EPA VICTORIA

Guideline for environmental management (GEM) – Rapid bioassessment methodology for rivers and streams

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EPA acknowledges Aboriginal people as the first peoples and Traditional custodians of the land and water on which we live, work and depend. We pay respect to Aboriginal Elders, past and present.

As Victoria's environmental regulator, we pay respect to how Country has been protected and cared for by Aboriginal people over many tens of thousands of years.

We acknowledge the unique spiritual and cultural significance of land, water and all that is in the environment to Traditional Owners, and recognise their continuing connection to, and aspirations for Country.

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1 Introduction

Due to the complexity of factors influencing stream and river health, a variety of physical, chemical and biological indicators can be used to describe the condition of waterbodies. An assessment based solely on the measurement of physical/chemical parameters may not detect adverse water quality conditions if:

- measurements have not been taken at the right time
- measurements have not been taken under the right conditions
- the right indicator has not been measured.

Furthermore, the effects of contamination on an aquatic ecosystem may last long after physical and/or chemical conditions have returned to normal.

Inclusion of holistic measures to assess the condition of an aquatic ecosystem has now become a standard approach in many jurisdictions, both in Australia and around the world. This biological assessment approach examines the state of the biological community of rivers and streams. It is used in conjunction with other factors such as:

- water quality
- in-stream habitat
- stream flows
- and riparian condition,

to assess the overall health of aquatic ecosystems.

The great value in directly monitoring the biological community is that it responds to all types of disturbances and toxicants. The net effect of all environmental factors – including impacts and stresses over a period of weeks, months or years – is captured, effectively summarising the history of conditions in the stream.

A form of biological assessment referred to as *rapid bioassessment* is now incorporated into a variety of State and Commonwealth policies, strategies and regulatory measures as a means for assessing:

- levels of attainment against established environmental refence standards
- progress towards meeting defined targets for improved environmental quality
- potential risks to aquatic ecosystems from the impacts of human activities
- the environmental condition, or health of aquatic ecosystems.

Recent advances in genetic analysis techniques, including DNA metabarcoding and environmental DNA analysis (eDNA), have enabled the creation of genetic libraries. DNA metabarcoding is now becoming increasingly available to supplement morphological collection and identification. These new techniques can facilitate rapid bioassessment and increase taxonomic resolution of invertebrates identified. The GEM provides a brief introduction into the use and sample preparation for the use of DNA barcoding in bioassessment.

1.1 Purpose

This Guideline for Environmental Management (GEM) describes the procedures for the rapid bioassessment of rivers and streams. The purpose of GEM is to provide the common techniques, methods and standards for sample collection, quality assurance and control for use by Victorian Government agencies, relevant persons and organisations. It updates and replaces *Rapid Bioassessment of Victorian Streams: The approach and methods of the Environment Protection Authority* (2003).

1.2 What is rapid bioassessment?

Aquatic ecosystems harbour a diversity of organisms, including macrophytes, algae, bacteria, invertebrates and fish. While any of these can, and have been used to monitor stream and river condition, Victoria's approach to biological assessment specifically uses aquatic invertebrates to assess stream and river health. Invertebrates are ideal organisms for biological monitoring because they are:

- found in all aquatic habitats
- typically fairly abundant
- easy to sample
- critical to stream functioning
- well-known to biologists on aspects of their biology and ecological requirements. Therefore, considerable data is available on how they respond to various forms of disturbance, such as pollution, and catchment land use change.

The rapid bioassessment approach currently used in Victoria has been elaborated and refined over the last twenty years (EPA Victoria 1998). It is based on similar methods developed independently in the United Kingdom (Wright et al. 1984) and the USA (Plafkin et al. 1989). Originally, a highly quantitative and costly approach focused on measuring density changes in invertebrate populations in response to impacts. The emphasis then shifted to a semi-quantitative (rapid) assessment of the diversity of the invertebrate community present at a site. This approach, still used to date in Victoria, underpinned the development, under the National River Health Program (CEPA 1994), of a predictive modelling tool for assessing river ecosystem health known as AUSRIVAS (Australian Rivers Assessment System).

The standard method for rapid bioassessment in rivers and streams involves the collection of two biological samples: a streambed, or benthic sample collected in fast flowing waters (the kick sample); and an edge, or littoral sample collected in slow flowing habitats and around in-stream vegetation (the sweep sample). Both samples are live sorted in the field, and the resulting invertebrate samples retained for identification in the laboratory. Kick and sweep samples can be collected in either of two seasons, autumn and spring. In addition to the biological samples, a suite of chemical (water quality) and physical (in-stream and riparian habitat) parameters are assessed at each site. Geographical information such as location, including latitude and longitude, altitude, slope and position within the river catchment, are also calculated in recognition of the significant influence of biogeography on aquatic ecosystems.

These parameters and measurements are used in the final assessment process, in which a range of invertebrate indices, including AUSRIVAS, is calculated to assess stream health. Some of the physical and chemical measures are used directly as predictor variables in AUSRIVAS models. Others are used to interpret index scores and diagnose possible causes of impaired environmental quality at a site.

1.3 Application of the Guideline (GEM)

The GEM must be followed wherever rapid bioassessment is undertaken for purposes required by the *Environment Protection Act 2017* including, but not limited to, works approvals, licence monitoring and where assessment against Environmental Reference Standards is required.

Under the *Environment Protection Act 2017*, EPA Victoria is responsible for developing environmental guidelines within the regulatory framework that encourages best practise. EPA requires that all rapid bioassessment undertaken for licensing and works approval purposes be conducted by an organisation accredited by training provided by the University of Canberra or previously provided by EPA (e.g. when approval or licensing is required for discharge of wastewater to waterways, EPA publication 1287). In addition, water quality measurements should be analysed in a facility accredited by the National Association of Testing Authorities (NATA). In special circumstances EPA may approve the use of a non-accredited organisation or business. Such approval must be sought prior to the commencement of the program.

The GEM should also be followed where rapid bioassessment is required under various State and national strategies, for example, as occurred in 2010 in the Index of Stream Condition: The third benchmark on stream condition (DELWP 2010).

The GEM does not provide directions on study design, site selection or reporting as these elements will vary in accordance with each individual study's objectives and assessment needs. It is also important to note that the rapid bioassessment method is not appropriate for all studies of stream impacts. This approach has been shown to work when large changes in community structure or changes in community composition occur. When an impact is suspected to cause only small changes in species composition or changes only to population density, a quantitative method of sampling may be more appropriate.

1.4 Audience

These guidelines are designed to be used by government agencies, private corporations, consulting firms, the scientific community and the broader community. Groups or organisations applying rapid bioassessment will need to have appropriate training to ensure quality control and accuracy. EPA can provide general assistance while the University of Canberra can assist with a formal training program. EPA can also provide assistance with voucher collections and other aspects of sampling detailed within this document.

3 Getting started

Preparation for a site visit requires forward planning. Issues such as prevailing weather conditions, transportation needs and field safety must be considered before embarking on a trip. Sufficient lead time should be allowed for gathering all the necessary information required to locate a site and the field equipment required for sampling.

3.1 Field safety

Risks may arise from any number of hazards associated with the tasks to be undertaken on a bioassessment field trip and the likely prevailing physical conditions at the time of sampling. These risks must be assessed prior to commencement of sampling, and appropriate risk management steps put in place. Always keep the following safety precautions in mind:

- Never sample a site alone; always work in teams of two or more.
- Do not attempt to sample in areas of excessively fast flows.
- Always test the water depth before entering a stream.
- It is recommended that a buoyancy vest be worn when working in swift or deep water.
- Wader suspenders should be worn over all other clothing, including buoyancy vests and wet weather gear, to enable the rapid removal of waders if required.
- Wash hands with antibacterial soap after sampling and before eating.
- Dependent on the likely pollutant inputs at proposed sampling sites, appropriate immunisations may be required prior to the start of a sampling season. Consult a qualified doctor.
- If sampling downstream of industrial sites, such as a sewage treatment plant, or in potential blue-green algal bloom sites, waders, safety glasses, gloves and long sleeve clothing should be worn.
- When sampling in urban sites, use extra caution as many hazardous objects (e.g. syringes, broken glass) often end up in these waterways.
- Chemicals used in the field (e.g. ethanol) should be transported and used in accordance with appropriate safe handling protocols.
- Sampling should not be undertaken when a stream is in spate or flood, or for four weeks after such an event. Therefore, it is a good idea to check the status of stream flows before visiting a site. Information on the status of stream flows throughout Victoria can be obtained from the Bureau of Meteorology website (www.bom.gov.au).

3.2 Preparing for a site visit

Prior to visiting a site, you will need to:

- Gather all sampling equipment. A complete checklist is provided in Appendix 1. Ensure the provision of sufficient sample jars, water chemistry bottles and field sheets for all the intended sites. Always carry spares.
- Obtain new sample bottles for the nutrient (water chemistry) samples according to appropriate protocols (EPA Victoria 2009). Pre-labelling sample vials and sampling bottles is also recommended to increase productivity in the field.

- Calibrate all field meters, and ensure the batteries are working and spares are available.
- Collect all necessary documentation for locating the sites to be visited. If a site has been sampled previously, make certain sufficient information is available to confirm the site's exact location. This should include the original sampling trip description of how to get to the site, and an accompanying diagram of where the survey reach is located (refer to Appendix 2 for an example of a detailed site map), as well as latitude and longitude readings (or AMGs) and a GPS or mobile phone map to confirm the site location. Photographs are also very useful for identifying a previously visited site.
- Obtain any permits needed for accessing or sampling at the site. These may include collecting permits (www.DELWP.vic.gov.au), permits for sampling within National Parks (www.parks.vic.gov.au), keys for access to closed catchments, or permission for entering private property.
- Collection of samples for DNA metabarcoding requires appropriate handling and preservation. Make sure to obtain sterile vials to collect samples, and only use ethanol as a preservative.

3.3 Ordering of tasks

The first consideration upon arrival at a site is the order in which different tasks will be carried out. This is necessary because collection of some samples, such as invertebrates which require disturbing the streambed, can interfere with the collection of others, such as water chemistry samples.

Perform the tasks that create the least amount of disturbance first, and always proceed in an upstream direction when sampling.

Given that the invertebrate samples are collected from a diversity of habitats (refer to Section 3), it may be necessary for the kick and sweep samplers to take turns sampling their respective habitats as they move upstream to avoid interfering with each other's efforts.

Figure 1 depicts the recommended order in which tasks should be undertaken in the field to minimise interference in the collection and measurement of the various samples and parameters.

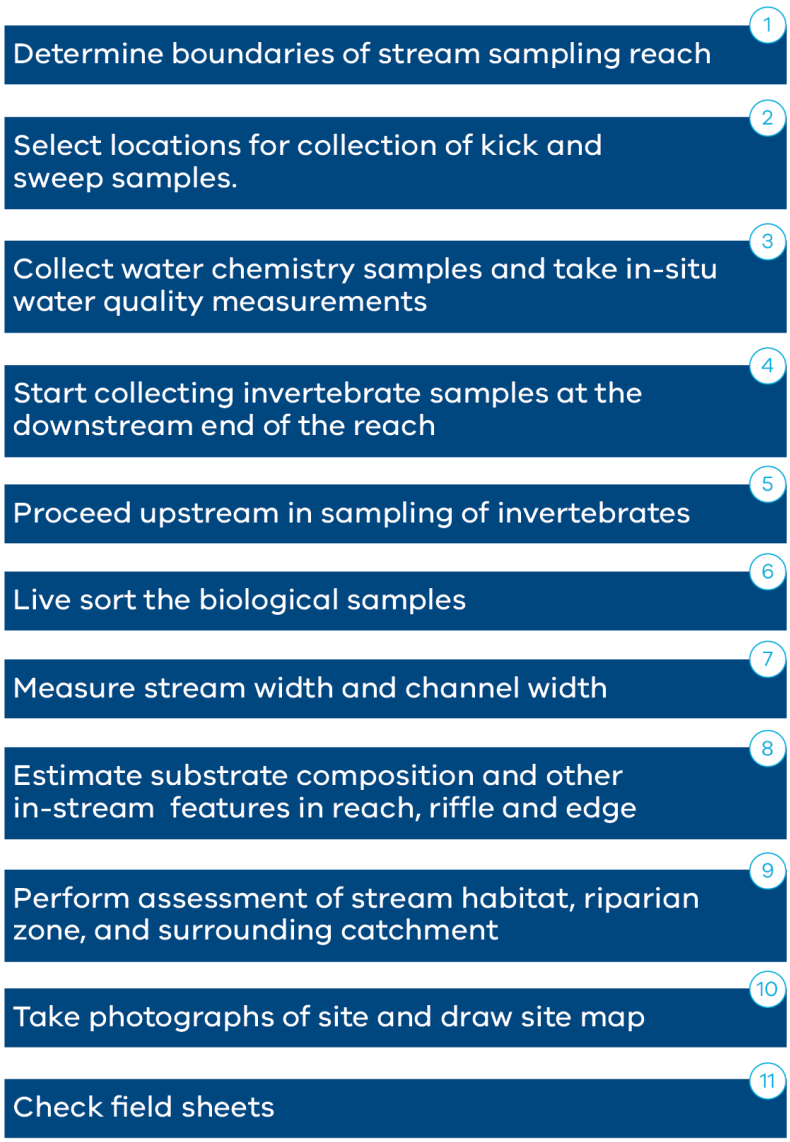

Figure 1: order of tasks to be undertaken in the field

4 Biological sampling

The key component of rapid bioassessment in rivers and streams is the collection of invertebrate samples. To ensure that results from different sites and different studies can be compared, the collection and sorting of the two biological samples required must follow the procedures in this guideline.

For a complete site assessment, samples can be collected in either autumn and spring seasons (i.e. within the same year, or in consecutive years).

When using the rapid bioassessment approach for broad-scale monitoring, keep in mind that the habitats in which invertebrate samples are collected must be representative of the types of habitats available on a larger scale at the site sampled and wherever possible, avoid sampling directly under or immediately upstream or downstream of a bridge or ford, or in the impounded waters of a small dam or weir. Refer to figure 1 for a brief overview of the biological sampling methods.

4.1 Kick sample (riffle habitat)

A kick sample is collected from wadeable areas of a stream where current velocities are moderate to high. Typically, these are rocky riffles where the flow is rapid and turbulent (Figure 2), but gravel or sand bars can also be sampled as long as there is adequate flow over these substrates. In the absence of a riffle, runs may be sampled.


Figure 2: examples of riffle habitats

A kick sample is not appropriate for pools or areas with muddy bottoms as it requires the presence of flow to divert dislodged invertebrates into the collecting net. If a riffle or run is not present at a site, or these habitats are too dangerous to sample, a kick sample should not be collected. Because current speed and stream depth are strongly affected by flow level, the ability to collect a kick sample at any given site may vary seasonally.

The term “kick sampling” comes from the actions made by the individual in collecting this sample. The streambed is disturbed by vigorously kicking or shuffling through the substrate while holding a collecting net pressed firmly against the streambed immediately downstream (figure 3). (Kick net dimensions: mesh size 250 µm; opening approximately 30 by 30 cm; net length 1 m.)

Figure 3: collecting a kick sample

Benthic invertebrates and organic debris dislodged by the kicking are collected in the net as the sampler slowly moves backwards in an upstream direction, while continually kicking the streambed.

A long net is used to reduce the chance of backwash carrying invertebrates back out of the net. In silty areas it may also be necessary to rinse the net frequently to reduce clogging and prevent backwash. If rocks are strongly embedded in the substrate, it may be necessary to dislodge them or brush their surface by hand in order to sweep invertebrates into the net. In sandy areas, the kicking action should be less vigorous to avoid filling the net with sand.

A kick sample must cover 10 m of streambed, and typically takes about five to ten minutes to collect. The aim is to maximise the range of microhabitats by sampling throughout the riffle at various points along its length. To achieve this, the sample is collected from several areas in the riffle, rather than a single transect along the streambed. This increases the range of depths, current velocities and substrate types sampled.

After collection, the sample is “washed” in the net to remove fine sediment and to concentrate all material at the bottom of the net. This will facilitate both the transfer of the sample to the sorting tray and the sorting of invertebrates from the sample. Sample “washing” is achieved by splashing water through the sides of the net and/or by dipping the net repeatedly into the water until the water from the net runs clear. Avoid submerging the lip of the net while dipping as this may allow either additional invertebrates to enter the net or collected invertebrates to escape. Large pebbles and cobbles should be removed from the net, taking care to ensure any items discarded are free of attached invertebrates.

At particularly silty sites, rinse the net by holding it with two hands, one placed at the end of the net, the other closing off the mouth of the net about half-way along its length. Agitate the net back and forth under water, lifting repeatedly to check until the water runs clear.

4.2 Sweep sample (edge habitat)

A sweep sample is collected by sweeping a collecting net along the edge of a stream in areas of little or no current. The aim is to sample a variety of slow flowing habitat types, including overhanging vegetation, snags and logs, backwaters, leaf packs, bare edges, and macrophyte beds (figure 4). All available edge habitat types at a site are included in the sweep sample, although not all habitat types will necessarily be present at all sites. The types of habitats sampled need to be recorded on the field sheets (refer to section 4.4.3), as these may aid in the interpretation of results.

Figure 4: examples of sweep habitats

A sweep sample can almost always be collected at a site. In small, fast flowing streams, sweep sample habitats may be scarce, nonetheless it is usually possible to collect an adequate sweep sample by sampling all slow water habitats, especially near the stream edge, over a long reach area.

The net used for collecting the sweep sample is similar to that used for kick sampling except that the net length is shorter (usually 40 cm), to avoid getting caught on snags and vegetation. The sweep sample must cover a total distance of 10 m, although in order to cover a diversity of habitats this will not typically be a continuous 10 m. Always work in an upstream direction.

When sampling around vegetation, agitate the net to help dislodge invertebrates from substrate surfaces (Figure 7). Take short, vigorous and fairly quick sweeps to help minimise the loss of invertebrates. In backwaters and along stream edges and logs, sweep the net just above the substrate surface. Where there are leaf packs, stir up the leaves enough to get the invertebrates out, but avoid collecting excessive amounts of leaves and twigs if possible, as this will make it harder to see the invertebrates during sorting. Be especially aware of fast-moving surface dwellers, such as water striders (Gerridae) and whirligig beetles (Gyrinidae), as these can be hard to collect. If they are observed in the areas sampled, but not successfully collected, it is important to record their presence on a separate label placed in the sample jar during live sorting.

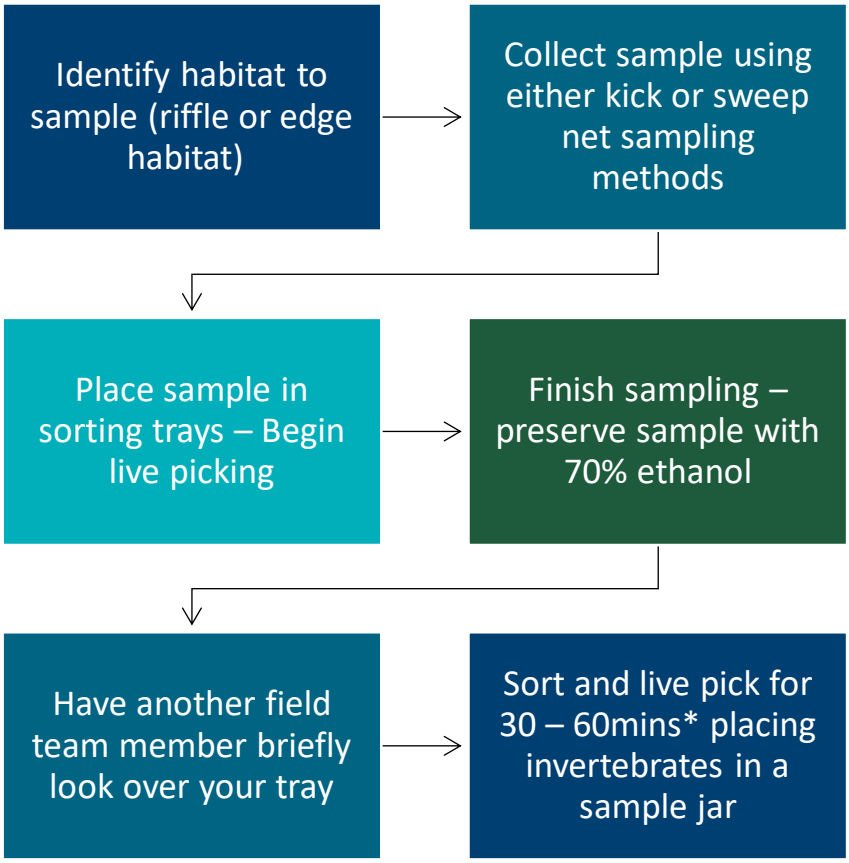

Figure 6: Flow chart of invertebrate sample collection procedures.

Refer to section 3.3 for details and tips on invertebrate collection. Note: if the sample has a particularly low abundance of invertebrates (e.g. fewer than 100 invertebrates have been found in 30 minutes), continue sorting for an additional 10 minutes. If no new taxa are found in the extra 10 minutes, cease sorting. If new taxa are found, continue as previously described for up to a maximum of 60 minutes.

After collection, "wash" the sweep sample in the net to remove fine sediments before placing it into the sorting tray. This is done in a similar fashion to the kick sample, by splashing water through the sides of the net and/or by repeatedly dipping the net in and out of the water until the water runs clear, while taking care not to submerge the rim. Large twigs and leaves can be removed from the sample after carefully examining them and picking off any attached invertebrates.

Note: Where conditions are inappropriate for the collection of natural substrate samples (i.e. natural riffle or pool) artificial habitats may be an option.

Figure 3: Collecting a sweep sample

Figure 4: Live picking in sorting trays

4.3 Live sorting – tricks and tips

After collecting and washing the sample in the net, it is then transferred to a large white tray for sorting (photographic or butchers' trays work well). To transfer the sample from the net to the sorting tray:

- Collect a bucket of stream water.
- Carefully invert the net over the tray (Figure 8) and use the water in the bucket to wash the invertebrates from the net (it helps to have someone assist with this step).
- Add enough stream water to the tray to allow the invertebrates to swim around, as this will make it easier to see them.

- If large amounts of leaves, wood or aquatic vegetation are collected, rinse these carefully to dislodge all attached invertebrates and remove them from the sample before sorting.
- If the water in the tray is cloudy due to clays or fine sediment in suspension, wash the sample back into the net and rinse in the stream until the water running from the net is clear.
- If large amounts of sand or coarse organic material are collected, put only a proportion of the whole sample into the tray at one time, bearing in mind that the entire sample needs to be sorted within the allotted time.
- After transferring the sample to the tray, check the sides of the net for any attached stray invertebrates and place these directly in the sample jar, then rinse the net thoroughly to prevent contamination of subsequent samples.

The primary objective of sorting is to collect as many different types of invertebrates as possible; the secondary objective is collect them in approximately in proportion to their abundance. Invertebrates are collected with the aid of forceps, pipettes and spoons and placed directly into sample jars (preferably wide-mouth glass or polyethylene jars, with a screw cap). Live sorting in the field must proceed in accordance with the following rules:

- Aim to collect approximately 200 invertebrates (plus or minus 40) in 30 minutes, although the final number can vary considerably depending on the quality of the site. The use of counters is strongly recommended to help keep track of the number of invertebrates picked.
- If new taxa are found in the last five minutes of the 30 minute sorting period, continue sorting for an additional 10 minutes beyond the original 30 minutes, focusing on the search for new taxa. If new taxa are found in this time then sorting continues for an additional 10 minutes. This can continue for up to a maximum of 60 minutes.
- If the sample has a particularly low abundance of invertebrates (e.g. fewer than 100 invertebrates have been found in 30 minutes), continue sorting for an additional 10 minutes. If no new taxa are found in the extra 10 minutes, cease sorting. If new taxa are found, continue as previously described for up to a maximum of 60 minutes.
- If a site has naturally low diversity, an experienced picker who can recognise separate taxa with confidence should not try to reach 200 individuals by collecting an excessive number of a single abundant taxon. In this case, it is acceptable to collect fewer than 200 invertebrates, with an accompanying note on the field sheets indicating the site had low diversity. However, inexperienced pickers who are uncertain about their ability to recognise different taxa are advised to pick the required 200 individuals. This strategy will ensure a diverse representation of invertebrates in the sample.
- Do not expend time picking out large numbers of individuals of abundant taxa. Only about 30 individuals of any single taxon need be picked. The remainder can then be ignored and sorting effort applied to the collection of other less obvious taxa.
- It is not necessary to pick out every individual of the large and conspicuous, but less abundant taxa.
- A useful strategy for collecting small invertebrates is to collect them from the edges and corners of the tray using a pipette.

- If possible, a minimum of 20 to 30 chironomids should be picked from every sample, to ensure that the sub-families are represented.
- Considerable effort needs to be directed to searching for small or cryptic taxa, particularly elmid larvae, oligochaetes, empidids, hydroptilids, small molluscs and ceratopogonids.
- If a collected specimen is too large to fit in the sample jar, as is often the case with yabbies and large bivalves, make a note of their presence on a separate label and place the label inside the sample jar. (Do NOT assume that writing this observation on the field sheets will suffice, as the field sheets may not be consulted in subsequent sorting and identification of the sample in the laboratory.)
- Other invertebrates observed during the collection of the sample, such as water striders (Gerridae) and whirligigs beetles (Gyrinidae), which move quickly and therefore are often hard to collect, are also noted on a label placed inside the sample jar if none are successfully collected in the sample.
- Once or twice during the sorting, strand animals by tilting the tray to one side, thus exposing a third to half of the tray bottom. Rapidly moving animals can be collected in this way, as well as snails and limpets which adhere to the bottom of the tray.
- If unsure of something (i.e. whether something is an invertebrate or not, or whether it is aquatic or terrestrial), collect it. It will be easier to determine what it is under the microscope in the laboratory.
- Always return the sample remaining in the sorting tray to the stream. Check for invertebrates clinging to the tray after the bulk of the sample is gone and add these to the sample jar. Leeches, flatworms, limpets, snails and psephenid beetles are often found this way.

Live sorting proceeds best when the invertebrates are alive and moving, and when light levels are relatively high. If a sample cannot be sorted immediately, place the net in a bucket of water in the shade. Do not delay the live sorting for long as the invertebrates will start to slow down or die fairly quickly, making them more difficult to see.

Placing the sorting tray in the sun while live sorting increases the visibility of invertebrates, particularly small and slow moving ones. If ambient light is low then artificial lighting may be required. Raindrops also adversely affect sorting performance. Umbrellas or tarps, ideally ones made of transparent or translucent material, should be used during rain.

Once sorting is completed, the live sorted sample is preserved in the field using 100 per cent ethanol. The final concentration in the sample jar should be 70 to 80 per cent ethanol. If there is too much water in the jar prior to preservation, carefully decant some without losing any of the invertebrates (e.g. use a pipette).

Place a label with the following information (written in pencil) inside the jar: name of the river or stream, the location name, site number or code, date, the type of sample (e.g. kick or sweep), and the name of the person who sorted the sample. Also record this information on the outside of the sample jar with a permanent marker.

5 Habitat assessment and water quality sampling

A standard set of habitat and water quality parameters are measured or assessed at all sites. The habitat assessments include physical measurements, descriptions of the habitats sampled, general observations of the site, and a characterisation of the riparian zone. Water quality is assessed through the measurement of chemical parameters, such as nutrients, turbidity and dissolved oxygen.

It is important to ensure the proper collection and effective data management of the habitat and water quality measurements. Use of the standard field sheets developed by EPA for the rapid bioassessment method or digital versions of these is strongly recommended. The following sections give detailed explanations of each item on the field sheets. A copy of the field sheets used by EPA is provided in Appendix 3.

Some of the physical/chemical parameters are essential inputs for the AUSRIVAS predictive models used in the assessment of ecosystem health and, therefore, must be measured at all sites. These are: latitude and longitude, alkalinity, stream width, reach and riffle substrate descriptions, percentage of reach covered by macrophytes, VEGCAT, shading, and riffle depth (if riffle habitat is present). (Refer to Appendix 3 and sections below for a complete description of these parameters.)

The remaining parameters are helpful for describing the site and interpreting results derived from the biological samples. Given that many of the parameters included in the habitat assessment are inherently subjective, and individuals invariably have their own biases, the habitat assessments must always be undertaken by at least two people. This will help ensure greater accuracy and consistency in the results.

Keep the following in mind when filling out the field sheets:

- If using paper, write legibly and always use pencil or waterproof ink.
- Do not leave blanks, as it is important to be able to distinguish "0" from "forgot to fill in". If there is any uncertainty about a particular item or parameter, then the people at the site are the best able to decide which of the options is the most appropriate and should do so.
- Some questions can have multiple responses while only one is possible for others. Make sure that only one response is filled in if only one is allowed.
- Before leaving the site, always have a second person check that all parts of the field sheets are filled in.

5.1 Site location information

Site notes, photographs and maps drawn on the first visit to a site are essential reference material for any intended future sampling at the same site. When arriving at a new site, prepare detailed information on the site, sufficient to enable others to locate the site at a later date. This includes detailed information for finding the site, map grid references and/or latitude and longitude if accurately established, and detailed drawings of the site itself.

Include in the site maps a large-scale perspective of the site location with notes for how to get there. In an accompanying diagram, draw the sampled reach and accurately indicate where the two biological samples were taken within the reach. Include physical characteristics of the reach,

such as bridges, river bars, large logs, riffles and pools, which are not likely to change before subsequent sampling events as these will help ensure re-sampling of the same locations within the stream. Refer to Appendix 2 for an example of a detailed site map. Photographs are also very useful for identifying the exact location of a site on future trips. Make a note of any requirements, such as keys for gates or the name and contact number of any person or office (e.g. Parks Victoria) whose permission is required for access to the site, and any general problems with access.

RIVER, CATCHMENT, LOCATION and LOCATION CODE: These parameters are important for locating the correct site in a broad area. River refers to the river or stream being sampled (e.g. Errinundra River); catchment refers to the major river catchment in which the stream or river is located (e.g. the Errinundra River is located in the East Gippsland catchment). Location name is particularly important for defining the exact location of the survey reach and as a unique identifier for the site (e.g. Errinundra River @ Errinundra). Each site should also be assigned a unique location code, which can be a number or series of letters, to help identify the site (i.e. EPA assigns a unique three letter location code to all sites). It is important that the same location name and code be used for the site each time it is sampled. If site name changes are proposed, both the new and old names should be recorded as well as the reasons for the proposed change. These basic site details are repeated at the top of each page of the field sheets as identifiers in case pages become detached or separated.

DATE and TIME: Date is important for distinguishing between site visits and determining which seasonal predictive models (AUSRIVAS) will be used for data analysis. It is also useful for relating back to prior events, such as high flow events, which may have influenced the invertebrate assemblage at the time of sampling. Time of day can aid in the interpretation of data, such as dissolved oxygen measurements, which can fluctuate significantly throughout the day.

PHOTOGRAPHS: At least two photographs should be taken at each site, one facing upstream and one facing downstream. This will assist in recognition of the correct sampling site on subsequent visits. Additional photographs should be taken of prominent features at the site and the access route to the site, if these will help locate the site on a future visit. Photograph numbers should be recorded on the field sheets to ensure the correct site names are assigned to the photographs, since more than one site may be visited on the same day or recorded on the same camera or mobile phone. It is also recommended that photographs be routinely taken on each site visit as particulars at the site may have changed since the last visit. Ensure that the site and location name or location code together with the date is recorded on each photograph as soon as they are processed to avoid potential misassignment of sites to photographs.

RECORDER(S) NAME: The name of the recorder(s) is important for following up any queries or issues that may arise when site data is analysed.

RECORDING SITE LOCATION: Latitude and longitude readings can be read directly off Google Maps on a smart mobile phone. Alternatively, The Map Grid of Australia (MGA) replaced the Australian Map Grid (AMG) system and is almost identical to co-ordinates used by the Global Positioning System (GPS). Latitude and Longitude can be determined using a 1:100,000 topographical maps or read directly from a hand-held GPS or from a mobile phone using Google Maps. If a GPS is used, readings should be confirmed on a 1:100,000 map as sometimes there may be few satellites available to provide an accurate position location. This information is essential for ensuring that the same site is sampled on future visits, and to assist in locating sites in difficult

terrain with few landmarks. If revisiting a historical site (1990's) where AMG co-ordinates were recorded a correction will need to be made to convert the site location to MGA co-ordinates.

Location Documentation Complete? is a prompt to ensure all details for finding and identifying a site are recorded.

5.2 Physical site characteristics

The parameters included in this section help provide a physical description of the site.

Always measure stream and channel width after the collection of the water chemistry and invertebrate samples to avoid disturbing the substrate and invertebrates (refer to Figure 1 for recommended ordering of tasks).

LENGTH OF SURVEYED REACH: The sampled reach should be approximately ten times the average width of the stream, but may be longer if for any reason (i.e. lack of suitable habitat) sampling needs to be carried out over a longer distance. The minimum reach size is 50 m; maximum reach size is 150 m. The surveyed reach must include all sampled areas. The length of the reach can be estimated or measured with a tape measure or calibrated range finder.

STREAM HABITAT IN SURVEYED REACH: This represents the estimated percentage of the three major habitat types (riffle, run, pool) in the surveyed reach. Note that riffle and run are combined into one category. Include all habitats with moderate to fast flowing water in this category. Pools are generally considered to have little or no flow and are usually deeper than riffles and runs. However, pools may consist of shallow slow-moving water in some streams, especially when stream flow levels are reduced.

STREAM WIDTH: This parameter refers to the width of the wetted surface of the stream itself, not the channel, and is measured from the edges of the water (Figure 7). Measure stream width at five regularly spaced transects within the surveyed reach. The ability to measure accurately is more important than regular spacing between transects, and therefore transects may be moved closer or further away from each other as required by the characteristics of the site.

Stream width measurements are made using a tape measure or a calibrated range finder. If using a tape measure and the opposite bank is inaccessible, wade into the stream as far as possible, measure that distance, and estimate the remaining distance. As a final alternative, stretch out an appropriate length (e.g. 10m) of the tape measure along the stream bank and use this as a reference to aid in the estimation of the stream width. Wherever possible, at least one measurement of stream width must be measured with a tape measure.

Accurate measurements should also be taken of the maximum and minimum stream widths of the surveyed reach and recorded on the field sheets. Note that the maximum and minimum stream widths may or may not correspond to one of the five transect measurements. Avoid using the area under a bridge or road crossing as a measuring point if it is noticeably different from the rest of the stream. Record the procedure that was used for measuring stream width (tape measure, range finder, estimate, or a combination of these).

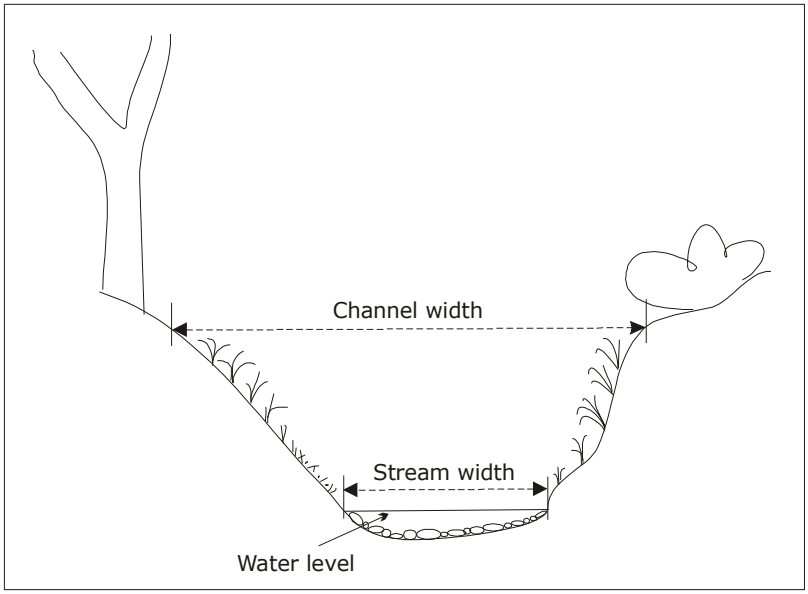

Figure 5: where to measure stream width and channel width

CHANNEL WIDTH: Channel width is the distance between the tops of the stream banks (figure 9), and can either be measured with a tape measure or calibrated range finder or estimated, depending on the site characteristics. Take five regularly spaced transect measurements within the surveyed reach. It is recommended that these measurements be taken in the same locations, and at the same time as the five stream width measurements. Record on the field sheets the procedure(s) used for measuring channel width.

5.3 Water quality measurements

Water quality measurements and water chemistry samples must be taken prior to disturbing the site. Special care should be taken to avoid disturbances to invertebrate sampling areas (refer to figure 1 for recommended ordering of tasks).

Unless otherwise indicated, the parameters listed below should be measured *in situ*, using calibrated field meters. Specific user instructions for meters vary considerably and it is essential that all staff involved in the fieldwork be adequately trained in the use of available meters. Operating manuals for the field meters should always be taken on sampling trips for consultation if problems arise. The make and model of the meter(s) used, as well as a unique identifying number given to each meter (e.g. serial number), should be recorded on the field sheets to enable tracking of calibration problems or meter faults.

Ideally *in situ* measurements are made in an area with at least a moderate current velocity. If there are no areas with adequate water movement, the meter's probe must be moved continuously through the water while measuring dissolved oxygen and pH, taking care not to disturb stream sediments. Avoid standing directly upstream of the meter probe.

When physical water samples are to be collected, please refer to *Sampling and analysis of Waters, Wastewaters, Soils and Wastes* (EPA publication 701, 2009).

- Water Temperature (°C) – most water quality meters will also measure water temperature; alternatively use a field thermometer. Allow sufficient time for the reading to stabilise. A probe or thermometer kept in a hot vehicle will need several minutes to adjust to cooler stream temperatures.

- Conductivity – conductivity is a measure of the ability of water to carry an electrical current and is used as an indication of salinity levels. Two units of measure (µS/cm and mS/cm) are provided on the field sheets as most meters can provide both unit measures. The units in which measurements are given will depend on the local concentration of ions. Freshwater generally has low conductivity levels and therefore will typically register concentrations in µS/cm. Ensure that the observed values are recorded against the appropriate unit. Conductivity is temperature dependent and should be measured at both ambient water temperature and at 25°C. Most good quality meters will provide both these values.
- Dissolved Oxygen (DO) – dissolved oxygen is essential for respiration by aquatic organisms. It is measured as both mg/L and percentage saturation. Most meters will provide DO readings in both units and both should be recorded. Several different types of DO meters exist, the most popular is now an optical sensor, which is more accurate than electrochemical or Clark DO electrodes. Ensure that observed values are recorded against the appropriate units. If flow rates are slow or the water body is not moving, agitate the probe in the water to create a current across the probe membrane. This is necessary because the probe actively removes oxygen from the surrounding water. If the probe is not moved around in relatively still waters, the DO readings will be lower than the actual DO levels in the stream due to depletion of the surrounding supply of oxygen. While moving the probe, it is important not to disturb the sediment as this could also remove oxygen from the water column.
- pH - pH is a measure of the acidity or alkalinity of a solution, ranging on a scale from 0 (acidic) to 14 (alkaline), with 7 being neutral. While measuring pH, be sure not to disturb sediments as decaying vegetation and organic matter found in some sediments can alter the pH of the surrounding water.
- Alkalinity – The alkalinity of water is its acid neutralising, or buffering capacity. Alkalinity is a required input for the AUSRIVAS predictive models used in assessing river condition and must be measured at all sites. Alkalinity can be measured in the field using a Hach kit or equivalent, or samples can be collected for laboratory analysis (APHA 1995, EPA Victoria 2009). If using a field kit, ensure that the specific instructions for the kit are followed. If a water sample is taken to measure alkalinity by laboratory analysis, collect at least 500 mL of water in a clean polyethylene bottle following the protocols detailed below for collection and storage of water samples. Record on the field sheets whether alkalinity was measured in the field or by laboratory analysis.
- Turbidity – Turbidity is a measure of water clarity, which is generally affected by the suspension of clay, silt or fine particulate organic and inorganic matter in the water column. Turbidity is often measured in the field with a turbidity meter or multi-probe, but can also be measured in the laboratory (APHA 1995, EPA Victoria 2009). Collect at least 100 mL of water in a clean polyethylene bottle in accordance with the protocols for collecting and storing water samples detailed below. Record on the field sheets whether turbidity was measured in the field or by laboratory analysis.
- Nutrients - Water samples should be collected for laboratory analysis of total phosphorus (TP), nitrates and nitrites (NOx), and total Kjeldahl nitrogen (TKN). Only two samples need to be collected, one for TP and NOx analysis, and one for TKN analysis. Collect the water samples in new (unused) polyethylene bottles as required by the laboratory conducting the specific analysis. Water samples for nutrient chemistry can be easily contaminated. It is

essential that the protocols described below are followed during their collection and storage.

Collection of water samples:

- Always collect the samples for water chemistry before the site is disturbed by other sampling activities.
- Collect the sample in an area of flow and upstream of the sample collector's position in the stream.
- For nutrient samples only, rinse the bottles and caps with stream water two to three times before filling them. Avoid touching the lips of the bottles and inside the caps as this can contaminate the sample. Do not fill the bottles to the top. Leave at least 3cm of air space to allow for expansion during freezing, otherwise the bottle will split.

Storage of water samples:

- *Alkalinity* and *Turbidity* – Keep samples in a dark and cold storage facility, such as an esky with ice or a portable refrigerator, but not frozen, until the samples can be analysed in the laboratory.
- *Nutrients* – Samples should be frozen immediately after collection. If this is not possible, they should be kept as cold as possible, by placing samples in a cooler with ice and frozen as soon as such facilities become available. Once frozen the samples should not be thawed until they are ready for analysis.

Sample labelling:

- Clearly label the outside of each sample bottle with the name of the river, location name, location code, date, and the type of analysis required for each.

A prompt is provided on the field sheet to confirm that the water samples have been collected.

If the collection of water samples prior to biological sampling was inadvertently forgotten, collect them from a site just upstream of the disturbed area.

5.4 Habitat descriptions

A description of the in-stream habitat within the surveyed reach, as well as some key features of the surrounding area are required at each site. Details on the types of habitat included in the kick and sweep samples are also recorded to aid in the interpretation of results.

5.4.1 Reach

All information in this section applies to the **ENTIRE** reach being sampled.

SUBSTRATE DESCRIPTION: Estimate the percentage cover of each of the substrate size categories listed on the field sheet (Appendix 3) over the entire reach. These percentages are estimated in plan view, based on surface cover, and must add up to 100 per cent. The substrate categories and the sizes they represent are:

- Bedrock: solid rock not broken into individual boulders

- Boulder: >256 mm
- Cobble: 64 – 256 mm
- Pebble: 16 – 64 mm
- Gravel: 2 – 16 mm
- Sand: 0.06 – 2 mm
- Silt/Clay: < 0.06 mm

The phi values listed next to the substrate size categories on the field sheets (refer to Appendix 3) are used in the calculation of one of the predictor variables for the AUSRIVAS predictive models. These are explained in more detail in section 6.

Figure 6: examples of cobble, pebble and gravel particle sizes

A useful diagram illustrating the size range of each substrate category is provided in Appendix 4. It is strongly recommended that this diagram be copied and taken on all sampling trips for easy reference. Figure 10 provides an example of the range of cobble, pebble and gravel sizes that may be encountered in the field.

Before assigning percentages to each substrate type, it is important to make a thorough survey of the entire reach area. If the streambed cannot be seen, feel around with hands or feet to get an idea of the substrate composition. If a site is dominated by fine sediment, be sure to feel the sediments to determine if they include sand (gritty in texture) or are mostly silt/clay (smooth in texture). Silt is included in the smallest size category only if it is clearly incorporated into the substrate.

Loose silt lying over the top of other substrates is not recorded in this section (it is assessed elsewhere). If the site is too deep and/or turbid, try using the pole of the sampling net to feel around and disturb the bottom (only after water chemistry and invertebrate sampling has concluded). As a last resort, use the bank substrate as a guide. Note any problems with determining substrate composition on the field sheet.

As estimates can involve a fair amount of subjectivity, three alternative procedures are described below to help increase the accuracy and objectivity of assessing percentage cover of substrate particles:

- *Method 1*: divide the reach up into habitats with similar substrate types, such as pools (which typically consist of fine sediments in the gravel, sand, and silt/clay categories) and riffle/runs (which are usually dominated by boulders, cobbles and pebbles). Estimate the percentage cover of each substrate type within each of these distinct habitats, then scale up to the whole reach, keeping in mind the percentage of the reach that was originally described as pool and riffle/run.

For example, if 50 % of the reach is pool, and sand makes up 50 % of the pools but is not found in the riffle/run habitats, then sand represents approximately 25 %of the substrate in the reach.

- *Method 2*: estimate the size of the area that would be filled in by a particular size of substrate if they were all gathered together. Divide this area by the total area of the reach, expressing it as the percentage of the reach occupied by that size of particle.

For example, if it is estimated that the total area covered by all boulders is 5 m by 20 m (100 m^2), and the total reach area is 100 m by 5 m (500 m^2), then 100 m^2 divided by 500 m^2 equates to 20 per cent of the reach covered by boulders. Repeat this process for each category.

- *Method 3*: using the percentage cover diagrams (Appendix 5) as an aid, separately assess the percentage cover of each size category as the stream reach is surveyed.

For example, visualise the size range of cobbles, then hold the percentage cover diagrams in view while surveying the streambed to help determine the percentage cover of cobbles within the reach. Repeat the process for each category.

OTHER STREAM FEATURES: Select the percentage category that best describes the abundance of the following stream features throughout the surveyed reach: willow roots, moss, filamentous algae (any visible algae growing on the stream bottom), loose silt lying over the substrate (includes both organic and inorganic silt), and macrophytes. Refer to section 4.4.3 for a more detailed description of these features.

All moss and macrophytes in the active stream channel are included in their respective categories, whether or not they are present in the waters of the stream itself. For example, any sedges and/or rushes growing at the stream edge or on the bank, or any moss growing on rocks on the stream bank, are included in the estimate of percentage cover of macrophytes and moss, respectively.

As with substrate composition, these percentages are estimated in plan view, based on surface cover. When assessing the presence of each feature, keep the following example in mind and apply the same logic to the site under evaluation. For a stream reach that is 100 m long and 5 m wide, 1 per cent of the reach is equivalent to an area 1 m long and 5 m wide, 10 per cent of the reach is equivalent to an area 10 m long and 5 m wide, and so on.

Note that the category numbers (e.g. 0-4) listed on the field sheets are used for inputting categorical data into the AUSRIVAS predictive models. Thus, it is important that these numbers not be changed.

ORGANIC MATERIAL: Record the number that corresponds to the percentage category that best describes the amount of organic material present throughout the reach (refer to Appendix 3). Organic material is divided into two categories. Coarse Particulate Organic Matter (CPOM) refers

to organic material (leaves and twigs) that is larger than 1 mm and wood smaller than 10 cm in diameter. Snags/Large Organic Material applies to all woody debris larger than 10 cm in diameter.

These percentages are estimated in plan view, based on surface cover within the wetted area of stream. Keep in mind the example given above (refer to Other Stream Features) when evaluating the percentage cover of organic material.

CURRENT VELOCITY IN REACH: For each of the following flow categories, choose the category that best describes the percentage of that flow velocity in the surveyed reach: no obvious flow, slow, medium/moderate, fast to very fast (refer to Appendix 3).

VEGCAT: The VEGCAT, or vegetation category, is the best summary of land use beyond the riparian zone (the vegetated area immediately adjacent to and extending up to 30 m perpendicular to the stream).

Only one category can be selected at any given site. If more than one category applies to a site, choose the category that best describes the overall site. When in doubt, consider the categories as a gradient of disturbance, from highly impacted (category 1) to unimpacted (category 5), and choose the most appropriate category accordingly. In cases where hobby farms dominate the land use, choose the category that best describes the primary form of agricultural activity on the hobby farm.

The following descriptions include the range of land uses covered by each category:

- Category 1- *urban*: this describes sites that are more than 50 per cent residential, with extensive paved surfaces producing run-off (figure 11). This category would apply to sites in the middle of a large city, such as Bendigo or Melbourne, but not to sites in smaller towns, such as Myrtleford.

Figure 7: VEGCAT 1 – Urban

Figure 8: VEGCAT 2 – Intensive agriculture

- Category 2 – *intensive agriculture*: intensive agriculture includes horticulture, viticulture, cropping, dairying, feed lots, etc (Figure 12). This category can also include small towns and residential areas. For example, the Ovens River immediately downstream of a town the size of Myrtleford would be included in this category.

- Category 3 – *mostly cleared, grazing*: This covers sites where the primary agricultural activity is grazing of livestock (Figure 13), although the degree of clearing at the site may vary from moderately to completely cleared. Hobby farms with little vegetation and several houses, but few paved surfaces and little intensive agriculture would be included in this category.

- Category 4 – significant patches of forest remaining, some forestry/agriculture: The presence of extensive amounts of remnant forest are necessary for this category rating. Small amounts of grazing or some forestry may be present, provided there is also a substantial cover of native forest (figure 14). This category also includes sites where one bank is cleared for grazing while the other bank is completely forested.

- Category 5 – native forest/natural vegetation: This category is reserved for uncleared forest or forested areas with only small clearings (e.g. picnic or camping sites) (figure 15).

Figure 9: VEGCAT 3 – mostly cleared, grazing

Figure 10: VEGCAT 4 – significant patches of forest remaining

Figure 11: VEGCAT 5 – native forest

SHADING: The percentage shading of the wetted river channel throughout the surveyed reach is estimated in plan view as it would appear at midday, with the sun directly overhead. The canopy is only considered solid if it actually is. If there are gaps in the canopy of any tree or shrub (e.g. gaps between leaves through which light penetrates) then this will reduce the amount of shading. Use the percentage cover diagrams as a guide (Appendix 5). Record the number corresponding to the appropriate shade category on the field sheet.

5.4.2 Riffle Habitat

This section applies only to the riffle/run area covered during collection of the kick sample.

Record the name of the person who collected the kick sample and the person who sorted the sample, as well as the time taken to pick the sample. If the sample covered an area less than 10 m, the length of the area sampled must be noted, with reasons for the variation from the standard 10 m. Record the approximate number of invertebrates picked during live sorting. If less than 150 invertebrates were picked, provide a brief explanation (e.g. heavily impacted site with low bug diversity).

If a riffle was not sampled, record the reason.

SUBSTRATE DESCRIPTION: Follow the guidance provided in the previous section for estimating reach substrate composition and percentage cover of other stream features and organic material. Remember that in this case the assessment is made only over the area included in the kick sample.

When evaluating the percentage cover of each feature keep in mind that 1 per cent of a 10 m kick sample is 10cm. As this is a very small area, the < 1 % category effectively refers to zero, or a very small amount. Using the same rationale, the 1-10 % category of a 10 m kick sample represents a length from 10 cm to 1 m, 10-35 % represents a length from 1m to 3.5 m, and so on.

DEPTH: Use a metre rule to measure depth at five representative points in the riffle or run where the kick sample was taken. No measurements are taken if a kick sample is not collected.

CURRENT VELOCITY: Indicate which of the current velocities listed on the field sheet (no flow, slow, medium/moderate, fast to very fast) were present in the area where the kick sample was taken (refer to Appendix 3). More than one current velocity can be selected if present.

5.4.3 Edge/Backwater Habitat

This section applies only to the edge/backwater area covered during collection of the sweep sample.

Record the name of the person who collected the sweep sample and the person who sorted the sample, as well as the time taken to pick the sample. If the sweep sample covered an area less than 10 m, the length of the area sampled must be noted, with reasons for the variation from the standard 10 m. Record the approximate number of invertebrates picked during live sorting. If less than 150 invertebrates were picked, provide a brief explanation (e.g. heavily impacted site with low bug diversity).

PERCENTAGE OF SAMPLED AREA COVERED BY: as sweep samples cover a variety of slow flowing habitat types, it is important to record the types and proportions of each sub-habitat sampled. These details may help in the interpretation of results. Identify the percentage category (refer to Appendix 3) which best describes the amount of each sub-habitat type included in the sweep sample. The total does not necessarily have to equal exactly 100 per cent. Sub-habitats that might be included in a sweep sample include:

- *Backwaters* – still or slow flowing areas, usually at the edge of a stream, that are separated from the main stream flow by an obstruction, such as a bar or log. Backwaters are a good habitat for surface dwelling invertebrates, and also tend to accumulate silt and leaf litter, which provide habitat for detritivorous insects.

- *Leaf packs/CPOM* – leaf packs are conglomerations of leaves that accumulate against rocks and vegetation in areas of flow, or at the bottom of pools and backwaters. CPOM (Course Particulate Organic Matter) refers to organic material (e.g. leaves) that is larger than 1 mm and wood smaller than 10 cm in diameter. Many invertebrates use leaf packs and CPOM as habitat and/or as a food source.

- *Undercut banks* – banks that overhang the water, typically resulting from erosion of finer sediments at the base of the bank. These areas are often shaded and have little flow, and provide excellent habitat for surface dwelling invertebrates and refugia for benthic invertebrates.

- *Roots* – trees and shrubs growing along the stream edge often extend their roots into the water, providing additional physical habitat structure for invertebrates. Roots often result in a slowing of the stream flow, which also creates habitat for surface dwelling invertebrates.

- *Bare edge* – these can be stream banks or beaches with little or no vegetation cover, or areas of exposed bedrock. These areas are often covered by a layer of fine silt, which serves as a primary habitat for some burrowing invertebrates. In unshaded sites, herbivorous invertebrates may be found feeding on the algae growing on submerged bedrock in edge habitats. Surface dwelling invertebrates are also found in the slower flows commonly present along bare stream edges.

- *Logs* – this category refers to wood greater than 10 cm in diameter. Some aquatic insects feed exclusively on wood, while others feed on the algae and microbes growing on the wood. Many invertebrates also live on or around submerged logs.

- *Trailing bank vegetation* – any vegetation growing on the stream bank (outside the water) or along the edges of the stream (in the water) that hangs into the water. This is the preferred habitat of some specialised invertebrates, but also provides a spatially complex habitat and food source for many generalist invertebrates.

- *Filamentous algae* – algae that is visible to the naked eye, formed from a chain-like series of cells, often found in strand-like clumps or mats. Filamentous algae provides both a food resource and habitat for invertebrates.

- *Macrophytes* – aquatic plants that are visible to the naked eye. Macrophytes can occur as submerged (e.g. ribbon weed, watermilfoil), emergent (e.g. sedges, rushes) or floating (e.g. lilies) forms. As with trailing bank vegetation, macrophytes are the preferred habitat of some invertebrates, but also provide a spatially complex habitat and food source for many types of invertebrates.

- *Moss* – a low-growing, leafy-stemmed plant typically appearing as tufts or mats on the surface of rocks. Some invertebrate taxa live exclusively in moss beds, others feed on it, and still others use it as an alternative habitat when preferred habitats are not available.

- *Loose silt lying on substrate* –the amount of loose silt lying over the entire area covered by the sweep sample, including both organic and inorganic silt material is estimated. This is an important habitat descriptor because silt often smothers habitats used by invertebrates and, therefore, may be an indicator of site degradation. In small amounts, silt can serve as a food source or refuge for some invertebrate taxa.

- *Other* – describe any other sub-habitat types that were included in the sweep sample and are not listed on the field sheet (e.g. old tyres).

CURRENT VELOCITY: Indicate which of the current velocities listed on the field sheet (no flow, slow, medium/moderate, fast to very fast) were present in the area where the sweep sample was taken (refer to Appendix 3). More than one current velocity can be selected if present.

5.5 Site observations

A series of general observations of the surveyed reach and surrounding area are recorded at each site (refer to Appendix 3). Note that left and right bank designations always refer to direction when facing downstream. For some of these parameters (as indicated in the following descriptions), more than one category can be selected.

WATER ODOURS, WATER OILS, and SEDIMENT ODOURS: Water odours and oils, and sediment odours, refer to the smells and visible signs of oils observed prior to or during sampling. Disturbance of the substrate during sampling often releases odours and oils and these should be included.

A range of odour and oil categories is included on the field sheets. Brief descriptions are provided below.

Odours:

- *Normal* – very little odour present, not offensive to the nose.
- *Sewage* – pungent wastewater smell, like dirty toilets.
- *Petroleum* – typically a diesel/oily smell or a strong petrol fume smell.
- *Chemical* – may be acidic or cause a burning sensation to the nose, like ammonia.
- *Anaerobic* – typically smelling like rotten egg gas or methane.
- *Stormwater* – similar to sewage but not as strong, a mixture of rotten garbage and sewage smells.
- *Musty* – smelling of damp towels, mould or mildew.

Oils:

- *Slick* – an expanse of a floating oily substance that stays connected, like wet paint.
- *Sheen* – appears as an iridescent rainbow-like reflection given off in direct sunlight.
- *Globs* – oil congealed into balls of elastic substance, like melted cheese or whipped thickened cream in water.
- *Flecks* – appears like fish food flakes sprinkled on the water, with angular edges.

If a new sediment odour is detected that is not listed, it should be marked as "*other*" and described. Note that more than one category may apply.

TURBIDITY: A visual assessment of stream turbidity should be made based on conditions existing prior to sampling. This parameter considers reduced water clarity due to suspended silt and clay only, and not dissolved leaf leachates, which gives water the appearance of black tea.

Brief descriptions of the turbidity categories listed on the field sheets are provided below.

Turbidity:

- *Clear* – stream bottom is visible in deep sunlit pools.
- *Slight* – most of the stream bottom is visible, but fine detail cannot be distinguished in deep waters.
- *Turbid* – little visibility even in full sunlight; stream bottom is not visible except near stream edge or in very shallow water.
- *Opaque/Liquid Silt* – no visibility, very muddy.

PLUME: plume is a coarse assessment of the presence of easily disturbed fine sediment in the riffle/run habitat only, and appears as a cloud or clouds of material released into the water when collecting the kick sample. If no riffle/run is sampled at a site, select "no riffle/run present".

FLOW LEVEL: flow level is an estimate of the water levels at the time of sampling. It is measured relative to the "water mark", which is a useful measure of typical flow levels (e.g. base flow) for the site. Establishing the location of the water mark is not always easy and requires some practice. A good indicator of the water mark is the limit of grasses and terrestrial vegetation, since the constant flow of water causes the loss of terrestrial vegetation below the water mark. Eroded areas that have resulted from the constant flow of water can also help identify the water mark. Moderate flow refers to a band above and below the water mark, not just the actual water mark. In practice this band is approximately 30 cm wide (i.e. 15 cm above and below the discrete water mark).

BARE GROUND: bare ground above normal inundation level shown by the "water mark" refers to ground devoid of vegetation and rocks or logs on the bank itself but not including the area below the "water mark". Only erodible ground is considered bare. Bedrock and boulders on the bank are not classed as bare ground. The percentage is estimated by eye but percentage cover diagrams (Appendix 5) may be useful. Keep the length of the reach in mind when assessing this feature as over-estimates are common. For example, what may seem like a large 5m bare section of bank equates to only 5 per cent over a 100 m reach. Note that left and right bank are evaluated separately.

Figure 12: heavy catchment erosion

Figure 13: heavy catchment erosion

LOCAL CATCHMENT EROSION: this refers to local erosion within the surrounding catchment, as visible from the site, and not just bank erosion. Refer to Figures 16 – 19 for examples of heavy, moderate, and slight catchment erosion.

LOCAL POINT SOURCE POLLUTION: list all potential as well as obvious sources of pollution that can be seen from the site. More than one source can be listed for a site.

Figure 14: moderate catchment erosion

Figure 15: slight catchment erosion

DAMS/BARRIERS: Record the presence of barriers that are likely to cause altered flow regimes at the site for a significant period of time. If in doubt, select "not known" and amend the information, with the use of maps or local knowledge, immediately upon returning from the field.

Dams refer to large dams used for irrigation, town water supply, flood mitigation or hydro-electric power generation. These are recorded as present if they are located anywhere within the upstream watershed, regardless of distance from site and even if they are located on a tributary. Small weirs and hydrologic gauging "V-notch" weirs are not considered as dams or barriers if upstream of the site. However, do record the presence of any small artificial barrier (e.g. weir) located within the surveyed reach or immediately downstream of the reach.

BRAIDING: Braiding refers to the presence of water flowing through more than one channel within the main stream channel (figure 20). Braiding is caused by erosive forces carving new channels within the streambed, erosion-resistant bedrock persisting in the main stream channel, or by large emergent deposits of stream substrates (e.g. bars). If braiding is present at the site, record the number of channels present.

SITE CLASSIFICATION: Site classification refers to the landscape surrounding the stream. A steep valley has obvious steep valley slopes within 500 m of the stream and is typical of upper catchments (figure 21). A broad valley has areas of high relief nearby, but generally at a distance greater than 500 m from the stream (figure 22). Broad valleys are generally the areas where streams begin to slow their descent to enter lowland areas. Plains are areas where little relief is apparent from the stream banks for many kilometres (figure 23). When classifying sites, care should be taken to include the larger watershed. For example, some rivers e.g. the Ovens River at Myrtleford, may appear to be located in a plain, is in fact located in a broad valley between two ranges.

Figure 16: braiding

Figure 17: steep valley

LAND USE: land use refers to the major land use or uses in the catchment. Consider the larger area, not only that immediately adjacent to the reach. Each bank should be assessed separately (remember: right and left bank refer to direction while facing downstream). More than one category can be listed for each bank. Intensive agriculture refers to piggeries, dairying, irrigated cropping, market gardens, orchards, vineyards and the like. Hobby farms should be considered in terms of their primary land use (e.g. forest, irrigation or grazing). Residential refers to moderate to highly built-up populated areas, not to a house on acreage. Native heath/grassland refers to alpine meadows, lowland and coastal wetlands dominated by tea trees, and dryland habitats dominated by native grasses.

BARS: bars are areas of the streambed that protrude from the water where significant deposition of stream substrates has occurred. These can be in the form of an island bar within the channel (figure 22), or banks/beaches that form a peninsula extending into the stream at a bend in the stream channel (figure 23).

Figure 18: island bar

Figure 19 : point bar

HEAVY RAIN OR SPATES IN LAST WEEK: this observation is recorded as it may help explain unexpected or depauperate invertebrate assemblages. Look for the presence of:

- flattened streamside vegetation
- recent debris dams
- flotsam and debris on overhanging vegetation
- and sediment depositions on the stream banks,

as these are all potential indications that a high flow event has occurred within the previous few days. Only heavy rainfall events or significant high flows are recorded. If unsure whether such an event has occurred recently, select "don't know".

5.6 Riparian characteristics

The riparian zone is the environment adjacent to the stream where vegetation interacts with the waterway, through fallen leaves or woody debris or by accessing underground stream water below the banks. Riparian zones contribute to energy flow within the streams by providing food and in-stream habitats through:

- leaves and woody debris stabilising banks, and reducing bank erosion and collapse
- providing habitat for aerial life stages of aquatic invertebrates.

Information on riparian zone characteristics helps interpret biological sampling results, and also provides a reference for future site visits. When assessing the riparian zone, examine the entire reach, not just the sampling access point as this may be dissimilar to other sections of the riparian zone. In cases where the structure and extent of the riparian vegetation varies along a stream reach, attempt to obtain an average value for each of the riparian parameters assessed.

WIDTH OF RIPARIAN ZONE: the riparian zone can be difficult to define and is often difficult to delineate in the field. While there are many interpretations of what constitutes the riparian zone, it is defined in this guideline as the area from the base of the bank for a width of up to 30 m, perpendicular to the stream. The average width of the riparian zone to a maximum of 30 m is recorded for each bank separately. Left and right bank refer to direction while facing downstream.

When assessing what constitutes riparian vegetation, keep in mind that this refers to woody vegetation that interacts in some way with the waterway. For example, at a site with a wide channel that has vegetation growing within the channel, but not right near the water's edge, this vegetation should be included in the measure of riparian width.

Grass is only included in the riparian width measurement in native grasslands and alpine and sub-alpine meadows.

If a break in the riparian zone is apparent (i.e. a sealed road), this would cause a break in any woody vegetation interacting with the waterway. Therefore, the riparian zone would end at the road, however if the break was small, and overhanging vegetation is present and continuous, the riparian zone may not be broken.

STRUCTURAL COMPOSITION OF RIPARIAN ZONE: the purpose of this section is to describe the physical appearance, or structural composition, of the riparian zone. It breaks riparian vegetation up into three structural categories - trees, shrubs and bushes, and ground cover – and assesses the percentage cover of each structural type to provide a more complete picture of the riparian zone. Each category should be evaluated as it appears at the time of sampling. For example, a plant that you know will become a large tree, but presently is only 2 m tall, and therefore is currently functioning as a shrub, should be included in the shrub category.

- *Trees* — woody vegetation over 5 m in height, usually with a single stem or trunk. This category can also be described as the overstorey, or canopy.
- *Shrubs and bushes* — woody plants less than 5 m in height, often consisting of multiple stems arising at or near the base. This category can also be described as the understorey. Tree ferns and blackberries are included in this category due to their structural function in the riparian zone.
- *Ground cover* — grasses, herbs, ferns and mosses growing close to the ground in riparian vegetation communities.

The schematics provided on the field sheets (refer to Appendix 3) give an approximate visual representation of three percentage cover categories for each structural component of the riparian zone. Using these as a guide, select the percentage category that best represents the vegetation cover in each of the structural categories. Assess the left and right banks separately (remember: left and right bank refer to direction while facing downstream).

EXOTIC VEGETATION: this section refers to the proportion of exotic (non-Australian) vegetation within the riparian zone as a whole. Note that left and right banks are not assessed separately. Indicate what percentage of each of the structural categories (trees, shrub layer and ground cover) is made up of exotic vegetation. Blackberries should be included in the shrub layer. Note that most grasses in disturbed environments are exotic, especially in agricultural areas.

LONGITUDINAL EXTENT OF RIPARIAN VEGETATION: the longitudinal extent of riparian vegetation is a measure of the continuity of the riparian vegetation on each bank. Select the category that best represents the continuity, or patchiness, of the tree and shrub layers. Ground cover is not included, except in native grasslands where trees and shrubs are not normally present. Note that the numbers in the descriptor column on the field sheets (Appendix 3) are used for data entry purposes by EPA and are not intended as indicators of riparian zone quality.

5.7 Macrophyte classification

Macrophytes are aquatic plants that can either be submerged, floating or emergent. Select the category that best represents what macrophytes are present. Appendix 3 provides figures to illustrate the physical characteristics of macrophytes that may be present.

6 Laboratory processing of invertebrate samples

This protocol provides details on how invertebrate samples collected in the field for the EPA biological monitoring program should be handled, sorted and identified in the laboratory. It also provides a list of the taxa to be ignored in the sorting process, discusses level of identification, and storing specimens and laboratory sheets.

The appropriate taxonomic level of identification for the EPA rapid biological monitoring program is family level. Other programs may require other methods and species level identification but are beyond the scope of this document.

6.1 Sorting invertebrates

Invertebrates from samples live sorted in the field are identified in the laboratory using a good quality stereomicroscope and relevant, up to sate taxonomic keys. Use EPA provided keys where accessible.

It is imperative that all personnel involved in this process are suitably trained and skilled in the identification of aquatic invertebrates.

Prior to commencement of sorting, sample details should be transferred accurately and legibly from the label in the sample jar to a laboratory count sheet and to labels in vials to be used for storage.

If there are problems with the field label, site details should be confirmed by checking the site details from field information. The following information is required: catchment, river/stream and location, site code, date, sample type (kick or sweep). Labels should be pre-printed or written in pencil or waterproof ink. It is important that the edge of the label sits below the rim of the vial to prevent wicking and drying of the contents. If printed laboratory sheets are used rather than a digital device, they should also be filled in at this time using pencil or waterproof ink. An example of a laboratory count sheet is given in Appendix 7 which could be used as a template for a digital files.

Details of invertebrates observed in the field (e.g. Gyrinid adults) or collected but released (e.g. crayfish) should be transferred from the sample lable to the laboratory count sheet prior to the commencement of sorting. Include a not indicating that the information was taken from the field label to explain why there is no corresponding specimen in the storage vial.

To avoid potential cross-contamination errors between samples, processing in the laboratory is undertaken on a sample by sample basis. Invertebrates are first separated from the organic and inorganic material in a sample and then identified and appropriately stored.

The processing of a sample begins with decanting the sample from the field sample jar into a Petri dish (or sorting tray), ensuring no invertebrates are spilled or remain in the sample jar.

Invertebrates are then removed from the sample by systematically scanning the Petri dish. Always ensure there is adequate ventilation at the laboratory bench when working with samples in alcohol.

The sample must be examined carefully to find individuals which may be very small or cryptic. The following information is important to ensure that all individuals are extracted and well preserved.

- Ensure that sufficient alcohol is in the Petri dishes to completely cover the invertebrates.
- Carefully check clumps of algae and detritus for hard to see invertebrates.
- Look for chironomids that love attached to the back of mayfly nymphs, small invertebrates caught in the mouths or gills or larger ones, and invertebrates that live in sticks.
- All shells and caddisfly cases need to be checked to confirm there is an animal inside. Some cases may contain more than one invertebrate as they often swim or crawl into cases in an attempt to avoid the alcohol in the sample jar.
- Do not identify or count empty shells or cases, shed skins/moults or invertebrates that were clearly dead (e.g. in a state of decay) at the time of sample collection.
- Most Trichoptera larvae can be left in their cases are these are often useful for identification. The exceptions ate those using sticks for cases; larvae should be removed from the case which is then discarded
- Scan the dish at least twice, once using bright field background and the second time using dark field background. This will help ensure that taxa are not overlooked.
- Aquatic invertebrates are always stored in 70 per cent alcohol (ethanol).

If leaving the laboratory before a sample is completed ensure that Petri dishes and sorting trays contain adequate ethanol and are completely covered to prevent drying. Place them in a safe place where they will not be knocked, lost, or confused with any other sample. Ensure that samples are adequately labelled.

At the completion of sorting, systematically scan the Petri dish twice to ensure no invertebrates remain.

The following taxa are to be excluded from assessment and discarded: Microturbellaria; Nematoda (roundworms); Rotifera; Gastrotricha; Bryozoa; Collembola (springtails); all millipedes and spiders; isopods in the family Oniscidae other than Haloniscus; beetles belonging to Staphylinidae, Caribidae and terrestrial families, adults of Scirtidae, Psephenidae, Ptilodactylidae and Chrysomelidae; terrestrial caterpillars and terrestrial snails; and the planktonic crustacean orders Choncostraca, Cladocera (water fleas), Ostracoda (seed shrimps) and Copepoda.

Procedural errors to avoid during laboratory sorting include:

- not writing labels immediately
- not writing all information on labels legibly or accurately
- using water instead of alcohol, or less than 70 per cent alcohol, for storage
- not tightening the lid on vials (sample may dry out)
- writing the wrong sample type on the laboratory sheet and/or vial labels
- not recording a taxon on the laboratory count sheet, or listing the name but not recording the count.

6.2 Identification of macroinvertebrate specimens

It is recommended that identification should proceed one order at a time. This will facilitate accurate identification of specimens as it will be easier to recognise the differences that distinguish families. Only one vial should be open at this stage. This is to ensure that specimens are placed into the correct vial. Having all specimens of one taxa in the one petri dish also means that they can be counted, and the count verified, prior to placing them in the vial, a method that results in more accurate counting.

Specimens should routinely be placed into two vials – Plecoptera, Ephemeroptera and Trichoptera in one vial and Miscellaneous Invertebrates in the second. If there are large numbers or large specimens of a particular group (e.g. shrimps, Odonata) these may be placed into additional vial(s) with the appropriate order label. From time to time you may also be requested to store other groups in a separate vial.

Keys have been developed by EPA for the identification of many orders of Victorian aquatic invertebrates. These keys should be used for consistent identifications. Routine identifications are to family level, with the following exceptions:

- Chironomidae larvae are always identified to sub-family, pupae to family.
- Oligochaeta (segmented worms), water mites, isopods of the sub-order Phreatoicidea, and Temnocephalidea should not be identified below these taxonomic levels.
- All Nematomorpha should be recorded as Family Gordiidae.

Note that for the beetle families, adults and larvae are always identified and counted separately.

As specimens are identified, the family name and count number are recorded on a prepared laboratory count sheet (see above). It is important that each new family found in a sample is recorded on the sheet as soon as it is identified, and that each count is recorded immediately. It is easy to be distracted after placing specimens into the vial and to forget to write down the name or the count. Rough sheets should not be used to record identifications or counts as this introduces the risk of transcription errors. For the beetle families, always list the adults and larvae separately as they may be performing different ecological functions and this information may be useful in the interpretation of results.

In order to ensure accuracy in the processing and identification of the biological samples, quality control must be carried out on a regular basis and samples are to be confirmed by a Victorian macroinvertebrate expert (Refer to section 8). This will allow for the correction of errors in identification, highlighting of problems that may require further training, and setting error levels on the data.

A maximum error rate of 3 per cent in family level invertebrate identifications must be maintained in order to undertake an assessment using the biological indices described in this Guideline.

6.3 Creating a voucher collection

The development and maintenance of a voucher collection of freshwater invertebrates is recommended for any laboratory or group undertaking aquatic bioassessment and is essential to both aid in correct identification of animals and to ensure everyone in the lab is using the same name for the same animal. A voucher collection is a reference collection of specimens that represent the variety of a taxon — family in this instance — that have had their identity confirmed by a senior taxonomist or a specialist in the field. A voucher collection is a useful tool for identification of invertebrates and complements taxonomic keys. It can help ensure that the same name is used for the same taxon by all individuals involved in sample processing, and can also aid in the correct identification of problematic specimens.

As new families are found and identified, they should be added to the voucher collection to create as complete a catalogue as possible of the aquatic invertebrates that may be encountered during a bioassessment program.

All specimens in a voucher collection should be stored in separate vials in 70 per cent alcohol and accurately labelled with the complete scientific name, the name of the river in which it was collected, the location name and date of collection.

Given that the specimens in a voucher collection serve as definitive family identifiers, it is essential that the integrity and quality of the collection be maintained. When using the voucher collection to aid with an identification, great care must be taken to ensure voucher specimens are not damaged or confused with specimens to which they are being compared. Good practice will include establishing any obvious differences, such as size or colour, before starting a detailed comparison.

When placing the voucher specimen into the Petri dish with other invertebrates for comparison purposes, exercise great diligence. The Petri dish may be accidentally knocked or differences in temperature and alcohol content can cause specimens to roll around in turbulence. Ensure that the voucher specimen, and not a sample specimen, is always returned to the voucher vial.

If a bioassessment program is discontinued, it is recommended that the voucher collection be lodged in the Museum of Victoria to provide a reference resource for future studies.

7 Physical predictor variables

Several geographical and physical parameters are required as predictor variables for the AUSRIVAS predictive models used to assess aquatic ecosystem condition. Some of these parameters are derived from 1:100,000 topographical maps, or digitally (e.g. GIS) if such facilities are available, while others are calculated from data collected in the field.

A complete list of the predictor variables required for the various AUSRIVAS models is provided in Appendix 9. Those parameters that have not been discussed in section 4 are described in more detail here.

LATITUDE and LONGITUDE: The latitude and longitude of a given site can be obtained from 1:100,000 topographical maps, with a hand-held Global Positioning System (GPS) and can also be recorded using Google Maps on a mobile phone. Readings taken from a map will most likely be Map Grid Australia (MGA), however older maps and sites visited pre mid 1990's may be Australian Map Grid (AMG) numbers. If this is the case, co-ordinates must be converted. and will be recorded as eastings and northings (always record the name and number of the map used). These must be converted into decimal degrees latitude and longitude for use in the AUSRIVAS predictive models. For input into the models, latitude must be recorded as a positive number.

ALTITUDE: Altitude is read directly from 1:100,000 topographical maps. Altitude is assigned from the topographical contour line closest to a site. If a site lies halfway between two contour lines, the midpoint value between the two lines is assigned as the altitude. Always check the contour line interval on the map being used before recording altitude (most maps use 20 m contour line intervals, but some use 40 m intervals). Altitude is recorded in metres above sea level. Some modern hand held GPS's have a function that allows the user to record altitude.

CATCHMENT AREA: Catchment area, also referred to as discharge area, is the total area of the catchment upstream of a given site. Catchment area is always measured and reported in square kilometres (km^2). Where access to GIS is available, this is the preferred procedure for calculating catchment area.

Alternatively, catchment area can be measured manually from 1:100,000 topographical maps using a digital or calibrated planimeter. The standard assumption for manual calculations of catchment area is that the ridges linking the highest points of the ranges dividing catchments, as shown by contour lines, are the boundaries of the catchments. Thus, all streams draining the high points towards the stream under study and upstream of the sample site are included in the calculation of catchment area for that site. The ridge lines can be traced onto tracing film or similar paper, and the planimeter applied to this. For sites encompassing large basin areas (e.g. five to six maps), such as in the Wimmera or the Goulburn catchments, smaller scale "basin maps" can be used. The planimeter will need to be recalibrated to this smaller scale before application. Estimates of catchment area taken from these smaller scale maps are not as accurate but are sufficient for use in the bioassessment models.

It is strongly recommended that two people independently calculate catchment area to ensure accuracy in the measure.

Another potential source for obtaining catchment area for sites near gauging stations is the Victorian Water Quality Monitoring Network, which maintains gauging stations around the state.

Catchment areas are available for all active gauging stations from the DELWP Water Measurement information System (http://data.water.vic.gov.au/monitoring.htm).

DISTANCE FROM SOURCE (DFS): As the name implies, this parameter measures the distance from a site to its uppermost headwaters. Functionally, this parameter acts as a surrogate for river size and the site's location within a catchment. The operational definition for distance from source is the longest possible distance that can be traced along a waterway following any continuous water course. Some estimation may be required in areas where headwater streams are intermittent or drain boggy or swampy land.

Distance from source is measured using a map distance measurement instrument (e.g. map wheel). Note that the bends in the river must be followed very carefully to document the full distance of the river. If a site is located on a distributary (a river that flows out of, or away from, a main river course), distance from source is still considered to be the distance to the headwaters, and not the distance to the main river channel from which the distributary diverged. Distance from source is measured in kilometres. As with catchment area, it is recommended that two people independently measure DFS to ensure accuracy in the measure.

SLOPE: Slope is determined using contour lines on a 1:100,000 topographical map. Always check the contour line interval on the map being used before calculating slope (most maps use 20 m contour line intervals, but some maps use 40 m intervals). To calculate slope at a site located between two contour lines, measure the length of the reach between the contour lines on either side of the site using a map wheel, and divide the contour line interval by this distance to give a slope in metres per kilometre (figure 24). If the site occurs directly on a contour line, use the two adjacent contour lines to determine slope (figure 25). Slope can also be determined using GIS. As with catchment area and DFS, it is recommended that two people independently measure slope to ensure accuracy in the measure.

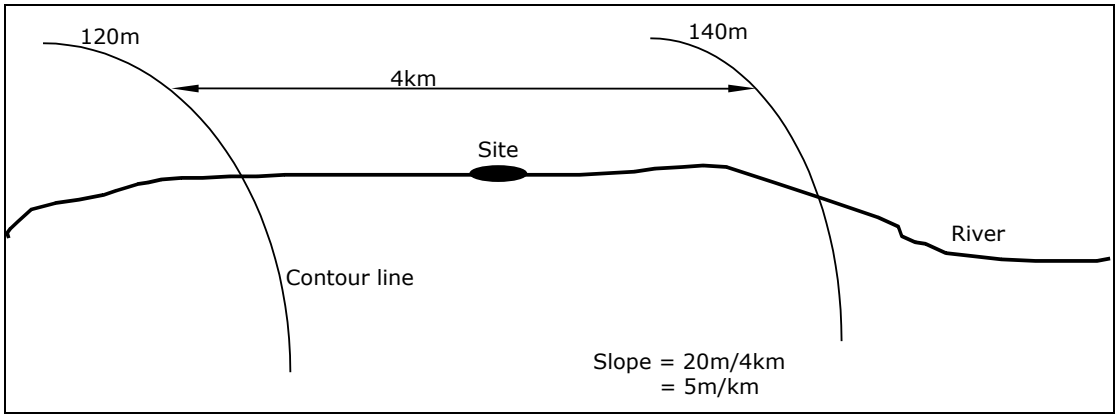

Figure 20: calculation of slope at a site located between two contour lines

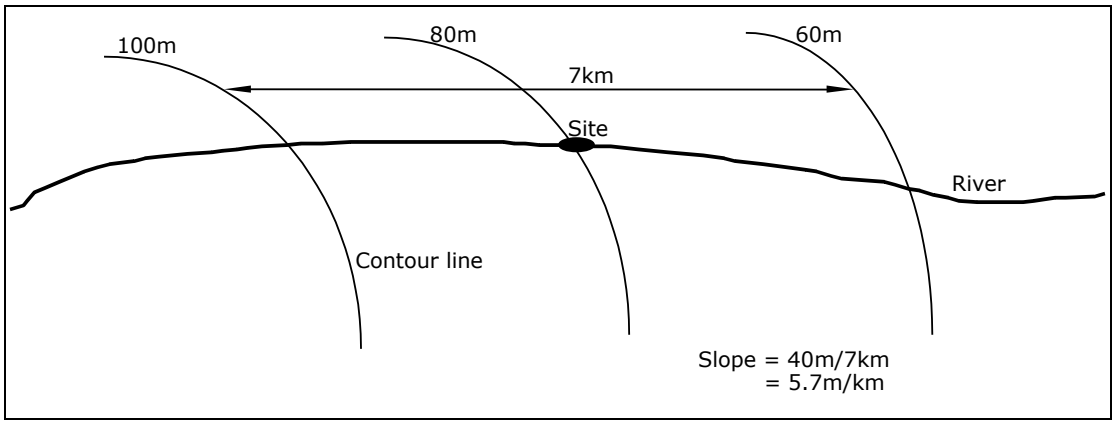

Figure 21: calculation of slope at a site that lies along a contour line

PHI: phi is an alternative, unit-less scale of sediment particle sizes commonly used by sedimentologists. It is used here as a way of representing the mean substrate particle size in a reach or riffle. Phi values for each of the substrate size categories are listed on the field sheets (refer to Appendix 3). To calculate mean substrate size in a reach in phi units, multiply the percentage of each substrate category present at a site by its phi value, divide by 100 to get a proportion, and then add them all together. Note that some phi values are negative. This parameter can also be calculated for the riffle habitat by using the percentages recorded for each substrate category in the riffle where the kick sample was collected.

SUBSTRATE HETEROGENEITY: substrate heterogeneity is a measure of the diversity of substrate sizes in a reach. This parameter is simply a sum of the number of substrate categories in the reach with percentage cover greater than 10. For example, if a reach contains 50 percent cobble, 40 percent pebble and 10 percent gravel, the substrate heterogeneity value for that reach equals 2.

8 Assessing the condition of aquatic ecosystems

There are many ways of analysing and interpreting invertebrate data to assess ecosystem condition. Ideally a number of methods are used, thus improving the robustness and reliability of conclusions. When results from different analyses are in accord, greater confidence may be placed in the outcome. Discrepancies, where they occur, can be used to indicate the type of environmental problem involved. Water quality and habitat condition also need to be considered for a complete assessment of aquatic ecosystem health.

The Environmental Reference Standards (ERS) includes four biological indices for assessing the condition of aquatic ecosystems. These were formerly in the SEPP (State Environment Protection Policy) (Waters) (EPA Victoria 2018) and have been transferred into the ERS. The indices fall into three categories:

- a measure of diversity — number of families
- biotic indices — the SIGNAL and EPT indices
- a measure of community composition — AUSRIVAS predictive models.

The development of these indices for assessing ecosystem condition has included the establishment of objectives to indicate agreed levels of environmental quality.

Aquatic communities will vary naturally across the State (fe.g. streams in the Wimmera will differ markedly from alpine streams). In recognition of this fact, the State has been characterised into five biological regions, called segments in the ERS. The objectives have been developed specific to the invertebrate communities within each region (Wells et al. 2002, EPA Victoria 2003a). Separate objectives had previously been developed for the Yarra and Western Port catchments (EPA Victoria 1999, 2001) but these are now replaced by the ERS.

A summary table of objectives and indicators from the ERS can be found in Table 1.

These biological indices and their associated objectives must be used wherever rapid bioassessment is undertaken for purposes required by the *Environment Protection Act 2017* including, but not limited to, works approvals, licence monitoring and assessment against environmental reference standards. In addition, the application of some but not all of these indices is required under various State and national strategies, for example, as occurred in 2010 in the Index of Stream Condition: The third benchmark on stream condition (DELWP 2010).

Table 1: Indicators and objectives to be used as environmental reference standards for rivers and streams in Victoria

Segment	Season	Habitat R = Riffle E = Edge	Indicators			
			EPT	SIGNAL2	Number of Families	AUSRIVAS Band
Highlands	Summer	R	9	6.1	17	N/A
		E	6	5	14	N/A
		ER	9	5.2	21	A
Uplands A	Autumn	R	8	5.6	19	A
		E	6	4.5	17	A
		ER	10	5.1	26	N/A
	Spring	R	7	5.6	17	A
		E	5	4.7	17	A
		ER	10	5.1	25	N/A
Uplands B	Autumn	R	7	5.2	18	A
		E	N/A	3.8	15	A
		ER	9	4.6	28	N/A
	Spring	R	8	5.5	18	A
		E	6	4.2	17	A
		ER	10	4.9	28	N/A
Central, Foothills and Coastal Plains	Autumn	R	5	4.5	16	A
		E	N/A	3.4	17	A
		ER	6	4.0	27	N/A
	Spring	R	5	4.5	16	A
		E	N/A	3.4	20	A
		ER	7	4.2	27	N/A
Urban	Autumn	R	4	3.9	13	B
		E	1	3.1	14	B
		ER	4	3.7	22	N/A
	Spring	R	3	4.2	13	B
		E	3	3.2	16	B
		ER	3	3.8	22	B

Segment	Season	Habitat R = Riffle E = Edge	Indicators			
			EPT	SIGNAL2	Number of Families	AUSRIVAS Band
Murray and Western Plains	Autumn	E	N/A	3.3	18	A
		ER	5	3.9	25	A
	Spring	R	N/A	4.4	14	N/A
		E	N/A	3.2	17	A
		ER	6	3.8	24	N/A

This guideline gives a description of each of the four biological indices to be used in Victoria and how each is calculated. However, no guidance or direction is provided on assessment or reporting as specific requirements will vary with, and be dependent on, the purposes for which an assessment is undertaken.

8.1 Using the biological indices

The application of the biological indices requires that sampling of the invertebrate community of a river or stream be conducted in accordance with the methods detailed in this guideline. Separate assessments are usually made for riffle and edge habitats but these new standards allow for assessment to be made with combined habitat data. The use of combined habitats data will be useful when using the EPT index. In some regions, the separate habits contain low numbers of taxa but when combined, the numbers of taxa are sufficiently large to reduce the impact of sampling error.

Biological samples can be collected once with either autumn or spring being suitable. Single season sampling will allow for flow intermittency (especially during autumn) and drought or dry conditions. The single season approach is supported by previous work by others which has found no significant differences between seasons and allowed for single season sampling (e.g. The Murray Darling Basin Authority's Sustainable Rivers Audit).

Biological samples should also be sampled from both riffle and edge habitats, separately. The data collected from the two habitats can later be combined for calculation of biological indices resulting in a larger number of families sampled, and a reduced influence of sample error.

In some instances, riffle habitats may not be present, or it may be impossible to sample them. Where the absence of riffle habitats is common, such as in lowland areas, environmental quality objectives have not been established for this habitat. A description of each biological index and how it is calculated is provided in sections 7.3 to 7.7. However, some essential taxonomic considerations must be addressed before proceeding with the calculation of the indices.

8.2 Taxonomic considerations

The biological indices require family level identification of invertebrates, with a few important exceptions:

- Chironomidae are always identified to sub-family.
- A higher than family level identification is adequate for a few select groups. Segmented worms need only be identified to Class Oligochaeta and all water mites are grouped together as Mites. Flatworms in the sub-order Temnocephalidea do not require further identification. For these taxa, this higher taxonomic level is treated as equivalent to family level. For example, the presence of mites in a sample would effectively contribute one to the Number of Families index.
- Recent major changes in taxonomy of the Odonata have not been incorporated into the biological indices. Therefore, the use of previously accepted family names is required. Specifically:
    - the families Aeshnidae and Telephlebiidae need to be summed to Aeshnidae
    - Synthemistidae, Austrocorduliidae, Cordulephyidae and Hemicorduliidae need to be summed to Corduliidae,

before calculating the indices. In addition, two families whose names have been changed must be listed under their old names when calculating the indices. These are Diphlebiidae (formerly Amphipterygidae) and Austropetaliidae (formerly Neopetaliidae).

- The following taxa are not included in the calculation of the biotic indices: Nematoda (roundworms); Rotifera; Gastrotricha; Bryozoa; Polychaeta; Collembola (springtails); all millipedes and spiders; isopods in the family Oniscidae; beetles belonging to Staphylinidae, Scaribidae and terrestrial families; terrestrial caterpillars and terrestrial snails; and the planktonic crustacean orders Cladocera (water fleas), Ostracoda (seed shrimps) and Copepoda.

It is important that these taxonomic considerations be addressed prior to the calculation of the indices because the inappropriate identification of any of these groups will alter the value of the indices, resulting in an erroneous site assessment.

8.3 Number of Families

The number of invertebrate families found at a site can give a reasonable representation of the ecological health of a stream as healthy streams generally have more families. The Number of Families index is calculated by simply summing the total number of 'families' of invertebrates present at a site.

Throughout a biological region, the expected number of families will vary according to quality of habitat and stream size, with larger streams, in general, supporting more taxa. Mild nutrient enrichment can increase the number of families due to an increase in food supply. Some streams may also be naturally diverse and could be considered as biodiversity 'hot spots.' Reduction in the expected number of families present can be caused by poor quality habitat and by various pollutants (e.g. toxicants).

8.4 The SIGNAL biotic index

SIGNAL (Stream Invertebrate Grade Number - Average Level) is an index of water quality based on the tolerance of aquatic biota to pollution (Chessman 1995). Using data from various studies of pollutants in south-eastern Australian streams, most, but not all, families of aquatic invertebrates have been assigned sensitivity grades according to their tolerance or intolerance to various pollutants. This index, now SIGNAL2, has since been revised for application to the entire of Australia (Chessman 2003). This update was based on survey data collected during the National River Health Program.

The list of invertebrate families (and sub-families) and SIGNAL 2 grades is included in appendix 8A. A number of orders, subclasses and phyla have also been assigned grades based on updated SIGNAL2 data (Chessman 2003), appendix 8B.

The SIGNAL index is calculated by summing together the sensitivity grades for each of the families found at a site that have been assigned a sensitivity grade, and then dividing by the number of graded families present. The output is a single number, between zero and ten, reflecting the degree of water pollution. Generally, high quality sites have high SIGNAL scores, and low quality sites have low SIGNAL scores. However, while SIGNAL is particularly good for assessing organic pollution, its usefulness for toxic impacts and other types of disturbance is less certain.

8.5 The EPT biotic index

The EPT index is the total number of families in the generally pollution-sensitive insect orders of Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). It is calculated by summing together the number of families in these three orders present at a site. Any loss of families in these groups usually indicates disturbance (Plafkin et al. 1989).

The EPT index cannot be used in all stream systems due to natural variations in the biogeographical distribution of the relevant taxa. For example, due to their ecological preference for well oxygenated, cool water streams, stoneflies and some mayfly families are naturally uncommon in the warmer, slower flowing waters that are typical of lowland regions. Therefore, the number of EPT families is not useful for assessing the ecosystem condition at these types of sites and objectives are not provided for the lowland biological regions.

8.6 AUSRIVAS

The Australian Rivers Assessment System, or AUSRIVAS, is a predictive modelling tool for assessing river ecosystem health (Davies 2000, Simpson and Norris 2000). Using a suite of mathematical models, AUSRIVAS predicts the invertebrates that should be present in specific stream habitats under reference conditions. It does this by comparing a test site with a group of reference sites which are as free as possible of environmental impacts, but which have similar physical and chemical characteristics to those found at the test site.

One of the products of AUSRIVAS is a list of the aquatic invertebrate families and their probabilities of being found at a test site if it was equivalent to reference quality. The sum of these probabilities of occurrence gives the number of taxa “expected” to be found at a site. By comparing the number of expected families with the number of families actually found, a ratio can be calculated for each test site. This ratio is expressed as the observed number of invertebrate families divided by the expected number of families at each site (the O:E score).

The value of the O:E score can range from a minimum of zero (none of the expected families were found at the site) to around one (all of the families which were expected were found). It is also possible to derive a score of greater than one, if more families were found at the site than were predicted by the model. A site with a score greater than one might be an unexpectedly diverse location, or the score may indicate mild nutrient enrichment by organic pollution, promoting increased colonisation of invertebrates.

The O:E scores derived from the models are compared to bands representing different levels of biological condition (Table 2). Although the precise scores that define each band may vary among regional models, band labels always correspond to the same classification (that is, band A always corresponds to "reference quality," band B to "below reference," and so on).

Sometimes the AUSRIVAS models do not produce an O:E score for a given site and instead describe the site as being 'outside the experience of the model'. This indicates that one or a combination of the environmental predictor variables places the test site beyond the scope of that encompassed by the reference sites used to build the model. In these cases, no assessment can be made using the AUSRIVAS index and assessments must be based on the other indices.

Previous versions of the GEM required the use of combined seasons, regional (i.e. segment) AUSRIVAS models for each habitat type for assessment against policy objectives. The new approach utilized within this guide, requires the use of single season, state-wide habitat based AUSRIVAS models for assessment against policy objectives. There are no combined habitat AUSRIVAS models at this stage.

Appendix 9 contains a complete list and brief descriptions of the predictor variables used in the state-wide AUSRIVAS models. Additional details on the measurement or calculation of the various predictor variables are provided in sections 4 and 6. When preparing the environmental predictor variables for inputting into AUSRIVAS be aware that some of them will consist of data collected in both the autumn and spring sampling seasons (e.g. alkalinity, stream width). The data to be used in the models is the average of the autumn and spring values. Note that not all predictor variables are required for all models.

The AUSRIVAS models and lists of the predictor variables required for each model, along with guidelines for running the models and the interpretation of results, are accessible on at the following URL: http://ausrivas.ewater.org.au.

Because invertebrate taxonomy can and does change, and every effort is made to keep up with these developments, it is essential that the taxonomy updates at this website be consulted before inputting data into the AUSRIVAS models.

Table 2: Band categories for AUSRIVAS O:E family scores

Band Label	Band Name	Comments
X	More biologically diverse than reference	More families found than expected; potential biodiversity 'hot spot'. Possible mild organic enrichment.
A	Similar to reference	Most/all of the expected familiesfound. Water quality and/or habitat condition roughly equivalent to reference sites. Impact on water quality and habitat condition does not result in a loss of macroinvertebrate diversity at the family taxonomic level.
B	Significantly impaired	Fewer families than expected. Potential impact either on water quality or habitat quality or both, resulting in loss of families. Possibly lost up to 40% of families.
C	Severely impaired	Many fewer families than expected. Loss of macroinvertebrate biodiversity due to substantial impacts on water and/or habitat quality. Possibly lost up to 70% of families.
D	Extremely impaired	Few of the expected families remain. Extremely poor water and/or habitat quality. Highly degraded. Possibly lost up to 100% of families

8.7 Rules for applying macroinvertebrate indicators

- *Assessments are to be made using three of the indices*

In some instances, all four biological indices may not be available for assessment. Therefore, EPA requires that at least three indices be used as the basis for an assessment, and it is recommended that SIGNAL2, EPT and AUSRIVAS are used as the primary indices.

Sometimes AUSRIVAS models cannot provide an assessment due to the input data being 'outside the experience of the model.' In these cases the Number of Families indicator is a suitable replacement.

- *Assessments are to be based on two samples collected from a site.*

All assessments against the objectives should be based on two samples collected at a site at a similar time. Where both habitats are present, they should be both included in the assessment and the combined habitats objectives used (refer to Table 1.).

- *Assessments should be based on combined riffle and edge habitats if these are present. If not, two edge habitat samples must be taken.*

In the wetter and more upland parts of the state, riffles are common and would usually be sampled, along with a sample from the edge of the stream. In the drier or more low-land areas of the state where riffles are uncommon or absent, two edge samples are to be taken. Under drought conditions the use of two edge samples will become more widespread. Where two edge samples are taken, the assessment against the edge objectives is based in the average of these two samples.

9 DNA barcoding approaches

Currently, rapid bioassessment involves taxonomic identification of aquatic macroinvertebrates based on morphology to mostly family level to assess stream and river health. This requires taxonomic expertise and can be made more difficult if specimens are fragmented, damaged or immature. However, employing DNA-based methods for identifying macroinvertebrates can greatly improve the reliability and taxonomic resolution of identification (Sweeney et al. 2011) and could provide a useful supplement for rapid bioassessment.

DNA-based identification can be used on any life stage and can provide species, rather than family, identification (e.g. Page, Choy & Hughes 2005; Hajibabaei et al. 2006a; Carew, Marshall & Hoffmann 2011). DNA barcoding, as proposed by Hebert et al. (2003), has been shown to be a powerful identification tool for identifying species (e.g. Ball et al. 2005; Hajibabaei et al. 2006a; Carew et al. 2007).

It has proven useful for understanding species diversity in many taxonomically difficult or poorly studied groups (e.g. Floyd et al. 2002; Blaxter, Elsworth & Daub 2004) and is often included in integrated taxonomic studies (e.g. Ferri et al. 2009; Tan et al. 2010). Typically, DNA barcodes can be easily obtained, analysed and interpreted and, with few exceptions, are highly accurate, identifying ~95% of invertebrate species (Hebert et al. 2003; Hajibabaei et al. 2006b). DNA barcoding can be especially useful for the routine detection of multiple species within and between studies, especially when species are morphologically cryptic or diversity is high (e.g. Sweeney et al. 2011).

The most useful gene for routine detection of species in animals is the mitochondrial COI gene, which is universally amplified in almost all animal taxa and shows strong species signal (Hebert et al. 2003.). Currently, there is a worldwide effort to DNA barcode animal life with the COI gene, with over 7 million DNA barcodes produced for invertebrates alone (http://www.boldsystems.org). While DNA barcodes can provide an excellent means for identifying species, until recently it has not been feasible to use them to routinely identify species for bioassessment as specimens required individual processing (Pfrender et al. 2010; Hajibabaei et al. 2011).

In contrast, next generation sequencing (NGS) has enabled DNA barcodes to be used for cost-effective bioassessment. In a single NGS instrument run, multiple species in many samples can be simultaneously DNA barcoded (and identified) (e.g. Aylagas et al. 2016; Elbrecht et al. 2017; Carew et al. 2018). This means that macroinvertebrate samples do not require individual sorting and identification as they can be bulk processed. This substantially reduces the time taken to identify species (Porter & Hajibabaei 2018) as well as providing more precise information on species composition. This process has become known as 'metabarcoding' (Yu et al. 2012).

Illumina's MiSeq Platform has been used for conducting metabarcoding, with increasingly well-developed protocols for its use in biodiversity assessment (Elbrecht et al. 2017; Porter & Hajibabaei 2018).

In contrast protocols using e-DNA, where water or sediment samples are used to detect macroinvertebrate diversity, are less developed and problematic (Roussel et al. 2015). Less macroinvertebrate diversity is detected with e-DNA when compared to metabarcoding because of the diffuse nature of invertebrate DNA in samples combined with the preferential detection of non-invertebrates (e.g. algae, zooplankton, fungi, bacteria) inhabiting water or sediment samples (Aylagas et al. 2016; Lim et al. 2016; Horton, Kershner & Blackwood 2017). Most current e-DNA protocols are targeted towards the detection of specific, often vertebrate, species and vertebrate biodiversity (e.g. Davy, Kidd & Wilson 2015; Civade et al. 2016; Robson et al. 2016).

Presently, metabarcoding is well suited for identifying macroinvertebrates collected using the rapid bioassessment protocols developed by EPA Victoria (Figure 15).

However, to use metabarcoding routinely in bioassessment more effort is needed to develop complete DNA barcode libraries where DNA barcodes are linked to voucher specimens which can be taxonomically verified (Carew et al. 2017). This is needed because the DNA barcodes generated by metabarcoding need to be compared to a reference DNA barcode library for identification. Online databases like the Barcode of Life's BOLD systems (http://www.boldsystems.org/), which includes data collected through the Murray-Darling Freshwater Research Centre (e.g. Shackleton & Rees 2015), and the National Centre for Biotechnology Information's GenBank (https://www.ncbi.nlm.nih.gov/genbank/) both contain DNA barcodes which can be used to identify specimens from metabarcoding. A recent analysis of the availability of DNA barcodes for Australian macroinvertebrates can be found in Carew et al. (2017). However, metabarcoding studies suggest while most widespread, common species in southern Australia are DNA barcoded, it is likely that few DNA barcodes are available for species in areas with high endemism, such as alpine areas (Carew et al. 2016; Carew et al. 2018).

To integrate metabarcoding and reference DNA barcoding into RBA protocols, sample preservation and handling require consideration (Carew et al. 2017). Currently, material collected under RBA protocols is picked in the field with 100% ethanol added to achieve 70-80% concentration in the final sample. Keeping the ethanol above 70% is also critical for short-term DNA preservation as DNA readily degrades in low percentage ethanol (Zimmermann et al. 2008; Baird et al. 2011).

After returning from the field, samples should be stored at 4°C and with ethanol preferably greater than 80% to further slow DNA degradation. Samples with debris stored in this manner typically do not show issues with DNA barcode amplification provided they are processed within ~6 weeks of collection (personal communication M. Carew).

Storing macroinvertebrate specimens in 70-80% ethanol is favoured for taxonomic work but for long-term DNA preservation samples should be stored in 100% ethanol at -20°C (Hajibabaei et al. 2005). Alternatively, Stein et al (2013) found that if samples were initially preserved in 95% ethanol, DNA barcoding was not affected by a low dilution ratio of 2:1 of 95% ethanol to water, transfer to 70% ethanol, the presence of abundant organic matter, or holding times of up to six months.

Considering DNA preservation when sampling macroinvertebrates ensures samples can be successfully used for metabarcoding and creating reference DNA barcodes. Further considerations when collecting macroinvertebrate samples especially for metabarcoding include not re-using ethanol and ensuring ethanol is not mixed between samples. Hajibabaei et al. (2012) showed that DNA barcodes can be obtained from preservation ethanol.

Furthermore, during sample sorting, care should be taken to ensure sample dishes are thoroughly cleaned (using detergent) between samples, to ensure no body-parts or residual ethanol from pervious samples remain. Metabarcoding is a highly sensitive technique which can detect species from trace quantities of DNA, so great care needs to be taken to avoid cross-contamination.

Macroinvertebrates families detected with metabarcoding are mostly analogous to those identified morphologically (e.g. Hajibabaei et al. 2011; Gibson et al. 2015; Carew et al. 2016). However, an increased number of species is detected with metabarcoding because cryptic species, and damaged and immature specimens can be easily recognised (Blaxter et al. 2004; Elbrecht et al. 2017; Carew et al. 2018; Porter & Hajibabaei 2018). Additional sources of variation exist between metabarcoding and morphological identification, that lead to slight differences in taxon detection. For example, metabarcoding has the ability to detect species from body parts, gut content, parasites, egg masses and exogenous sources. This means taxa not present morphologically can be detected from these sources using metabarcoding.

Alternatively, some taxa found morphologically are not detected with metabarcoding. These are often small taxa represented by single individuals or species from certain taxonomic groups with difficult to amplify DNA barcodes (Hajibabaei et al. 2012; Elbrecht et al. 2017; Carew et al. 2018). The detection of additional taxa and the failure to detect some taxa generally occurs at a low level, so that metrics calculated from either morphological or metabarcoding data produce similar site assessments (see Emilson et al. 2017; Carew et al. 2018).

Currently, metabarcoding for biological assessment is best suited for detecting species presence/absence as bias in factors, such as DNA barcode amplification and organisms size, effect the ability to quantify taxa (Hajibabaei et al. 2012; Elbrecht & Leese 2015).

EPA encourages the integration of metabarcoding into bioassessment. EPA views this new technology as a future tool for conducting through stream and river rapid bioassessment. It is becoming cost neutral compared with family-level morphological identification (Elbrecht et al. 2017; Porter & Hajibabaei 2018). Protocols are sufficiently developed; the limitations are understood and there are moves towards standardised methods with appropriate levels of replication and inclusion of negative and positive controls to ensure data quality.

In the short-term, more DNA barcoding is needed to develop reference DNA barcode libraries in Australia. Building these libraries will provide a valuable taxonomic and DNA resource and are a worthy investment. Initially, duplicate sampling of sites would provide the necessary macroinvertebrate material to allow individual DNA barcoding to build reference libraries and

allow widespread validation of metabarcoding against morphological methods. By metabarcoding first, taxa without DNA barcode taxa are discovered and these taxa can then be targeted for reference DNA barcoding. Once DNA barcode libraries and validation against morphological methods are largely complete, metabarcoding could be solely used for macroinvertebrate identification and will allow diagnostic species level metrics and indices to be developed for bioassessment.

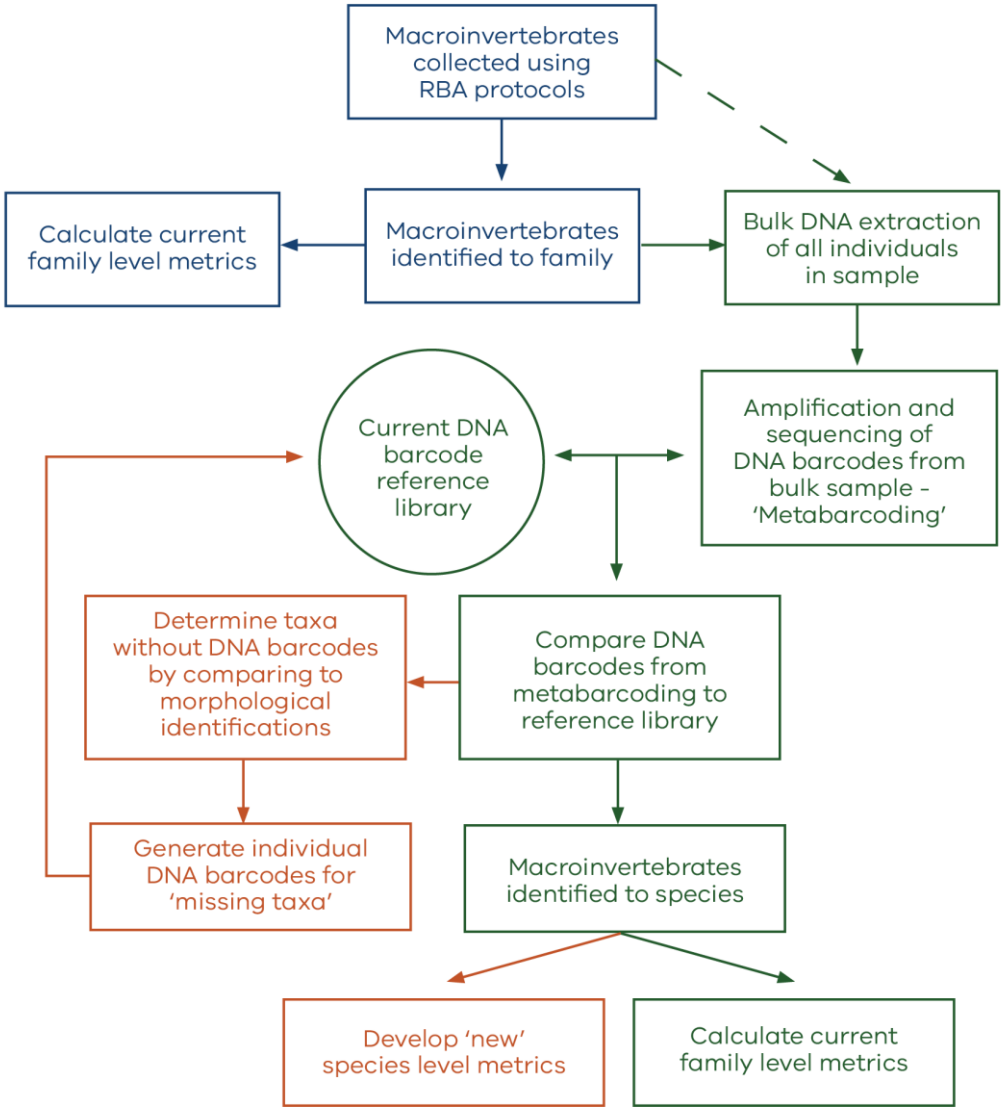

Figure 22. Workflow showing how metabarcoding (in green) of macroinvertebrate samples can be integrated into existing RBA protocols (in blue).

*Areas requiring further research are given in orange.

Currently, DNA barcode reference libraries are incomplete, so additional taxa as determined by metabarcoding will be required for individual DNA barcoding. In addition, there is a new opportunity through metabarcoding to develop diagnostic species-level metrics for routine use. Over time metabarcoding could be used solely for macroinvertebrate identification.

10 Quality assurance systems

The method described in this guideline requires consistency and quality in its implementation. The conduct and maintenance of quality assurance systems ensures that an assessment or monitoring program and management decisions are supported by data of the type and quality required and expected for their intended use.

The terms 'quality assurance' (QA) and 'quality control' (QC) are often confused. Quality assurance is all of the actions, procedures, checks and decisions undertaken to ensure the accuracy and reliability of data. Quality control is those parts of QA which serve to monitor and measure the effectiveness of QA procedures against previously agreed acceptance/rejection criteria.

For all biological assessment or monitoring purposes, a comprehensive and documented quality assurance system is required. The goals of a quality assurance program are precision, accuracy, completeness, comparability, reliability and representativeness of data. A QA system must address operational procedures and methods, staff training, validation and reporting of data, and review and corrective processes.

In general, users of sampling and analytical methodologies such as those described in this Guideline, must:

- perform some initial demonstration of capability and conduct ongoing demonstrations of capability
- maintain complete and accurate written records of all program data, and operational and performance testing
- demonstrate that procedures are being conducted and are operating within quality control limits
- perform all quality control operations according to a scheduled program
- establish the ability to generate data of acceptable accuracy and precision;
- participate in audits and performance evaluation studies
- unless otherwise prescribed by legislation or contractual obligation, retain all records for a period of not less than three years.

This guideline is not intended to provide detailed guidance for establishing and implementing a quality assurance system. For more information on developing a QA system, readers are referred to documentation available from NATA (e.g. NATA 1995).

There are, however, a number of processes that are specifically identified quality issues for the rapid bioassessment method. These are described in the following sections and appropriate practices addressing these issues must be included in any bioassessment QA system established to meet the needs of this Guideline.

10.1 Staff competence

Individuals engaged in bioassessment sampling and analyses require special expertise. Training and supervision of staff must be undertaken by experienced personnel with appropriate tertiary qualifications in relevant disciplines. Staff must be trained to a sufficient standard to ensure they are capable of undertaking the relevant tasks in a competent manner within the required quality control limits. The training program may consist of extensive 'on the job' instruction, in-house training programs, and/or specialist training courses conducted by external organisations where available. Records of all training received by staff (including 'on the job' training) and the level of skill attained must be maintained.

10.2 Field procedures

It is essential the quality assurance procedures be developed and implemented for the field component of rapid bioassessment to ensure the integrity of data collected in the field. The key areas that require attention are detailed below.

Site Location Information: prior to actual sampling, it may be necessary to select a new site and provide sufficient information for others to find it in the future, or to successfully find a previously sampled site.

It is surprisingly easy for a sampling team to think they are at the correct site when they are not, and it is important to have appropriate documentation procedures in place to avoid this.

An example of a complete and well-documented site location information sheet is given in Appendix 2. It is not necessary to follow this precise format, but it is essential that all the relevant data for finding the site (including map references and site drawings) are available in some form that can easily be used by others. These site documentation sheets are produced after the first visit to a site, using information collected by the first sampling team.

Biological Sampling: the collection and live sorting of biological samples must only be undertaken by experienced staff. New staff must be adequately trained and supervised in sampling a range of different stream types, until it is determined that they are conducting procedures with sufficient accuracy and reliability. Collecting the residue from a sample after live picking by an inexperienced sampler, and sorting part of this residue in the laboratory is an informative and useful exercise for determining what particular invertebrate groups are routinely being missed in the live picking technique.

Habitat and Water Quality Assessments: field sheets must be filled out by two experienced persons to ensure that a consensus is reached on the estimated and/or more subjective habitat variables. An inexperienced person can be involved, but only as a third person for the purposes of providing 'on the job' training in these aspects. This should remain in force until they are judged as proficient and meet the expectations of an 'experienced' person. Field sheets should be checked for completeness in the field, before leaving the site.

All measurement of water quality parameters must be made, and instruments calibrated, maintained and operated in accordance with the instrument manufacturer's instructions as well as standard scientific methods and the procedures outlined in this guideline.

10.3 Laboratory processing of invertebrate samples

Quality assurance and quality control of laboratory identifications are essential for correcting errors in identification and enumeration, to highlight problems that may require further training, and to establish error levels on the data.

In order to ensure accuracy in the processing and identification of the biological samples, a subset of all samples must be re-identified and re-counted by a Victorian macroinvertebrate expert on a periodic basis. This guideline requires a maximum error rate of 3 per cent for invertebrate identifications at the family level. Any individual or laboratory undertaking rapid bioassessment using the method described in this Guideline must be able to show that invertebrate identifications are within this error limit.

Laboratory QA is performed on a per-individual basis. Initially, all invertebrate samples sorted and identified by staff in training must be re-identified and re-counted by a senior taxonomist until a 3 per cent error rate in identification is established and maintained. With more experienced staff, an appropriate time to conduct QA checks is after an individual processor has completed a series of samples, such as those from one or two catchments comprising between 10 and 30 samples. A senior taxonomist then randomly selects 10 per cent of these samples, ideally including both kick and sweep samples, for re-identification and re-counting. The individual who performed the original processing and identification of the samples must not know in advance which samples will be checked.

A QA assessment sheet is completed following the re-identification of a sample. An example of such a sheet is provided in Appendix 11. The information recorded on these sheets includes: any incorrect identifications at family level (or higher, as required), any overlooked taxa, or taxa put into vials but inadvertently not recorded on the laboratory count sheet, any discrepancies in enumeration, and any discrepancies in the level of resolution of the identification. A record is made of any actions required by the individual who originally processed and identified the sample to correct errors found as a result of the re-identification (e.g. re-identification of problem taxa in other samples from that catchment).

To calculate an individual's sample level error rate, the number of miss-identifications and the number of taxa not recorded on the count sheet from a given sample are added together, and expressed as a percentage of the total number of taxa identified in the sample. Other errors, such as enumeration errors, are not included in the calculation of identification error rates.

Because samples typically do not have more than 30 families represented, one or more errors in any given sample can produce an error rate greater than 3 per cent. Therefore, an individual's *actual* error rate should be calculated over all of an individual's samples that have been re-identified in a given year (or other appropriate time period). This is done by adding together the mis-identification and recording errors (as described above) across all samples processed by the individual and dividing by the total number of taxa in those samples.

It is essential that the re-identification and re-counting of invertebrate samples be carried out regularly, rather than at the end of a program, to enable rapid correction of the data and prevent repeated mistakes.

It is also important that quality assurance of sorted samples be completed prior to data entry, to reduce the potential number of changes (and associated transcription errors) required to the database.

It is also strongly recommended that all data entry be independently validated by a second individual.

10.4 Calculating the biological indices

There are several places where errors can occur during the calculation of the biological indices. To minimise the risk of errors, it is strongly recommended that the data be retrieved, and indices calculated independently by two persons, and then compared to ensure they match. Two people should also independently measure the various geographical parameters used in the AUSRIVAS models to ensure the accuracy of these measures.

Although this practice requires an additional investment of time and resources, ensuring the accuracy and integrity of a dataset should not be treated as a trivial issue. As an absolute minimum, a second person with experience in the calculation of the parameters and indices, should critically appraise the dataset to identify any potential discrepancies or errors.

Glossary

Algae – Various chiefly aquatic, photosynthetic organisms, ranging in size from single-celled forms to the giant kelp (singular is alga).

Alkalinity – The alkalinity of water is its acid neutralizing, or buffering, capacity. In surface waters it is primarily a function of carbonate, bicarbonate, and hydroxide concentrations.

Ambient – Existing in the surrounding environment or area, as in 'ambient temperature.'

AMG (Australian Map Grid) – The Australian Map Grid (AMG) is a spatial coordinates system used to pinpoint locations in Australia, and is similar in operation to latitude and longitude systems.

Anaerobic – The absence of oxygen. Anaerobic sediments have a distinctive sulfurous or "rotten egg" odour due to the action of anaerobic bacteria.

AUSRIVAS (Australian River Assessment System) – This is a rapid biological assessment system for streams and rivers that generates region-specific predictions of the invertebrate fauna expected to be present in the absence of environmental stress. Predicted or expected fauna are obtained from modelling data collected from a number of reference sites. The predicted fauna are then compared to the observed fauna lists and the resulting ratio is used to indicate the extent of the anthropogenic impact.

Backwater – An area of still water in a stream caused by an obstruction or an opposing current in a river channel, or across a river bar, usually with little or no flow.

Bar – An exposed expanse of more or less linear deposits of sand, gravel or coarser substrates in a river.

Bedrock – The solid continuous rock that underlies loose rock, sand, clay, or gravel. Bedrock is often exposed in streambeds and appears as large flattened or contoured expanses of rock.

Benthic – Relating to or happening on the bottom; under a body of water.

Bioassessment – The systematic use of biotic responses to stressors to estimate and evaluate changes in the environment.

Biological Index – A measure or indicator of biological condition. In the Guideline it refers to the different measures of aquatic invertebrate community composition used in assessing the ecological condition of rivers and streams.

Biological Region – a defined geographical region that is characterised by having a particular biological community and similar physical and environmental conditions throughout.

Boulder – Stream substrate particles that are greater than 256mm in diameter (excluding bedrock).

Braiding – Stream channel morphology consisting of interwoven channels flowing around islands or bars of coarse sediments and/or sandbanks, instead of a single channel.

Catchment – The total land area above a specific point on a river from which water drains towards the river.

Channel – The bed of a stream or river between the crest of its flood banks; it is usually wider than and includes the stream's wetted area.

Channel Sinuosity – A bend, or a series of bends and turns in the stream channel.

Clay – A soft earth (particle size less than 0.004mm in diameter), which is sticky, plastic, or may be moulded with the hands.

Cobble – Stream substrate particles ranging from 64 to 256 mm in diameter; usually naturally rounded by erosional forces.

Conductivity – A measure of the ability of water to carry an electrical current which depends on the presence of ions in solution. It is used as an indication of salinity. The conductivity of a solution depends on temperature and is typically measured at both ambient temperature (the actual temperature of the water) and adjusted to a standard 25°C.

CPOM (Coarse Particulate Organic Matter) – The leaves and leaf fragments greater than 1 mm in size and small sticks less than 10cm in diameter that make up organic detritus in freshwater bodies.

Cryptic – Concealed or camouflaged.

Current Velocity – The speed at which water is flowing in a stream, usually measured in metres/second (m/s); may vary considerably over small spatial scales.

Detritus – A generic term referring to organic material formed from decomposing plant litter and other organisms. Often forms deposits in pools and backwaters of streams.

Discharge – The total volume of water flowing past a given point in a river over a given amount of time. Units are either megalitres/day or metres^3/hour.

Dissolved Oxygen (DO) – Oxygen that is dissolved in water, measured as mg/L or percentage saturation. DO levels in natural waters depend on physical, chemical and biological activity in the water body and will vary with temperature and altitude, and also with light levels due to photosynthetic activity. Dissolved oxygen is essential for the respiration of aquatic organisms; low DO can result in fish kills and reductions in invertebrate fauna.

DNA – Deoxyribonucleic acid, organismal building blocks of life

Edge – One of the two stream habitats sampled in running waters using the rapid bioassessment method. Edge habitats may consist of a combination of bare edges, macrophyte beds, overhanging vegetation, undercut banks, logs, backwaters, etc.

eDNA – Environmental DNA is a exogenous DNA that is released into the environment by an organism. Sources include shed skin and hair, faeces, mucous and carcases.

Embeddedness – The degree to which larger substrate particles, such as boulders and cobbles, are surrounded or tightly held in place by finer substrate particles.

EPT – A biological index that consists of the sum of all families within the insect orders of Ephemeroptera (mayflies), Trichoptera (caddisflies) and Plectoptera (stoneflies) found at a site. As members of these orders are generally pollution sensitive, any loss of families from these groups usually indicates disturbance.

Erosion – A natural or anthropogenically driven process, including weathering, abrasion, corrosion, and transportation, by which material is worn away from the earth's surface.

Fauna – Animals, especially the animals of a particular country, region, or time, considered as a group or community.

Filamentous Algae – Algae that is visible to the naked eye and is formed from a chain-like or thread-like series of cells; often forms mats or clumps in streams.

Flora – Plants, especially the plants of a particular country, region, or time, considered as a group or community.

FPOM (Fine Particulate Organic Matter) – The very small (less than 1mm) organic detritus particles in freshwater bodies, produced through the action of mechanical and/or biological grinding forces on leaves, twigs and small sticks.

GIS (Geographic Information System) – A computer mapping software system for manipulating, analysing and displaying data related to locations on the Earth's surface.

GPS (Global Positioning System) – A system of satellites, computers, and receivers that is able to determine the exact location of a receiver on Earth by calculating the difference in time required for signals from different satellites to reach the receiver.

Gravel – Stream substrate particles ranging from 2 to 16mm in diameter.

Groundcover – Small plants, such as mosses, ferns, grasses, and herbs, growing close to the ground (does not include saplings).

Habitat – The area or environment where an organism or ecological community normally lives or occurs.

Headwater – The source and upper part of a stream.

Inorganic – Of, or relating to, compounds not containing hydrocarbon groups; not composed of organic matter.

In-situ – A Latin term meaning 'in its original place.' For example, measuring water quality *in-situ* refers to taking water quality measurement directly in the body of water of interest.

In-stream – Within the wetted channel of a river or stream.

Invertebrate – An animal, such as an insect, worm, crustacean or mollusc, which lacks a backbone or spinal column.

Kick Sample – One of two biological samples that form the core of the rapid bioassessment method. Kick samples are collected in riffle or run habitats.

Latitude – The angular distance north or south of the earth's equator, measured in degrees, minutes and seconds along a meridian.

Littoral – Of, or relating to, a coastal or shore region of a body of water; the region of the shore of a lake, river or ocean.

Longitude – Angular distance on the earth's surface, measured east or west from the prime meridian, expressed in degrees, minutes, and seconds.

Macrophyte – An aquatic plant that is visible to the naked eye, but not including filamentous algae, mosses or liverworts.

Metabarcoding – DNA extraction, amplification and DNA barcoding of bulk samples of invertebrates.

Microhabitat – A very small, specialised habitat, such as within a clump of leaf litter or in the space between rocks.

Monitoring Programs – Sampling programs undertaken to identify changes in the quality or health of an ecosystem or environment over time.

Moss – A low-growing, leafy-stemmed plant typically growing in tufts or mats on the surface of rocks or logs.

Next Generation Sequencing – also known as high-throughput DNA sequencing, is DNA sequencing method that allows simultaneous sequencing of many different species from multiple samples.

Nutrients – Chemical compounds that are necessary for the growth and reproduction of organisms. In limnology, the term generally refers to the different nitrogen and phosphorous compounds dissolved in water that are essential for the growth of plants and algae.

O:E Score – For AUSRIVAS - The number of expected macroinvertebrate taxa collected (observed) divided by the number expected to occur at a site

Organic – Derived from living organisms.

Organism – An individual form of life, such as a plant, animal, or fungus.

Overstorey – The tallest plants, generally trees, which form the canopy in a vegetation community.

PCR – Polymerase chain reaction is a method for measuring eDNA, qPCR (quantitative polymerase chain reaction) is the preferred method.

Pebble – Stream substrate particles ranging from 16 to 64mm in diameter.

pH – A measure of the acidity or alkalinity of a solution. The pH scale commonly in use ranges from 0 to 14, with neutral solution having a pH of 7. pH increases in value with increasing alkalinity and decreases with increasing acidity. The pH of natural waters is generally between 6 and 8.

Plume – The cloud of suspended sediment formed while disturbing a stream's substrate in fast flowing areas.

Point Source Pollution – Pollution entering a waterway through a single identifiable source, such as a drain or roadway.

Pool – A still or slowly flowing section of a stream that is usually deep but can be shallow.

Quality Assurance (QA) – The actions, procedures, checks and decisions undertaken to ensure that data is accurate and reliable.

Quality Control (QC) – Those parts of a quality assurance system which serve to monitor and measure the effectiveness of QA procedures against previously agreed acceptance/rejection criteria.

Range Finder – Any of various optical, electronic, or acoustical instruments used to determine the distance of an object from the observer.

Rapid Bioassessment – A biological assessment approach that is designed to reduce costs (effort and financial) while producing results that are readily interpretable by non-specialists such as managers, decision-makers and the general public.

Reach – An expanse of stream or river under study. For rapid bioassessment purposes, reach is defined as ten times the average stream width, from a minimum of 50m to a maximum of 150m.

Reference DNA barcode – DNA barcoding of individual specimens that are typically vouchered and taxonomically verified. These can be used to generate libraries of DNA barcodes which can be used to identify taxa in metabarcoding or e-DNA surveys

Reference Site – A relatively unimpacted or best available site on a river or stream used as a reference or benchmark with which to compare test sites; used in the generation of AUSRIVAS models and the development of environmental quality objectives for the biological indices used in assessing stream condition in Victoria.

Regulated River – A river or stream that has been subjected to flow regulation by dams, reservoirs, weirs, diversions or other large stream works.

Riffle – A stretch of choppy water in a stream or river caused by shallow fast flows over rocks, a shoal or a sandbar; a rapid.

Riparian – Relating to or located on the banks of a river or stream, especially in terms of vegetation interacting with the stream.

Run – Fast flowing water with an unbroken surface in a stream or river, usually deeper than riffles.

Salinity – The relative proportion of salt in a solution.

Sand – Substrate particles ranging from 0.06 to 2mm in diameter; can be distinguished from silt by its coarse texture.

Sediment – Solid fragments of inorganic material that come from the weathering of rock and are carried and deposited by water; can include gravel, sand and silt.

SIGNAL and SIGNAL2 (Stream Invertebrate Grade Number – Average Level) – An index of stream health based on the relative pollution tolerances of various invertebrate families. The presence or absence of particular invertebrate families is used as an indication of water quality at a site. Generally used in conjunction with other biotic indices, such as AUSRIVAS.

Silt – Very fine sediment particles ranging from 0.004 to 0.06mm in diameter; can be distinguished from sand by its smooth texture. Silt can be either organic or inorganic in origin.

Snag – A tree or part of a tree that has fallen into a river, forming habitat for aquatic organisms.

Substrate – A surface on which an organism grows or is attached. In streams, the material that makes up the river bottom.

Survey – A comprehensive inspection or investigation; a gathering of a sample of data considered to be representative of a whole.

Sweep Sample – One of two biological samples that form the core of the rapid bioassessment method. Sweep samples are collected in edge habitats.

Taxon – A taxonomic category or group, such as a phylum, order, family, genus, or species (plural is taxa).

Taxonomic – Pertaining to, or involving, taxonomy, or the laws and principles of arranging species or groups into a system exhibiting their relationship to each other and their places in a natural classification.

Toxicant – A poison or poisonous agent.

Turbidity – A measure of water clarity, turbidity is caused by material such as clay, silt, or fine particulate organic and inorganic matter becoming suspended in the water column; not to be confused with colour caused by dissolved organic matter such as tannins and lignins. High turbidity is usually associated with erosional processes.

Understorey – The plants in a vegetation community that comprise the layer below the canopy, generally shrubs, tree ferns, vines and the like.

VEGCAT – Vegetation category. A land use category used in AUSRIVAS models. It is the best summary of land use for the area surrounding the sampled reach, within sight of or as ascertained from approaching the site, but not including the riparian zone.

Voucher Collection – A reference collection of specimens that includes examples of all reported taxa and which represents the variability present within a given taxon. The purpose of a voucher collection is to aid in identifications and ensure taxonomic consistency.

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Appendix 1: Field equipment checklist

- ✓ Site location information sheets and photos
- ✓ Maps – Google maps, 1:100,000 paper map sheets, HEMA digital maps etc.
- ✓ Field sheets and spares (and extra field sheets printed on waterproof paper) or digital devices
- ✓ Guideline for Environmental Management: Rapid Bioassessment Methodology for Rivers and Streams
- ✓ Camera or mobile phone
- ✓ GPS
- ✓ Mobile field communications
- ✓ Percentage cover diagram
- ✓ Particle size diagram
- ✓ Clipboards (2)
- ✓ Waders
- ✓ Kick net (and spares) [dimensions: 250µm mesh, length 1m, opening around 30 by 30cm]
- ✓ Sweep net (and spares) [dimensions: 250µm mesh, length 40cm, opening around 30 by 30cm] Plastic buckets (2)
- ✓ Sorting trays for live-picking (can also use ice-cube trays) (2)
- ✓ Table and stools
- ✓ Live pick jars [e.g. wide-mouth, screw cap glass or polyethylene jars] (2 per site and spares)
- ✓ 100 per cent ethanol
- ✓ Squeeze bottles
- ✓ Plastic pipettes
- ✓ Nutrient bottles [250mL polyethylene bottles] (2 per site and spares)
- ✓ Turbidity bottles* [100mL or larger polyethylene bottles] (1 per site and spares)
- ✓ Alkalinity bottles* [500mL polyethylene bottles] (1 per site and spares)
- ✓ Plastic bags (ziplock, whirlpak etc.) for vegetation samples
- ✓ Water chemistry meters/kits (and operation manuals and spare batteries)
- ✓ Esky and ice for water samples
- ✓ Portable car refrigerator/freezer for nutrient samples (if available)
- ✓ Metre rule
- ✓ Measuring tape (50 or 100m)
- ✓ Range finder (if available)

* Sample bottles for turbidity and alkalinity only required if not measured with meters in the field.

Appendix 2: Example of a site location information sheet

CATCHMENT: EAST GIPPSLAND

RIVER/STREAM: Errinundra River
SITE LOCATION: Errinundra
SITE CODE: ZZP

Latitude: 37.3752 o S Longitude: 148.8997 o E
Map co-ordinates: VicRoads 68G8 1:100 000 Map 8623 Bendoc 6682 58618
Altitude: 220 metres

Longterm Reference Site [ ]
AUSRIVAS Reference Site [X]
Test Site [ ]

Invertebrate Sampling History

A = Autumn ; S = Spring

Year	Season Sampled	Year	Season Sampled
1993	-	2000	-
1994	A, S	2001	-
1995	A	2002	A, S
1996	S	2003	
1997	-	2004	
1998	-	2005	
1999	-	2006	

Appendix 3: Field sheets

EPA FIELD SAMPING AND HABITAT ASSESSMENT SHEETS

RIVER..... CATCHMENT .....

LOCATION..... DATE..... TIME .....

LOCATION CODE..... PHOTOGRAPH(S) .....

RECORDER(S) NAME(S) .....

AUSTRALIAN MAP GRID REFERENCE

LATITUDE .....LONGITUDE .....

is location documentation complete? .....

REACH DIMENSIONS

LENGTH OF SURVEYED REACH ..... m
This is defined as 10X the average stream width, to a maximum of 150m. Minimum reach length is 50m.

Stream Habitat in surveyed reach (%) Riffle/Run ..... % Pool .....%

Stream Width from edges of water. Take 5 evenly spaced measurements within surveyed reach; also record maximum and minimum.

1.....m 2.....m 3.....m 4.....m 5.....m Max.: .....m Min.: .....m

Method used: tape measure [ ] range finder [ ] estimate [ ]

Channel width from tops of banks 1.....m 2.....m 3.....m 4.....m 5.....m

Method used: tape measure [ ] range finder [ ] estimate [ ]

WATER QUALITY MEASUREMENTS: Instrument(s) make, model and number .....

Water Temperature (ºC) ..... pH .....

Conductivity (ambient) ..... µS/cm ..... mS/cm Alkalinity (mg/L):

Measured in lab..... [ ] or field.....[ ]

Conductivity (@ 25 ºC) ..... µS/cm ..... mS/cm Turbidity (NTU).....

Measured in lab..... [ ] or field.....[ ]

Dissolved Oxygen (mg/L) .....
(agitate probe if flow <5 cm/sec)

% Sat. Dissolved Oxygen .....
(agitate probe if flow <5 cm/sec)

Water samples collected for: Nutrient analysis..... Yes [ ] No [ ]
Turbidity..... Yes [ ] No [ ]
Alkalinity..... Yes [ ] No [ ]

RIVER ..... DATE ..... LOCATION CODE .....

REACH: All information in this section refers to the entire reach.

SUBSTRATE DESCRIPTION (% cover): USE PARTICLE SIZE DIAGRAM.

			phi
Bedrock	.....	[.....]	(-9.5)
Boulder	(>256mm)	[.....]	(-9.0)
Cobble	(64-256mm)	[.....]	(-6.5)
Pebble	(16-64mm)	[.....]	(-4.5)
Gravel	(2-16mm)	[.....]	(-2.0)
Sand	(0.06-2mm)	[.....]	(2.0)
Clay/Silt	(<0.06mm)	[.....]	(8.0)
	Total	.....100%.....	

OTHER STREAM FEATURES

Percent of reach covered by...	<1%	1-10%	10-35%	35-65%	65-90%	>90%
Willow Roots	0a	0b	1	2	3	4
Moss	0a	0b	1	2	3	4
Filamentous algae	0a	0b	1	2	3	4
Loose silt lying on substrate (organic & inorganic)	0a	0b	1	2	3	4
Total macrophytes	0a	0b	1	2	3	4

(For macrophytes, include those which are out of the water but in the active channel.)

ORGANIC MATERIAL (% cover of organic material)

Coarse Particulate Organic Material (leaves and wood < 10cm diameter)	1. <5%	2. 5 – 20%	3. >20%	[ ]
Snags/Large Organic Material (wood >10cm diameter)	1. <5%	2. 5 – 20%	3. >20%	[ ]

CURRENT VELOCITY IN REACH: Choose one percentage category for each flow category in the reach:

	0%	1-10%	11-40%	41-60%	>60%
No obvious flow	0	1	2	3	4
Slow	0	1	2	3	4
Medium/moderate	0	1	2	3	4
Fast to very fast	0	1	2	3	4

VEGCAT (Landuse category for AUSRIVAS.) Land use beyond the riparian zone (30m).

1. Urban
2. Intensive agriculture
3. Mostly cleared, grazing
4. Significant patches of forest remaining, some forestry/agriculture (eg, grazing)
5. Native forest/natural vegetation [ ]

SHADING of stream channel, as at mid day (shading category for AUSRIVAS). USE % SHADING DIAGRAMS.

1. <5% 2. 6-25% 3. 26-50% 4. 51-75% 5. >76% [ ]

RIVER .....DATE..... LOCATION CODE .....

RIFFLE /RUN: All information in this section refers only to the riffle/run area sampled.

Invertebrates collected by ..... Invertebrates picked/ sorted by .....

Length of riffle/run sampled: 10 metres [ ] Other ..... metres. Time taken to pick sample .....mins.
Approx. # of invertebrates picked: 200[ ]...150[ ]...100[ ]...50[ ], if <150, why?.....

SUBSTRATE DESCRIPTION (% cover): USE PARTICLE SIZE DIAGRAM.

			phi
Bedrock	.....	[.....]	(-9.5)
Boulder	(>256mm)	[.....]	(-9.0)
Cobble	(64-256mm)	[.....]	(-6.5)
Pebble	(16-64mm)	[.....]	(-4.5)
Gravel	(2-16mm)	[.....]	(-2.0)
Sand	(0.06-2mm)	[.....]	(2.0)
Clay/Silt	(<0.06mm)	[.....]	(8.0)
	Total	....100%..	

IF RIFFLE/RUN NOT SAMPLED, WHY NOT:
1. Not present [ ]
2. Too shallow [ ]
3. Too small [ ]
4. Too dangerous [ ]
5. Other ..... [ ]

Percentage of sampled area covered by:	<1%	1-10%	10-35%	35-65%	65-90%	>90%
Willow Roots	0a	0b	1	2	3	4
Moss	0a	0b	1	2	3	4
Filamentous algae	0a	0b	1	2	3	4
Macrophytes	0a	0b	1	2	3	4
Loose silt lying on substrate (organic & inorganic)	0a	0b	1	2	3	4

Coarse Particulate Organic Material (leaves and wood < 10cm diameter) 1. <5% 2. 5 – 20% 3. >20% [ ]
Snags/Large Organic Material (wood >10cm diameter) 1. <5% 2. 5 – 20% 3. >20% [ ]

DEPTH 1.....cm 2 .....cm 3.....cm 4.....cm 5.....cm
Take 5 representative measurements from the riffle/run habitat over the range of depths in the kick sample.

CURRENT VELOCITY in sampled area. Tick boxes for each current velocity present; more than 1 box can be ticked:
Kick sample: 0. no flow [ ] 1. slow [ ] 2. medium/moderate [ ] 3. fast to very fast [ ]

EDGE / BACKWATER: All information in this section refers only to the edge area sampled.

Invertebrates collected by ..... Invertebrates picked/ sorted by .....
Length of edge sampled: 10 metres [ ] Other ..... metres. Time taken to pick sample .....mins.
Approx. # of invertebrates picked: 200[ ]...150[ ]...100[ ]...50[ ], if <150, why?.....

Percentage of sampled area covered by :	<1%	1-10%	10-35%	35-65%	65-90%	>90%
1. Backwaters	0a	0b	1	2	3	4
2. Leaf packs/CPOM	0a	0b	1	2	3	4
3. Undercut banks	0a	0b	1	2	3	4
4. Roots	0a	0b	1	2	3	4
5. Bare edge	0a	0b	1	2	3	4
6. Logs (wood >10cm)	0a	0b	1	2	3	4
7. Trailing bank vegetation (including grasses)	0a	0b	1	2	3	4
8. Filamentous algae	0a	0b	1	2	3	4
9. Macrophyte	0a	0b	1	2	3	4
10. Moss	0a	0b	1	2	3	4
11. Loose silt lying on substrate (organic & inorganic)	0a	0b	1	2	3	4
12. Other.....	0a	0b	1	2	3	4

CURRENT VELOCITY in sampled area. Tick boxes for each current velocity present; more than 1 box can be ticked:
Sweep sample: 0. no flow [ ] 1. slow [ ] 2. medium/moderate [ ] 3. fast to very fast [ ]

RIVER ..... DATE ..... LOCATION CODE .....

SITE OBSERVATIONS (Indicate appropriate number in brackets at right; some may consist of >1 category.)

WATER ODOURS: 1. normal 2. sewage 3. petroleum 4. chemical 5.stormwater 6. musty [ ]

WATER OILS: 1. none 2. slick 3. sheen 4. globs 5. flecks [ ]

TURBIDITY: 1. clear 2. slight 3. turbid 4.opaque/liquid silt (clay like) [ ]

PLUME: (ONLY in riffle/run; amount of easily disturbed fine sediment)
1. little or none 2. some 3. lots 4. no riffle /run present [ ]

SEDIMENT ODOURS:
1. normal 2. sewage 3. petroleum 4. chemical 5. anaerobic 6. Other .....[ ]

FLOW LEVEL: (relative to "water mark", i.e. normal inundation level shown by limit of terrestrial grasses, by eroded area, or by boundary in bank sediment types)
1. No flow (dry / isolated pools) 2. Low (<<water mark) 3. Moderate (around water mark) 4. High (>>water mark) 5. Flood [ ]
DO NOT SAMPLE IN HIGH FLOW OR FLOOD.

BARE GROUND above normal inundation level shown by "water mark": Left bank ..... %
(This refers only to erosional ground; bedrock is not erosional ground.) Right bank ..... %

LOCAL CATCHMENT EROSION: (within sight of site) 1. none 2. slight 3. moderate 4. heavy [ ]

LOCAL POINT SOURCE POLLUTION: 1. None 2. Gravel road/track/ford 3. Tip 4. Quarry 5. Drain 6. Fish farm 7. Earthworks 8. Mine 9. Stock access point 10. Culvert 11. STP 12. Other .....[ ]

DAMS / BARRIERS:
1. present upstream 2. small artificial structure (e.g. weir) at or immediately downstream of site 3. absent 4. not known [ ]

BRAIDING: 1. yes, no. of channels ..... 2. no [ ]

SITE CLASSIFICATION: 1. steep valley 2. broad valley 3. plains [ ]

LEFT BANK AND RIGHT BANK REFER TO DIRECTION FACING DOWNSTREAM.

LANDUSE: Left Bank 1. Native forest 2. Forestry 3. Native heath/grassland 4. Grazing 5. Cropped 6. Residential 7. Industrial 8. Recreational 9. Intensive agriculture [ ]

LANDUSE: Right Bank 1. Native forest 2. Forestry 3. Native heath/grassland 4. Grazing 5. Cropped 6. Residential 7. Industrial 8. Recreational 9. Intensive agriculture [ ]

BARS: (bed surface protruding from water & forming a bar within the channel) ..... %

HAVE THERE BEEN HEAVY RAINS OR SPATES IN THE LAST WEEK? 1. yes 2. no 3. not sure [ ]

RIVER ..... DATE ..... LOCATION CODE .....

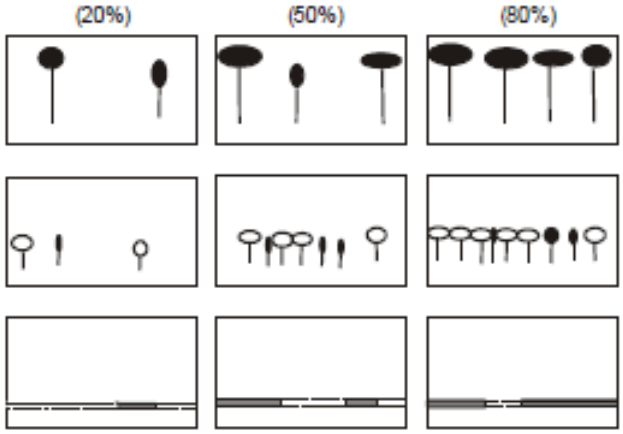
ALL LEFT BANK AND RIGHT BANK REFER TO DIRECTION FACING DOWNSTREAM.

RIPARIAN CHARACTERISTICS OF SURVEYED REACH

WIDTH of riparian zone (to a maximum of 30 m perpendicular to flow)

Left bank ..... m Right bank ..... m

STRUCTURAL COMPOSITION of riparian zone Using the diagrams below as a guide, tick the box corresponding to the percentage category that best describes the percent cover of each vegetation category; evaluate left (L) and right (R) bank separately.



	0	1	2	3	4
	0%	< 20%	20-50%	50-80%	> 80%
Trees L	☐	☐	☐	☐	☐
Trees R	☐	☐	☐	☐	☐
Shrubs and bushes, including blackberries L	☐	☐	☐	☐	☐
Shrubs and bushes, including blackberries R	☐	☐	☐	☐	☐
Ground covers including grasses, ferns & herbs L	☐	☐	☐	☐	☐
Ground covers including grasses, ferns & herbs R	☐	☐	☐	☐	☐

EXOTIC VEGETATION What percentage of each of the following categories is made up of exotic vegetation?

	0%	1-10%	11-40%	41-60%	>60%
Trees	0	1	2	3	4
Shrub Layer	0	1	2	3	4
Ground cover	0	1	2	3	4

LONGITUDINAL EXTENT of riparian vegetation Choose one category for each bank. Do not include ground cover layer except where site is in native grassland.

	Left bank	Right bank
0. None	☐	☐
1. Isolated / scattered	☐	☐
2. Regularly spaced	☐	☐
3. Occasional clumps	☐	☐
4. Semi-continuous	☐	☐
5. Continuous	☐	☐

RIVER .....DATE .....LOCATION CODE.....

MACROPHYTES IN REACH

Indicate which macrophyte structural types are present in the reach with the following abundances:

I = Isolated, S = Scattered, B = In beds, C = Choking the stream

Submerged

 ☐

Feather like e.g. Myriophyllum, Ceratophyllum & Chara

 ☐

Broad strap-like E.g. Vallisneria, Triglochin

 ☐

Grass like

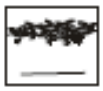
 ☐

Branched form. E.g. Elodea, Potamogeton

Floating

 ☐

Attached, Lilly-like

 ☐

Free floating Small plant e.g. Azolla, Duckweed

 ☐

Free floating large Plant. E.g. Salvinia, Water hyacinth

Emergent

 ☐

Reed like. E.g. Typha, Phragmites

 ☐

Unbranched, broad leaf form. E.g. Curled dock, Water plantain, Arrowhead

 ☐

Grass- like E.g. Paspalum

 ☐

Tussok/ rush like E.g. Juncus, Cyperus Carex

 ☐

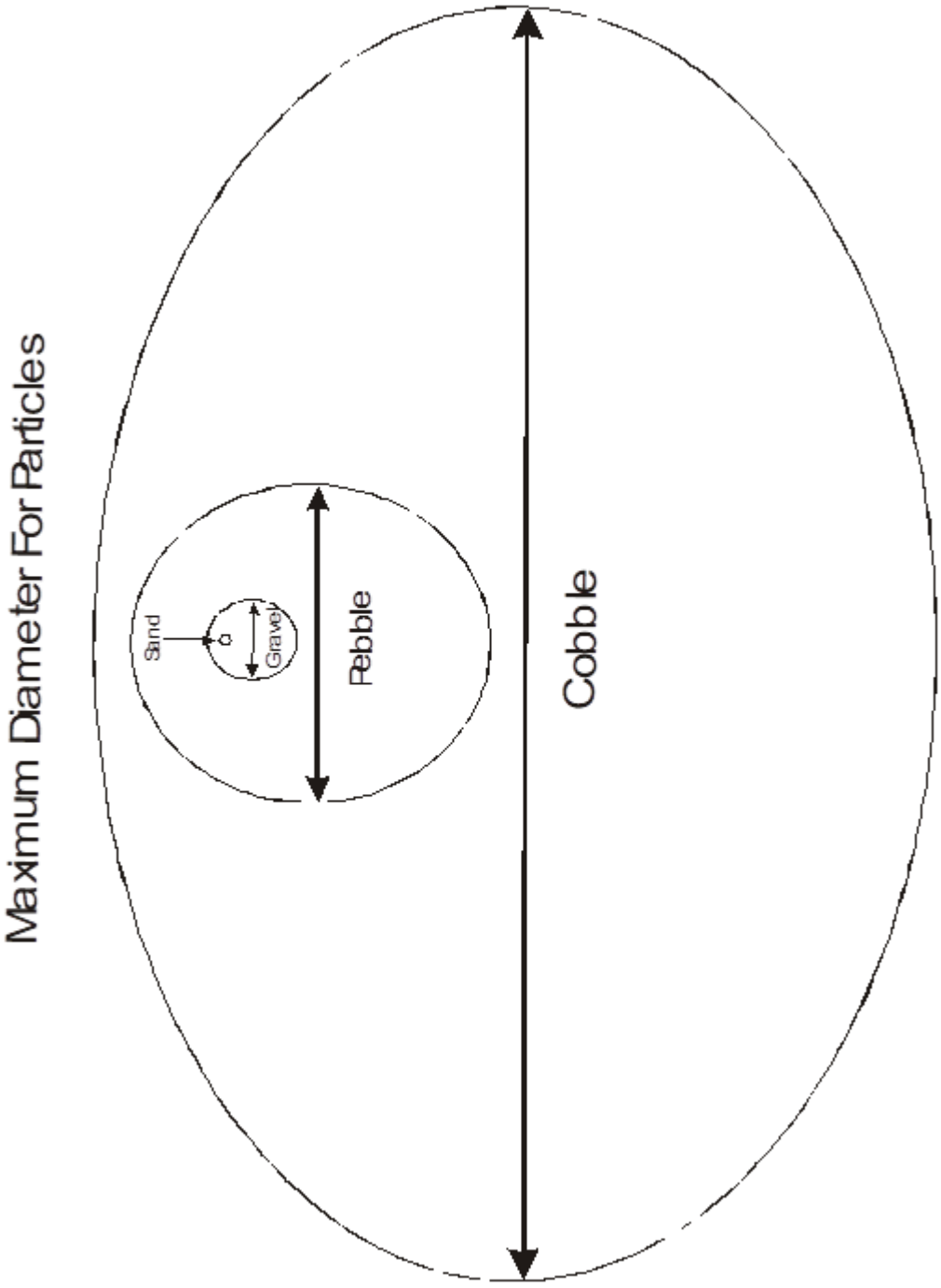
Branched form E.g. Ranunculus, Polygonum

Vegetation samples collected for identification in the lab: Yes [ ] No [ ]

ARE ALL SPACES FILLED IN ON ALL SHEETS? Yes [ ] Checked by

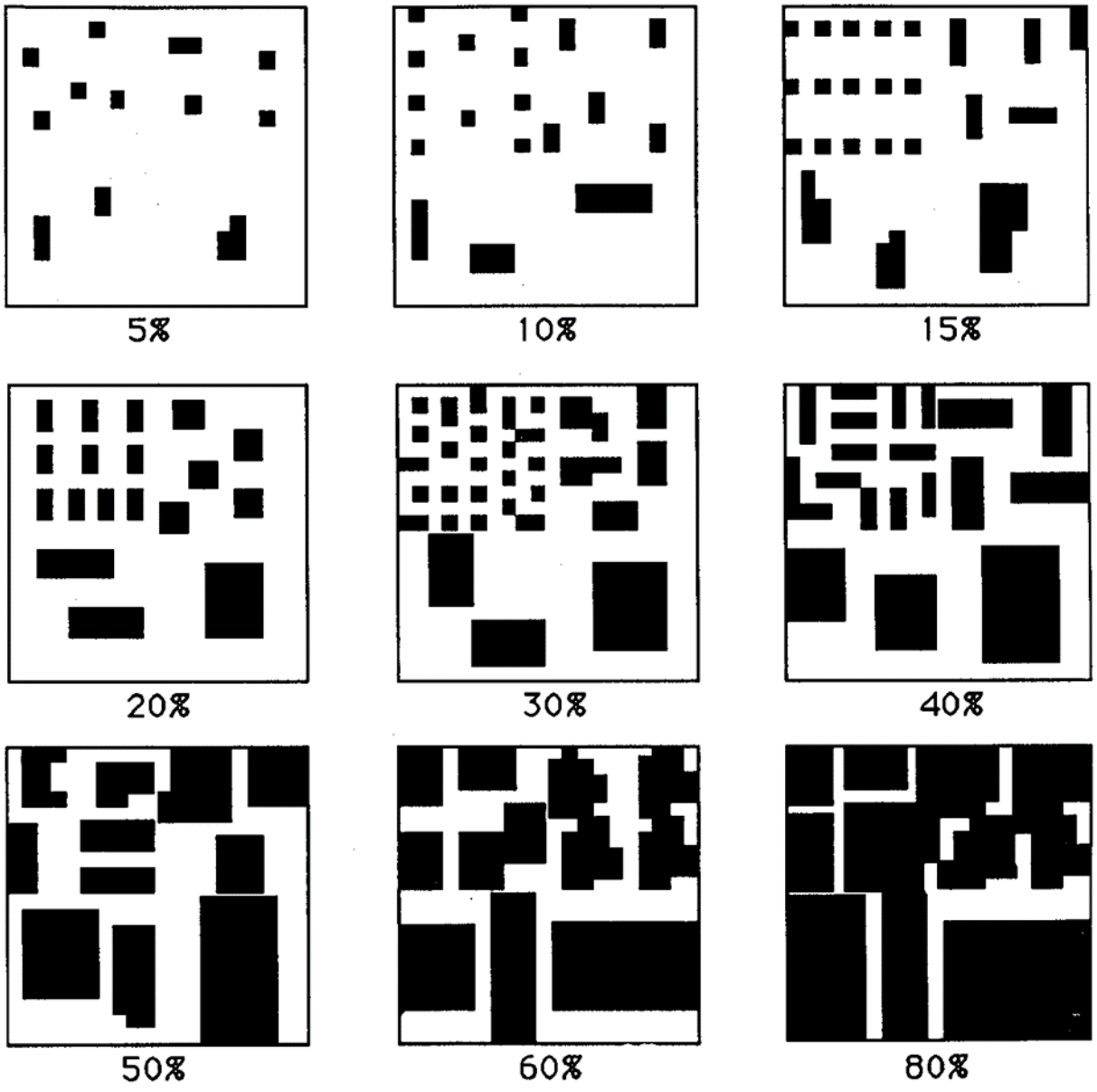
.....

Appendix 4: Particle size diagram



Appendix 5: Percentage shading diagrams

(Reproduced from Gordon et al. 1992 with permission from John Wiley & Sons Ltd, as modified from Northcote 1979 with permission from Rellim Technical Publications).



Appendix 6: Recommended taxonomic identification keys

The following keys are those recommended for identification of aquatic invertebrates to the appropriate taxonomic level required for the rapid bioassessment of rivers and streams as described in this Guideline.

Be aware that taxonomic changes can and do occur, which means that old keys are often incorrect and the keys listed here may become obsolete. It is important to keep up to date on the literature and use new keys as they become available.

For overviews and keys to order, the most useful keys are:

- Williams (1980) This is not as useful at the family level for many groups because many of the keys are outdated. However, for some families, as indicated below, this is the best available reference for Australia.
- Gooderham and Tsyrlin (2002) This guide has excellent photographs and interesting information about many of the aquatic invertebrate groups. The keys to families can be helpful when used as a supplement to those listed below, but they often do not enable positive identification of all families from Victoria.
- Australian Aquatic Invertebrates (2012) Lucid Key http://keys.lucidcentral.org/keys/lwrrdc/public/Aquatics/main.htm
- Hawking JH, Smith LM, LeBusque K, Davey C (editors) (2013) Identification and Ecology of Australian Freshwater Invertebrates. http://www.mdfrc.org.au/bugguide

The following is a list of the most appropriate taxonomic keys (listed by major taxonomic grouping) for the aquatic invertebrates likely to be encountered in the sampling of rivers and streams. Note that this list includes only those taxonomic groups that are used in the calculation of the biological indices described in this Guideline.

- Porifera [Only one family: Spongillidae]
- Cnidaria (Williams 1980) No key is provided but only two families are likely to be encountered: F. Clavidae (the colonial hydroids) and F. Hydridae (individual, non-colonial hydroids). Recognition of these two families is possible from the text and figures in this reference.
- Tricladida [Only one family: Dugesiidae]
- Temnocephalidea [Identify to this level only]
- Nemertea [Only one family: Tetrastemmatidae]
- Nematomorpha [Only one family: Gordiidae] It was historically thought that Nematomorpha were represented in Victoria by the single family Gordiidae. This, however, may not be appropriate, and due to difficulties in family level identification, a future recommendation may be made for identification to the order level only.
- Hirudinea (Govedich 2001)
- Oligochaeta [Identify to this level only]
- Mites [Identify to this level only]
- Gastropoda (Smith 1996)
- Bivalvia (Smith 1996)
- Crustacea (Williams 1980) This reference provides a key to the major groups of Crustacea, but only the following groups are used in the Victorian indices, for which family level identification is required:
- Syncarida [Only one family: Koonungidae]

- Decapoda (Williams 1980)
- Amphipoda (Bradbury and Williams 1999, Horwitz et al. 1995; do not use Williams 1980)
- Isopoda (Williams, 1980) Note the following exceptions:
- Phreatoicidae: For historical reasons, all families within the sub-order Phreatoicidea are placed in the family Phreatoicidae. However, the family level taxonomy of this sub-order is currently under review and future recommendations may reflect any changes.
- Oniscidae: The family Oniscidae, a primarily terrestrial family with one salt lake representative, may accidentally occur in freshwater samples and thus are ignored.
- Ephemeroptera (Dean and Suter 1996) If not available use Peters and Campbell 1991.
- Odonata (Hawking and Theischinger 1999, Theischinger and Hawking 2006)
- Plecoptera (Theischinger 1991a, Williams 1980)
- Hemiptera (Williams 1980)
- Megaloptera (Theischinger 2000, Theischinger 1991b)
- Neuroptera (Williams 1980)
- Coleoptera (Lawrence 1992 if available, also Williams 1980, Gooderham and Tsyrlin 2002)
- Mecoptera [Only one family: Nannochoristidae]
- Diptera (Cranston 1995, Williams 1980)
- Trichoptera (Dean et al. 1995, Neboiss 1991)
- Lepidoptera (Hawking 2001)

Taxonomy reference list

Bradbury, J.H. and Williams, W.D. (1999) Key to and checklist of inland aquatic amphipods of Australia. Technical Reports of the Australian Museum 14:1-21.

Cranston, P.S. (1995) Keys to aquatic Diptera families. Taxonomy Workshop, Murray-Darling Freshwater Research Centre, February 1995, Albury, Australia.

Dean, J.C. and Suter, P.J. (1996) Mayfly nymphs of Australia, a guide to genera. Co-operative Research Centre for Freshwater Ecology, Identification Guide No. 7, Albury, Australia.

Dean, J.C., St Clair, R.M. and Cartwright, D.I. (1995) A key to late instar larvae of Australian Trichoptera families. In: Monitoring River Health Initiative Taxonomy Workshop Handbook (ed. J.H. Hawking) Chapter 6, pp 66-101. Taxonomy Workshop, Murray-Darling Freshwater Research Centre, Albury, Australia.

Gooderham, J. and Tsyrlin, E. (2002) The Waterbug Book, a guide to the freshwater macroinvertebrates of temperate Australia. 240 pp. CSIRO Publishing, Melbourne.

Govedich, F.R. (2001) A reference guide to the ecology and taxonomy of freshwater and terrestrial leeches (Euhirudinea) of Australasia and Oceania. Co-operative Research Centre for Freshwater Ecology, Identification Guide No. 35, Albury, Australia.

Hawking, J.H. (2001) An introduction to the identification of aquatic caterpillars (Lepidoptera) found in Australian inland waters. Co-operative Research Centre for Freshwater Ecology, Identification Guide No. 37, Albury, Australia.

Hawking, J.H. and Theischinger, G. (1999) Dragonfly larvae (Odonata), a guide to the identification of larvae of Australian families and to the identification and ecology of larvae from New South Wales. Co-operative Research Centre for Freshwater Ecology, Identification Guide No. 24, Albury, Australia.

Hawking J.H., Smith L.M., LeBusque K., Davey C. (editors) (2013) Identification and Ecology of Australian Freshwater Invertebrates. http://www.mdfrc.org.au/bugguide

Horwitz, P. (1995) A preliminary key to the species of Decapoda (Crustacea: Malacostraca) found in Australian inland waters. Co-operative Research Centre for Freshwater Ecology, Identification Guide No. 5, Albury, Australia.

Horwitz, P., Knott, B. and Williams, W.D. (1995) A preliminary key to the Malacostracan families (Crustacea) found in Australian inland waters. Co-operative Research Centre for Freshwater Ecology, Identification Guide No.4, Albury, Australia.

Lawrence, J.F. (1992) Australian aquatic Coleoptera (adults and larvae). Taxonomy Workshop, Murray-Darling Freshwater Research Centre, February 1992. Albury, Australia.

Neboiss, A. (1991) Trichoptera. In: *The Insects of Australia*. 2nd Edition, Eds. CSIRO Division of Entomology, pp. 787-816, Melbourne University Press, Carlton, Australia.

Peters, W.L. and Campbell, I.C. (1991) Ephemeroptera. In: *The Insects of Australia*. 2nd Edition, Eds. CSIRO Division of Entomology, pp. 279-293, Melbourne University Press, Carlton, Australia.

Smith, B.J. (1996) Identification keys to the families and genera of bivalve and gastropod molluscs found in Australian inland waters. Co-operative Research Centre for Freshwater Ecology, Identification Guide No.6, Albury, Australia.

Theischinger, G. (1991a) Plecoptera. In: *The Insects of Australia*. 2nd Edition, Eds. CSIRO Division of Entomology, pp. 311-319. Melbourne University Press, Carlton, Australia.

Theischinger, G. (1991b) Megaloptera. In: *The Insects of Australia*. 2nd Edition, Eds. CSIRO Division of Entomology, pp. 516-520, Melbourne University Press, Carlton, Australia.

Theischinger, G. (2000) Australian alderfly larvae and adults (Insecta: Megaloptera). Co-operative Research Centre for Freshwater Ecology, Identification Guide No.29, Albury, Australia.

Theischinger, G and Hawking J.H. (2006) The complete field guide to dragonflies of Australia. CSIRO Publishing, Clayton, Australia.

Williams, W.D. (1980) Australian Freshwater Life. 2nd Edition, Macmillan, Melbourne, Australia.

Appendix 7: Laboratory invertebrate identification sheet

Basin/Catchment: **Project:**

Site Code:

River:

Location:

Sample Date:

No. of pages: **Sorted and ID'd by:** **QA/QC by:**

Data entry - date : **by:** **Data validation - date:** **by:**

Taxon	Kick/Sweep

Appendix 8A: SIGNAL2 Biotic index grades (adapted from Chessman 2003)

Taxon	Grade	Taxon	Grade	Taxon	Grade
Aeshnidae	4	Gomphidae	5	Osmylidae	7
Ameletopsidae	7	Gordiidae	5	Palaemonidae	4
Amphisopidae	1	Grapsidae	7	Paracallipidae	3
Amphipterygidae	8	Gripopterygidae	8	Paramelitidae	4
Ancylidae	4	Gyrinidae	4	Parastacidae	4
Antipodoeciidae	8	Haliplidae	2	Pelecorchynchidae	10
Aphroteniinae	8	Hebridae	3	Perthiidae	4
Athericidae	8	Helicophidae	10	Philopotamidae	8
Atriplectididae	7	Helicopsychidae	8	Philorheithridae	8
Atyidae	3	Hemicorduliidae	5	Phreatoicidae	4
Austrocorduliidae	10	Heteroceridae	1	Phreatoicopsidae	2
Austroperlidae	10	Hydraenidae	3	Physidae	1
Baetidae	5	Hydridae	2	Planorbidae	2
Belostomatidae	1	Hydrobiidae	4	Pleidae	2
Bithyniidae	3	Hydrobiosidae	8	Podonominae	6
Blephariceridae	10	Hydrochidae	4	Polycentropodidae	7
Branchipodidae	1	Hydrometridae	3	Pomatiopsidae	1
Brentidae	3	Hydrophilidae	2	Prosopistomatidae	4
Caenidae	4	Hydropsychidae	6	Protoneuridae	4
Calamoceratidae	7	Hydroptilidae	4	Psephenidae	6
Calocidae	9	Hygrobiidae	1	Psychodidae	3
Carabidae	3	Hymenosomatidae	3	Ptiliidae	3
Cecidomyidae	1	Hypolestidae	9	Ptilodactylidae	10
Ceinidae	2	Hyriidae	5	Pyralidae	3
Ceratopogonidae	4	Isostictidae	3	Richardsonianidae	4
Chaoboridae	4	Janiridae	3	Saldidae	1
Chironominae	3	Kokiriidae	3	Scatopsidae	1
Chrysomelidae	2	Koonungidae	1	Sciaridae	6
Cirolanidae	2	Leptoceridae	6	Sciomyzidae	2
Clavidae	3	Leptophlebiidae	8	Scirtidae	6
Coenagrionidae	2	Lestidae	1	Sialidae	5
Coloburiscidae	8	Libellulidae	4	Simuliidae	5
Conoesucidae	7	Limnephilidae	8	Siphlonuridae	10
Corbiculidae	4	Limnichidae	4	Siphonotidae	6
Corduliidaen	7	Lindeniidae	3	Sisyridae	3
Corixidae	2	Lymnaeidae	3	Sphaeriidae	5
Corophiidae	4	Macromiidae	8	Sphaeromatidae	1
Corydalidae	7	Megapodagrionidae	5	Spongillidae	3
Culicidae	1	Melitidae	7	Staphylinidae	3
Curculionidae	2	Mesemphisophidae	3	Stratiomyidae	2
Diamesinae	6	Mesoveliidae	2	Sundatelphysidae	3
Diphlebiidae	6	Microsporidae	7	Synlestidae	7
Dipseudopsidae	9	Muscidae	1	Synthemidae	2
Dixidae	7	Nannochoristidae	9	Syrphidae	2
Dolichopodidae	3	Naucoridae	2	Tabanidae	3
Dugesiidae	2	Neoniphargidae	4	Talitridae	3
Dytiscidae	2	Nepidae	3	Tanyderidae	6
Ecnomidae	4	Neurorthidae	9	Tanypodinae	4
Elmidae	7	Noteridae	4	Tasimiidae	8
Empididae	5	Notonectidae	1	Telephlebiidae	9

Ephydridae	2	Notonemouridae	6	Teloganodidae	9
Erpobdellidae	1	Ochteridae	2	Temnocephlidae	5
Eusiridae	7	Odontoceridae	7	Tetrastemmatidae	7
Eustheniidae	10	Thiaridae	4	Thaumaleidae	7
Oeconesidae	8	Oniscidae	2	Triopsidae	1
Gerridae	4	Oniscigastridae	8	Urothemistidae	1
Glossiphoniidae	1	Ornithobdellidae	1	Veliidae	4
Glossosomatidae	9	Orthocladiinae	4	Viviparidae	4

Appendix 8B: SIGNAL2 Biotic index grades for macroinvertebrate orders, subclasses, classes and phyla

Taxon	Grade
Acarina	6
Amphipoda	3
Anaspodacea	6
Anostraca	1
Bivalvia	3
Branchiura	1
Bryozoa	4
Coleoptera	5
Collembola	1
Conchostraca	1
Decapoda	4
Diplopoda	4
Diptera	3
Ephemeroptera	9
Gastropoda	1
Hemiptera	2
Hirudinea	1
Hydrozoa	1
Isopoda	2
Lepidoptera	2
Mecoptera	10
Megaloptera	8
Nematoda	3
Nemertea	3
Neuroptera	6
Nematomorpha	6
Notostraca	1
Odonata	3
Oligochaeta	2
Plecoptera	10
Porifera	4
Trichoptera	8
Tubellaria	2

Appendix 9: Predictor variables for AUSRIVAS models

Predictor Variable	Explanation
Latitude[1]	Latitude at a given site measure in decimal degrees. Latitude must be in positive units.
Longitude[1]	Longitude at a given site measured in decimal degrees.
Altitude[1]	Height above sea level at a given site (m).
Catchment Area[1]	Total catchment area upstream of a site (km^2); data usually transformed to log10(x).
Distance From Source[1]	Distance from a site to its uppermost headwaters (m); data usually transformed to log10(x).
Slope[1]	The inclination, or degree of deviation from the horizontal, at a site (m/km); data usually transformed to log10(x).
Reach Phi[1,3]	Mean substrate particle size in the entire reach measured in phi units.
Riffle Phi[1,3]	Mean substrate particle size in the riffle habitat measured in phi units.
Substrate Heterogeneity[1,3]	Number of substrate size categories in the reach with percent coverage >10.
Alkalinity[2,3]	Alkalinity (total carbonates) measured at a site (mg/L); data usually transformed to log10(x).
Stream Width[2,3]	Mean width of the wetted stream channel (m); data usually transformed to log10(x).
VegCat[2,3]	Land use category surrounding the reach beyond the riparian zone.
Shading[2,3]	Category representing percentage shading of the stream reach at midday.
Bedrock[2,3]	Percent bedrock in reach (%); for some models data is transformed to log10(x+1).
Boulder[2,3]	Percent boulder in reach (%).
Cobble[2,3]	Percent cobble in reach (%).
Pebble[2,3]	Percent pebble in reach (%); for some models data is transformed to log10(x+1).
Gravel[2,3]	Percent gravel in reach (%); for some models data is transformed to log10(x+1).
Depth[2,3]	Mean water depth in riffle habitat (cm); for some models data is transformed to log10(x).
Macrophyte Category[2,3,4]	Category representing percentage of reach covered by macrophytes.

[1] Detailed explanations of the calculation or measurement of these parameters can be found in section 6.
[2] Detailed explanations of the calculation or estimation of these parameters can be found in section 4.
[3] Use the mean of autumn and spring values for these parameters.
[4] Macrophyte categories 0a and 0b should be treated as a single category (0=<10%) for AUSRIVAS purposes.

Appendix 10: Quality assurance sheet for taxonomic identifications

CATCHMENT(S) / BASIN(S) :

SEASON/YEAR:

Number of sites: **Number of samples:**

Processed by: **Checked by:** **QC Date:**

	Sample 1	Sample 2	Sample 3
Site			
Sample date			
Habitat	Kick/Sweep	Kick/Sweep	Kick/Sweep
No. of families (or higher taxa)			
No. of discrepancies in family level IDs			
No. of families not recorded			
Family level discrepancies as % of total taxa			
No. of taxa with enumeration discrepancies of 1-2 specimens			
No. of taxa with enumeration discrepancies of >2 specimens			
No. of taxa with different level of resolution			

Details of ID discrepancies:

Sample: Identified by processor as: Identified by checker as: