

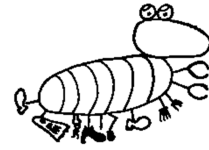
The ALT Method

(Agreed Level Taxonomy)



Prepared by [The Waterbug Company](#)

for DSE Victoria



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Cover photo: Juvenile *Ranatra* in a bucket

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Introduction

The ALT (Agreed Level Taxonomy) method uses invertebrates to assess river or wetland health. The "Agreed Levels" referred to in the name describe the fact that the taxonomic levels to which invertebrates are identified are agreed upon by the people using the method. This contrasts with most existing methods that restrict identifications to the taxonomic level of family (for example, 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.).



 1	 2	Taxonomic Level	1	2
		KINGDOM	Animalia	Animalia
		PHYLUM	Arthropoda	Arthropoda
		CLASS	Insecta	Insecta
		ORDER	Coleoptera	Coleoptera
		FAMILY	Elmidae	Dytiscidae
		genus	<i>Notriolus</i>	<i>Onychohydrus</i>
		species	<i>Notriolus quadriplagiatus</i>	<i>Onychohydrus scutellaris</i>

Figure 1. Taxonomic levels. 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 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.

ALT identifications can be made reliably in the field using live specimens and without a microscope. The use of live specimens means that a suite of live characteristics such as colour and movement can be used in the identification process. This alters the way animals are identified quite profoundly. The following manual draws heavily upon existing taxonomic keys, but has been totally re-crafted to provide identification guides that will reliably help operators to identify live animals in the field.

The ALT method is designed to be carried out almost completely in the field. A site assessment will take slightly over two hours and provide the practitioner with an idea of the health of the site in terms of its invertebrate (waterbug) fauna. A number of extra observational data sets allow further interpretation of the site, and provide a data set that can assist with quality control.

The main driver for this method is an attempt to avoid killing and preserving large numbers of invertebrates. This has been combined with the need for quality ecological data to provide methods that are as rigorous and testable as possible within these constraints.

The ALT method is mainly concerned with the processes for sorting and identifying animals, although this manual also outlines a series of methods for sampling. The data yield from these processes will eventually be interpreted using SIGNAL 2 (Chessman 2003) or an equivalent.

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 bad consider sampling on another day, or taking the sample indoors to process (and bringing the animals back to the site afterwards).

DO NOT RETURN ANIMALS TO A DIFFERENT SITE AS THIS MAY SPREAD DISEASES OR PEST SPECIES (see the section on waterway hygiene - p11).

Equipment

ALT sampling requires a net and waders (or gumboots if the river or wetland is shallow). In addition to this, ALT picking and identifying requires the following list of equipment:

- picking tray (roughly 30cm x 50cm and 10cm deep) 2 or 3 of these allow samples to be split (light coloured kitty litter trays are good)
- small clear vials (specimen jars will do - remove the label so you can see in)
- ALT data sheet
- 3 or 4 'ALTered' ice cube trays
- plastic spoons
- plastic pipettes
- 4 X magnifying glass (or better)
- small LED torch

Sampling in a River

Most of the information in a sample of bugs comes from the diversity of bugs present. This information is solidly linked to the sub-habitats that the sample has been taken from. Leaf packs provide an important food source/habitat in faster flowing rivers. These are collections of leaves and bark that become lodged behind rocks in the stream and slowly build up as they ensnare other passing detritus and become small hotspots for diversity. In a superficially homogenous riffle, different sizes of rock and subtly different hydraulic conditions can favour distinctly different animals. The patchiness of these habitats makes it important to try and sample as broad a range of sub-habitats as possible to make sure you have a good sample of the diversity available at a site.

The ALT data sheet has a list of possible sub-habitats, when sampling you should fill in the data sheet to record how many of these were included in your sample.

Table 1. Riverine sub-habitats and some fauna commonly found in them.

Sub-habitat description	Likely fauna (examples only)
coarse substrate	predatory stoneflies, dragonflies, crayfish, net-spinning caddis
gravels	predatory maggots, worms, chironomids
depositional areas	vulture caddis, other caddis
leaf packs	amphipods, some caddis
wood	shrimp, elmids, some chironomids
plants	amphipods, damselflies

The habitats described in Table 1 above 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 edgewater, where vegetation exists in slower moving waters offer a potentially different faunal assemblage to the fast flowing riffles. The question is whether to keep these samples separate, or to combine them to create a sample that is characteristic of the whole river at the sampling site.

Historically, AUSRIVAS and SIGNAL samples have been separated into either "pool/edges" and "riffles" samples. We would suggest that the time spent processing two different habitats might be better invested in a replicate sample of the combined habitats, to allow some estimate of repeatability and quality control to be incorporated into the sampling. This might even be two sets of people working on the same sample, but not sharing their results until they finish the identification process. This approach is one way of ameliorating the fact that the samples are not kept (and so can't be checked later).

The recommended sampling method for rivers is a 10m combined sample covering as many habitats as possible. As a rough guide, spend about a minute on every metre you sample. Waterwatch nets have an overly fine mesh however, so care should be taken to empty the net into a tray when it fills. This will prevent reversion (the sample being washed from the net because it has become clogged). Reversion can be minimised by coarsening the mesh size to about 1mm, and the resulting samples will be easier to sort through. However, the method for ALT is robust to slight differences in the net used so long as care is taken to sample from the different habitats as outlined above.

Sampling in a wetland

Wetlands can be just as diverse as rivers, so a similar approach is recommended, with samples from a number of identifiable sub-habitats being combined. Wetlands produce large amounts of organic matter however, so a 5m sample will produce enough organics to sort through. Table 2 shows examples of wetland sub-habitats and fauna that are likely to be found in each one.

Table 2. Wetland sub-habitats and some fauna commonly found in them.

Sub-habitat description	Likely fauna (examples only)
aquatic vegetation	different types of water weed can host different invertebrates
edgy vegetation	damselflies, stick caddis
open water	back swimmers, mites,
depositional areas	worms, chironomids
wood	large dragonflies, shrimp, some caddis

ALT Picking Method

The ALT method relies heavily upon your ability to see all of the invertebrates in your sample. Because of this, two things are really important before you start picking:

1. rinse your sample properly - until the water running through it in the net is clear
2. don't put so much stuff in your tray that all the animals can hide from you. If necessary, split the sample across 2 or 3 trays and split your time/attention between them.

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 more commonly forgotten animals will actually have their names written on the ice cube trays as the first reminder of what to start looking for. 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. Do this for 15 to 20 minutes until you have lots of separate filled cells that you recognise as different animals. Now look back over the list on the ALT data sheet and see if there are any groups you might have obviously missed. Continue picking for another 10 -15 minutes.


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.

Keep the sample in your sorting trays while you ID, and then go back for a final 10 minutes once you have ID'd everything and check for things you might have missed.

ALT ID-ing (identifying)

Start with one cell of an ice cube tray. Looking at a large example of your animal, identify it using the ALT keys. Use the size guides on your ice cube tray to help give you an idea of how big the animal is. 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 this 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 4-5 animals in each cell to make sure that they are the same.

Once you are happy with your ID, find it on the ALT data sheet and tick one of the boxes next to it. There are different boxes depending on how abundant the animals are:

- 1 - 5 animals
- 5 - 20 animals
- lots of animals

Work in pairs and check that you agree on the IDs. When possible, pair inexperienced people with experienced people. This allows the knowledge to be passed on effectively, and sidesteps 'traps for young players'.

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 leaches or small snails stuck to your tray.

Helpful tools: Using a ruler and a fine point, waterproof. permanent marker you should draw three graticules (lines of specific length) at one end of each ice cube tray to help you with size classes when you are identifying animals. One should be 5mm, one 10mm and the last 15mm

5mm



10mm



15mm



These will help you when the ALT key uses length as a character.

Interpreting ALT data

Waterwatch sites tend to be in places that community members care about; near farms that they own, public facilities that they use, or industries that they are concerned about. This placement of sites is slightly different to the methods employed by state agencies that set out to cover specific geographic areas, land-use gradients or biogeographical patterns, all of which have known effects on faunal composition. State agencies are usually intending to establish broad-scale patterns across the landscape, so they need the samples at each of the sites that they choose to be as comparable as possible. These needs result in the AUSRIVAS riffle and edge samples; neat, clearly defined habitats that can be identified at multiple sites. In designing the ALT sampling methodology we have surrendered the fixed habitat approach in favour of an attempt to catalogue as much biodiversity as possible in sites that are chosen by the community because they are important. It is entirely possible that this will result in low biodiversity or SIGNAL scores because of a lack of suitable habitats. However, this is assumed to be part of what is being assessed and will be apparent in the ALT data; it is a source of information about the health of a site, rather than a problem with the sampling design.

ALT data sheets record data at two levels; a measure of habitat diversity, and a measure of biotic diversity (calculated alongside a SIGNAL score). This allows the data to be interpreted and likely impacts such as water quality or physically homogenous sites can be diagnosed. It should be noted that some sites are naturally physically homogenous, and that this needs to be acknowledged as it will often also be reflected in the faunal diversity.

Some sites may score consistently low when compared alongside more habitat rich locations. However the ALT method will hopefully provide enough resolution to allow assessment of these sites against themselves over time and diagnosis of trends in the habitat diversity and associated fauna.

QA/QC in ALT

When possible, we suggest that groups have enough people in them to do two replicate samples at a site. The people involved in the sampling can discuss where to take samples, and may even share a large sample split between them. However, for the pair of samples to be useful, there should be minimal exchanges between the groups during the IDing stage, so as not to influence one another's results. These paired samples will eventually give us an error value for the method.

If people are keen, the ALT method will work fine with a voucher collection. These offer a further chance for QC and calibration of users, but are by no means mandatory.

Testing the abilities of operators to identify animals correctly could be done using either of the two following methods:

1. While people are working at a site they preserve representatives of each of the taxa they identify and label them for verification with an expert (either a coordinator or an external authority depending on circumstances). Multiple examples of each taxa would need to be preserved to check for consistency in the ID as well as accuracy.
2. A standard RAP sample (as taken by the EPA) is performed at the site and live picked, preserving all the animals found for comparison with the ALT results.

Testing days

We recommend state coordinated testing days, where multiple people sample the same site and compare their results. This allows calibration between testers, and can also serve as an occasion to network and possibly perform the more formal quality control measures outlined above.

Training and Accreditation

The training of ALT identification tends to need live animals for it to work, so training days will necessarily be held in the field with all the materials to hand, or possibly as intensive workshops (to be discussed). One of the ancillary products of the ALT method is a DVD with footage of some of the more readily identifiable invertebrates. This provides good examples to demonstrate the characters used in ALT ID, but is no substitute for field based training and expert training. Eventually, testing results over time will need to be formalised into some form of accreditation, but this will need to be discussed by the coordinators based on what levels of knowledge they see as useable. This will also need to be assessed in light of the method's ability to detect various impacts to aquatic ecology, something that is being examined in parallel work. It is possible that this work will result in ALT SIGNAL values needing to be re-calculated to maintain sensitivity to impacts, rather than using standard SIGNAL 2 (Chessman 2003) scores.

Waterway Hygiene - combating ecological threats

Currently in Australia, there are a range of unpleasant 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.

It is bad form to transport 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.



Physa acuta (USA)

Mosquito fish

Potamopyrgus

Contagion between sites is more likely for people sampling wetlands than rivers (rivers tend to be linked together while wetlands are often separate from one another), but it is unwise to assume that your work is safe from these problems. Control measures only work if everyone follows them.

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 they aren't getting a free ride to the next site. However, stopping algae, fungi and bacteria requires you to be a lot more vigilant. 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, is drying the equipment out. 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, for drying to work you have to be sure that all of the equipment has dried out totally. 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 meths), can be cheap, effective and quick, so you can do it between sites. Equipment needs to be fairly dry so the ethanol or methanol doesn't become too diluted to work. Ethanol or methanol

is a flammable liquid, so it 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 in 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 time to soak the equipment. Once clean, rinse off the detergent, so you don't end up killing off 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 meths.

(see also AQIS import case details "Fresh Water Articles and Equipment - Used" at www.aqis.gov.au)

Home ponds and classroom aquariums: The most important rule with these is to always return the animals to the place where you found them. If it is too far away, and you aren't going to be able to bring them back, then don't take them in the first place.

