

AUSTRALIAN CAPITAL TERRITORY

AUStralian RIVer Assessment System



AUSRIVAS

SAMPLING AND PROCESSING MANUAL



**AUSTRALIAN CAPITAL TERRITORY, AUSTRALIAN RIVER Assessment System
(AUSRIVAS), SAMPLING AND PROCESSING MANUAL**

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Front Cover Photograph:

Nerida Davies collecting an edge sample. Photo by Phil Sloane.

TABLE OF CONTENTS

INTRODUCTION: BIOLOGICAL ASSESSMENT OF RIVER HEALTH	1
AUSRIVAS.....	1
SAMPLING PROGRAM AND SITE SELECTION.....	2
WHEN TO SAMPLE	3
HABITATS SAMPLED	3
PREPARING FOR A FIELD TRIP	5
GENERAL SAFETY.....	7
DATA SHEETS	7
Site Information Sheet.....	7
Field Sampling Sheet	10
<i>General Information</i>	<i>10</i>
<i>Measurements</i>	<i>11</i>
<i>Stream Measurements.....</i>	<i>15</i>
<i>Riffle, Pool, Run, Edge and Macrophytes.....</i>	<i>17</i>
<i>Depth and Velocity Measurements.....</i>	<i>19</i>
<i>Riparian Vegetation.....</i>	<i>20</i>
<i>Observations</i>	<i>25</i>
<i>Instream Characteristics.....</i>	<i>29</i>
<i>Macrophytes.....</i>	<i>33</i>
<i>Habitat Assessment.....</i>	<i>34</i>
<i>Checking Field Sampling Sheets.....</i>	<i>35</i>
MACROINVERTEBRATE SAMPLING	36
Riffle Sampling.....	36
Edge Sampling	36
Sample Transfer and Preservation.....	37
MACROINVERTEBRATE SAMPLE PROCESSING	39
Sub-Sampling	39
Sorting.....	39
MACROINVERTEBRATE IDENTIFICATION.....	40
Identification	40
Macroinvertebrate Identification Sheet.....	42
Labelling	42
Macroinvertebrate Scan.....	43
QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES	43
QA/QC - Sorting	43
QA/QC - Identification.....	45
<i>Percent Taxa Error.....</i>	<i>45</i>
<i>Percent Incorrect Identifications</i>	<i>46</i>
REFERENCES	49
APPENDICES.....	52

INTRODUCTION: BIOLOGICAL ASSESSMENT OF RIVER HEALTH

The term 'river health' is an umbrella term, as is human health, both can be measured and assessed by a range of different indicators. The analogy with human health provides an insight into the complexities of aquatic ecosystem damage and in turn evokes awareness and concern about human impacts on rivers. Traditionally, water quality guidelines have focused on physical and chemical indicators of stream condition. Water quality indicators have undergone a shift in focus with the emphasis toward biological assessment of river condition (Norris and Norris, 1995; Wright, 1995; Resh *et al.*, 1996; Hart *et al.*, 1999; Norris and Thoms, 1999; ANZECC and ARMICANZ, 2000). These recent approaches to river health assessment acknowledge the importance of physical, chemical and biological interactions, recognising the biological end point resulting from the various stressors added to and modifying aquatic systems.

Aquatic macroinvertebrates (i.e., animals without backbones that can be seen with the naked eye, e.g., shrimps, worms, crayfish, aquatic snails, mussels, aquatic stage of some insect larvae, such as dragonfly larvae, mayflies, caddisflies, etc.) are commonly used biological indicators for freshwater resources (Rosenberg and Resh, 1993). Macroinvertebrates are widely used because they are generally abundant throughout the study area, easy to collect and identify, have relatively long life-cycles, and are sensitive to various changes in water and habitat quality. Even if all the appropriate chemical and physical water quality data could be collected at a site it would only be indicative of the status of the river at the moment of sampling. However, measurements of aquatic biota are a more meaningful measure of water quality in that they summarize the preceding river conditions for weeks or months before their collection. For example, an episodic pollution event such as a chemical spill may go undetected by periodic water sampling regimes but damage to the macroinvertebrate assemblage can be detected long after the cause of the impact has passed. 'Rapid' biological assessment methods have been designed so that macroinvertebrate sample collection, processing, and data analysis is fast and easily done (Resh *et al.*, 1995). Rapid bioassessment methods have facilitated multi-site, regional assessments that were prohibitive to the traditional, intensive-sampling techniques (Parsons and Norris, 1996; Smith *et al.*, 1999; Turak *et al.*, 1999; Marchant *et al.*, 1999).

AUSRIVAS

The broad scale assessment of Australia's rivers required the development of a nationally comparative, standardized sampling protocol (Davies 1994; Schofield and Davies, 1996; Davies, 2000; Simpson and Norris, 2000). The AUStralian RIVer Assessment System (AUSRIVAS) was developed for Australia's National River Health Program (Davies, 1994; Schofield and Davies, 1996; Coysh *et al.*, 2000; Simpson and Norris, 2000). AUSRIVAS is a modification of the British RIVPACS bioassessment approach (Wright *et al.*, 1984; Moss *et al.*, 1987; Wright, 1995; Wright, *et al.*, 2000). Standardized sampling methods, computer software and predictive models are all components of AUSRIVAS. Macroinvertebrate assemblages, as assessed by AUSRIVAS, have also been identified as an environmental indicator for 'State of the Environment' (SoE) reporting (ANZECC, 2000).

AUSRIVAS assessments of river condition are based on the collection of a representative sample of the macroinvertebrate assemblage at a site. Macroinvertebrate samples from various habitats (e.g., riffle and stream edge) have a distinctive faunal composition. Parsons and Norris (1996) found that macroinvertebrate samples from the same habitat at different sites were more similar than samples from different habitats at the same site. If all habitats were sampled to produce a composite sample, detection of biological damage may be confounded because of habitat-effort bias if all habitats are not present at a site or are not present in the same proportions at all sites (Parsons and Norris, 1996). Sometimes, a particular human activity may impact one particular habitat but not the other, which is why AUSRIVAS samples the two major habitats within specific regions. Therefore, stratified sampling of habitats (e.g., riffle or stream edge) is an important component of the AUSRIVAS bioassessment technique, and may actually reduce sample variability and optimise the detection of impacts. One sample is taken from 10 metres of each single habitat (e.g., riffle or stream edge) for AUSRIVAS assessments. All animals have their preferred niche and the sample from 10m of habitat should incorporate all the micro-habitats within the main habitat, which potentially produce variability among samples (e.g., areas behind, in front and on the surface of large rocks where velocities may vary, macrophyte stands within the habitat, the shallow and deeper water, etc.). Natural seasonal variation is also accounted for by season-specific sampling and predictive models, thus, the collection of macroinvertebrates is restricting to particular seasons (Coysh *et al.*, 2000; Simpson and Norris, 2000).

A D-framed pond net is used to collect the macroinvertebrates and the animals require sorting from detritus, plant matter and sediment also collected. Sorting and counting all the animals from the large samples that are usually collected is time consuming and costly, thus negating one of the 'rapid' components of bioassessment. Therefore, sub-sampling of the preserved material, using sample splitters in the laboratory and fixing the number of animals counted, is also employed. All these components of the AUSRIVAS sampling procedure require rigid standardization to reduce bias and make accurate site assessments and sample comparisons.

The AUSRIVAS methods have been standardized within regions (i.e., states/territories) but may vary between them. The following manual describes the AUSRIVAS, Australian Capital Territory, sampling methods developed by the Cooperative Research Centre for Freshwater Ecology, University of Canberra.

SAMPLING PROGRAM AND SITE SELECTION

Site selection, study design, sampling frequency, replication, etc., should be based on the objectives of your particular study.

Selection of reference and test sites in the ACT (under the NRHP) followed the protocols outlined in the River Bioassessment Manual (Davies 1994). 'Reference sites' are those 'least disturbed' sites sampled for the production of a database used in the construction of AUSRIVAS predictive models. The reference sites cover the range of stream types found in and surrounding the ACT. 'Test site' is a term used to describe a site in unknown condition and/or identified to be of importance for

assessing the condition of a river potentially experiencing damage from poor water quality or habitat degradation.

WHEN TO SAMPLE

Sites must be sampled in either **spring** (September to November) or **autumn** (March to May) if the site is to be assessed using a single-season AUSRIVAS predictive model. If a combined-seasons AUSRIVAS model is to be used for assessment then the site should be sampled in both spring and autumn (in the same habitat). Sampling **MUST NOT** be conducted if streams are in flood. Sampling should resume 4 weeks after the floods subside.

HABITATS SAMPLED

In the ACT, models have been created for both **riffle** and **edge** habitats. Areas of riffle and edge habitats that are representative of the reach should be chosen for sampling. The **reach** is defined as five times the mode bank-full width either side of the riffle sampling area, unless the bank-full width is less than 10m, then the minimum reach length is 100m (i.e., 50m either side of the riffle sampling site).

The **riffle** habitat is one of flowing broken water over gravel, pebble, cobble or boulder, with a depth greater than 10 cm.



Riffle Habitat

The **edge** habitat consists of slow flowing or still waters adjacent to the bank, preferably with overhanging or emergent vegetation, undercut banks, root mats or other suitable habitat providing cover and refuge for macroinvertebrates.



Edge Habitat



Edge Habitat

PREPARING FOR A FIELD TRIP

Before embarking on a field trip, some preparation is needed. The following should be done well in advance:

- Wash water sample bottles
- Calibrate Hydrolab (or similar device used to measure water quality variables)
- Charge batteries e.g., Hydrolab battery
- Buffer and stain Formalin (not required if using ethanol as the preservative)
- Gather sampling equipment (full equipment list given in Appendix 1)
- Organize transport

How to wash water sample bottles:

1. Prepare a 1% to 3% solution of a phosphate-free detergent (e.g. Decon Neutracon) and tap water. Ensure the solution is well mixed.
2. Fully immerse the sample bottles (including lids) and allow them to soak for 24 hours.
3. After scrubbing, remove the bottles and lids from the detergent solution and rinse thoroughly (three agitated rinses under flowing tap water), to ensure all traces of contamination and cleaning solution have been removed.
4. Rinse each bottle and lid three times using de-ionized water.
5. Drain thoroughly and replace lids.
6. Store separately from unwashed bottles in a container labeled “washed and (de-ionized) rinsed”.

How to buffer and stain Formalin:

Formalin is the preferred solution for preserving macroinvertebrate samples, however, it is extremely hazardous, and therefore strict handling procedures apply. A Materials Safety Data Sheet (MSDS) for Formalin can be obtained from <http://msds.pdc.cornell.edu>. Health and safety implications will prevent its use if suitable storage and handling facilities (e.g., fume hood, airtight transport container, safety goggles, protective gloves) are unavailable. If suitable storage and handling facilities are unavailable, 70% ethanol should be used to preserve samples.

Buffering Formalin prevents calcareous shells dissolving.

Buffering - Add 1 gram of Calcium Carbonate (CaCO_3) to 5 Litres of Formalin.
Mix well.

Staining the Formalin turns the insects pink, which aids sorting.

Staining - Add 1 gram of Rose Bengal stain to 5 L of Formalin.

A range of equipment is used to collect physical, chemical, habitat and biological data. A full list of sampling equipment can be found in Appendix 1



a) Net, sieve, containers and bucket



b) Flow meter and float



c) Hydrolab



d) Alkalinity kit



e) Global Positioning System



f) Tape measure, data sheet, and water plant identification book

GENERAL SAFETY

Know the safety guidelines and requirements of the particular organisation you work for.

- Inform someone of your field trip e.g. where you are going, when you'll return.
- Take someone with you. Never go sampling alone.
- Take care when moving in and around water.
- Always take a first aid kit with you.
- If you are working in a very remote area, it may be advisable to take an Emergency Position Indicating Radio Beacon (EPIRB) with you as well.

DATA SHEETS

Two types of data sheets are used in the ACT to record information about a site. These are the Site Information Sheet and the Field Sampling Sheet. It is necessary to fill out both data sheets when assessing a site.

The Site Information Sheet is used to record location and catchment scale characteristics, which are largely derived from 1:100 000 maps, as well as access details and photographs of a site. The site information sheet need only be filled out once for each site.

The Field Sampling Sheet is used to record the physical, chemical and habitat characteristics measured at a site. The field sampling sheet must be filled out each time you visit a site, even if that site has been sampled before.

Definitions of the variables found on the ACT site information and field sampling sheets are given in the following section. Also included are the methods used to obtain the data. It is essential that data is recorded for all variables on the site information and field sampling sheets, as missing data may prevent the testing of a site. The ACT site information and field sampling sheets can be found on the AUSRIVAS web site at <http://ausrivas.canberra.edu.au>.

Site Information Sheet

River: Name of river or stream sampled e.g., Murrumbidgee River

Location Code: Drainage division|Basin|2|Site number. Drainage division and basin number are from Review of Australia's Water Resources 1975 (AWRC 1976). All sites within the ACT will have a site number beginning with 4102, e.g., Where 4 = Murray-Darling Drainage division, 10 = Murrumbidgee River Basin, 2 = a running water site (1 designates dams etc), followed by the three digit site number.

Location: Describe where the site is on the river e.g., Murrumbidgee River 100m upstream of Uriarra Crossing

Catchment: Name the major river catchment (Stream Order ≥ 4) the stream belongs to. e.g., Sullivans Creek (Stream Order 3) belongs to the Molonglo River catchment (Stream Order 6).

Stream Order: Hierarchical ordering of streams based on the degree of branching (Armantrout 1998). Stream order should be derived from a 1:100 000 scale map following the methods of Strahler (1952). The most exterior streams are the first-order streams and a second-order stream is formed by the junction of two first-order streams; the junction of two second-order streams forms a third-order stream etc. (Figure 1a).

Distance from source (DFS) km: The distance, in kilometres, between the source of the stream and the site being assessed. Measure the distance along the drainage lines to the furthest point upstream (Figure 1b). DFS is measured from 1:100 000 scale maps using a map wheel or other map-measuring device.

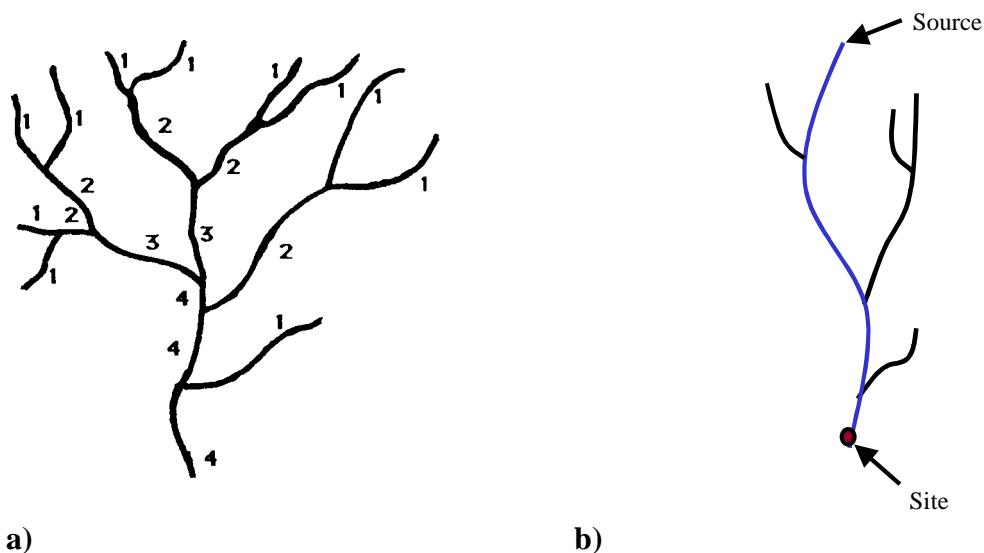


Figure 1 a) The Strahler (1952) stream ordering method (Gordon *et al.* 1992) used to determine stream order; **b)** Distance from source is the distance in kilometres, between the source of the stream and the site being assessed.

Catchment area upstream (km^2): The area above a specific point on a stream from which the water drains towards the stream (Gordon *et al.* 1992). Catchment area is measured with a planimeter or from a GIS with a digital terrain model.

Altitude: Height above sea level recorded in metres. Altitude is derived from a map.

If required for interpretation, the Coefficient of variation in annual discharge and mean annual discharge should be calculated from monthly data if available.

GPS datum: (as set on the GPS unit at the time you record your position). The datum is a mathematical surface on which a mapping and coordinate system is based. WGS84 or GDA 94 should be used.

Latitude and Longitude: should be recorded to the nearest second using a GPS in the field. The ACT AUSRIVAS models require latitude and longitude to be entered as degrees and minutes, rounded to the nearest minute. For example, latitude 35° 04' 46" would be entered into AUSRIVAS as 3505, and a longitude of 148° 48' 10" would become 14848. Note that no decimal places or spaces are included in the number.

Zone: Grid Zone Designation e.g., the ACT grid zone designation is 55H.

Easting and Northing: should be measured using a GPS in the field. If determining grid reference using a map, the easting and northing should be recorded as accurately as possible. Information on how to calculate a site's grid reference is given in Appendix 2.

Record the details of the map on which the site is located, including map name, scale, number, and edition. The names and numbers of adjoining maps should also be recorded.

The directions, sketch of access route, sketch of reach, and photographs (showing access to site, and the river upstream and downstream of site) are essential for locating the site for re-sampling at later dates. The sketch of the access route should include: the river; flow direction; site location; roads; crossings; access details (location of turnoffs, distances and landmarks leading to site); and a northing. The sketch of the reach should include: the location of riffle, run, pool, edge and macrophyte habitats within the reach; flow direction; access point; riffle and edge sampling locations; and a northing.

If permission from the landowner, or a permit is required to access the site then indicate this on the site-information sheet, along with the name and contact details of the land-owner/manager. If a key is required to access the site, record the key number and the contact details of the person from whom the key is available.

At least three colour photographs (showing access to the site, and river upstream and downstream of the site) should be taken at each site sampled and attached to the site information sheet. The photos will aid location of the site during subsequent sampling trips and aid in later data interpretation.

Field Sampling Sheet

General Information

Date: Must be written on every page

Time: Time sampling occurred

Location Code: Drainage division|Basin|2|Site number. Drainage division and basin number are from Review of Australia's Water Resources 1975 (AWRC 1976). All sites within the ACT will have a site number beginning with 4102, e.g., Where 4 = Murray-Darling Drainage division, 10 = Murrumbidgee River Basin, 2 = a running water site, followed by the three digit site number. The location code must be written on every page.

River: Name of river or stream sampled e.g., Murrumbidgee River. Must be written on every page.

Location: Describe where the site is on the river e.g., Murrumbidgee River, 100m upstream of Uriarra Crossing

Project: The name of the project or monitoring program.

Test/Reference: Indicate whether the site is a test or reference site.

Recorders Name: The name of the person recording the data. Must be recorded so queries arising later about the data can be answered.

Photograph Number (s): The negative number (s) of any photographs taken at the site.

Weather: A general description such as fine/sunny, overcast etc.

Air Temperature: °C at the time of sampling.

Cloud Cover %: Estimate percent cloud cover over the region of the site.

Rain in last week?: Was any rain recorded in the catchment in the week before sampling.



Measurements

Water Samples and Basic Water Measurements

The water sample and basic water measurements are the first measurements to be collected before disturbing the stream by collecting macroinvertebrates etc.

Water samples are generally collected for the analysis of total nitrogen (TN), total phosphorus (TP) and nitrates/nitrites (NOX). Samples should be placed on ice immediately and frozen as soon as possible if the water is not analysed immediately for TP and TN (A.P.H.A. 1992). Where water samples are taken for NOX or ammonia analysis they should be filtered using 0.45µm sterile syringe filters in the field before transport and storage.

Water samples may be used to measure alkalinity and turbidity if it is not measured in the field. Turbidity and alkalinity should be analysed as soon as possible on return to the laboratory.

Taking water samples for TN and TP

1. Sample bottles should be washed and prepared in accordance to the washing sample bottles section.
2. 250ml bottles are usually used for collecting water samples for TN and TP.
3. Label the water sample bottle with the site number, river, date and collector's name. Ensure the lid is also labelled with as much information as possible (e.g at least site and date).
4. Samples may be collected by hand or using a sampling rod.
5. Do not open the bottle until it is ready to be filled.
6. The water sample should be taken from the riffle or from the centre of the stream if no riffle is present.
7. To prevent contamination, take care not to disturb the sediment or the sides of the bank when taking the water sample. If the water has been disturbed, sample upstream where it has not been disturbed.
8. Hold the bottle at its base (if sampling by hand). Remove the screwcap ensuring that your fingers do not come into contact with the neck of the bottle or the inner surface of the lid.
9. Plunge the open bottle (neck down) into the water and then turn the bottle around to face upstream.
10. To ensure a representative sample, it is important that the sample bottle is fully submersed to avoid collecting particles that may be floating on the water surface.
11. Fully submerge and fill the bottle and rinse three times with sample water (replacing the lid and shaking vigorously each time) before taking the final sample.
12. Fill the bottle completely to exclude air and replace the screwcap tightly taking care not to contaminate the neck or inner surface of the lid.
13. If the water samples are to be frozen, leave room for expansion (approximately 10%).
14. Samples should be immediately placed on ice and frozen as soon as possible if the water is not analysed immediately for total phosphorus (TP) and total nitrogen (TN; A.P.H.A. 1992).

Taking water samples for NOX (filtered samples)

1. Sample bottles should be washed and prepared in accordance to the washing sample bottles section.
2. 60ml water bottles can be used for the final filtered sample.
3. Label the water sample bottle with the site number, river, date and collector's name. If the sample is to be filtered for NOX this should be recorded on the outside of the bottle. Ensure the lid is also labelled with as much information as possible (e.g at least site and date). A spare clean water sample bottle is required to assist in filtering.
4. Samples may be collected by hand or using a sampling rod.
5. Do not open the spare bottle until it is ready to be filled.
6. The water sample should be taken from the riffle or from the centre of the stream if no riffle is present.
7. To prevent contamination, take care not to disturb the sediment or the sides of the bank when taking the water sample. If the water has been disturbed, sample upstream where it has not been disturbed.
8. Hold the spare water sample bottle at its base (if sampling by hand). Remove the screwcap ensuring that your fingers do not come into contact with the neck of the bottle or the inner surface of the lid.
9. Plunge the open bottle (neck down) into the water and then turn the bottle around to face upstream.
10. To ensure a representative sample, it is important that the spare sample bottle is fully submersed to avoid collecting particles that may be floating on the water surface.
11. Fully submerge and fill the spare bottle and rinse three times with sample water (replacing the lid and shaking vigorously each time) before filling the spare sample bottle with water.
12. Using a disposable syringe extract 10ml of water out of the spare sample bottle. Place a 0.45µm sterile syringe filters on the end of the syringe. Force the water through the filter into the final collection bottle.
13. Recap the bottle and rinse thoroughly. Discard this water and repeat step 12 **another two** times.
14. Extract and filter water into the final collection bottle filling the bottle completely to exclude air and replace the screwcap tightly taking care not to contaminate the neck or inner surface of the lid.
15. If the water samples are to be frozen, leave room for expansion (approximately 10%).
16. Samples should be immediately placed on ice and frozen as soon as possible if the water is not analysed immediately.

Water sample taken: tick yes or no and number of water samples taken.



a) Collecting water sample



b) Measuring water quality variables

Water measurements in the field - If measuring water quality variables in the field ensure the measuring device (e.g. Hydrolab) is correctly calibrated before your field trip. Place the Hydrolab (or similar measuring device) in flowing water upstream of where you are standing and record measurements on the field sampling sheet after allowing sufficient time for the reading to stabilize. Record on the field sampling sheet the make and model of the instrument used to measure the variables. The following measurements must be taken before sampling macroinvertebrates and disturbing the streambed:

Water Temperature: °C at the time of sampling.

Electrical Conductivity (EC): is a measure of the total concentration of inorganic ions (salts) in the water (ANZECC/ARMCANZ 2000).

pH: is a measure of the acidity or alkalinity of water and has a scale from 0 (extremely acid) to 14 (extremely alkaline), with 7 being neutral (ANZECC/ARMCANZ 2000).

Dissolved Oxygen concentration (mg l^{-1}): reflects the equilibrium between oxygen consuming processes (e.g. respiration) and oxygen releasing processes (e.g., photosynthesis; ANZECC/ARMCANZ 2000).

Dissolved Oxygen Percent Saturation (% Sat.): Dissolved oxygen levels relative to the atmospheric concentration at a given altitude and temperature (Boulton and Brock 1999). Supersaturation (DO % sat. >100%) can occur as a result of stream turbulence or photosynthesis in algae rich ponds during sunny, windless days (Boulton and Brock 1999). If the measuring apparatus does not calculate percent saturation, barometric pressure must be recorded to calculate this variable.

Turbidity: is a measure of the waters ‘muddiness’, and is caused by the presence of suspended particulate and colloidal matter consisting of suspended clay, silt, phytoplankton and detritus (ANZECC/ARMCANZ 2000). If the measuring apparatus does not measure turbidity, it must be measured from the water sample on return to the laboratory.

Alkalinity: an expression of the buffering capacity of water, measured as the milliequivalents of hydrogen ions neutralised by a litre of water (expressed as CaCO₃ in mg L⁻¹; Boulton and Brock 1999). Alkalinity may be measured in the field or from a water sample on return to the laboratory.

Alkalinity can be determined in the field by acid titration to pH end point 4.5 using Methol Red indicator.

Standard Method (APHA, 1992): Place 100ml of river water in a beaker with two drops of indicator. While swirling the water in the beaker, use a syringe to add Sulfuric (H₂SO₄ 0.02 N) acid (drop by drop) until the water colour just turns and remains pink. Record on the data-sheet the amount of acid used. Alkalinity, mg CaCO₃ l⁻¹ calculated by:

$$\frac{A * N * 50000}{\text{water sample (ml)}}$$

where: **A** = ml H₂SO₄
N = Normality of acid (0.02N)



a) Before adding acid - water is green



b) When titration reaches end point
- water is pink

Stream Measurements

Bank height (m): from moderate water level to top of bank-full height, an estimated average for the reach. Bank-full height (Figure 2) may be determined in the field from the presence of trash lines (flood deposits), the limits of lichen growth on rocks, an abrupt change in the slope of the bank, scour lines and vegetation limits (Gordon *et al.* 1992).

Bank-full width (m): measured from top of one bank (bank-full height) to top of the other bank (Figure 2). Measure or estimate made for the reach.

Stream width (m): measure/estimate from the edges of the water (Figure 2). Measure or estimate the minimum, maximum and mode (most common value) stream widths along the reach. Estimates are acceptable where measurements cannot be made.

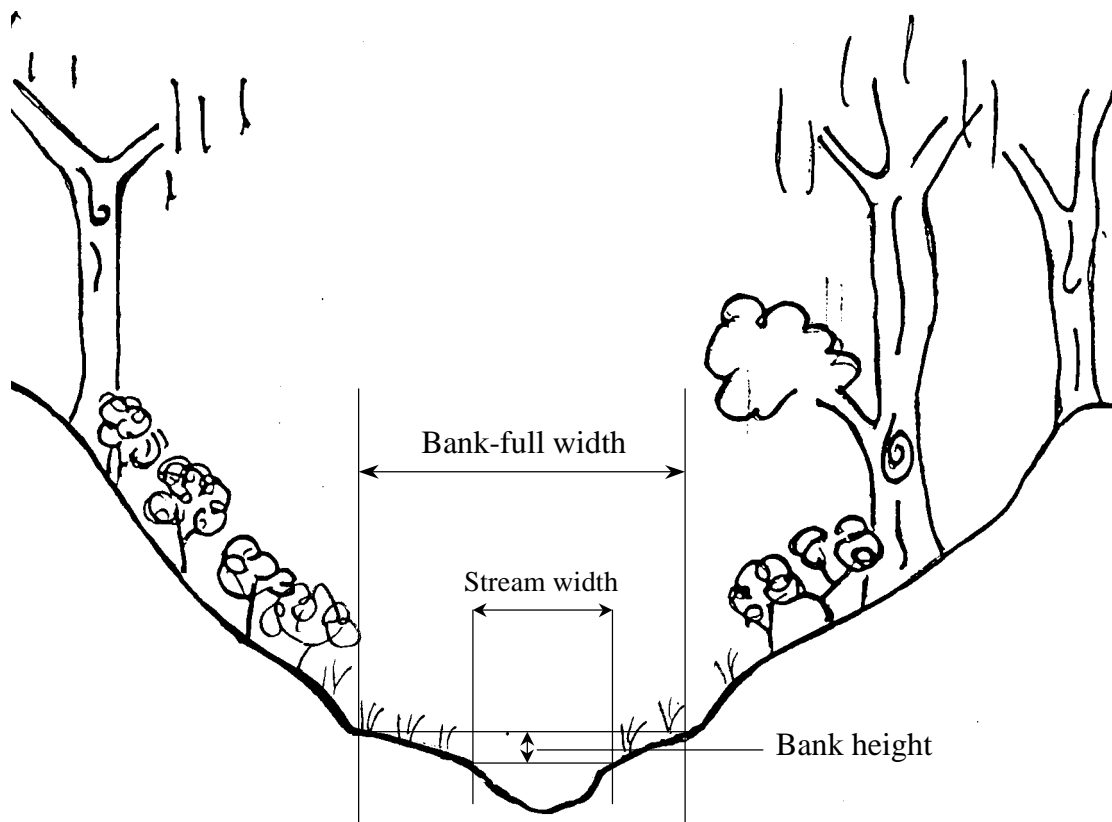


Figure 2 Example of bank height, bank-full width, and stream width.

Length of reach: 5 times the mode bank-full width on either side of the riffle sampling area, i.e. 10 times mode bank-full width, unless the bank-full width is less than 10m, then the minimum reach length is 100m (ie. 50m either side of the riffle sampling site; Figure 3).

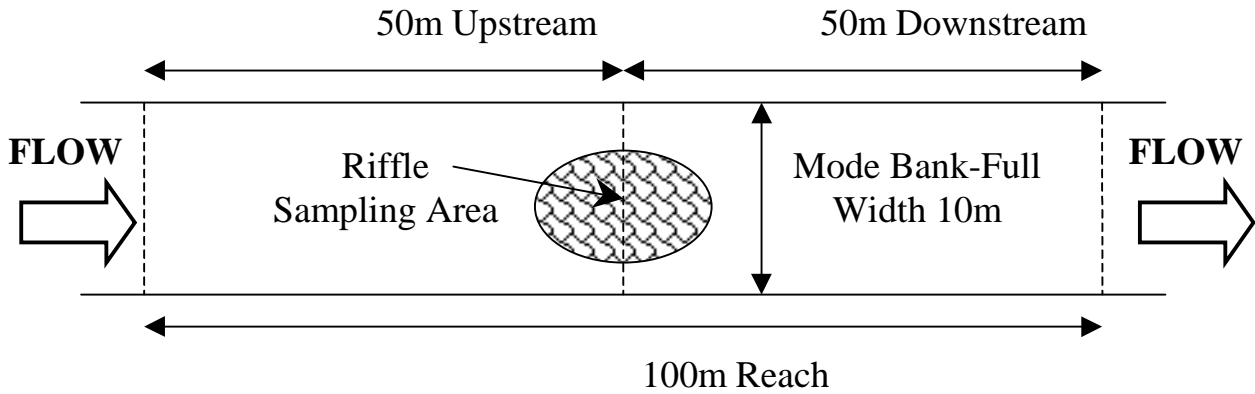


Figure 3 Example of how to calculate the length of a reach. In this example, the mode bank-full width is 10m, therefore the length of the reach is 100m (ie. 5 times the mode bank-full width on either side of the riffle sampling area, or 10 times the mode bank-full width). (Note: diagram not drawn to scale).

Riffle, Pool, Run, Edge and Macrophytes

Record the percent riffle, pool, run, edge, and macrophyte areas within the reach, defined as follows:

Riffle: riffle habitat is one of flowing broken water over gravel, pebble, cobble or boulder, with a depth greater than 10 cm.

Pool: deeper areas, very slow flowing or not flowing.

Run: relatively fast flowing, unbroken water not classified as a riffle.

Together the riffle, run, and pool in a typical stream total 100 % (Figure 4).

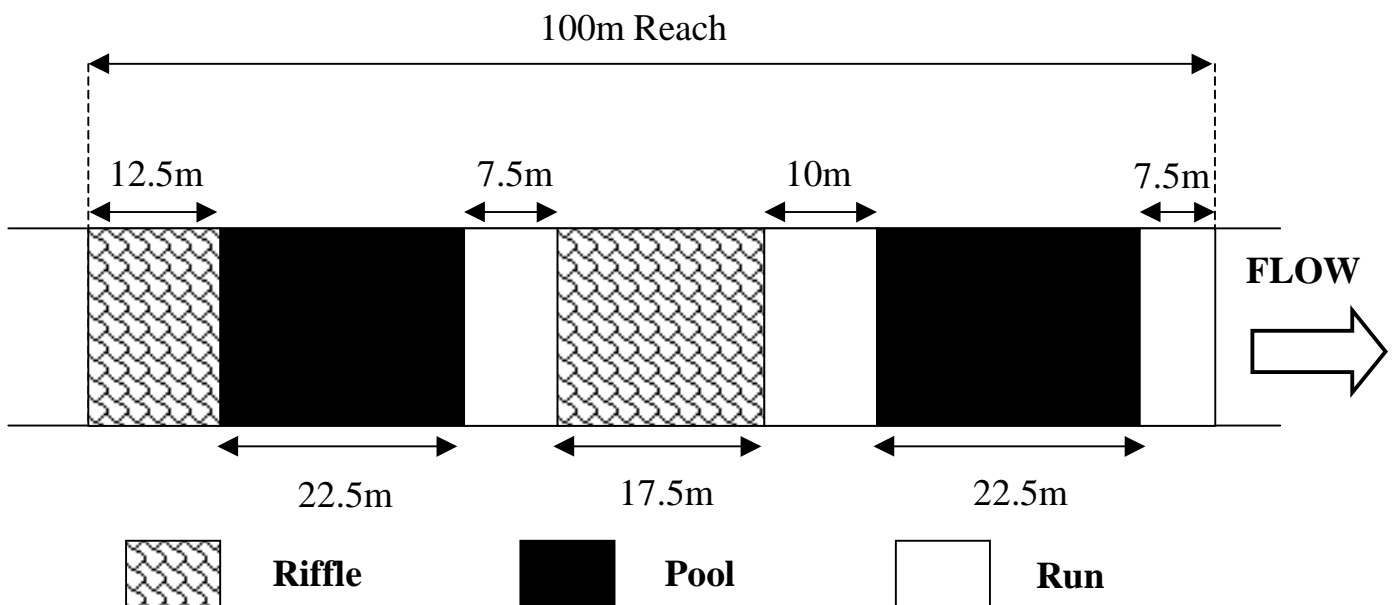


Figure 4 Example of how to calculate percent riffle, pool, and run habitat in the reach. (Note: In reality habitats may or may not span entire width of stream; diagram not drawn to scale).

Percent riffle habitat: $12.5\text{m} + 17.5\text{m} = 30\text{m}$ of riffle. Divide 30m by 100m (the length of the reach) = **30% riffle habitat in reach.**

Percent pool habitat: $22.5\text{m} + 22.5\text{m} = 45\text{m}$ of pool. Divide 45m by 100m (the length of the reach) = **45% pool habitat in reach.**

Percent run habitat: $7.5\text{m} + 10\text{m} + 7.5\text{m} = 25\text{m}$ of run. Divide 25m by 100m (the length of the reach) = **25% run habitat in reach.**

Edge: percent of both banks in the reach with good edge habitat that may be effectively sampled with a sweep net (Figure 5). The edge habitat consists of slow flowing or still waters adjacent to the bank, preferably with overhanging or emergent vegetation or other suitable habitat providing cover and refuge for macroinvertebrates. **Note:** Macrophytes located along the banks are included as part of the edge habitat.

Macrophyte: Submerged, floating and emergent plants (Figure 5), see photos page 31.

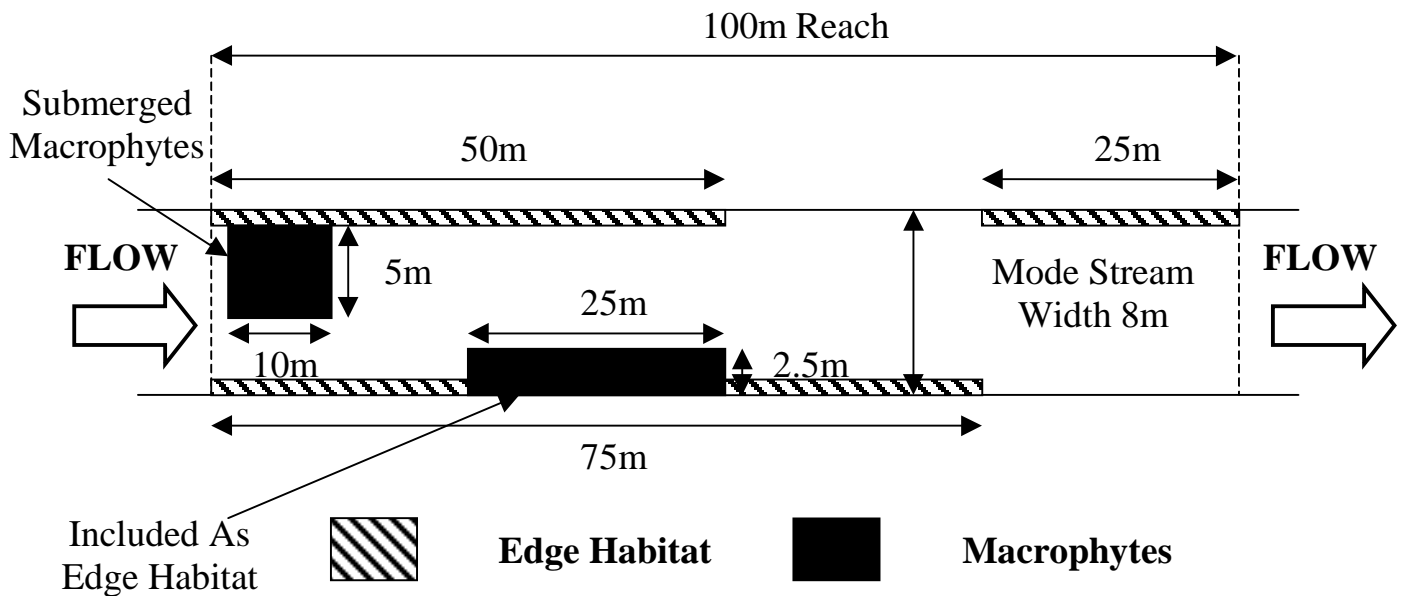


Figure 5 Example of how to calculate percent edge and macrophyte habitat in the reach. (Note: diagram not drawn to scale).

Percent edge habitat that may be effectively sampled with a sweep net: $50\text{m} + 25\text{m} + 75\text{m} = 150\text{m}$ (note: macrophytes growing along the edge are included as edge habitat). Divide 150m by 200m (the length of the reach x 2 banks) = $0.75 \times 100 = 75\%$ **edge habitat in reach.**

Percent macrophyte habitat (includes submerged macrophytes): $25\text{m} \times 2.5\text{m} + 10\text{m} \times 5\text{m} = 62.5\text{m}^2 + 50\text{m}^2 = 112.5\text{m}^2$. Divide 112.5m^2 by 800m^2 (i.e. the total reach area, $100\text{m} \times 8\text{m}$) = $0.1406 \times 100 = \text{approx. } 14\%$ **macrophyte habitat in reach.**

Depth and Velocity Measurements

Stream velocity may be measured in a variety of ways e.g., flow meter or by timing a floating object over a specified distance. Indicate on the field sampling sheet the units used to record flow.

Flow meter: Three measurements of velocity and depth are taken at each of the riffle and edge habitats, if both these habitats are sampled. The three measurements should encompass the flow variability within the sampled habitats. If depth is <30 cm flow is measured at half depth only, if depth is ≥ 30 cm, measure lower flow (L) at $1/5$ depth and upper flow (U) at $4/5$ depth. Calculate mean velocity in metres per second from all measurements taken at each habitat.

Bubble float: Three replicate measurements of velocity are timed over a 10m stretch of river at each habitat. Ensure float is partially submerged to avoid influence by wind. Calculate mean velocity in metres per second from all measurements taken at each habitat.



Riparian Vegetation

The riparian zone is the area from the water's edge to a distance from the bank where the stream still interacts with and influences the type and density of bank-side vegetation. The following observations are taken from the reach ie 5 times the mode bank-full width upstream and downstream of the sampling area.

Width of riparian zone: measured separately for the left and right bank (left and right bank derived by facing downstream) - may be measured or estimated. It is preferable to measure distances at a number of sites until estimates can be made with accuracy.

Percent cover of riparian zone: Percent cover of riparian zone by four vegetation types (ie trees >10m, trees < 10m, shrubs and grasses/ferns/sedges) is estimated from a plan view within the reach and includes both the left and right bank riparian zones viewed as a whole. Percent cover for categories trees >10m and trees <10m when summed, should not exceed 100%. Percent cover for categories shrub and grasses/ferns/sedges are treated separately and may each total 100%. The sum total of all four categories may be greater than 100 % because of the 'layering' effect found within the riparian zone (i.e., shrubs and grasses can grow under trees etc.). The schematic diagrams in Figure 6 may help determine the percent cover. List the species present in each category if possible. Note: A shrub is defined as a woody perennial plant, smaller than 6m, that does not have a main trunk and which branches from the base.

Shading of river: Estimate the percentage of river within the reach shaded by riparian vegetation when the sun is directly overhead (plan view). The schematic diagrams in Figure 6 may help determine the percent cover.



a) <5% Shading



b) >76% Shading

Percent Native/Exotic Vegetation: Percentage cover (plan view) of native and exotic vegetation in the reach. The two values must total 100 %. The schematic diagrams in Figure 6 may help determine the percent cover.

1% COVER

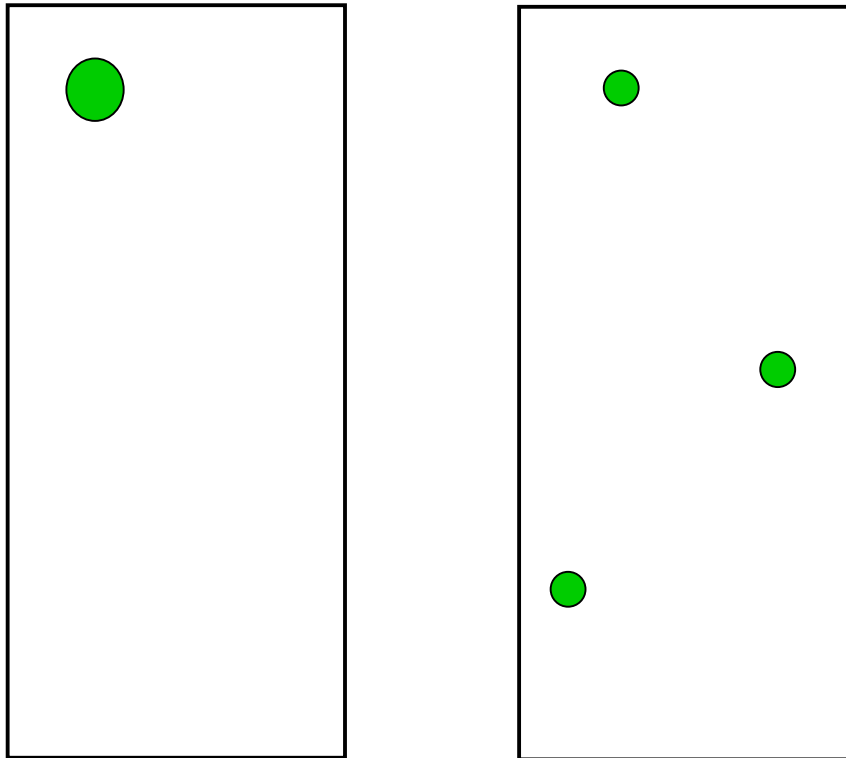
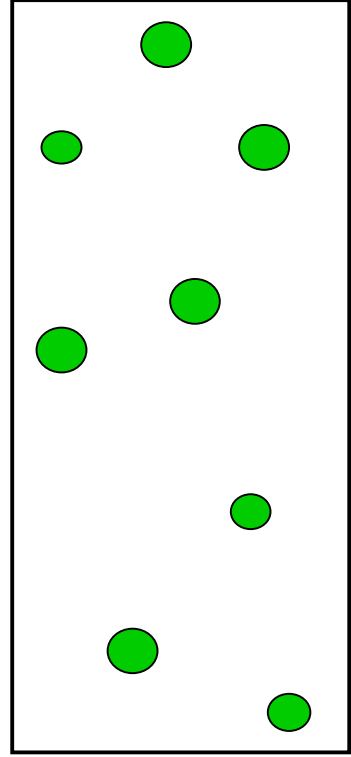
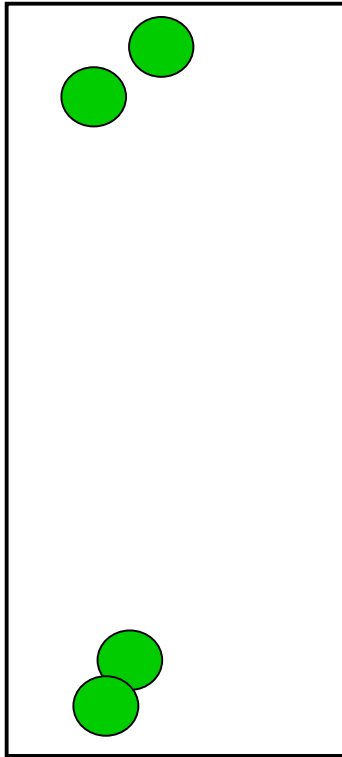


Figure 6. Schematic diagrams of 1%, 5%, 10%, 20%, 40%, 60% and 80% vegetative cover within the riparian zone. These schematic diagrams are used in conjunction with the riparian zone composition and native and exotic riparian vegetation variables. Drawings are from Parsons, Thoms and Norris (2001) modified from schematic diagrams presented in White and Ladson (1999), and are reproduced with kind permission of the Department of Natural Resources and Environment, Victoria.

5% COVER



10% COVER

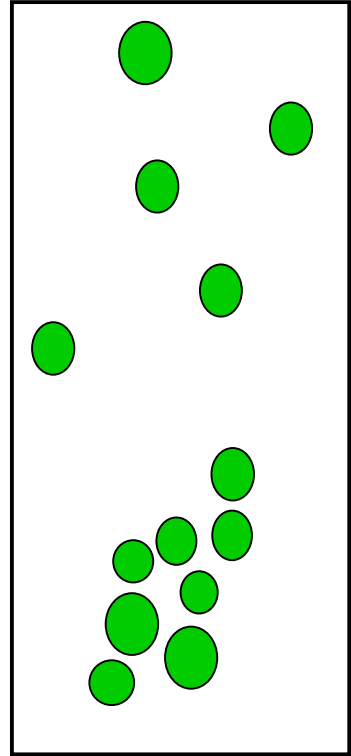
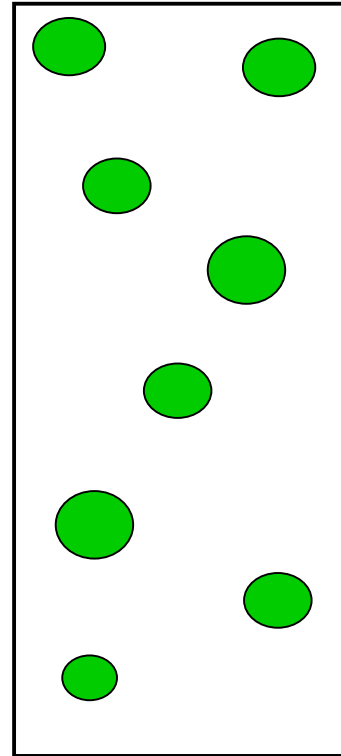
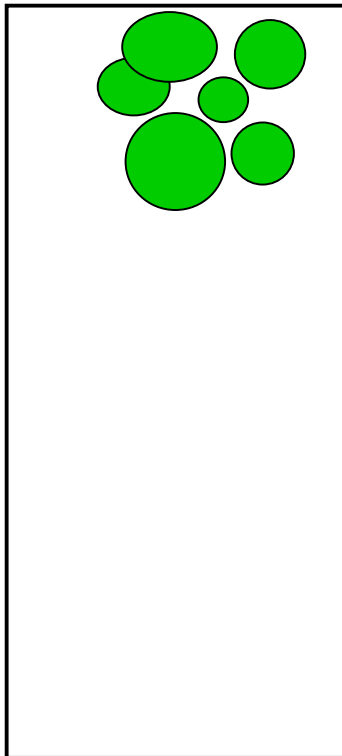
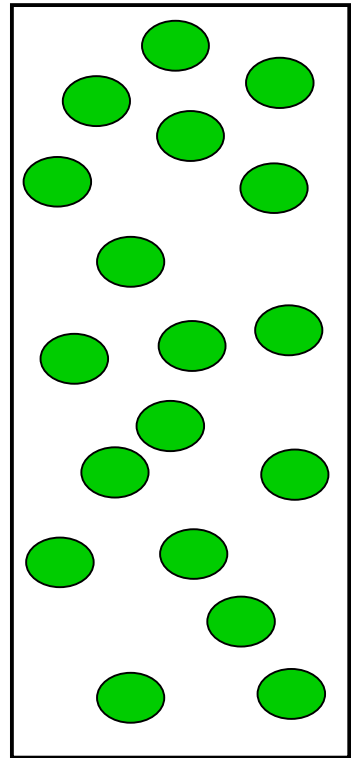
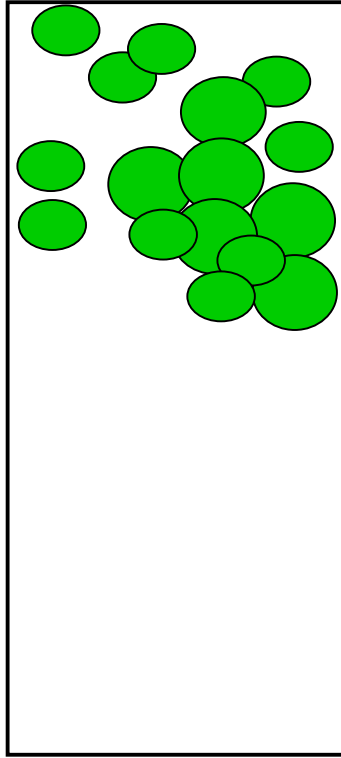
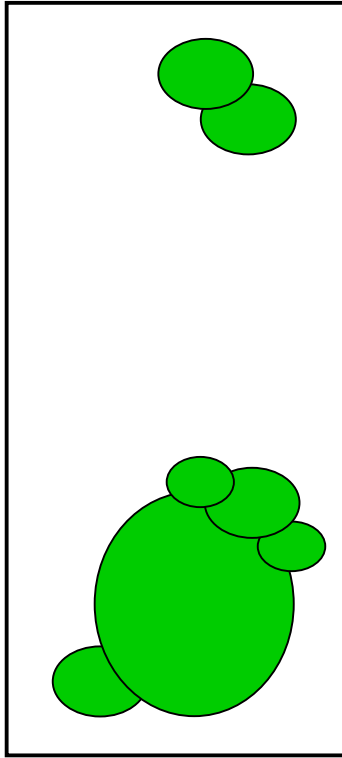


Figure 6 (cont.)

20% COVER



40% COVER

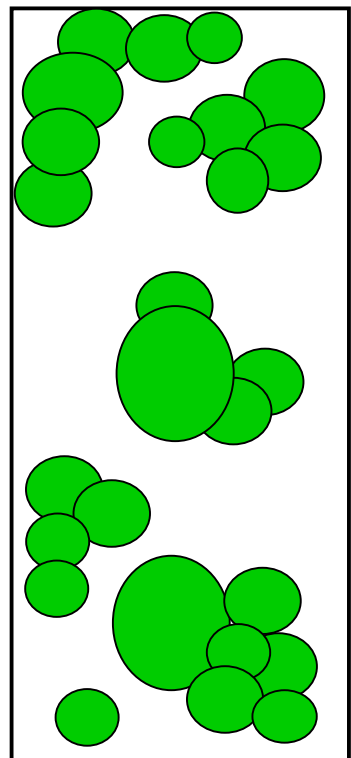
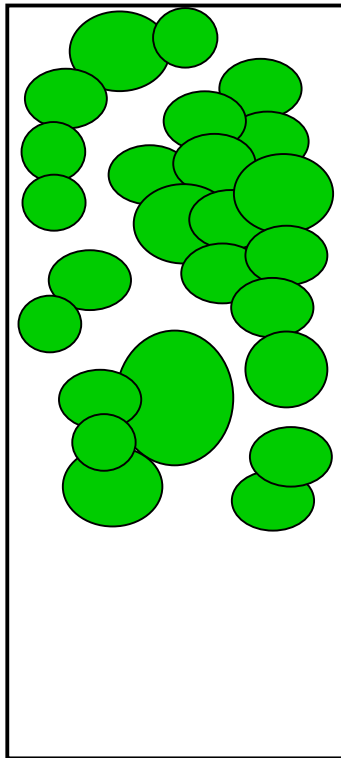
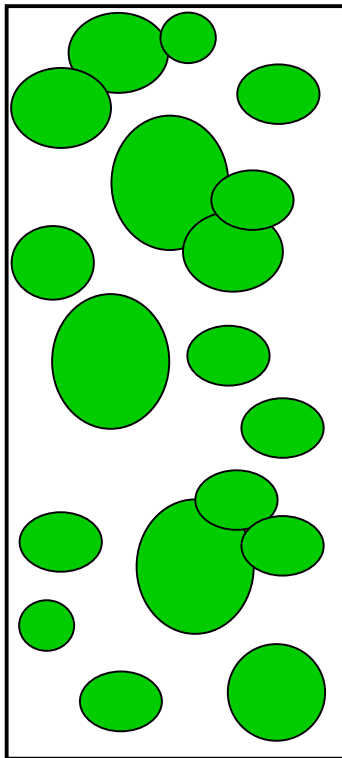
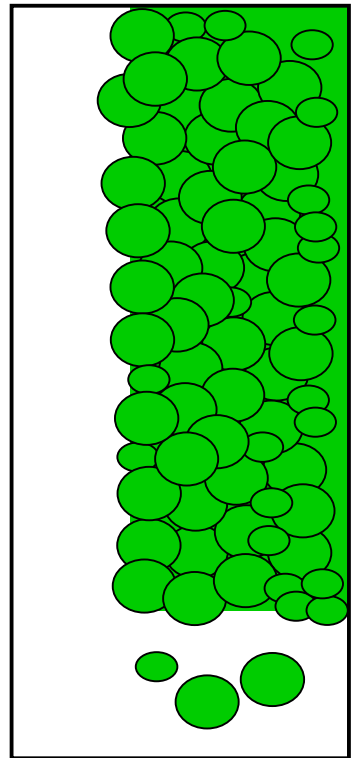
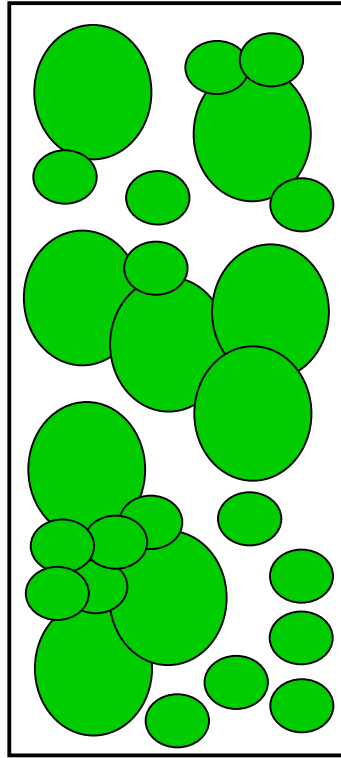
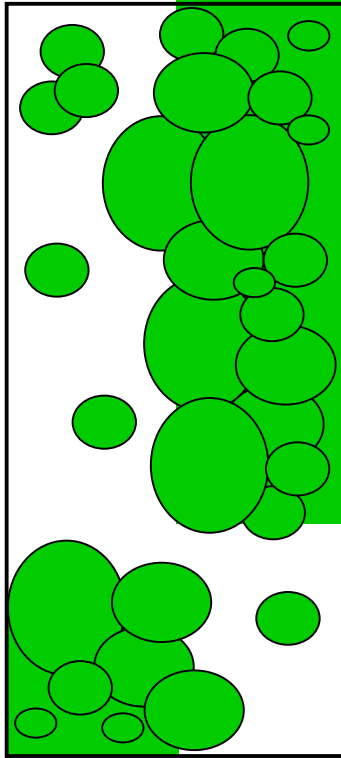


Figure 6 (cont.)

60% COVER



80% COVER

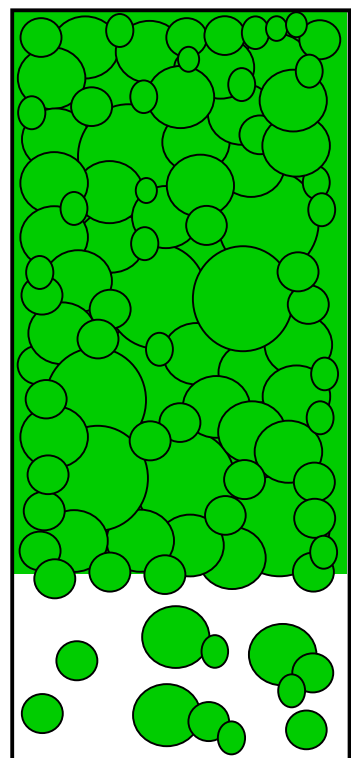
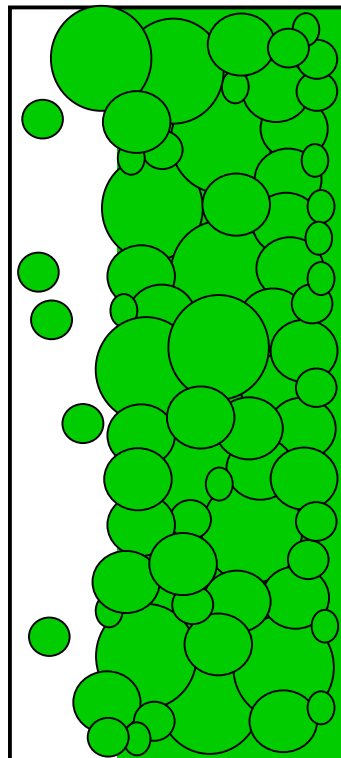
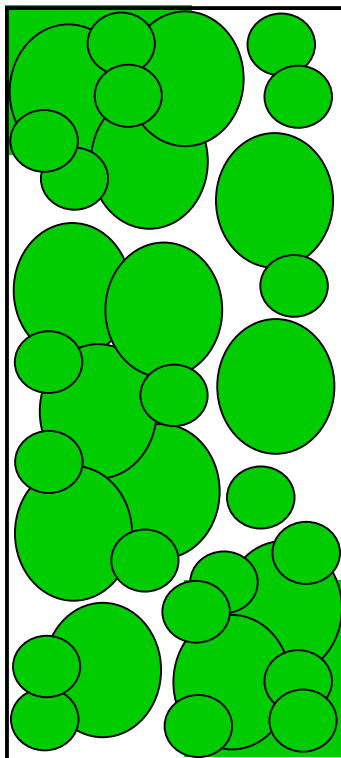


Figure 6 (cont.)

Observations

The following observations are recorded to give an indication of the conditions at the site. These are intended to give a basic description of the site; an indication of factors that may affect water quality; and provide indicators of poor water and habitat quality.

Water Odours: note any noticeable water odours at the site.

Water Oils: note the presence and type of oils (e.g., slick, sheen, globs, flecks, none) on the surface of the water.

Turbidity: visually examine water sample collected in the bottle to assess turbidity, because substratum may influence the appearance of turbidity.

Plume: the amount of fine suspended-sediment generated when disturbing the substratum to sample macroinvertebrates. Note the plume while taking the macroinvertebrate sample from the riffle.



a) Plume

Sediment Oils: examine sediment in the stream and take a handful out of the streambed for visual examination.

Sediment Odours: smell sediment taken out of the streambed. Hydrogen Sulphide (H_2S or 'rotten egg gas') is an odour commonly encountered in deoxygenated sediments.

Flow Level: relative to usual water level or ‘water mark’ evident from terrestrial grasses, eroded areas or boundary/line in bank sediment.



a) Low flow



b) Flood

Bare ground above water mark: Facing downstream, estimate for the bank on your left and right the percentage of bare ground for the entire reach within the riparian zone. Bare ground includes eroded areas or those not supporting vegetation but otherwise expected to be vegetated (Note: bare ground does not include areas covered by boulders or bedrock).

Underside of stones not deeply embedded, black: Examine a number of stones from the streambed. Black indicates anaerobic (without air) conditions.

Sediment Deposits: sediment deposits other than those that may naturally occur.

Local catchment erosion: erosion in the surrounding catchment for one kilometre upstream and downstream of the site, evident from gully erosion, sheet erosion, riverbank erosion etc.



a) Some erosion



b) Heavy erosion

Local PS (Point Source) Pollution: Pollution discharging to the stream from a definite source e.g., Sewage effluent discharge. Note the obvious or potential point sources of pollution within the reach.



a) Point Source Pollution

Local NPS (Non Point Source) Pollution: Pollution not coming from a definite source, e.g., widespread livestock grazing, crop fertilising. Note the obvious or potential non-point sources of pollution within the reach.

Dams/Barriers: Barriers may be natural e.g., waterfall, blocking of flow by fallen trees, or man made e.g., low level weir. Examine local area within one kilometre upstream and downstream of the site.



a) Dam



b) Barrier

River Braiding: Number of separate braids formed by the river within the reach.

Site Classification: Is the site situated in a steep valley, broad valley etc. May circle more than one category if present.



Steep Valley



Broad Valley

Land Use: Observed the land use 100m from the river over the entire reach for both left and right banks. May circle more than one category if present.



Land use: Forestry

Bars: Percent of bed surface protruding from normal water level and forming a bar within the reach (Note: does not include stream bars that would be submerged during normal flows).



Bar

Instream Characteristics

The following in-stream visual estimates for the **reach**, **riffle** and **edge** are taken to characterise the reach and the sampled areas. In-stream substratum characteristics are essential for providing habitat and food sources for macroinvertebrates. **Note:** The following estimates are repeated for the **reach** and for each habitat where the macroinvertebrate samples were collected (e.g., **riffle** and **edge** habitats).

Substratum description: Visually estimate the composition of river substratum (to a depth of 10cm) into the following substrate categories: % bedrock, % boulder >256mm, % cobble 64-256mm, % pebble 16-64mm, % gravel 2-16mm, % sand 0.06-2mm, % silt 0.004-0.06mm, and % clay <0.004mm within the reach or habitat, whichever is applicable. The sum of all the substrate categories must total 100 %. Examples of gravel, pebble and cobble are shown in Figure 7a-c, with various substrate compositions shown in Figure 8a-c. When drawn to scale, Figure 9 is a useful visual aid to help beginners estimate the substrate categories.



Figure 7 a) Gravel



b) Pebble



c) Cobble



Figure 8 a) Gravel & sand



b) Pebble, gravel & sand



b) Cobble & pebble

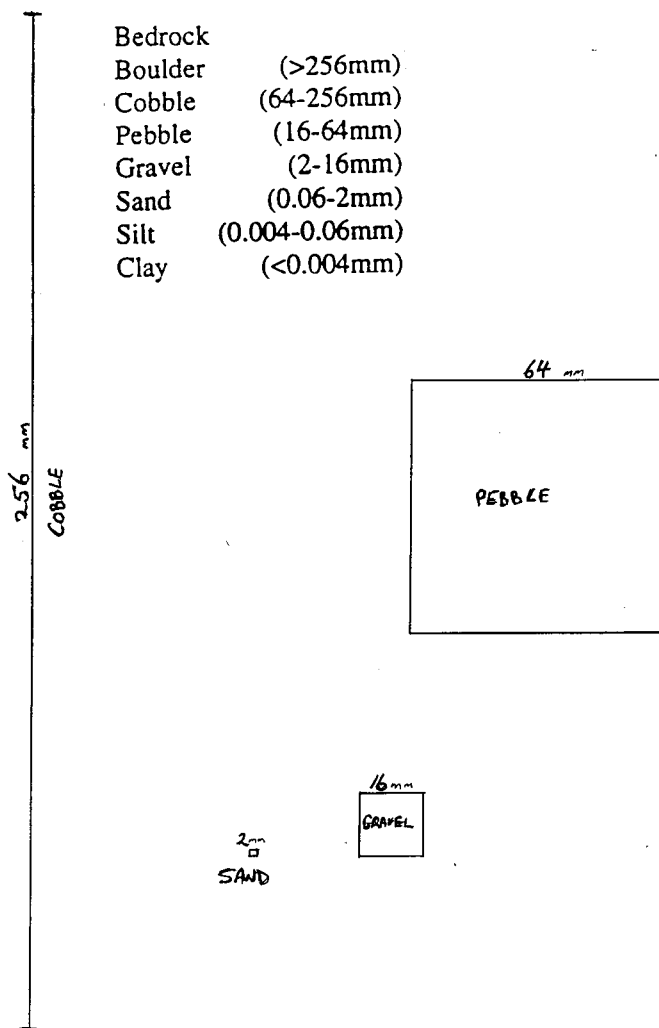


Figure 9. When drawn to scale the above diagram is a useful visual aid to help beginner samplers estimate the substrate categories.

Organic substratum:

Detritus: Percent cover of sticks, wood and Coarse Particulate Organic Matter (CPOM) covering the river substratum.

Muck/Mud: Percent cover of dark or black fine organic material covering the river substratum.

Percent of Reach covered by:

Periphyton: microfloral growth on river substratum (Note: includes algae and diatoms, not moss, filamentous algae or macrophytes).



Periphyton cover >90% (category 5)

Moss: Non-vascular, spore-bearing green plants.



Filamentous algae: Stringy or thread-like algae that may grow in clumps or mats.



Filamentous algae

Macrophytes: Submerged, floating and emergent plants.



Emergent macrophytes



Submerged macrophytes

Trailing bank vegetation: the amount of bank vegetation hanging over or in the water within the edge habitat. Note: Not recorded in reach or riffle habitats.

Macrophytes

Note the presence of macrophytes in the reach and identify them. “A Field Guide to Waterplants in Australia” (Sainty and Jacobs, 1994) will aid in macrophyte identification. If species is unknown, collect a sample of the macrophyte for identification by an expert.



Epiphyte cover on macrophytes: Epiphyte cover is the biofilm that lives on the surface of submerged plants but does not derive nourishment from them (e.g., periphyton). Examine submerged sections of macrophytes in the reach and note the amount of epiphyte cover.



Epiphyte cover on submerged plant

Habitat Assessment

The habitat assessment quantifies various aspects of the site and assesses the quality of the habitat available for macroinvertebrates. Observations are taken from the reach.

Bottom substratum/available cover: Assess the amount of substratum available as macroinvertebrate habitat e.g., pebble, gravel, cobble, boulder, logs, undercut banks. Choose category and circle appropriate score within the category.

Embeddedness: Assess how embedded gravel, cobble and boulders are. Choose category and circle appropriate score within the category. Excellent embeddedness category is defined as minimal fine sediment surrounding substratum that may block interstitial spaces and thus reduce macroinvertebrate refugia within the substratum.

Velocity/depth category: Assess how many velocity/depth categories are present (ie. slow deep, slow shallow, fast deep, fast shallow), where slow = $<0.3 \text{ ms}^{-1}$ and deep = $>0.5 \text{ m}$. Choose a category and circle appropriate score within the category.

Channel alteration: Assess the degree of channelization and bar formation within the reach. Choose category and circle appropriate score within the category.

Bottom scouring and deposition: an assessment of substratum stability. Larger substratum (larger than gravel/pebble) is not subject to scouring and deposition in usual flows. However, smaller substratum (gravel/sand/silt/clay) is subject scouring and deposition. Choose category and circle appropriate score within the category.

Pool/riffle, run/bend ratio: Calculate the distance between the riffles divided by the stream width. Choose category and circle appropriate score within the category.

Bank stability: Observe the bank side slope and amount of bank erosion within the reach. Use this information to determine the bank stability of the site. Choose category and circle appropriate score within the category.



Moderately unstable bank



Unstable bank

Bank vegetative stability: Determine the percentage of the riparian zone covered by vegetation. Choose category and circle appropriate score within the category.

Streamside cover: Determine the dominant vegetation within the riparian zone. Choose category and circle appropriate score within the category.



Dominant vegetation is grass (poor streamside cover).



Dominant vegetation is of tree form (excellent streamside cover).

Checking Field Sampling Sheets

It is critical that once the field sampling sheet has been completed, it is re-checked to ensure no measurements have been missed. The discovery of missing data once back in the laboratory can mean returning to the site, a costly mistake in both time and money, and one that could have been easily avoided. It is also critical that measurements of zero are recorded as such on the field sampling sheet and not left blank. A blank value may be interpreted as missing data when being entered into a database by another person and can result in the site not being assessed.

MACROINVERTEBRATE SAMPLING

Riffle Sampling

Samples are taken with a 250µm mesh rectangular or D-framed net with a 350mm wide aperture at the base. Facing downstream, the operator should place the net directly on the substratum in front of the feet and vigorously disturb and dislodge the substratum by kicking and twisting the feet to a depth of approximately 10cm, slowly moving upstream employing this method. If fine particles block the flow of water through the net, stop and rinse the net before continuing. Separate lengths of riffle may be sampled if a continuous 10m section is not present. Record the collector's name on the field sampling sheet along with the total length of riffle sampled, if less than 10m.

Edge Sampling

Samples are taken with a 250µm mesh rectangular or D-framed net with a 350mm wide aperture at the base. Macroinvertebrates are collected by vigorously sweeping from a distance of approximately one metre from the bank to the bank edge, disturbing the emergent and overhanging vegetation in the water if present. The operator should slowly move upstream for a distance of 10 metres employing this method. Separate lengths of edge may be sampled if a continuous 10m section is not present. Record the collector's name on the field sampling sheet along with the total length of edge sampled, if less than 10m.



Riffle sampling



Edge sampling



Edge sampling

Sample Transfer and Preservation

Before transferring the sample to a sealable container, both the container and lid should be labelled with the site number, river, habitat type, collector's name and date. A dry container is much easier to write on than a wet one!



Labelled containers

Note: If more than one container is needed to store the sample please record this information on the sample container e.g., if two containers are required label them 1 of 2 and 2 of 2.

An efficient way to transfer samples from the net to the sampling container is described below:

1. Rinse net sides to consolidate sample at the bottom of the net.
2. Place a medium sized tray on the ground, and turn the net inside out, so that the sample falls into the tray.
3. Place material collected in the tray into the sample container. If any large stones have been collected, rinse thoroughly before discarding.
4. Pour water into the tray and rinse net to remove any remaining material.
5. Tip water from tray through a 250 μ m sieve to collect remaining material. Rinse tray if necessary to remove all material. Place material collected in the sieve into the sample container.
6. Using forceps, check the net for any remaining invertebrates and place into sample container.



Step 2 – Turning net inside out



Step 3 – Putting sample into container

Fill the sample container to $\frac{3}{4}$ full to allow room for preservative. If more than one container is required to store the sample, clearly indicate this when labelling the containers, e.g., if two containers are required, label the containers 1 of 2 and 2 of 2. The sample should then be preserved with 10 % formalin or 70 % alcohol.

Note: Formalin is the preferred solution for preserving macroinvertebrate samples, however, it is extremely hazardous, and therefore strict handling procedures apply. A Materials Safety Data Sheet (MSDS) for Formalin can be obtained from <http://msds.pdc.cornell.edu>. Health and safety implications will prevent its use if suitable storage and handling facilities (e.g., fume hood, airtight transport container, safety goggles, protective gloves) are unavailable. If suitable storage and handling facilities are unavailable, 70% ethanol should be used to preserve samples.



Adding preservative to macroinvertebrate sample

After collecting a sample, it is essential to wash the net thoroughly to avoid any cross sample contamination between habitats or sites.

MACROINVERTEBRATE SAMPLE PROCESSING

Sub-Sampling

Place the sample in a 250 µm mesh sieve under a fume hood and rinse with water to remove preservative and fine sediment. The sample is sub-sampled to obtain a 200 organism sub-sample. The sub-sampler consists of a box divided into 100 cells, 3.3 cm diameter by 3 cm deep, based on a sub-sampler designed by Marchant (1989). More information on the sub-sampling boxes available at <http://www.marineaction1.cjb.net/>.

Evenly distribute the sample in the sub-sampler box by hand and fill with water until the water level is at the top of the cells. Holding the box by one side, vigorously shake the box until the sample is evenly distributed. Rotate the box and shake again. Repeat this process a number of times (typically 8 -12) until the sample is evenly distributed throughout the cells in the box. A 200-organism sub-sample is required.

Use a vacuum pump to remove the contents of two or three randomly selected cells (randomly chosen using two 10-sided dice). After vacuum extraction, sand may be removed from cell contents using a saturated CaCl₂ (Calcium Chloride) solution, to aid and speed sorting and identification. The macroinvertebrates and organic matter will float in this solution and can be poured off into a sieve. The macroinvertebrates should be rinsed and then transferred to a channel tray or similar for sorting.



250 µm mesh sieves



Taking a macroinvertebrate sub-sample

Sorting

Sort the samples under a stereo microscope (X 10 magnification), removing and counting all macroinvertebrates. Some taxa are not used in the ACT AUSRIVAS predictive models. These taxa have been outlined in Table 1. Calculate the number of cells needed to obtain 200 organisms and extract these from the sub-sampling box. Extracted cells must be completely sorted even if the 200-organism count is reached, to enable estimates of relative and total abundance.

Table 1. List of taxa to count and not to count for the ACT AUSRIVAS predictive models.

DO COUNT	DON'T COUNT
TURBELLARIA (flatworms) MOLLUSCA – Gastropoda (snails) & Bivalvia (mussels) ANNELIDA – Oligochaeta (worms), Hirudinea (leaches) ARACHNIDA – Acarina (mites) CRUSTACEA Amphipoda (scuds) Isopoda Decapoda INSECTA Coleoptera (beetles) Diptera (true flies) Ephemeroptera (mayflies) Hemiptera (bugs) Mecoptera (scorpionflies) Lepidoptera (moths) Me.g.,aloptera (alderflies/dobsonflies) Neuroptera (lacewings) Odonata (damselfly & dragonflies) Plecoptera (stoneflies) Trichoptera (caddis flies)	CNIDARIA NEMATODA (roundworms) NEMATOMORPHA (horsehair worms) ROTIFERA CRUSTACEA Anostraca (fairy shrimps) Notostraca (tadpole shrimps) Conchostraca (clams shrimps) Cladocera (water-fleas) Ostracoda (shell shrimps) Copepoda COLLEMBOLA (springtails)

MACROINVERTEBRATE IDENTIFICATION

Identification

All macroinvertebrates are to be identified to family level except for Oligochaeta (Class), Acarina (Order) and Chironomidae (Sub-family) using the keys recommended by Hawking (2000). A reference list of the family level dichotomous keys is also available on the AUSRIVAS web site (<http://ausrivas.canberra.edu.au>). Adults and larvae for each family are combined for the purposes of data entry and analysis. All samples should be separated into Orders and placed in separate vials to eliminate any high level discrepancies. This is also required for future curatorial preservation and storage.

When an identification problem is encountered a decision tree (Hawking and O’Conner 1997) for identifications should be followed (Figure 10). Very small, damaged, immature animals or pupae that cannot be identified with confidence should be noted as such (e.g., Trichoptera juvenile). These animals are counted and placed in separate vials for each category. The counts for unidentified animals are not included in the 200-organism sub-sample.

Damaged animals should be identified if possible, with both head and tails counted and the highest number recorded and placed in the appropriate vials. If a specimen cannot be identified it should be noted as such (e.g., Ephemeroptera damaged) and placed in the appropriate vials. The same procedures apply to the identification of Oligochaeta.

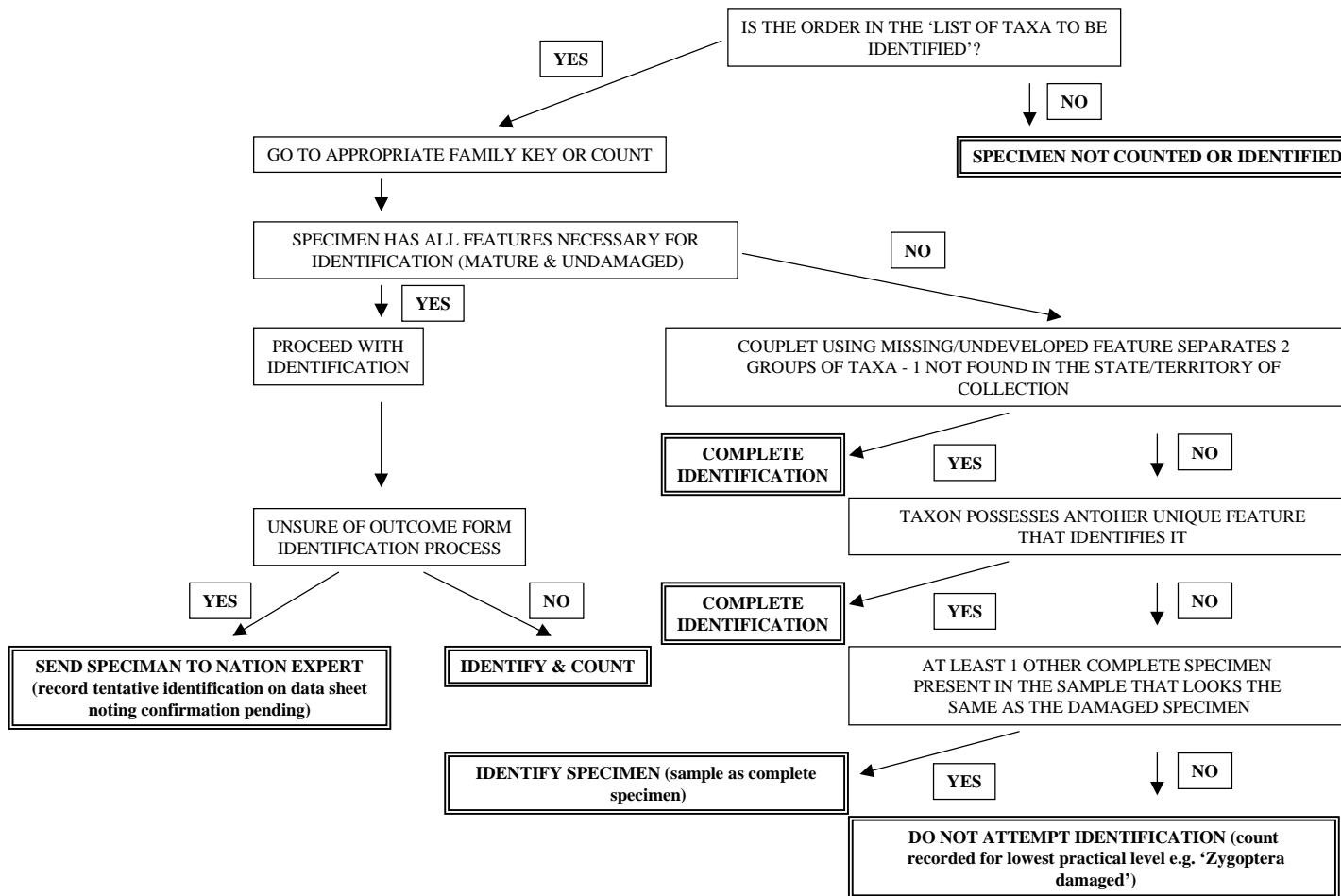


Figure 10 Decision tree to be followed when an identification problem is encountered (Hawking and O'Conner 1997).

Recent changes to the classification of the order Odonata has resulted in a number of “new” Australian families being recognised (Hawking and Theischinger 1999). This reclassification of the order Odonata has implications for AUSRIVAS because the predictive models were constructed using the old classification system, and thus, will not predict these “new” families to occur. It is therefore necessary to note on the data sheet the taxon’s former family name, next to the taxon’s “new” family name. When entering data into AUSRIVAS all reclassified Odonata families MUST be entered under the taxon’s former name in order for AUSRIVAS to calculate an accurate observed to expected ratio.

Macroinvertebrate Identification Sheet

When recording information on the Macroinvertebrate Identifications Sheet please check that:

- The former names of reclassified Odonata families have been recorded next to the “new” family names;
- All tallies have been transferred to the count column;
- The counts have been summed and the total count is correct;
- The number of taxa have been summed and is correct. Note: If a taxon is represented in the sample by both juvenile and adult forms, count them as a single taxon when recording the total number of taxa;
- The number of vials used is recorded;
- The percentage of sample used is recorded.

The Macroinvertebrate Identification Sheet can be downloaded from <http://ausrivas.canberra.edu.au>.

Labelling

Ensure that each of the vials contains a label. Write on the labels in pencil to ensure that the information will not be removed by the ethanol. The front of the label should include: the site number; river name; date the sample was collected; name of the person identifying the sample; and the habitat that the sample came from (e.g. riffle or edge). The back of the label should include: the name of the taxon in the vial; number of animals in the vial; and the vial number in reference to the total number of vials (e.g. vial 3 of 7). Labels can be found at <http://ausrivas.canberra.edu.au>.

Example label:

Site: 003 River: <i>Cotter River</i> Date: 22.9.94 Name: <i>Joe Bloggs</i> Habitat: <i>Riffle</i>

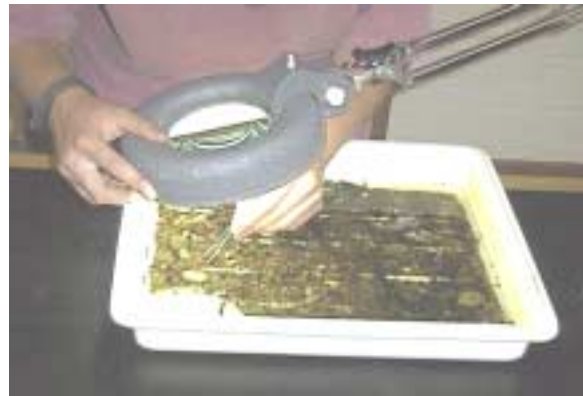
Front of label

Taxon: <i>Trichoptera</i> No.: 78 _3_ of _7_
--

Back of label

Macroinvertebrate Scan

Once the 200 organism sub-sample has been completed, place the unsorted sample residue into a large tray and scan for 15 minutes, collecting any taxa not found in the sub-sample. By conducting a scan, a more complete taxa list can be obtained, incorporating large and rare taxa that may not have been collected in the 200 organism sub-sample. Macroinvertebrates collected during the scan ARE NOT used in AUSRIVAS assessments and should be kept separate from macroinvertebrates collected in the sub-sample and recorded on a SEPARATE data sheet. The Scan Identification Sheet can be down-loaded from the AUSRIVAS web site at <http://ausrivas.canberra.edu.au>.



QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

Quality control/quality assurance (QA/QC) procedures are designed to establish an acceptable standard of macroinvertebrate sorting and identification. The quality control component reduces the level of error in sorting and identification, while the quality assurance component provides potential users with the assurance that the accuracy of results is within controlled and acceptable limits.

QA/QC - Sorting

For new persons, projects, or sampling runs, quality control staff should check the residues of the first five samples sorted for missed organisms. In order to pass the QA/QC process, $\geq 95\%$ of the total number of organisms in the sub-sample must be recovered. If one of the first five samples fails the QA/QC protocol, the process begins again until five consecutive samples have passed.

Following the initial five samples, a random selection of two samples in the following ten, two samples in the following 30 and two samples in the following 50, will be checked. If any of these samples fail the QA/QC protocol, the person must again pass five consecutive samples.

Staff checking samples will have adequate experience in sorting. Where possible, QA/QC of sample residues should be conducted by more than one person to avoid bias and increased workloads. If less than 95% of the organisms are recovered from the sub-sample, commonly missed taxa should be shown to the person and suitable instruction given to rectify the problem. Laboratory sample record sheets are to be fully completed by the person conducting the QA/QC check. Error/action codes are to be recorded on laboratory sample record sheets if appropriate (Table 2).

Table 2 QA/QC error and action codes

Error codes	
CC	Number of organisms recovered from the sub-sample represents less than 95% of the total number of organisms in the sub-sample.
IE	Identification error (i.e., “Percent Taxa Error” and/or the “Percent Incorrect Identifications”) greater than 5%.
LE	Labelling error
SE	Sub-sampling error – e.g., if the sample was stored in more than one sample container and only one container was sorted.
WE	Washing error – some sample bypassed sieve when rinsing
DE	Data entry error on data sheet
CE	Calculation error – mathematical error on data sheets
Action codes	
LC	Labels corrected – contact person who collected the sample if error is on the original sample label
SC	Sample re-sampled, processed, re-checked and data sheets corrected
WC	Material bypassing the sieve caught in washbasin, sample combined and rewashed
WI	Material bypassing sieve lost, partial sample processed
DC	Data entry corrected (strike out incorrect entry with one line and write in the correct entry, initial).

QA/QC - Identification

Two methods are used to calculate a sample's identification QA/QC result. These are the "Percent Taxa Error" and the "Percent Incorrect Identifications". A sample must pass both methods to achieve an overall pass.

Percent Taxa Error

A "Taxa Error" occurs when a mis-identification results in the loss or addition of a taxon. The "Percent Taxa Error" is the "Number of Taxa Errors" divided by the "Total Number of Original Taxa", multiplied by one hundred. Samples pass if the "Percent Taxa Error" is less than or equal to 5% at the family level (10% at species level).

Percent Taxa Error	
Number of Taxa Errors (a)	
Total Number of Original Taxa (b)	
Percent Taxa Error ($[a/b] \times 100$)	
Pass or Fail? (Pass if $\leq 5\%$ Family Level)	

Examples of how to calculate "Taxa Error" for different scenarios are shown below.

Example A: One Taxa Error – Empididae has been mis-identified as Athericidae.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	3	0 (-3)
	Ceratopoginidae	2	2
	Empididae		3 (+3)
	Simuliidae	2	2
	Tipulidae	1	1

Example B: One Taxa Error – Two Empididae have been mis-identified as Athericidae.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	3	1 (-2)
	Ceratopoginidae	2	2
	Empididae		2 (+2)
	Simuliidae	2	2
	Tipulidae	1	1

Example C: Two Taxa Errors – Both Empididae and Tipulidae have been mis-identified as Athericidae.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	3	0 (-3)
	Ceratopoginidae	2	2
	Empididae		2 (+2)
	Simuliidae	2	2
	Tipulidae		1 (+1)

Example D: Two Taxa Errors – Empididae has been mis-identified as Athericidae and Tipulidae.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	2	0 (-2)
	Ceratopoginidae	2	2
	Empididae		3 (+3)
	Simuliidae	2	2
	Tipulidae	1	0 (-1)

Percent Incorrect Identifications

The “Percent Incorrect Identifications” is the "Number of Organisms Incorrectly Identified" divided by the "Total Number of Organisms in the Original Count", multiplied by one hundred. Samples pass if the "Percent Incorrect Identifications" is less than or equal to 5% at the family level (10% at species level).

Percent Incorrect Count	
Number of Organisms Incorrectly Identified (a)	
Total Number of Organisms in Original Count (b)	
Percent Incorrect Identifications ($[a/b] \times 100$)	
Pass or Fail? (Pass if $\leq 5\%$ Family Level)	

Examples of how to calculate the “Number of Organisms Incorrectly Identified” for different scenarios are shown below.

Example A: Number of Organisms Incorrectly Identified = 3. Three Empididae have been mis-identified as three Athericidae.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	6	3 (-3)
	Ceratopoginidae	2	2
	Empididae		3 (+3)
	Simuliidae	2	2
	Tipulidae	1	1

Example B: Number of Organisms Incorrectly Identified = 3. Two Empididae and one Tipulidae have been mis-identified as three Athericidae.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	3	0 (-3)
	Ceratopoginidae	2	2
	Empididae	2	4 (+2)
	Simuliidae	2	2
	Tipulidae	2	3 (+1)

Example C: Number of Organisms Incorrectly Identified = 4. Three Empididae have been mis-identified as Athericidae and one organism was not found in the QA/QC count.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	4	0 (-4)
	Ceratopoginidae	2	2
	Empididae		3 (+3)
	Simuliidae	2	2
	Tipulidae	1	1

Example D: Number of Organisms Incorrectly Identified = 3. Two Empididae have been mis-identified as Athericidae and one additional Simuliidae was found in the QA/QC count.

CLASS/ORDER	FAMILY	ORIGINAL COUNT	QA/QC COUNT
DIPTERA	Athericidae	2	0 (-2)
	Ceratopoginidae	2	2
	Empididae		2 (+2)
	Simuliidae	1	2 (+1)
	Tipulidae	1	1

For new persons, projects, or sampling runs, quality control staff should check the first five samples identified. If one of the first five samples fails the QA/QC protocol, the process begins again until five consecutive samples have passed.

Following the initial five samples, a random selection of two samples in the following ten, two samples in the following 30 and two samples in the following 50, will be checked. If any of these samples fail the QA/QC protocol, the person must again pass five consecutive samples.

Staff checking samples will have adequate experience in identification. Where possible, QA/QC of sample identifications should be conducted by more than one person to avoid bias and increased workloads. All mis-identifications will be shown to the person and suitable instruction given to rectify the mis-identification. Other samples containing taxa that were mis-identified are then checked for identification errors by the original identifier. Laboratory sample record sheets are to be fully completed by the person conducting the QA/QC check. Error/action codes are to be recorded on laboratory sample record sheets if appropriate (Table 2).



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APPENDICES

Appendix 1 Field Equipment List

TO DO WELL IN ADVANCE	CHECK
Vehicle 4WD - Book in advance	
Hydrolab - book in advance & calibrate	
Hydrolab spare battery - (charge a day or two prior)	
Travel forms - to be completed prior to travel	
Book - flow metre, GPS, digital camera, phone, large esky, waders, backpack etc.	
Bottles - Wash in advance	
Formalin - buffer and stain	

ITEM	QUANTITY	CHECK
ACT Sampling and Processing Manual		
Kicknets 250 mm mesh	2	
Sieves, 250 mm mesh for "rapid" sampling	2	
Small plastic bucket	1	
Medium white tray	1	
Formalin - buffered & with Rose Bengal stain		
Gloves - for handling formalin	1	
Bug sampling containers		
Bottles - 250ml plastic for water quality samples		
Flow meter	1	
Float (if flow meter fails)	1	
Waders + spare	2	
Wader repair kit	1	
Hydrolab	1	
Alkalinity kit	1	
Backpacks for Hydrolab, equipment etc	2	
Spare battery	1	
Spare DO membranes & O ₂ probe solution		
Buffer solutions 4 & 10 for Hydrolab calibration		
Hydrolab calibration instructions		
Barometer	1	
GPS		
Digital camera		
Mobile phone		
Field Sampling Sheets		
Site Information Sheets		
Time sheets		
Clipboard	1	
Maps		
Marker pens/ pencils	3	
Esky (large) & Ice	1	
First Aid Kit	1	
4WD recovery equipment		
Shovel	1	
EPIRB- Emergency Position Indicating Radio Beacon if working in remote areas	1	
Tape measure 100m	1	
Calculator		
Camera & film		
Sunscreen		
Toilet paper		
Jackets		
Plastic bags for veg. samples		
Reference - "A Field Guide to Waterplants in Australia" Sainty & Jacobs 1994		

Appendix 2 How to Calculate A Site's Grid Reference

Grid References - Eastings & Northings

Every map - scale up to 1:100,000 will have a 1km grid square lines marked on the map as follows:

1:100,000 1km grid = 1cm X 1cm

1:50,000 1km grid = 2cm X 2cm

1:25,000 1km grid = 4cm X 4cm

1:4,000 1km grid = 25cm X 25cm

smaller scale maps 1:250,000, 1:500,000 etc. will have grid lines that are greater than 1km.

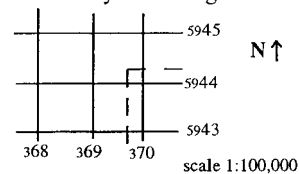
To generate a 9 figure grid reference (4 figure Easting & 5 figure Northing) these are the steps.

4 figure Easting - the first 3 digits denote the 1km grid line (referred above) the last digit is determined by calculating how far along/within the grid square in *tenths* is the point or site.

5 figure Northing - the first 4 digits denote the 1km grid line, the last digit is determined by calculating how far along/within the grid square in *tenths* is the point or site.

This example the Grid Reference would be 3697 59443

The 9 figure grid reference produces an accuracy to within 100 metres.



To generate a 13 figure grid reference (6 figure Easting & 7 figure Northing) the steps are the same as above but you need to calculate within the grid square in *thousandths*.

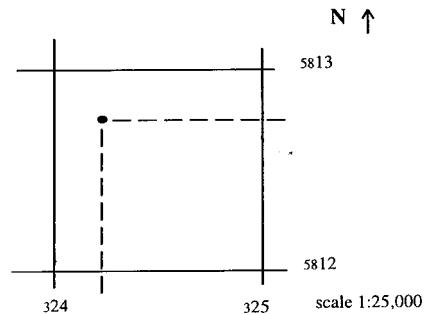
this means that for a 1:100,000 map (commonly used) dividing a grid square of 1cm X 1cm into thousandths (visually possible??), even with a larger scale map 1:4,000 this would be dividing up a grid square of 25cm X 25cm into thousandths (guess work!!)

Example

Using a 1:25,000 map (map that is quite detailed, many areas in Australia would not have areas mapped down to this scale) this grid square would be as appears on the map 4cm X 4cm

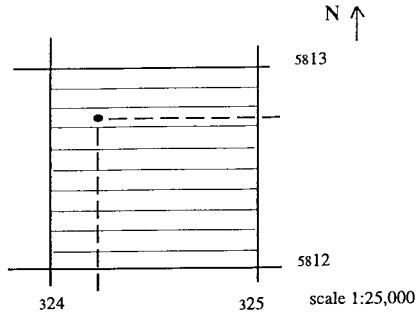
To determine the Northing for this example:

- i. the first four digits are straight forward 5812 on the grid line
 - ii. Next divide the grid square in tenths (4mm segments) - the site is greater than 7/10 up the square and less than 8/10 - therefore 58127
 - iii. find the point down to 1/100 (a segment 0.4mm) divide this segment up into tenths = determine what fraction the site lies between one point and the next just 0.04mm apart.
 - iv. find the point down to 1/1000 (a segment 0.04mm) determine what fraction the site lies between one point and the next just 0.004mm apart.
- Step iii & iv are not really visually possible!!

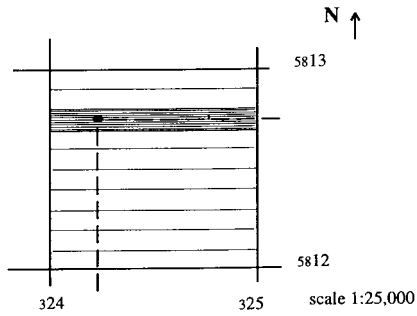


The Easting would be determined the same way as the Northing except that in step I three digits are obtained rather than four - 324. Imagine trying to determine 13 figure grid ref. with 1:100,000 map - step iii determine what fraction the site lies between one point and the next - just 0.01mm apart iv 0.001mm apart

9 Figure Grid Reference
 This example looking at
 determining the Northing
 58127



11 Figure Grid Reference
 This example looking at
 determining the Northing
 581275



13 Figure Grid Reference
 This example looking at
 determining the Northing
 581275 - The 7th figure
 not possible to visually
 see/accurately determine.

