

Appendix B

Literature Review: QA/QC Methodology for Rapid Bioassessment Programs

National River Health Program
**AusRivAS Quality Assurance and
Quality Control Project**

Literature Review
**QA/QC methodology for rapid
bioassessment programs**



for

Australian Government
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Heritage**

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Foreword

WATER ECOscience was engaged by the Department of the Environment and Heritage to undertake a comprehensive study of Quality Assurance / Quality Control (QA/QC) issues and procedures associated with the Australian AusRivAS program.

One component of this study involves a review of available literature dealing with QA/QC methodology and criteria associated with the physical collection of site biological and environmental data, the identification of macroinvertebrates and data processing and storage.

This document reviews such bioassessment programs from a number of countries, assessing and comparing their QA/QC methods and procedures to those used in the AusRivAS program. Physical habitat assessment QA/QC procedures within the AusRivAS program have also been collated and reviewed with particular emphasis given to data used as predictor variables in AusRivAS models.

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Summary

This literature review was undertaken as part of the AusRivAS Quality Assurance and Quality Control Project. The review examines QA/QC methods used overseas and their applicability to Australian conditions and the Australian River Assessment System (known as “AusRivAS”).

The three major national programs for rapid bioassessment of river health practiced in the UK, USA and Canada are summarised and compared with AusRivAS in the key areas of:

- Selection of reference and test sites;
- Sampling procedures;
- Habitat assessment;
- Macroinvertebrate collection and identification; and
- Quality assurance and quality control for data collection and sample processing.

The review provides a comprehensive overview of QA/QC practices and concludes that Australian AusRivAS QA/QC is comparable with, or better than, overseas practices. However, due to the degree of independent development by each state / territory lead agency there are often substantial differences in the level of documentation and application of QA/QC practices. A consistent approach is important for a national program such as AusRivAS.

Future research and development needs were also identified, with specific QA/QC recommendations incorporated into the other relevant components of this project.

1 Introduction

1.1 Rapid Bioassessment

Rapid biological assessment (ie. rapid bioassessment - RBA) can be used to describe two very different types of biological monitoring (Norris and Norris 1995). The first is a continual monitoring situation to detect trigger or alarm levels of organisms or toxicants. The second, and the subject of this review, refers to expeditious sampling of biota with rapid delivery of assessment results (Norris and Norris 1995). Benthic macroinvertebrate rapid bioassessment techniques and procedures were developed in 1977 in conjunction with the commencement of the RIVPACS program in October of the same year (Wright 1997, Davies 2001 *pers comm*). The United States Environmental Protection Authority further developed and expanded the RBA techniques to include fish and the work of Plafkin *et al.* (1989) was later used by several other countries (eg. Australia and Canada) to develop their own rapid bioassessment procedures (Norris and Norris 1995).

Rapid bioassessment offers several advantages over the more traditional macroinvertebrate sampling methods, which involve a relatively large expenditure of time to collect, process and identify biological samples (Lenat & Eaton 1991). Rapid bioassessment reduces sampling effort, and therefore cost, by taking a relatively large sample instead of several individual replicates and reduces the number of organisms that must be processed by using a standardised sub-sampling procedure. In addition, rapid bioassessment programs often employ more efficient methods of data analysis than traditional biological assessment programs and produce results that are presented and summarised in a manner readily understood by non-specialists (Resh *et al.* 1995).

Although rapid bioassessment of freshwater systems is now used in a number of different countries, only the United States, the United Kingdom, Canada and Australia conduct integrated, large-scale programs using comprehensive models that integrate macroinvertebrate and physico-chemical data to compare test sites to a benchmark or reference condition.

The bioassessment programs used by these four countries vary in the extent to which they are applied and in their base method. However, all are based on similar theory and all require established and documented quality assurance and quality control systems to ensure that the integrity and veracity of the models used, and the results they produce, are maintained.

This review concentrates on the Quality Assurance/ Quality Control systems for bioassessment programs employed in the USA, Canada and the UK, as the continental scale of these programs make them most relevant to Australia.

1.2 AusRivAS- National and International Context

The Australian River Assessment System (known as “AusRivAS”) was developed by the Cooperative Research Centre for Freshwater Ecology, in partnership with state and territory river management agencies, under the auspices of the National River Health Program (NRHP) funded by the Commonwealth Government. The NRHP was established in the Prime Minister’s Environment Statement in 1992 (O’Connor *et al.* 1996).

The objectives of National River Health Program are to:

- provide a sound information base on which to establish environmental flows;
- undertake a comprehensive assessment of the health of inland waters, identify key areas for the maintenance of aquatic and riparian health and biodiversity, and identify stressed inland waters;
- consolidate and apply techniques for improving the health of inland waters, particularly those identified as stressed;
- develop community, industry and management expertise in sustainable water resources management and raise awareness of environmental health issues and the needs of our rivers.

The NRHP, initially called the National River Processes and Management Program commenced in December 1992 (Davies 1994; O’Connor *et al.* 1996). The Monitoring River Health Initiative (MHRI) – a key component of the NRHP – used aquatic invertebrates to assess on a national level the ecological condition of Australian rivers (Smith & Kay 1998). As part of the MHRI more than 1500 reference sites were sampled across all states and territories during 1994/96 to establish the predictive AusRivAS models.

The second phase of the NRHP utilised the AusRivAS models to undertake the First National Assessment of River Health (FNARH) (Smith & Kay 1998), later referred to as the Australia-wide Assessment of River Health (AWARH). The FNARH - AWARH commenced in 1997 and nearly 6000 sites have been assessed nation-wide. Australia is the first country in the world to undertake such a continental-scale assessment of the ecological health of its rivers (PIE 1998).

The AusRivAS models are based on the RIVPACS models (River InVertebrate Prediction And Classification System) developed at the Institute of Freshwater Ecology (IFE) in the United Kingdom (Wright 1995).

Although AusRivAS models are based on British models, the appreciable differences between the two countries in factors that influence stream ecology make comparison difficult. Australia is the driest inhabited continent, with much of its low relief landscape subject to high temperatures, high evaporation and low precipitation (Lake 1995). River discharges are usually low and temporary (intermittent) streams are a prominent feature of the landscape. Australian streams are generally more variable in their flow and have mean peak annual floods an order of magnitude higher than the world stream average (McMahon 1982a cited in Lake *et al.* 1985).

Despite these differences, the UK RIVPACS approach was preferred over similar North American schemes because it used a regional framework, as opposed to uniform national standards, and had been successfully used to assess river

condition on a national scale (Smith *et al.* 1999). The objectives of both the AusRivAS and RIVPACS models and their associated sampling programs are to predict the composition of the macroinvertebrate community at a river site in the absence of any anthropogenic impacts (Expected taxa [E]) and compare this to the macroinvertebrate community actually present (Observed taxa [O]).

The current project, the *National River Health Program - AusRivAS Quality Assurance and Quality Control Project*, provides a national, external audit of data collected by the various state and territory government agencies using AusRivAS to assess river health in Australia. Information from this project augments previous quality assurance and quality control (QA/QC) work conducted under the NRHP and focuses on QA/QC during field operations, during subsequent laboratory sample processing and data entry, for both biological and environmental data associated with AusRivAS bioassessment for the NRHP.

1.3 Project Outline

The *National River Health Program - AusRivAS Quality Assurance and Quality Control Project* was part of the Toolbox component of the Australia-Wide Assessment of River Health and involved a national, external audit of data collected by the various state and territory agencies using AusRivAS to assess river health.

The objectives of the project were:

5. Assess and report on state agency performance for macroinvertebrate sampling, processing and identification procedures against existing criteria.
6. Develop criteria for assessing agency performance in the collection of environmental and habitat field data.
7. Assess and report on agency performance in the collection of environmental and habitat data and macroinvertebrate data.
8. Provide feedback and advice on the problems of staff performance in AusRivAS methods to state / territory agencies and to the new Training and Accreditation Project of AusRivAS.

The project includes liaison with two other toolbox projects:

- AusRivAS error analysis project - provide advice on actual error magnitudes in environmental data to enable evaluation of the consequences of errors associated with this type of data.
- Training and Accreditation Project - liaison with the Principal Investigator to ensure deficiencies detected in the implementation of AusRivAS methods by lead agency staff can be addressed in any proposed training program.

The *AusRivAS Quality Assurance and Quality Control Project* involves two types of audit, broadly categorised as: i) Veracity of macroinvertebrate sample processing and taxonomic identification, and ii) Collection and entry of field environmental (ie. habitat) data. This component of the project, the Literature Review, puts the AusRivAS program into an international context and compares the Quality Assurance and Quality Control procedures of the AusRivAS approach with that of similar programs.

Much of the available information concerning Australian and overseas bioassessment QA/QC programs is in the form of government reports, internet web pages and manuals produced by state and federal agencies not all of which has

been subjected to peer review or published in scientific journals. In this review, every effort was made to use the original source reference.

2 AusRivAS, BEAST, RIVPACS and RBP

2.1 Basics of each Program

AusRivAS is used throughout Australia, however state and territory agencies have developed different AusRivAS models that are specific to the environments found in their jurisdictions and their own needs. Consequently, AusRivAS protocols vary marginally across the country. Despite this variability, the different state and territory models are all based on the same modelling program and are collectively referred to as AusRivAS.

AusRivAS, like the RIVPACS system on which it is based, consist of a series of multivariate models that compare the biota of a test site to that at a number of relevant reference sites. The models produce scores based on the ratio of the number of observed macroinvertebrate taxa to the number of taxa expected in the absence of human impact (Coysh *et al.* 2000). Users of the program input macroinvertebrate, physico-chemical and habitat data - the specific variables depending on the model used (ie. area, season, habitat). The major habitat types recognised for routine sampling under the MRHI river bioassessment program are riffles, run, pool, rocks, macrophytes, edge / backwater and logs / sticks (Davies 1994). Soft or hard sediment of deep water pool / runs may also be sampled. Each state / territory has constructed models for single season data as well as data for combined seasons.

In contrast to the Australian system, the RIVPACS program involves sampling all major habitat types at a site in one composite sample, with different habitats sampled in proportion to their occurrence at each site (Murray-Bligh 1999a). As in Australia, the RIVPACS system is based on data collected in different seasons to ensure a comprehensive taxa list (Wright 1995, Davies 1994). The rationale behind habitat specific sampling in Australia is that each habitat has a characteristic macroinvertebrate community (Humphries *et al.* 1996) and, within a given region, differences between habitats at a site may be greater than differences between sites (Parsons & Norris 1996). Consequently, unless comparisons between sites are based on the same habitat types they may be confounded by habitat differences within a sample at each site (Parsons & Norris 1996).

RIVPACS is used throughout the United Kingdom and Northern Ireland. The first comprehensive RIVPACS program, referred to as RIVPACS II, was developed in 1990 and has been constantly refined, resulting in the RIVPACS III program that is currently in use (Murray-Bligh 1999a). RIVPACS provides a single biotic index of water quality, and is the basis of a number of other similar programs used around the world. The model, which requires water quality, habitat and macroinvertebrate datasets from representative habitats, can be applied at a variety of taxonomic resolutions. The RIVPACS model produces a number of outputs based on a variety of biotic indices which compare the macroinvertebrate fauna of the test site to what would be expected in the absence of environmental stress (Murray-Bligh 1999a).

BEAST (Benthic Assessment of Sediment), the system used in Canada, is again based on RIVPACS. The BEAST system was first used to assess the benthos in the Great Lakes (Reynoldson *et al.* 1995) and over time it has been refined and applied to lotic systems. It has been used in the bioassessment of the Fraser River catchment (Reece *et al.* 2001, Reynoldson *et al.* 2001) and in assessing the

impacts of mine effluent on the aquatic environment (Environment Canada 2001). BEAST, like RIVPACS uses the reference condition concept, and requires the measurement of a number of environmental variables in addition to macroinvertebrate community data. The main differences between this method and RIVPACS / AusRivAS involve the statistical methods employed to determine reference sites applicable to test sites (Barbour *et al.* 1999).

Rapid Bioassessment Protocols (RBP I, II and III) are used to varying degrees by water resource agencies throughout the USA, although the protocols may not always be applied to computer-based bioassessment models (Summary of State Bioassessment Programs, 1998). The majority of North American states using the bioassessment protocols apply them using a multimetric approach, although a multivariate approach is used in some states. The multivariate approach relies on statistical analyses to develop models for predicting expected conditions at other sites. The multivariate models are built from data collected at reference sites, whereas the multimetric approach is based on a number of different measurable indicators of human influence. Scores based on these indicators are compared to the distribution of scores from appropriate reference sites to determine the degree of human influence (<http://www.epa.gov/waterscience/biocriteria/multibio.html>).

Although the Canadian BEAST and Australian AusRivAS bioassessment programs are based on the United Kingdom RIVPACS system, differences in approach and application set them apart, with possibly the BEAST system having departed more from its RIVPACS heritage. The USA RBP systems, with their lack of uniformity across the country, are different again, further increasing the gamut of variability amongst the bioassessment programs of the four countries. Despite these differences, all of the programs are based on the same underlying premise: that aquatic macroinvertebrates can be used as indicators to assess water quality against a referential benchmark. This not only allows for the health of a particular site to be assessed, but also enables spatial comparison with sites in other river systems (where appropriate reference sites exist).

2.2 Selection of Reference and Test Sites

Reference Sites

One of the most successful outcomes of bioassessment programs has been the development of the Reference Condition Approach (RCA) (Rosenberg 1998). With this technique a group of minimally disturbed sites, related by selected physical, chemical and biological characteristics, are identified / established and used as a comparison for data collected from monitoring sites. AusRivAS, BEAST and RIVPACS all use similar methods and criteria in establishing reference sites.

The establishment of the reference condition is a significant development in biomonitoring because it solves the problem of trying to locate nearby or control reference sites when studying an impacted system. Instead of using upstream and downstream (and possibly before and after) reference sites in a river system, an array of biologically and physico-chemically similar, least impacted sites scattered throughout a catchment or region is used. Once the reference condition is established, any site suspected of being impacted can be assessed by comparing it to the reference data and its status determined (Rosenberg 1998).

In Australia it is accepted that most of the larger lowland rivers are subject to some degree of impact from land use practices (Marchant *et al.* 1994), rendering it difficult

or impossible to obtain reference sites in pristine condition. Accordingly, the development of a definitive protocol for the selection of Australian reference sites is not possible. Given these difficulties, the principle of 'least disturbed site selection', which recognises that a set of reference sites may include sites with a degree of disturbance, has been used as a compromise (Davies 1994). The choice of reference sites for developing the AusRivAS models involved singling out regions or catchments of priority concern in each state, determining the general characteristics of rivers in those regions and / or catchments, selecting reaches in each that had least human disturbance and then identifying and sampling sites within each reach (Davies 1994). The final set of reference sites selected was also examined after analysis of biological data from the reference sites was completed. The process employed to select the reference sites used in development of the AusRivAS model is outlined in Figure 1.

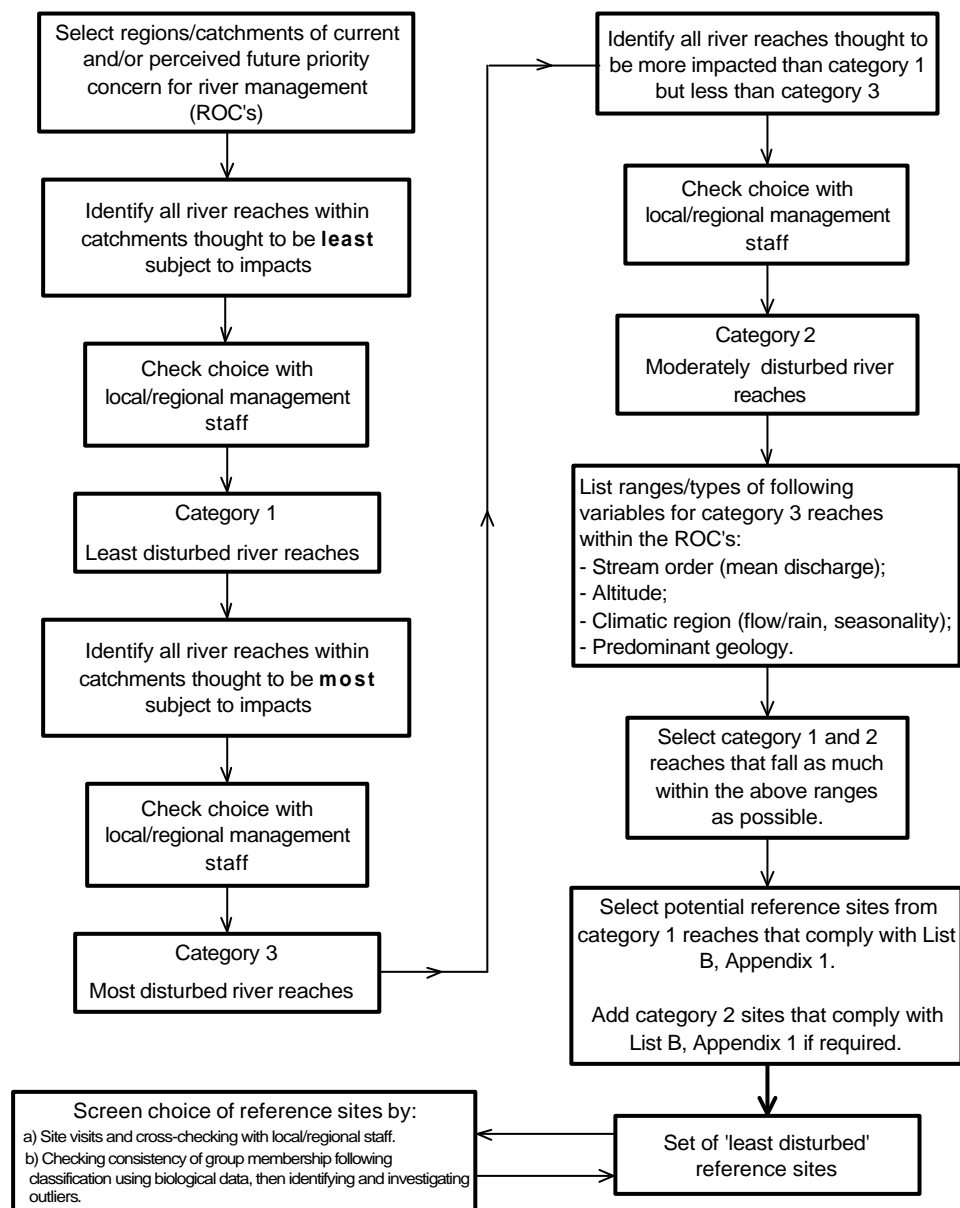


Figure 1 Decision tree used in Phase 1 of the NRHP to select reference sites for use in AusRivAS model development (Davies 1994; Davies 2001, pers. comm.).

In Britain, the selection of sites of high biological quality for the RIVPACS system was aided by discussions with local water industry biologists who had detailed knowledge of appropriate reference sites in their area (Wright 1994). The spatial relationship between reference and test sites is similar to those for AusRivAS in that they do not need to be on the same rivers, or even the same systems (Moss *et al.* 1999). The development of RIVPACS required a wide range of reference sites of high biological quality to ensure that the system met the standards, or targets, required for use in the assessment of site quality (Moss *et al.* 1999). With each successive version of RIVPACS, more stringent criteria were established for the acceptance of a site as a reference site. During later studies to develop alternate techniques for initial site classification and prediction, reference sites were located on rivers that were largely free from serious pollution and had a good record of physical and chemical conditions (Moss *et al.* 1999).

In Canada, reference sites are chosen to represent the area of interest, as currently models are created for specific areas or programs (Environment Canada 2001).

In the USA, two types of reference conditions are commonly used in biological surveys: regional reference (similar to that for AusRivAS and RIVPACS) and site-specific (Barbour *et al.* 1996). Site-specific reference conditions generally consist of measuring of conditions upstream of a discharge point or from a 'paired' waterbody. Regional reference conditions involve measurements from a population of relatively unimpaired sites within a homogenous region and habitat type (Barbour *et al.* 1999). The appropriateness of the regional reference approach versus the site-specific control approach is assessed by the responsible agency before inclusion into a specific program. In the situation where the bioassessment program is being used in an upstream-downstream study to assess impact sources, regional reference criteria may be less important if an unimpacted site-specific control site can be sampled. Alternatively, when a "snapshot" or trend monitoring survey is being conducted in a watershed or river basin, use of regional criteria may be the only means of assessing impact (Barbour *et al.* 1999).

Other criteria that have been used in selecting regional reference sites in the USA include:

- Drainage wholly within the eco-region of interest,
- No upstream impoundments,
- No known discharges or contaminants in place,
- No known spills or other pollution incidents,
- Low human population density,
- Low agricultural activity,
- Low road and highway density,
- Drainage is on public lands,
- Minimal non-point source problems (agriculture, mining, feedlots, logging etc),
- No known intensive fish stocking (Resh *et al.* 1995).

Test Sites

In Australia, Canada and some USA programs, *test* or *monitoring* sites can include sites with a known history of problems or where it is known that future events will either improve or adversely affect the waterbody (Coysh *et al.* 2000, Reynoldson *et al.* 2001).

In the USA this is referred to as *'targeted'* selection, where the selection of a site is relevant to specific studies focussing on particular issues. The other site selection method utilised in the majority of studies in the USA is *'probabilistic'* (random) selection, which provides information of the overall status or condition of the watershed, basin or region (Barbour *et al.* 1999).

In the UK, the underlying strategy is for sites to be positioned approximately 5, 10, 20, 30 and 40 kilometres downstream of the water source and at 20 kilometre intervals thereafter (Wright 1995). This approach takes into account that both environmental features and macroinvertebrate assemblages change more rapidly near the source of the river system. Industry biologists with extensive local knowledge also provide advice on the most appropriate locations for sampling sites.

In Australia, monitoring sites for the MRHI river bioassessment program included sites for which management actions to improve water quality and catchment health (eg. reduction of nutrient inputs from sewerage treatment plants) are planned (Davies 1994). The range of river types and conditions covered by monitoring sites was also incorporated into the selection of reference sites and preferably covered the entire range of river types in each state / territory (Davies 1994). In addition, sampling sites were selected through "a consultative process with the Department of the Environment and Heritage, other relevant state agencies, local governments, water management utilities, industry, catchment organisations and communities having regard to key river and catchment management issues and ensuring state-wide coverage of the assessment" (Bruce Gray, EA, *pers. comm.*).

2.3 Sampling Procedures

In Australia, each state / territory agency has devised its own manual of procedures relating to the conditions and habitats sampled in their region (AusRivAS 2001). The manuals vary in the degree of detail provided, but generally describe procedures for site selection, collection of environmental data and macroinvertebrate sorting and enumeration. In addition, the national protocol provides further generic guidance. The national manual, plus all state / territory manuals are available on the AusRivAS web site (<http://ausrivas.canberra.edu.au/macroinvertebrates/man/sampling>) and on the Commonwealth web site (<http://www.deh.gov.au/water/rivers/nrhp/monitoring.html#pro>).

The UK Environment Agency has a procedures manual detailing best practice for obtaining samples compatible with RIVPACS (Murray-Bligh 1999a). The handbook includes detailed equipment specifications and procedures for site selection, sampling, collection of environmental data (in the field and from maps) and macroinvertebrate sorting and enumeration.

In the USA, the EPA uses the Rapid Bioassessment Protocols (Plafkin *et al.* 1989) as the basis for sampling procedures. Individual agencies undertaking sampling for the EPA may modify these protocols for particular regions, however any changes must be approved by the US EPA in the form of a QA project plan (discussed in the QA/QC section below).

In Canada, the Metal Mining EEM Guidance Document (Environment Canada 2001) outlines recommended freshwater field methods for the sampling of benthic invertebrate communities to assess the effects of mine effluent on freshwater aquatic environments. Specific procedures are also recommended for the processing of samples in the laboratory. Recommendations encompass the sampling equipment required for different habitats, specimen preservation techniques, site environmental variables to be measured, laboratory methods and quality control procedures for field and laboratory operations. Quality control procedures for field and laboratory operations will be discussed in detail in later sections.

Habitat Assessment

It has been recognised for several decades that assessment of stream habitat quality is an important factor in the interpretation of biological data (Hynes 1970 cited in Resh *et al.* 1995). Incorporation of habitat data is an integral component of the majority of available bioassessment programs and models.

In the UK, Australia and Canada, certain habitat characteristics (predictor variables) are used to predict the fauna expected at a test site by matching it to appropriate reference sites. Each program measures different habitat characteristics and employs different methods. In Australia, both the methods used and the variables measured differ between the states and territories. Although the Canadian BEAST program recommends a number of habitat parameters for assessment (Environment Canada 2001), it is not an established national approach and other methods are also employed. Consequently, it is likely that habitat variables measured will differ across Canada (Reynoldson *et al.* 1995, Reynoldson *et al.* 2001). In the USA, habitat assessments are used in *a posteriori* analyses to assist with classification and interpretation of aquatic faunal data and to determine whether it is poor water quality or degraded habitat that is stressing the invertebrate community (Resh *et al.* 1995). To a lesser extent, habitat data may also be used in the USA in the prediction of biotic communities.

Each of the four countries documents the assessment method it employs, although some are more comprehensively detailed than others (UK: Murray-Bligh 1999a, Australia: AusRivAS and Commonwealth websites, Canada: Environment Canada 2001).

The agencies responsible for conducting bioassessment in the four countries all advocate assessing a range of environmental variables at each site in addition to those used in the model to assist in the interpretation of bioassessment model outputs. To this purpose each country except Canada has specific field sheets to be completed at each site (see AusRivAS website for most recent manuals including habitat assessment sheets, Barbour *et al.* 1999, Murray-Bligh 1999a). This approach ensures that all necessary information is recorded in a standardised (for each country) manner, rather than relying on field staff to remember to assess all potential issues.

Macroinvertebrate Collection and Identification

The AusRivAS predictive system and associated sampling methods have advantages over traditional assessment techniques in that the sampling methods are standardised, easy to perform, and require minimal equipment (Coysh *et al.* 2000). AusRivAS and other bioassessment systems such as RIVPACS, BEAST

and RBP have documented procedures for collecting and identifying macroinvertebrates. A number of reviews of the different methods used within the Australian states and territories have been conducted (Humphrey and Thurtell 1996 and WATER ECOscience, 2002) and have addressed quality control and quality assurance issues.

Overall, collection methods vary between the different country programs, with nets of different mesh size used, streams sampled using either standard distances or set time periods, macroinvertebrates picked in the field or in the laboratory, and macroinvertebrates identified to different taxonomic levels. Common to bioassessment programs in Australia, the UK, USA and Canada (lotic) is the use of a dip (pond) or kick net for sampling all habitat types. AusRivAS sampling programs involve the use of a 250 μ m mesh net over a cumulative distance of 10 metres, whilst in RIVPACS sampling a 900 μ m net is used for a total of three minutes at each site. A medium naturalist's dredge has also been used in RIVPACS sampling where the use of a pond net was inappropriate (Wright 1995). In a study of 30 different sampling protocols used across the USA, Resh and Jackson (1993) found that kick nets with a mesh size of 400-600 μ m were used in 75% of the studies. In the USA and some Australian states and territories, samples are live picked in the field (typically timed or a certain number of animals picked). In RIVPACS, samples are preserved in the field, with sorting and identification completed in the laboratory.

In Canada, the Metal Mining EEM Guidance Document (Environment Canada 2001) recommends different sampling approaches for different habitats. A Ponar or Ekman grab is suggested for depositional zones in freshwater lakes or rivers, a Neill-Hess cylinder type sampler or U-net sampler in erosional areas, and a kick net sampler in shallow lotic systems with good flow (Environment Canada 2001).

Within each country, variability in the bioassessment program is lower and only the level of taxonomic resolution used in the model differs to any degree. This relative uniformity makes each country's QA/QC methods relatively easy to document and adhere to. The level of taxonomic resolution used in the models is often determined by a combination of the study objectives and taxonomic knowledge, but for programs like AusRivAS there are only a limited number of models for genus or species level application. With RIVPACS prediction models, invertebrate identifications are usually taken to species level (where adequate keys were available), although a significant proportion of biological assessments in the UK are conducted at the Biological Monitoring Working Party (BMWP) family level (Wright 1995). With AusRivAS, macroinvertebrate identification is normally to family-level. A number of international and national studies have demonstrated that family level identification provides adequate taxonomic discrimination from which to group sites based on their macroinvertebrate communities (eg. Plafkin *et al.* 1989, Marchant *et al.* 1995, Hewlett 2000).

The level of taxonomic resolution (order, family, genera or species) in USA programs varies depending on the study objectives and the Rapid Bioassessment Protocol used. Rapid Bioassessment Protocol I, which involves a screening or reconnaissance assessment with limited data generation, uses order/family level identifications. Rapid Bioassessment Protocol II involves field collection and analysis of major taxa and utilises family level identifications. Rapid Bioassessment Protocol III involves systematic field collection and lab analysis to detect subtle impairments and uses the lowest possible taxonomic level (ie. genera or species) (Plafkin *et al.* 1989).

2.4 Quality Assurance and Quality Control (QA/QC)

General QA/QC Procedures

Effective quality assurance and quality control procedures and a clear delineation of QA/QC responsibilities are essential to ensure the validity of environmental monitoring data. However, as pointed out in Dines & Murray-Bligh (2000) quality assurance has only recently been applied to the collection of biological information. For the purposes of this discussion the term “*quality control*” refers to the routine application of procedures for measurement and control of errors in the monitoring and measurement process. The term “*quality assurance*” refers to the overall management strategy to ensure quality, including sampling, staff proficiency, laboratory facilities, equipment, methodologies, and materials; which make up an integrated QA program (OECD 1998). These two activities are referred to in combination simply as QA/QC.

Macroinvertebrate QA/QC has been defined by the Northern Territory’s Lands, Planning Environment as follows:

“Quality control / quality assurance procedures are designed to establish an acceptable standard of macroinvertebrate sorting and taxonomic identifications. The quality control component minimises the variation in sorting and identification efficiency. Quality assurance provides potential users with the assurance that the accuracy of results is within controlled and acceptable limits.”

QA/QC procedures are important features in all bioassessment programs and each of the models investigated has documented procedures, although the level of documentation varies between and within countries.

In Australia, each state and territory using AusRivAS for bioassessment have developed their own QA/QC procedures. These QA/QC procedures are outlined in the relevant methods manuals of the lead agency in each state / territory and include QA/QC procedures for field work, sample sorting, taxonomic identification and data entry / validation. The extent of documented QA/QC procedures, and the level of detail with which they are described, varies among the states / territories (see Table 1). This variation will be discussed in more detail in later sections.

The UK Environment Agency has adopted national QA procedures for macroinvertebrate sampling and analysis to ensure that data is of known and appropriate quality and suited for use in the various iterations of RIVPACS. Formal quality control systems are limited to laboratory analyses (in particular sorting and identification). However, the UK Environment Agency has produced detailed specifications for sampling and analytical equipment, together with standard methodologies for sample collection and analysis (Dines & Murray-Bligh 2000, Murray-Bligh 1999a, Murray-Bligh 1999b).

Table 1 Documentation of QA/QC procedures for Australian states and territories.

Unless indicated otherwise, specified information comes from AusRivAS manuals for each state. Level of detail in provided information varies widely between the states and territories. (X indicates that no procedures exist or that no information is available; P/C refers to physico-chemical; Biol refers to biological samples, and N/A refers to not applicable).

Documented Procedures	QLD	NSW	ACT	VIC	TAS	SA	WA	NT
Field sheet procedures (ie. Detailed instructions with field sheets)	✓	✓	✓	✓	✓	✓	✓	✓
<u>Field QA/QC (P/C)</u>								
- Site assessment	✓ ¹	✓ ²	✓ ¹	✓ ³	X	X	X	X
- Water sampling	✓	✓ ⁴	✓ ⁴	X ⁵	✓ ⁶	X ⁷	X ⁸	✓ ⁹
<u>Field QA/QC (Biol)</u>								
- Sample collection	X	X	X	X	X	X	X	X
- Live sorting	✓	✓	N/A	✓	✓	N/A	✓	N/A
<u>Laboratory QA/QC (Biol)</u>								
Sample sorting and/or Identification	✓	✓	✓	✓	✓	✓	✓	✓
Data entry/validation	✓	✓	X	✓ ¹⁰	X	X	✓	✓

Explanation of Superscript references:

Note	Comment
1	QA methods relate only to checking of field sheets to ensure that no measurements or tasks have been missed
2	Information obtained from Waddell (2001). Some visual / subjective assessments regularly checked using quantitative methods and comparison between different team leaders.
3	Second person reviews field readings and site position/elevation details
4	Information obtained from Waddell (2001). Detailed procedures on sample collection/ transport and calibration requirements for water quality measuring devices.
5	Manual states only that samples should be collected according to standard protocols.
6	Information on water sample collection and transport.
7	Details relate only to sample container requirements and sample transport procedures.
8	Manual states only that water sample bottles should be pre-washed with nutrient free detergent.
9	No procedures for sample collection, however, details procedures for specific analysis types; requirement to calibrate water quality measuring devices appropriate for the range of conditions expected and registration system for samples.
10	Information provided by Stephen Periss, EPA (<i>pers. comm.</i>). After data is entered, it is checked against data sheets. However, this is not documented in the Victorian RBA manual.

The USA EPA QA/QC program requires that all USA EPA national program offices, USA EPA regional offices, and USA EPA laboratories participate in a centrally planned, directed, and coordinated agency wide QA/QC program. This requirement also applies to efforts carried out by the states and interstate agencies that are supported by the EPA through grants, contracts, or other formalised agreements. Under the US EPA's QA/QC program, each office or laboratory generating data must implement, at a minimum, the prescribed procedures to ensure that precision, accuracy, completeness, comparability and representativeness of data are known and documented (Barbour *et al.* 1999). Each US agency has a designated QA/QC officer (or person in charge of the program) responsible for maintaining consistency among investigators. At regularly scheduled intervals, the QA/QC officer reassesses previously assessed sites and analysed data to determine whether reproducible results are being attained (Barbour *et al.* 1999).

Field Operations QA/QC

Habitat Assessment QA/QC

Despite extensive literature searches, very little information was unearthed concerning quality control and quality assurance for the collection of habitat data. This limited information suggests that the UK, Canada and the USA do not have specified QA/QC procedures for habitat assessments. WATER ECOscience, on behalf of the Department of the Environment and Heritage, is conducting an audit of all Australian state / territory agency field methods and laboratory procedures. This involves physical, environmental and chemical data collection, macroinvertebrate sample processing/identification and data entry procedures. This audit found that of the eight Australian state / territory lead agencies, only four (VIC, QLD, ACT and NSW) have established QA/QC procedures to audit habitat assessments (Table 1).

Australia, the UK and the USA have specific field sheets to be completed at each site (for the most recent Australian field sheets, refer to the AusRivAS website: <http://ausriv.as.canberra.edu.au/>). This is to ensure that all necessary information is recorded in a standardised (for each country) manner, rather than relying on field staff to remember to assess all potential issues.

Plafkin *et al.* (1989) indicates that the habitat description stage within the USA Rapid Bioassessment Protocols is predominantly a subjective evaluation and, as such, variability among operators will potentially influence the final conclusions. To minimise this, the protocols recommend that investigators should be appropriately trained in the evaluation techniques and, to encourage consistency among investigators, periodic cross checks should be conducted.

Murray-Bligh (1999a) also recognises that the surveyor or investigator can introduce systematic bias and recommends that whenever possible environmental data should be collected by more than one person on each sampling occasion, and the average values noted.

Sample Collection QA/QC

Documentation specifying protocols for the field collection of macroinvertebrate samples serves to standardise collection practices and minimise errors and variation in the sampling procedure. In broad terms, such documentation forms part of a sample collection QA/QC system. However, for fully effective quality assurance, it must be complemented with procedures to check that the sampling protocols are being correctly applied.

As outlined previously, formal quality control systems in the UK for macroinvertebrate samples collected in accordance with the standard methods for RIVPACS are limited to laboratory analyses (Murray-Bligh 1999b). The UK considers that achieving standards of quality control for field collection (including the collection of environmental data for RIVPACS) equivalent to that for sample analysis would be costly, impractical and require the collection and analysis of replicate samples by an auditor or AQC inspector (Murray-Bligh, 1999b). Consequently, quality assurance for sample collection for UK RIVPACS studies relies on procedures manual BT001 (“Procedures for collecting and analysing macroinvertebrate samples”), a training video and training workshops.

Murray-Bligh (1999b) indicates that documentation is considered an important component of quality assurance in the UK and stresses the importance of being able to identify the precise location of a sampling area, particularly by different staff.

This ensures that comparisons of samples reflect environmental changes rather than differences between sites.

The Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish (Barbour *et al.* 1999) used in the USA also outline quality control procedures appropriate for the collection of benthic macroinvertebrates. The protocols recommend that sample labels should be properly completed with all relevant details (eg. sample identification code, date, sampling location, stream name, collectors name), with the outside of the sample contained also displaying the same information. If required, chain-of-custody forms should also include the same information as the sample labels. In addition, when sampling is completed at a site all sampling equipment (eg. nets, pans, and trays) used to collect samples should be thoroughly rinsed and examined carefully. Any organisms retained on the equipment should be placed in the relevant sample container. All equipment should be checked prior to usage at the next site. To monitor the variability associated with the sampling technique or collection team, it is recommended that replicates should be collected from 10% of sites (Barbour *et al.* 1999).

In Canada, QA/QC procedures for benthic invertebrate field and laboratory operations to assess the effects of mine effluent on the aquatic environment are outlined in The Metal Mining EEM Guidance Document (Environment Canada 2001). The document recommends that QA/QC procedures for benthic invertebrate surveys be defined during development of the study design and should be followed exactly to ensure the collection of high quality data. Field sampling equipment should be specified in addition to any protocols appropriate to the study. While a QA/QC plan for field sampling can have many components, the main procedures recommended in the Metal Mining EEM document are:

- Field sampling should be undertaken by personnel who have appropriate training and experience with field equipment and objectives.
- All safety measures should be identified, understood and adhered to.
- Appropriate collection equipment for the specific waterbody and selected invertebrate group should be used.
- *A priori* criteria for accepting samples obtained and clear direction should acceptability guidelines fail.
- In order to describe sediment colour, odour, texture and debris, visual descriptions of benthic grab samples should be recorded.
- Trip blanks and equipment rinsates to check chemical sampling contamination.
- Field sieving to occur as soon as possible after retrieval of samples.
- Appropriate containers and preservative should be used for the storage of samples.
- Sample contained appropriately labelled.
- Appropriate shipping and storage procedures should be used in addition to chain-of-custody forms.

All Australian states and territories have established and well-documented procedures for the collection of macroinvertebrate samples. In all cases the type of habitat(s) to be sampled, the type of sampling equipment to be used, the distance to be sampled, and the sampling technique to be employed are clearly outlined. However, to form part of a fully effective QA/QC program the procedures need to be

complemented with an audit process to provide assurance that the practices are being followed. The AusRivAS requirement to retain 10% of samples for review partly fulfils this quality assurance requirement.

Available information indicates that none of the other three countries (United Kingdom, USA or Canada) have such quality assurance requirements for auditing biological sample collection for their RBA programs. However, all countries have documented procedures for biological sample collection that would serve to minimise the chances of errors occurring.

Sample Processing QA/QC

In the UK, the procedures manual BT001, “Procedures for collecting and analysing macroinvertebrate samples”, outlines instructions for the quality assurance associated with sample traceability (Murray-Bligh 1999a). This includes the maintenance of unambiguous sampling schedules, emphasis on the importance of documentation and indicating the stage to which a sample has been analysed in order to trace samples that have been damaged, destroyed or subject to other accidents.

Standard data record sheets are also used in the UK as part of the quality assurance procedures for data archiving and analysis (Murray-Bligh 1999b). Data should be entered on these standard data sheets or straight onto the computer. To minimise transcription errors when data is entered onto regional databases, data should be checked against the original data sheets.

In the UK, the assessment of errors in sorting and identification of macroinvertebrates to family level was introduced in 1990 (Murray-Bligh 1999b). At present, some audits are conducted on species level samples, however, to date a standard procedure has yet to be finalised or documented.

Current methods of sample analysis in the UK are such that QA/QC procedures for the estimation of abundances are not possible. An Institute of Freshwater Ecology audit of the Environmental Agency and other similar UK organisations found that the majority of errors result from taxa being missed during sample sorting (Dines & Murray-Bligh 2000). These errors were identified during the subsequent audit and are referred to as ‘gains’ because they result in additions to the list of macroinvertebrate taxa originally recorded. A ‘loss’ is recorded when a taxon is identified that the auditors cannot find. These errors are much less frequent than ‘gains’ and are usually related to recording mistakes or misidentification (Murray-Bligh 1999a). The annual mean number of ‘gains’ per sample is used to measure performance at laboratory and regional level. The target quality is two actual gains (as opposed to net gains). After determining the precision of this mean, and studying the distribution of the errors, confidence limits can be calculated for the annual mean (Murray-Bligh 1999b). The RIVPACS III+ program refers to net gains (gain minus loss) in its calculations of accuracy (Murray-Bligh 1999a). It has been determined that sixty samples per year provide an acceptably reliable estimate of performance (Dines & Murray-Bligh 2000)

An Analytical Quality Control (AQC) scheme is also in operation within the UK Environment Agency and is designed to provide rapid feedback to the analysts, enabling corrective action to be undertaken quickly (Murray-Bligh 1999a, 1999b). The AQC team consists of highly experienced biologists who have demonstrated their ability and consistency through previous audit results. This scheme operates in addition to the external audits conducted by the IFE). Although work conducted

by outside contractors for the UK Environment Agency should theoretically be subjected to audits, the extent to which this actually occurs varies (Murray-Bligh 1999b).

In Australia, QA/QC procedures for sample processing vary in type and detail between the states and territories (AusRivAS). Each has developed its own manual outlining QA/QC procedures for sample processing and most have detailed their procedures on purpose-designed laboratory sheets. These are discussed in detail in the macroinvertebrate sampling audit (Appendix E). Relevant QA/QC procedures for each state and territory are presented in appendix B:A.

Although QA/QC procedures in the Australian states and territories vary, all tend to recheck 5-10% of processed samples for identification accuracy (AusRivAS state manuals 2001). In states that use live-picking procedures (Victoria, New South Wales, Tasmania, Queensland and Western Australia), samples are selected randomly and the residues from live picking are preserved and taken back to the lab for quality control of live picking efficacy. States and territories that use lab-picking procedures (Australian Capital Territory, South Australia and the Northern Territory) conduct quality control checks for missed taxa in the picked residue. Australian states and territories are also subject to external QA procedures conducted by independent organisations under instruction from the Commonwealth Department of the Environment and Heritage.

Canada has no specific written procedures for conducting QA/QC on macroinvertebrate identification, however relevant authorities do suggest issues that should be investigated, such as error trigger values, and the minimum number of samples that should be assessed (Environment Canada, 2001).

The Metal Mining EEM Guidance Document (Environment Canada 2001) outlines QA/QC procedures for invertebrate sorting and identification. The procedures include the training of staff involved in the laboratory component of surveys (sample processing and analysis) and the maintenance of detailed sample processing notes. In addition, it is recommended that at least ten percent of all samples (randomly selected) be resorted. Environment Canada (2001) defines the criteria for an acceptable sort as "less than or equal to ten percent of the total number of organisms were missed". If >10% of the total number is found during the checking procedure, then all samples within the group of samples should be resorted. Furthermore, if an entire group of invertebrates is missed by the sorter (ie. if it was consistently being incorrectly identified) then a re-sort would also be required, even though the missed organism may have been less than ten percent of the total (Environment Canada 2001).

If sub-sampling has been necessary, the influence of this process on estimates of abundance may also be examined. This would involve a minimum of 10% of the samples and documentation on the effects of subsampling on sample estimates.

Environment Canada (2001) also recommends that a complete reference collection for each mine site be established. The collection should be verified by an external taxonomic expert and be updated when necessary. Habitat and geographic location should influence the choice of appropriate taxonomic references to be used in the identification of invertebrates.

In the USA, laboratories performing work for the US EPA must provide both program and project plans for QA procedures (Plafkin *et al.* 1989). The QA project plan describes specific objectives, network design, procedures, methods, and

controls that will be applied to a specific project to ensure that data produced is of known and acceptable quality.

In addition, the USA Rapid Bioassessment Protocols outline procedures for quality control of sorting and identification (taxonomy). Quality control for sorting involves the examination by appropriate personnel (eg. QC personnel or experienced co-workers) of 10% of samples in each lot. A lot is defined as “a special study, basin study, entire index period or individual sorter” (Barbour *et al.* 1999). Quality control involves an appropriate individual examining the grids chosen and the tray used for sorting and searching for any organisms that may have been missed by the original sorter. If any organisms are located, they are added to the relevant sample vials. Less than ten organisms (or 10% for larger subsamples) found by the second sorter is regarded as a ‘pass’, if more than 10 (or 10%) are located, the sample ‘fails’. Further, if the first 10% of the sample lot do not pass this criterion, a second 10% is rechecked by an appropriate person. Qualified personnel will also check 100% of the samples sorted by new personnel or ‘sorters in training’ until it is deemed that ‘training’ is complete (Barbour *et al.* 1999).

As for field equipment, once sample processing is complete all equipment involved in the processing process (sieves, pans, trays etc) and that have come in contact with the sample is to be rinsed thoroughly and picked free of organisms or debris. Any remaining organisms are added to the sample residue (Barbour *et al.* 1999).

Information on collected samples is to be recorded in a “sample log” notebook to enable progress tracking of each sample within the sample lot. Tracking of each sample is to be updated following completion of each step (eg. subsampling and sorting, mounting of midges and worms, taxonomy).

Quality control procedures for taxonomy include the establishment and maintenance of a voucher collection incorporating properly labelled and preserved specimens. A second taxonomist, not responsible for the original identifications, should randomly check samples to verify identifications. If specimens are sent to external sources for verification, they should be recorded in a specially designated notebook that includes the label information and date of sample dispatch. When samples are returned to the laboratory the date received, the name of the external identifier and the result should be recorded in this “taxonomy validation notebook” (Barbour *et al.* 1999).

It is also recognised that taxonomists should participate in periodic training on specific taxonomic groups to ensure accurate identifications and a library of taxonomic literature should be maintained and updated as required.

2.5 Training

Training is an important aspect of QA/QC management, as staff properly trained in procedures produce higher quality work and are less likely to make mistakes than untrained personnel. Documented training procedures ensure that all staff receive similar training, reducing ambiguities and therefore the likelihood of QA/QC errors occurring.

In the UK, inexperienced or new Agency staff undergo two phases of training involving different levels of checking and supervision. The results produced by inexperienced staff are subjected to an audit before they are accepted for operational use. Agency staff are usually required to possess a relevant degree or

equivalent. The Agency recognises that implementing more structured training to ensure that staff develop and maintain the necessary skills may be necessary (Dines & Murray-Bligh 2000). A training video for collecting macroinvertebrates was developed for the 1990 National Survey (Murray-Bligh, 1999a). Training workshops are also held to ensure that all Agency staff conducting biological assessments understand and adopt QA protocols

In the USA, personnel conducting assessments are trained in a consistent manner, often by the same person within each agency, to ensure that assessments are conducted in a proper and standardised manner (Plafkin *et al.* 1989). Training workshops similar to those in the UK are also conducted.

In Australia, AusRivAS training is controlled by the individual state and territory agencies undertaking the sampling. National workshops were held regularly to ensure consistency. A national AusRivAS training and accreditation course has been established to maintain the accuracy and precision of AusRivAS assessments conducted by individuals (<http://www.ausrivas.canberra.edu.au/training/>). Participation in the course, while not mandatory, is considered a prerequisite for all personnel using AusRivAS and / or the associated AusRivAS models. The course is very comprehensive and consists of four modules involving a training component, an accreditation component, and authorisation to conduct training and confer accreditation. The accreditation component is intended to provide quality control by reducing error and variation in results, while the accreditation component will provide quality assurance by ensuring the accuracy of user results is within controlled and acceptable limits. Different AusRivAS training and accreditation courses have been developed for each state and territory to accommodate their different sampling methods, and a publicly accessible national register of trained and accredited operators is maintained by the Department of the Environment and Heritage (Bruce Gray *pers. com.*, 2004) (<http://www.deh.gov.au/water/rivers/nrhp/monitoring.html#register>).

2.6 Data Inputs

For AusRivAS, state and territory specific variables have been incorporated into each of the different models, with each variable considered to be unaffected by anthropogenic impacts (AusRivAS 2001). Operators are required to record values for a number of variables as defined on the field sheet attached to the sampling and processing manual relevant to the state or territory. The models usually require between 10 and 20 variables, which are most often a combination of chemical values, such as alkalinity, physical values, such as stream width, habitat values, such as riparian cover and locality values, such as longitude. Variables should be measured at the same time as macroinvertebrate sampling. Frequently, variables in addition to those required by the AusRivAS models are measured at each site and these often prove useful in helping to explain unusual results, or for assessing temporal changes at particular sites. It could be argued that habitat variables, such as pH, electrical conductivity, stream cover and percentage algae cover, used by AusRivAS models (AusRivAS Version 2.2) may be influenced by anthropogenic activity. However, even if this were the case the habitat selection process would ensure that such variables are still adequate discriminators between sites (AusRivAS 2001).

RIVPACS models require two types of variables: environmental and locality. Environmental variables consist of stream width, depth, substrate characteristics and alkalinity. Locality variables comprise altitude, distance from source, slope,

national grid reference and discharge category (Murray-Bligh 1999a). Selection of these variables for inclusion in the models was based on the assumption that they are unimpacted by human activity. Environmental data should be averaged over a range of seasons and over as many years as possible, while locality data should be averaged from at least two independent measurements, preferably by two different operators. The RIVPACS procedure manual contains examples of the field data sheets, which can be customised for each laboratory, required to be completed for each site on every sampling occasion (Murray-Bligh 1999a). The manual also contains an acceptable range and standard error for each of the variables assessed to ensure reliability of the RIVPACS output.

The USA models do not require habitat variable inputs, but they do use habitat information *post hoc* as an aid in interpreting results (Barbour *et al.* 1999). The general RBP procedures manual contains habitat assessment sheets as well as comprehensive guidelines for their completion.

The Metal Mining EEM Guidance Document (Environment Canada 2002) also outlines 'explanatory variables' that should be quantitatively measured at each replicate station at the same time as benthic invertebrate sampling. Although this data is not included in the models, it is useful as an aid in interpreting results. This enables environmental correlates to be linked with invertebrate community structure and is particularly recommended for "reference condition approaches" (Environment Canada 2002). The environmental parameters assessed should include variables from each of the categories listed below (Environment Canada 2001):

- Habitat description: stream order, drainage area.
- Physical-chemical measurements: water temperature, dissolved oxygen, pH, dissolved oxygen, conductivity, turbidity, and alkalinity.
- Morphometric measurements: bankfull width, wetted width, depth and slope.
- Flow characteristics: velocity and stream hydrograph.
- Substratum characteristics: embeddedness and, substratum composition (eg. % gravel, cobble etc).
- Sediment chemistry: fine sediment particle size and TOC.
- Riparian zone characteristics: riparian vegetation and, canopy cover.

2.7 Data Outputs

The basic output from the AusRivAS model is the ratio of Observed to Expected taxa (O/E ratio) (Coysh *et al.* 2000) and includes a band allocation representing different levels of biological impairment (Table 2). This allows an assessment of the extent to which a site has been impacted, as well as characterising the general health of that part of the river being investigated (Environment Protection Authority Victoria 1998). AusRivAS produces a variety of other outputs, such as group probabilities, number of taxa, taxa probabilities and an O/E SIGNAL score that indicates the level of water quality impairment at a site.

The SIGNAL (Stream Invertebrate Grade Number - Average Level) score is based on the British BMWP system where individual families are allocated a score from 1-10 based on the family's tolerance to pollution. Calculation of SIGNAL scores involves summing the grades of all families present and then dividing the total by the number of families to provide an average grade per family (Chessman 1995; Coysh *et al.* 2000; Chessman 2002). A limitation of SIGNAL scores that must be

considered when interpreting results is that species within a single family can often show considerable variation in their response to pollutants and some families are sensitive to certain types of pollution, yet tolerant to others (Environment Protection Authority Victoria 1998).

The AusRivAS model indicates when the test site cannot be compared to suitable reference sites, or where the accuracy cannot be guaranteed (Coysh *et al.* 2000). For example, where the probability of site belonging to a site group is less than or equal to 5% prudence is required when interpreting results.

The RIVPACS model produces three different outputs that, when examined together, provide an assessment of what may be affecting water quality at a particular site. Each of the three outputs, BMWP-score (Biological Monitoring Working Party), ASPT (Average Score Per Taxon, BMWP divided by the number of taxa) and N-taxa (number of taxa), is compared to scores predicted by RIVPACS. The resulting output is the Environmental Quality Index (EQI). The manual suggests that individual indices, rather than EQI scores, should be used to assess changes at a site (when a reference site is not required) and that the EQI should be used when comparing different sites, which does require a reference condition (Murray-Bligh 1999a). The RIVPACS EQI scores can be graded into different classes (A-F) representing different levels of biological condition (Table 3).

As with the AusRivAS models, caution is required when interpreting results where the probability of site belonging to a site group is less than or equal to 5%.

Outputs from the Canadian BEAST program consist of total invertebrate density, taxon richness, Simpson's diversity index and the Bray-Curtis similarity index. Benthic macroinvertebrate communities at test sites are deemed impacted if they differ significantly (as determined by statistical methods appropriate to the study design, such as an ANOVA, ANCOVA, regression) from reference sites (as defined by the environmental variables) (Environment Canada 2001). The nature of this assessment does not allow for general classes or bands to be applied to the data. The accuracy of results relies on the appropriateness of the study design and methods of data analysis.

*Table 2 Legend for mapping AusRivAS scores
 (taken from <http://www.ea.gov.au/water/rivers/nrhp/scores.html>)*

AusRivAS band	AusRivAS O/E score	Band description	Aquatic Macroinvertebrate Biodiversity Status	Interpretation Guide
X	>1.2	More biologically diverse than reference sites (needs further investigation)	Over 112% more biodiversity than reference sites	More macroinvertebrate families found than expected Potential biodiversity “hotspot” Possible mild nutrient enrichment
R	Approx. 1.00	Reference site	Natural or near natural levels of biodiversity	Reference site used in the construction of the AusRivAS models Presumed to be in reference condition Undisturbed/natural/pristine or least disturbed/impacted
A	0.85 to 1.15	Reference condition	Similar levels of biodiversity to reference sites	Most or all of the expected families were found at the site Water quality and/or habitat condition roughly equivalent to reference sites Impact on water quality and habitat condition does not result in a loss of macroinvertebrate biodiversity
B	0.55 to 0.84	Significantly impaired	Approximately 16% to 45% of macroinvertebrate biodiversity has been lost	Several expected families not found Water quality and/or habitat condition significantly impaired Ecologically and statistically significant loss of macroinvertebrate biodiversity
C	0.25 to 0.54	Severely impaired	Approximately 46% to 75% of macroinvertebrate biodiversity has been lost	Many expected families not found Water quality and/or habitat condition severely impaired Severe loss of macroinvertebrate biodiversity
D	0 to 0.24	Extremely impaired	Approximately 76% to 100% of macroinvertebrate biodiversity has been lost	Few of the expected macroinvertebrate families found Poor water and/or habitat quality Loss of macroinvertebrate biodiversity Highly degraded
?	-	Beyond the capacity of current AusRivAS models	Not assessable	Could not be assessed using current AusRivAS models

Table 3 *United Kingdom RIVPACS EQI biological classification scheme (taken from Murray-Bligh 1999a)*

Class	Description	Lower Class Limits	
		EQI ASPT	EQI N-taxa
A	Very good	1.00	0.85
B	Good	0.90	0.70
C	Fairly good	0.77	0.55
D	Fair	0.65	0.45
E	Poor	0.50	0.30
F	Bad	0.00	0.00

In the majority of USA bioassessment programs, decisions concerning stream health classification ultimately depend on results obtained using several different types of measures (or metrics) - this is often referred to as the 'multimetric' approach (Resh *et al.* 1995). The available metrics are split into four different categories: richness (number of total taxa), composition (% Chironomidae), tolerance (% dominant taxon) and trophic/habitat (% filterers) (Barbour *et al.* 1999). The metrics used are presumed to represent ecological features of interest and also have a range of sensitivities to different kinds of stress (Resh *et al.* 1995).

Taxa richness and EPT (*Ephemoptera*, *Plecoptera*, *Trichoptera*) richness are the most widely used measures in bioassessment programs in the USA (95% of all programs; Tetra Tech 1993 cited in Resh *et al.* 1995). Evaluation of measures used in USA bioassessment programs has commenced, however a more appropriate bioassessment approach may involve comparing groups of reference and test sites with similar habitat characteristics using standard parametric tests or multivariate procedures (Resh *et al.* 1995). Both of these approaches avoid the widely criticised strategy of relying upon a single index.

3 Future research and development needs

Bioassessment programs such as AusRivAS, RIVPACS, BEAST and RBP I, II & III offer advantages over traditional methods in that they are rapid and standardised, allow for a rapid turnaround of results, provide outputs tailored for a range of users, allow comparisons between different sites and, in the case of AusRivAS and RIVPACS, allow for the classification of water quality at sites using macroinvertebrates.

Norris *et al.* (1998) outlined some of the benefits of using the AusRivAS models for the assessment of Australian streams:

- a) The standardised methods used for AusRivAS assessments enable spatial and temporal comparisons that are rarely possible between studies using other techniques.
- b) The models are quick and because the impairment of the biota is measured directly, sampling for specific pollutants is not necessary.
- c) The existence of a reference sites database allows the sampling of new test sites without the need for new or modified experiments.
- d) The prediction of a 'target community' provides managers with a goal to aim for and the banding system allows them to monitor the progress of remediation measures.

Despite the advantages that these programs provide, each have limitations and areas where further research and development are required.

Under the AusRivAS program, research is necessary to determine the spatial and temporal variability of macroinvertebrate data and hence the variability of the models and their outputs. This has to some degree been addressed by Barmuta *et al.* (2001) in the AusRivAS Errors Analysis.

Also, it has been determined that combined seasons data is more reliable than single season data. The Victorian Environmental Protection Authority (EPA) is currently investigating how a site changes over time, between seasons (short term) and over years (long term). The Victorian EPA is also researching model spatial variability and to date has established that riffle habitat models are more reliable than edge habitat models, as there is a greater variety, and hence variability, of microhabitats in the edge habitat.

Halse *et al.* (2002) indicate that although spring and autumn AusRivAS models for Western Australia appear to provide a sensitive and reliable assessment of river condition, further development would improve assessments in particular river types. They found that degradation levels in the wheatbelt region tended to be underestimated due to an insufficient number of undisturbed sites to provide a true baseline for modelling. It was suggested that additional reference site sampling would improve assessments of naturally saline and short coastal streams. Halse *et al.* (2002) also suggest that the need for more reference sites is a shortcoming of any reference-type approach in situations where impacts occur throughout the region of interest. Wright (2000, cited in Halse *et al.* 2002) indicates that the "development of river assessment methods is an iterative process and continuing research and development is essential".

Halse *et al.* (2002) identified four areas where further research and development would be beneficial in Western Australia:

1. Additional reference sites in naturally saline streams of the South Coast and short coastal streams of the south-west so that the AusRivAS models have the ability to recognise these streams as distinct types.
2. Additional autumn sampling of reference sites with a 60 minute live pick. The current autumn model is regarded as sub-optimal, largely due to its lack of autumn sampling in a large proportion of the south-west and it being largely based on a 30 minute pick.
3. Construction of models based on field sub-sampling with a box sampler may result in an increase in sensitivity and reliability of AusRivAS.
4. Quantification of the frequency and likely magnitude of assessment errors. Previous work has indicated that one-off site assessments with AusRivAS can lead to an incorrect assessment of river condition.

A number of the above recommendations are incorporated into the Final Report for this project.

AusRivAS has been developed primarily for use in freshwater lotic environments and future research and development is required for application of the models in lentic, estuarine and wetland systems (AusRivAS 2004).

In the USA, rapid bioassessment protocols are currently used only in wadeable streams. A major research and development priority is the development of techniques that enable bioassessment to be applied to large rivers and lakes. The USA EPA Lake and Reservoir Bioassessment and Biocriteria Technical Guidance Document is part of an effort to advance the use of biological assessment in this area (Gerritsen *et al.* 1998).

Other areas identified by Resh *et al.* (1995) for future research and development include:

- further refinement of ecological principles underlying choice of metrics,
- inclusion of chemical criteria and toxicity tests to establish thresholds that indicate impairment,
- development of criteria indicating the necessity for implementation of quantitative assessment studies.

Although the value of the RIVPACS approach was revealed during the 1990 River Quality Survey, some weaknesses of the system were also identified. The RIVPACS II data set proved inadequate for some stream types and geographical areas, with the faunal richness of chalk streams underpredicted by RIVPACS. At present, bioassessment models use only presence / absence community composition data and therefore opportunities for detecting changes in abundance prior to loss of taxa are limited to crude assessments. It is recommended that the Department of the Environment and Heritage consider (prior to the use of AusRivAS models for NAP and NHT II regional planning purposes) the outcome of the AusRivAS Errors Analysis. In particular, the finding that the AusRivAS output is sensitive to the categorical variables specific to each state or territory; and the recommendation that further information is gathered on real 'errors' associated with variations between trained AusRivAS operators (Barmuta *et al.* 2001).

Rapid bioassessment protocols utilising macroinvertebrates have been established to reduce the cost and effort associated with some of the more traditional

approaches to sampling and data analysis. In the USA rapid bioassessment programs are based on the multimetric approach, where several indices related to ecological values are used to present the findings. In contrast, the approach in the UK and Australia has been to use models based on multivariate statistics to assess biological impact by predicting the fauna expected to occur at a test site.

Irrespective of the RBA approach employed, the validity and usefulness of site assessment results are dependent on the efficacy of the model used and the quality of data input to the model. For the majority of users, there is little or no control over the model itself and the reliability of model outputs is a function of the reference site data and data quality more generally. Maintaining data quality requires experienced and diligent operators working within strict quality boundaries. The importance of due diligence to this approach is recognised by the lead agencies of the four countries with large-scale RBA programs (Australia, Canada, the UK and the USA) and each has established QA/QC programs, although their level of development and application varies.

3.1 Quality Assurance and Quality Control

Australian QA/QC procedures are generally comparable with those of the other three countries using RBA protocols, however there are still areas requiring improvement. Australian QA/QC procedures are specific to each state and territory and these vary in their level of development, documentation and the extent to which improvement is required. All Australian states and territories have demonstrated their commitment to establishing demonstrably effective programs that maximise the quality and consistency of AusRivAS data both within and between states / territories. This latter aspect is especially important given that AusRivAS is intended as a 'national' approach to riverine biological assessment.

The basic elements of a laboratory QA/QC program (ISO/IEC 1999) are listed below together with the assessed status of the relevant components of the AusRivAS program.

1. *Documented and validated methods followed by all operators* – adequate procedures are available for most agencies, but they need to be brought to a common standard, especially those for QA/QC.
2. *Maintained and calibrated equipment suitable to the task* – some calibration is undertaken but there appears to be no consistency in approach between agencies.
3. *Targets for acceptable levels of uncertainty for each variable* – currently some target criteria are available for macroinvertebrate identification (Waddell 2001), in addition to the WISE criteria (see Appendix E); and Murray-Bligh 1999 provides further targets for field parameters. These could be expanded to cover all variables measured in the field as well as macroinvertebrate identification and counting.
4. *Monitoring of errors to ensure that they are within limits* – whilst agencies generally undertake internal checking, these results are not readily available nor are they interpreted as part of QA/QC. Some internal assessment of agency performance has been undertaken by Waddell, 2001.
5. *Tracking of sample identification and status* – this is often done poorly with inadequate information to uniquely identify the sample. Some agencies have

prepared standard sample labels which could form the basis of a minimum sample identification procedure.

6. *Checking of data transcriptions/calculations through to final storage* – this aspect is variable between agencies. There needs to be a uniform approach with periodic auditing of agency compliance with agreed protocols.
7. *Training of operators with testing of competency* – training is undertaken through the national program, however the extent to which it includes QA./QC aspects and the assessment of trainee competencies is uncertain.
8. *External auditing of agency proficiency* – this Project is the first such program for physico-chemical and habitat variables but a previous program for macroinvertebrates has been undertaken (Humphrey and Thurtell, 1997). The benefits of this external audit is apparent in the Macroinvertebrate Sample Processing Error Report (Appendix E) undertaken as part of this project.

Whilst application of these requirements to environmental assessment is often more complex than for the laboratory situation, most of the above can be applied in some form to AusRivAS procedures.

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Appendix B:1 Australian State and Territory QA/QC methods for Macroinvertebrate Sample Processing and Identification

The internal QA/QC protocols vary between the states and territories and this is reflected in each agencies method manual. The level of detail below is indicative of the detail in the manual, guideline or regulatory publications of each state / territory.

QUEENSLAND

The residues of ten percent of all samples taken in the field are retained for analysis. Half of these samples are put aside for external analysis; the other half are subsampled and 10% of each sample is analysed by the unit's staff. The data is analysed, compared to the sample picked in the field, reports written, and presented as part of the milestone reports.

- Internal QA/QC checks are performed on staff, by staff, on a regular basis. At each round of QA/QC, a person is assigned to analyse a sample identified by another.
- Samples identified during the previous fortnight are selected at random and re-identified. The resultant taxa lists are compared and discrepancies in identification checked by other staff in the unit. Any errors are discussed with the original identifier (both misidentifications and errors of enumeration) and a report prepared which is read and signed by all members that underwent the QA/QC check.

NEW SOUTH WALES

The following information has been extracted from Waddell (2001)

An internal quality control and quality assurance program was undertaken on a subset of all AWAHR samples collected in NSW to ensure an acceptable standard of macroinvertebrate identification was being achieved. The guidelines presented in Hawking and O'Connor (1997) were followed for macroinvertebrate identification and quantification quality control and quality assurance.

- If an uncertainty in identification exists, such as for immature or unknown specimens, a decision tree presented in Hawking and O'Connor (1997a) was followed to determine if the specimen could be correctly identified.
- Five percent of samples for each season were selected using a stratified/random basis for re-identification. Selected samples included all identifiers and a range of habitats and biogeographic regions.
- Quality assurance staff re-identified samples following the guidelines presented in Hawking and O'Connor (1997a).
- Error rates were calculated as outlined in Hawking and O'Connor (1997b) and included Percentage of New Taxa and the Bray Curtis similarity index.
- To provide an alternative estimate of dissimilarity between the original and QA samples, Sorensen's Index (Bennison *et al.*, 1989) was also used. Sorensen's Index uses total taxa numbers, opposed to relative abundance data, and was considered more appropriate than Bray Curtis for reflecting

errors that have the potential to affect AusRivAS results based on presence-absence data.

- A <10% error (as used in the MRHI QA/QC program (Hawking and O'Connor, 1997c)) was considered acceptable. Samples with a 'new taxa' percentage of 10% or greater and/or a Sorrensen's index of less than 0.91 failed the QA test.
- Identification errors were compiled and follow-up action was implemented to correct mistakes and improve identification performance. An external QA/QC program was also conducted on samples collected during the MRHI phase and follow-up action was undertaken to tackle identified identification issues.

AUSTRALIAN CAPITAL TERRITORY

QA/QC checking procedures are to be carried out by experienced persons for samples sorted by new personnel and/or from each sampling run, ie. from any new project or any new batch of samples for a continuing project.

Sorting

- Following the completion of sample sorting QA/QC personnel are to check the sample remains for missed organisms. Checking of sample remnants is to continue until >90% of the total number of organisms in the sample are consistently removed.
- Error code(s) and/or action code(s) are to be recorded on laboratory sample record sheets if appropriate.
- Laboratory sample record sheets are to be fully completed by the person conducting the QA/QC check.

Table of QA/QC error and action codes

Code	Code Description
<u>Error codes</u>	
CC	Number of organisms recovered from sample represents more than 10% of the total number of organisms recovered from the sample.
IE	Number of organisms incorrectly identified or counted is more than 10% of the total number of organisms.
LE	Labelling error
SE	Sub-sampling error- eg. if the sample was contained in more than one sample and not all the sample used.
WE	Washing error- some sample bypassed washing sieve
DE	Data entry error on data sheet
CE	Calculation error- mathematical error on data sheets
<u>Action codes</u>	
LC	Labels corrected- contact person who collected the sample if error is on the original sample label
SC	Sample re-subsampled, processed, re-checked and data sheets corrected
WC	Material bypassing the sieve caught in washbasin, sample combined and rewashed
WI	Material bypassing sieve lost, partial sample processed
DC	Data entry corrected (strike out incorrect entry with one line and write

Code	Code Description
	in the correct entry, initial).

Identification

- All samples are separated into Orders and placed in separate vials to eliminate any high level discrepancies. This is also required for future curatorial preservation and storage.
- When an identification problem is encountered a decision tree (Hawking and O'Connor 1997) for identifications should be followed.
- Very small, damaged, immature animals or pupae that cannot be identified with confidence should be noted as such (eg. Trichoptera juvenile). These animals are counted and placed in separate vials for each category. The counts for unidentified animals are not included in the 200-organism sub-sample.
- Damaged animals should be identified if possible, with both head and tails counted and the highest number recorded and placed in the appropriate vials. If a specimen cannot be identified it should be noted as such (eg. Ephemeroptera damaged) and placed in the appropriate vials. The same procedures apply to the identification of Oligochaeta.
- For new persons, projects, or sampling runs, quality control staff should check the first five samples identified.
- A misidentification error of < 10 % of the total number of animals is deemed acceptable at Family level, this is the error rate used by the Murray Darling Freshwater Research Centre who conducted external quality control checks of all state agencies. If the error is 10 %, misidentifications are corrected under the guidance of quality control staff. All misidentifications will be shown to the person and suitable instruction given to rectify the misidentification. Other samples containing taxa that were misidentified are then checked for identification errors by the original identifier.
- Following the checking of the initial five samples, a random selection of two samples in the following ten, two samples in the following 30 and two samples in the following 50, will be checked.
- Persons checking samples will have adequate experience in identification. Where possible different samples will be checked by more than one person to avoid bias and increased workloads.

VICTORIA

- The Victorian EPA will retain five per cent of residue samples for purposes of internal quality control of live-picking efficacy.
- Each residue sample shall be randomly sub-sampled using a box sub-sampler and ten per cent recovered for picking and identification of macroinvertebrates under a dissecting microscope. Macroinvertebrates shall be identified to family as for the live-picked samples and where taxonomic resolution allows. Results from the residue samples will be recorded and entered onto the EPA biological database under method code 31 for sweep sample residues and method code 32 for kick sample residues.

- For quality control purposes, and in particular to ensure consistency across laboratory staff, a random selection of 10% of all sorted macroinvertebrate samples shall be re-identified by a senior taxonomist or ecologist. Errors at family level should be less than 1% and errors at species level should be less than 10%. Sample processors are responsible for ensuring that their identifications are checked for quality.
- For each sample processor, quality control checks shall be undertaken on 10% of samples identified to species level and independently on 10% of samples identified to family. Samples from different catchments can be grouped for QC testing, providing that all samples have been identified by the same processor at one level of taxonomic resolution.
- The checker shall select in a random process the sample(s) that will be quality controlled. Appropriately qualified staff shall undertake quality control:
- For all discrepancies between processor and checker identifications, an appropriate third person shall be consulted and a consensus decision made on the actual identification.
- The checker will complete the standard QC report form and discuss the results with the processor. The form shall then be placed into the laboratory QC folder, and details of the QC check entered onto the front index page of the folder.
- As a minimum, 10% of all sample residues are to be preserved for quality assurance/quality control assessment, using the same procedures (envelopes etc) as conducted under MRHI Phase 1 rounds 3 and 4. Sample selection is to be organised by the lead agency. Stratified random allocation of samples must be conducted with respect to operator, catchment and habitat. The person with knowledge of specific sites/operators for which samples require preservation must not be involved in sample sorting. Half of these samples (ie. 5% of the total sample number) must be sorted and an assessment made of individual operator quality assurance/quality control performance. A proportion of the remaining samples must be forwarded for external quality assurance/quality control (to be arranged).
- Additional internal quality assurance/quality control is to be conducted as agreed with the Program Coordinator.
- 5% of all processed samples, accompanied by their data sheets, must be forwarded for external quality assurance/quality control for taxonomic identification (to be arranged).

TASMANIA

The QA/QC program aims to assess the effectiveness of individual operator sorting procedures using as its basis, comparison of the composition of live-picked samples with associated residues. The MRHI Bioassessment manual states that the aim of the live pick procedure is to ensure that the broadest range of biota are collected at a site. This implies that the taxa list derived from a live pick will encompass more taxa than would be expected if a random sample of animals of equivalent number to the live sort total were drawn from the sample (ie. 'whole sample estimate').

- Approximately 10% of all riffle and edgewater residues are to be preserved from each sampling round. Half of these (5%) are to be processed so that operator sorting efficiency can be assessed

- Analysis is carried out as per previous external audits conducted by ERISS
- Approximately 5% of the samples collected each round are cross-checked by persons with adequate identification experience.
- Samples are selected to cover a broad range of biogeographical regions, habitats and staff.
- A mis-identification error of < 10 % of the total number of animals is deemed acceptable at the Family level. This is the error rate used by the Murray Darling Freshwater Research Centre who conducted external quality control checks of all state agencies
- In all cases, identification problems are to be resolved with additional training of staff. Past samples containing taxa that were found to be misidentified are to be rechecked and, where appropriate, the database updated.

SOUTH AUSTRALIA

As part of a national QA/QC program involving the identification of macroinvertebrates, the Murray-Darling Freshwater Research Centre independently checked samples that had been sorted and identified by the team in S.A. Samples were assessed for the 1994, 1995 and 1997 surveys. The results from these showed the high performance of the approach used in S.A. and indicated that no further work was needed to improve the identification of specimens from this state.

- All new staff are trained in the use of the sub-sampler and identification keys used in S.A. The experienced team members have also assisted new staff to identify organisms that they are not familiar with and check difficult taxa.

The addition of new staff during the program led to the development of a more rigorous internal training protocol in 1998. This included:

- Random checks of sorting trays of new members to ensure all specimens were being collected and more importantly that novel taxa were not being overlooked.
- All staff involved with the project to process and identify a contrived sorted sample to provide a check on counting and identification skills.

WESTERN AUSTRALIA

- As a minimum, 10% of all sample residues are to be preserved for quality assurance/quality control assessment
- Stratified random allocation of samples must be conducted with respect to operator, catchment and habitat
- The person with knowledge of specific sites/operators for which samples require preservation must not be involved in sample sorting.
- Half of these samples (ie. 5% of the total sample number) must be sorted and an assessment made of individual operator quality assurance/quality control performance
- Additional internal quality assurance/quality control should be performed

NORTHERN TERRITORY

Quality control / quality assurance procedures are designed to establish an acceptable standard of macroinvertebrate sorting and taxonomic identifications. The quality control component minimises the variation in sorting and identification efficiency. Quality assurance provides potential users with the assurance that the accuracy of results is within controlled and acceptable limits.

Sorting Efficiency

- All new staff are to be trained in the NT laboratory procedures including sub-sampling, sorting and sample storage.
- Once sorting has commenced the residues of the first five samples are checked by quality control staff.
- An assessment is made following the first five samples whether sorting efficiency is acceptable.
- If not satisfactory the checking is continued for every sample until the problem is rectified.
- If the sorting for the first five samples is acceptable a random selection of two samples in the following ten, two samples in the following 30 and finally 1 sample in every 20 will be checked by quality control staff.
- The protocol is repeated for the commencement of new projects and sampling runs as well as new staff.
- A sorting efficiency of > 90% is deemed acceptable. Sorted samples are resorted and missed animals are identified and enumerated and compared to the animals collected from the original sort. If the number of animals of a particular Family counted in the re-sort is $\geq 10\%$ of the total count (original plus re-sort count for that Family) the person is given suitable instruction to ensure that particular Family is adequately collected in future samples.
- It is important when sorting to ensure all different taxa in a sample are collected. If a particular Family contributes significantly to the count (> 10% of the total count) an error in the count of $\geq 10\%$ is less important than in a Family with a lower count. Where errors occur in the count of Families which contribute significantly to the total count, sorting staff are cautioned and made aware of these groups to reduce the chance of significant errors in future samples. Taxa not collected in the original count but collected in the re-sort are treated as a significant error with the appropriate instruction to correct the problem.

Identification and Enumeration Efficiency

- All new staff are to be trained in the NT laboratory procedures including sub-sampling, sorting, identification, enumeration, sample storage and archiving.
- Once sample identification has commenced the identification results of the first five samples are checked by quality control staff.
- An assessment is made following the first five samples whether identification and enumeration accuracy is satisfactory.
- If not satisfactory appropriate instruction is given to the person as described in the boxed section below.

- If identification and enumeration for the first five samples is acceptable a random selection of two samples in the following ten, two samples in the following 30 and finally 1 sample in every 20 will be checked by quality control staff.
- The protocol is repeated for the commencement of new projects and sampling runs as well as new staff.
- A misidentification error of < 10 % of the total number of animals is deemed acceptable. This is the error rate used by the Murray Darling Freshwater Research Centre who conducted external quality control checks of all state agencies participating in the AusRivAS program. If the error is > 10 %, misidentifications are corrected under the guidance of quality control staff. All misidentifications will be shown to the person and suitable instruction given to rectify the misidentification. Other samples containing taxa that were found to be misidentified are then checked by the original identifier for misidentification errors.
- When an identification problem is encountered a decision tree for identifications should be followed as is figured in Hawking and O'Connor (1997).
- Very small, damaged, immature animals or pupae that cannot be identified with confidence should be noted as such (eg. Trichoptera juvenile). These animals are counted and placed in separate vials for each category. The counts for unidentified animals are not included in the 200 organism subsample.
- Damaged animals should be identified if possible, with both head and tails counted and the highest number recorded and placed in the appropriate vials. If a specimen cannot be identified it should be noted as such (eg. Ephemeroptera damaged) and placed in the appropriate vial. The same procedures apply to the identification of Oligochaeta.
- When identifying the samples, the taxa are separated into Orders and placed in separate vials to eliminate any high level discrepancies. This is also required for future curatorial preservation and storage.