

6. Biological monitoring methods



Contents

Introduction	6-2
Overview of this chapter	6-2
Why this chapter is important	6-2
How to use this chapter.....	6-2
Introduction to macro-invertebrates	6-3
What they are	6-3
Types and classification	6-4
Where they live.....	6-4
Life cycles.....	6-6
Factors affecting macro-invertebrates.....	6-6
Monitoring macro-invertebrates	6-8
What they indicate	6-8
Strengths and limitations	6-8
How information can be used.....	6-9
Designing your study	6-10
Where to place monitoring sites in the catchment.....	6-10
Where to sample in the stream.....	6-10
When to sample.....	6-10
Choosing your monitoring methods	6-10
Safety considerations	6-14
Method 1 for monitoring macro-invertebrates	6-14
Method overview.....	6-14
Equipment	6-14
Where to sample.....	6-14
Data confidence procedures.....	6-15
Collecting your sample	6-15
Sorting your sample.....	6-15
Processing your sample	6-15
Calculating results	6-15
Interpreting results	6-15
Method 2 for monitoring macro-invertebrates	6-16
Method overview.....	6-16
Equipment	6-16
Where to sample.....	6-16
Data confidence procedures.....	6-16
Collecting your sample	6-16
Sorting your sample.....	6-18

Processing your sample	6-18
Calculating results	6-19
Interpreting results	6-19
Method 3 for monitoring macro-invertebrates	6-20
Method overview.....	6-20
Equipment	6-20
Where to sample.....	6-21
Data confidence procedures.....	6-21
Before collecting your sample	6-21
Collecting your sample	6-22
Sorting your sample.....	6-23
Before you leave the field site	6-24
Processing your sample	6-24
Calculating results and indexes	6-24
Interpreting results	6-25
Bibliography	6-27
Further reading	6-28
Macro-invertebrate identification guides ...	6-28
Guide to identifying macro-invertebrates	

Figures

Figure 6-1 Examples of macro-invertebrates found in Queensland waterways.....	6-3
Figure 6-2 Plan view and cross-sections of a pool, riffle and run habitats	6-5
Figure 6-3 A simplified example of the role of macro-invertebrates in a stream food web	6-5
Figure 6-4 Example of where kick sampling and sweep sampling should occur at a site ..	6-17, 6-22

Tables

Table 6-1 Steps in developing a monitoring plan	6-2
Table 6-2 Linnean classification of the common freshwater shrimp	6-4
Table 6-3 Methods guide for biological monitoring	6-12
Table 6-4 Interpretation of SIGNAL 2 scores	6-19, 6-25
Table 6-5 Biological monitoring values for South East Queensland.....	6-25



Introduction

Overview of this chapter

Chapter 6 assists you to undertake biological monitoring to assess the ecological health of freshwater rivers and streams. It provides guidance on monitoring methods for biological indicators. The chapter focuses on the use of macro-invertebrates ('water bugs') as biological indicators, rather than other stream biota such as fish.

Specifically, this chapter provides technical information to assist you to:

- understand the biological components of your waterway (particularly macro-invertebrates)
- understand the value of macro-invertebrates as biological indicators of waterway health
- choose a monitoring method that is right for your needs
- carry out your chosen monitoring method
- interpret your data to determine the ecological health of your waterway.

Why this chapter is important

This chapter will help you understand the value of biological monitoring and help you decide whether it is an appropriate tool to answer your monitoring objectives. Monitoring the biological community of a waterway can give a direct insight into the overall ecological health of the waterway, including both the water quality, and stream condition and habitat.

This chapter also explains how to undertake biological monitoring using three different methods. It is important to choose and carry out an appropriate monitoring method to ensure that the data you collect is suitable to answer your project objectives and that this is done within your available resources (budget, time and skills).

The chapter focuses on the use of macro-invertebrates as biological indicators of water quality and stream health. The omission of other biological stream indicators (such as freshwater fish) in this chapter is in no way a reflection of their value as biological indicators, but rather a consideration of the practical elements involved in community waterway monitoring. Macro-invertebrates are also the biological indicators most widely used by professional scientists for a number of practical and scientific reasons, outlined in this chapter.

How to use this chapter

Chapter 6 has been developed to support Question 9 in the development of a monitoring plan (see Table 6–1). Use the information in this chapter to help you carry out planning and procedures for biological monitoring.

Table 6–1 Steps in developing a monitoring plan

Key steps	Monitoring plan questions
Set monitoring objectives	Q1 Why are you monitoring? Q2 Who will use your data? Q3 How will the data be used? Q4 What data quality do you require?
Develop a study design	Q5 What is your study type? Q6 What will you monitor? Q7 Where will you monitor? Q8 When and how often will you monitor?
Choose monitoring methods and procedures	Q9 What methods will you use?
Plan data management, interpretation, reporting and communication	Q10 Who will be involved and how? Q11 How will the data be managed and reported? Q12 How will you ensure confidence in your data?



This chapter includes sections on:

- macro-invertebrates—what they are, why they are important, where they live and what affects them
- how to design your biological study
- how to choose and carry out biological monitoring methods
- safety considerations.

It provides instructions for carrying out three different monitoring methods. Method 1 produces introductory-level, *demonstrative* biological data. Method 2 produces intermediate-level, *indicative* biological data. Method 3 produces complex, *analytical* biological data.

For each monitoring method, the following information is provided:

- method overview
- where to sample
- equipment
- data confidence procedures
- sampling procedures
- sorting procedures
- processing procedures
- how to calculate results
- how to interpret results.

Record sheets for each biological monitoring method are provided in Chapter 10.

Introduction to macro-invertebrates

What they are

An aquatic macro-invertebrate, or water bug, is an animal without a backbone (invertebrate) that spends all or part of its life in water, and can be seen by the naked eye (macro). Many kinds of macro-invertebrates inhabit Australian waterways, including worms, snails, mites, bugs, beetles, yabbies and various insect nymphs and larvae. Although some *Euastacus* species of crayfish can reach lengths up to twenty centimetres or so, macro-invertebrates are generally between less than a millimetre and seven centimetres long. See Figure 6–1 for some examples of aquatic macro-invertebrates found in Queensland waterways. A group of macro-invertebrates living together is called a biological community.

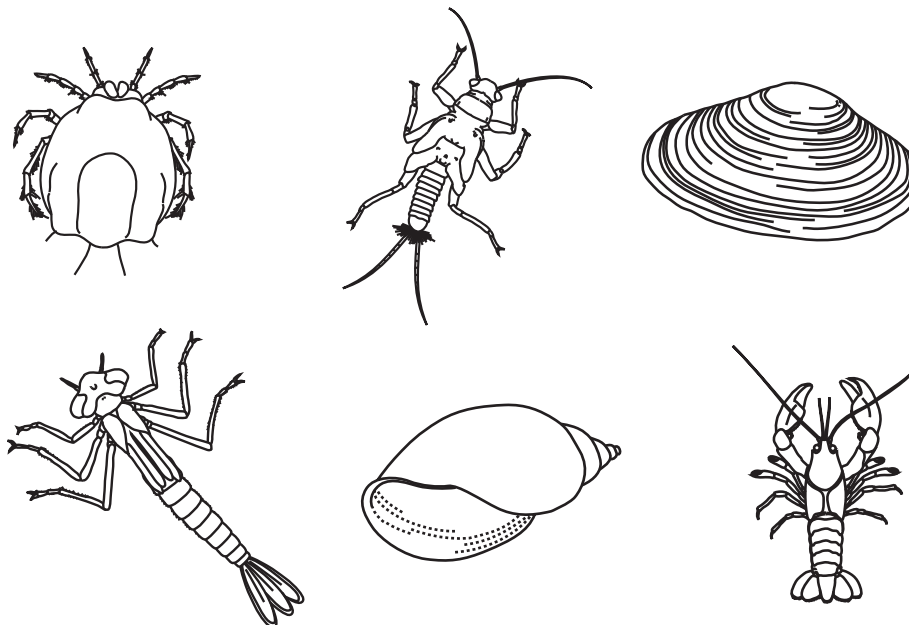


Figure 6–1 Examples of macro-invertebrates found in Queensland waterways (Department of Infrastructure, Planning and Natural Resources 2000)

Top row: Water mite (Acariformes), stonefly nymph (Plecoptera), freshwater mussel (Bivalvia)

Bottom row: Damselfly nymph (Odonata), freshwater snail (Gastropoda), freshwater yabby (Decapoda)



Types and classification

When conducting macro-invertebrate monitoring, it is essential to have a good understanding of the biological classification (Linnean) system used to categorise all species of organisms. The Linnean classification system is hierarchical, with seven main divisions—*kingdom*, *phylum*, *class*, *order*, *family*, *genus* and *species*—each of which provides progressively more specific information about a particular organism. Therefore, within the animal kingdom there are several phyla, each phylum contains several classes, and so on down to the genus and species, the formal name for every living thing. Genus and species names are written in italics, with the species name always in lower case. Table 6–2 shows the Linnean classification of the common freshwater shrimp.

Table 6–2 Linnean classification of the common freshwater shrimp

Kingdom	Animalia
Phylum	Arthropoda
Class	Crustacea
Order	Decapoda
Family	Atyidae
Genus	<i>Paratya</i>
Species	<i>australiensis</i>

Examples of common aquatic macro-invertebrate classes include Gastropoda (snails), Arachnida (spiders and mites), Crustacea (crustaceans), Insecta (insects), Turbellaria (flatworms) and Oligochaeta (segmented worms). Identifying macro-invertebrates to species level can be difficult, due to the minute size of some organisms and their lack of distinguishing features. Some groups, like the true flies, or Diptera, contain many undescribed (unclassified) species, making species-level identification impossible. Furthermore, up-to-date taxonomic identification keys are not available for some macro-invertebrate species. Therefore, the majority of macro-invertebrate identification for the purposes of waterway monitoring occurs at the class, order or family level.

Where they live

Macro-invertebrates inhabit all types of water, from rushing, rocky rivers to sandy-bottomed streams, densely vegetated ponds and murky farm dams. This biological abundance is because many insect species require water during their early life stages; many insect larvae are aquatic. Aquatic habitats can be broadly categorised into moving water (rivers, creeks and streams) or still water (waterholes, wetlands, backwaters, lakes and pools). Most macro-invertebrate families are found in one type of habitat or the other, as macro-invertebrates possess special adaptations for the specific aquatic habitat that they live in. Flowing water habitats have both fast- and slow-moving sections. In fast-moving water, macro-invertebrates must be able to grip to a surface while feeding. Adaptations to allow for this include streamlined bodies, suction parts, hooks and fine filters for sieving food from the passing water. In slow-moving or still water, macro-invertebrates do not need to hang on to a surface and their food is not provided by the water current. Consequently, macro-invertebrates in still or slow-flowing water have a wider range of shapes and sizes and are more mobile.

Within flowing water, four different macro-invertebrate habitat types are recognised: riffles, runs, pools and stream edges. These different macro-invertebrate habitats occur due to the varying flows, depths and substrates of the stream. See Figure 6–2 for a plan view and cross-section of a waterway showing pool, riffle and run habitats.

Riffles are shallow, rocky sections of a waterway with fast-flowing, turbulent water. The water surface in riffle habitats is broken (the surface is not continuous and the water forms spray, foam and droplets), leading to increased levels and mixing of oxygen in the water. Rocks provide a large surface area onto which macro-invertebrates can attach and the fast-flowing water provides a continual supply of food. Some species of macro-invertebrates stay on the underside of rocks for protection from the flow. A high diversity of macro-invertebrates can be supported in riffles due to the living spaces and food provided.

Runs are areas of deeper, flowing water with a smooth water surface and smaller particles settled on the bottom (substrate). Food is suspended in the water, deposited on the bottom or growing in the waterway bed. Occasional floods can wash macro-invertebrates downstream. Due to the lack of stability and living places, fewer macro-invertebrates inhabit runs than riffles.

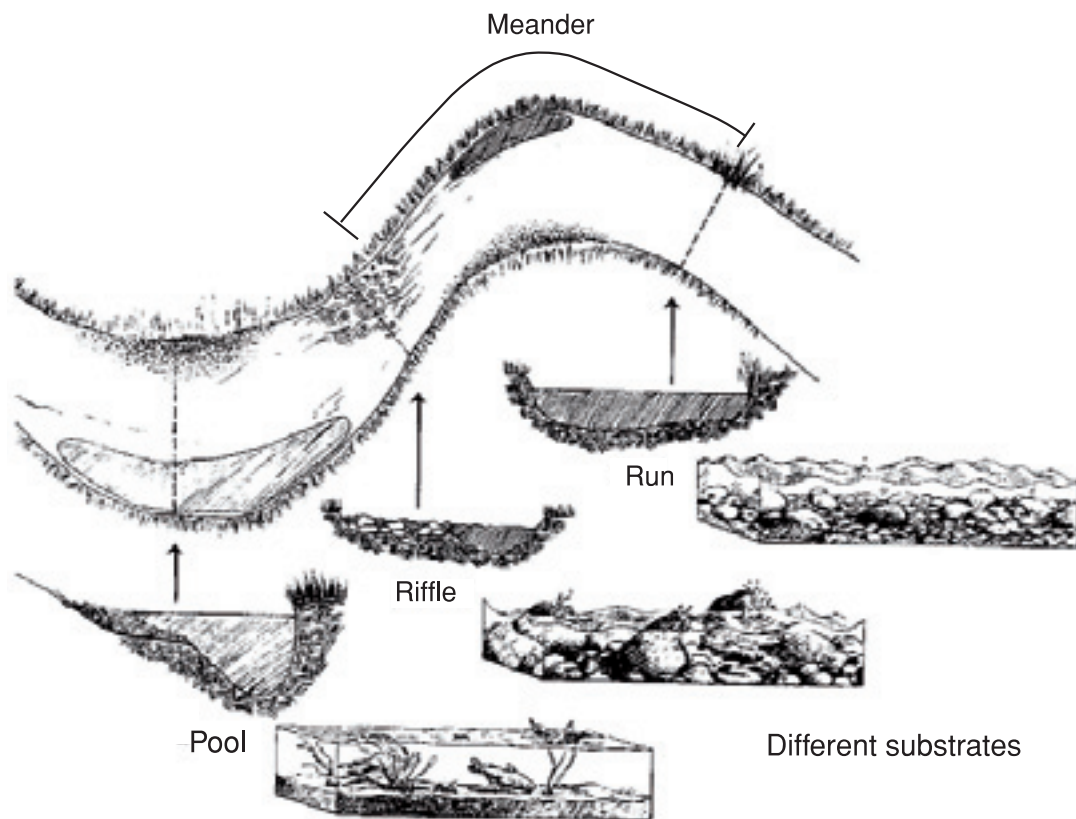


Figure 6-2 Plan view and cross-sections of pool, riffle and run habitats (Lyon 1995)

Pools are deeper areas where water is still or flows slowly. Pools have sandy or muddy bottoms, so macro-invertebrates are able to attach themselves to plant stems, roots, logs and other submerged objects. Fewer types of macro-invertebrates inhabit pools than riffles.

Edge habitats occur along stream edges near the water surface. Edge habitats incorporate overhanging vegetation from the stream banks, emergent aquatic plant (macrophyte) beds, sheltered overhangs, root mats and leaf packs. The macro-invertebrate communities found in edge habitats differ from those in riffles and pools, and survive best in places that provide protection, camouflage and food sources.

Macro-invertebrates form a vital link in the food web of a river ecosystem. Many are grazers, eating leaf litter, weeds and algae and thus funnelling sunlight energy to fish and other larger predators. Other macro-invertebrates are predators themselves, preying on smaller macro-invertebrate species. The presence or absence of macro-invertebrates will generally determine the presence or absence of animals higher up the food chain. For an example of the role that macro-invertebrates play in an aquatic food web, refer to Figure 6-3.

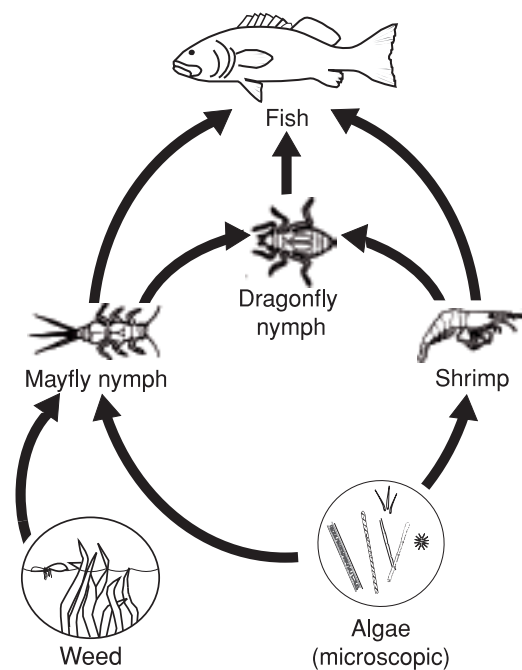


Figure 6-3 A simplified example of the role of macro-invertebrates in a stream food web. Arrows point to the consumers.



Life cycles

Many species of macro-invertebrates go through several changes in appearance throughout their life cycles. The majority of macro-invertebrates undergo either complete or incomplete metamorphosis during the process of developing from an egg to an adult. Complete metamorphosis comprises four distinct stages: the egg stage, larval stage, pupal stage and adult stage. A number of the macro-invertebrates that undergo complete metamorphosis are aquatic during their egg and larval stages, but terrestrial as adults. Examples of macro-invertebrates that go through complete metamorphosis include caddisflies (Trichoptera), beetles (Coleoptera) and true flies (Diptera). Incomplete metamorphosis comprises only three stages: the egg stage, nymph stage and adult stage. Macro-invertebrates that go through incomplete metamorphosis include mayflies (Ephemeroptera), stoneflies (Plecoptera) and true bugs (Hemiptera). While a number of macro-invertebrates that undergo incomplete metamorphosis live in terrestrial environments during their adult stage, others such as water striders and water scorpions spend their entire lives in aquatic ecosystems.

6-6

Factors affecting macro-invertebrates

Many macro-invertebrates are highly sensitive to environmental changes. Some species have, over time, adapted to such a narrow range of habitat conditions that even the smallest change in environmental conditions can have devastating effects. The conditions influencing the composition of macro-invertebrate communities include physical, chemical, and biological waterway conditions, and human influences.

Physical habitat

Riffle, edges, pools, and runs—Variability in physical conditions (including flows and depths), and availability of each, influences the macro-invertebrate community composition.

Current velocity—How fast the water moves will impact on macro-invertebrate communities. A current velocity of 0.5 metres per second in riffles will support the most diverse communities, while floods may flush macro-invertebrates and plants downstream. Higher flow velocities also increase dissolved oxygen levels, so high-velocity habitats can support a greater richness of macro-invertebrates.

Bottom composition—The bottom of a waterway is made up of different materials (substrates) such as various rocks (with sizes ranging from that of marbles to basketballs), mud, sand, silt and gravel. Highly diverse substrates containing a variety of rock sizes provide the best habitat for macro-invertebrates.

Aquatic plants—Known as macrophytes, aquatic plants provide habitats for many different types of macro-invertebrates. Aquatic plants also assist to camouflage some macro-invertebrates from predators and protect them from the water flow.

Flow (discharge)—The amount of water in the channel determines how much of the river bed is exposed to air. When the waterway is drying up, macro-invertebrates will converge in remaining water holes and populations will become more concentrated. Some macro-invertebrates are better at coping with these conditions than others.

Depth and water clarity—These factors affect whether light can penetrate through the water column to the bottom, allowing plants to grow and therefore provide food and shelter for macro-invertebrates.

Shading—Streamside vegetation provides shading to regulate extreme water temperatures during summer and provides food (leaves, branches and bark) for macro-invertebrates.

Temperature—Some macro-invertebrates cannot tolerate wide variations in temperature or warm water. Dissolved oxygen levels decrease as water temperature increases, which places macro-invertebrates under stress.

Water chemistry

The chemistry of waterways is affected by rainwater, the geology of the catchment, animals in the water and human activities. Chapter 5 details how to use physico-chemical parameters to measure water quality. The following chemical characteristics have an impact on macro-invertebrates.

pH—This is the acidity or alkalinity of the water, measured on a scale of 0 to 14 units. A pH of less than 5 or more than 9 units can be toxic to macro-invertebrates.

Dissolved oxygen (DO)—DO is added to water by plant photosynthesis and by water mixing with air as it flows over rocks. Macro-invertebrates take up this oxygen, which is dissolved in water. In still or slow-flowing water with a high density of



aquatic animals, DO can be lowered to dangerous levels (less than 5 milligrams per litre) due to biological activity. This disrupts the metabolism of macro-invertebrate species, leading to death.

Nutrients (phosphorus and nitrogen)—These nutrients are essential for life. Still or slow-moving water traps nutrients and silt. If nutrient levels are low, water is clear and macro-invertebrates are sparse, whereas high concentrations of nutrients promote plant growth and abundance of grazing macro-invertebrates.

Salinity (concentration of dissolved salts)—The natural salinity level varies between waterways, though human activities can lead to dramatic increases in salinity. Macro-invertebrates vary in their tolerance to salinity, thus the salinity level at a site will influence the types of macro-invertebrates that are present.

Biological factors

Available food—Food sources for macro-invertebrates come from small aquatic organisms, algae, streamside vegetation, and decaying food particles in the water from upstream. Algal growth is affected by sunlight and nutrients. Overhanging vegetation cover changes along the length of a waterway, varying the availability of this food source, hence the macro-invertebrate composition also changes.

Seasons—Most macro-invertebrates hatch in summer and mature from egg to adult by the next summer, so are easier to identify in spring sampling. Most macro-invertebrates have several life-cycle stages in developing from an egg to a mature adult, at which many live in water. Macro-invertebrates are generally easier to sample when they are further developed, as they are larger and thus easier to catch and identify.

Human factors

Human activities in a catchment or waterway can significantly modify macro-invertebrate communities and, consequently, affect animals higher in the food chain. Effects of such activities include changing the sediment load, clearing stream vegetation and increasing nutrient and effluent input.

Suspended solids—The reduction in light penetration due to suspended solids limits primary productivity and photosynthesis, resulting in

reduced DO levels. Sediment deposited on the waterway bed can smother bottom-dwelling communities and fill in holes and depressions, reducing habitat diversity and availability.

Riparian vegetation—Removing riparian vegetation reduces the input of organic matter such as leaves and bark, which are food sources for macro-invertebrates. Additionally, it will increase the amount of light reaching the waterway (that was previously shaded), possibly resulting in increased algal production and increased surface water temperatures—both of which can affect the number and diversity of macro-invertebrates.

Removal of woody debris—Woody debris provides habitat and food sources for some macro-invertebrates. Reducing the variety of habitats available for colonisation decreases macro-invertebrate communities and can destabilise the bed and banks of the waterway.

Barriers—Barriers such as dams alter natural flow, water temperature and water chemistry. They also restrict the movement of aquatic animals, often obstructing downstream transport, and disrupt the various life stages of many macro-invertebrates.

Run-off—Increases in nutrients from catchment run-off (such as through erosion, salinity or sedimentation) promote algal production, and this change in food supply supports an abundance of grazing macro-invertebrates; however, increased nutrient levels can also increase the suspended solids in the waterway.

Sewage and industrial effluent—Toxic substances such as heavy metals and pesticides can kill macro-invertebrates. Sewage effluent also leads to reduced levels of DO in the waterway, which can affect the macro-invertebrate community. Toxic contaminants and reduced DO levels can result in decreased macro-invertebrate diversity, but may not affect the relative abundance of organisms. This is because some species of macro-invertebrates are tolerant of certain toxicants and/or low levels of dissolved oxygen. Some may even flourish in such conditions, as competition from less tolerant species is reduced. Thus, moderate organic pollution will decrease diversity but the abundance of tolerant species may actually increase.



Monitoring macro-invertebrates

Macro-invertebrate monitoring can provide an insight into human impacts on waterways. This information can provide a basis for making informed decisions about the health of aquatic ecosystems and whether action is required to address certain human impacts.

What they indicate

Biological monitoring using macro-invertebrate sampling can tell a story about the health of your waterway, which is indicated by the abundance, diversity and composition (whether pollution-tolerant taxa are present) of the macro-invertebrate community. Collecting physico-chemical (water quality) samples from the site can only provide information on the conditions at the time of sampling. However, macro-invertebrate sampling can indicate the long-term water quality and physical conditions at the site. This is because most macro-invertebrates spend all or part of their life in-stream and have a limited spatial distribution. If the water quality or physical habitat at a site deteriorates, certain types of macro-invertebrates may become extinct from the site. Therefore, macro-invertebrates can provide evidence of pollution that is not present at the time of monitoring.

Macro-invertebrate monitoring can also provide meaning to your water quality results. For example, you may record an electrical conductivity (EC) reading of 2000 microsiemens per centimetre (mS/cm) at a site; however, it can be difficult to tell whether this EC level is suitable for the health of the aquatic ecosystem, as this differs greatly between different types of waterways. Directly assessing components of the ecosystem, such as the macro-invertebrate community, will give you a more direct measure of the health of the aquatic ecosystem and whether the water quality is acceptable.

Abundance—This is the total number of macro-invertebrates. A high abundance indicates water enriched with nutrients, while a low abundance indicates erosion, toxic pollution or a recent flood event. Most methods do not measure macro-invertebrate abundance because of the time involved.

Diversity (richness)—This relates to the number of different types (taxa) of macro-invertebrates present. Healthy waterways generally have higher diversity than degraded waterways. Communities with high diversity are generally more stable over time.

Composition—This is the proportion of different types of macro-invertebrates (taxa) living together. Healthy waterways generally contain a high proportion of mayflies, stoneflies and caddisflies (Ephemeropterans, Plecopterans and Trichopterans), while degraded waterways tend to contain a high proportion of worms (Nematodes), water boatmen (Hemipterans) and midge fly larvae (Chironomids).

Pollution tolerance—This refers to the ability of macro-invertebrate taxa to withstand organic pollution from sewage, industrial effluent, heated water and other pollution types. For example, worms are quite tolerant of pollution, while most stonefly families are very sensitive and do not survive in polluted waters. Consequently, pollution-intolerant macro-invertebrates are unlikely to be present in degraded water.

Strengths and limitations

Macro-invertebrates are good indicators of waterway health because they:

- are affected by physical, chemical and biological conditions, and can therefore represent the overall ecological health of the stream
- are a critical part of the aquatic food web, as they feed on plants and are eaten by predators
- cannot easily escape pollution, and can consequently indicate the effects of pollution events, intermittent pollution or long-term changes
- are abundant, and easily sampled and identified
- are relatively cheap and time-effective to monitor



- live long enough to provide a record of environmental quality
- respond quickly and are capable of a graded response to a broad range of stress factors
- are found in most aquatic habitats
- tend to inhabit small-order streams that do not support other biological indicators such as fish
- only need to be sampled twice a year, with results staying valid for a two or more years.

Macro-invertebrates are good indicators of trends in waterway health, as they can reflect various changes in water quality and physical condition, including bank and bed stability, riparian vegetation and in-stream habitat. However, it is also important to be aware of the limitations of using macro-invertebrates as waterway health parameters. These limitations include the following:

- They are unable to indicate the cause of an impact.
- They do not respond to all impacts.
- They do not indicate the presence of micro-organisms that can cause disease in humans.
- They are not as sensitive to altered river flows as other aquatic biological indicators, such as fish, because many macro-invertebrates have wings at some stage of their life cycle.
- Natural seasonal variations prevent comparisons being made between samples taken at different times of the year.

How information can be used

Macro-invertebrate sampling can be used for many different purposes, including the following.

Education and awareness—Macro-invertebrate monitoring is an excellent activity for learning about waterways and promoting stewardship of the environment.

Identification of ecological health—Macro-invertebrates can be used for baseline monitoring to develop a picture of stream health over the whole catchment, and to show changes from the headwaters to the mouth. Comparing results with those from reference sites (those not affected by humans, or minimally so) enables you determine

whether sites are in reference (good) condition. This assists with the effective planning of restoration actions. The *Queensland water quality guidelines* (Environmental Protection Agency 2006) include biological (macro-invertebrate) guidelines for South East Queensland that have been developed using data from reference sites in the region, to allow the comparison and interpretation of biological monitoring results from that region. There is potential for this to occur in other regions of Queensland.

Identification of waterway health trends—The results of macro-invertebrate monitoring at a site can be used to identify worsening or improving conditions over time.

Identification of pollution impacts—Changes in the abundance and diversity of macro-invertebrates can show the impact of point-source or diffuse-source pollution on the waterway.

Restoration activities—When stream restoration or rehabilitation projects are undertaken, or when pollution control efforts are made, macro-invertebrate sampling can be used to assess the effectiveness of such efforts.

Early warning detection of human impacts—By the time decreases in the health of the waterway (such as decreased water quality or physical condition) are noticeable to the human eye, the impacts may be severe or permanent. Macro-invertebrate monitoring can detect these impacts early and therefore trigger the necessary management actions.



Designing your study

The following questions should be addressed prior to selecting your monitoring methods:

- Where should I place my monitoring sites in the catchment?
- Where should I sample in the stream?
- When should I sample?

Where to place monitoring sites in the catchment

The placement of your monitoring sites within the catchment will depend largely on your monitoring objectives. It is also important to consider health and safety issues as well as practical issues, such as whether access to the site is suitable. For general guidance on selecting waterway monitoring sites, refer to Chapter 4.

Where to sample in the stream

Deciding where to sample in the stream is important, as the sample you collect must be representative of the macro-invertebrate community present at the site. As macro-invertebrates are known to prefer specific habitats in the stream, you need to sample the full range of these. In flowing waters, three habitats that have been identified as providing good areas for macro-invertebrate sampling are riffles and runs, pools, and stream edges.

Riffles provide habitat for a diverse range of macro-invertebrates, making them highly desirable sampling sites. Sampling should take place in areas of the stream where the velocity of the current is moderate to high. Conventionally, these are rocky riffles where the flow is rapid and turbulent, but gravel and sand bars can also be sampled as riffle if the water surface is broken.

Stream edge habitats should also be sampled. Your aim when sampling edge habitats should be to include a variety of slow-flowing habitat types. Such habitats include overhanging vegetation, undercut banks, snags and logs, backwaters, leaf packs and bare edges.

Pools provide habitat for a number of macro-invertebrates that do not occur in riffle or stream edge habitats. Although pool habitats do not

support species diversity as high as riffle or edge habitat, they should still be sampled whenever present at a site because the macro-invertebrate taxa found in pools will usually be different to those found in the other habitat types.

No matter which habitats are present at your site, you should sample in roughly the same place each monitoring session to enable genuine comparisons to be made between data collected during different monitoring sessions.

When to sample

Sampling should occur twice a year, preferably in spring and autumn. To minimise physical damage to the site, sampling should occur no more than four times a year. Spring samples will contain specimens of insects hatched the previous summer, making them easier to identify (as adult insects generally have more distinguishing features). Autumn samples will be representative of lower flows and higher temperatures, when pollution inputs may have a higher impact. Regardless of what time of year you decide to sample, it is important that sampling occur at roughly the same time in future years to enable data comparison.

For general guidance on deciding when to monitor, refer to Chapter 4.

Choosing your monitoring methods

Once you have decided where and when to monitor, you need to choose the monitoring methods that you will use. Your choice of method will be affected by the level of data quality (macro-invertebrate identification) you require to achieve your project objectives, the level of skill your group possesses in identifying macro-invertebrates, and your available resources.

This manual provides three methods to choose from. Method 1 generates a demonstrative level of data quality, as only selected macro-invertebrates are identified to order level. These assessment methods are most suited for groups who are focused on increasing education and awareness.

Methods 2 and 3 are suitable for generating indicative and analytical data, respectively; however, the success of these methods will depend on the expertise and experience of the persons collecting the data, and the quality control measures undertaken.



The field sampling protocols for methods 2 and 3, and the sorting protocols for Method 3, are based on the Queensland version of the Australian River Assessment System (AUSRIVAS) (Conrick & Cockayne 2001). This methodology is currently used by the Department of Environment and Resource Management for biological monitoring across the state. AUSRIVAS is a nationally recognised system used to quickly and efficiently assess the biological health of Australian rivers and streams. AUSRIVAS monitoring uses a macro-invertebrate sampling method known as 'rapid biological assessment'. The AUSRIVAS method is currently the most common method used by state agencies, universities and others across Australia to assess the biological health of waterways. To find out more about AUSRIVAS, visit the website <<http://ausriv.as.canberra.edu.au>>.

The only main difference between Method 3 in this manual and the Queensland AUSRIVAS methodology is the way that results are analysed and interpreted. AUSRIVAS results are normally interpreted using specially designed models that predict the macro-invertebrate fauna expected to occur at a site in the absence of environmental stress, to which the fauna collected at a site can be compared. The AUSRIVAS models calculate a rating score for a site based on how the fauna collected at the site compare to the fauna expected to be present by the model—this is known as an AUSRIVAS score. AUSRIVAS models have been developed for each Australian state and territory, including several regions within Queensland, and apply to freshwater streams. No models have been developed for lakes, estuaries or wetlands.

The methods in this manual do not require AUSRIVAS models to analyse and interpret results. Instead, results for Method 3 are calculated and interpreted using another set of indexes commonly used by state agencies in Queensland to measure stream health: macro-invertebrate family richness, SIGNAL 2 (stream invertebrate grade number average level) and PET (Plecoptera-Ephemeroptera-Trichoptera) richness.

To choose the most appropriate method for your project, refer to Table 6–3. Consider your required data quality and your available resources (time, budget and skills).

If you are aiming to measure a change over time (baseline condition and trend monitoring, impact assessment or restoration assessment) or to compare sites to one another, it is especially important to think about the level of data resolution and sensitivity that you require. Are you hoping to detect differences of small or large magnitudes? The method that you use, particularly the level of macro-invertebrate identification that you undertake, will influence the ability of your data to indicate differences between sites or over time. This is because macro-invertebrate taxa have varied tolerances to poor water quality.

For example, mayflies belong to the order Ephemeroptera (of which there are six families in Queensland). Most mayflies are sensitive to poor water quality; however, a small number of families are fairly tolerant. Therefore, it would be necessary to identify which families are present in your sample to determine whether any sensitive taxa are present. If you only identified your sample to order level, rather than family level, you may conclude that the water quality was good at the site even though only the tolerant mayfly families may be present.

In such cases, Method 1 will not generally be able to detect changes to the biological health of the waterway over time or across the catchment. Method 2 may be able to detect major changes only, and Method 3 should be able to detect more minor changes.

However, rapid bioassessment methods in general (on which all the methods in this manual are based) are only designed to detect broad-scale changes to the health of the waterway.

If you aim to detect minor changes to the macro-invertebrate community at a site, quantitative monitoring methods that measure the abundance and diversity of macro-invertebrates at a site and identify them to species level are probably the best option. However, keep in mind that this type of monitoring can be very costly, and requires a very high degree of experience and training in macro-invertebrate identification.

Table 6-3 Methods guide for biological monitoring

	Method 1	Method 2	Method 3
Data quality produced	Demonstrative—only selected macro-invertebrates are identified to order level, using their common names (e.g. freshwater shrimp, water boatman, dragonfly nymph).	Indicative—all macro-invertebrates are identified to order level.	Analytical—macro-invertebrates are identified to family level.
Who is to use data	Internal sharing of data	External sharing of data with: <ul style="list-style-type: none"> • catchment, Landcare or Waterwatch groups • landholders • schools • industry groups 	External sharing of data with: <ul style="list-style-type: none"> • regional bodies • state agencies • universities • private consultants • local government • regional bodies • industry groups
Suitability for type of monitoring	Baseline monitoring (only snapshot assessment)	Baseline monitoring Ambient monitoring	Baseline monitoring Impact assessment (indicative use only) Restoration assessment (indicative use only) Compliance monitoring
Reasons for monitoring	<ul style="list-style-type: none"> • Increase community understanding and awareness • Increase community skills 	<ul style="list-style-type: none"> • Increase community understanding and awareness • Increase community skills • Establish a baseline (identify current condition) • Monitor trends through time to identify decline or improvement in condition 	<ul style="list-style-type: none"> • Increase community understanding and awareness • Increase community skills • Establish a baseline (identify current condition) • Monitor trends through time to identify decline or improvement in condition • Assess impact of a land use or pollution source (indicative use only) • Assess effectiveness of a management action (indicative use only)



Knowledge and skills	Beginner—groups with no or little experience (e.g. new community groups, primary and secondary students).	Some experience—groups with some experience and training who can identify macro-invertebrates to order level (e.g. community groups and upper secondary students trained by a group coordinator or equivalent). Only major body features need to be identified.	Significant experience—groups with extensive experience, skills and training who can identify macro-invertebrates to family level (e.g. experienced community groups trained by an aquatic ecologist or group facilitator with equivalent knowledge). Requires patience, knowledge and identification on the basis of subtle differences.
Time	Sampling procedure takes less than 1 hour but the activity may take longer due to education and training content. There is no requirement for how often sites should be visited.	Sampling procedure takes approximately 1–2 hours. Processing sample (identification of macros) in field takes a further 1–2 hours. Sites should be visited twice in a year (autumn and spring) for a single assessment.	Sampling procedure takes approximately 2 hours. Processing samples in laboratory takes approximately 2 hours per site sample for an experienced picker. Sites should be visited twice in a year (autumn and spring) for a single assessment.
Approximate equipment costs	<p>\$70 for:</p> <ul style="list-style-type: none"> • sampling net • white plastic sorting tray or bucket • tweezers, plastic pipette or small paint brush • ice cube tray • magnifying glass 	<p>\$120 for:</p> <ul style="list-style-type: none"> • triangular dip net • white plastic sorting tray • ice-cube tray • tweezers, pipette, plastic spoons or paint brushes • magnifying glass • latex rubber gloves • gumboots • identification resources 	<p>\$160 for:</p> <ul style="list-style-type: none"> • triangular dip net • white plastic sorting tray • tweezers or forceps • rubber gloves • 70% methylated spirits • vials and vial labels • gumboots • identification resources <p>+ \$200 for a low-power (×20) binocular microscope</p>

The above methods are based on the rapid bioassessment methods currently used for most biological assessment across Australia. Rapid bioassessment is designed to enable swift evaluations of stream health to be made using biological indicators (normally macro-invertebrates), through cost effective, scientifically valid procedures. Rapid bioassessment is not suitable when the objective of the study is to collect high-level data for the following reasons:

- to assess the impact of a land use or pollution source (impact assessment)
- to assess the effectiveness of a management action (restoration assessment)
- to investigate causes of a particular water quality or river health problem (investigative studies).

To meet the above objectives using biological (macro-invertebrate) monitoring methods, identification of specimens to species level would be required. This requires both a large amount of time and a prohibitively high level of expertise in macro-invertebrate identification.





Safety considerations

Personal safety while conducting macro-invertebrate sampling is based on management of potential risks. Always remember that personal safety comes first and that no task is so important that safety should be compromised. Some considerations for your safety when undertaking sampling include the following:

- Always let someone know where you are and how long you will be.
- Do not undertake monitoring alone or at night.
- Choose a site to sample that is safe and is easy to access.
- Do not sample in areas that are heavily polluted or have toxic algae outbreaks.
- Wear appropriate clothing and footwear.
- Wear gloves when sampling—even though the water may look clean, it may not be safe.
- Take fresh drinking water with you—never drink the water that you are sampling.

Estuarine crocodiles inhabit freshwater streams and rivers in much of northern Queensland. Do not conduct monitoring in areas that crocodiles are known to frequent or where evidence of crocodiles (nests, tracks or warning signs) can be seen. Take care when sampling in areas of crocodile distribution, and acquire local knowledge where possible to correctly identify crocodile areas. If sampling must be conducted at sites within the range of crocodile distribution, take appropriate safety precautions. Develop a safety plan and ensure that all field workers are adequately trained in crocodile safety procedures. Some wildlife parks offer training in crocodile safety, which may be of use.

For a more comprehensive review of health and safety risk management, refer to the *Health and safety guidelines for community-based waterway monitoring* (Department of Natural Resources and Water 2006).

Method 1 for monitoring macro-invertebrates

Method overview

The purpose of this method is to provide an introduction to biological monitoring through simple macro-invertebrate sampling and analysis. The sampling and sorting procedures are designed so that a minimal level of expertise or formal training is required. Identification and analysis of sampled macro-invertebrates is carried out using the Waterwatch Queensland *Stream quality slide* (included with this manual) or the macro-invertebrate record sheet for Method 1 (provided in Chapter 10), which uses selected easy-to-identify macro-invertebrates as indicators of stream health. Identifying which of the selected fauna are present at a site enables you to assess the stream health. This method is only capable of producing demonstrative data and would be appropriate for primary and secondary school groups wanting to learn about macro-invertebrates, or community groups wanting to gain an insight into the value of biological water monitoring.

Equipment

For this method, you will need:

- a sampling net (for example, a kitchen strainer, fish tank net or homemade stocking net)
- a white plastic sorting tray or bucket (for example, an ice cream container)
- tweezers, plastic pipette, and plastic spoon or small paint brush
- an ice-cube tray
- a magnifying glass
- the *Stream quality slide* or Method 1 record sheet (see Chapter 10)
- gumboots.

Where to sample

Different types of macro-invertebrates live in specific parts of the waterway. These habitats include stream edges, rocky and sandy pools, aquatic plants, and riffles. Macro-invertebrates that occur in stream edges are generally found clinging to overhanging vegetation from the banks or on tree roots. In pool habitats, macro-invertebrates tend to occur around woody debris and snags or on the leaves of aquatic plants. In riffle habitats,



water bugs are usually found on the underside of rocks or clinging tightly to rocks on the upper side. To gain a broad understanding of the macro-invertebrates present at your site, it is a good idea to sample all of the habitats present in your stream.

Data confidence procedures

As this method is designed to provide demonstrative data, there is no need to conduct any data confidence procedures other than equipment maintenance checks.

Collecting your sample

1. Start downstream and work upwards along the water's edge to prevent disturbing any habitat that you have not yet sampled. Allow the flow to carry the animals into your net. When there is not enough flow to wash the macro-invertebrates into your net, sweep it through the water as you walk upstream.
2. Sweep the net for five minutes through each different aquatic habitat present, including the bottom and along the edge of your site. When sampling among water plants, beat gently and scrape your net against the base of the plants to bump off any macro-invertebrates clinging to the surface.
3. Empty the contents of your net into your sorting tray or bucket. When sampling along the stream bed, remove any leaves from your net and place them in your sorting tray. Remove leaves one at a time and look closely for the presence of animals.

Sorting your sample

1. Place a small amount of stream water into the sorting tray or bucket and empty the contents of your net into the tray. Wash down the sides of your net with water to ensure that your entire sample is transferred into the tray. Keep the tray in a shady area, as the shade encourages the macro-invertebrates to emerge.
2. If your sample has high levels of sediment, let the water settle for approximately 10 minutes before sorting.
3. Sort the captured water bugs into groups of similar-looking bugs using a pipette, tweezers or small paint brush. Place the animals of each group into separate sections in the ice-cube tray. Only one specimen of each water bug is required.
4. Return all extra water bugs to the stream in a shady spot.

Processing your sample

The Waterwatch Queensland *Stream quality slide* identifies ten water bugs that commonly occur in waterways throughout Queensland and can be used to provide a general measure of waterway condition. These ten water bugs have been divided into groups according to their pollution tolerances. Once you have sorted through your sample, identify which of the ten key water bugs used on the *Stream quality slide* are present at your site. A basic record sheet (Method 1) using the same ten macro-invertebrates has been developed as an alternative to the *Stream quality slide*. This sheet can be found in Chapter 10 of this manual.

Calculating results

Once you have identified any water bugs in your sample that are on the Waterwatch Queensland *Stream quality slide*, move the slide accordingly to match the animals found at your site. This should give your site a rating of excellent, good, fair or poor based on the tolerance of the water bugs found at your site. The basic survey sheet uses the same rating system.

Interpreting results

This overall stream rating method produces demonstrative data to give a general indication of the stream health at your site; however, it can indicate stream health only in relation to the presence or absence of ten selected organisms, so you must keep this in mind when interpreting your data. The lack of formal identification also restricts the value of data interpretation. The data interpretation from this method should be restricted to educational purposes, with the quality of data being insufficient to monitor trends in waterway condition.



Method 2 for monitoring macro-invertebrates

Method overview

The objective of this method is to increase community understanding and awareness of stream health and, at the same time, monitor major trends to identify a decline or improvement in stream condition. To successfully achieve this, training by an individual experienced in the study of macro-invertebrates is required before commencing sampling. Identification of specimens is primarily carried out to the order level of taxonomy, drawing on numerous macro-invertebrate identification resources. Order-level data is used to calculate a SIGNAL 2 score that provides an indication of stream health at the sampling site, based on modelled sensitivities of the macro-invertebrates collected. This method can produce indicative, intermediate-quality data when identification is carried out accurately. Ideally, this level of monitoring would be appropriate for high school students or catchment, Landcare, and Waterwatch groups aiming to monitor broad trends in stream condition over time and roughly assess the general health of the waterway.

This field sampling protocol has been adapted from the Queensland AUSRIVAS methodology (Conrick & Cockayne 2001) currently used by the Department of Environment and Resource Management for biological monitoring across the state.

Equipment

For this method, you will need:

- a triangular dip net (250 mm × 250 mm × 250 mm)
- a white plastic sorting tray
- tweezers and pipettes
- rubber gloves (for polluted sites)
- waders
- a bucket (for collecting stream water for sorting tray)
- an ice-cube tray
- a pencil (for writing on field sheets)
- a copy of sampling procedures
- the Method 2 record sheet (see Chapter 10).

Where to sample

Separate sampling of each habitat type is recommended because each habitat has a potentially distinct array of fauna. Riffle habitat generally has high levels of both abundance and diversity, making it ideal for macro-invertebrate sampling. Therefore, whenever riffle habitat is present at a site, it should be sampled. Edge and pool habitats are the other major types of habitat where macro-invertebrates tend to occur in large numbers. As edge habitat is always present, it can provide valuable data for comparing macro-invertebrates at different sites. Regardless of which types of habitat you sample at a site, it is important to conduct all sorting and analysis of each habitat separately. Generally, if a habitat makes up more than 10% of the stream reach, it should be considered for sampling.

Data confidence procedures

To ensure that your data is of the desired intermediate standard, the following measures are recommended:

- Check sampling nets for holes, and repair if necessary.
- Collect as close to 200 fauna specimens as possible so that the macro-invertebrates collected provide a fair representation of those present at the site.
- Sample in the same habitat areas for each monitoring session at a given site.
- Compare your findings with those of other groups within the region
- Have at least one experienced and trained person present for each monitoring session.

Collecting your sample

All macro-invertebrate samples should be collected with a triangular mesh dip net. The two types of sampling methods to be used are kick and sweep sampling. Kick sampling is primarily used for sampling bed and riffle habitats, while sweep sampling is used for sampling edge habitats. A total distance of 10 metres should be sampled across each habitat (bed and edge) covering a variety of velocities and stream features.

Figure 6-4 shows an example of where sweep and kick sampling should occur at a site.

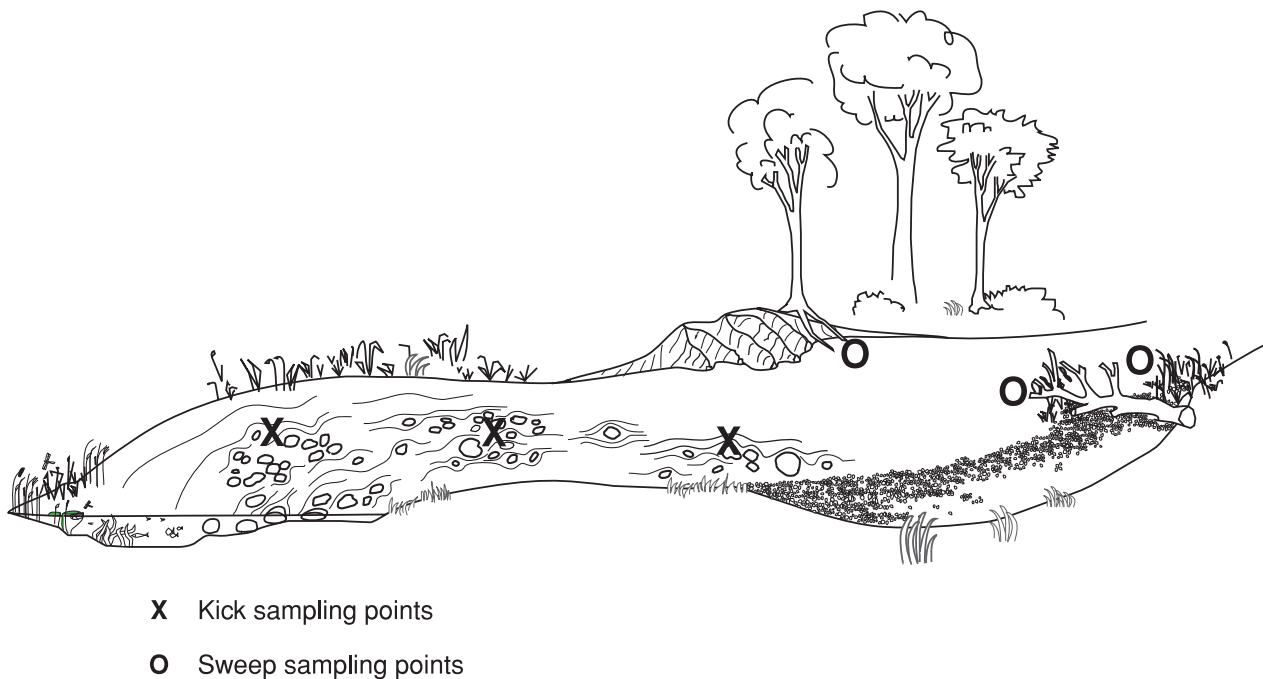


Figure 6-4 Example of where kick sampling and sweep sampling should occur at a site

Sweep sampling

This method is best suited to stream edge, pool and backwater habitats with vegetation overhanging from the stream bank, aquatic plant (macrophyte) beds, undercut banks, root mats, leaf packs, woody debris, and other stream features that provide suitable living places for macro-invertebrates.

The following steps are recommended in order to gain the truest representation of a site's macro-invertebrate community when conducting sweep sampling:

1. Insert the net among the key features of the habitat (such as overhanging vegetation) and use a short, upward-sweeping movement at right angles to the bank. Stir up the bottom while doing so, ensuring that bottom-dwelling animals are suspended and then caught when sweeping through the cloud of suspended material. Continue this procedure along 10 m of the habitat to sample the full range of stream features.
2. Stop regularly to rinse mud and fine silt out of your net. The sample should be free of sediment prior to sorting. An easy way to rinse your net without losing your sample is to wash water into the side of your net while you are holding it with the mouth of the net pointing upwards above the water.

3. Once finished, scoop the net out of the water in a forward motion to prevent fauna from escaping. Flush the net with water to remove any remaining sediment prior to sorting.
4. Empty the contents of the net gently into about 2 cm of clear stream water in a white sorting tray. Rinse down the sides of your net into the tray to ensure that you transfer the full sample into the tray. Pick off any stray macro-invertebrates still clinging to the net using a pair of tweezers.

Kick sampling

This method is designed for sampling stream bed habitats and can be used to sample a range of depths and flows. It is performed most effectively in riffles—fast-flowing, rocky sections of the stream bed—where the highest diversity of macro-invertebrates is generally found. However, kick sampling is also an effective method for sampling silty or sandy beds and rocky or gravel beds in slightly slower-flowing sections of the stream. All types of bed habitat present at a site should be sampled using the kick method.



The following steps are recommended in order to gain the truest representation of a site's macro-invertebrate community when conducting kick sampling:

1. Approach the sampling area from downstream to prevent disturbing fauna prior to collecting.
2. Holding the net downstream with its mouth facing the sampling area upstream, disturb the stream bed by digging your foot underneath the stones and turning them over. Macro-invertebrates will become suspended in water and will be pushed into the net by the flow of the stream. If there is little or no flow, you will have to use a short sweeping action with the net while stirring up the bed.
3. If you encounter large rocks, place them in the net by hand and rub off fauna any into the net before placing the rock back on the stream bed.
4. Stop regularly to rinse mud and fine silt out of the net.
5. Repeat these procedures over a distance of 10 m, aiming to sample the full range of flow velocities and key features of the bed habitat.
6. Once finished, scoop the net out of the water in a forward motion to prevent fauna from escaping. Flush the net with water to remove any remaining sediment prior to sorting.
7. Empty contents of the net into 2 cm of clean water in the white sorting tray.
8. Before taking another sample, rinse the net so that all fauna and debris are removed.

Sorting your sample

Keep your kick and sweep samples separate. Mixing samples from different habitats is not recommended, as each habitat supports a distinct community of macro-invertebrates. This enables comparisons to be made between the same types of habitat at different sites.

Sample sorting should be conducted at the site, thus enabling the return of all organisms to the waterway once they have been noted on your record sheet. To make certain that adequate quantities of fauna are collected from the sample, 100 to 200 specimens should be moved from the sorting tray to the ice-cube trays. Sorting generally takes an individual between 30 minutes and 1 hour, depending on their experience and the fauna abundance of the site. Effective sorting of your sample can be accomplished by adhering to the following guidelines:

1. Distribute your sample out over the bottom of a white tray. Spend a moment to observe the movement, body shape and colour of the fauna within the sample.
2. Pour 1 cm of water into each of the wells of your ice-cube tray. Pick through the sample in your sorting tray and use a pipette, tweezers, spoon or brush to transfer your fauna to the wells in the ice-cube tray.
3. Transfer any fauna that you see from the sorting tray into the ice-cube trays during the initial 20 minutes. Place each macro-invertebrate type into a separate well of your ice-cube tray. Only one specimen of each taxon is needed.
4. For the following 10 to 20 minutes, focus particularly on fauna that are uncommon. If, after 30 minutes, you discover a macro-invertebrate you had not previously seen, sort for a further 10 minutes. If, in that 10 minutes, another new specimen has been found, sort for another further 10 minutes. Continue until no new specimens are found.
5. Return the fauna to the water once you have finished, as close to the collection site as possible.

Processing your sample

Identify the macro-invertebrates in your sample to order level using the macro-invertebrate identification booklet provided at the end of this chapter, or other identification resources listed on page 6–28. Group your specimens together based on the taxa grouping used for the Method 2 macro-invertebrate record sheet, located in Chapter 10 (for the majority of organisms this is to the taxonomic level of order). If you come across a macro-invertebrate that you cannot identify, record this on your record sheet, describing what it is you have found. Check your findings with a local macro-invertebrate expert—such as an aquatic ecologist, a macro-invertebrate taxonomist or your monitoring coordinator—and various macro-invertebrate guides. Once you have identified each specimen using the identification booklet, record which fauna were found at your site by filling out the Method 2 macro-invertebrate record sheet. Remember, it is best to keep the data for each habitat type separate.



Calculating results

SIGNAL 2 score—order

The impact of pollution on Australian macro-invertebrates can be assessed using the SIGNAL 2 Index. Each taxon has been given a sensitivity score from 1 to 10 based on its modelled sensitivity to pollution. The higher the sensitivity score of a taxonomic group of macro-invertebrates, the greater its pollution sensitivity.

The SIGNAL 2 score is calculated by averaging the pollution sensitivity scores of the macro-invertebrate groups (generally, orders) present at the site. The SIGNAL 2 score should be calculated separately for each sample from a different habitat type. To determine the overall score for the site, the resulting scores for each habitat type should be averaged. The Method 2 macro-invertebrate record sheet provides the sensitivity grades for common macro-invertebrate orders. The survey sheet is designed to assist you to calculate the SIGNAL 2 scores for your site.

Interpreting results

The table below is a summation of stream health based on the value of a SIGNAL 2 score at a site. It is important to remember that only samples collected from the same type of habitat using the same sampling methods should be compared.

Table 6–4 Interpretation of SIGNAL 2 scores
(adapted from Gooderham & Tsyrlin 2002)

SIGNAL 2 score	Habitat quality
Greater than 6	Healthy habitat
Between 5 and 6	Mild pollution
Between 4 and 5	Moderate pollution
Less than 4	Severe pollution

Developing a SIGNAL 2 score using specimens identified to order level is a far more accurate means of assessing waterway health than the basic method used in this chapter. However, it is worth noting that macro-invertebrate orders may consist of many different families, therefore an assessment will be far more accurate with identification to family level. It is also important to take into account that many waterways naturally have macro-invertebrate communities with low SIGNAL 2 scores—this is often the case in ephemeral streams—so caution needs to be taken when assessing stream health based entirely on SIGNAL 2 score results.

This method could be used to increase community awareness, identify current conditions and monitor trends in biological communities over time. The quality of data produced would be insufficient to assess the impact of a land use, the effect of a pollution source, or the effectiveness of a management action; or to document minor changes over time.



Method 3 for monitoring macro-invertebrates

Method overview

This complex monitoring method promotes a high level of community understanding and knowledge of stream health through the monitoring of biological (macro-invertebrate) parameters. The data collected from this method has the potential to be of high quality and value, enabling accurate assessments of waterway condition and the detection of long-term (over several years) trends. This is particularly so when combined with high-level water quality data, as the local water quality can greatly influence or explain patterns in macro-invertebrate communities. The method can also be used for rough assessments of restoration actions and pollution impacts.

This level of monitoring would be suitable for state agencies, universities, private consultants and community groups with extensive experience in macro-invertebrate identification. A high level of expertise within the group, and prior training from an aquatic ecologist or highly experienced person, is essential. Processing samples (identifying macro-invertebrates) is the most difficult part of this method, therefore some groups may wish to outsource this component of the monitoring to a more experienced operator (a state agency, university or consultant). This approach would suit those community-based groups who have limited experience in macro-invertebrate identification but have an interest in the level of data that this method can generate.

Method 3 involves identifying most specimens to a family level of taxonomic classification, using a dissecting microscope and scientific identification resources. For these reasons, and because potentially volatile preserving solutions (like methylated spirits) are used, identification of macro-invertebrates is normally undertaken in a laboratory. Analysis of data is carried out by calculating a number of indexes or rating scores. As with the Method 2 procedure outlined in this chapter, a SIGNAL 2 score is calculated. However, for the complex method, SIGNAL 2 values are assigned to each macro-invertebrate family rather than order. To complement the SIGNAL 2 score of a site, family richness and PET richness calculations are recommended. PET richness looks

at the number of stonefly (Plecoptera), mayfly (Ephemeroptera) and caddisfly (Trichoptera) families present at the site, as these groups or orders of aquatic insects are known to be highly sensitive to environmental stress.

The field sampling and sorting protocols for this method are based on the Queensland version of AUSRIVAS (Conrick & Cockayne 2001). This methodology is currently used by the Department of Environment and Resource Management for biological monitoring across Queensland.

The only main difference between Method 3 and the Queensland AUSRIVAS methodology is the way that results are analysed and interpreted. AUSRIVAS results are normally interpreted using specially designed models that predict the macro-invertebrate fauna expected to occur at a site in the absence of environmental stress (to which the fauna collected at a site can be compared).

The methods in this manual do not require AUSRIVAS models to analyse and interpret results. Instead, results for Method 3 in this manual are calculated and interpreted using indexes commonly used by state agencies in Queensland to measure stream health: macro-invertebrate family richness, the SIGNAL 2 score, and PET richness.

Equipment

Required **field** equipment includes:

- a triangular dip net (250 mm × 250 mm × 250 mm)
- a white plastic sorting tray
- tweezers and pipettes
- rubber gloves (for polluted sites)
- 70% methylated spirits
- sample jars (big enough to fit large macro-invertebrates such as yabbies)
- sample jar labels (to go inside each jar)
- waders
- or bucket (for collecting stream water for sorting tray)
- record sheets—field cover sheet, Method 3 record sheet, physico-chemical field sheet, Method 2 stream condition and habitat record sheet (see Chapter 10)
- a permanent marker (for writing labels on sample jar lids)
- a pencil (for writing on field sheets and labels)
- a copy of sampling procedures.



Required **laboratory** equipment includes:

- identification resources (refer to the list on page 6–28)
- a microscope
- tweezers and pipettes
- a Petri dish
- small vials
- vial labels
- the Method 3 laboratory record sheet
- a pencil.

Where to sample

If a particular type of habitat makes up more than 10% of the stream within your site, it should be considered for sampling. In Queensland, stream edge and bed areas are the only two types of habitat sampled by AUSRIVAS (Conrick & Cockayne 2001). The first choice of bed habitat is riffle. Failing this, a rocky bed should be sampled; however, if nothing else is available, a sandy bed should be used. Separate sampling of individual habitats is required, as each habitat can potentially have its own unique fauna.

AUSRIVAS models have been developed for different regions within Queensland, including coastal and far-inland models. There are separate models for both edge and pool habitats for autumn and spring sampling seasons, as well as a combined seasonal–annual model.

Data confidence procedures

To make certain that your data is of the highest possible accuracy and precision, the following measures are recommended:

- Have all participants involved in sampling and identification trained by a suitably experienced person to within a predetermined tolerable error range.
- Check the sampling net for holes and repair if necessary prior to sampling.
- Collect a minimum of 200 fauna specimens, or as close to 200 as possible, so that the diversity of macro-invertebrates collected is representative of that present at the site.
- Sample the same habitats during each monitoring session at a given site. It is essential to accurately record the sampling site location whenever the site is assessed.
- Make sure that all sample jars and vials are correctly and adequately labelled. Sample jars from the field should have a label on the lid as well as inside the jar, in case one of the labels becomes smudged or damaged.

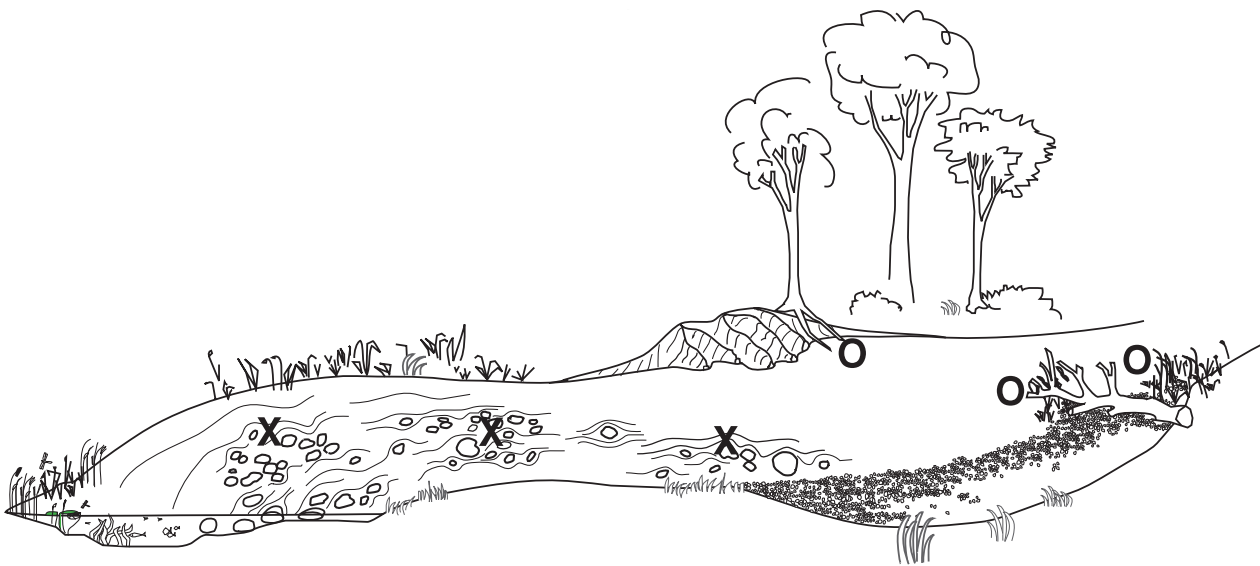
- Wash each net and tray thoroughly after use to prevent contamination of subsequent samples by the presence of macro-invertebrates collected at previous sites.
- Have 10% of macro-invertebrate samples and sample identifications checked (reidentified) by an aquatic ecologist or suitably experienced person, to check the error rates of sampling and determine your achieved data quality.
- Compare your samples and identification with that of other groups within the region.

To further ensure confidence in your data, it is recommended that the macro-invertebrate quality assurance sheet (provided in Chapter 10 of this manual) be used following the reidentification of a sample. This sheet is designed to detect any incorrect specimen identification and any taxa overlooked. By using the sheet, identification and processing errors can be easily identified and addressed.

Before collecting your sample

Prior to collecting your macro-invertebrates, information needs to be collected about your sampling site. This includes information about the whole reach (100 m section of the river), the habitats sampled and the riparian vegetation. To ensure that you collect the required information, both the Method 3 macro-invertebrate method field sheet and the generic field cover sheet need to be completed prior to conducting your sampling. These field sheets can be found in Chapter 10.

To further enhance your understanding of the health of your stream, it is strongly recommended that basic-level physico-chemical sampling and intermediate-level stream condition and habitat monitoring be conducted prior to your macro-invertebrate sampling. Physico-chemical testing needs to be carried out before macro-invertebrate sampling, as macro-invertebrate sampling will disturb the stream bed and affect the water composition. The data collected through basic physico-chemical and stream condition monitoring may help to explain the biological findings at your site. Physico-chemical parameters can restrict the types of macro-invertebrate families present due to their tolerance levels. The record sheets for physico-chemical and stream condition and habitat monitoring can be found in Chapter 10. For guidance on how to conduct basic physico-chemical sampling and intermediate-level stream condition assessment, refer to chapters 5 and 7 of the manual.



- X Kick sampling points
O Sweep sampling points

Figure 6-4 Example of where kick sampling and sweep sampling should occur at a site

Collecting your sample

All macro-invertebrate samples should be collected with a triangular mesh dip net. The two types of sampling methods to be used are kick and sweep sampling. Kick sampling is primarily used for sampling bed habitats and sweep sampling is used for sampling edge habitats. An overall distance of 10 m should be sampled in each habitat (bed and edge) covering a variety of velocities and stream features. Figure 6-4 shows where kick sampling and sweep sampling should occur at a site.

Sweep sampling

This method should be used for sampling edge habitats with vegetation overhanging from the stream bank, aquatic plant (macrophyte) beds, undercut banks, root mats, leaf packs, woody debris and other stream features that provide suitable living places for macro-invertebrates.

Note: Macrophytes (aquatic plants) are not sampled as part of the edge habitat under the Queensland AUSRIVAS protocols (Conrick & Cockayne 2001). This is because macrophytes are naturally unevenly distributed throughout Queensland, and samples including macrophytes cannot be compared to sites without them.

The following steps are recommended in order to gain the truest representation of the macro-

invertebrate community at a site when sampling in stream edge habitats:

1. Insert the net among the key features of the habitat (such as overhanging vegetation) and use a short, upward-sweeping movement at right angles to the bank. Stir up the bottom while doing so, ensuring that bottom-dwelling animals are suspended and then caught when sweeping through the cloud of suspended material. Continue this procedure along 10 m of the habitat to sample the full range of stream features.
2. Stop regularly to rinse mud and fine silt out of your net. The sample should be free of sediment prior to sorting. An easy way to rinse your net without losing your sample is to wash water into the side of your net while you are holding it with the mouth of the net pointing upwards above the water.
3. Once finished, scoop the net out of the water in a forward motion to prevent fauna from escaping. Flush the net with water to remove any remaining sediment prior to sorting.
4. Empty the contents of the net gently into about 2 cm of clear stream water in a white sorting tray. Rinse down the sides of your net into the tray to ensure that you transfer the full sample into the tray. Pick off any stray macro-invertebrates still clinging to the net using a pair of tweezers.



Kick sampling

This method is designed for sampling stream bed habitats and can be used to sample a range of depths and flows. It is performed most effectively in riffles (fast-flowing rocky sections of the stream bed), where the highest diversity of macro-invertebrates is generally found. However, kick sampling is also an effective method for sampling silt/sand beds and rock/gravel beds in slightly slower-flowing sections of the stream. All types of bed habitats present at a site should be sampled using the kick method.

The following steps are recommended in order to gain the truest representation of a macro-invertebrate community at a site when sampling in stream bed habitats:

1. Approach the sampling area from downstream to prevent disturbing fauna prior to collecting.
2. Holding the net downstream with its mouth facing the sampling area upstream, disturb the stream bed by digging your foot underneath the stones and turning them over. Macro-invertebrates will become suspended in water and will be pushed into the net by the flow of the stream. If there is little or no flow, you will have to use a short sweeping action with the net while stirring up the bed.
3. If you encounter large rocks, place them in the net by hand and rub off fauna any into the net before placing the rock back on the stream bed.
4. Stop regularly to rinse mud and fine silt out of the net.
5. Repeat these procedures over a distance of 10 m, aiming to sample the full range of flow velocities and key features of the bed habitat.
6. Once finished, scoop the net out of the water in a forward motion to prevent fauna from escaping. Flush the net with water to remove any remaining sediment prior to sorting.
7. Empty contents of the net into 2 cm of clean water in the white sorting tray.
8. Before taking another sample, rinse the net so that all fauna and debris are removed.

Sorting your sample

You should sort your sample in the field to enable excess fauna to be returned to the waterway and to minimise time spent sorting through the sample in the laboratory later. Keep samples from each habitat type (edge and bed) in separate sorting trays and then continue to treat them separately.

This enables comparisons to be made between the same types of habitat at different sites.

To sort your sample, the following procedure should be followed:

1. Half-fill a sample jar with 70% methylated spirits.
2. Write a label on the lid of your sample jar using a permanent marker. This should include the site name, site code, date, name of the person who collected the sample, and the type of sample (kick/bed or sweep/edge). Place a label with the same information inside the sample jar (a handwritten label using a pencil and normal paper will be fine in methylated spirits solution).
3. Pick macro-invertebrates out of the sample tray using tweezers or a pipette and place them in a sample jar. Aim to collect about 200 individuals with at least one of every type of macro-invertebrate family present. Try to collect up 10 individuals of each type. However, for non-biting midge (Chironomidae) larvae, collect at least 30 individuals to ensure adequate representation of the subfamilies (these are a special group that need to be identified to subfamily level later on). If you are unsure of the identity of a particular individual, collect it anyway. The following process for picking should be followed:
 - a. In the first 5 minutes, pick all the common and abundant taxa. After that, the major picking effort should be directed at finding the less common and more cryptic taxa. These can be found by checking clumps of algae and detritus for hard-to-see macro-invertebrates and looking for chironomids that live attached to mayfly nymphs and dragonfly larvae. After 10 minutes, no more taxa should be picked unless it is suspected that a particularly common specimen is actually more than one family.
 - b. If you get 200 animals, then stop at the end of 30 minutes. If, at the end of 30 minutes, 200 fauna have not been sorted, continue for a further 10 minutes. If any new taxa are found in that 10 minutes, extend the picking time for a further 10 minutes. Continue this procedure until either no new taxa are found, 200 animals have been collected or 60 minutes have been spent on picking.
4. Record the total picking time on your field record sheet.



Before you leave the field site

1. Return leftover fauna to the stream and wash down trays.
2. Rinse nets thoroughly in the stream and check that they are clean.
3. Top up sample jars with more methylated spirits if required.
4. Fill out all sections on the field sheet relating to macro-invertebrate sampling activities.
5. Check that all other sections of field sheets are complete.

Processing your sample

Take your samples to a laboratory that has microscope facilities. All specimens need to be identified to family level with the exception of sponges (Porifera), nematodes (Nematoda), ribbon worms (Nemertea), Gordian or horsehair worms (Nematomorpha), freshwater worms (Oligochaeta), mites (Acarina), wheel animalcules (Rotifera), springtails (Collembola), and microcrustacea (Ostracoda, Copepoda, Cladocera).

Use the Method 3 laboratory sheet as a guide to the level of identification required for each specimen. For a list of recommended macro-invertebrate identification resources, refer to page 6–28. Another means of identifying your samples is to build a macro-invertebrate reference collection over time. To build a reference collection, place up to three archetypal specimens (good representatives) of each taxon in a vial. Add a label that records the type of macro-invertebrate plus the date and site of collection. Have your reference collection identified by an aquatic ecologist to ensure accurate identification. Sampled specimens can be compared to those in the reference collection for identification.

To process your sample and identify your specimens, the best procedure is:

1. Empty the contents of your sample jar into a Petri dish.
2. Remove macro-invertebrates systematically from the Petri dish and place them immediately into water-filled Petri dishes, or vials, arranged by taxonomic order.
3. Once all macro-invertebrates have been removed from the sample, identify each taxonomic order one at a time. This is done most effectively by having all specimens belonging to a single order in one Petri dish.

4. Once a specimen has been identified, place it in a labelled, alcohol-filled storage vial.
5. Record each family present in your sample on the Method 3 laboratory sheet.

If you are uncertain about the identity of a specimen, obtain a second opinion from a more experienced colleague or an aquatic ecologist. Once a specimen has been identified, record it on the laboratory sheet. After all specimens have been identified and recorded, calculate the total for each family. Place all vials in an evaporation-proof container, checking that they are well-sealed, and store the container in a suitable location. Transfer this data to an electronic spreadsheet or database, and have the database cross-checked against the data sheets to ensure accuracy. Ensure that this data is securely stored and backed up.

Calculating results and indexes

Identifying macro-invertebrates to family level enables you to calculate a number of indexes. The following sections explain what each index measures and how it is calculated.

Note: The term family is used, even though some taxa are only identified to order level, while others are identified to subfamily level.

Number of families (richness)

Richness is calculated by counting the number of macro-invertebrate families present at a site. The total number of taxa (identified to the stated level) present at the site is the richness score for the site.

SIGNAL 2 score—family

Each family has been given a grade from 1 to 10, with lower grades indicating high levels of pollution tolerance, and high grades indicating low levels of pollution tolerance. A complete list of SIGNAL 2 scores for Australian macro-invertebrates has been provided on the Method 3 macro-invertebrate record sheet. The SIGNAL 2 score is calculated by averaging the pollution sensitivity grade numbers of the macro-invertebrate groups (generally family) present at the site.



Note: Not all macro-invertebrate taxa potentially found in Queensland have been given a SIGNAL 2 grade. For example, rotifers, two families of dragonflies and damselflies, and a family each of snail, hemipteran, and caddisfly found in Queensland have no SIGNAL 2 grade.

PET richness

PET richness is a measure of stream condition that focuses on three ecologically sensitive orders of aquatic insects. They are the orders of Plecoptera (stoneflies), Ephemeroptera (mayflies) and Trichoptera (caddisflies). PET richness is the sum of the number of families belonging to these three orders present at your site.

Interpreting results

Number of families (richness)

Totalling the number of macro-invertebrate families present at a site can provide a reasonable representation of the health of a waterway, with healthy waterways generally containing more families than polluted waterways. Be sure not to count the same family twice if it occurs in both the edge and bed samples.

SIGNAL score—family

Calculating a SIGNAL 2 score is an effective method of analysing stream health at your site. The higher the value of the SIGNAL score, the healthier the condition of the site (refer to Table 6-4).

Table 6-4 Interpretation of SIGNAL 2 scores (adapted from Gooderham & Tsyrlin 2002)

SIGNAL 2 score	Habitat quality
Greater than 6	Healthy habitat
Between 5 and 6	Mild pollution
Between 4 and 5	Moderate pollution
Less than 4	Severe pollution

PET richness

In-stream presence of macro-invertebrates belonging to PET orders is generally considered to indicate healthy conditions. High PET scores indicate high levels of aquatic health, while low PET scores indicate poor stream conditions.

Data interpretation should always account for the local context, as scores could be naturally low. This could be done through one of two approaches. In one approach, comparisons are made between your data and any available local, regional or state guidelines. Use the smallest scale guidelines available for your waterway. South East Queensland regional biological guidelines have been established for richness, PET and SIGNAL 2 scores in the *Queensland water quality guidelines* (Environmental Protection Agency 2006). These guideline values are for slightly to moderately disturbed waterways in South East Queensland only (see Table 6-5). It is likely that future versions of the guidelines will include biological index values for all regions of the state.

Table 6-5 Biological monitoring values for South East Queensland (developed and used by EHMP)

Indicator	Water type			
	Wallum and north coastal	Lowland	Upland	South coastal
Richness	12	17	18	6
PET taxa	2	3	4	0
SIGNAL 2 score	3.32	3.5	4.2	3.5



The second approach is to compare data to a range of values from a number of reference sites for that year only. This method takes into account natural influences on macro-invertebrate communities that may have occurred, such as droughts and floods. An example of a biological monitoring program that applies this approach is the Queensland Ambient Biological Monitoring and Assessment Program (ABMAP).

However, using either of these two approaches will only enable interpretation of data for that site. For advice on how to interpret data as part of a wider study, such as restoration assessment, refer to Chapter 8 of this manual.

The combination of richness, PET and SIGNAL 2 scores (at family level) can help you develop a thorough understanding of waterway health. Data from Method 3 can be used not only to increase awareness, identify current trends and monitor trends through time, but also to assess the impact of a land use or a pollution source and assess the effectiveness of a management strategy.

Linking biological assessment to other waterway monitoring is strongly recommended, to increase the weight of evidence to support your conclusions. This may consist of combining biological indicators with physico-chemical tests or habitat assessment data, particularly if you wish to utilise the AUSRIVAS models.



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