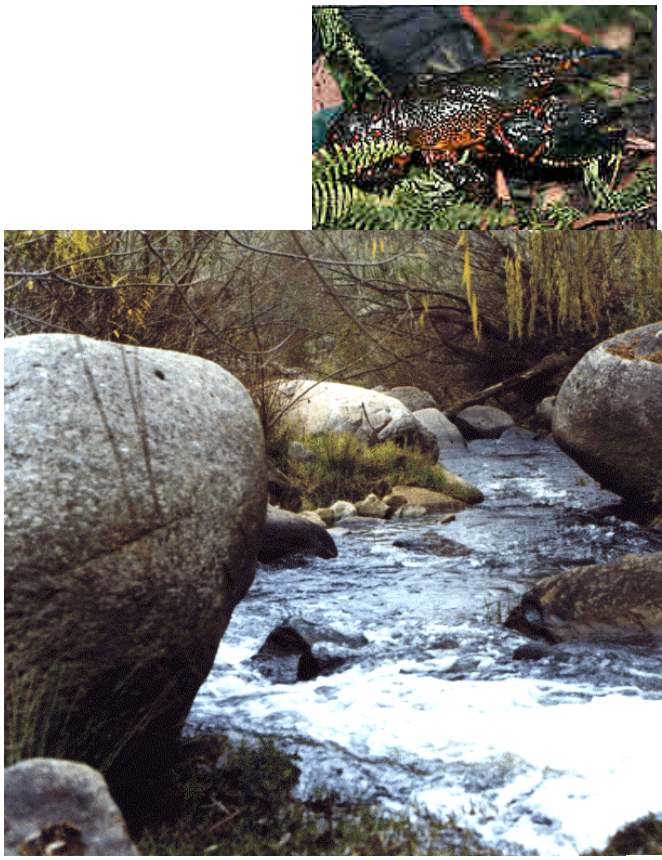
SIGNAL 2

A SCORING SYSTEM FOR MACRO-INVERTEBRATES ('WATER BUGS') IN AUSTRALIAN RIVERS



User manual

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Table of contents

What is SIGNAL 2?

SIGNAL stands for 'Stream Invertebrate Grade Number – Average Level.' It is a simple scoring system for macro-invertebrate ('water bug') samples from Australian rivers.

A SIGNAL score gives an indication of water quality in the river from which the sample was collected. Rivers with high SIGNAL scores are likely to have low levels of salinity, turbidity and nutrients such as nitrogen and phosphorus. They are also likely to be high in dissolved oxygen.

When considered together with macro-invertebrate richness (the number of types of macro-invertebrates), SIGNAL can provide indications of the types of pollution and other physical and chemical factors that are affecting the macro-invertebrate community.

SIGNAL was first developed in 1993 for use in the Hawkesbury–Nepean River system near Sydney, and especially for assessing the impacts of discharges from sewage treatment plants. The original SIGNAL was a preliminary version, because knowledge of the distributions and tolerances of Australian river macro-invertebrates was limited at that time. Most of the knowledge that did exist was for south-eastern Australia, and the original SIGNAL was difficult to apply in northern, western and inland Australia.

Since 1994, biologists from government agencies around Australia have been sampling macro-invertebrates and measuring water and habitat quality in hundreds of rivers. This work, under the National River Health Program and other projects, provided data that were used to produce a more versatile and reliable version of SIGNAL. The new version is called SIGNAL 2.

The original version of SIGNAL required all macro-invertebrates to be identified to the taxonomic (classification) level of family. This is the level routinely used by most agency biologists. Although species-level identification provides more information, especially on conservation values, it is a specialised and laborious task. Community groups such as those in the national Waterwatch program often cannot take identification to family level. Typically, these groups identify to the taxonomic levels of order, class and phylum, depending on the type of macro-invertebrate.

SIGNAL 2 has versions to suit both family and order-class-phylum identification.

The purpose of this manual

This manual provides practical advice on how to calculate and interpret a SIGNAL 2 score. It includes a brief introduction to the relevance of aquatic macro-invertebrates in river and stream assessment, and some advice on sampling and identification. These matters are not treated in detail because several excellent guides on sampling and identification already exist. References are provided for those wishing to pursue these topics further.

Readers who are familiar with macro-invertebrate biology and sampling can skip the introductory sections.

Why study macro-invertebrates?

Vertebrates are those animals with a backbone like mammals, birds, reptiles, frogs and fish. Invertebrates is the name given to remainder of the animal kingdom. Although invertebrates all lack a backbone, they differ from one another in many respects.

Macro-invertebrates are those invertebrates that can be seen without the aid of a microscope or magnifying glass. Aquatic macro-invertebrates are those that spend all or part of their life cycles in water. They include many insects, crustaceans, mites, molluscs and worms.

The term 'water bugs' is often used as shorthand for aquatic macro-invertebrates. However, scientifically speaking the word 'bug' applies only to insects of the order Hemiptera (often called 'true bugs').

Around the world, various groups of animals and plants are used for the assessment of river condition. However, it is the macro-invertebrates that are used most often. There are several reasons for this.

1. Macro-invertebrates are found in almost every water body, even rivers and ponds that dry from time to time.

2. Macro-invertebrates are easy to catch with simple hand nets and relatively easy to identify.

3. There are many different types of macro-invertebrates. Each type requires particular environmental conditions in order to survive, grow and reproduce. Some types are tolerant of water pollution whereas others are very sensitive. So biologists can tell a lot about the environmental conditions in a water body from the types of macro-invertebrates present and their abundances.

4. Some macro-invertebrates are mobile but many, such as mussels, are sedentary. A sedentary macro-invertebrate collected from a particular site on a river may have been living there for months or even years. For the sedentary macro-invertebrate to survive, conditions must have been suitable throughout this period. If a pulse of severe pollution flows through a site it many be many months before new animals colonise and the macro-invertebrate community recovers, even if water quality returns quickly to normal. So studying macro-invertebrates provides an indication of past conditions as well as present conditions. In contrast, a spot water quality measurement provides information only on conditions at the time of sampling.

5. Macro-invertebrates are a major component of biological diversity. About 99% of animal species are invertebrates. Understanding the effects of human activity on aquatic macro-invertebrates helps in finding ways to conserve them.

6. A healthy macro-invertebrate community is important to the normal functioning of a water body. Macro-invertebrates occupy a central position in the food webs of rivers and streams. Almost every type of organic matter is eaten by some macro-invertebrate or another; algae, water plants, dead leaves and wood are all food for some types of invertebrates. In turn, macro-invertebrates are eaten by one another and by most types of aquatic vertebrates including fish, frogs, turtles, birds, platypus and water rats.

Macro-invertebrate types and classification

As for other animals and plants, the classification system for macro-invertebrates is hierarchical. Within the animal kingdom, macro-invertebrates belong to various phyla. Each phylum comprises several classes, each class comprises several orders, and so on down to genus and species. As an example, here is the classification of a common freshwater shrimp.

Kingdom:	**Animalia**
Phylum (plural phyla):	**Arthropoda**
Class:	**Crustacea**
Order:	**Decapoda**
Family:	**Atyidae**
Genus (plural genera):	***Paratya***
Species:	***australiensis***

By convention, genus and species names are always written in italics. The species name is entirely in lower case.

The table on the next page lists the phyla, classes and orders of aquatic macro-invertebrates that are likely to be distinguished by community groups.

Table 1. List of phyla, classes and orders of aquatic macro-invertebrates

Phylum	Class	Order	Common name
Porifera			Freshwater sponges
Cnidaria	Hydrozoa		Hydras; freshwater jellyfish
Platyhelminthes	Turbellaria		Flatworms
Nemertea			Proboscis worms
Aschelminthes	Nematoda		Roundworms; nematodes
	Nematomorpha		Horsehair worms; gordian worms
Bryozoa			Pipe-mosses
Mollusca	Bivalvia		Mussels; clams
	Gastropoda		Snails
Annelida	Hirudinea		Leeches
	Oligochaeta		Segmented worms
	Polychaeta		Bristle worms
Arthropoda	Acarina		Mites
	Crustacea	Amphipoda	Side swimmers; scuds
		Anaspidacea	(Shrimp-like animals)
		Anostraca	Brine shrimps; fairy shrimps
		Branchiura (sub-class)	Fish lice
		Conchostraca	Clam shrimp
		Decapoda	Shrimps; yabbies; crayfish; crabs
		Isopoda	Freshwater slaters
		Notostraca	Shield shrimps; tadpole shrimps
	Diplopoda		Aquatic millipedes
	Insecta	Coleoptera	Beetles
		Collembola	Springtails
		Diptera	True flies
		Ephemeroptera	Mayflies
		Hemiptera	True bugs
		Lepidoptera	Moths
		Mecoptera	Scorpion flies
		Megaloptera	Alder flies; dobson flies
		Neuroptera	Lacewings
		Odonata	Dragonflies; damsel flies
		Plecoptera	Stoneflies
		Trichoptera	Caddis flies

Designing a macro-invertebrate study

Before rushing out to sample macro-invertebrates, it is important to decide on the objectives of the project.

Collecting some invertebrates in a dip net and calculating a SIGNAL 2 score provides only a simple or rapid assessment, particularly if identification is taken only to order-class-phylum level. It gives some indication of what the condition of the site may be, but is not an absolute measure of how 'good' or 'healthy' the site is. A lot of information, covering the physical and chemical environment and several groups of plants and animals, is needed to make a judgement about 'health'.

The idea of 'goodness' or 'health' also implies a community vision or target for what the site should be like. This target might be the 'natural' condition or the condition that existed before Europeans arrived in Australia. In many cases, however, it may be difficult to know just what the site was like naturally, because it has changed so much. Also, it may not be possible to return to that condition because so much development has occurred in the catchment. In this case, the aim may be to manage the river as well as possible and restore some features that are known to have been lost, for example by replanting native trees on the banks and providing better treatment for wastewater discharged to the river.

Examples of objectives for a macro-invertebrate study could be:

1. To gain a better understanding of the different types of macro-invertebrates, their ecological roles and their use in biological assessment.

2. To compare the study river with similar rivers that are still in their natural condition, in order to estimate how much the macro-invertebrates in the study river have changed.

3. To compare the macro-invertebrates of the study river with others in the region, to obtain an indication about whether its management needs to be improved.

4. To monitor the study river to see how its macro-invertebrate community changes as the management of the river is improved.

Once the objective has been decided, an appropriate study can be designed. For example, if the objective were the second of those above it would be necessary to sample the study river plus a number of natural 'reference rivers'. If the fourth objective were applicable, it would be necessary to sample many times, probably over a period of several years.

Sampling macro-invertebrates

Macro-invertebrates are usually collected with pond nets with mesh sizes ranging from 0.25 to 0.5 millimetres, although there are many other types of collecting gear. The Web site of the Australian River Assessment Scheme (AUSRIVAS), a part of the National River Health Program, has macro-invertebrate sampling manuals for each State and Territory (http://ausrivas.canberra.edu.au/). Waterwatch manuals also give detailed instructions on sampling procedures. The Waterwatch Web site is at http://www.waterwatch.org.au/.

It is important to choose the sampling areas carefully. Bare area such as sand banks and muddy beds without stones, wood or vegetation usually support few macro-invertebrates. Areas that have recently been flooded should not be sampled because macro-invertebrates may not yet have colonised them. For safety reasons, deep water and very fast-flowing water should also be avoided.

A good sampling area in most streams is a sheltered **alcove**, or still area at the edge of the stream, like the one shown below. Preferably some combination of aquatic plants, vegetation trailing into the water from the banks, overhanging banks and submerged logs will be present. The net should be used to sweep among stones and logs, into the shore and under overhangs. Practice is needed to develop a technique that will scoop up macro-invertebrates from the stream bed without collecting large quantities of sediment.

Plate 1. Sampling macro-invertebrates from a stream alcove

Riffles and similar areas of flowing water generally make good macro-invertebrate habitat, especially if stones of various sizes are present. An example is pictured below. The kick-sampling technique can be used in flowing water, digging the feet well into the stream bed and holding the net downstream to catch the disturbed invertebrates. It is important to move about to cover areas with both fast and slow flow.

Plate 2. A rocky riffle is a good sampling area for macro-invertebrates

It is important to allow enough time to collect an adequate sample of the macro-invertebrates present in each sampling area. Generally between 3 and 10 minutes per area will do, depending on the size of the net. With a small net it may be necessary to take several small samples rather than one big one, in order to prevent clogging. Aim to cover at least 10 metres of stream. It is also important to keep samples from different areas (alcoves and riffles) separate. The SIGNAL score from an alcove and a riffle at the same site will generally not be the same.

After sampling, the net will contain macro-invertebrates plus sediment and debris such as leaf fragments. The contents of the net can be spread in large white trays to allow the macro-invertebrates to be picked out with pipettes. The picked macro-invertebrates can be placed in containers, such as ice-cube trays filled with stream water, for later identification.

It is very important that the macro-invertebrates can be seen clearly in the trays. In muddy streams, it is a good idea to rinse the net thoroughly in the water so that any mud is washed through it. In some streams, large quantities of sand can be a problem. If the tray contains too much sand, add water and shake the tray, then quickly pour the water (with invertebrates and other light debris) into another tray.

Macro-invertebrates are seen more easily if the debris is not spread too thickly in the tray. The tray should appear white with dark blotches rather than dark with the occasional white patch. If there is a lot of debris, use several trays or process it bit by bit. A magnifying glass may help in locating the smaller macro-invertebrates. It also helps to shake the tray from time to time, in order to make the macro-invertebrates move. Some will stick to the tray and can be seen more easily if it is tilted up occasionally.

In order to calculate a reliable SIGNAL 2 score, it is desirable to collect at least 100 macro-invertebrates and preferably 150-200. This may be difficult if the stream is so polluted that few macro-invertebrates are present. However, it should not be difficult in most places. It will generally take half an hour to an hour for one person to reach this total, depending on the person's experience and the nature of the site.

The box below gives a summary of sampling recommendations.

Checklist for macro-invertebrate sampling

- Sample an alcove/sheltered edge and a riffle if both are present
- If possible choose areas with good macro-invertebrate habitat (stones, logs, vegetation)
- Take each sample thoroughly (at least 3 minutes and 10 metres of stream). Be sure to disturb the bed material
- Spread the samples out well in big trays so that small invertebrates that hide and don't move can be seen
- Aim to pick at least 100 macro-invertebrates per sampling area, and preferably 150-200. Try to find as many types as possible
- Keep invertebrates from each sampling area separate

Identifying macroinvertebrates

Macro-invertebrates can be identified either by comparing collected specimens with illustrations of the various groups, or by using identification keys. Keys generally consist of a series of paired descriptions of particular bodily features. Each pair, called a couplet, is numbered. The user begins with the first couplet and selects the description that best fits the specimen being 'keyed'. The chosen alternative will direct the user to another numbered couplet. This process continues until enough wrong alternatives have been eliminated to positively identify the specimen.

Some Waterwatch manuals contain keys or pictorial guides to macroinvertebrates. The following books and compact disc are also recommended.

Davis, J.A. and Christidis, F. (1997). *A guide to wetland invertebrates of southwestern Australia*. Western Australian Museum: Perth.

Hawking, J.H. and Smith, F.J. (1997). *Colour guide to invertebrates of Australian inland waters.* Identification guide No. 8. Co-operative Research Centre for Freshwater Ecology: Albury.

Ingram, B.A., Hawking, J.H. and Shiel, R.J. (1997). *Aquatic life in freshwater ponds* Identification guide No. 9. Co-operative Research Centre for Freshwater Ecology: Albury.

CSIRO (1999). *Interactive guide to Australian aquatic invertebrates.* 2nd edition. CSIRO Division of Entomology: Canberra (CD ROM).

For those wishing to undertake detailed identification, the following reference lists all the relevant keys in the scientific literature.

Hawking, J.H. (2000). *Key to keys*. *A guide to keys and zoological information to identify invertebrates from Australian inland waters.* Identification guide No. 2, 2nd edition. Co-operative Research Centre for Freshwater Ecology: Albury.

Calculating a SIGNAL 2 score

Once all the specimens are identified to either the family or the order-class-phylum level, the SIGNAL 2 score can be calculated.

Each type of macro-invertebrate has a 'grade number' between 1 and 10. These are listed in Appendix 1 at the back of this manual. A low grade number means that the macro-invertebrate is tolerant of a range of environmental conditions, including common forms of water pollution. A high number means that the macro-invertebrate is sensitive to most forms of pollution. The higher the number, the greater the average sensitivity.

Generally, grades for the family and order-class-phylum levels of identification should not be mixed in the same calculation. However, in family-level studies, a few groups that are more difficult to take to family level are often left at order-class-phylum level, for example mites (Acarina) and segmented worms (Oligochaeta). In these cases the order-class-phylum grades can be used in the family-level calculation. However, this must be done consistently if valid comparisons are to be made between SIGNAL 2 scores for different samples.

SIGNAL 2 scores can be calculated with or without abundance weighting. If no weighting is used, the SIGNAL score is the average of the grade numbers for those macro-invertebrate types collected. If abundance weighting is used, a weight factor is derived for each type of macro-invertebrate. Various weighting schemes are possible. The one used in this manual has been employed for several years in the New South Wales Streamwatch 'Water Bug Survey'.

Table 2 gives an example of how to calculate a score for the order-class-phylum version of SIGNAL 2 and Table 3 gives an example for the family version. These calculations proceed by the following steps.

Step 1: A list of the macro-invertebrate types found in the sample is made at either the family or order-class-phylum level, depending on how far the identification is taken.

Step 2: The relevant grade number is entered alongside each type of macro-invertebrate in the list. If a type has been recorded that has no grade number assigned, it should be deleted from the list. This will happen only rarely.

Step 3: The number of specimens of each macro-invertebrate type that were collected (abundance) is entered alongside the grade number.

Step 4: The weight table is used to determine the weight factor for each type of macro-invertebrate, according to the number of specimens collected. The weight factors are tabulated next to the abundance values.

Step 5: The grade number for each macro-invertebrate type is multiplied by the corresponding weight factor and the results are tabulated.

Step 6: The weight factors for all macro-invertebrate types are added.

Step 7: The products of grade numbers and weight factors are added.

Step 8: The second of these totals is divided by the first to produce the abundance-weighted SIGNAL 2 score.

Table 2. How to calculate a SIGNAL 2 score for a survey site. An example using the order-class-phylum version

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

CALCULATION TABLE					
Invertebrate orders, classes and phyla collected at the site	Common name	SIGNAL 2 sensitivity grade	Number of specimens	Weight factor	Grade x weight factor
Acarina	Mites	6	10	3	18
Coleoptera	Beetles and beetle larvae	5	5	2	10
Decapoda	Yabbies, prawns and shrimps	4	1	1	4
Diptera	True fly larvae	3	35	5	15
Ephemeroptera	Mayfly nymphs	9	8	3	27
Hemiptera	True bugs and their nymphs	2	17	4	8
Nemertea	Proboscis worms	3	2	1	3
Odonata	Dragonfly and damselfly nymphs	3	3	2	6
Oligochaeta	Segmented worms	2	8	3	6
Plecoptera	Stonefly nymphs	10	12	4	40
Trichoptera	Caddis fly larvae	8	22	5	40
Turbellaria	Flatworms	2	4	2	4
TOTALS				**35**	**181**

SIGNAL SCORE = TOTAL OF GRADE x WEIGHT FACTOR / TOTAL OF WEIGHT FACTOR = 181/35 = 5.2

Table 3. How to calculate a SIGNAL 2 score for a survey site. An example using the family version

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

CALCULATION TABLE				
Invertebrate families collected at the site	SIGNAL 2 sensitivity grade	Number of specimens	Weight factor	Grade x weight factor
Atyidae	3	8	3	9
Baetidae	5	15	4	20
Caenidae	4	12	4	16
Chironomidae (subfamily Chironominae)	3	22	5	15
Chironomidae (subfamily Orthocladiinae)	4	16	4	16
Coenagrionidae	2	4	2	4
Corixidae	2	2	1	2
Dytiscidae	2	3	2	4
Hydrophilidae	2	5	2	4
Hydropsychidae	6	35	5	30
Leptoceridae	6	12	4	24
Notonectidae	1	7	3	3
Physidae	1	6	3	3
Planorbidae	2	1	1	2
Simuliidae	5	42	5	25
TOTALS			**48**	**177**

SIGNAL SCORE = TOTAL OF GRADE x WEIGHT FACTOR / TOTAL OF WEIGHT FACTOR = 177/48 = 3.7

Plotting the results

In order to interpret the SIGNAL 2 score, the next step is to plot both the score and the number of types of macro-invertebrates recorded on a graph with two axes (a biplot). Figure 1 below is an example of a biplot for the order-class-phylum (OCP) version using the data in Table 2.

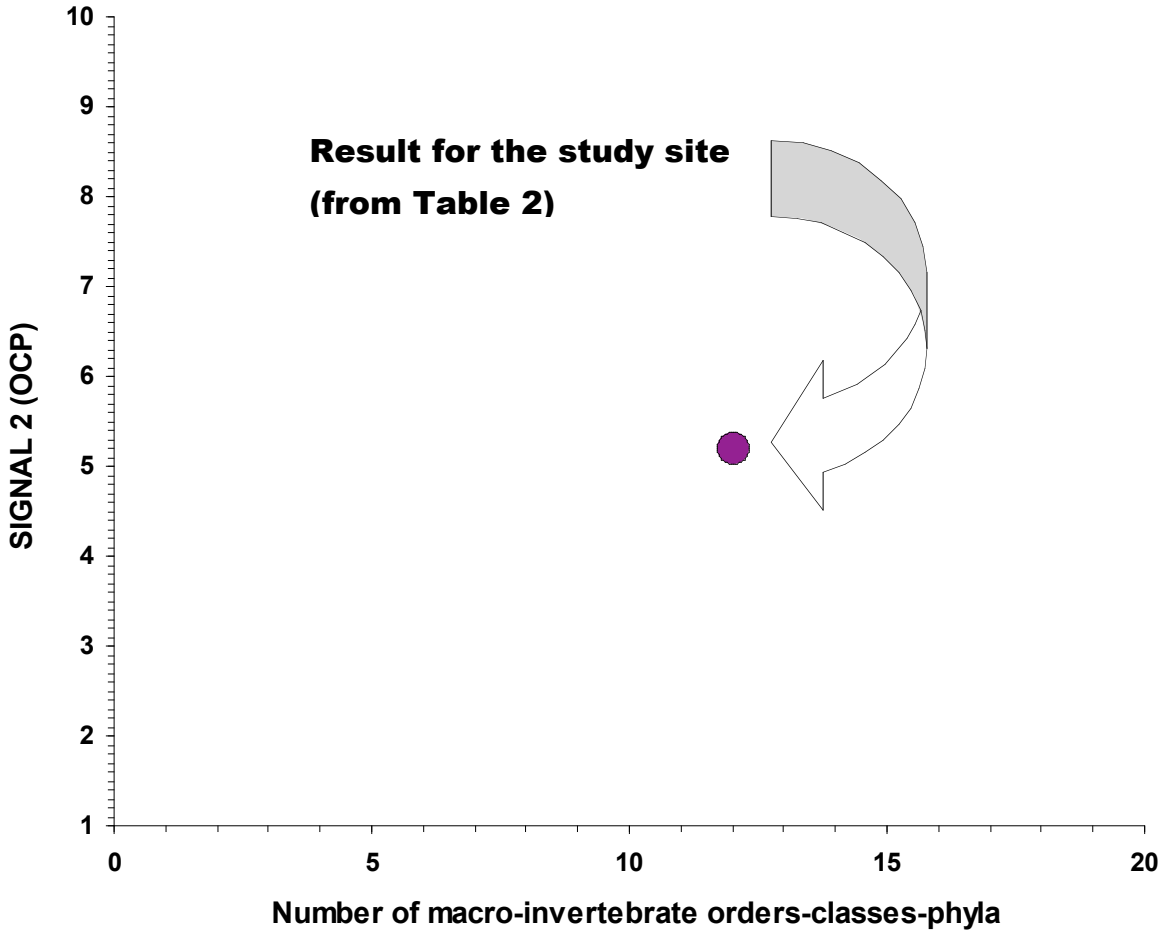

Figure 1. A biplot of the SIGNAL 2 score and number of macro-invertebrate types for a survey site. An example using the order-class-phylum (OCP) version and the data in Table 2

The vertical axis (SIGNAL 2 (OCP) score) ranges from 1 to 10, since these are the minimum and maximum possible scores respectively. However, it most cases, scores will lie between 3 and 7. The maximum possible number of orders-classes-phyla varies among regions of Australia, but it is very rare to collect more than 20.

Figure 2 below is an example of a biplot for the family version using the data in Table 3. The maximum number of families shown is 40, because it is very rare to record more than this.

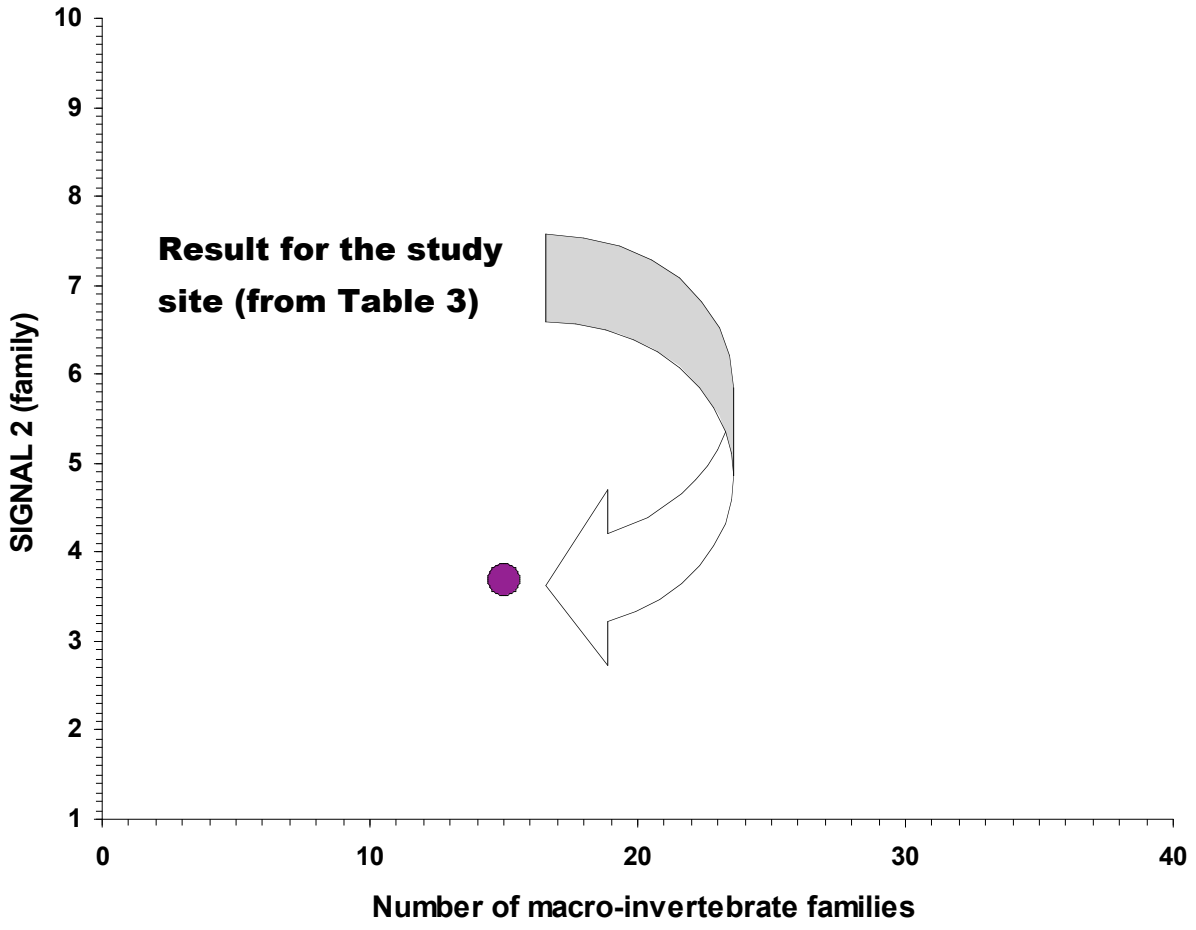

Figure 2. A biplot of the SIGNAL 2 score and number of macro-invertebrate types for a survey site. An example using the family version and the data in Table 3

Interpreting results – the quadrant diagram

A point on a biplot means little by itself. A quadrant diagram is used to place the result in context.

The basic quadrant diagram is shown in Figure 3 (for the order-class-phylum version of SIGNAL 2) and Figure 4 (for the family version). The area of the biplot is divided into four quadrants. The appropriate boundaries between the four quadrants will differ between geographic regions of Australia because of natural variation in macro-invertebrate assemblages. They will also vary according to sampling effort and the types of habitats sampled. For this reason, numbers on the axes corresponding to the quadrant boundaries are not shown. The setting of appropriate boundaries for each particular case is discussed in the next section.

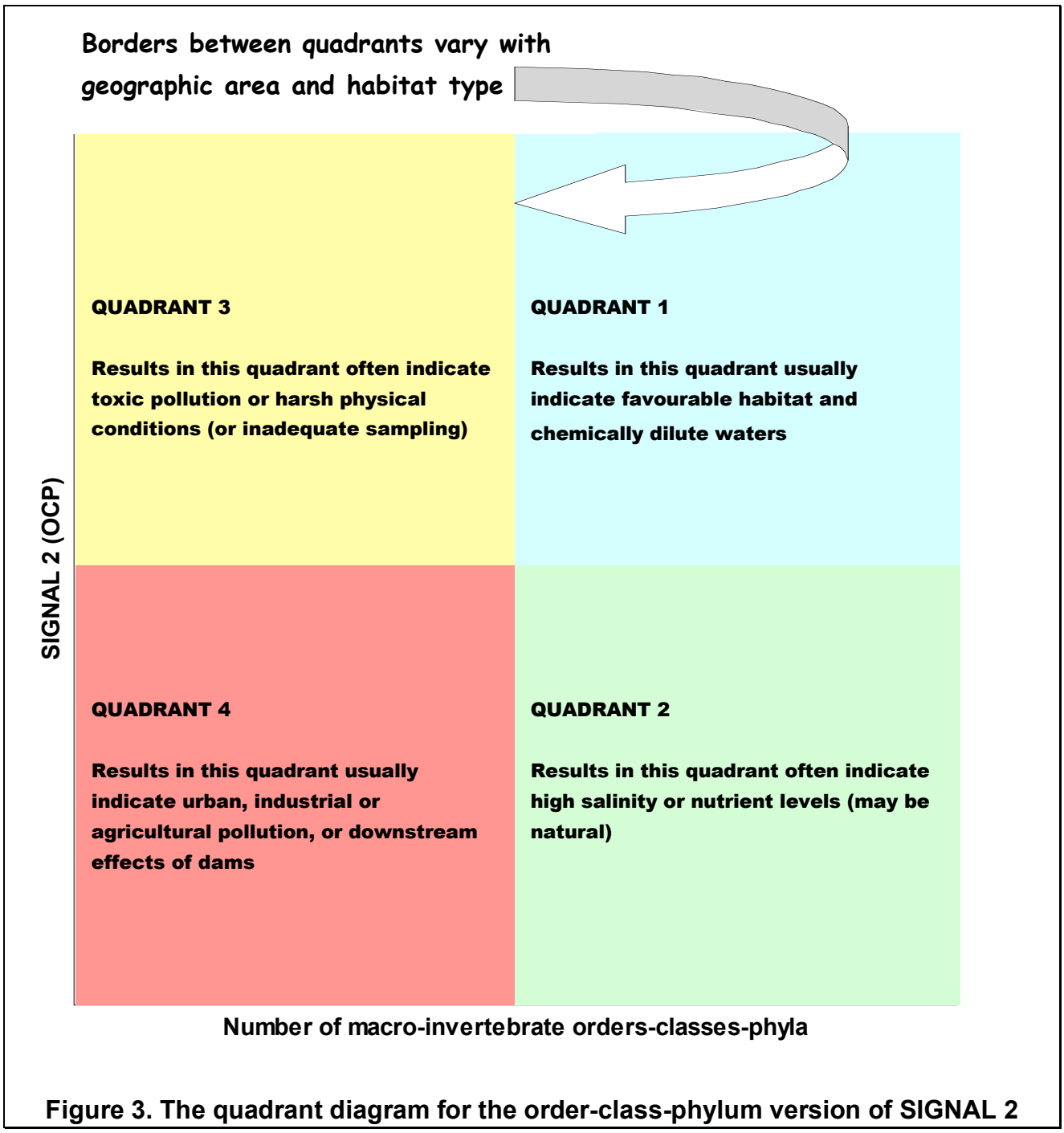

Figure 3. The quadrant diagram for the order-class-phylum version of SIGNAL 2

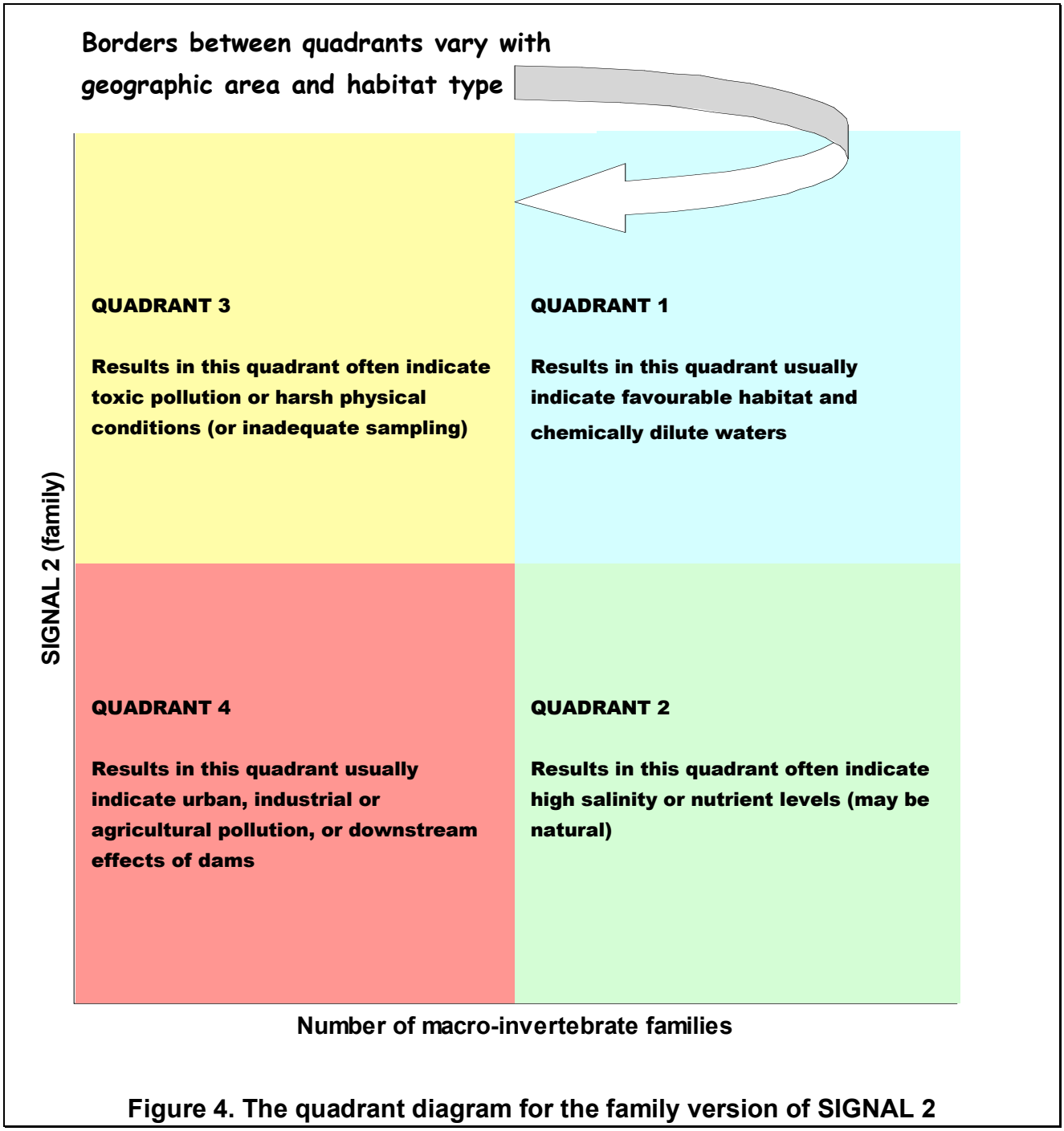

Figure 4. The quadrant diagram for the family version of SIGNAL 2

Quadrant 1 (at the top right) represents high values of both SIGNAL 2 and the number of macro-invertebrate types. The presence of large number of types suggests that the diversity of physical habitats is high and that stress factors like toxic chemicals and harsh physical conditions are not present. The high SIGNAL 2 scores suggests that turbidity, salinity and nutrient concentrations are low. Streams in undisturbed native forest will often fall in this quadrant.

Quadrant 2 (at the bottom right) represents lower SIGNAL 2 scores and a high diversity of macro-invertebrate types. Sites falling in this quadrant are likely to have higher levels or turbidity, salinity or nutrients than those in quadrant 1, as suggested by the lower SIGNAL 2 score. These levels may be high either naturally, because of local geology and soil types, or as a result of human activities. The high number of macro-invertebrate types suggests that physical conditions are still benign and toxic chemicals are not present in large amounts. Many agricultural streams without severe impacts fall into this quadrant.

Quadrant 3 (at the top left) represents high values of SIGNAL 2 but few macro-invertebrate types. Sites with toxic pollution, such as those with below old mine sites where acid rock drainage can result in low pH and high concentrations of trace metals, usually fall either in this quadrant or in quadrant 4. This occurs because the tolerances of some macro-invertebrate types differ according to the type of pollution. For example, snails and segmented worms are tolerant of most forms of pollution but sensitive to metals. Certain caddis fly families, such as the Leptoceridae, stonefly families such as the Gripopterygidae and Notonemouridae, and the alderfly family Corydalidae are tolerant of metals even though they are sensitive to many other forms of pollution. SIGNAL 2 is designed to respond to the most common forms of water quality variation, such as organic and nutrient enrichment and salinity. Sites with unusual forms of pollution may still have high SIGNAL scores.

Harsh physical conditions can also result in sites falling in quadrant 3. A very simple habitat structure, such as occurs on mobile sand beds or bare muddy beds, can result in few macro-invertebrate types being able to colonise and survive, even if water quality is suitable for them. Extreme floods can wash macro-invertebrates away, so that few types are collected if sampling occurs soon after the flood has receded. Streambeds that have recently filled with water after drought may also harbour few types of macro-invertebrates, until the macro-invertebrates have had time to colonise and breed. Poor sampling technique or inadequate sampling effort can also result in a site falling in quadrant 3, because few macro-invertebrates are collected even though many are present.

Quadrant 4 (at the bottom left) represents low values of both the SIGNAL 2 score and the number of invertebrate types. Most sites falling into this quadrant will be suffering from one or more forms of human impact.

Setting boundaries on the quadrant diagram

It is preferable to set the boundaries on the quadrant diagram individually, in order to suit each study region and the local sampling methods.

Figure 5 shows a simplified example of how this can be done. The coloured dots in the biplot represent three macro-invertebrate samples taken from alcove/edgewater habitat at each of six sites in the upper Macquarie River catchment in the central west of New South Wales. One of the sites (blue dots) was in a forest area with very little human disturbance. Two others (pale and dark green dots) were in agricultural areas where high levels of stream turbidity, nitrogen, phosphorus and salinity are common. One site (yellow dots) was downstream of a disused metal mine, another was immediately downstream of a large dam (dark red dots) and the last site was on a small stream below the discharge point of a sewage treatment plant. The boundaries were set so that the samples from undisturbed sites fall in quadrant 1.

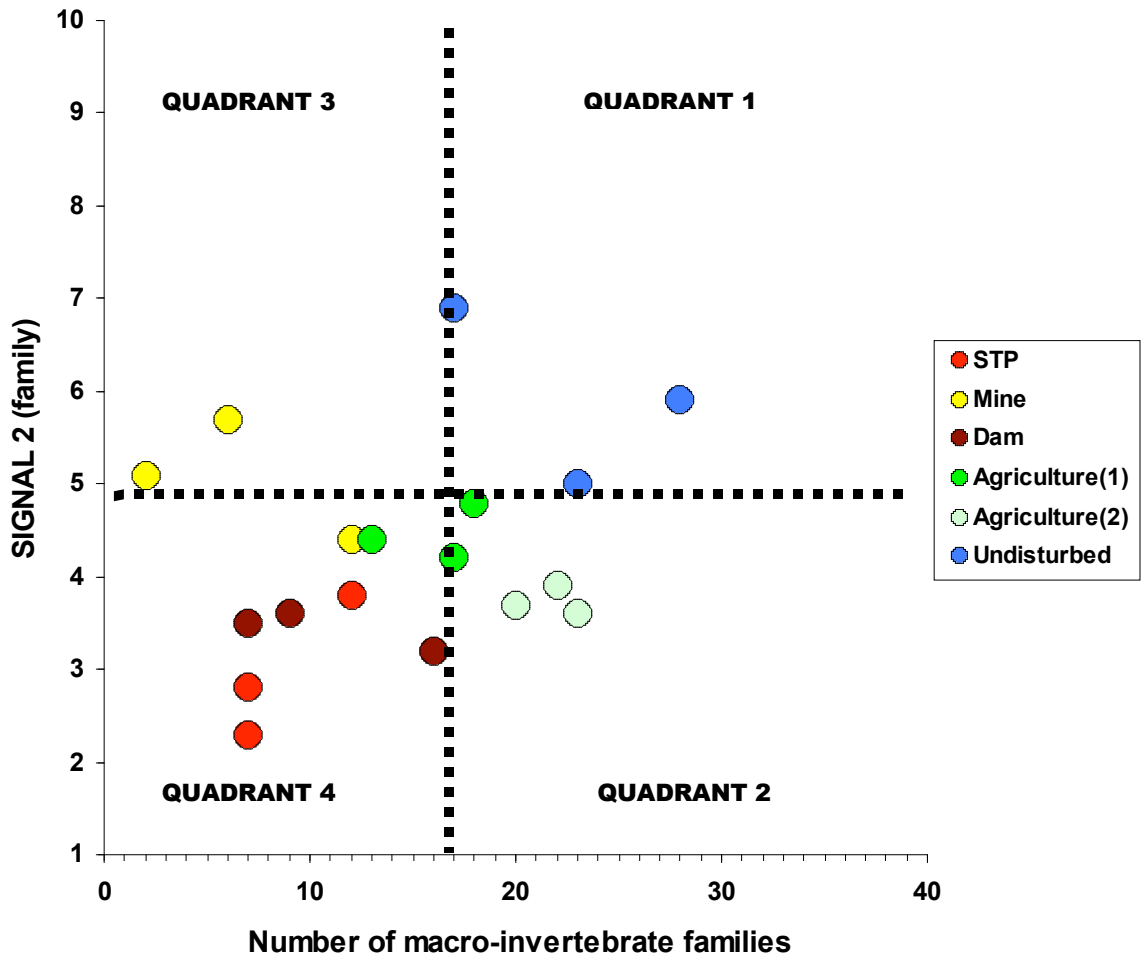

Figure 5. Example of a biplot of SIGNAL 2 (family) scores from 3 samples from each of 6 sites in the upper Macquarie River catchment, New South Wales. The dotted lines represent possible quadrant boundaries

With large numbers of samples, there will often be some overlap between disturbed and undisturbed sites. In these cases, setting the boundaries so that **all** samples for undisturbed sites are in quadrant 1 may result in too many disturbed samples being in this quadrant. On the other hand, setting the boundaries of quadrant 1 to **exclude** all samples from disturbed sites may result in many samples from undisturbed sites being in quadrants 2, 3 and 4. In these cases, a compromise must be found between the two extremes.

In many regions, there may be no undisturbed sites. A considerable amount of judgment is needed in such cases. If the best available sites are considered to be in good condition and well managed, the boundaries could be set so that such sites are in quadrant 1. If even the best sites available are considered to be degraded and poorly managed, the boundaries should be set so that these sites are somewhat outside quadrant 1.

Since many users may lack the data or experience to set their own quadrant boundaries, **suggested** quadrant boundaries are listed in Tables 4 and 5 for various regions and the two principal habitats recommended for sampling. Because it is very difficult to provide general guidelines that deal with all local circumstances in different parts of Australia, it is highly recommended that users verify the appropriateness of these boundaries for local application.

Table 4. Suggested interim quadrant boundaries for different regions of Australia for alcove/edgewater habitat

Region	Boundary for number of orders-classes-phyla	Boundary for SIGNAL 2 (OCP)	Boundary for number of families	Boundary for SIGNAL 2 (family)
Murray Darling basin above 400 m elevation; coastal basins of Victoria and NSW; Tasmania	7.5	5.5	19.5	5.0
Murray Darling basin between 400 m and 200 m elevation	6.5	5.0	17.5	4.5
Queensland east of the Great Dividing Range	7.5	4.5	19.5	4.0
Remainder of Australia	5.5	4.5	15.5	4.0

Table 5. Suggested interim quadrant boundaries for different regions of Australia for riffle habitat

Region	Boundary for number of orders-classes-phyla	Boundary for SIGNAL 2 (OCP)	Boundary for number of families	Boundary for SIGNAL 2 (family)
Murray Darling basin above 400 m elevation; coastal basins of Victoria and NSW; Tasmania	6.5	6.0	17.5	6.0
Murray Darling basin between 400 m and 200 m elevation	6.5	5.5	16.5	5.5
Queensland east of the Great Dividing Range	6.5	5.0	17.5	5.0
Remainder of Australia	6.5	5.0	15.5	5.0

Dealing with variability

Every macro-invertebrate sample taken from a stream is different. Catching macro-invertebrates is a haphazard business. Not every spot in the stream has exactly the same animals in it, so depending exactly where the sample is taken, a different group of macro-invertebrates will be caught. Also, each person samples a little differently, and some animals will get caught in the net while others will manage to escape.

As Figure 5 shows, different samples from the same site will be scattered on the biplot. Therefore, it is unwise to draw conclusions from a single sample. Initially, at least three samples should be taken, preferably by different people or groups. If these show a wide scatter, or give contradictory indications, more samples should be taken until a clear picture emerges.

The further away results are from the borders between quadrants, the more likely they are to signify the conditions represented by the quadrant in which they lie. Results close to quadrant boundaries indicate the need for more sampling.

Relating macro-invertebrate results to other information

It is always important to remember that SIGNAL 2 scores and biplots are a simple, rapid assessment and not a comprehensive assessment of a stream or even of its macro-invertebrates. The biplot provides an **indication** of things that may be affecting the macro-invertebrates at the site, such as water and habitat quality.

Linking SIGNAL 2 assessments to other types of information will increase the weight of evidence and lead to more confident conclusions. Such information might include water quality test results, physical habitat assessments and assessments of other life forms, such as vegetation. Waterwatch manuals provide methods and guidance on how to undertake these types of assessments.

It is also important to understand what may be influencing the stream: the land use in its catchment, the human activities that may be affecting it, and the infrastructure present, such as dams, drains and wastewater discharge points. It is difficult to interpret results from a single site in isolation.

And what about wetlands?

Most of the macro-invertebrate groups that occur in streams also occur in freshwater lakes, farm dams and other wetlands. However, the applicability of SIGNAL 2 to wetlands has not been tested. Some of the macro-invertebrate orders that have the highest SIGNAL 2 sensitivity grades are rare in wetlands, for example stoneflies and to a lesser extent mayflies and caddis flies. Therefore, wetlands are likely to have naturally lower scores than streams in the same region.

SIGNAL and AUSRIVAS

The Australian River Assessment Scheme (AUSRIVAS) is a set of computer models that compare a macro-invertebrate family list from a sampling site (test site) with a data base from a large number of reference sites throughout Australia. The reference sites are those in each region of Australia that are believed to be least damaged by human activity. The AUSRIVAS models match the test site to appropriate reference sites for similar types of streams in the same State or region. If the test site is lacking the macro-invertebrate families that are expected to occur, according to the reference site data base, it is likely that the test site is in worse condition than the reference sites.

The AUSRIVAS Web site can be visited at http://ausrivas.canberra.edu.au/. The site contains lots of information about macroinvertebrates and biological monitoring. A user name and password are needed to use the computer models.

The AUSRIVAS models can also calculate a reference SIGNAL score for comparison with the actual score at a test site. However, AUSRIVAS is currently using the old version of SIGNAL, and there is some debate in the scientific community about how the AUSRIVAS should calculate the reference score. Model outputs involving SIGNAL should be interpreted with caution until the next version of the AUSRIVAS is on line.

It is also important to remember that in many regions of Australia, even the least damaged reference sites are far removed from their natural state. Therefore the AUSRIVAS reference data base does not represent a pristine condition.

Scientific papers on SIGNAL

The following articles in scientific journals provide more information on the origin and development of SIGNAL. A paper on SIGNAL 2 is currently in preparation.

1. Chessman, B.C. (1994). The use of macroinvertebrates for the rapid biological assessment of streams in the Sydney region, New South Wales, Australia. pp. 235-245 in *Classification of rivers and environmental health indicators. A joint South African/Australian workshop* (ed. M.C. Uys). Water Research Commission: Pretoria, South Africa. Report No. TT 63/94.
2. Chessman, B.C. (1995). Rapid assessment of rivers using macroinvertebrates: a procedure based on habitat-specific sampling, family-level identification, and a biotic index. *Australian Journal of Ecology,* 20, 122-129.
3. Growns, J.E., Chessman, B.C., McEvoy, P.K. and Wright, I.A. (1995). Rapid assessment of rivers using macroinvertebrates: case studies in the Nepean River and Blue Mountains, NSW. *Australian Journal of Ecology,* 20, 130-141.
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5. Chessman, B.C., Growns, J.E. and Kotlash, A.R. (1997). Objective derivation of macroinvertebrate family sensitivity grade numbers for the SIGNAL biotic index: application to the Hunter River system, New South Wales. *Marine and Freshwater Research*, 48, 159-172.
6. Chessman, B.C. and McEvoy, P.K. (1998). Towards diagnostic biotic indices for river macroinvertebrates. *Hydrobiologia*, 364, 169-182.

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Appendix 1: SIGNAL 2 grades

Table A1. Order-class-phylum grades, arranged alphabetically

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

Table A2. Family grades, arranged alphabetically by order-class-phylum

Acarina	Arrenuridae	5
Acarina	Aturidae	6
Acarina	Eylaidae	5
Acarina	Hydrachnidae	4
Acarina	Hydrodromidae	5
Acarina	Hydryphantidae	5
Acarina	Hygrobatidae	7
Acarina	Limnesiidae	6
Acarina	Limnocharidae	7
Acarina	Mideopsidae	6
Acarina	Momoniidae	5
Acarina	Notodromadidae	1
Acarina	Pionidae	4
Acarina	Torrenticolidae	6
Acarina	Unionicolidae	6
Amphipoda	Ceinidae	2
Amphipoda	Corophiidae	4
Amphipoda	Eusiridae	7
Amphipoda	Melitidae	7
Amphipoda	Neoniphargidae	4
Amphipoda	Paracalliopidae	3
Amphipoda	Paramelitidae	4
Amphipoda	Perthiidae	4
Amphipoda	Talitridae	3
Anaspidacea	Koonungidae	1
Anostraca	Branchipodidae	1
Bivalvia	Corbiculidae	4
Bivalvia	Hyriidae	5
Bivalvia	Sphaeriidae	5
Coleoptera	Brentidae	3
Coleoptera	Carabidae	3
Coleoptera	Chrysomelidae	2
Coleoptera	Curculionidae	2
Coleoptera	Dytiscidae	2

Coleoptera	Elmidae	7
Coleoptera	Gyrinidae	4
Coleoptera	Haliplidae	2
Coleoptera	Heteroceridae	1
Coleoptera	Hydraenidae	3
Coleoptera	Hydrochidae	4
Coleoptera	Hydrophilidae	2
Coleoptera	Hygrobiidae	1
Coleoptera	Limnichidae	4
Coleoptera	Microsporidae	7
Coleoptera	Noteridae	4
Coleoptera	Psephenidae	6
Coleoptera	Ptiliidae	3
Coleoptera	Ptilodactylidae	10
Coleoptera	Scirtidae	6
Coleoptera	Staphylinidae	3
Decapoda	Atyidae	3
Decapoda	Grapsidae	7
Decapoda	Hymenosomatidae	3
Decapoda	Palaemonidae	4
Decapoda	Parastacidae	4
Decapoda	Sundatelphusidae	3
Diplopoda	Siphonotidae	6
Diptera	Aphroteniinae (subfamily)	8
Diptera	Athericidae	8
Diptera	Blephariceridae	10
Diptera	Cecidomyidae	1
Diptera	Ceratopogonidae	4
Diptera	Chaoboridae	2
Diptera	Chironominae (subfamily)	3
Diptera	Culicidae	1
Diptera	Diamesinae (subfamily)	6
Diptera	Dixidae	7
Diptera	Dolichopodidae	3
Diptera	Empididae	5

Diptera	Ephydridae	2
Diptera	Muscidae	1
Diptera	Orthocladiinae (subfamily)	4
Diptera	Pelecorhynchidae	10
Diptera	Podonominae (subfamily)	6
Diptera	Psychodidae	3
Diptera	Scatopsidae	1
Diptera	Sciaridae	6
Diptera	Sciomyzidae	2
Diptera	Simuliidae	5
Diptera	Stratiomyidae	2
Diptera	Syrphidae	2
Diptera	Tabanidae	3
Diptera	Tanyderidae	6
Diptera	Tanypodinae (sub-family)	4
Diptera	Thaumaleidae	7
Diptera	Tipulidae	5
Ephemeroptera	Ameletopsidae	7
Ephemeroptera	Baetidae	5
Ephemeroptera	Caenidae	4
Ephemeroptera	Coloburiscidae	8
Ephemeroptera	Leptophlebiidae	8
Ephemeroptera	Oniscigastridae	8
Ephemeroptera	Prosopistomatidae	4
Ephemeroptera	Siphlonuridae	10
Ephemeroptera	Teloganodidae (formerly Ephemerellidae)	9
Gastropoda	Ancylidae	4
Gastropoda	Bithyniidae	3
Gastropoda	Glacidorbidae	5
Gastropoda	Hydrobiidae	4
Gastropoda	Lymnaeidae	1
Gastropoda	Physidae	1
Gastropoda	Planorbidae	2
Gastropoda	Pomatiopsidae	1
Gastropoda	Thiaridae	4

Gastropoda	Viviparidae	4
Hemiptera	Belostomatidae	1
Hemiptera	Corixidae	2
Hemiptera	Gelastocoridae	5
Hemiptera	Gerridae	4
Hemiptera	Hebridae	3
Hemiptera	Hydrometridae	3
Hemiptera	Mesoveliidae	2
Hemiptera	Naucoridae	2
Hemiptera	Nepidae	3
Hemiptera	Notonectidae	1
Hemiptera	Ochteridae	2
Hemiptera	Pleidae	2
Hemiptera	Saldidae	1
Hemiptera	Veliidae	3
Hirudinea	Erpobdellidae	1
Hirudinea	Glossiphoniidae	1
Hirudinea	Ornithobdellidae	1
Hirudinea	Richardsonianidae	4
Hydrozoa	Clavidae	3
Hydrozoa	Hydridae	2
Isopoda	Amphisopidae	1
Isopoda	Cirolanidae	2
Isopoda	Janiridae	3
Isopoda	Mesamphisopidae	3
Isopoda	Oniscidae	2
Isopoda	Phreatoicidae	4
Isopoda	Phreatoicopsidae	2
Isopoda	Sphaeromatidae	1
Lepidoptera	Pyralidae	3
Mecoptera	Nannochoristidae	9
Megaloptera	Corydalidae	7
Megaloptera	Sialidae	5
Nemertea	Tetrastemmatidae	7
Neuroptera	Neurorthidae	9

Neuroptera	Osmylidae	7
Neuroptera	Sisyridae	3
Nematomorpha	Gordiidae	5
Notostraca	Triopsidae	1
Odonata	Aeshnidae	4
Odonata	Austrocorduliidae (formerly part of Corduliidae)	10
Odonata	Coenagrionidae	2
Odonata	Cordulephyidae (formerly part of Corduliidae)	5
Odonata	Corduliidae	5
Odonata	Diphlebiidae (formerly Amphipterygidae)	6
Odonata	Gomphidae	5
Odonata	Hemicorduliidae (formerly part of Corduliidae)	5
Odonata	Hypolestidae (formerly Lestoideidae)	9
Odonata	Isostictidae	3
Odonata	Lestidae	1
Odonata	Libellulidae	4
Odonata	Lindeniidae (formerly part of Gomphidae)	3
Odonata	Macromiidae (formerly part of Corduliidae)	8
Odonata	Megapodagrionidae	5
Odonata	Protoneuridae	4
Odonata	Synlestidae	7
Odonata	Synthemistidae (formerly part of Corduliidae)	2
Odonata	Telephlebiidae (formerly part of Aeshnidae)	9
Odonata	Urothemistidae (formerly part of Libellulidae)	1
Oligochaeta	Enchytraeidae	4
Oligochaeta	Lumbriculidae	1
Oligochaeta	Naididae	2
Oligochaeta	Phreodrilidae	2
Oligochaeta	Tubificidae	2
Plecoptera	Austroperlidae	10
Plecoptera	Eustheniidae	10
Plecoptera	Gripopterygidae	8
Plecoptera	Notonemouridae	6
Porifera	Spongillidae	3
Trichoptera	Antipodoeciidae	8

Trichoptera	Atriplectididae	7
Trichoptera	Calamoceratidae	7
Trichoptera	Calocidae	9
Trichoptera	Conoesucidae	7
Trichoptera	Dipseudopsidae	9
Trichoptera	Ecnomidae	4
Trichoptera	Glossosomatidae	9
Trichoptera	Helicophidae	10
Trichoptera	Helicopsychidae	8
Trichoptera	Hydrobiosidae	8
Trichoptera	Hydropsychidae	6
Trichoptera	Hydroptilidae	4
Trichoptera	Kokiriidae	3
Trichoptera	Leptoceridae	6
Trichoptera	Limnephilidae	8
Trichoptera	Odontoceridae	7
Trichoptera	Oeconesidae	8
Trichoptera	Philopotamidae	8
Trichoptera	Philorheithridae	8
Trichoptera	Polycentropodidae	7
Trichoptera	Tasimiidae	8
Turbellaria	Dugesiidae	2
Turbellaria	Temnocephala	5

Appendix 2: Example results and calculation sheet

Water Bug Survey Results Sheet

USE THIS VERSION ONLY FOR:

Alcove/edgewater habitat

Murray Darling basin above 400 m elevation; coastal basins of Victoria and NSW; Tasmania

Group name: ..... **Number in group:** .....

Survey site: ..... **Date sampled:** .....

Step 1: Enter the number of specimens (i.e. how many) of each bug found in column 1
Step 2: Refer to the weight table for the correct weight factor for the number found
Step 3: Enter the correct weight factor for each bug in column 2
Step 4: Multiply the weight factor (column 2) by the bug grade (column 3) and enter the answer in column 4
Step 5: Add up column 2 (weight factors)
Step 6: Add up column 4 (bug value x weight factor)
Step 7: Divide total column 4 by total column 2 to calculate your SIGNAL score
Step 8: Add up the total number of bug types you found (NOT specimens)
Step 9: Use the interpretation chart to get an indication of the likely condition of your sampling area

Weight table

Number of specimens of bug type (column 1)	Weight factor (column 2)
1 – 2 →	1
3 – 5 →	2
6 – 10 →	3
11 – 20 →	4
> 20 →	5

WATER BUG TYPE	Column 1 Number of specimens	Column 2 Weight factor	Column 3 Bug grade	Column 4 Weight factor x bug grade
Very sensitive to most pollutants				
Stonefly nymph			10	
Mayfly nymph			9	
Alder fly larva			8	
Caddis fly larva			8	
Sensitive to most pollutants				
Horsehair worm			6	
Water mite			6	
Moderately tolerant of most pollutants				
Beetle or beetle larva			5	
Yabby or shrimp			4	
Dragonfly or damselfly nymph			3	
Fly larva or midge			3	
Mussel or clam			3	
Nematode			3	
Side swimmer			3	
Very tolerant of most pollutants				
Flatworm			2	
Freshwater slater			2	
Moth caterpillar			2	
Segmented worm			2	
True bug or true bug nymph			2	
Leech			1	
Snail			1	
	TOTALS			

SIGNAL score = total column 4 / total column 2 = ____ =

Bug types found that are not on list:

Total No. of bug types found =

Interpretation chart

SIGNAL score	0 - 7 Number of bug types	More than 7 Number of bug types
Above 5.5	Suggests toxic pollution or poor habitat	Suggests good habitat and water quality
Below 5.5	Suggests pollution	Suggests high salinity or nutrient levels (may be natural)