Biological assessment

Version: June 2018

Aquatic macroinvertebrate sampling, processing and index calculation

1 Purpose and scope

This document describes methods for aquatic macroinvertebrate sampling. Methods for both live picking and laboratory picking are presented. However, when assessing whether a site meets the aquatic macroinvertebrate water quality objectives (WQOs) prescribed under the Environmental Protection (Water) Policy (2009), the live picking method must be followed, as this method was used derived the current published WQOs. Local WQOs may be derived using the laboratory picked method, provided adequate reference site data are available (in terms of number of sites and over time) (see EHP 2009 for guidance). The method used to define any WQOs should be clearly stated.

2 Associated documents

Sampling design and preparation:

- *Permits and approvals*
- *Record keeping, including taking field photographs and videos*

Biological assessment: Background to aquatic macroinvertebrate sampling and index calculation.

3 Health and safety

Before following the methods contained in this document, a detailed risk management process (identification, assessment, control and review of the hazards and risks) must be undertaken. All work carried out must comply with the Queensland Work Health and Safety legislative obligations.

4 Permits and approvals

Permits and approvals may be required to conduct activities involving animals, plants and/or in protected areas (for example National Park/Regional Park, State Forest or State Marine Park). See *Permits and approvals* for more information on requirements.

Note: Crustaceans (e.g. crabs, prawns, crayfish and lobsters) are considered fish under the *Fisheries Act 1994* and therefore relevant permits and approvals need to be sought.

5 Skills, training and experience

There are four components in this method: sample collection, live picking, laboratory picking, and laboratory taxonomic identification. Skills, training and/or experience required to understand and/or undertake each of these components are:

- **Aquatic macroinvertebrate sample collection:** In house training by an accredited or equivalently trained operator at a range of habitats and sites.
- **Field (live) picking:** Completion of an aquatic macroinvertebrate ecology course and/or equivalent AUSRIVAS accreditation in **live** picking. In house training may be provided by an accredited or equivalently trained operator. The QA/QC procedure outlined in this document can be used to assess

competency.

- **Laboratory picking:** Completion of an aquatic macroinvertebrate ecology course and/or equivalent AUSRIVAS accreditation in **laboratory** picking. In house training by an accredited or equivalently trained operator at a range of habitats and sites.
- **Aquatic macroinvertebrate identification:** Completion of an aquatic macroinvertebrate identification course and/or equivalent AUSRIVAS accreditation in aquatic macroinvertebrate identification. In house training may be provided by an accredited or equivalently trained operator. The QA/QC procedure outlined in this document can be used to assess competency.

6 Equipment

See Appendix 1 for example equipment checklist.

7 Procedure

Nationally, two methods are used for collecting organisms: field picking and laboratory picking. The choice of which method to adopt will be influenced by considerations of the objective of the study, precision required, time, cost and balance of effort in the field versus laboratory. Either method may be used, although it is critical to maintain the same technique for all sites when comparing data.

For Queensland, the field picking option has been adopted as the preferred standard method. This is the method that was used to collect data for the development of macroinvertebrate water quality objectives (WQOs) that are defined for a number of river basins under the Environmental Protection (Water) Policy (2009) (EPP (Water))

Note: If the stream being considered is experiencing high flow, or has recently filled after being dry, macroinvertebrate sampling should not be conducted. Once the high flow has subsided, a period of 4–6 weeks should be left before conducting sampling in order for hydrological conditions to stabilise and aquatic macroinvertebrate populations to recover.

7.1 Habitat selection

7.1.1 Sampling site and aquatic habitat

The site is defined as 50m upstream and 50m downstream of the entry point (total of 100m of the stream reach). Aquatic habitats are defined using a combination of visual identification and velocity to depth ratio validation. The key visual identifiers for each habitat type are summarised in Table 1. The velocity to depth table (Appendix 2) can be used to confirm the identification of a habitat type based on flow. Velocity should be calculated using a flow meter at several points along a stream cross section. Alternatively a visual estimator can be used, where the time it takes for an object to move down a known length of stream is measured. Depth can be measured using a depth measuring pole.

Table 1: Key descriptors of different habitat types

Habitat type	Key descriptor	Depth	Flow
Edge	Habitats along bank, approximately 0.5m from bank. May be terrestrial or semi-aquatic vegetation (e.g. paragrass and sedges), tree roots or bare area.		Little or no current
Backwater	Pool of water away from the main channel (e.g. ox-bow, off-cut channel). May have a silty bed with accumulated plant litter (e.g. leaves, twigs etc.)		Circular or back flow
Riffle (bed)	Broken water over stony beds	Shallow (<0.3m)	Fast (≥0.2m/s)
Run (bed)	Unbroken water	Deep	Fast flowing
Pool (bed)	Unbroken, main channel	Relatively deep	Stationary/slow
Macrophyte (bed)	Areas where emergent, submergent and floating macrophytes or aquatic plants are present	Relatively shallow	Slow or fast

Habitats to be sampled in each region to align with the EPP (Water) WQOs for macroinvertebrates are summarised in Table 2.

Table 2: Habitats to be sampled in each region to align with the EPP (Water) Water Quality Objectives (WQOs)

Areas with aquatic macroinvertebrate water quality objectives (WQOs)[1]	Habitats assessed
Wet Tropics[a]	Edge and riffle
Fitzroy Basin waters	Edge and composite of all bed habitats
Mackay-Whitsundays[b]	Edge and riffle
Capricorn-Curtis Coast region[c]	Edge
Moreton Bay/south-east Queensland	Edge

except [a] *Barron River Basin,* [b] *Burrum, Gregory, Isis, Cherwell and Elliott rivers, and* [c] *Curtis Island, Calliope River and Boyne River basins where WQOs are not available for aquatic macroinvertebrates.*

7.2 Sampling aquatic macroinvertebrates using a dip net

7.2.1 Preparation

1. Check sampling area is of a wading depth i.e. maximum depth of 1–1.5m. For Workplace Health and

[1] Schedule 1 of the Environmental Protection (Water) Policy 2009

Safety reasons, ensure it doesn't have a strong current and isn't slippery.
2. Check nets for damage or holes. Only use nets that are free of damage or holes.
3. Wash nets and buckets prior to sampling each site to remove animals left from previous sampling.
4. Record site information (for example site location, site code, date, time etc.).
5. Record GPS co-ordinate of site.

7.2.2 Sampling habitats

1. Sample 10m of each of the chosen habitats using the techniques described below. The 10m sample for each habitat type can be fragmented within the 100m reach of the site.
2. Within each habitat, there are usually several different microhabitats present (e.g. an edge habitat may have tree roots, paragrass, bare banks, or leaf litter). As many of these microhabitats as possible should be sampled.

7.2.2.1 Edge/backwater

1. Locate an edge area with little or no aquatic vegetation (stands of paragrass, *Urochloa* sp., are acceptable as edge habitat). An alcove or backwater with abundant benthic leaf litter is also acceptable. Suitable areas include tree roots, fine organic/silt deposits, and trailing vegetation, and are often indicated by the presence of surface-dwelling insects. Bare edges should also be sampled.
2. Working upstream when possible, use short forward and upward sweeping movements at right angles and towards the bank and continue sampling until the desired edge length/distance is reached. Stir up the benthos while doing so, ensuring that benthic animals are suspended and then caught when sweeping through the cloud of suspended material.
3. There may be aquatic plants along the banks and in backwaters. Avoid sampling these areas as part of an edge sample.

Note: Avoid clogging the net with fine particulate material. If necessary, flush the net with water without losing macroinvertebrates already captured.

7.2.2.2 Riffle/runs

1. While holding the net with its mouth facing upstream, disturb the substratum with the feet so that macroinvertebrates are washed into the net. Turn and rub cobbles and rocks by hand to dislodge organisms.
2. Continue this process walking backwards and working upstream, covering both the fastest and slowest flowing sections of the riffle/runs until a distance of 10m is sampled.
3. Place several cobbles into the net and then transfer them into a bucket for direct picking of animals from the cobbles.

Note: Do not include material from macrophytes and/or wood debris located in the riffle.

7.2.2.3 Pools

1. Disturb the substratum by kicking with your feet.
    - If the stream is flowing, hold the net downstream of feet with the mouth facing upstream into the area being disturbed.
    - If there is no flow use a short sweeping action with the net to stir up the bed.
2. Continue this process walking backwards and working upstream over the required distance.
3. If the bed is rocky and the rocks are too large to kick over, in addition to sweeping over a distance of 10m, remove about 10 rocks of a range of sizes and wash the macroinvertebrates from them into the net by scrubbing gently with the hands or a light brush. Leave the rocks out of the water to allow cryptic specimens to emerge. These can then be hand-picked, using tweezers. Leaving the rocks in the sun for too long will dry out and kill the animals.

Note: Both Runs and Pools can have silty/sandy and rocky/gravel beds. When sampling silty/sandy beds, select an area with plant litter or periphyton (not macrophytes) rather than an area of clean sand.

7.2.2.4 Composite bed

1. Sample 5m of each available bed habitat at the site, one after the other into the one net, using the

methods described in Section 7.2.2.2 and 7.2.2.3.

Note: If there is only one type of bed habitat present at the site, sample 10m of this habitat.

7.2.3 Completion of sampling

7.2.3.1 For live picking of sample

1. After sampling a habitat, empty the contents of the net into a bucket by inverting the net.
2. Splash water onto the inverted net to wash any remaining animals into the bucket.

7.2.3.2 For laboratory picking of sample

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 the sample falls into the tray.
3. Place material collected in the tray into a sample container. Large plastic screw-top jars or heavy-duty plastic bags stored in a polydrum are suitable containers.
4. Rinse the net into the tray to remove any remaining material.
5. Tip water from tray through a 250µm sieved 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
7. Ensure a completed label is placed in the vial noting the project name, site number and name, sampling date, habitat sampled, sample collector and picker and any relevant notes (Figure 1). Label must be completed using pencil or alcohol-proof ink and should be on waterproof paper. Labels should also be printed in alcohol-proof ink.
8. 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.
9. Preserve the sample with 70 % alcohol.

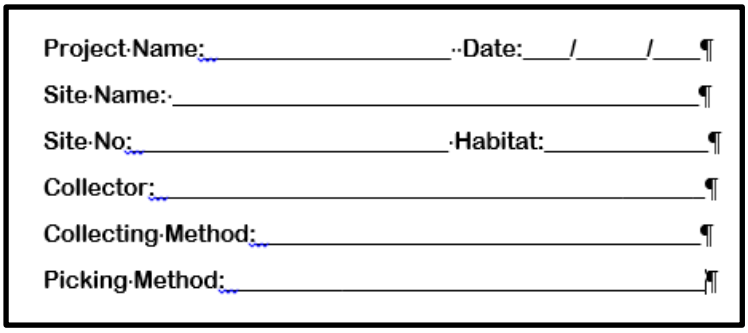

Figure 1: Label for laboratory picked sample

7.3 Live picking aquatic macroinvertebrates

7.3.1 Sample preparation

If the sample has a large amount of detrital material, it is advisable to separate it into two using a 1cm panning sieve. Place each fraction in a separate bucket or tray; making sure that there is water in the bucket to prevent animals from desiccating.

Note: Samples should be picked as soon as possible after collection. Keep samples wet, cool and tightly covered if there is any delay in picking. A single operator picks the sample.

7.3.2 Method

1. Set up table and chairs with timer, white trays, picking tool and vial or jar half filled with 70% ethanol or methylated spirits. For one of the first three samples collected in a sampling program, the procedure in Section 7.3.4 must be followed.
2. Place a small amount of the sample material and water into a white tray.

3. Start the timer.
4. Pick aquatic macroinvertebrates from the tray using tweezers, spoon and pipettes, and transfer into the vial with the ethanol (or methylated spirits). Suck up small taxa, e.g. mites, with a pipette.
    - Aim to collect only 10 individuals of any one taxon, i.e. do not spend a large proportion of picking time concentrating on animals that all appear to be the same. When uncertain of the identity, then collect all of the organisms. At least 30 midge larvae (Chironomidae) should be collected to ensure adequate representation of the sub-families. The aim of live-picking is essentially to collect the greatest number of taxa as possible from the sample. Ten individuals of apparent taxa provides some indication of abundance and assists in collecting taxa that are visually similar.
    - At the start of the live pick, the common and abundant taxa should be targeted for the first 5 minutes. After that, the major picking effort should be directed at finding the less common, inconspicuous taxa.
5. Work progressively through the sample, replacing picked material with fresh sample.
6. After picking for 30 minutes, search specifically for new taxa for 10 minutes. If any new taxa are found in these 10 minutes, extend the picking time by another 10 minutes.
7. Follow this procedure until either no new taxa are found or a maximum of 60 minutes have been spent on picking.
8. Record the picking time, and add to the vial label (Figure 2).
9. Record if the sample has very few animals and any likely reasons why that may be so (e.g. very silty/sandy bed). Add note to vial label (Figure 2).

Note:

- Ensure that you search through all components of the sample, including the sediment at the bottom of the bucket. Very occasionally, the collected sample is so large that it cannot be sorted adequately in the given time. In these circumstances, ensure that you have picked over all the different types of detritus, substrate and water-borne material present in your sample in the 60 minutes.
- If it is raining, cold or extreme heat, or conditions of poor light exist due to cloud cover or approaching twilight, the sample must be taken back to the vehicle/motel/camp etc. for sorting under cover and with improved light conditions. It is best to avoid these situations in the first place.

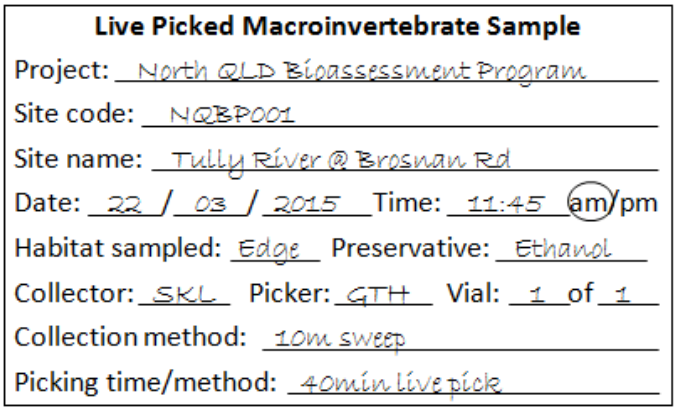

Figure 2: Example of a vial label for aquatic macroinvertebrate samples picked in the field

7.3.3 Curation and storage

1. When picking is completed, remove some of the liquid in the vial (which is usually diluted by water that has been added with the animals) using a mesh-covered syringe (Figure 3).
2. Refill the vial with fresh 70% ethanol or methylated spirits to the top and ensure the lid is tightly screwed on.
3. Ensure the vial you use is large enough. If the animals you collect take up more than 30% of the volume, use a larger container. Alternatively, use several vials clearly labelled to indicate that more than one vial was used e.g. ‘vial 1 of 3’.
4. A label must be placed in the vial, an example of the kind of information that should accompany the

sample is presented in Figure 2. Label must be completed using pencil or alcohol-proof ink. Labels should also be printed in alcohol-proof ink.

5. Samples should be packed upright, in rigid lidded containers. Foam inserts are useful to ensure samples remain upright.

Figure 3: Mesh covered syringe

7.3.4 Quality assurance and quality control of live-picked aquatic macroinvertebrates

7.3.4.1 Field-based review of live picking

For one of the first three live picks of a sampling round, the following procedure must be followed:

1. The sample is live picked by the operator (Section 7.3.2). During the pick, a field aquatic macroinvertebrate record sheet is filled in (Appendix 3).
2. An immediate re-pick is then undertaken by a second operator on the residue of the sample picked by the first operator. The second operator must be trained and accredited. The re-pick is undertaken in the same way as a normal field live-pick and the field aquatic macroinvertebrate record sheet completed by the first operator is also filled in by the second operator.
3. The field aquatic macroinvertebrate record sheet is immediately checked for inconsistencies, which are discussed between the two pickers to reduce systematic errors in subsequent live picks. If errors are large a check of a second sample should be considered.
4. Both samples are then preserved and transported to the laboratory for identification and further analysis.

7.3.4.2 Laboratory assessment of live pick

1. Samples from both operators are processed as outlined in Section 7.4.
2. Two statistical measures are then applied to the data:
    - The ratio of the live pick taxa richness from operator 1 to the total QA/QC sample taxa richness (sample from operator 1 + sample from operator 2) must be >0.90.
    - The Bray-Curtis Dissimilarity Index between samples picked by operator 1 and the total QA/QC sample taxa richness (sample from operator 1 + sample from operator 2) must be <0.2. Bray-Curtis Dissimilarity Index is based on presence/absence data.

Failure to meet these standards triggers a review of the operator's skills including consideration of re-training as required. Results of the quality assurance and quality control program should be presented in any report as evidence of quality in the results.

7.4 Laboratory picking aquatic macroinvertebrates

1. Tip the preserved sample into a series of 10mm and 250mm sieves and thoroughly wash the sample.
2. If there are large coarse fractions (sticks, leaves, etc.) wash these over the sieves and place them into a sorting tray. Examine these coarse fractions preferably using a magnifying glass, for approximately 10 minutes, ensuring that any macroinvertebrates attached to the coarse fractions are collected.

Note: keep an eye out for stick and leaf-cased Trichoptera.

3. Evenly distribute the remaining smaller fractions from the sieves into a subsampler. The subsampler used and recommended is a modified Marchant subsampler (Marchant, 1989) (Figure 4). This subsampler contains 100 circular cells, each 3.5cm in diameter x 3.5cm deep.
4. Fill the subsampler until the water level reaches the top of the cells, secure the lid, and rotate vigorously in both directions until the sample is distributed throughout the cells. Using a vacuum pump, subsample 10% (10 cells) of the whole sample, ensuring every 1% (1 cell) of the subsample is stored in separate containers. Label each container.
5. Sort and identify the subsamples (see sections 7.5.2 and 7.5.3 for a detailed method details), noting how many new taxa there are in every 1% subsample sorted.

Note: Other types of subsamplers may be used if a Marchant subsampler is not available. Overall, 10 % of the sample should be subsampled.

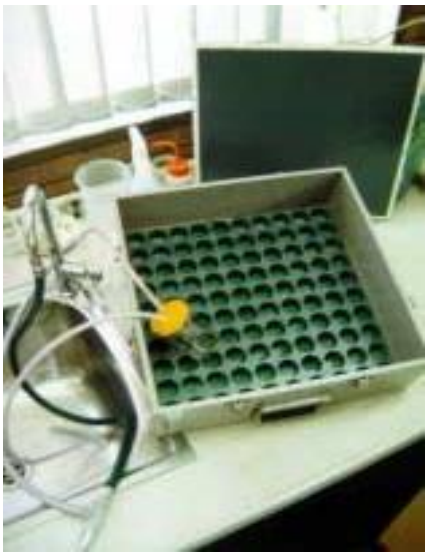

Figure 4: Modified Marchant subsampler

7.5 Identification and enumeration of aquatic macroinvertebrates to family level

7.5.1 Sample preparation

1. Rinse the preservative from the sample by placing it in a 250µm sieve and gently running water through it, or use a plastic 250ml specimen jar with a mesh insert in the lid (Figure 5a).
2. Using a water squeeze bottle, flush the sieved contents into a large petri dish or sorting tray lid (Figure 5b). If the sample is large flush the sieve into a 250mL specimen jar. Small amounts of the sample and water can then be poured into a sorting tray or petri dish for processing. If there is excess water in the sample it can be removed using a 250µm mesh-covered syringe.

Note: Always ensure that there is enough water left to completely cover all animals. When finished or if processing is interrupted for an extended period of time (e.g. overnight) replace the water with preservative (70% ethanol/methylated spirits and 3% glycerol).

7.5.2 Sample processing

1. Place sorting tray under a dissecting microscope (minimum 10x magnification) and search systematically for aquatic macroinvertebrates.
2. Remove the animals from the sorting tray under the lowest magnification and place in water in small petri dishes or glass specimen blocks for later identification. Use a separate dish for each type of taxa. It may be desirable to separate the adults, larvae, and pupae of some taxa such as beetles (adults and larvae) and dipterans (larvae and pupae), for ease of identification.

3. Work your way through the sorting tray until all specimens have been removed.
4. Adjust the microscope to a higher magnification and pass over the sorting tray once more. This will ensure that the smaller specimens that may be missed at a lower magnification are seen.
5. Discard any non-macroinvertebrate material that may have been collected.
6. Select a polypropylene vial or glass jar that is large enough to fit the entire sample and a label. Use pencil or alcohol-proof ink to fill in details. The label must have all the information presented in Figure 6.
7. Place the label in the vial and half fill the vial with preservative (70% ethanol (or methylated spirits)/3% glycerol/27% water).
8. Select a group of specimens and identify each specimen using the appropriate taxonomic keys. Organisms are identified to various taxa level as outlined in the example *Aquatic Macroinvertebrate Field Record Sheet* presented in Appendix 3. If uncertain about the identity, obtain a second opinion from a colleague or local specialist. If a new taxon is suspected, contact a taxonomic specialist or alternatively the Queensland Museum.
9. When the specimen has been identified, place it in the vial and tally in the identification sheet (see example sheet provided in Appendix 4).
10. Once all taxa have been identified and placed in the vial, fill it with preservative (70% ethanol (or methylated spirits)/3% glycerol/27% water) and screw the lid on firmly.
11. Record the total tally for each taxon.

(a) (b)

Figure 5: Example laboratory equipment used for aquatic macroinvertebrate sorting – (a) 250mL specimen jar with a mesh insert in the lid and (b) sorting tray and fine forceps.

Identified Macroinvertebrate Sample

Project: North QLD Bioassessment Program

Site code: NQBP001

Site name: Tully River @ Brosnan Rd

Date: 22 / 03 / 2015 Time: 11:45 am/pm

Habitat sampled: Edge Taxa: All

Collector: SKL Picker: GTH

ID by: PRT ID date: 01 / 04 / 2015

Vial 1 of 1 Preservative: 70% Ethanol

Figure 6: Example laboratory vial label

7.5.3 Quality assurance of taxonomic identification of aquatic macroinvertebrates to family level

7.5.3.1 QA/QC requirements

For each person undertaking aquatic macroinvertebrate identification (the operator) the following checks should occur:

1. The first five samples processed from a sampling round are to be re-checked by someone who is accredited (or equivalent) to identify aquatic macroinvertebrates.
2. Ten per cent of each operator's samples must be randomly selected for re-identification. These must not include the initial five samples.

For the samples that are undergoing a QA/QC check:

3. A QA/QC laboratory tally sheet (Appendix 5) is used to record the identities and numbers of all taxa in a sample. Two columns are provided so that the original tallies can be recorded alongside the checked tallies. Original tallies should only be obtained after the check has been completed.
4. If there are discrepancies in either identification these should be checked. This may entail checking that all animals were flushed from the vial and label, that all the animals were removed from the sorting tray to the holding dishes, and that there are no small animals hidden by or attached to larger ones.

7.5.3.2 Calculation of quality assurance results

Determining whether or not an operator meets laboratory QA/QC standards can be derived from two statistical measures:

- Ratio of taxon richness in the original sample identification (by the operator) and QA identification (by accredited person) (see section 7.5.3.1) must be >0.90
- Number of correct identifications (>90%).

Failure to meet these standards triggers a review of the operator's skills including consideration of re-training as required.

7.6 Long-term sample preservation and storage

1. Samples in small vials can be permanently stored in an evaporation-proof container such as a large 2L glass jar (polypropylene containers are also appropriate) filled with preservative to ensure individual samples do not dry-out. If a jar is under-filled, the remaining space should be filled with cotton wool to reduce movement of the vials in the jar.
2. The jar must be labelled with a unique number, with the project name, dates and sample identifiers (e.g. Samples 45-104) written on a label so it can be seen from the outside of the jar.
3. Details of the contents and location of each jar should be stored in a central location, ideally in an electronic format to enable samples to be located at a future date.
4. The jars must be checked annually and topped up with the preservative if required. A local schedule for maintenance should be implemented to ensure this occurs.

7.7 Calculation of richness, SIGNAL, PET taxa richness, per cent sensitive taxa and per cent tolerant taxa

7.7.1 Richness (taxa) index

Count the number of different taxa at the relevant level of taxonomic resolution (as outlined in the aquatic macroinvertebrate laboratory identification sheet – Appendix 4).

7.7.2 Average SIGNAL index calculation

1. Allocate a SIGNAL grade number to each taxon in the sample. SIGNAL grade numbers for version 2.4 are available from the SIGNAL manual (Chessman 2003) which can be found at http://www.environment.gov.au/resource/signal-2iv-scoring-system-macroinvertebrates-water-bugs-

australian-rivers.

2. The SIGNAL Index is calculated for each sample by averaging the SIGNAL grade numbers of all of the aquatic macroinvertebrate taxa collected in a sample.
3. Taxa that do not have a SIGNAL grade number, for example Copepoda, Cladocera and Ostracoda, are not used in the calculation of the SIGNAL Index.

7.7.3 PET taxa richness

PET taxa richness is the count of families (or genera/species) that belong to the following three orders of aquatic macroinvertebrates: Plecoptera (stoneflies), Ephemeroptera (mayflies) and Trichoptera (caddisflies).

7.7.4 Calculating the % sensitive taxa index using presence/absence data

1. Count the number of taxa in a sample that have SIGNAL grade numbers.
2. Count the number of taxa that are sensitive (SIGNAL grade ≥8).
3. Calculate the per cent sensitive taxa using the formula below:

$$\%\ \text{Sensitive Taxa Index} = \frac{\text{Number of taxa with SIGNAL grade numbers} \geq 8}{\text{Total number of taxa in sample with SIGNAL grade numbers}} \times 100$$

7.7.5 Calculating the % tolerant taxa index using presence/absence data

1. Count the total number of taxa in a sample that have SIGNAL grade numbers.
2. Count the number of taxa that are sensitive (SIGNAL grade ≤3).
3. Calculate the per cent sensitive taxa using the formula below:

$$\%\ \text{Tolerant Taxa Index} = \frac{\text{Number of taxa with SIGNAL grade numbers} \leq 3}{\text{Total number of taxa in sample that have SIGNAL grade numbers}} \times 100$$

8 References and additional reading

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Appendix 1 Equipment checklist

Table 1 Aquatic habitat identification

Equipment	✓
Flow current meter	
Depth measuring device (ruler or pole with tape measure)	

Table 2 Sampling aquatic macroinvertebrates using a dip net

Equipment	✓
1 x net (Triangular 250mm x 250mm x 250mm opening, 1-1.5m handle, Mesh: 250µm with depth of 50-75cm). Spare net or equipment to mend net.	
3 x buckets	
Note book or field sampling sheet	
Pens, pencils, waterproof markers	
Waders	

Table 3 Live picking aquatic macroinvertebrates

Equipment	✓
Large white plastic trays (minimum 2) (approx. 40cm x 30cm x 6cm)	
Picking tools: very fine forceps, plastic Pasteur pipettes, plastic spoon	
Timer	
Buckets (minimum 2 per sample)	
Sieve (1cm mesh, 30cm diameter)	
Table and two chairs	
Alcohol stable 30mL, 70mL, 120mL and 250mL plastic screw top vials/jars	
Note book or field sampling sheet	
Alcohol stable sample labels (Figure 1)	
Mesh-covered syringe	
250µm mesh dip net, or 250µm mesh sieve	
Pencil or alcohol-proof ink	
Container with lid for picked sample vials preferable with foam insert	
70% ethanol or methylated spirits	

Table 4 Identification of aquatic macroinvertebrates

Equipment	✓
250µm sieve, or plastic 250ml specimen jar with a mesh insert in the lid	
Dissecting stereomicroscope (x6 to x50 magnification)	
Sorting tray or large glass petri dish	
Glass petri dishes/glass specimen blocks (watchglasses)	
Fine forceps	
Alcohol and water wash bottles	
Storage vials (polypropylene) 5ml, 25ml	
Glass storage jars (wide mouth) 70ml	
Vial and curatorial jars labels	
Laboratory identification sheet (Appendix 4)	
Taxonomic (identification) keys	
Pencil or alcohol-proof ink	
Cotton wool or cardboard filler, if required	
70% ethanol or methylated spirits	
Mesh covered syringe	

Appendix 2 Velocity: Depth table

For use in identifying aquatic habitats based on Velocity to Depth ratio.

- V:D > 0.032 = riffle (R=Riffle in Table 5)
- V:D < 0.0124 = pool (P=Pool in Table 5)
- V:D 0.0124 – 0.032 = run (Run in Table 5)

Table 5 The Velocity: Depth table to identify stream habitat types (R = riffle and P = pool)

Depth (cm) \ Velocity (m/sec)	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5
5	P	run	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
10	P	P	run	run	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
15	P	P	run	run	run	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
20	P	P	P	run	run	run	run	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
25	P	P	P	P	run	run	run	run	run	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
30	P	P	P	P	run	run	run	run	run	run	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
35	P	P	P	P	P	run	run	run	run	run	run	run	R	R	R	R	R	R	R	R	R	R	R	R	R	R
40	P	P	P	P	P	run	run	run	run	run	run	run	run	R	R	R	R	R	R	R	R	R	R	R	R	R
45	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	R	R	R	R	R	R	R	R	R	R	R
50	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	R	R	R	R	R	R	R	R	R
55	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	R	R	R	R	R	R	R	R
60	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	R	R	R	R	R	R
65	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	R	R	R	R	R
70	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	run	run	R	R	R
75	P	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	run	run	run	R
80	P	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	run	run	run	run
85	P	P	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	run	run	run
90	P	P	P	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	run	run
95	P	P	P	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	run	run
100	P	P	P	P	P	P	P	P	P	P	P	P	P	run	run	run	run	run	run	run	run	run	run	run	run	run

Appendix 3 Example aquatic macroinvertebrate field record sheet

(appears on following page)

Aquatic Macroinvertebrate Field Record Sheet

Project:_____ Vial: ___ of ___ *(e.g. 2 of 3)* Collectors initials: __ __ __

Site code: _____ Habitat: _____ *(e.g. Edge)*

Site name: _____ Picker 1 name: _____ Picker 2 name: _____

Sampling run: _____ Picker 1 (original picker) signature: Picker 2 (re-picker) signature:

Sampling date: __ __ / __ __ / 20_____ _____ _____

Picker 1 (original picker): tally number of specimens in column "1" next to the correct taxonomic group
Picker 2 (re-picker): tally number of specimens in column "2" next to the correct taxonomic group

	1	2		1	2		1	2		1	2		1	2
Lower Phyla			Glacidorbidae			s-f Tanypodinae			Georissidae			**Zygoptera**		
Rotifera			Tateidae [ex-Hydrobiidae]			Culicidae			Gyrinidae			Argiolestidae [ex-Megapodagrionidae]		
Porifera			Lymnaeidae			Dixidae			Haliplidae			Calopterygidae		
Hydridae			Neritidae			Dolichopodidae			Heteroceridae			Chorismagrionidae		
Clavidae			Physidae			Empididae			Hydraenidae			Coenagrionidae		
Dugesiidae			Planorbidae			Ephydridae			Hydrochidae			Diphlebiidae		
Temnocephaloidea			Thiaridae			Muscidae			Hydrophilidae			Isostictidae		
Nemertea			Viviparidae			Psychodidae			Hygrobiidae			Lestidae		
Nematoda			Corbiculidae			Sciomyzidae			Limnichidae			Platycnemidae [ex-Protoneuridae]		
Gordiidae			Hyriidae			Simuliidae			Nanophyidae [ex-Brentidae]			Synlestidae		
Oligochaeta			Sphaeriidae			Stratiomyidae			Noteridae			**Epiprocta**		
Polychaeta			**Megaloptera**			Tabanidae			Psephenidae [adults terrestrial]			Aeshnidae		
Hirudinea			Corydalidae			Tanyderidae			Ptilodactylidae			Austrocorduliidae		
Glossiphoniidae			Sialidae			Thaumaleidae			Scirtidae			Cordulephyidae		
Hirudinidae			**Ephemeroptera**			Tipulidae			Spercheidae			Corduliidae		
Erpobdellidae			Ameletopsidae			**Hemiptera**			Sphaeriusidae [ex-Microsporidae]			Gomphidae		
Acarina			Baetidae			Aphelocheiridae			Staphylinidae			Hemicorduliidae		
Collembola			Caenidae			Belostomatidae			**Trichoptera**			Libellulidae		
Sminthurididae			Ephemerellidae			Corixidae			Antipodeciidae			Lindeniidae		
Crustacea			Leptophlebiidae			Gelastocoridae			Atriplectididae			Macromiidae		
Spinicaudata			Prosopistomatidae			Gerridae			Calamoceratidae			Pseudocorduliidae		
Cladocera			**Neuroptera**			Hebridae			Calocidae			Synthemistidae		
Ostracoda			Neurorthidae			Hydrometridae			Conoesucidae			Telephlebiidae		
Copepoda			Osmylidae			Leptopodidae			Dipseudopsidae			Urothemistidae		
Notostraca			Sisyridae			Mesoveliidae			Ecnomidae			**Moths**		
Psammaspididae			**Plecoptera**			Micronectidae			Glossosomatidae			Crambidae [ex-Pyralidae]		
Amphi-Chiltoniidae [Ex. Ceinidae]			Austroperlidae			Naucoridae			Helicophidae					
Amphi-Corophiidae			Eustheniidae			Nepidae			Helicopsychidae			**OTHER TAXA**		
Amphi-Eusiridae			Gripopterygidae			Notonectidae			Hydrobiosidae					
Iso-Sphaeromatidae			Notonemouridae			Ochteridae			Hydropsychidae					
Iso-Corallanidae			**Diptera**			Pleidae			Hydroptilidae					
Atyidae			Athericidae			Saldidae			Leptoceridae					
Palaemonidae			Blephaceridae			Veliidae			Odontoceridae					
Parastacidae			Ceratopogonidae			**Coleoptera**			Philopotamidae					
Parathelphusidae			Corethrellidae			Carabidae			Philorhreithridae					
Trogloplacidae			Chaoboridae			Chrysomelidae			Polycentropodidae					
Mollusca			Chironomidae			Curculionidae			Psychomyiidae					
Ancylidae [part Planorbidae]			s-f Chironominae			Dytiscidae			Tasimiidae					
Bithyniidae			s-f Orthocladiinae			Elmidae			Stenopsychidae					

Appendix 4 Example of a laboratory identification sheet

(appears on following page)

Aquatic Macroinvertebrate Identification Sheet

Project:_____

Site code: _____ Vial: ___ of ___ *(e.g. 2 of 3)* I.D. by: __ __ __

Site name: _____ Habitat: _____ *(e.g. Edge)* I.D. date: __ __ / __ __ / 20____

Sampling run: _____ Collectors initials: __ __ __ Entered by: __ __ __

Sampling date: __ __ / __ __ / 20_____ Picker initials: __ __ __ Entry checked by: __ __ __

Counts by taxa

Lower Phyla		Glacidorbidae		s-f Tanypodinae		Georissidae		**Zygoptera**	
Rotifera		Tateidae [ex-Hydrobiidae]		Culicidae		Gyrinidae		Argiolestidae [ex-Megapodagrionidae]	
Porifera		Lymnaeidae		Dixidae		Haliplidae		Calopterygidae	
Hydridae		Neritidae		Dolichopodidae		Heteroceridae		Chorismagrionidae	
Clavidae		Physidae		Empididae		Hydraenidae		Coenagrionidae	
Dugesiidae		Planorbidae		Ephydridae		Hydrochidae		Diphlebiidae	
Temnocephaloidea		Thiaridae		Muscidae		Hydrophilidae		Isostictidae	
Nemertea		Viviparidae		Psychodidae		Hygrobiidae		Lestidae	
Nematoda		Corbiculidae		Sciomyzidae		Limnichidae		Platycnemidae [ex-Protoneuridae]	
Gordiidae		Hyriidae		Simuliidae		Nanophyidae [ex-Brentidae]		Synlestidae	
Oligochaeta		Sphaeriidae		Stratiomyidae		Noteridae		**Epiprocta**	
Polychaeta		**Megaloptera**		Tabanidae		Psephenidae [adults terrestrial]		Aeshnidae	
Hirudinea		Corydalidae		Tanyderidae		Ptilodactylidae		Austrocorduliidae	
Glossiphoniidae		Sialidae		Thaumaleidae		Scirtidae		Cordulephyidae	
Hirudinidae		**Ephemeroptera**		Tipulidae		Spercheidae		Corduliidae	
Erpobdellidae		Ameletopsidae		**Hemiptera**		Sphaeriusidae [ex-Microsporidae]		Gomphidae	
Acarina		Baetidae		Aphelocheiridae		Staphylinidae		Hemicorduliidae	
Collembola		Caenidae		Belostomatidae		**Trichoptera**		Libellulidae	
Sminthuridídae		Ephemerellidae		Corixidae		Antipodeciidae		Lindeniidae	
Crustacea		Leptophlebiidae		Gelastocoridae		Atriplectididae		Macromiidae	
Spinicaudata		Prosopistomatidae		Gerridae		Calamoceratidae		Pseudocorduliidae	
Cladocera		**Neuroptera**		Hebridae		Calocidae		Synthemistidae	
Ostracoda		Neurorthidae		Hydrometridae		Conoesucidae		Telephlebiidae	
Copepoda		Osmylidae		Leptopodidae		Dipseudopsidae		Urothemistidae	
Notostraca		Sisyridae		Mesoveliidae		Ecnomidae		**Moths**	
Psammaspididae		**Plecoptera**		Micronectidae		Glossosomatidae		Crambidae [ex-Pyralidae]	
Amphi-Chiltoniidae [Ex. Ceinidae]		Austroperlidae		Naucoridae		Helicophidae			
Amphi-Corophiidae		Eustheniidae		Nepidae		Helicopsychidae		**OTHER TAXA**	
Amphi-Eusiridae		Gripopterygidae		Notonectidae		Hydrobiosidae			
Iso-Sphaeromatidae		Notonemouridae		Ochteridae		Hydropsychidae			
Iso-Corallanidae		**Diptera**		Pleidae		Hydroptilidae			
Atyidae		Athericidae		Saldidae		Leptoceridae			
Palaemonidae		Blephaceridae		Veliidae		Odontoceridae			
Parastacidae		Ceratopogonidae		**Coleoptera**		Philopotamidae			
Parathelphusidae		Corethrellidae		Carabidae		Philorhreithridae			
Trogloplacidae		Chaoboridae		Chrysomelidae		Polycentropodidae			
Mollusca		Chironomidae		Curculionidae		Psychomyiidae			
Ancylidae (part anorbidae))		s-f Chironominae		Dytiscidae		Tasimiidae			
Bithyniidae		s-f Orthocladiinae		Elmidae		Stenopsychidae			

NOTES [e.g. taxa kept aside/sent away for expert ID]: _____

Appendix 5 Example of a QA/QC laboratory identification sheet

(appears on following page)

Aquatic Macroinvertebrate Identification Sheet – QA/QC

Project:_____ Vial: ___ of ___ *(e.g. 2 of 3)* QA/QC date: __ __ / __ __ / 20____

Site code: _____ Habitat: _____ *(e.g. Edge)* QA/QC I.D. by: __ __ __

Site name: _____ Picker initials: __ __ __ Verification by: __ __ __

Sampling run:_____ Original I.D. by: __ __ __ Entered by: __ __ __

Sampling date: __ __ / __ __ / 20_____ I.D. date: __ __ / __ __ / 20____ Entry checked by: __ __ __

Counts by taxa

	QA			QA			QA			QA			QA	
Lower Phyla			Glacidorbidae			s-f Tanypodinae			Georissidae			**Zygoptera**		
Rotifera			Tateidae [ex-Hydrobiidae]			Culicidae			Gyrinidae			Argiolestidae [ex-Megapodagrionidae]		
Porifera			Lymnaeidae			Dixidae			Haliplidae			Calopterygidae		
Hydridae			Neritidae			Dolichopodidae			Heteroceridae			Chorismagrionidae		
Clavidae			Physidae			Empididae			Hydraenidae			Coenagrionidae		
Dugesiidae			Planorbidae			Ephydridae			Hydrochidae			Diphlebiidae		
Temnocephaloidea			Thiaridae			Muscidae			Hydrophilidae			Isostictidae		
Nemertea			Viviparidae			Psychodidae			Hygrobiidae			Lestidae		
Nematoda			Corbiculidae			Sciomyzidae			Limnichidae			Platycnemidae [ex-Protoneuridae]		
Gordiidae			Hyriidae			Simuliidae			Nanophyidae [ex-Brentidae]			Synlestidae		
Oligochaeta			Sphaeriidae			Stratiomyidae			Noteridae			**Epiprocta**		
Polychaeta			**Megaloptera**			Tabanidae			Psephenidae [adults terrestrial]			Aeshnidae		
Hirudinea			Corydalidae			Tanyderidae			Ptilodactylidae			Austrocorduliidae		
Glossiphoniidae			Sialidae			Thaumaleidae			Scirtidae			Cordulephyidae		
Hirudinidae			**Ephemeroptera**			Tipulidae			Spercheidae			Corduliidae		
Erpobdellidae			Ameletopsidae			**Hemiptera**			Sphaeriusidae [ex-Microsporidae]			Gomphidae		
Acarina			Baetidae			Aphelocheiridae			Staphylinidae			Hemicorduliidae		
Collembola			Caenidae			Belostomatidae			**Trichoptera**			Libellulidae		
Sminthuridídae			Ephemerellidae			Corixidae			Antipodeciidae			Lindeniidae		
Crustacea			Leptophlebiidae			Gelastocoridae			Atriplectididae			Macromiidae		
Spinicaudata			Prosopistomatidae			Gerridae			Calamoceratidae			Pseudocorduliidae		
Cladocera			**Neuroptera**			Hebridae			Calocidae			Synthemistidae		
Ostracoda			Neurorthidae			Hydrometridae			Conoesucidae			Telephlebiidae		
Copepoda			Osmylidae			Leptopodidae			Dipseudopsidae			Urothemistidae		
Notostraca			Sisyridae			Mesoveliidae			Ecnomidae			**Moths**		
Psammaspididae			**Plecoptera**			Micronectidae			Glossosomatidae			Crambidae [ex-Pyralidae]		
Amphi-Chiltoniidae [Ex. Ceinidae]			Austroperlidae			Naucoridae			Helicophidae					
Amphi-Corophiidae			Eustheniidae			Nepidae			Helicopsychidae			**OTHER TAXA**		
Amphi-Eusiridae			Gripopterygidae			Notonectidae			Hydrobiosidae					
Iso-Sphaeromatidae			Notonemouridae			Ochteridae			Hydropsychidae					
Iso-Corallanidae			**Diptera**			Pleidae			Hydroptilidae					
Atyidae			Athericidae			Saldidae			Leptoceridae					
Palaemonidae			Blephaceridae			Veliidae			Odontoceridae					
Parastacidae			Ceratopogonidae			**Coleoptera**			Philopotamidae					
Parathelphusidae			Corethrellidae			Carabidae			Philorhreithridae					
Trogloplacidae			Chaoboridae			Chrysomelidae			Polycentropodidae					
Mollusca			Chironomidae			Curculionidae			Psychomyiidae					
Ancylidae [part Planorbidae]			s-f Chironominae			Dytiscidae			Tasimiidae					
Bithyniidae			s-f Orthocladiinae			Elmidae			Stenopsychidae					