclined from the valve margin towards the valve ends (plural: costae).
costa	in diatoms, ribs of silica that run across the valve and appear under LM as heavy lines.
dorsal	top or upper.
eukaryote	refers to organisms in which cell inclusions are enclosed within membranes (includes all the algae except for cyanobacteria).
false	in cyanobacteria, branches that form when a new trichome grows within the
branching	same sheath as an existing trichome, rather than branches that form when cells divide in a different direction.
frustule	the entire silica cell wall of a diatom.
genus	the unit of classification next most detailed after species. Subsequent units are family, order, class, division.
girdle	(also called the cingulum) bands of silica that join together the two parts (valves) of a diatom frustule.
girdle view	diatoms seen from the side (i.e., you see the side of each valve, plus the girdle in between).
heterocsyt	in cyanobacteria, cells within the trichome that are different from the normal vegetative cells; the site of nitrogen fixation.
hormogonium	in cyanobacteria, part of the trichome that detaches as a reproductive body (plural hormogonia).
intercalary	in cyanobacteria, in amongst the vegetative cells.
keel	in diatom species whose valve is sharply angled at the raphe, the edge of the ridge where the raphe is situated.
keel puncta	openings through the keel that look like a series of dots or lines under the light microscope
lamellated	in layers.
monoraphid	term for diatoms that have a raphe system on one valve only.
motile	capable of moving.
naviculoid	in diatoms, shaped like *Navicula*: symmetrical, lens-shaped.
parietal	referring to chloroplasts: lying against the inside of the cell wall.
prokaryote	refers to organisms that do not have cell inclusions (such as chloroplasts) contained in membranes, e.g., cyanobacteria.
pseudoraphe	in araphid diatoms, a break in the striae as if a raphe was present.
puncta	in diatoms, small openings or depressions that make up the striae.
pyrenoid	structure within a cell where starch is stored.

radiate	in diatoms, striae that are inclined from the valve margin towards the centre of the valve.
raphe	in diatoms, a pair of slits in the valve face which appears as lines under the light microscope.
reticulate	referring to chloroplasts, with holes, like lace or netting.
rimoportula	in diatoms, an isolated hole lying at the edge of the valve face.
septum	in diatoms, plates of silica within the girdle bands, lying parallel to the valve face (plural: septa) .
setae	hair-like extensions from cells.
species	usually the most detailed unit of biological classification (though some species are further split into varieties).
stigma	in diatoms, isolated hole(s) on the valve face (plural stigmata).
stria	in diatoms, a row of areolae, under low power LM appearing like a line (plural: striae).
taxon	any taxonomic unit (species, genus, family, etc.)
thallus	the overall form of an alga.
trichome	in cyanobacteria, a multi-celled filament.
true branching	branches that form by cells dividing in a different direction (compare with false branching in cyanobacteria).
valve	in diatoms, one of the pair of silica structures that fit together to form the frustule.
valve view	diatoms seen from the top (i.e., the face of the valve).
vegetative cells	the ordinary cells of a filament (or a trichome in cyanobacteria), i.e., not reproductive cells, holdfast cells, etc.
ventral	bottom or lower.

10.6   Recently re-named diatom genera, with their equivalent traditional names

The following table lists diatom genera mentioned in this manual which have new names as a result of re-classification of exisiting genera, or new descriptions. These changes are just a small proportion of the many revisions currently being applied in diatom taxonomy.

Revised classification	Traditional classification
Achnanthidium	*Achnanthes minutissima* group
Aulacoseira	some freshwater *Melosira* species
Brachysira	*Anomoeoneis vitrea* group
Cyclostephnos	*Stephanodiscus dubius* group
Encyonema	*Cymbella cesatii* group
Eunophora	newly described
Fragilariforma	*Fragilaria viriscens* group
Placoneis	*Navicula gastrum* group
Planothidium	*Achnanthes lanceolata* group
Pseudostaurosira	*Fragilaria brevistriata* group
Reimeria	*Cymbella sinuata*
Rossithidium	*Achnanthes linearis* group
Sellaphora	*Navicula pupula* group
Staurosira	*Fragilaria construens* group
Staurosirella	*Fragilaria leptostauron* group

10.7 Selection of texts that may be useful in stream periphyton identifications

Bourelly, P. 1981: Les Algues d'eau douce: Initiation à la systématique. Tome II: Les Algues jaunes at brunes. Boubee, Paris.

Bourelly, P. 1985: Les Algues d'eau douce: Initiation à la systématique. Tome III: Les Algues bleues et rouge. Boubee, Paris.

Bourelly, P. 1988: Les Algues d'eau douce: Initiation à a systématique. Compléments Tome I: Algues vertes. Boubee, Paris.

Bourelly, P. 1990: Les Algues d'eau douce: Initiation à la systématique. Tome I: Les Algues vertes. Boubee, Paris.

Cox, E.J. 1996. Identification of freshwater diatoms from live material. Chapman & Hall.

Croasdale, H. and Flint, E.A. 1986. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume I. Christchurch: DSIR Botany Division.

Croasdale, H. and Flint, E.A. 1988. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume II. Christchurch: DSIR Botany Division.

Croasdale, H., Flint, E.A. and Racine, M.M. 1994. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume III. Christchurch: DSIR Botany Division.

Drouet, F. 1981: Revision of the Stigonemataceae with a summary of the classification of the blue-green algae. Nova Hedwigia 66: 1–221.

Entwistle, T.J.; Kraft, G.T. 1984. Survey of freshwater red algae (Rhodophyta) of south-eastern Australia. *Australian Journal of Marine and Freshwater Research 35*: 213–259.

Entwistle, T.J., Sonneman, J.A.; Lewis, S.H. 1998: Freshwater algae of Australia. a guide to the conspicuous genera. Sainty and Associates, NSW, Australia.

Etheredge, M.K.; Pridmore, R.D. 1987. The Freshwater Planktonic Blue-greens (Cyanophyta∕Cyanobacteria) of New Zealand. A taxonomic guide. Water & Soil Miscellaneous Publication No. 111. Ministry of Works and Development, Wellington.

Foged, N. 1979: Diatoms in New Zealand. The North Island. *Bibliotheca Phycologica. Band 47.* J. Cramer, Vaduz.

Krammer, K.; Lange-Bertalot, H. 1991–1997. Bacillariophyceae. Vols 1–4. (Susswasserflora von Mitteleuropa; Bd. 2). Fischer, Stuttgart.

Patrick, R.; Reimer, C. W. 1966: The diatoms of the United States. Vol 1. Academy of Natural Sciences, Philadelphia.

Patrick, R.; Reimer, C. W. 1975: The diatoms of the United States. Vol. 2. Academy of Natural Sciences, Philadelphia.

Prescott, G. W. 1973: Algae of the Western Great Lakes area. Wm C. Brown, Iowa.

Prescott, G. W. 1954: How to know the freshwater algae. Wm C. Brown, Iowa.

Whitford, L. A.; Schumacher, G. J. 1973: A manual of freshwater algae. Spars Press, Raleigh, North Carolina.

10.8   List of diatom species with authorities and selected references for identification

Abbreviations used for references:

P&R   Patrick and Reimer

KLB   Krammer and Lange-Bertalot

page

Achnanthes exigua Grunow   158
   P&R 1966. p. 257. pl 16, f. 21-22.
Achnanthes inflata (Kutzing) Grunow   156
   P&R 1966. p. 279, pl. 19, figs 15-16
Achnanthes oblongella Oestrup   158
   KLB 1991-1997. vol. 2/4, p. 29, pl 16/1-14
Achnanthidium lanceolatum Brebisson   157
   P&R 1966. p. 269, Pl 18, f 1-10 (as *Achnanthes
   lanceolata*; now *Planothidium lanceolatum* (see
   Round and Bukhityarova 1996)
Achanthidium linearis W. Smith   158
   P&R 1966. p. 251, pl.16, f3-4 (as *Achnanthes
   linearis*; now *Rossithidium linearis* (see Round
   and Bukhityarova 1996).
Achnanthidium minutissimum (Kutzing) Czarnecki   157
   P&R 1966. p. 253, pl. 16 figs 9-10 (as *Achnanthes
   minutissima*).
Amphora ovalis (Kutzing) Kutzing   160
   KLB 1991-1997. vol. 2/1, p. 345, pl.149.
Amphora veneta var *capitata* (Haworth)
   KLB 1991-1997. vol. 2/1, p. 349, pl.151/8.   160
Asterionella formosa Hassall
   KLB 1991-1997. vol. 2/3, p. 103, pl. 103-104
Aulacoseira granulata (Ehrenberg) Simonsen 1979   148
   KLB 1991-1997. vol. 2/3, p.22, pl 16/10); Foged
   1979, pl. I (as *Melosira granulata*).
Brachysira serians (Brebisson in Kutzing) Round & Mann 161
   Cox 1996. p. 84, fig. 27b, P&R 1966, p. 378, pl 33/
   1,2 (var acuta) (as *Anomoeoneis*).
Brachysira vitrea (Grunow) Ross   161
   KLB 1991-1997. vol. 2/1, p. 256, pl 94/21-28 (as
   Anomoeoneis). (also listed in Foged 1979 as
   Anomoeoneis exilis var. *lanceolata*).
Cocconeis pediculus Ehrenberg   162
   P&R 1966. p. 240, pl 15, figs 3-4.
Cocconeis placentula Ehrenberg   162
   P&R 1966. p. 240, pl 15, fig 7
Cyclotella meneghiniana Kutzing1   163
   KLB 1991-1997. vol. 2/3, p. 44, pl 44/1-10; Foged
   1979, pl. III, fig. 6.
Cyclotella stelligera Cleve & Grunow   163
   KLB 1991-1997. vol. 2/3 p. 50, pl 49/1-4;
   Foged 1979, p. 36, pl III, figs 8,9,11
Cymbella aspera (Ehrenberg) Peragallo   164
   KLB 1991-1997. vol. 2/1, p. 319, pl 131/1;
   Foged, pl. 35, fig. 14
Cymbella cistula (Hempr.) Grunow var *gracilis* Hustedt   165
   Foged 1979, p. 37, pl 35, fig. 12
Cymbella kappii Cholnoky   164
   Foged 1979. p. 39, pl. 34, figs 8-10
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11   References

Aloi, J.E. 1990: A critical review of recent freshwater periphyton field methods. *Canadian Journal of Fisheries and Aquatic Sciences 47*: 656–670.

Anagnostidis, K.; Komàrek, J. 1985. Modern approach to the classification system of cyanophytes, 1 - Introduction. *Archiv fur Hydrobiologie /* Suppl. 71, *Algological Studies 38-39*: 291–302.

Anagnostidis, K.; Komàrek, J. 1988a. Modern approach to the classification system of cyanophytes, 3 - Oscillatoriales. *Archiv fur Hydrobiologie /* Suppl. 80, *Algological Studies 50-53*: 327–472.

Anagnostidis, K.; Komàrek, J. 1988b. Modern approach to the classification system of cyanophytes, 5 - Stigonematales. *Archiv fur Hydrobiologie /* Suppl. 86, Algological Studies 59: 1–73.

APHA 1995: Standard Methods for the Examination of Water and Wastewater (19th Edition). American Public Health Association, Washington, DC.

Barber, H.G.; Haworth, E.Y. 1981. A guide to the structure of the diatom frustule. Freshwater Biological Association. Biological Publication No. 44.

Biggs, B.J.F. 1985: The use of periphyton in the monitoring of water quality. In: Pridmore, R.D.; Cooper, A.B. Biological Monitoring in Freshwaters: proceedings of a seminar. Ministry of Works and Development, Wellington, NZ.

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. 1988a: A periphyton sampler for shallow, swift rivers. *New Zealand Journal of Marine and Freshwater Research 22*: 189–199.

Biggs, B.J.F. 1988b: Artificial substrate exposure times for periphyton biomass estimates in rivers. *New Zealand Journal of Marine and Freshwater Research 22*: 189–199.

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

Biggs, B.J.F. 1990: Use of relative specific growth rates of periphytic diatoms to assess enrichment of a stream. *New Zealand Journal of Marine and Freshwater Research 24*: 9–18.

Biggs, B.J.F. 1995: The contribution of disturbance, catchment geology and landuse to the habitat template of periphyton in stream ecosystems. *Freshwater Biology 33*: 419–438.

Biggs, B.J.F. 1996: Patterns in benthic algae of streams. In: Stevenson, R J.; Bothwell, M.L.; Lowe, R.L. . Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.

Biggs, B.J.F. 2000a: New Zealand Periphyton Guideline: Detecting, Monitoring and Managing the Enrichment of Streams. Ministry for Environment Publication, Wellington, 151 pp.

Biggs, B.J.F. 2000b: 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.; Close, M.E. 1989: Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. *Freshwater Biology 22*: 209–231.

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

Biggs, B.J.F.; Lowe, R.L. 1994: Responses of two trophic levels to patch enrichment along a New Zealand stream continuum. *New Zealand Journal of Marine and Freshwater Research 28*: 119–134.

Biggs, B.J.F.; Price, 1987. A survey of filamentous algae proliferations in New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research 21*: 175-191.

Biggs, B.J.F.; Shand, B.I. 1987: Biological communities and power development in the Lower Clutha River, Otago. Publication No. 10 of the Hydrology Centre, Christchurch. Ministry of Works and Development, Christchurch, NZ.

Biggs, B.J.F.; Stokseth, S. 1996: Hydraulic habitat preferences for periphyton in rivers. *Regulated Rivers: Research and Management 12*: 251–261.

Biggs, B.J.F.; Goring, D.G.; Nikora, V.I. 1998a: Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. *Journal of Phycology 34*: 598–607.

Biggs, B.J.F.; Kilroy, C.; Lowe, R.L. 1998b: Periphyton development in three valley segments of a New Zealand grassland river: Test of a habitat matrix conceptual model within a catchment. *Archiv fur Hydrobiologie 143*: 147–177.

Biggs, B.J.F.; Kilroy, C.; Mulcock, C.M. 1998c: New Zealand Stream Monitoring and Assessment Kit. Stream Monitoring Manual. Version 1. *NIWA Technical Report 40.* 150 p.

Biggs, B.J.F.; Smith, R.A.; 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.

Boston, H.L.; Hill, W.R. 1991: Photosynthesis-light relations of stream periphyton communities. *Limnology and Oceanography 36*: 644–656.

Bothwell, M. L. 1985: Phosphorus limitation of lotic periphyton growth rates: an intersite comparison using continuous-flow troughs (Thompsen River system: British Columbia). *Limnology and Oceanography 30*: 527–542.

Bothwell, M.L. 1988: Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: the influence of temperature and light. *Canadian Journal of Fisheries and Aquatic Sciences 45*: 261–270.

Bott, T.L. 1996: Primary productivity and community respiration. In: Hauer, F.R.; Lamberti, G.A. Methods in Stream Ecology. Academic Press, San Diego.

Bourelly, P. 1981: Les Algues d'eau douce: Initiation à la systématique. Tome II: Les Algues jaunes at brunes. Boubee, Paris.

Bourelly, P. 1985: Les Algues d'eau douce: Initiation à la systématique. Tome III: Les Algues bleues et rouge. Boubee, Paris.

Cassie, V. 1984: A checklist of the freshwater diatoms in New Zealand. *Bibliotheca Diatomologica 4*: 1–129. J. Cramer.

Cassie, V. 1984: Checklist of freshwater algae in New Zealand (excluding the diatoms and charophytes). Parts 1 and 2. Water & Soil Technical Publications Nos 24 and 25. Ministry of Works and Development, Wellington.

Cassie, V. 1989: A contribution to the study of New Zealand Diatoms. *Bibliotheca Diatomologica. Band 17.* J. Cramer: Berlin, Stuttgart.

Cattaneo, A.; Amireault, M.C. 1992: How artificial are artificial substrata for periphyton? *Journal of the North American Benthological Society 11*: 244-256.

Cattaneo, A.; Ghittori, S.; Vendegna, V. 1975: The development of benthonic phytocoenosis on artificial substrates in the Ticino River. *Oecologia 19*: 315–327.

Cattaneo, A.; Roberge, G. 1991: Efficiency of a brush sampler to measure periphyton in streams and lakes. *Canadian Journal of Fisheries and Aquatic Sciences 48*: 1877–1881.

Chessman, B.C.; Hutton, P.E.; Burch, J.M. 1992: Limiting nutrients for periphyton growth in sub-alpine, forest, agricultural and urban streams. *Freshwater Biology 28*: 349–361.

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

Corkum, L.D. 1996: Patterns of nutrient release from nutrient diffusing substrates in flowing water. *Hydrobiologia 333*: 37–43.

Cox, E.J. 1987. *Placoneis* Mereschowsky: the re-evaluation of a diatom genus originally characterized by its chloroplast type. *Diatom Research 2*: 145-157.

Cox, E.J. 1996. Identification of freshwater diatoms from live material. Chapman & Hall.

Croasdale, H. and Flint, E.A. 1986. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume I. Christchurch: DSIR Botany Division.

Croasdale, H. and Flint, E.A. 1988. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume II. Christchurch: DSIR Botany Division.

Croasdale, H., Flint, E.A. and Racine, M.M. 1994. Flora of New Zealand: freshwater algae, chlorophyta, desmids. Volume III. Christchurch: DSIR Botany Division.

Davies-Colley, R.J.; Hickey, C.W.; Quinn, J.M.; Ryan, P.A. 1992: Effects of clay discharges on

streams. 1. Optical properties and epilithon. *Hydrobiologia 248*: 215–234.

Dodds, W. K.; Brock, J. 1998: A portable flow chamber for in-situ determination of benthic metabolism. *Freshwater Biology 39*: 49–59.

Drouet, F. and Daily, W.A. 1956. Revision of the coccoid Myxophyceae. Butler Univ. Bot. Stud. 12: 1–218. Facsimile Edition (1973). Hafner Press (Macmillan), New York.

Drouet, F. 1981: Revision of the Stigonemataceae with a summary of the classification of the blue-green algae. *Nova Hedwigia 66*: 1–221.

Dube, M.G.; Culp. J.M.; Scrimgeour, G.J. 1997. Nutrient limitation and herbivory: processes influenced by bleached kraft pulp mill effluent. *Canadian Journal of Fisheries and Aquatic Sciences 54*: 2584–2595.

Elliott, J. M. 1977: Some methods for the statistical analysis of benthic invertebrates. Scientific Publication 25, Freshwater Biological Association, Cumbria, England.

Entwistle, T.J.; Kraft, G.T. 1984. Survey of freshwater red algae (Rhodophyta) of south-eastern Australia. *Australian Journal of Marine and Freshwater Research 35*: 213–259.

Fairchild, G.W.; Lowe, R.L. 1984: Artificial substrates which release nutrients: effects on periphyton and invertebrate succession. *Hydrobiologia 114*: 29-37.

Fairchild, G.W.; Lowe, R.L.; Richardson, W.B. 1985: Algal periphyton growth on nutrient-diffusing substrates: an in-situ bioassay. *Ecology 66*: 465-472.

Foged, N. 1979: Diatoms in New Zealand. The North Island. *Bibliotheca Phycologica. Band 47*. J. Cramer, Vaduz.

Francoeur, S.N.; Biggs, B.J.F.; Lowe, R.L. 1999a: Direct inhibition of stream algal biomass accrual by malathion from insecticide-diffusing substrata (NDS). *Journal of Freshwater Ecology 14*: 179-186.

Francoeur, S.N.; Biggs, B.J.F.; Smith, R.; Lowe, R.L. 1999b: Nutrient limitation of algal biomass accrual: Seasonal patterns and comparison of methodology. *Journal of the North American Benthological Society 18*: 242–260.

Geitler, L. 1925: Synoptische Darstellung de Cyanophyceen in morphologischer und systematischer Hinsicht. *Beih. bot. Cbl. 2*, 41: 163–184.

Geitler, L. 1932: Cyanophyceae. In: Rabenhorst, L. Kryptogamen-Flora von Deutschland, Osterreich und der Schweiz. 14: 1–1196.

Geitler, L. 1942: Schizophyta (Klasse Schizophyceae). In: Engler and Prantl: Nat. Pflanzenfam 1b. 232 p. Berlin.

Gibeau, G.G.; Miller, M.C. 1989: A micro-bioassay for epilithon using nutrient-diffusing artificial substrata. *Journal of Freshwater Ecology 5*: 171–176.

Gilbert, R. O. 1987: Statistical Methods for Environmental Pollution Monitoring. J. Wiley & Sons, New York.

Goldman, J. C. 1980: Physiological processes, nutrient availability, and the concept of relative specific growth rates in marine phytoplankton ecology. In: Falkowski, P. G. (ed.) Primary Productivity in the Sea. Brookhaven Symposia in Biology. Plenum Press, New York.

Graham, L.E.; Wilcox, L.W. 2000 Algae. Prentice-Hall, Upper Saddler River, New Jersey.

Green, R. H. 1979: Sampling Design and Statistical Methods for Environmental Biologists. J. Wiley & Sons, Toronto.

Hickey, C. W. 1988: Benthic chamber for use in rivers: testing against oxygen mass balances. *Journal of Environmental Engineering 114*: 828–845.

Hill, W. 1996: Effects of light. In: Stevenson, R. J.; Bothwell, M.L.; Lowe, R.L. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.

Holm-Hansen, O.; Reimann, B. 1978: Chlorophyll *a* determination: improvements in methodology. *Oikos 30*: 438–447.

Hynes, H.B.N. 1974: The Biology of Polluted Waters. University of Toronto Press, Toronto.

Jacobsen, T.R.; Rai, H. 1990: Comparison of spectrophotometric, fluorometric and high performance liquid chromatography methods for the determination of chlorophyll a in aquatic samples: effects of solvent and extraction procedures. *Internationale Revue der gesamten Hydrobiologie 75*: 207–217.

Kershaw, K.A. 1973: Quantitative and Dynamic Plant Ecology. Edward Arnold, London.

Kociolek, J.P.; Stoermer, E.F. 1987. Ultrastructure of *Cymbella sinuata* and its allies (Bacillariophyceae) and their transfer to *Reimeria* gen. nov. *Syst. Bot. 12*: 451–459.

Komàrek, J.; Anagnostidis, K.1986. Modern approach to the classification system of cyanophytes, 2 - Chrooococcales. *Archiv fur Hydrobiologie /* Suppl. 73, *Algological Studies 43*: 157-226.

Komàrek, J. ; Anagnostidis, K. 1989. Modern approach to the classification system of cyanophytes, 4 - Nostocales. *Archiv fur Hydrobiologie /* Suppl. 82, *Algological Studies 56*: 247-345.

Krammer, K. 1992. *Pinnularia*: eine Monographie der europaischen Taxa. *Bibliotheca Diatomologica, Band 26.* J. Cramer, Berlin, Stuttgart.

Krammer, K.; Lange-Bertalot, H. 1991–1997. Bacillariophyceae. Vols 1–4. (Susswasserflora von Mitteleuropa; Bd. 2). Fischer, Stuttgart.

Levin, S.A. 1994: The problem of pattern and scale in ecology. *Ecology 73*: 1943–1967.

Loeb, S. 1981: An in-situ method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. *Limnology and Oceanography 26*: 394–399.

Lorenzen, C.J. 1966: A method for the continuous measurement of in vivo chlorophyll concentration. *Deep Sea Research 13*: 223–227.

Lowe, R.L.; LaLiberte, G.D. 1996: Benthic stream algae: distribution and structure. In: Hauer, F.R.; Lamberti, G.A. Methods in Stream Ecology. Academic Press, San Diego.

Lowe, R.L.; Pan, Y. 1996: Benthic algal communities as biomonitors. In: Stevenson, R.J.; Bothwell, M.L.; Lowe, R.L. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.

Lowe, R.L.; Golladay, S.W.; Webster, J.R. 1986: Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. *Journal of the North American Benthological Society 5*: 221–229.

Mann, D.G. 1989: The diatom genus *Sellaphora*: separation from *Navicula. British Phycological Journal 24*: 1-20.

Marker, A. F. H. 1972: The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. *Freshwater Biology 2*: 361–385.

Margulis, L.; Corliss, J.O.; Melkonian, M.; Chapman, D.J. (eds). Handbook of Protoctista. Jones and Bartlett Publishers. Boston.MfE 1997: Environmental Performance Indicators: Proposals for air, freshwater and land. Ministry for the Environment, Wellington, New Zealand.

Mook, D. H.; Hoskin, C. M. 1982: Organic determinations by ignition: Caution advised. *Estuarine and Coastal Shelf Science 15*: 697–699.

Necchi, O.; Branco, L. H. .Z.; Branco, C. C. 1995. Comparison of three techniques for estimating periphyton abundance in bedrock streams. *Archiv fur Hydrobiologie 134*: 393–402.

Norris, R.H.; Georges, A. 1993: Analysis and interpretation of benthic macroinvertebrate surveys. In: Rosenberg, D.M.; Resh, V.H. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman & Hall, New York.

Nusch, E.A. 1980: Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv fur Hydrobiologie 14*: 14–36.

Patrick, R.; Reimer, C. W. 1966: The diatoms of the United States. Vol 1. Academy of Natural Sciences, Philadelphia.

Patrick, R.; Reimer, C. W. 1975: The diatoms of the United States. Vol. 2. Academy of Natural Sciences, Philadelphia.

Peterson, C.G.; Grimm, N.B. 1992: Temporal variation in enrichment effects during periphyton succession in a nitrogen-limited desert stream ecosystem. *Journal of the North American Benthological Society 11*: 20-36.

Porter, S.D.; Cuffney, T.F.; Gurtz, M.E.; Meador, M.R. 1993: Methods for collecting algal samples as part of the national water-quality assessment program. U.S. Geological Survey Open-File Report 93-409.

Pringle, C.M.; Bowers, J.A. 1984: An in-situ substratum fertilization technique: diatom colonization on nutrient-enriched, sand substrata. *Canadian Journal of Fisheries and Aquatic Sciences 41*: 1247–1251.

Riemann, B. 1978: Absorption co-efficients for chlorophyll *a* and *b* in methanol and a comment on interferance of chlorophyll b in determinations of chlorophyll a. *Vatten 3*: 187–194.

Reimann, B.; Ernst, D. 1982: Extraction of chlorophylls *a* and *b* from phytoplankton using standard extraction techniques. *Freshwater Biology 12*: 217–223.

Ridley-Thomas, C.I.; Austin, A.; Lucey, W.P.; Clark, M.J.R. 1989: Variability in the determination of ash-free dry weight for periphyton communities: a call for a standard method. *Water Research 23*: 667–670.

Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M.; Stanier, R.Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology 111*: 1-61.

Round, F.E.; Bukhtiyarova, L. 1996. Four new genera based on *Achnanthes* (*Achnanthidium*) together with a re-definition of *Achnanthidium. Diatom Research 11*: 345-361.

Round, F.E.; Crawford, R.M.; Mann, D.G. 1990. The diatoms. Biology and morphology of the genera. Cambridge University Press.

Sandgren, C.D.; Robinson, J. V. 1984: A stratified sampling approach to compensating for non-random sedimentation of phytoplankton cells in invertebed microscope settling chambers. *British Phycological Journal 19*: 67–72.

Sartory, D.P. 1982: Spectrophotometric analysis of chlorophyll in freshwater phytoplankton. Technical Report TR 115, Hydrology Research Institute, Department of Environment Affairs, South Africa. 163 p.

Sartory, D.P. 1985: The determination of algal chlorophyllous pigments by high performance liquid chromatography and spectrophotometry. *Water Research 19*: 605–610.

Sartory, D.P.; Grobbelaar, J.E. 1984: Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia 114*: 177–187.

Scrimgeour, G.J.; Chambers, P.A. 1997: Development and application of a nutrient-diffusing bioassay for large rivers. *Freshwater Biology 38*: 221–231.

Snedecor, G. W.; Cochran, W. G. 1980: Statistical Methods. The Iowa State University Press, Ames, Iowa, U.S.A.

Snelder, T.; Biggs, B.J.F.; McDowall, R.M.; Shankar, U.; Stephens, T.; Boothroyd, I. 1998: Physically based ecotyping of New Zealand rivers for water resources management. NIWA Consultancy Report MFE80505. 60 p.

Sokal, R.R.; Rohlf, F.J. 1981: Biometry. W.H. Freeman, SanFrancisco.

Stanier, R.Y.; Cohen-Bazire, G.: 1977. Phototrophic prokaryotes: the Cyanobacteria. In: Starr, M.P.; Ingraham, J.L.; Balows, A. (eds). *Annual Review of Microbiology 31*: 225–274.

Steel, R.G.D.; Torrie, J.H. 1960: Principles and Procedures of Statistics: with special reference to biological sciences. McGraw-Hill, New York.

Stevenson, R. J. 1996: An introduction to algal ecology in freshwater benthic habitats. In: Stevenson, R. J.; Bothwell, M.L.; Lowe, R.L. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, SanDiego.

Stevenson, R.J.; Bothwell, M.L.; Lowe, R.L. 1996: Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego.

Tate, C.M. 1990: Patterns and controls of nitrogen in tallgrass prairie streams. *Ecology 71*: 2007–2018.

Underwood, A.J. 1997: Experiments in Ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, England.

Vyverman, W., Sabbe, K., Mann, D., Kilroy, C., Vyverman, R., Vanhoutte, K. and Hodgson, D. (1998). Eunophora *gen nov. (Bacillariophyta)* from Tasmania and New Zealand: description and comparison with Eunotia and amphoroid diatoms. *European Journal of Phycology 33: 95–111.*

Ward, R.C.; Loftis, J.C.; McBride, G.B. 1990: Design of Water Quality Surveys. Van Nostrand Reinhold, New York.

Webb, D.J.; Burnison, B.K.; Trimbee, A.M.; Prepas, E.E. 1992: Comparison of chlorophyll a extraction with ethanol and dimethyl sulfoxide/acetone, and concern about spectrophotometric phaeopigment correction. *Canadian Journal of Fisheries and Aquatic Sciences 49*: 2331–2336.

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.

Winterbourn, M.J. 1990: Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream. *Freshwater Biology 23*: 463–474.

Williams, D.M.; Round, F.E. 1987. Revision of the genus *Fragilaria. Diatom Research 2*: 267-288.

Williams, D.M.; Round, F.E. 1988. *Fragilariaforma*, nom. nov., a new generic name for *Neofragilaria* Willams & Round. *Diatom Research 3*: 265–267.

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

Wolman, M.G. 1954: A method of sampling coarse river bed material. *Transactions, American Geophysical Union 35*: 951–956.

Zar, J. H. 1996: Biostatistical Analysis. Prentice-Hall International, Inc.

Nonparametric methods and equivalence tests

Graham McBride, NIWA, Hamilton, May 2000

This manual has discussed a number of statistical techniques that may be thought of as "standard"; certainly they are in wide use. However there are two items about which some elaboration has been thought desirable. The first bears on the increasing use of "nonparametric" techniques. These are of wide applicability because they require fewer assumptions than the more traditional methods and can be fruitful in some cases. The second bears on more fundamental questions of how we may, or may not, make useful inferences using the standard null hypothesis testing procedure. An alternative procedure – equivalence tests – is outlined.

Nonparametric methods

Many statistical procedures are built on the idea of sampling from a known distribution-commonly the normal distribution. This distribution has two "parameters" – the mean and the standard deviation. It is completely defined once these two parameters are specified. Such "parametric" procedures are always the best to use when we have strong grounds to believe that we are indeed sampling from distributions that are reasonably normal.

But we often encounter datasets that suggest that we are sampling from a distinctly non-normal distribution. Most usually they are right-skewed, i.e., having a small number of very high values with the rest being much smaller. Such a pattern may compromise the behaviour of the normal methods and other more appropriate methods may give better results. Two alternative approaches may be taken to this problem.

The first uses transformations. Most typically we may take logarithms of the data, adding 1 to each datum first if any zeroes are present (the logarithm of zero is undefined). This is appropriate when the data are right-skewed and so the logarithms' distribution becomes much more normal-looking (logarithms have the effect of reducing large numbers to much smaller numbers, but small number are reduced much less, e.g., $\log_{10}(100) = 2$ and $\log_{10}(10) = 1$). Parametric methods are then applied to these transformed data, and, by taking the antilog of the result, we get the final answer in the original measurement scale of the data. Some (at times overlooked) features of this approach should be noted:

- the parameter about which hypotheses are made is typically not the arithmetic mean but the geometric mean;
- when transforming data back to the original scale some statistical bias is introduced (Gilbert 1987: 149), for which correction procedures are available (Gilbert 1987: 165).

The second alternative notes that we may have data that look neither reasonably normal nor lognormal. While in some cases other transformations could possibly be found, it can be wiser to dispense with the need to invoke any distribution at all. This is done by replacing each datum by its rank, i.e., the largest datum is assigned rank 1, the next rank is assigned rank 2, etc. This approach, while preserving the relative order of the data, replaces the actual differences between adjacent data by 1 ranking unit. It therefore loses information, but has the advantage of not requiring a particular distribution to be assumed. Methods based on this approach are called nonparametric (parameters are not required, because a distribution is not assumed), or, equivalently, distribution-free. They require fewer assumptions than do

normal methods, but they do invoke some (for which the interested reader may refer to Conover 1980 or Johnson 1995).

If sampling is from the normal distribution, parametric tests will always be more powerful than nonparametric tests. But if sampling is from distinctly non-normal distributions, nonparametric tests tend to have more powe – sometimes much more (e.g., in the case of highly skewed distributions, typical of microbiological concentrations). It is worth noting that the hypotheses tested by nonparametric procedures are often in terms of some form of rank statistic, usually the median. Since the true median and geometric mean of a lognormal distribution are identical (Gilbert 1987) there seems good reason to prefer the more generally-applicable nonparametric approaches as we may often be dealing with distributions that are neither particularly normal nor lognormal.

Details of how to perform these tests are available in good texts (Conover 1980; Sokal & Rohlf 1981; Iman & Conover 1983; Zar 1984, 1996; Gilbert 1987). A brief summary for commonly encountered tests on means and medians is given below.

Type of data	Parametric test	Equivalent nonparametric test
Two sets of samples	t-test	Wilcoxon Rank Sum test*
A set of paired samples	Paired t-test	Wilcoxon Signed Rank test
Several sets of samples	Anova	Kruskal-Wallis test

*This is essentially the same as the Mann-Whitney test. It is sometimes called the Wilcoxon–Mann-Whitney test.

Note also that there are nonparametric statistics that measure correlation. The commonly-quoted correlation coefficient (r") is actually Pearson's (parametric) correlation coefficient which measures the degree of linear correlation; if x,y pairs of data fall exactly on an upward-sloping straight line then $r = 1$ (if the line is downward-sloping then $r = -1$). If the y data tend to increase as x increases, but in a jagged or curvilinear fashion, r may be considerably lower than 1, yet the data are strongly correlated. In this situation it is appropriate to use the nonparametric analogue of Pearson's r. This is Spearman's rho (sometimes denoted by rS). It measures the degree of monotonicity in the relationship between x and y, whereas r measures the degree of linearity. One can obtain a rather higher value of rS than of r, showing that relying on r alone as the "correlation coefficient" can be misleading.

Because most modern software includes some nonparametric options it is a good idea to use both parametric and nonparametric procedures on the same dataset and compare the results obtained. This has the desirable effect of forcing the investigator to explain why any differences arise and which result is to be relied upon.

Equivalence tests

There has been a long tradition of using two-sided "null hypothesis" tests in many of the sciences. The tested hypothesis posits that there is no difference whatsoever between tested parameters. These tests are usually presented (e.g., by Zar 1996) as clear-cut, well-established procedures that adequately answer many questions relating to inferences about our environment.

Some question this view (e.g., Johnson 1999, Germano 1999). In particular, what relationship is there between the null hypothesis and a research hypothesis? Let's take the case of a study

of the impact of gold mining operations on stream invertebrates (McBride 1998, 1999) – it could equally well apply to stream periphyton. The former hypothesis, being "null", posits that the difference in species richness between upstream and downstream sites is exactly zero. But a research hypothesis is not "null". It will be along the lines of "whatever difference is present, it is not ecologically important, and so we will not infer that an impact has occurred". Procedures for such hypotheses do exist, and are often called "equivalence tests". They require the investigator to state the minimum value of the difference that would be considered to be environmentally important. This must be done before the test can be performed. Contrast this with the situation in performing a null hypothesis test (e.g., a *t*-test) using standard software-the investigator is not required to state the null test value (it is assumed to be zero).

Fundamental nature of the hypotheses

A two-sided null hypothesis cannot be true: the probability that two parameters (e.g., mean periphyton densities) are exactly equal is vanishingly small. In contrast, either a one-sided hypothesis or a two-sided interval hypothesis can be true. That is, the difference could be either less than or greater than a critical value, or it may be within or beyond a critical interval.

Interpreting the test result

If the null hypothesis is rejected "impact" will be inferred, with the investigator using the phrase that a "statistically significant difference" has been found. That hypothesis may not be rejected of course, in which case it is often mistakenly inferred that there is "no difference".

If the research hypothesis is rejected one might use the language of being "confident that an ecologically important impact has occurred". If it is not rejected one may regard the upstream and downstream sites as "equivalent" (yet recognising that they are in fact different), so saying there probably was not an impact. Accordingly, one can argue that this procedure, and not the null hypothesis testing procedure, is relevant to examination of the research hypothesis.

Performing equivalence tests

Procedures for testing equivalence hypotheses do exist.

If one is concerned about only increases (or decreases) in periphyton then one-sided tests (parametric or nonparametric) can be performed. These tests are well explained in current texts.

If there is interest in either increases or decreases then two-sided tests of an interval are appropriate. Procedures for performing these tests have been developed in the drugs-testing agencies (Chow & Liu 1992) but are only just becoming available for environmental scientists (McBride 1998, 1999). A reliable spreadsheet calculator (with accompanying commentary) for the stream invertebrate species richness case described in these two articles is available from the author (g.mcbride@niwa.cri.nz), and will be available at our website later (http://www.niwa.cri.nz/_private/pgsf/stats/index.html). It is easily extensible to other data. Nonparametric equivalence tests procedures also exist, though are not embedded in the above software.

Why the fuss?

At the risk of being repetitious, the two-sided null hypothesis procedure tests a barren hypothesis. This posits that there is no impact whatsoever, i.e., mean upstream and downstream invertebrate densities or periphyton densities are exactly equal. It is barren because it cannot be true. There will always be some impact, however small. Yet the procedure assumes the hypothesis to be true and only rejects it if data are in some way convincing to the

contrary. That "convincing" is encapsulated in the test's *p*-value, which is the probability of getting data at least as extreme as was obtained if the hypothesis were true. This *p*-value has the unfortunate property of tending to get ever smaller as the number of samples is increased, so that with a very large number of samples one would almost always reject the null hypothesis – precisely because it isn't true. As a consequence we have to note these important features:

- null hypotheses may be rejected but should never be "accepted", merely "not rejected";

- the "minimum detectable difference" tends to become ever-smaller as the number of samples is increased, and as a consequence;

- comparisons of the "statistical significance" attained in various studies is only valid if the numbers of samples are the same (or nearly so) in each case.

If one tests an interval (equivalence) hypothesis these difficulties are very much diminished, precisely because we are then testing a hypothesis that can be true. We do not invoke a barren hypothesis and attempt to shoehorn it into a meaningful scientific research programme; either hypothesis (i.e., the tested hypothesis or its alternative) is potentially fruitful and this can be argued to be more in conformity with actual science practice (Chalmers 1978, Veiland & Hodge 1998).

Isn't all this a bit contorted?

One can argue that it is indeed so. Questions that may arise to an enquiring mind include:

- Why is the calculation of the p-value based on all data at least as extreme as was obtained? (it is based on a consideration of data that was not obtained?!)

- How then does the *p*-value actually constitute "evidence"?

- Why can't the procedures directly address the real question: the probability of there having been an environmentally important impact given the actual data obtained (and not more extreme data that were not obtained).

Such matters can be addressed using different statistical methods, known as likelihood and Bayesian methods (Hilborn & Mangel 1997, Royall 1998, Lee 1999). These can be applied to equivalence testing. While not appropriate to give all details here, readers should be aware of the potential for new and fruitful statistical methods becoming more readily available for environmental studies in the coming years. A Bayesian method is available in the above-noted spreadsheet.

References

Chalmers, A.F. 1978: What is this thing called Science? University of Queensland Press, St. Lucia, Queensland, Australia.

Chow, S.C.; Liu, J.P. 1992: Design and Analysis of Bioavailability and Bioequivalence Studies. Marcel Dekker: New York.

Conover, W.J. (1980). Practical Nonparametric Statistics. 2nd ed. Wiley: New York.

Conover, W.J.; Iman, R.L. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. *The American Statistician 35*(3): 124–129.

Germano, J.D. (1999). Ecology, statistics, and the art of misdiagnosis: The need for a paradigm shift. *Environmental Reviews 7*: 167–190.

Gilbert, R.O. (1987). Statistical Methods for Environmental Pollution Monitoring. Van Nostrand Reinhold: New York.

Goodman, S.N. (1993). p values, hypothesis tests, and likelihood: implications for epidemiology of a neglected historical debate. *American Journal of Epidemiology 137*(5): 485–501 (includes discussion).

Harlow, L.L., Muliak, S.A., and Steiger, J.H. (eds) (1997). What If There Were No Significance Tests? Lawrence Erlbaum: Mahwah, New Jersey.

Hilborn, R.; Mangel, M. (1997). The Ecological Detective-Confronting Models with Data. Princeton University Press: Princeton, New Jersey.

Iman, R.L.; Conover, W.J. (1983). A Modern Approach to Statistics. Wiley: New York.

Johnson, D.H. (1995). Statistical sirens: the allure of nonparametrics. *Ecology 76*(6): 1998–2000.

Johnson, D.H. (1999). The insignificance of statistical significance testing. Journal of Wildlife Management 63(3): 763-772.

Lee, P.M. (1999). Bayesian Statistics: An Introduction. 2nd ed. Edward Arnold: London.

McBride, G.B. (1998). When differences are equivalent. *Water & Atmosphere 6*(4): 21–23.

McBride, G.B. (1999). Equivalence tests can enhance environmental science and management. Australian & New Zealand Journal of Statistics 41(1): 19-29.

Morrison, D.E.; Henkel, R.E. (1970). The Significance Test Controversy-A Reader. Aldine: Chicago.

Royall, R.M. (1997). Statistical Evidence: a Likelihood Paradigm. Chapman & Hall, London.

Sokal, R.R., and Rohlf, F.J. (1981). Biometry. 2nd ed. W.H. Freeman & Co.: New York.

Veiland, V.J.; Hodge, S.E. (1998). Review of Statistical Evidence: A Likelihood Paradigm. By Richard Royall. American Journal of Human Genetics 63: 283-289.

Zar, J.H. (1984). Biostatistical Analysis. 2rd ed. Prentice-Hall: Englewood Cliffs, NJ.

Zar, J.H. (1996). Biostatistical Analysis. 3rd ed. Prentice-Hall: Upper Saddle River, NJ.

Appendix 2

Predominant periphyton community types commonly found in New Zealand streams during summer low flows and their habitats
(secondary and filamentous taxa listed in decreasing order of abundance that they are usually found in communities)

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa — Understorey taxa	Range in peak AFDM (g/m²)	Range in peak chl. a (mg/m²)	Typical habitat	Conductivity (μS/cm)	Comment
Oligotrophic habitats							
O1: Moderate to thin mats of light green to violet coloured filaments	*Audouinella hermanii*	*Cymbella kappii, Synedra ulna, Fragilaria vaucheriae*	< 15	< 40	Widespread over unenriched to moderately enriched streams in hard rock catchments with no to extensive pastoral agriculture.	50 - 200	*Audouinella*-dominated communities require a very stable substrata. Often found mixed with bryophytes and willow roots submerged in the streams. Can form a violet-red mat on bedrock and boulders. Usually firmly attached.
O2: Forms a light brown-green film on rocks.	*Lyngbya* sp.	*Synedra ulna, Gomphoneis, Navicula avenacea*	< 35	< 150	Moderate to unenriched foothills-fed streams draining tussock or beech forest catchments, generally with hard rock geology. Predominantly low biomass on cobbles in swifter waters.	< 100	Appears highly resistant to removal by small floods and grazing. Strongly attached basal pad of cells. Can be found quite abundantly in mesotrophic – eutrophic streams subject to high grazing pressure. Taxonomic designation of this representative of *Lyngbya* a little uncertain. Looks somewhat like *Amphithrix*.
O3: Small dark brown-black patches	*Schizothrix/ Calothrix/ Lyngbya*		< 5	< 10	Unenriched, stable bed foothills streams in forested catchments with hard rock geology.	< 80	These communities appear to be highly grazer resistant and may also dominate mesotrophic or eutrophic streams subject to high grazing pressure.
O4: Short to long mats of green filaments	*Ulothrix zonata*	*Spirogyra* spp., *Oedogonium* spp. — *Synedra ulna, Cymbella kappii, Gomphoneis, Gomphonema parvulum, Fragilaria vaucheriae*	< 40	< 200	Common in unenriched streams draining bush and alpine catchments. May dominated more enriched streams in winter.	< 100	Tolerant of cold water and often forms large green filamentous mats along the periphery of high country streams. May occasionally form high biomass where groundwater discharges into streams or in winter in enriched streams.

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa / Understorey taxa	Range in peak AFDM (g/m²)	Range in peak chl. a (mg/m²)	Typical habitat	Conductivity (μS/cm)	Comment
O5: Mucilaginous olive green or dark	Nostoc *sp.*	*Microspora* sp., , *Phormidium* spp., *Audouinella hermanii* *Gomphoneis minuta* var. *cassieae, Synedra* spp., *Navicula* spp.	< 20	< 100	Unenriched streams, predominantly flowing form foothills areas (more commonly with tussock landuse and hard rock geology such as schist).	< 100	Forms conspicuous dark green mucilaginous balls on rocks in low velocity areas. May also proliferate in very damp/ partially inundated grassy areas where it often becomes dark green/black as the out mucilage of the ball dries.
Mesotrophic habitats							
M1: Mats of yellow-green filaments	*Cladophora* sp.	*Oedogonium* spp., *Melosira varians* *Gomphonema parvulum, Cymbella kappii, Synedra ulna, Cocconeis placentula, Navicula rhyncocephala*	25 - 35	100 - 300	Mixed scrub/pastoral tussock and or exotic forest with some Tertiary sediments in catchment. Mod. - low vel. runs, entangled on rocks/ projections in v. shallow cobbly streams	100 - 250	Conspicuous in some unshaded high country streams of Hawkes Bay, Marlborough, and Canterbury in mid-late summer. Often collects near surface of stream wrapped around projections; coarse feel.
M2: Thin yellow-green film	*Fragilaria* spp./ *Gomphonema tenellum Synedra ulna/ S. rumpens/ Encyonema minutum/ Gomphoneis*		< 35	< 200	Foothills or spring-fed streams that are moderately enriched. Catchments of unimproved tussock, scrub, or bush with hard rock geology.	< 80	Often these communities are maintained at a low biomass through intense invertebrate grazing. Overall these are the most commonly observed throughout the year in moderate to unenriched streams (thin films often dominated by *G. tenellum* in the unenriched streams).
M3: Thick white-brown mucilages (with olive-green surface)	*Gomphoneis/ Cymbella kappii/ Synedra ulna*	*Ulothrix zonata, Stigeoclonium lubricum* *Cymbella kappii, C. minuta, Synedra ulna, Fragilaria vaucheriae*	< 50	50 - 200	Ubiquitous, but most common in foothills, mountain and spring-fed streams draining improved tussock catchments. Proliferates in riffles with localised enrichment from groundwater.	50 - 120	Often prolific in riffles. Also commonly attaches to macrophytes in swift springs and submerged willow tree roots. This community may also dominate highly disturbed systems regardless of degree of enrichment (e.g. glacier fed rivers), but at a very low biomass.

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa ——— Understorey taxa	Range in peak AFDM (g/m²)	Range in peak chl. a (mg/m²)	Typical habitat	Conductivity (µS/cm)	Comment
M4: Small - large mats of olive green filaments	*Oedogonium/ Microspora/ Zygnema* spp.	*Spirogyra* spp., *Melosira varians, Microspora* sp. ——— *Synedra ulna, Cocconeis placentula, Navicula rhyncocephala*	10 - 40		Wide range of habitats. Conspicuous in moderately enriched foothills and lowland-fed gravel/cobble bed streams (with greywacke and/or basaltic geology) throughout New Zealand.	80 - 250	*Oedogonium* tends to be more dominant in the North Island in these habitats, whereas *Zygnema* and *Microspora* tend to be more dominant in the South Island. Difficult to discern dominant taxon without a microscope, though small *Oedogonium* mats may occur as small tufts of filaments (like small patches of green cotton wool) to forming huge mats with filaments metres long.
M5: Skin of dark brown - black on mucilage	*Phormidium* spp.	*Synedra ulna, Cymbella kappii, Gomphoneis, Cocconeis placentula, Gomphonema parvulum, Cymbella minuta*	< ? 20		Wide range, but more commonly in foothills streams with low - moderate enrichment from pastoral agriculture (greywacke and/or basaltic geology).	50 - 120	Conspicuous in generally low velocity areas on stable cobbles to silt throughout New Zealand after long periods (several months) without floods. Forms a dark brown skin over a mucilage base that usually has abundant diatoms and may be up to 1 cm thick.
M6: Loosely entwined mat of light to dark slimy green filaments	*Spirogyra* spp.	*Oedogonium* spp., *Cladophora* sp., *Phormidium* spp, *Stigeoclonium lubricum* ——— *Gomphoneis, S. ulna, Cymbella kappii, Gomphonema parvulum, A. lanceolatum, Cocconeis placentula*	< 20		Ubiquitous community, most commonly dominates moderately enriched to unenriched habitats	50 - 200	Most conspicuous as mats along the periphery of stream channels/braids during flow recessions. May form clouds of bright green filaments in pools or backwater areas. Filaments have a "slimy" feel.
M7: Bright green tufts of filaments	*Stigeoclonium lubricum*	*Gomphonema parvulum, Gomphoneis, Cymbella minuta, Cymbella kappii, Synedra ulna*	< 20	< 70	Often in moderate - unenriched foothills-fed cobble bed rivers, in moderately developed catchments generally with hard rock geology.	50 - 120	Forms bright green tree-like tufts on cobbles, particularly late in summer. Often associated with diatom mucilage.
M8: Light to dark green fibrous mats	*Vaucheria* spp.		20 - 80	200 - 1500	Wide distribution. Silty banks in oligotrophic streams to gravels in eutrophic habitats. Mostly mesotrophic habitats.	100 - 400	This taxon is easily identified in the field by its macroscopic, fibrous, matted growth form. The mats are generally attached by "rhizoid-like" structures. While some diatoms may be deposited in the mats, they are generally monospecific.

Macroscopic appearance	Dominant taxon/taxa	Secondary filamentous taxa	Range in peak AFDM (g/m²)	Range in peak chl. a (mg/m²)	Typical habitat	Conductivity (µS/cm)	Comment
		Understorey taxa					
Enriched habitats							
E1: Fluffy-brown filaments	*Melosira varians*	*Oedogonium* spp. *Synedra ulna, Cocconeis placentula, Navicula cryptocephala, N. rhynchocephala*	20 - 35		Intensively developed pastoral and/or exotic forestry catchments with greywacke/hard sediment alluvium geology. Also common in some areas with andesitic volcanics and pastoral agriculture.	100 - 250	Often forms "sludgy brown" mats covering the sediments (sometimes as mats of very fragile blue/brown filaments). Common throughout N.Z., but particularly in Taranaki, Rangitikei/Wanganui, Wairarapa, Wellington, Nelson and Canterbury.
E2: Mats of yellow-green filaments	*Cladophora glomerata*	*Rhizoclonium* sp. *Epithemia sorex, Cocconeis placentula, Synedra ulna, Cymbella kappii, Gomphoneis*	40 - 80	400 - 1000	Intensively developed pastoral catchments and/or catchments with a high proportion of Tertiary sediments.	200 - 450	Conspicuous in unshaded gravel-bed and lowland streams of Hawkes Bay/Gisborne, Manawatu/Wanganui and the Wairarapa in mid-late summer. Can form large floating mats and beds, as well as extensive mats along the periphery of channels. Blooms in low velocity runs and pools, particularly where temp. > 15 oC
E3: Mats of coarse green filaments	*Rhizoclonium* spp.	*Melosira varians* *Cocconeis placentula, Synedra ulna, Cymbella kappii, Navicula avenacea, Rhoicosphenia curvata*	> 50	> 400	Intensively developed pastoral catchments with a high proportion of Tertiary sediments.	200 - 450	Conspicuous in unshaded lowland streams of Hawkes Bay/Gisborne, Manawatu/Wanganui and the Wairarapa in mid-late summer. Can form large floating mats and beds, as well as extensive mats along the periphery of channels. Mats have a very coarse wiry feel. Blooms in low velocity runs and pools, particularly where temp. > 15 oC
E4: Mats of whitish, fluffy, often branch slime	*Sphaerotilus natans, Zoogloea*	*Stigeoclonium tenue, Melosira varians* *Cocconeis placentula, Cymbella* spp., *Fragilaria* spp., *Gomphonema parvulum, Nitzschia palea*	10 - 20	10 - 80	Associated with waste discharges high in low molecular weight BOD. Most likely to be found in lowland and spring-fed streams.		Commonly called "sewage fungus". Dominant organisms are actually filamentous bacteria. A rare occurrence now as most discharges are regulated to prevent proliferations of these communities.

Periphyton field identification chart. Part 1
(from the New Zealand Stream Health Monitoring and Assessment Kit)

For more information on each periphyton type, see page 45 in this Manual.

Thin mat or film (less than 0.5 mm thick)

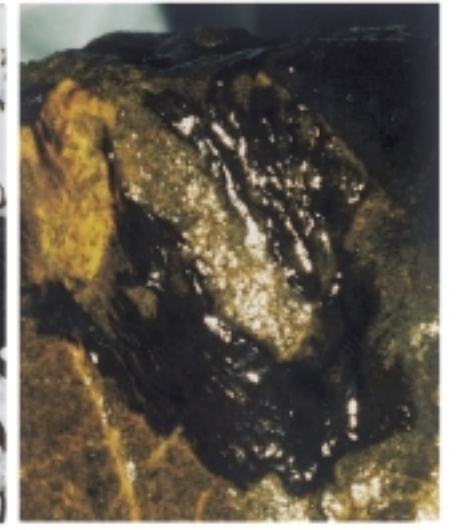Green | Light brown | Black/dark brown

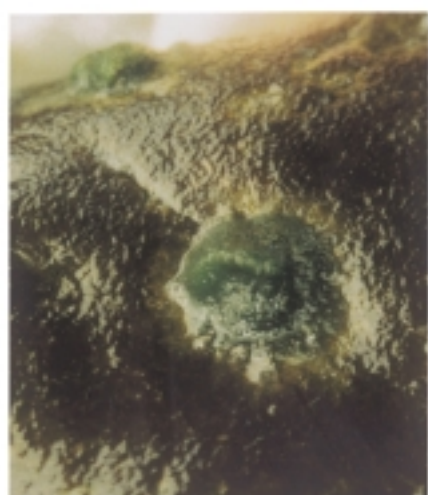## Medium mat (0.5 to 3 mm thick)

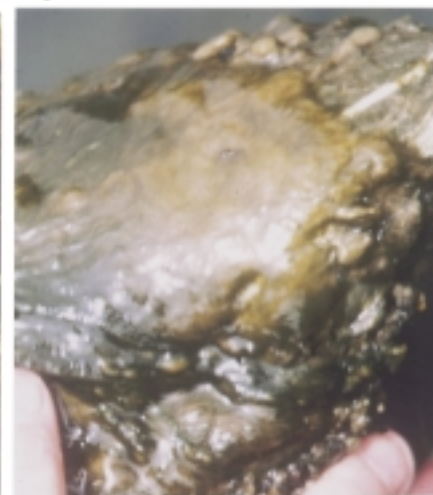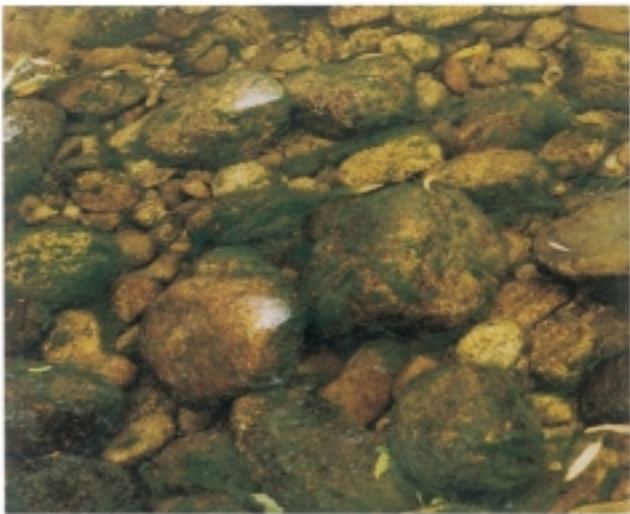Green | Light brown | Black/dark brown

Thick mat (more than 3 mm thick)

Green | Light brown | Black/dark brown

Periphyton field identification chart. Part 2
(from the New Zealand Stream Health Monitoring and Assessment Kit)
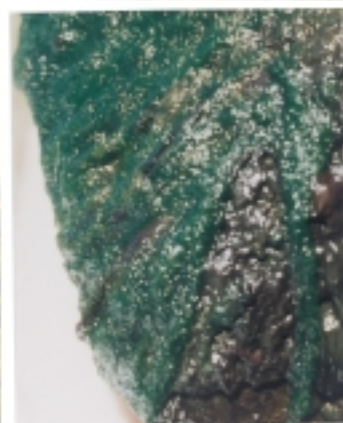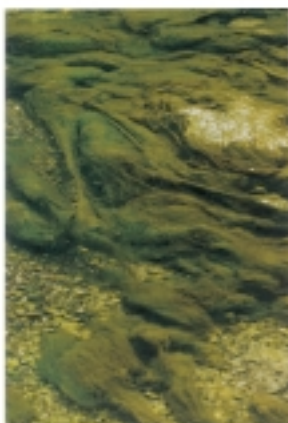
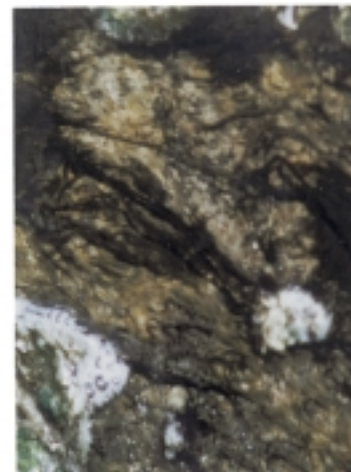## Short filaments (less than about 2 cm long)
Green

Brown/reddish

Long filaments (more than about 2 cm long)
Green

Brown/reddish

Method Summary: Rapid Assessment Method 1 (RAM-1)

Percentage cover of a site by filamentous green/brown algae for assessing compliance with aesthetic/recreational guidelines for proliferations

Equipment:

1. 2 tape measures (50 m and 20 m long).
2. 4 pegs (>20 cm long) and mallet.
3. Sampling quadrat.
4. Glass or clear plastic bowl.
5. Field data sheet.

Procedure:

- Select a reference point at the downstream end of your site and mark with a peg.

- Attach the 50 m tape to the peg and lay taut for its full distance (or 5 times the stream width).

- Mark 10 equally spaced intervals along the tape.

- Attach the end of the 20 m tape to a third peg and stretch across the stream and anchor.

- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals

- Place the sampling quadrat on the stream bed centred on the selected interval

- Hold the glass bowl on the surface of the water to obtain a clear view of the stream bed.

- Estimate the percentage cover within the quadrat of filamentous green/brown algae which have filaments >3 cm long and record on your field sheet. Move to the next point.

- Complete the transect then move the transect upstream for the pre-selected interval and repeat the recordings.

- When complete, enter the data onto a spreadsheet and calculate the mean percentage cover of the site by filamentous algae >3 cm long.

Method Summary: Rapid Assessment Method 2 (RAM-2)

Percentage cover of substrates by different categories of periphyton for general resource surveys and assessing broadscale effects of perturbations

Equipment:

1. 2 tape measures (10 m and 20 m long).

2. 4 pegs (>20 cm long) and mallet.

3. Small tea strainer (approximately 8 cm in diameter).

4. Field data sheet (preferably of water proof paper).

5. Periphyton field identification chart.

Procedure:

- Select a reference point at the downstream end of your site and mark with a peg.

- Attach the tape measure to the peg and lay it out taut for a distance of 10 metres (or 5 times the stream width, whichever is the smaller).

- Divide the distance along the tape into thirds and mark the tape.

- Attach the 20 m tape measure to a third peg at the location of the reference peg stretch the tape across the stream.

- Divide the width of the stream (water's edge to water's edge) into 5 equally spaced points.

- At the first point close your eyes and pick up the first stone that you touch.

- If the stream bottom is gravel, sand or silt, take a scooped sample with the tea strainer.

- Estimate the percentage cover of the stone in by periphyton in each category (± 5%) according to the field identification chart and enter this on the field data sheet.

- Complete the transect then move the tape upstream for the second transect at one-third interval and repeat the recordings.

- When complete, calculate the mean percentage cover of sampling points for each category of periphyton.

Method Summary: Quantitative Method 1a (QM-1a)

Whole cobble/gravel sampling

Equipment:

1. 20–30 m tape.
2. 2 pegs (>20 cm long) and mallet.
3. Deep sided laboratory tray or container.
4. Brushes.
5. Scalpel.
6. Squirt bottle.
7. Sample containers.
8. Calipers.

Procedure:

- Select a reference point and drive a peg into the ground.

- Attach the tape measure to the peg and lay it out taut across the stream.

- Divide the width of the stream (water's edge to water's edge) into 10 equally spaced intervals.

- Move out to the first point and with closed eyes pick up the first retrievable stone touched.

- Return it to the stream bank.

- Scrape off thick periphyton with scalpel and rinse stone and place in sample container.

- Then scrub the stone thoroughly with the brush and rinse.

- Transfer the contents of the white tray into your sample container.

- Finally, rinse the tray into the sample container until no trace of periphyton remains.

- Store the labelled container on ice in a chilli-bin (cooler) for transport to the laboratory.

- Measure the x, y and z dimensions of the stone with the calipers.

- Proceed to the next sampling point and repeat the above procedures.

Method Summary: Quantitative Method 1b (QM-1b)

Scraping or brushing a sample from a defined area on the top of a stone

Equipment:

1. 20–30 m tape measure.
2. 2 pegs (>20 cm long) and mallet.
3. Deep sided laboratory tray or container.
4. Scalpel.
5. Small scrubbing brushes.
6. Sample containers.
7. Pipettes/'eye-dropper'.
8. Squirt bottle.
9. Sampling ring.

Procedure:

- Select a reference point in the middle of your site 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.

- Divide the width of the stream into 10 equally spaced intervals.

- Move out to the first point across the transect and with eyes closed pick up the first retrievable stone you touch.

- Return the stone to the stream bank.

- Define a circle on the top of the stone.

- Scrape away all the surrounding periphyton from the outside of the ring.

- Remove the ring and then scrape off periphyton with scalpel and rinse into a container.

- Scrub the sample area with a toothbrush then remove the slurry with the small pipette and some additional drops of water.

- Store the labelled container on ice for transport to the laboratory.

- For a mat of filaments slide your hand underneath the filaments, lift and press the ring down through the mat onto your palm to cut out a circle of the mat for the sample.

Method Summary: Live Algal Biomass

Chlorophyll *a*: ethanol – spectrophotometer method

Equipment:

1. Data sheets.
2. 90% Ethanol.
3. Filter papers.
4. Centrifuge tubes.
5. Forceps.
6. Vacuum filter.
7. Pipettes (5 ml and 0.1 ml).
8. Squirt bottle.
9. 0.36 molar HCl.
10. Water bath.
11. Spectrophotometer.
12. Pipettes set to 4 ml and 0.1ml.
13. Tissues.
14. 0.36-M hydrochloric acid

Procedure (filtering):

- Place 5 ml of 90% ethanol in tubes.
- Place fresh filter papers in each filtering chamber.
- Blend sample, shake the bottle and filter ~3 ↔ 5 ml aliquots (shake bottle between each).
- Remove fragments of leaves, mosses, invertebrates etc from filter paper with forceps.
- Apply suction.
- Record sub-sample volume.
- Rinse pipette.
- After filtering fold the paper in half, loosely roll up and place in centrifuge tube with ethanol.

Procedure (spectrophotometer analysis):

- Pre-heat water bath to 78°C (boiling point of ethanol).
- Immerse the racks of tubes in the bath for exactly five minutes.
- Place the racks in the refrigerator overnight.
- Warm up spectrophotometer.
- Set the wavelengths at peak for chlorophyll a for your spectrophotometer and 750 nm.
- Clean cuvette and take blank readings using 90% ethanol.
- Compress filter papers to the bottom of the centrifuge tubes, and re-close firmly.
- Centrifuge at 6000 rpm for 10 minutes.
- Pipette 4 ml of extract of first sample into the cuvette.
- Read absorbances at 665 (or wherever the chlorophyll *a* peak is on your spectrophotomer) and at 750 nm.
- Insert 0.1 ml of 0.3-M HCl in the cuvette, shake and analyse again at 665 and 750 nm.

Calculations:

Chlorophyll *a* (mg/sample) =

$$[(\text{abs.665 before} - \text{abs.665 after}) * 28.66 * \text{sample vol.} * \text{extractant vol.}]$$
$$/ \text{ (filtered sub-sample vol.)}$$

(Subtract the respective turbidity blanks read at 750 nm from each reading first.)

Method Summary: Total Biomass

Ash-free dry mass

Equipment:

1. Data sheets.
2. Filter papers.
3. Crucibles, with number/ID mark, on metal trays.
4. Forceps.
5. Vacuum filter.
6. Glass beakers, stoppered bottles (marked at 50 ml intervals).
7. Blender.
8. Automatic pipettes (5 ml).
9. Squirt bottle.
10. Desiccator.
11. Muffle furnace
12. Drying oven
13. Precision balance.

Procedure (filtering and weighing):

- Place a filter paper in each crucible.
- Pre-ash in the muffle furnace at 400°C for two hours.
- If dry weight is required cool in desiccator and weigh.
- Place fresh filter papers in each filtering chamber.
- Blend sample, shake the bottle and filter ~3 ↔ 5 ml aliquots (shake bottle between each).
- Remove fragments of leaves, mosses, invertebrates etc from filter paper with forceps.
- Apply suction.
- Record sub-sample volume.
- Rinse pipette.
- After filtering fold the paper in half and place crucible.
- Dry the sub-samples for 24 hours at 105°C.
- Weigh each crucible after cooling in a desiccator.
- Ash for 4 hours at 400°C.
- Cool in desiccator and weigh.

Calculations:

Ash-free dry mass (g/sample) = [{(weight of crucible + filter + sample after drying)
− (weight of crucible + filter + sample after ashing)} * sample volume]
/ [volume of filtered sub-sample]

Dry mass (g per sample) = [{(weight of crucible + filter after drying)
− (weight of crucible + filter + sample after drying)} * sample volume]
/ [volume of filtered sub-sample]

Periphyton – the slimy coating that grows on the beds of streams and rivers – is an essential component of stream ecosystems, but can also be a management issue.

This *Stream Periphyton Monitoring Manual* is a follow-up to the *New Zealand Periphyton Guideline: Detecting, Monitoring and Managing Enrichment of Streams.* The latter publication reviews periphyton communities and their use as environmental indicators, and presents guidelines to prevent proliferations. This manual describes a standard set of methods to enable water managers and researchers to collect and analyse data on periphyton. Included are field sampling systems, laboratory procedures, microscope studies, data analysis techniques and an illustrated guide to the common types of periphyton found in New Zealand. The methods – based on internationally accepted protocols – have been used within NIWA for many years and are suitable for most streams and rivers in New Zealand.

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