# WATERWATCH

Environmental leader guide to running an ALT macroinvertebrate survey

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#### Introduction

Aquatic macroinvertebrates (or waterbugs) are animals without a backbone that are visible to the naked eye. They are a very important part of waterway ecosystems and have varying tolerances to pollution so they can be used as a biological indicator of waterway health.

Monitoring macroinvertebrates in your local area in a systematic way is an excellent hands-on activity for school-age groups such as environment clubs or Scout groups. Participants can track the health of waterways across different sampling sites and monitor changes over time.

This guide is designed to be used by groups of school-age children such as school environment clubs, STEM (Science, Technology, Engineering and Mathematics) clubs, after school programs and community groups such as Scouts.

#### Macroinvertebrates

Macroinvertebrates are a diverse group of insects, worms, molluscs, crustaceans and other invertebrates. Depending on the species, they live in different parts of a waterway such as the water surface, in aquatic vegetation or in the sediment on the bottom of the waterway. You can find them living in ponds, wetlands, creeks and streams. How many and what you find will be related to both the available habitat and overall health of the waterway.

There is plenty of food available in freshwater environments and macroinvertebrates have evolved many different feeding strategies to survive. Within this group of animals there are predators, herbivores, algae grazers, filter feeders and many animals that have more than one feeding style. This makes them a fascinating group to observe and study. The different habitat needs of these animals means they can be impacted by a loss of plants and reduction in water quality from issues such as erosion. The diversity (number of species) and abundance of macroinvertebrates in a waterway can be improved by revegetation and managing the quality of the stormwater that flows into the waterway.

#### Sensitivity and SIGNAL scores

There is likely to be some kind of macroinvertebrate in almost any aquatic environment, but healthy waterways tend to have more macroinvertebrates of different types than polluted waterways. Note that some environments such as still or slow flowing waters will naturally have lower scores, due to the available physical habitat and changes in water quality.

Because different types of macroinvertebrates vary in their tolerance to changes in their environment (e.g. temperature, pH and pollutant levels) we can use them to as a biological indicator of water quality. To do this, we use the SIGNAL (Stream Invertebrate Grade Number - Average Level<sup>1</sup>) scores of macroinvertebrates. This is a simple biotic index that indicates its level of tolerance to pollution. Macroinvertebrates with high SIGNAL scores are very sensitive to changes in their environment, while those with low signal scores are very tolerant of environmental change. Consequently, an abundance of different macroinvertebrates, including those with high and low SIGNAL scores indicates that the waterway ecosystem is healthy.

<sup>&</sup>lt;sup>1</sup> Chessman BC 2003, 'New sensitivity grades for Australian river macroinvertebrates', *Marine and Freshwater Research*, vol. 54, pp.95-103.



#### Agreed Level Taxonomy method

This guide explains how to conduct a rigorous macroinvertebrate survey using the Agreed Level Taxonomy (ALT) method. This methodology was developed by the Waterbug Company and detailed information about it can be found at <<u>www.thewaterbug.net/ALT.html</u>>.

The following videos provide an accessible introduction to conducting a macroinvertebrate monitoring survey.

#### The Waterbug Census video

<<u>www.youtube.com/watch?time\_continue=3&v=uaRsl4AdA7E</u>> [2:24] is a simple overview of macroinvertebrate monitoring. It explains that waterbugs can be used as biological indicators of the health of a river. They are an important part of the food chain for larger animals that live in the waterway such as platypus. It shows how a sample is collected from different areas of a site, how the sample is sorted and the waterbugs identified using poster charts and a key. A datasheet is used to record data and calculate a sensitivity rating for the waterbugs.

A guide to the Waterbug Census for citizen scientists <<u>www.youtube.com/watch?v=-</u> <u>I2Iyd1SntA></u> [5:17] provides a more detailed introduction to the ALT method of sampling. The video describes how the ALT sampling method is used for the Waterwatch Waterbug Census programs. It includes a habitat assessment, sweep and kick sampling and sorting techniques. The macroinvertebrates in a sample can be identified with ALT keys and recorded on a data sheet which is used to calculate a score to assess the health of the waterway.

The ALT method<sup>2</sup> uses macroinvertebrates to assess river or wetland health. The term 'Agreed Levels' describes the fact that the taxonomic levels to which macroinvertebrates are identified has been agreed upon by the people using the method. Animals are identified based on their features and the way they move while alive, rather than being preserved. This also means they can be returned to the waterway after the data has been collected. ALT identifications result in data sets of mixed taxonomic levels, some at genus or species, and others at higher levels.

This approach contrasts with most existing methods that restrict identifications to the taxonomic level of family (see Figure 1). In many cases, ALT will be at coarser taxonomic resolution than family level; however, in some cases, people will recognise distinctive species or genera, thus refining the taxonomic resolution (see Figure 2).

<sup>&</sup>lt;sup>2</sup> The Waterbug Company 2010, *The ALT method*, <<u>www.thewaterbug.net/attachments/ALT\_methods\_v1.2.pdf</u>>. Accessed 25 April 2019.

		Taxonomic Level	1	2	
A star MA	KINGDOM	Animalia	Animalia		
		PHYLUM	Arthropoda	Arthropoda	
	CLASS	Insecta	Insecta		
	ORDER	Coleoptera	Coleoptera		
		FAMILY	Elmidae	Dytiscidae	
		genus	Notriolus	Onychohydrus	
1	2	species	Notriolus	Onychohydrus	
	۷. ۲		quadriplagiatus	scutellaris	

**Figure 1**: The elmid beetle (1) and the diving beetle (2) can both be described using different levels of taxonomy. Notice how they share the same Kingdom, Phylum, Class and Order but diverge at the level of Family. (Figure courtesy of The Waterbug Company)



**Figure 2:** An example where resolution would be refined to genus level. The leafy water scorpion Laccotrephes (left) is readily distinguished from the slender water scorpion Ranatra (right). At family level both animals would be listed as Nepidae and this distinction would be lost. (Images courtesy of the The Waterbug Company)

The benefits of the ALT method are that:

- identification can be completed in the field without microscopes. An ALT survey is likely to take two hours in the field and can provide you with an assessment of the health of the site in terms of macroinvertebrate populations.
- macroinvertebrates are kept alive. Colour and movement can assist with the identification of live animals. Existing taxonomic keys have been modified for ALT to include these features. This method is designed to be rigorous without the need to preserve specimens.

• data can be equivalent to SIGNAL 2 scores. This means that data can be compared between different sites and over time. Longitudinal data collection can used to track changes in waterway health over time.

#### Waterwatch Waterbug Census

Across Melbourne, the Waterwatch program works with the community to collect information on macroinvertebrates, to improve the understanding of waterway health and contribute to ongoing research. Consider attending a Waterbug Census event or training day to gain field based experience in macroinvertebrate sampling and identification using ALT methods.

Register your interest to be notified when sessions are planned in your area by emailing <u>waterwatch@melbournewater.com.au</u>.

Training days usually cover the following:

- Identifying a suitable site and habitats for sampling
- Safely collecting a sample of macroinvertebrates from a waterway
- Using equipment to sort animals into taxa groups for identification
- Identifying and recording the macroinvertebrate sample using ALT methodology and tools.

#### Before you start

The ALT method assumes that the sample will be processed in the field so it is sensitive to weather conditions. Rain or a lack of light have the potential to cause discomfort and may affect your ability to make accurate identifications. If field conditions are poor, sample on another day or take the sample indoors to process (and return the animals to the same site afterwards).

### Do not return animals to a different site as this may spread disease or pest species (Refer to Appendix 1 Waterway hygiene).

#### Equipment

#### Sampling

- a fine-meshed sampling net on a pole
- a bucket
- waders or gumboots if the river or wetland is shallow

#### Picking

- 2-3 white trays; roughly 30cm x 50cm and 10cm deep
- small clear vials (specimen jars will do remove the label so you can see in)
- 1-2 ice cube trays (per tray)
- plastic spoons
- plastic pipettes
- 4 X magnifying glass (or better)
- small LED torch

### ALT Identification keys and guides

These identification tools are available for ALT sampling:

ALT higher level key <<u>www.thewaterbug.net/attachments/ALT highkey little.pdf</u>>. This dichotomous key is used to identify distinct taxa or to key out macroinvertebrates to their taxonomic Order level. Once the taxa is identified on the chart:

- Animals in a red box are not keyed any further and can be recorded on the data sheet
- Animals in a blue box need to then be keyed out using the ALT keys v1.5 to identify the animals to the agreed taxonomic level.

The chart can also be used as a standalone identification tool by upper primary or middle years-aged students to classify animals primarily to Order level.

ALT keys v1.5 <<u>thewaterbug.net/attachments/ALT KEYS v1.5 withorderkeyback.pdf</u>>. This key is used with the ALT higher level key to enable participants to key out macroinvertebrates to their agreed level. It is suitable for interested extension middle years' students and for senior secondary students.

<u>The waterbug app</u> (Apple or Android). <u>The waterbug app</u> is a very useful tool for identifying and uploading waterbug data. Students can identify waterbugs using a dichotomous key with engaging cartoon diagrams to explain structural features. It is the app version of the ALT dichotomous keys used above and is the identification tool of choice for the National Waterbug Blitz <u>www.waterbugblitz.org.au/</u>, a country-wide citizen science initiative which surveys the waterbugs in local waterways in spring each year.

#### Habitat assessment

ALT habitat sheet <<u>www.thewaterbug.net/attachments/ALT\_HABITAT\_SHEET\_v1.5.pdf</u>> This data sheet describes the range of potential macroinvertebrate habitats and the weather conditions for sampling.

#### **Recording ALT data**

ALT bug sheet <<u>thewaterbug.net/attachments/ALT\_BUG\_SHEET\_v1.5.pdf></u> This data sheet is used to record the types of macroinvertebrates found and their abundance in the sample.

#### Additional resources

A beginners guide to waterbug identification, Melbourne Water <<u>www.melbournewater.com.au/media/117/download</u>> This guide assists students to identify the more common waterbugs found in the Melbourne area. It also provides information about the taxonomy of the waterbugs, their anatomy, distribution, diet and sensitivity to pollution.

*Teacher guide: How to run a waterbug session with students*, Melbourne Water <<u>https://www.melbournewater.com.au/media/8431/download</u>> This introductory guide is designed to assist teachers to conduct effective waterbug monitoring sessions in which students act as scientists to identify and pose questions, gather and analyse data and draw conclusions to make evidence-based arguments about the health of a waterway. It also provides instructions for teachers collecting a waterbug sample for classroom use.

### Safety

#### Your safety is of the highest importance!

- Never sample alone
- Always check the weather forecast before going out to monitor
- Always wear waders correctly
- Never enter the water if you feel unsafe at the site.

Make sure that you fully comply with your organisation's risk assessment and safety procedures.

#### **Extreme weather**

Always check the forecast before going out to monitor. As a guideline, consider the following:

Weather event	Monitoring consideration
Temperature	30°C-35 °C: Consider alternative arrangements 35 °C +: Cancel event
Fire	<b>Low – Very High rating:</b> take into account bushfire risk. If in doubt modify/postpone/cancel.
	<b>Severe rating:</b> Avoid going into high risk areas. If in doubt modify/postpone/cancel.
	Extreme and Code Red rating: Cancel event.
Wind	Greater than 50km/hour: Cancel event.
Rainfall	Noticeable change in waterway conditions: Cancel e.

#### Table 1: Extreme weather considerations

#### Habitat assessment

When you arrive onsite, complete the *ALT habitat sheet*.

#### Sampling for waterbugs

#### Never sample alone!

Most of the information in a sample of macroinvertebrates comes from the diversity of animals present. This information is solidly linked to the types of sub-habitats that the sample was taken from. Habitats are often diverse,

as shown in Table 2, so it is important that you sample broadly across the sub-habitats, to collect a diverse range of animals.

The habitat assessment sheet can help you to break down your habitats, so that you are collecting a representative sample from the habitats present. A **riffle** is a shallow part of a waterway that is usually pebbly or rocky.

Habitat type	Description	Benefits
Leaf packs	Collections of leaves and bark that become lodged behind rocks in the stream and slowly build up.	Provide an important food source/habitat in faster flowing rivers. Tend to become diverse habitats.
Rocks	Pebbles and boulders.	Rocks create different flow conditions which can be beneficial for different bugs that prefer to live within a riffle.
Aquatic vegetation	Vegetation growing in the water.	Often the habitat that will yield the most bugs. Plant eating bugs will live in this area, as it provides food and good protection.
Woody debris	Dead logs and wood that are in-stream.	Provides habitat for bugs that breakdown dead organic material. Can be overlooked, but provides important habitat.
Sand and depositional areas	In still areas of the creek where sediment has washed in and settled.	These can be small habitats within flowing systems that will be home to very different bugs to those living within the riffle area.
Sediment	Usually the floor of the creek, often a limited habitat in fast flowing areas.	Bugs living in this habitat, such as worms, will most likely not be found in other sub-habitats in the same area.

#### Table 2: Habitat types and their benefits

### Sampling method for both running systems and wetlands

- 1. Complete a habitat assessment (*ALT habitat sheet*) for the site.
- 2. Sample at site for 10 minutes.
- 3. Sample all habitats present, allocating equivalent time to percentage covered as listed in your habitat sheet. For example, if aquatic vegetation (water plants) cover is 20%, sample the aquatic vegetation for 2 minutes.
- 4. Collect your sample in a bucket with water and remove from site for identification.

### Sampling a river or creek

#### The habitats described in

Table below are all typical of fast flowing water. Further habitats become available when you turn your attention to slower flowing parts of the river, such as the pools, or the edge. Deep waters are unsafe to sample, so these shouldn't be attempted, but edge waters, where vegetation exists in slower moving waters offer a potentially different faunal assemblage to the fast flowing riffles.

Sub-habitat description	Likely fauna examples
Coarse substrate	Predatory stoneflies, dragonflies, crayfish, net- spinning caddisfly
Gravels	Predatory maggots, worms, chironomids
Depositional areas	Vulture caddisfly, other caddisflies
Leaf packs	Amphipods, some caddisflies
Wood	Shrimp, elmids, some chironomids
Plants	Amphipods, damselflies

#### Table 3: Riverine sub habitats and some common fauna found there

The recommended sampling method for rivers is a 10 metre combined sample covering as many habitats as possible. As a rough guide, spend about a minute on every metre you sample. It does not need to be a continuous stretch of stream.

Nets with a very fine mesh should be emptied into the net into a tray when it fills. This will prevent reversion (the sample being washed from the net because it has become clogged).

#### Sampling a wetland

Wetlands can be just as diverse as rivers. The wetland sub-habitats are outlined in Table 4. A similar approach is recommended, combining samples from a number of identifiable sub-habitats.

Wetlands produce large amounts of organic matter, so a 5 metre sample will produce enough organics to sort through.

Sub-habitat description	Likely fauna examples
Aquatic vegetation	Different types of water weed can host different invertebrates
Edge vegetation	Damselflies, stick caddisflies
Open water	Backswimmers, mites
Depositional areas	Worms, chironomids
Wood	Large dragonflies, shrimp, some caddisflies

#### Table 4: Wetland sub-habitats and some fauna commonly found in them.

### ALT picking method

- 1. **Prepare your sample** Make sure it is rinsed in the net, so that you are not bringing in too much sediment and try to avoid too much organic material.
- 2. **Split your sample across trays** so that the whole sample can all be viewed.
- 3. For 30 minutes pick out as many different animals as you can find During this time, focus on diversity and don't identify anything as you go. Put macroinvertebrates that look the same in the same cell of the ice cube tray.
- 4. **If you find something new in the last 5 minutes** add another five minutes to your picking time. Repeat this until you haven't found anything new in your sample.
- 5. **Once picking is completed** move on to the identification process.

The main aim of live picking is to pick a diversity of animals. To help you with this, the data sheet is laid out with all the most likely fauna named, so you can browse this to check if you are missing things. The overall process of picking is to sort samples from the sorting tray into the ice cube trays, putting similar animals in the same cells of the ice cube trays.

Principles for picking:

- DIVERSITY! Look for it.
- If there are lots of something, pick at least half a dozen examples (maybe fill a couple of cells).
- If there are large and small versions of something get both and put them in separate cells. They might be different things.
- Grab little things with the pipette, it's quicker.

### ALT identifying method

Start with one cell of an ice cube tray. Look at a large example of your animal and identify it using the ALT keys. If the animal is particularly fast moving, try stranding it by only giving it a drop of water to move in.

If necessary transfer the animal to a clear vial so you can observe it from the side. You may need to use a hand lens to see some features. These will be marked in the keys with the magnifying glass symbol. A small torch can also help to see finer details.

If you have multiple animals in one ice cube tray cell, have a look at more than one animal to make sure that they all show the same characteristics that allowed you to identify the first one. If they don't you will need to separate them out to different cells and then identify them separately. Check at least 4 or 5 animals in each cell to make sure that they are the same.

Once you are happy with your identification, find it on the *ALT bug sheet* and tick one of the boxes next to it. The groupings for the abundance of the animals found are:

- 1-2 animals
- 3-5 animals

- 6-10 animals
- 11-20 animals
- more than 20 animals.

Work in pairs and check that you agree on the identification. If possible, pair inexperienced people with experienced people to enable knowledge transfer.

Write notes on things you think might be important, such as two obviously different things that key out as the same thing. The last column of the data sheet is set aside for this sort of information.

Once you have finished and are ready to put the sample back, tip out all the organics, and check one last time for small animals in the sand and gravel (some caddis have sand cases and can be panned for – a bit like gold). Finally check there are no leeches or small snails stuck to your tray.

The Waterbug app can be used to identify and upload waterbug data (see Equipment).

### Interpreting ALT data

There are two parts to your ALT data collection: habitat diversity and biotic (or faunal) diversity. Keeping records of the changing habitat at your site can assist you with tracking the health of your site and its associated macroinvertebrates. This allows the data to be interpreted and likely impacts such as water quality or changes to the site can be diagnosed. It should be noted that some sites are naturally physically homogenous. This needs to be acknowledged as it will often also be reflected in the faunal diversity. Some sites may score consistently low when compared with more habitat-rich locations. However the ALT method will (hopefully) provide enough resolution in the data to assess changes at these sites over time and to diagnose trends in the habitat diversity and associated fauna.

### Calculating the SIGNAL score for your site

A SIGNAL score for your site can be calculated from the number of taxa collected and their SIGNAL scores. The formula for calculating the SIGNAL score of your site is:

SIGNAL SITE SCORE = (Sum of SIGNAL SCORE x WEIGHT FACTOR) / TOTAL OF WEIGHT FACTOR

On your *ALT bug sheet*, the number of collected taxa are grouped. These groupings are for weighting factors to calculate your SIGNAL score, as shown in Table 5.

### Table 5: Weight table

Number of specimens	1-2	3-5	6-10	11-20	>20	
Weight factor	1	2	3	4	5	

The following example shows how to calculate the site SIGNAL score from the data sheet (Table 6).

### Table 6: Example data sheet

	SIGNAL	raw					
ALT Identification	score	count	1-2	3-5	6-10	11-20	>20
ALT Animals without keys							
Phylum Turbellaria (flatworms)	1	10			$\checkmark$		
Molluscs							
Family Physidae, Species Phsa acuta	1	25					$\checkmark$
True fly larvae							
Family Chironomidae (chironomids)	3	40					$\checkmark$
True bugs							
Family Corixidae, Genus Micronecta	2	90					$\checkmark$
Damselfly & dragonfly							
Families Lestidae and Family							
Coenagrionidae	2	5		$\checkmark$			
Caddisfly							
Family Leptoceridae, Genus Notalina	6	15				$\checkmark$	

Then calculate the weight factors for each of your taxa (type of animal) present and calculate the SIGNAL x weight factor (Table 7):

### Table 7: Example weight factor calculations

	SIGNAL	Weight	SIGNAL x
ALT Identification	score	factor	weight factor
ALT Animals without keys			
Phylum Turbellaria (flatworms)	1	3	3
Molluscs			
Family Physidae, Species Phsa acuta	1	5	5
True fly larvae			
Family Chironomidae (chironomids)	3	5	15
True bugs			
Family Corixidae, Genus Micronecta	2	5	10
Damselfly & dragonfly			
Families Lestidae and Family Coenagrionidae	2	2	4
Caddisfly			
Family Leptoceridae, Genus Notalina	6	4	24
TOTAL		24	61

Finally calculate the site score:

SIGNAL SITE SCORE = (TOTAL OF SIGNAL SCORE x WEIGHT FACTOR) / TOTAL OF WEIGHT FACTOR = 61/24 = 2.54

The score for this site is **2.54** 

#### **Other resources**

There are other resources that can assist you with your bug identification or citizen science in general.

**Waterbug Face:** A Facebook group run by the Waterbug Company, where you can post pictures of unknown bugs for identification.

You can contact the Melbourne Water Waterwatch team for assistance and advice at <u>Waterwatch@melbournewater.com.au</u>

### Appendix 1 Waterway hygiene<sup>3</sup>

Currently in Australia, there are a range of organisms that threaten the ecology of our rivers, lakes and wetlands. They range in size from vertebrates such as cane toads and mosquito fish to invasive algal species, water based fungi and bacteria. While it is easy to demonise these species, the main reason they are a problem is people. Waterwatchers have the potential to make these issues worse simply because we travel between sites. The simple act of returning a tray of bugs to a different wetland (so the bugs don't die) can be responsible for the spread of any of the invasive species above.

Avoid transporting organic material (animals, plants or soil) between wetlands or rivers (or even between different sites along the same river). Many of the invasive invertebrate species we have in Australia are linked with the transport of aquatic plants. For example, Canadian waterweed, which is a widespread invasive plant throughout south-eastern Australia, often harbours eggs or immature animals which are then introduced to new habitats when the weed is moved (thus introducing both foreign fauna and flora to the waterway). This demonstrates one of the more common ways that pest organisms are distributed.

It is fairly easy to avoid transporting larger organisms between locations. Fish, tadpoles, macroinvertebrates and aquatic plants are large enough that you can check visually that they aren't stuck in nets. Make sure larger organisms aren't getting a free ride to the next site.

However, stopping algae, fungi and bacteria requires a lot more vigilance. Any nets or field equipment that come into contact with water, mud or soil have the potential to transport infection.

There are a number of ways of sterilising equipment between sites that vary in their simplicity and effectiveness:

**Air drying**: The simplest method is drying the equipment. This is a good option over summer if you are only visiting a single location each day and is good housekeeping at the end of any sampling trip. Unfortunately, you must be sure that all the equipment is totally dry. The whole process is useless if there is a patch of net that doesn't dry or if the waders have a soggy notebook in the front pocket.

**Methanol/ethanol**: Spraying equipment with ethanol (or methylated spirits) can be cheap, effective and quick, so you can do it between sites. Equipment needs to be fairly dry to ensure that the ethanol or methanol doesn't become too diluted to work. Ethanol or methanol is a flammable liquid and should be kept away from open flame. It is also a mild solvent, so you need to be careful with it around some plastics and it will make marker pen rub off. The appropriate Material Safety Data Sheets (MSDS) should be complied with and stored with it for access in an emergency.

**Nappy cleaner/salt/antiseptic/bleach**: If you have access to a sink or bath between trips, another simple method is to clean your gear with a strong solution (5%) of nappy cleaner, salt or antiseptic, or 2% bleach solution. This method works best if you have the

<sup>&</sup>lt;sup>3</sup> The Waterbug Company 2010, *The ALT method*, pages 11-14

<sup>&</sup>lt;www.thewaterbug.net/attachments/ALT\_methods\_v1.2.pdf>

time to soak the equipment. Once it is clean, rinse off the detergent so you don't end up killing any bugs in your next wetland.

**Hot water**: Soaking items in hot water (above 45°C) for 40 minutes will also kill most problem organisms and can be useful if you have equipment you don't want to bleach or spray with methylated spirits.

For further information refer to the AQIS Aquavetplan: Operational procedures manual – Decontamination (2008) at <<u>www.agriculture.gov.au/SiteCollectionDocuments/animal-plant/aquatic/aquavetplan/decontamination-manual.docx</u>>.