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Appendix 1

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 *r*S). 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 *r*S 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.

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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/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity ($\mu S/cm$)	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/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity ($\mu S/cm$)	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/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity ($\mu S/cm$)	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 / Understorey taxa	Range in peak AFDM (g/m^2)	Range in peak chl. a (mg/m^2)	Typical habitat	Conductivity ($\mu S/cm$)	Comment
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

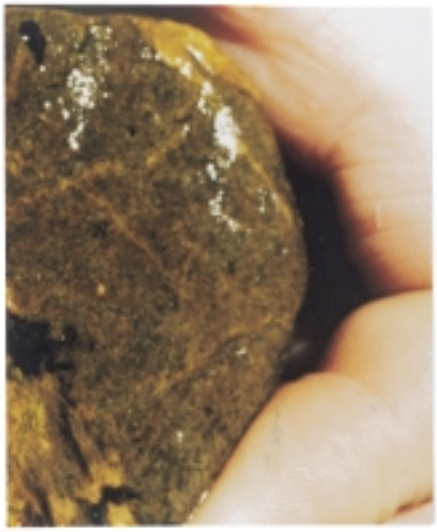
(from the New Zealand Stream Health Monitoring and Assessment Kit)

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



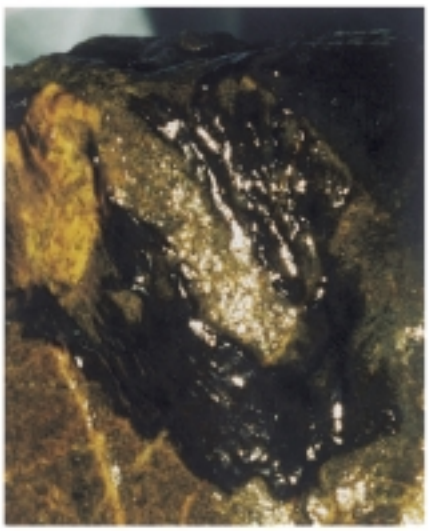
Black/dark brown

Medium mat (0.5 to 3 mm thick)

Green

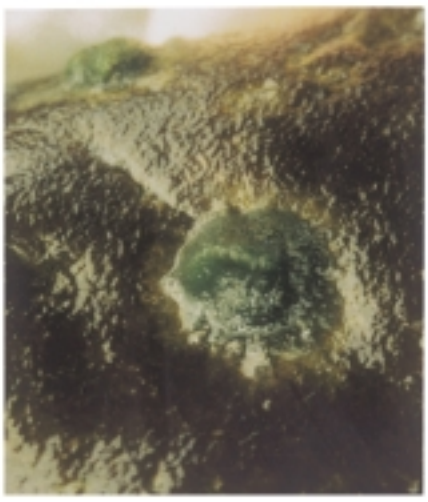
Light brown

Black/dark brown

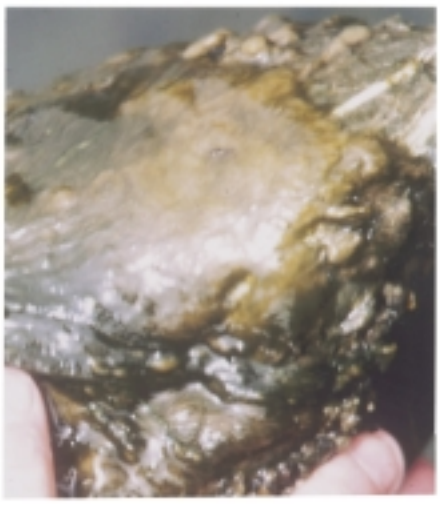


Thick mat (more than 3 mm thick)

Green



Light brown



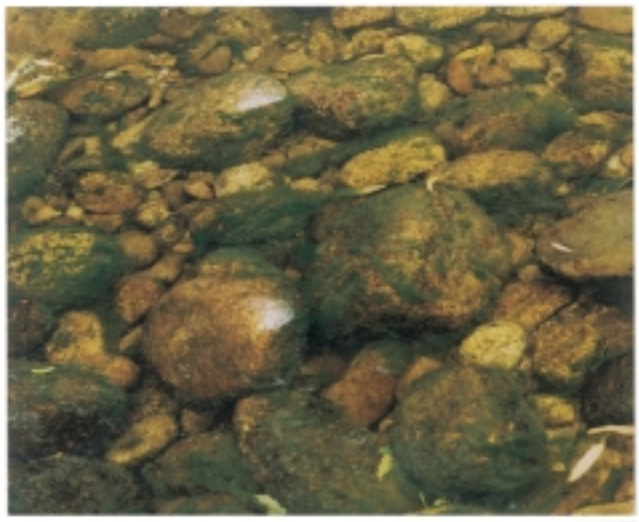
Black/dark brown

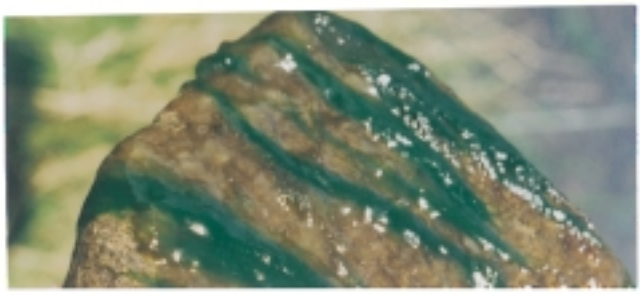
Periphyton field identification chart. Part 2

(from the New Zealand Stream Health Monitoring and Assessment Kit)

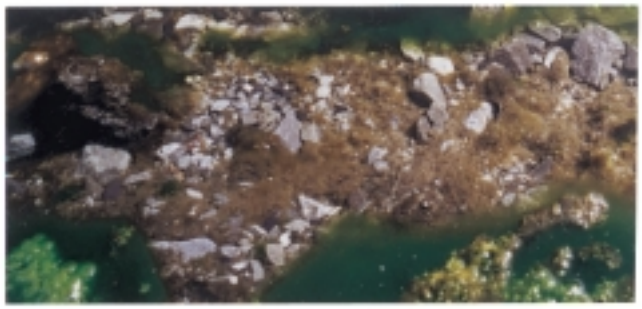
Short filaments (less than about 2 cm long)

Green



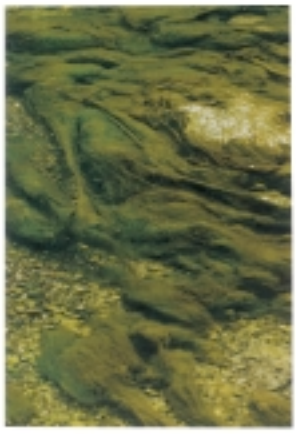


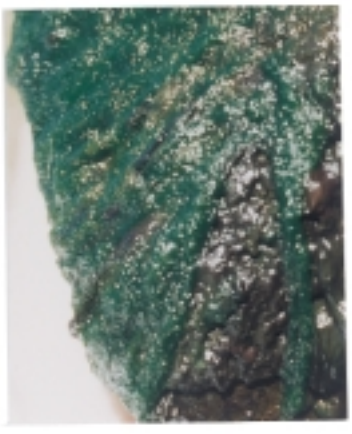
Brown/reddish



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.

For a full version of this method, see Section 6.4.2 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

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.

For a full version of this method, see Section 6.4.3 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

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.

For a full version of this method, see Section 6.5.4 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

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.

For a full version of this method, see Section 6.5.5 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

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) =

[(abs.665 before – abs.665 after) * 28.66 * sample vol. * extractant vol.]
/ (filtered sub-sample vol.)

(Subtract the respective turbidity blanks read at 750 nm from each reading first.)

For a full version of this method, see Section 7.1 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.

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):

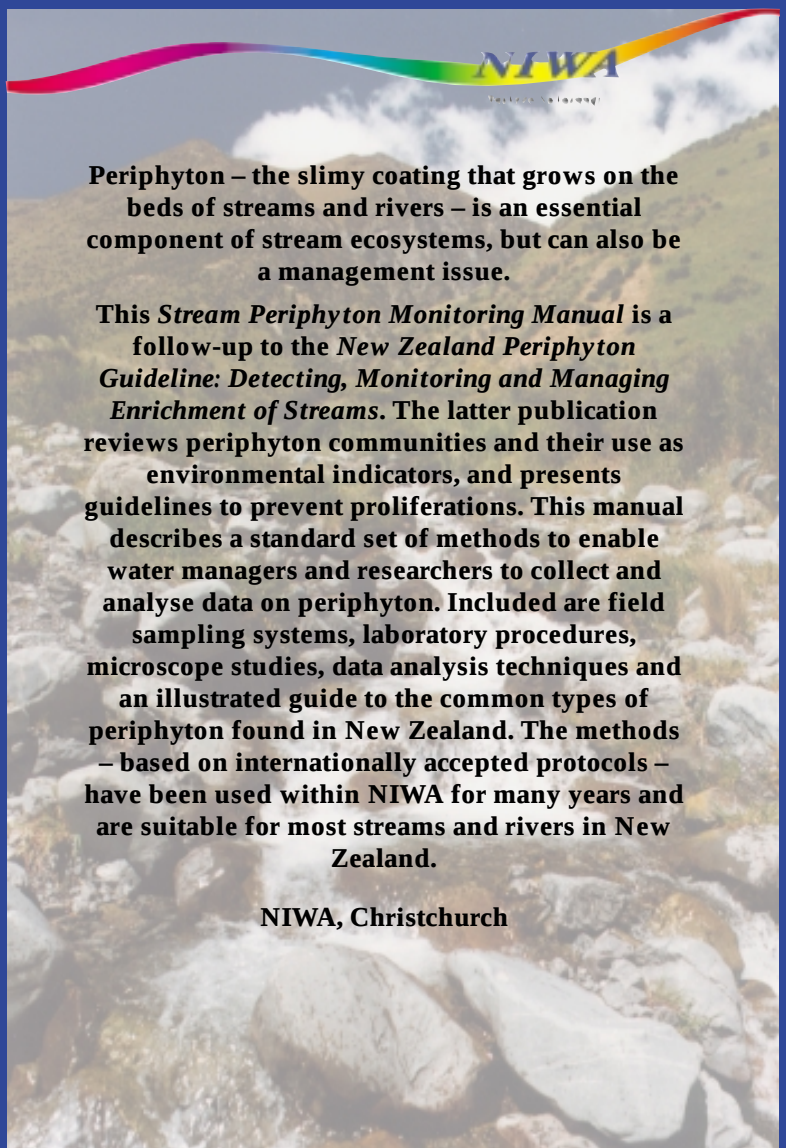
- Place a filter paper in each crucible.
- Pre-ash in the muffle furnace at 400°C for two hours.
- If dry weight is required cool in desiccator and weigh.
- Place fresh filter papers in each filtering chamber.
- Blend sample, shake the bottle and filter ~3 ↔ 5 ml aliquots (shake bottle between each).
- 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]

For a full version of this method, see Section 7.2 in: Biggs, B.J.F.: Kilroy, C. 2000. Stream Periphyton Monitoring Manual. Published by NIWA for Ministry for the Environment.



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.

NIWA, Christchurch