

New sensitivity grades for Australian river macroinvertebrates

Bruce C. Chessman

Centre for Natural Resources, Department of Sustainable Natural Resources,
PO Box 3720, Parramatta, NSW 2124, Australia. Email: bchessman@dlwc.nsw.gov.au

Abstract. The SIGNAL biotic index for river macroinvertebrates, originally developed and tested in eastern Australia, was revised for application to the entire continent. Macroinvertebrate survey data from the National River Health Program were used to set grade numbers between 1 and 10 to represent the water-quality sensitivities of 210 taxa. Grades were assigned at the taxonomic levels customarily used by government agencies (predominantly family level) and by community groups (mainly order). A new index version using these grades, SIGNAL2, was correlated with water temperature, turbidity, electrical conductivity, alkalinity, pH, dissolved oxygen, total nitrogen and total phosphorus. Because of natural spatial variation in water quality, index scores need to be interpreted in a local context or against site-specific predictions generated by the Australian River Assessment System (AUSRIVAS).

Extra keywords: bioassessment, biological monitoring, biotic index, pollution, water quality.

Introduction

The use of biotic indices based on macroinvertebrates to assess water pollution and other human impacts on rivers has a long history (Washington 1984). Biotic indices continue to be developed and have been incorporated within more complex approaches framed around multimetric assessment (Karr and Chu 1999) and assemblage prediction (Wright *et al.* 2000). SIGNAL (Stream Invertebrate Grade Number Average Level) is a simple biotic index for river macroinvertebrates, developed initially for application to eastern Australia, especially the Hawkesbury–Nepean river system (Chessman 1995). It was adapted from the Average Score Per Taxon (ASPT) version of the Biological Monitoring Working Party (BMWP) system used in Great Britain (Hawkes 1997). The SIGNAL score for a macroinvertebrate sample is calculated by averaging the pollution sensitivity grade numbers of the families present, which may range from 10 (most sensitive) to 1 (most tolerant). Chessman (1995) associated bands of SIGNAL scores with indicative levels of water quality in the Hawkesbury–Nepean river system (clean water, possible mild pollution, probable moderate pollution and probable severe pollution).

Initial testing of SIGNAL in the Sydney and Blue Mountains regions of New South Wales showed that it was responsive to sewage pollution and urban runoff and little affected by gradients in natural factors such as stream size and elevation (Growth *et al.* 1995, 1997). Subsequent studies in both New South Wales and Victoria showed that SIGNAL was the most sensitive to anthropogenic disturbance of a large suite of macroinvertebrate metrics (Metzeling *et al.* 2003).

SIGNAL has been applied for various purposes, sometimes with modification. For example, it has been included as one of five elements in the Victorian Index of Stream Condition (Ladson *et al.* 1999) and used in setting environmental quality objectives in the draft State Environment Protection Policy (Waters of Victoria) prepared by the Victorian Environment Protection Authority (Anon. 2001a). It has also been incorporated in the Australian River Assessment System (AUSRIVAS), a derivative of the British RIVPACS (River Invertebrate Prediction and Classification System: Wright *et al.* 2000) developed as part of Australia's National River Health Program (NRHP: Davies 2000). AUSRIVAS is a software system that compares the macroinvertebrate families collected from a river with those found at physically matched reference sites. AUSRIVAS can be used to generate reference SIGNAL values for comparison with observed values (Ransom *et al.* 2003).

In the original version of SIGNAL, grade numbers were set subjectively for just 111 families, mainly with information from 12 published case studies (listed in Chessman 1995). Subsequently, efforts were made to improve the performance of the index by developing extended and more accurate grading systems. Chessman *et al.* (1997) devised an algorithm for setting grade numbers objectively, and tested it using survey data from the Hunter River system, New South Wales. However, they warned that the improved grade numbers derived for that system might not be applicable in other regions. Chessman and McEvoy (1998) addressed the issue of differential sensitivity to different types of pollutants such as sewage and trace metals. They showed that grades derived objectively for different pollutants were sometimes

significantly different, but many families were about equally sensitive to a range of factors.

To date, development and testing of SIGNAL have focussed on the south-eastern coastal drainage division. Consequently, SIGNAL grades have not been defined for many families that are rare or absent in the south-east. The uncertainty surrounding application of Chessman's (1995) scheme to northern, western and inland Australia (e.g. Bunn and Davies 2000) has made it difficult to use the index nationally, either on its own or as part of AUSRIVAS. And SIGNAL has been of little use for river bioassessment by groups of community volunteers, such as those under the national Waterwatch umbrella (Chalkley *et al.* 1999). SIGNAL has operated only at the taxonomic level of family, whereas community groups seldom take identification below order level.

This paper describes a project designed to broaden SIGNAL's applicability by deriving grades at the family and higher levels for 210 taxa from the whole of Australia. The NRHP provided the data needed to derive SIGNAL grades across the continent. These data comprise counts of individuals of macroinvertebrate taxa (mostly families) for more than 15 000 samples collected from defined mesohabitats (e.g. riffles, edgewater and macrophyte beds) at over 4000 sites in spring and autumn during the years 1994–2000, together with a range of environmental measurements.

Materials and methods

A modification of the objective method of Chessman *et al.* (1997) was used to derive pollution sensitivity grade numbers from NRHP data provided by State and Territory government agencies. This method requires data sets in which the dominant gradients are those resulting from human disturbance rather than natural spatial and temporal variation. In order to reduce the influence of natural gradients, analyses were done separately for specific combinations of geographic region, mesohabitat and season. Australia was divided into 24 regions for this purpose (Appendix 1). Most States were subdivided according to published distributional analyses (Turak *et al.* 1999; Wells *et al.* 2002) and the advice of State agency biologists. The smallest jurisdictions (the ACT and Tasmania) were not subdivided, and the Northern Territory was kept as a whole because of the relatively small amount of data and lack of prior biogeographic analyses. Amalgamation of data from different States and Territories was not considered because adjacent jurisdictions often used somewhat different methods of sampling and sample processing. Thus the 24 regions were defined by a combination of natural and political boundaries. Data from the initial sampling rounds (1994–1996) were excluded from this component of the analysis, because this period was dominated by assessment of reference sites with lesser degrees of human disturbance (Davies 2000).

Most taxa in the NRHP data sets were families but chironomids were always split into subfamilies. For Odonata the recent family redefinitions summarized by Hawking and Theischinger (1999) had generally not been used. The following taxa were not taken to family in at least one State or Territory, and in many cases everywhere: Acarina, Amphipoda, Branchiura, Bryozoa, Cladocera, Collembola, Conchostrecha, Copepoda, Diplopoda, Hirudinea, Isopoda, Nemertea, Nematoda, Oligochaeta, Ostracoda, Polychaeta, Porifera, Rotifera, Tardigrada, Temnocephala and Turbellaria. Family grades were derived for whatever

familial and sub-familial taxa were included in the data sets from each jurisdiction.

Each data set for a particular combination of geographic region, mesohabitat and season was treated as follows. Firstly, rank correlation coefficients were calculated between scores on the original version of SIGNAL (hereafter SIGNAL1) and the abundances of each taxon across all samples in the data set. Since rare taxa with few occurrences cannot possibly achieve a large positive or negative correlation (Chessman *et al.* 1997), each correlation coefficient was expressed as a proportion of the maximum correlation that is mathematically possible for a taxon recorded from the same proportion of samples. The adjusted correlation coefficients were then used to assign grades to the taxa. The taxon with the highest positive adjusted correlation was assigned a grade of 10 and the taxon with the lowest negative adjusted correlation was assigned a grade of 1. The other taxa were scaled between these extremes in proportion to their correlation coefficients. The assigned grades were then used to derive revised sample scores. This process was repeated up to 40 times until grades stabilized. Sets of overall grades for each of the 24 regions were derived by averaging grades obtained from the individual data sets. Average grades were rescaled to range from 1 to 10. Finally, grades were averaged across the 24 regions, and the averages were rescaled to range from 1 to 10. Standard errors were calculated for the grades of those taxa that were recorded from more than one region, in order to provide a measure of confidence in the averaged national SIGNAL2 grades.

In order to develop grade sets for use by community groups, NRHP data were amalgamated into those higher taxa that are characteristically distinguished by such groups: mainly orders plus some phyla and classes, and one sub-class (Branchiura). Order/class/phylum (OCP) grades were derived from the amalgamated data in the same manner as described above. A few common but small-bodied taxa (Cladocera, Copepoda, Rotifera and Tardigrada) were recorded in some States but ignored in most. These taxa were excluded from the OCP grades. Polychaeta occurred rarely in the NRHP data and were excluded from the OCP grades because the class is essentially marine and estuarine.

In order to assess the behaviour of SIGNAL2, index scores were calculated from both the family and OCP grades. Since the NRHP data sets included non-family taxa, it was necessary to use both family and OCP grades to calculate 'family' scores. SIGNAL2 scores were calculated as count-weighted means of the grades of the taxa present in each sample. To prevent this calculation from being overwhelmed by the most frequent taxa, taxon counts were first transformed to the fourth root, except for Queensland data. Queensland counts had been limited to a maximum of 10 specimens per taxon, and therefore a less severe square root transformation was used.

Both family and OCP scores were correlated with several water-quality variables that were measured by agency staff in all or the great majority of regions (Table 1). These variables reflect widespread human impacts on Australian rivers such as eutrophication, salinization, erosion and sedimentation (Anon. 2001b; Ball *et al.* 2001; Harris 2001; Jolly *et al.* 2001; Prosser *et al.* 2001). Correlations were also calculated for two natural variables that were recorded in most regions and are well known to associate strongly with patterns of stream macroinvertebrate communities: altitude and distance from the source of the river. Values of water-quality variables that were below analytical detection limits were converted to half the limit for correlation analysis and production of scatterplots. Those variables that had highly skewed distributions were logarithmically transformed before correlation analysis.

Results

SIGNAL2 grades were derived for 171 families and six chironomid subfamilies (Appendix 2) and 33 higher taxa (Appendix 3). Standard errors of grades were generally less

Table 1. Coefficients of Pearson correlations between environmental variables and SIGNAL2 scores at the family and order/class/phylum (OCP) levels

Correlations are based on individual samples from various seasons, habitats and regions. All correlations are highly significant ($P < 0.001$)

Variable	Family	OCP
Altitude	0.28	0.27
Distance from the source ^A	-0.36	-0.25
Water temperature	-0.42	-0.34
Turbidity ^A	-0.36	-0.30
Electrical conductivity ^A	-0.55	-0.46
Alkalinity ^A	-0.50	-0.44
pH	-0.32	-0.25
Dissolved oxygen	0.37	0.33
Total nitrogen ^A	-0.50	-0.45
Total phosphorus ^A	-0.49	-0.46

^A Variable logarithmically transformed before analysis.

than one unit; the higher standard errors were usually for the rarer taxa. Most of the higher grades were assigned to the orders Ephemeroptera, Plecoptera and Trichoptera and their constituent families. At the OCP level, many taxa had grades in the range 1–3.

Correlations of SIGNAL2 scores with environmental variables were weaker for the OCP version than for the family version (Table 1). On average, SIGNAL2 scores were most strongly correlated (in a negative direction) with salinity indicators (electrical conductivity and alkalinity) and nutrients (total nitrogen and phosphorus). SIGNAL2 scores had a positive correlation with site elevation and a negative correlation with distance from the source (Table 1). The relationships between SIGNAL2 scores and environmental variables often followed a triangular pattern, whereby scores were variable at one extreme of the range of the environmental variable, but constrained to a narrow band at the opposite extreme (Figs 1 and 2).

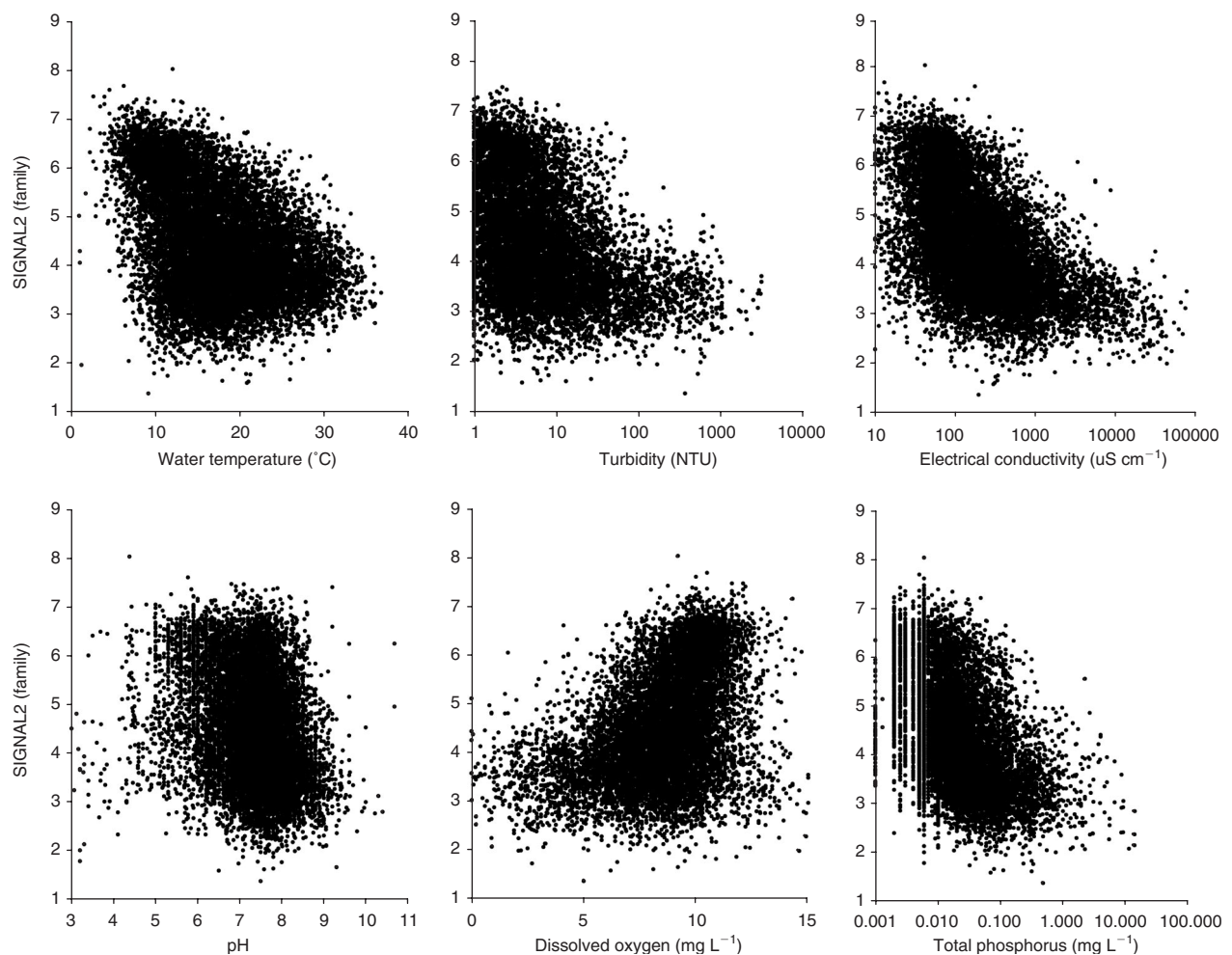


Fig. 1. Relationships between SIGNAL2 scores (family version) and selected water-quality variables. Data are for individual samples from various seasons, habitats and regions.

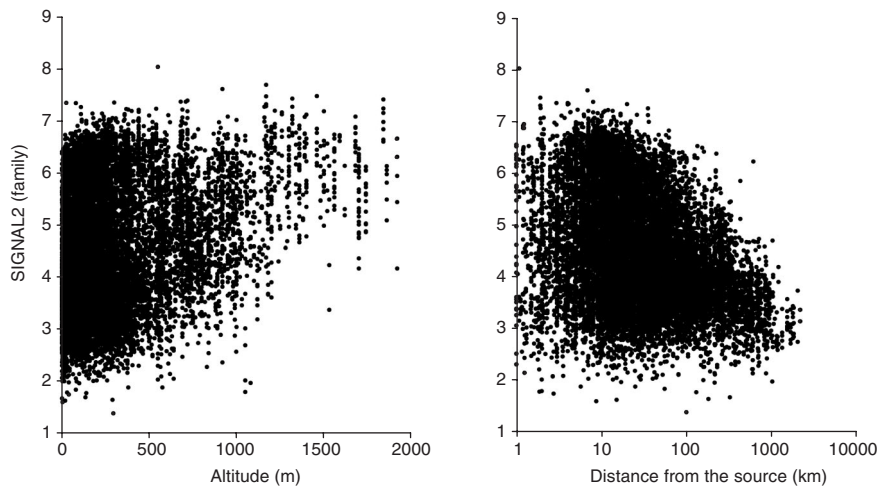


Fig. 2. Relationships between SIGNAL2 scores (family version) and site altitude and distance from the source of the river. Data are for individual samples from various seasons, habitats and regions.

Discussion

The relationships of SIGNAL2 to the water-quality variables imply that SIGNAL2 scores are limited by multiple factors. Low SIGNAL2 scores occurred across the entire range of each water-quality variable, but high SIGNAL2 scores occurred only when temperature, turbidity, conductivity, alkalinity and nutrient concentrations were low, and dissolved oxygen was high (Table 1, Fig. 1). Thus a high score is indicative of cool water with low quantities of suspended and dissolved substances, but a low score may indicate any of several kinds of physical and chemical enrichment or contamination.

Low SIGNAL2 scores were seldom found at high altitudes, but were the norm in rivers far from their sources (Fig. 2). However, the converse was not true; rivers at low altitudes did not necessarily have low scores and rivers close to their sources did not necessarily have high scores. Probably several factors are responsible for these relationships. High-elevation areas in Australia are often conserved as national parks or state forests, and usually lack major pollution sources other than localized ski villages, old mine sites and roads. Accumulation of salts and nutrients is uncommon in upland areas because of weathering and leaching by relatively high rainfall on steeper gradients. Rivers remote from their sources are inevitably in flat landscapes and are highly likely to have multiple human disturbances in their catchments. In addition, such rivers are more turbid and saline because they flow through depositional terrain where intrusion of saline groundwater is naturally common and often accentuated by agricultural activities (Hart and McKelvie 1986).

At a continental scale, the water-quality variables with which SIGNAL2 is correlated vary enormously (see abscissae in Fig. 1). Such variation arises both naturally and as a result of human activity. Consequently, raw SIGNAL2 scores

cannot be used as an indicator of human impact at large spatial scales. Many taxa with the high grades are adapted to the cool, perennial streams of the south-east. Such taxa cannot survive in the naturally warmer, non-perennial and more physically and chemically enriched rivers that characterize most of the remainder of the continent. For example, Boulton *et al.* (2000) noted that few of the families with the highest SIGNAL1 grades occur in intermittent streams, even if they are in near-pristine condition. The comparison of raw scores is applicable only at smaller spatial scales, for example to contrast disturbed and pristine sites within a district or to compare sites upstream and downstream of a wastewater discharge (cf. Metzeling *et al.* 2003). Any such comparison should be made for the same season and habitats.

One option for dealing with natural variation is to use AUSRIVAS to generate site, season and habitat-specific reference SIGNAL2 scores. Table 2 shows how this can be done, by means of a hypothetical example. The reference score is calculated as a weighted average of the grades of all families (and other taxa) predicted by the relevant AUSRIVAS computer model. The predicted probabilities of occurrence are used for weighting. This reference score can be compared with the actual score. Because AUSRIVAS predicts only probability of collection, not abundance, the actual score should not be abundance-weighted. It should be calculated as a simple arithmetic average of the grades of all taxa collected.

A limitation of this approach arises because large tracts of Australia are affected by multiple, broad-scale human disturbances (Cocks 1992; Cullen and Lake 1995; Kingsford 2000; Rutherford 2000; Schofield *et al.* 2000; Ball *et al.* 2001). As a consequence, pristine examples no longer exist for many river types. AUSRIVAS therefore relies on 'least disturbed' reference sites (Davies 2000). In practice, it seems that only

Table 2. Simplified hypothetical example of the suggested use of AUSRIVAS computer model outputs to calculate a predicted SIGNAL2 score

Eight taxa (families A–H) have non-zero probabilities of collection according to model outputs. Of these, four (A, D, E and F) are actually collected. Two families (I and J) have zero probabilities, and of these, one is actually collected (family J). The expected score is obtained by multiplying the grade of each taxon by its probability of collection, summing the products, and dividing by the sum of the probabilities. In this case, the expected score is $24.4/4.0 = 6.1$. The observed score is the sum of the grades of the families collected divided by the number of families collected, i.e. $(5 + 7 + 8 + 4 + 1)/5 = 25/5 = 5.0$

Taxon	SIGNAL2 grade	Probability of collection	Grade \times probability	Taxon collected?
Family A	5	1.0	5.0	Yes
Family B	3	0.8	2.4	No
Family C	10	0.6	6.0	No
Family D	7	0.6	4.2	Yes
Family E	8	0.5	4.0	Yes
Family F	4	0.3	1.2	Yes
Family G	7	0.1	0.7	No
Family H	9	0.1	0.9	No
Family I	5	0.0	0.0	No
Family J	1	0.0	0.0	Yes
Sum		4.0	24.4	

certain types of disturbance are excluded when ‘least disturbed’ sites are chosen, possibly because these disturbances are more conspicuous or less widespread. For example, in New South Wales, Turak *et al.* (1999) considered that ‘in an area where all large rivers have dams in their upper reaches as well as some grazing, the reference sites would be selected primarily on the basis of riparian zone integrity and absence of major point sources of pollution’. Many AUSRIVAS reference sites in New South Wales are affected by disturbances such as geomorphic change since European settlement, altered flow regimes, cold water releases from the hypolimnetic zone of reservoirs, diffuse runoff and groundwater discharge from agricultural and urban areas and the presence of alien fish, invertebrate and plant species (B. Chessman, personal observation).

Because many AUSRIVAS reference sites are subject to human disturbance, use of AUSRIVAS outputs will probably underestimate natural SIGNAL2 scores. The degree of underestimation is likely to be low for regions or river types for which near-pristine examples remain, but could be substantial in areas with extensive and severe disturbance, such as the sheep-wheat belts of south-eastern and south-western Australia (see, for example, Kay *et al.* 2001). An additional limitation is that AUSRIVAS does not operate at the order/class/phylum level, and so cannot be used to set reference values for the OCP version of SIGNAL2. An alternative approach is to rate SIGNAL scores differently in different regions (Newall and Wells 2000). For example, in Victoria Ladson and White (1999) rated a SIGNAL1 score of 7 in

upland river reaches as equivalent to 6 in lowland reaches, 6 in uplands as equivalent to 5 in lowlands, and so on. The draft State Environment Protection Policy (Waters of Victoria) sets different SIGNAL1 objectives for different regions and habitats (Anon. 2001a).

Although SIGNAL2 is sensitive to a wide range of water-quality characteristics, and consequently to a wide range of human disturbances, no single index can be expected to mirror all types of human impact on river systems. Consequently, SIGNAL2 should be used as part of a suite of bioassessment metrics, covering macroinvertebrates and other key groups of biota (Chessman 2002). The OCP version was not as closely related to water quality as the family version, but the difference was not huge (Table 1). Therefore it seems that SIGNAL2 can be usefully, if less accurately, applied at the order/class/phylum level favoured for assessment by community groups.

Acknowledgments

The development of SIGNAL2 was funded by Land and Water Australia and Environment Australia as part of the National River Health Program. I am grateful to Peter Davies, NRHP co-ordinator, for his support of this project, and to the following people and agencies for providing the raw data: Greg Keen, Department of Urban Services, ACT; Eren Turak and Grant Hose, Environment Protection Authority, NSW; Jane Suggitt and Peter Dostine, Lands, Planning and Environment, NT; Satish Choy and Diane Conrick, Natural Resources and Mines, Qld; Peter Goonan, Environment Protection Authority, SA; Tom Krasniski, Department of Primary Industries and Fisheries, Tas.; Leon Metzeling and Ros StClair, Environment Protection Authority, Vic.; and Stuart Halse and Winston Kay, Department of Conservation and Land Management, WA. I thank Bruce Gray, Environment Australia, Peter Goonan, Stuart Halse and Leon Metzeling for comments on a draft manuscript.

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Appendix 1. Australian regions defined for the derivation of SIGNAL2 grades

Region	Description
Australian Capital Territory	Murrumbidgee River basin above Lake Burrinjuck in Murray-Darling drainage division
New South Wales eastern plains	New South Wales part of South-east Coast drainage division below 100 m elevation
New South Wales eastern slopes	New South Wales part of South-east Coast drainage division from 100 to 1000 m elevation
New South Wales high country	New South Wales part of Murray-Darling and South-east Coast drainage divisions above 1000 m elevation
New South Wales western plains	New South Wales part of Murray-Darling drainage division below 200 m elevation
New South Wales western slopes	New South Wales part of Murray-Darling drainage division from 200 to 1000 m elevation
Queensland central coast	Basins 20–35 in North-east Coast drainage division
Queensland Darling basin	Queensland part of Murray-Darling drainage division
Queensland gulf and cape	Basins 1–6 in North-east Coast drainage division and Queensland part of Gulf of Carpentaria drainage division
Queensland internal basins	Queensland parts of Lake Eyre and Bulloo-Bancannia drainage divisions
Queensland south-east	Basins 36–46 in North-east Coast drainage division
Queensland wet tropics	Basins 7–19 in North-east Coast drainage division
South Australian north	South Australian parts of Lake Eyre and Western Plateau drainage divisions 10 and 12
South Australian south	South Australian Gulf drainage division and South Australian parts of Murray-Darling and South-east Coast drainage divisions
Tasmania	Tasmanian drainage division
Victoria region 1	See Wells <i>et al.</i> (2002)
Victoria region 2	See Wells <i>et al.</i> (2002)
Victoria region 3	See Wells <i>et al.</i> (2002)
Victoria region 4	See Wells <i>et al.</i> (2002)
Victoria region 5 (north-draining part)	See Wells <i>et al.</i> (2002)
Victoria region 5 (south-draining part)	See Wells <i>et al.</i> (2002)
Western Australian north	Indian Ocean drainage division and Western Australian part of Timor Sea drainage division
Western Australian south	South-west Coast drainage division
Northern Territory	Northern Territory parts of Gulf of Carpentaria and Timor Sea drainage divisions

Appendix 2. SIGNAL2 grades for macroinvertebrate families and subfamilies

Standard errors are given for all taxa recorded from more than one region

Taxon	Grade	Standard error of grade	Taxon	Grade	Standard error of grade	Taxon	Grade	Standard error of grade
Aeshnidae (<i>sensu lato</i>)	4	0.6	Gordiidae	5	0.6	Paracalliopidae	3	1.3
Ameletopsidae	7	1.2	Grapsidae	7	3.2	Paramelitidae	4	0.3
Amphisopidae	1		Gripopterygidae	8	0.4	Parastacidae	4	0.4
Ancylidae	4	0.4	Gyrinidae	4	0.4	Pelecorynchidae	10	
Antipodoeciidae	8	1.4	Haliplidae	2	0.6	Perthidae	4	0.9
Aphroteniinae	8	0.7	Hebridae	3	0.6	Philopotamidae	8	0.4
Athericidae	8	0.4	Helicophidae	10	0.3	Philorheithridae	8	0.7
Atriplectididae	7	0.5	Helicopsychidae	8	0.5	Phreatoicidae	4	0.7
Atyidae	3	0.5	Hemicorduliidae	5	1.1	Phreatoicopsidae	2	
Austrocorduliidae	10		Heteroceridae	1	0.4	Physidae	1	0.3
Austroperlidae	10	0.3	Hydraenidae	3	0.3	Planorbidae	2	0.4
Baetidae	5	0.2	Hydridae	2	0.4	Pleididae	2	0.5
Belostomatidae	1	0.8	Hydrobiidae	4	0.4	Podonominae	6	0.6
Bithyniidae	3	1.8	Hydrobiosidae	8	0.3	Polycentropodidae	7	0.5
Blephariceridae	10	0.3	Hydrochidae	4	0.9	Pomatiopsidae	1	0.9
Branchipodidae	1	0.3	Hydrometridae	3	0.5	Prosopistomatidae	4	
Brentidae	3	0.8	Hydrophilidae	2	0.2	Protoneuridae	4	0.6
Caenidae	4	0.4	Hydropsychidae	6	0.3	Psephenidae	6	0.4
Calamoceratidae	7	0.5	Hydroptilidae	4	0.4	Psychodidae	3	0.6
Calocidae	9	0.3	Hygrobiiidae	1	1.3	Ptiliidae	3	0.5
Carabidae	3	0.8	Hymenosomatidae	3	1.2	Ptilodactylidae	10	0.4
Cecidomyiidae	1	1.5	Hypolestidae (formerly	9	1.4	Pyrilidae	3	0.5
Ceinidae	2	0.7	Lestoideidae)			Richardsonianidae	4	0.6
Ceratopogonidae	4	0.2	Hyrriidae	5	0.8	Saldidae	1	0.8
Chaoboridae	2	0.8	Isostictidae	3	0.3	Scatopsidae	1	2.1
Chironominae	3	0.2	Janiridae	3	0.9	Sciaridae	6	2.3
Chrysomelidae	2	0.8	Kokiriidae	3		Sciomyzidae	2	0.8
Cirolanidae	2	0.9	Koonungidae	1	2.1	Scirtidae	6	0.3
Clavidae	3	1.8	Leptoceridae	6	0.2	Sialidae	5	0.7
Coenagrionidae	2	0.5	Leptophlebiidae	8	0.3	Simuliidae	5	0.3
Coloburiscidae	8	0.6	Lestidae	1	0.6	Siphonuridae	10	0.9
Conoesucidae	7	0.7	Libellulidae (<i>sensu lato</i>)	4	0.4	Siphonotidae	6	0.8
Corbiculidae	4	0.4	Limnephilidae	8	0.6	Sisyridae	3	0.8
Corduliidae (<i>sensu lato</i>)	5	0.4	Limnichidae	4	1.0	Sphaeriidae	5	0.5
Corixidae	2	0.4	Lindeniidae	3		Sphaeromatidae	1	1.3
Corophiidae	4	0.8	Lymnaeidae	1	0.5	Spongillidae	3	0.9
Corydalidae	7	0.5	Macromiidae	8		Staphylinidae	3	0.3
Culicidae	1	0.3	Megapodagrionidae	5	0.6	Stratiomyidae	2	0.3
Curculionidae	2	0.6	Melitidae	7	2.7	Sundatelpusidae	3	1.1
Diamesinae	6	0.4	Mesamphisopidae	3		Synlestidae (<i>sensu lato</i>)	7	0.7
Diphlebiidae (formerly	6	1.0	Mesoveliidae	2	0.4	Synthemistidae	2	0.9
Amphipterygidae)			Microsporidae	7	1.0	Syrphidae	2	0.9
Dipseudopsidae	9	1.3	Muscidae	1	0.6	Tabanidae	3	0.2
Dixidae	7	0.5	Nannochoristidae	9	0.5	Talitridae	3	0.7
Dolichopodidae	3	0.3	Naucoridae	2	0.7	Tanyderidae	6	1.3
Dugesiidae	2	0.3	Neoniphargidae	4	1.4	Tanypodinae	4	0.3
Dytiscidae	2	0.2	Nepidae	3	0.6	Tasimiidae	8	0.5
Ecnomidae	4	0.3	Neurorthidae	9	0.6	Telephlebiidae	9	1.8
Elmidae	7	0.3	Noteridae	4	0.7	Teloganodidae (formerly	9	0.4
Empididae	5	0.3	Notonectidae	1	0.4	Ephemerellidae)		
Ephydriidae	2	0.6	Notonemouridae	6	0.4	Temnocephalidae	5	0.6
Erpobdellidae	1	0.6	Ochteridae	2	1.0	Tetrastemmatidae	7	0.4
Eusiridae	7	0.8	Odontoceridae	7	0.5	Thaumaleidae	7	0.9
Eustheniidae	10	0.2	Oconesidae	8		Thiaridae	4	0.7
Gelastocoridae	5	0.7	Oniscidae	2	0.7	Tipulidae	5	0.3
Gerridae	4	0.4	Oniscigastridae	8	0.5	Triopsidae	1	0.1
Glacidorbidae	5	2.1	Ornithobdellidae	1		Urothemistidae	1	
Glossiphoniidae	1	0.5	Orthoclaidiinae	4	0.3	Veliidae	3	0.3
Glossosomatidae	9	0.2	Osmyliidae	7	1.1	Viviparidae	4	1.1
Gomphidae (<i>sensu lato</i>)	5	0.4	Palaemonidae	4	0.5			

**Appendix 3. SIGNAL2 grades for macroinvertebrate orders,
subclasses, classes and phyla**

Standard errors are given for all taxa

Taxon	Grade	Standard error of grade
Acarina	6	0.3
Amphipoda	3	0.6
Anaspidacea	6	0.2
Anostraca	1	0.5
Bivalvia	3	0.6
Branchiura	1	3.0
Bryozoa	4	5.2
Coleoptera	5	0.4
Collembola	1	0.6
Conchostraca	1	0.6
Decapoda	4	0.5
Diplopoda	4	1.5
Diptera	3	0.3
Ephemeroptera	9	0.3
Gastropoda	1	0.5
Hemiptera	2	0.5
Hirudinea	1	0.7
Hydrozoa	1	0.7
Isopoda	2	0.7
Lepidoptera	2	0.5
Mecoptera	10	0.7
Megaloptera	8	0.6
Nematoda	3	0.8
Nemertea	3	0.9
Neuroptera	6	0.8
Nematomorpha	6	0.7
Notostraca	1	0.4
Odonata	3	0.4
Oligochaeta	2	0.4
Plecoptera	10	0.3
Porifera	4	1.3
Trichoptera	8	0.3
Turbellaria	2	0.3