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LWRRDC Final Report: Development and implementation of QA/QC protocols for sample processing components of the MRHI agency bioassessment program.

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August 1998

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Development and implementation of QA/QC protocols for sample processing components of the MRHI agency bioassessment program

by

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FINAL REPORT TO

LAND AND WATER RESOURCES RESEARCH AND DEVELOPMENT CORPORATION (Reference No. ARR2)

December 1997

LWRRDC Final Report

Project ARR2

Project title: Development and implementation of QA/QC protocols for sampling and sorting components of the MRHI agency bioassessment program

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Date of submission: December 1997

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Abstract

One of the support R&D projects for the first funding round of the Monitoring River Health Initiative comprised external Quality Assurance/Quality Control (QA/QC) audits of MRHI State/Territory agency sample processing procedures (laboratory subsampling and sorting of preserved samples, and field live-sorting procedures), together with research components to refine QA/QC protocols and acceptance criteria. Sample processing errors have been quantified for the 3rd and 4th sampling rounds of the agency program. These data may be used to assess the degree to which the sensitivity of derived models has been compromised by such errors.

The external QA/QC audits have confirmed the potential of the live-sort procedure to result in 'high' error rates. For these samples, two main sources of error were identified from the assessments, (i) under-representation of taxa, and (ii) different taxa recovery rates depending upon the efficiency of the operator. Factors contributing to poor taxa recovery in live-sorted samples included low live-sort sample size, operator inexperience, and commonly-occurring taxa missed in samples and across sites. Some preliminary simulations were conducted in the course of the study to evaluate the implications of live sorting errors for model development and sensitivity. Variable findings arose from these simulations, from the inability to derive models from error-ridden data to unexpected consequences for UPGMA classification arising from data sets upon which 'few' and 'many' errors were superimposed. Regardless, all of the simulations indicated the potential for live-sort error to adversely affect the sensitivity of models.

Changes have already been made to live-sorting protocols to reduce sample processing errors whilst additional changes to the protocol will follow as the results of additional R&D come to hand. Improvement in the procedures for taxa recovery in ongoing MRHI studies would only stand to benefit monitoring programs if the quality of data for existing reference sites are also improved through a re-sampling, data replacement and re-modelling program. A sensitivity analysis is required in future to more comprehensively determine the sizes of various sources of error and variation in sample processing, and their effects on the rates of misclassification to quality bands.

Development and implementation of QA/QC protocols for sample processing components of the MRHI agency bioassessment program

Final report to LWRRDC

1 Introduction

In 1993 the Commonwealth of Australia funded the 'National River Health Program' (NRHP) to assess and monitor the health of the nation's rivers and streams (Schofield & Davies 1996). Part of this program is the 'Monitoring River Health Initiative' (MRHI), involving government agencies from all Australian States and Territories in a national program to develop a standardised and coordinated rapid bioassessment approach to biological monitoring of water quality in Australian streams and rivers.

The success of such an extensive program will depend on the development, acceptance and implementation of standard protocols encompassing all aspects of data acquisition (Davies 1994). Even though standard protocols have been adopted across all States and Territory agencies, there may be differences in the way these protocols are interpreted and implemented by different personnel/organisations. Such variations may affect the integrity of the data gathered. Therefore, there is a need for continual review of the quality of the data being collected. The accepted approach for reviewing data quality is to implement an ongoing Quality Assurance/Quality Control (QA/QC) program.

QA/QC is recognised as an essential component of any large project involving many different parties. Its purpose is to ensure that methods for data collection are standardised, that data are of a consistent and high quality and that this quality is maintained throughout the project (Plafkin et al 1989).

Given the objectives and broad scale nature of the MRHI, and the similarity of the rapid bioassessment protocols to those used overseas (Plafkin et al 1989, Wright 1995, Cuffney et al 1993a), the development and implementation of QA/QC programs were seen as logical and necessary components of the Australian program. Of the many aspects of the MRHI bioassessment protocol that warranted QA/QC, only two components, namely sample identifications and sample processing procedures, were targeted for *external* audits by independent agencies in the first funding phase of the MRHI. The present study was concerned with an external QA/QC program of the two standardised procedures used by agencies to process samples: (i) field live sorting conducted by QLD, NSW, VIC, TAS and south-west WA, and (ii) laboratory subsampling and sorting conducted by NT, SA, ACT and for north-west WA.

Given the infancy of bioassessment programs applied at a national level across Australia, and the paucity of any information on QA/QC of sample processing procedures as applied to the MRHI protocols, this QA/QC program was conducted in two parallel parts, namely: (i) implementation of routine external audits, with (ii) underpinning by literature review and R&D to evaluate methods and to devise and refine the criteria upon which the quality of agency samples was to be assessed. Specifically, the project objectives were to:

1. provide a literature review (and summary of relevant opinion) on QA/QC methodology and criteria for sampling and sorting of macroinvertebrate samples;
2. identify and recommend components of agency sampling and sorting protocols for

internal and external QA/QC audit;

3. recommend interim QA/QC methods and acceptance/rejection criteria for internal and external audit;
4. implement internal and external QA/QC programs;
5. evaluate and adopt suitable laboratory subsampling methodology;
6. undertake R&D to refine acceptance/rejection criteria;
7. recommend protocols and criteria for internal and external QA/QC of ongoing and future National/State monitoring programs; and
8. assess and report on agency QA/QC performances to the NRHP committee.

2 Methods

During the course of this study, additional investigations were conducted to evaluate the implications of errors associated with live sorting for model development and sensitivity. As a consequence, objectives 6 and 7 were expanded to incorporate aspects of these studies. Objectives 1 - 8 are reported in the following sections under the broader collective aims, and in the logical order:

- review of practices conducted elsewhere from which interim criteria were derived, and delimitation of internal and external QA/QC responsibilities (objectives 1-4 from above);
- evaluate and adopt suitable laboratory subsampling methodology;
- assess and report on QA/QC performances to agencies and the NRHP committee;
- undertake R&D to refine acceptance/rejection criteria. This was combined in the latter stages of the study with an evaluation of the implications for model development and sensitivity of errors associated with live sorting; and
- recommend protocols and criteria for internal and external QA/QC of ongoing and future National/State monitoring programs.

A brief description of methods used for the study components follows.

2.1 Literature review, derivation of interim criteria, and internal vs external QA/QC

A complete description of practices conducted elsewhere from which interim assessment criteria were derived, and delimitation of internal and external QA/QC responsibilities, are contained in the report by Storey & Humphrey (1996). The review draws largely on similar work being conducted in the UK and USA, with interim assessment criteria and thresholds for agency data acceptance/rejection being modified from comparable QA/QC being conducted in these countries (Cuffney et al 1993b, van Dijk 1994).

2.2 Evaluation and adoption of suitable laboratory subsampling methodology

A comparative statistical evaluation of results from processing three 'mega-samples', of known macroinvertebrate community structure, through four different subsampling devices is contained in the report by Storey & Humphrey (1997a). The work aimed to investigate and quantify sources of error in subsampling devices used by State and Territory agencies that preserve samples in the field for subsequent laboratory sorting.

2.3 Assess and report on QA/QC performances to agencies and the NRHP committee

Sample residues left after agency sample processing (field live sorting and laboratory subsampling and sorting) were selected at random and transported to ***eriss*** for external QA/QC processing. Processing entailed subsampling and sorting of residues and comparison of macroinvertebrate community composition and structure data present in an estimate of the

'whole sample' (live-sort + residue) with those present in the agency component. Agency data were assessed against the degree of departure in taxa number and community composition from whole sample estimates (WSE). Complete descriptions of this work are contained in Humphrey & Thurtell (1997).

2.4 Refine acceptance/rejection thresholds for assessment criteria and evaluate the implications for modelling of errors associated with live sorting

The approach adopted here was to simulate and introduce sample processing errors into an agency UPGMA classification and model that was relatively 'error-free' (ie derived from a lab subsampled and sorted data set) and determine a threshold error rate at which misclassification and mis-banding (model output) occurred. These thresholds, based upon live sort - WSE taxa number ratios and dissimilarity, would then serve to assess the adequacy of agency data when applied to various assessment criteria. Two types of simulated sample processing error were introduced into the agency model, namely that which preserved in a systematic manner, the pattern of error (Storey & Humphrey 1997b) and that which represented actual error (taxa biases) (Humphrey et al in draft) as observed in agency data. By replacing the entire 'error-free' agency data set with error-ridden data, both approaches could also be used to evaluate, in a preliminary manner, the implications for model development and sensitivity of errors associated with sample processing. Other approaches were used to derive acceptance/rejection thresholds for assessment criteria and were based simply on UPGMA mean within to between group dissimilarities (Storey & Humphrey 1997c).

2.5 Recommend protocols and criteria for internal and external QA/QC of ongoing and future National/State monitoring programs.

Such was the concern expressed at the degree and extent of error arising in live-sorting after the 3rd and 4th sampling rounds of the MRHI that recommendations were made that the live-sort protocol be revised (Attachment 3). As a consequence, some important changes have been made to the protocol whilst others will be implemented following additional R&D. A refinement of assessment criteria and acceptance/rejection thresholds for these criteria have also been recommended (Humphrey & Thurtell 1997).

3 Results

In the following sections, a summary of results, their interpretation, practical significance and a comparison of results against project objectives, are provided.

3.1 Literature review, derivation of interim criteria, and internal vs external QA/QC

In the report by Storey & Humphrey (1996), a number of objectives, in common with those of the project objectives are met, namely:

1. A summary of QA/QC programs applied to overseas rapid bioassessment protocols and a review of methods/approaches used in these programs.
2. Identification of all aspects of the MRHI protocol that should be subjected to QA/QC. Here internal QA/QC by agencies was identified as necessary for *all* aspects of the MRHI protocol. A major advantage of internal QA/QC identified in the UK lies in its potential to provide rapid feedback to staff, allowing corrective action to be taken in good time (Dines & Murray-Bligh in draft). It was recommended that external QA/QC be restricted to taxonomic identifications and efficiency of procedures used to subsample and sort samples, ie in line with practices adopted overseas (UK and USA).
3. Recommendation of basic approaches, designs and analyses to be applied by agencies when implementing QA/QC programs as part of the MRHI.
4. Selection of interim criteria (data quality objectives) for acceptance/rejection of QA/QC

conditions as applied in overseas studies, and a summary of remedial action recommended in the event of non-compliance.

5. A summary of QA/QC programs currently being undertaken as part of the Australian Monitoring River Health Initiative.

Storey & Humphrey (1996) recommended that formal arrangements be put in place for internal QA/QC and that their report should form the basis of a manual detailing all aspects of the MRHI protocol and QA/QC procedures.

3.2 Evaluation and adoption of suitable laboratory subsampling methodology

The design of the study evaluating the performance of different subsampling devices used by MRHI agencies was statistically rigorous, with results from the three mega-samples processed showing comparably high precision and accuracy of each of the devices in characterising community composition and structure (Storey & Humphrey 1997a). There was inherent variability for each device, and this tended to be influenced by sample composition (e.g. amount of detritus and proportion of 'rare' taxa in the community sampled). Whilst some devices are much faster (more economic) to use than others (Storey & Humphrey 1997a), the results, nevertheless, indicate that subsampling devices used by the different agencies that preserve samples in the field for subsequent laboratory sorting are adequate for this purpose. The results are also applicable to setting of 'best-possible' criteria for acceptance/rejection of agency subsampling and sorting data as assessed under a QA/QC program (see Humphrey & Thurtell 1997). The term, 'interim' in the title of the report by Storey & Humphrey (1997a) is a misnomer as the submitted report represents a completed study.

3.3 Assess and report on QA/QC performances to agencies and the NRHP committee

The report in Attachment 1 by Humphrey & Thurtell (1997) describes results for this aspect of the project. Feedback to agency staff on their performance has been progressive and relevant correspondence from ***eriss*** to agencies is enclosed in the report. Sorted and identified animals from residue and corresponding live-sort components from 95 live-sort agency samples, representing 5 habitats, together with 40 subsampling agency samples and residues, representing 6 habitats, were used in an assessment of the efficiency of agency sample processing procedures.

For agencies using a live-sort method for sample processing, two main sources of error were identified from the assessments, ie (i) under-representation of taxa; and (ii) different taxa recovery rates depending upon the efficiency of the operator. Factors contributing to poor taxa recovery in live-sorted samples included (i) low live-sort sample size, (ii) operator inexperience, and (iii) taxa commonly occurring in samples and across sites being missed (Humphrey & Thurtell 1997). For agencies using a lab subsampling and sorting method for sample processing, the main errors were associated with poor taxa recovery at low sample size, a consequence mainly of proportional subsampling (Humphrey & Thurtell 1997).

As reported in section 4 below and as a consequence of the above findings, aspects of the live-sort protocol were revised for implementation during the First National Assessment of River Health in Australia. The 30 minute time limit for sampling was replaced by a target sample size of 200 animals or sorting to one hour, whichever was reached first. In addition, agency staff were made aware of the taxa commonly missed in samples so that training programs could be implemented to redress deficiencies. Additional changes to the protocol will follow as the results of further R&D come to hand. For laboratory subsampling agencies, some recommended changes leading to standardisation of protocols have been made. These include (i) an emphasis on maximising taxa recovery (including 'large rares') through a coarse-screen search of the entire sample, and (ii) fixed-count subsampling.

3.4 Refine acceptance/rejection thresholds for assessment criteria and evaluate the implications for modelling of errors associated with live sorting

Two 'whole-model' simulations were conducted in this study to assess the effects of live-sort errors upon model construction and model-output sensitivity. The basis of the first of these was the superimposition of pattern of community structure and preservation of dissimilarity and taxa number ratio between 'live-sort' and 'whole sample estimate' found in live-sort samples assessed under the QA/QC program, upon an existing data set (derived from laboratory subsampling of preserved samples) with a new model constructed on the simulated data (Storey & Humphrey 1997b). The performance of the 'original' and the 'error' models could then be compared. In the event, it was not possible to derive an 'error' model from the 'error' data. Identified failings included breakdown in the biological structure of the classification which resulted in poor discriminant function analysis, reference sites appearing as impacted due to the effects of errors, impacted test sites appearing as more severely impacted due to errors, impacted test sites appearing as unimpacted, and classification of samples to incorrect groups in the classification.

There was a limitation in this approach arising mainly because at the time of conducting the study, there was insufficient data available to include also the *nature* of the errors, ie types of taxa typically missed or overrepresented in live-sort data. The second simulation was designed to overcome this deficiency by superimposing *actual* error (taxa biases) as observed in agency data (Humphrey et al in draft). This study was concerned with the consequences of missed common taxa for UPGMA classification, representing only one step in the full assessment and evaluation that would be required to address this issue. For this, the taxa in an AUSRIVAS data set for which data on taxa commonly-occurring across sites were well represented (ie the same ACT subsampled and sorted data set as used for the first simulation) were altered to match the bias observed in live-sort data. Two sets of live-sort data were used in the simulations: NSW, one of the poorer performing agencies, and for the average bias observed across eastern states, QLD, NSW, VIC and TAS. The average bias was not as severe as that for the single agency. Deletion of taxa was performed at random from actual occurrences in the original ACT data set, until the occurrences matched that of the bias represented in the two data sets. The deletions involved 16 out of a total of 39 taxa. For each of the single agency and average agency data, 3 separate simulations and classifications were run.

In the original ACT classification, 6 clearly defined groups were identified, and a model was successfully constructed by the CRC for Freshwater Ecology after applying Discriminant Function Analysis. For the classifications derived after error rates for the single agency were applied to the ACT data, dissimilarity cut-offs for the groups were found to be higher in the altered data indicating introduction of errors. Even so, in 2 of the 3 classifications, some preservation of the original classification was evident, even if there was a loss of one or two groups from the new classifications; only in one classification was there evidence of breakdown or "chaining" in classification structure.

In the eastern states classifications, however, there was less evidence of preservation of group structure and all exhibited 'chaining'. Interspersion of the original group sites was also more evident in the eastern states classifications. This is counter-intuitive to expectations, ie the better quality data produced more poorly-defined classifications. No diagnosis of the classifications has been conducted as yet to indicate why this result might have occurred, suffice it to say that it may represent a weakness in cluster analysis as a basis for group definition. Moreover, the level of taxonomic resolution used for MRHI, family-level presence-absence, may be so coarse that any structure present in the classification may be easily lost. This might be exacerbated in data sets from small geographical regions, such as the ACT, where group definition based upon family-level level presence-absence data could be expected to be quite subtle and vulnerable to introduction of even small errors.

Regardless, all of the classifications produced in the study appeared to contain greater inherent variability (as assessed by higher dissimilarity cut-offs for the groups) and the potential, therefore, to adversely affect the sensitivity of models.

In the first simulation described above, revised thresholds for QA/QC acceptance criteria were derived by feeding error samples one-by-one into the 'original' model and determining the incidences of misclassification and mis-banding. The recommended criteria (a Bray-Curtis dissimilarity value on presence-absence data of ~ 0.35, and a taxon number ratio of approximately 0.86 between live-sort and WSE data) are conservative but certainly achievable for data gathered under a revised sorting protocol (Storey & Humphrey 1997b). Note, however, that changes to the live-sort protocol (section 3.5) would necessitate a partial revision at least, of QA/QC assessment criteria. In Attachment 2, Storey & Humphrey (1997c) describe other approaches used to derive acceptance/rejection thresholds for assessment criteria that are based on UPGMA within to between group mean dissimilarities, giving a Bray-Curtis dissimilarity of approximately 0.38.

Further R&D by way of a sensitivity analysis is required to determine the consequences to model development, sensitivity and outputs, of data of the type found in this QA/QC program.

3.5 Recommend protocols and criteria for internal and external QA/QC of ongoing and future National/State monitoring programs.

Elements of the revised live-sort protocol were described in section 3.3 with further details provided in Attachment 3 (correspondence from Storey and Humphrey) and in Attachment 1 (Humphrey & Thurtell 1997). Revised protocols for sample processing procedures would also require revised QA/QC assessment criteria. A number of such criteria are proposed by Humphrey & Thurtell (1997) but would require refinement and testing.

4 Adoption of results

Communication for this project has focused on reporting of progress at NRHP technical advisory and steering committee meetings, a special MRHI meeting of agencies and TAC staff (February 1997), annual MRHI workshops (Canberra) and correspondence to agencies with feedback to them on their performance.

Over the period November 1996 to February 1997, the commissioned QA/QC team for MRHI sample processing procedures (***eriss*** and UWA) recommended changes to future live sorting and forwarded to agencies and technical experts for MRHI, draft protocols for live sorting. At a MRHI meeting held in Canberra in February (1997), the recommendations and proposed changes to the live sort protocol were essentially endorsed though there was concern expressed by some that the level of scrutiny and detail being proposed were driving the approach away from the essence of rapid assessment. Nevertheless, from this meeting it was decided to implement some of the recommended changes to the protocol immediately and implement others as results of additional R&D came to hand.

5 Publication titles

Apart from milestone and final reports to LWRRDC listed in section 7 below, two external publications have been prepared from the work conducted in this project:

Humphrey CL, Storey AW & Thurtell L In draft. AUSRIVAS - operator sample processing errors and temporal variability: implications for model sensitivity. *Proceedings of International RIVPACS Workshop, 16-18 September 1995, Jesus College, Oxford,* Organised and funded by Institute of Freshwater Ecology and Environment Agency (UK) and the Land and Water Resources Research and Development Corporation (Australia).

Storey AW & Humphrey CL In draft. Assessment of the efficiency of four types of device for subsampling of aquatic macroinvertebrate samples. *Hydrobiologia.*

6 Additional information

Recommendations as to future requirements for sample processing procedures and associated R&D are summarised according to short-term (immediate) and ongoing needs in Humphrey & Thurtell (1997). Additional information can be obtained in the milestone and final reports to LWRRDC listed in section 7 below, or by contacting the authors directly.

Acknowledgments

This project was jointly funded by the Commonwealth Environment, and Primary Industry and Energy Departments through the Land and Water Resources Research and Development Corporation. We thank Robyn Graham, Daryl Lehmann and their staff of ***eriss*** for managing the finances of the project.

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Storey AW & Humphrey CL 1997b. Refinement of QA/QC acceptance criteria for MRHI sorting procedures and a preliminary assessment of the effect of live-sort errors on the robustness of MRHI models: A conservative analysis through data simulation. *In* Development and implementation of QA/QC protocols for sampling and sorting components of the MRHI agency bioassessment program, by C Humphrey, A Storey & L Thurtell, Milestone Report 2 to Land and Water Resources Research and Development Corporation (Reference No. ARR2), Feb 1997.

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Additional attachment

Attachment 3. Proposed revised protocol for MRHI sample processing procedures and rationale, as distributed to agency staff and technical experts in January 1997.

ATTACHMENT 1

External QA/QC of MRHI agency subsampling and sorting procedures: Results for 1995 and 1996

FINAL REPORT

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Summary

External Quality Assurance/Quality Control (QA/QC) audits of MRHI State/Territory sample processing procedures (laboratory subsampling and sorting of preserved samples, and field live-sorting procedures) were conducted in order to quantify sample processing errors for the 3rd and 4th sampling rounds of the agency program.

The external QA/QC audits confirmed the potential of the live-sort procedure in particular, to result in 'high' error rates. For these samples, two main sources of error were identified from the assessments, (i) under-representation of taxa, and (ii) different taxa recovery rates depending upon the efficiency of the operator. Factors contributing to poor taxa recovery in live-sorted samples included low live-sort sample size, operator inexperience, and commonly-occurring taxa missed in samples and across sites. A small proportion of laboratory subsampled and sorted samples were also characterised by poor recovery of taxa, a consequence of low organism abundance in fixed-proportion subsamples.

Recommendations as to future requirements for sample processing procedures and associated R&D may be summarised according to short-term (immediate) and ongoing needs:

Short-term

1. Some changes to the MRHI live-sorting protocol have been made and implemented immediately, including fixed-count sorting (200 animals or one-hour sorting whichever is reached first) and staff training for recognition and recovery of small common/cryptic taxa. These changes were written into agency contracts for sampling under the FNARH. We note that improvement in the procedures for taxa recovery in ongoing MRHI studies would only stand to benefit monitoring programs if the quality of data for reference sites are also improved through a re-sampling, data replacement and re-modelling program.
2. It is not possible to re-process live-sorted samples as these are not preserved after sampling. However, it has been recommended to agency staff that poorly-'sampled' sites - as identified for example by low sample size - be re-sampled to replace reference site data of dubious quality.
3. It would be prudent to be cautious in the promotion of AUSRIVAS for site-specific assessments until the sensitivity of the method has been fully assessed and data quantity increased and quality improved.

Further needs including R&D

1. A sensitivity analysis is required to determine the sizes of various sources of error and variation and their effects on the rates of misclassification to quality bands (sensu Clarke et al 1996).
2. An extensive R&D project is required to fully revise live-sort protocols ensuring more discipline, prescription and training elements to future sampling and sample processing.
3. Internal and external QA/QC must accompany all future sampling and sample processing by MRHI agencies.

External QA/QC of MRHI agency subsampling and sorting procedures: Results for 1995 and 1996

1 Introduction

The Monitoring River Health Initiative (MRHI), quality assurance and control (QA/QC) for components of the bioassessment protocol have been categorised into 'external' and 'internal'. Internal QA/QC is the responsibility of the individual State and Territory agencies (hereafter S/T agencies) conducting the bioassessment program while external QA/QC is being carried out by independent agencies not involved with collection of data for this program. The rationale for separate external and internal QA/QC is provided in Storey and Humphrey (1996). A critical component of the MRHI protocol requiring external QA/QC is the efficiency of S/T agencies in sample processing procedures, ie subsampling and/or sorting. The ***eriss*** was commissioned by the National River Health Program committee to conduct such an external audit.

Two sample processing protocols are currently employed by agencies, either field sorting of live samples or laboratory subsampling and sorting of preserved samples. The external QA/QC study reported here, aimed to cross-check community composition and structure reported by agencies (from live-sorting or laboratory subsampling and sorting) against pre-defined benchmarks of community composition and structure of the whole sample. At the onset, it was recognised that most of the error arising in agency sorting procedures would arise in live sorting - as opposed to laboratory subsampling and sorting of preserved samples. Consequently, the majority of effort and reporting herein pertain to QA/QC results for live-sorted samples.

2 Methods for QA/QC of agency live-sorting procedures

2.1 Selection and procurement of residues for processing

Agencies that incorporate live-sorting in the MRHI protocol are VIC, NSW, QLD, south-west WA and TAS. For external QA/QC, a percentage of the residues remaining after field sorting of samples from rounds 3 and 4 of the first phase of the MRHI program was retained by agencies and preserved for later laboratory processing. These samples were randomly selected on the basis of geographical region, catchment, habitat and/or operator in such a manner that the sorting operator was only made aware of which residues were required for QA/QC processing *after* field sorting was completed. For the current QA/QC, this selection procedure was carried out by way of sealed envelopes sent from the external auditor (***eriss***) to the MRHI agency operators, the contents of which indicated whether or not the samples were required for processing (~5% of samples). For the most part, a sealed envelope was associated with, and accompanied, every sample sorted in the field since August 1995. Sample identity (location and habitat) was labelled on the outside of the envelope and after completion of field sorting, the operator opened the envelope to determine whether preservation of the sample residue was required.

Table A1 (Appendix 1) lists the sites and habitats from which agency residues were requested, the percentage of samples requested from the total number of habitats, and the number of

residues actually processed for external QA/QC. Community structure data from the live-sort (LS) component of samples was sought in either of two ways: (i) the preferred approach, by way of receipt of the actual LS sample with identification and enumeration then performed by the external auditor; or (ii) by way of the data being forwarded directly from the agency concerned. Those samples processed/identified in entirety (live-sort and residue) are indicated in Table A1. For each of the live-sorted samples processed for QA/QC assessment, data on the 'experience' of the sorting operator was requested from agencies in terms of number of years experience at live sorting. Agencies sorting preserved subsamples (ACT, SA, NT and northwest WA) were presented with a list of residues to retain from rounds 3 and 4 for external QA/QC audits (Table A1). These residues were forwarded to ***eriss*** following removal and processing of required subsamples by agencies.

Table 1 lists the final suite of samples which were assessed, and for which results are reported herein. Where possible, a minimum of several samples from each habitat and for each agency was processed across the 3rd and 4th sampling rounds. This provided a 'baseline' to assess performance (improvement or maintenance of standards) of MRHI agency data.

Table 1. Number of agency samples completed for external QA/QC assessment, in relation to habitat.

Agency	Habitat						
	Riffle	Edge	Pool	Macrophyte	Channel	Sand	TOTAL
NSW	7	9	1	2	-	-	19
TAS	8	7	-	-	-	-	15
QLD	7	6	4	5	-	-	22
VIC	9	8	-	-	-	-	17
WA (LS)	3	-	2	7	11	-	23
WA (Lab.)	2	-	1	1	2	-	6
ACT	5	5	-	-	-	-	10
NT	-	5	-	-	-	5	10
SA	3	3	4	3	-	-	13
Total	44	43	12	18	13	5	135

2.2 Processing of residues and analysis of data

Assessment of the efficiency of agency subsampling and sorting procedures required community composition and structure of agency subsampled and/or sorted samples to be compared to those of residues preserved and processed further after agency sorting.

2.2.1 QA/QC assessment of field live-sorted samples

Two possible criteria were available upon which to assess the efficiency of agency live-sorting procedures, ie the criterion that the agency-processed samples (i) were *representative* of the whole sample in terms of community composition and structure, or (ii) contained the *broadest range of biota* captured/collected at a site - the latter being the aim of live-sorting as stated in the MRHI Bioassessment Manual (Davies 1994). Field live-sorted samples were partially assessed in the external QA/QC program against both criteria, using the following procedures:

Representativeness of live-sorted samples

Using the assessment objective of 'representativeness', the aim in QA/QC audits of field live-sorted samples was to compare the live-sorted component with an equivalent-sized component representative of the whole sample (prior to sorting). Specifically, the live-sorted component was compared to a 'Proportional Estimate of the Whole Sample' or 'WSE', where the WSE is

an estimate of community composition and/or structure present in the original unsorted sample had the same number of animals as was live-sorted been derived from laboratory subsampling. The WSE was estimated from the addition of taxa information derived from a subsample of the residue and a 'subsample', to a similar proportion as that taken from the residue, of the Live-sort (LS) component. Details of procedures used to estimate WSE are provided in Appendix 2. For LS samples with sample abundance <100, WSE was also standardised to an abundance of 100 animals and LS data compared to assessment criteria derived from this (WSE_{100}) data set, as well as that derived conventionally for an N equivalent to the live-sort sample size. Subsamples of the residue were taken using a modified Marchant subsampler. (See Storey & Humphrey (1997a) for details of the relative efficiency of this device.)

QA/QC criteria to adopt in the assessment of field live-sorting representativeness were modified from Storey and Humphrey (1996). Thresholds for acceptance/rejection of agency results for QA/QC criteria based upon presence/absence data, follow generally from those preliminary values derived from associated R&D (Storey & Humphrey 1997b,c; see also section 3.3 below). For relative abundance data, liberal thresholds were set on the basis that if these values could not be met (ie B-C dissim > 0.5), it would not be possible to use the data in models based upon rank abundance. Thus:

1. The number of taxa encountered in the live-sort (LS) component must not fall below 20 percent of the number of taxa recorded in the best proportional estimate of the whole sample (WSE) (ie taxa number ratio, LS/WSE, must be ≥ 0.8); and

2. The community similarity index (Bray-Curtis) comparing the LS component and WSE must be at least 50 percent for relative abundance data and 65 percent for presence-absence data.

The LS/WSE ratio used in the assessments is in some ways analogous to the RIVPACS observed/expected ratio.

Recovery of as broad a range of taxa as possible

Adherence to this objective by agency staff would imply that the taxa list derived from sorting would 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 original sample (the latter equivalent to the WSE). In maximising taxa recovery, there would be an expectation that taxa would not necessarily be recovered in proportion to their relative abundance in the whole sample.

QA/QC criteria to adopt in the assessment of field live-sorting that aimed to maximise taxa recovery were only partially developed - and hence assessment against this objective only partially achieved - for this study. The sorting objective is one based upon taxa recovery only, with little consideration, if any, given to recovery of relative abundance data. Hence, QA/QC criteria are based only upon presence/absence data. The principle of QA/QC criteria developed for this live-sorting objective is that taxa number derived from live sorting should exceed that derived from a 'whole sample estimate' as defined above. Further, because of the *additional* taxa that would be expected in the LS component (above those found in the WSE), these taxa would not be used in the criterion assessing the degree of similarity of community composition between LS and WSE samples. Compositional similarity in this case would be reduced and restricted to only those taxa commonly-occurring in the whole sample.

Possible QA/QC criteria and thresholds for acceptance/rejection of agency results based upon maximisation of taxa recovery are stipulated only for taxa number at this stage, thus:

- The number of taxa encountered in the live-sort (LS) component must exceed the number of taxa recorded in the best proportional estimate of the whole sample (WSE) (ie taxa number ratio, LS/WSE, must be ≥ 1.0).

A criterion based upon compositional similarity of LS and WSE components for the live-sort objective (based upon maximisation of taxa recovery) that could be applied in future QA/QC for MRHI, is discussed in section 4.2.4.

2.2.2 QA/QC assessment of laboratory subsampled and sorted samples

It was assumed that the aim in laboratory subsampling and sorting was to obtain a sample *representative* of the whole sample in terms of community composition and structure. The principles of the QA/QC procedures used to assess agency results were similar to those used to assess representativeness of live-sorted samples, as described above. The QA/QC procedure adopted for assessing the representativeness of laboratory subsampled and sorted (preserved) samples used as its basis, subsampling of another fraction of equivalent size from the residue, sorting and identifying animals from the second fraction, then comparing community composition and structure of the first (agency) and second (externally-derived) fractions. The procedures used to make this comparison were similar to those used to assess live-sorted samples and are detailed in Appendix 2.

In comparing an additional same-sized subsample from the residue, the external QA/QC subsampling took into account the fraction already removed by the agency, whilst additional taxa data derived in the agency sample from a coarse screening of the whole sample following subsampling (ie usually rare, conspicuous taxa) were not included in the QA/QC assessment. Subsamples of the residue for the externally-derived fraction were taken using a modified Marchant subsampler.

Interim QA/QC criteria and thresholds for acceptance/rejection of the criteria were modified from Storey and Humphrey (1996), thresholds generally being more conservative than those used to assess field live-sorting efficiency. Thus:

1. The number of taxa encountered in the second (external QA/QC) subsample must lie within 20 percent of the number of taxa recorded in the agency subsample; and
2. The community similarity index (Bray-Curtis) comparing the original subsample and the second subsample must be at least 70 percent for relative abundance data and 65 percent for presence/absence data.

2.2.3 Taxonomic resolution

The only difference in taxonomic resolution adopted in the QA/QC program that differed from that of MRHI agency procedures was the identification for QA/QC of Chironomidae to family and not subfamily level. This procedure was adopted in order to expedite the number of samples that could be processed. Thus, for all comparisons of external auditor vs agency results, agency chironomid subfamilial data have been combined.

2.2.4 Modifications to approaches described in earlier milestone reports describing external QA/QC assessment

In a past report (Thurtell & Humphrey 1996), we compared results for analysis of data with and without 'rare' taxa (ie taxa occurring uncommonly in the sample). The conclusions drawn from this comparison were that there were essentially no improvements to data quality either with or without the inclusion of taxa occurring uncommonly in the sample. In any case, analysis associated with MRHI modelling decides on the issue of rarity (= relative occurrence

across sites) and for these reasons, analysis has been conducted using all taxa encountered in the LS (or agency laboratory subsample) and residue components of the sample.

In previous reports, an additional criterion based upon Spearman Rank correlations, was applied to rank abundance data (Thurtell & Humphrey 1996, Humphrey & Thurtell 1997). However, rank correlation methods are sensitive to sample size in determining statistical significance and across regions of naturally-varying taxa richness, therefore, could lead to misleading conclusions. Rather, the Bray-Curtis dissimilarity measure was used to describe the degree of similarity in samples for both presence-absence and relative abundance data. As this measure is the basis of UPGMA classification of MRHI data for model development, dissimilarity values derived in agency vs external QA/QC comparisons are potentially well suited to assessing the degree of agency performance.

2.2.5 Data analysis

As described above and in detail in Appendix 2, the WSE (for live-sort data and its analogue for laboratory subsampled and processed data) was estimated from addition of taxa information derived from a subsample of the residue and a 'subsample', to a similar proportion, of the live-sort component. The principle was adapted from similar assessments performed by Davies et al (1997). 'Subsampling' of the LS component, and standardisation of the combined residue and live-sorted fractions to the same N as the live-sort component (or to 100 for LS abundance < 100), were achieved by proportional scaling, with particular rules governing the rounding of taxa fractions (< 1) (Appendix 2). The accuracy with which the WSE is derived from these calculations was assessed by comparing taxa number derived after scaling down the combined residue and 'subsampled' LS components to the required WSE using (i) scaling procedures derived in this study (Appendix 2), and (ii) the 'rarefaction' (= 'scaling down') formula of Heck et al (1975). The 'rarefaction' formula of Heck et al (1975), is based upon the hypergeometric distribution, and calculates expected taxa number (together with confidence limits) in a random sample of n individuals from a collection containing N individuals and S taxa. The method of Heck et al (1975) was assumed to be the most accurate for estimating taxa number and in this respect was the benchmark for assessing accuracy of the proportional scaling method used in the present study. Community structure data from twenty live-sort samples and associated residues, encompassing a wide spread of LS taxa number values, was used in this comparison.

Paradox for Windows (1994) computer macros were used to derive all calculations for QA/QC assessments and the rarefaction estimates of Heck et al (1975). The Bray-Curtis dissimilarity measure was calculated in the computer macro, for two samples, j and k, based on taxa 1 to N (indexed by i) as:

$$\left(\sum_i |x_{ij} - x_{ik}|\right) / \left[\sum_i (x_{ij} + x_{ik})\right]$$

where x_{ij} is the abundance for taxon i in sample j.

Summary data for QA/QC assessments were generally calculated and plotted using median and quartile values, owing to the skewed nature of results. Medians and percentiles, being resistant to extreme values/outliers, are appropriate summary statistics for data of this type. Regression analyses conducted in this study were performed using the MINITAB software package (MINITAB 1995). All boxplots defined lower and upper quartiles divided at the median, with vertical lines showing the range of values that fall within 1.5 times the interquartile range. Outliers are points outside these limits and are plotted with asterisks.

3 Results

3.1 Verification of procedures used to derive WSE data

3.1.1 Comparison of scaling procedures

The accuracy of the scaling-down calculations used to derive WSE data in the present study was determined by comparing estimates of expected taxa number using rarefaction procedures adopted in this study with those of Heck et al (1975). For 20 samples encompassing a wide spread of LS taxa number values, taxa number derived after scaling down the combined residue and 'subsampled' LS components to the required WSE were compared for the two methods using regression analysis. The relationship between taxa number estimated using the two methods in shown in Figure 1. The regression equation describing this relationship is given as:

$$E(S_n) = -0.077 + 0.964\ MPS_n \qquad (P = 0.000,\ R^2 = 0.98)$$

where $E(S_n)$ = expected number of taxa using rarefaction formula of Heck et al (1975), and MPS_n = taxa number using modified proportional scaling method adopted in the present study.

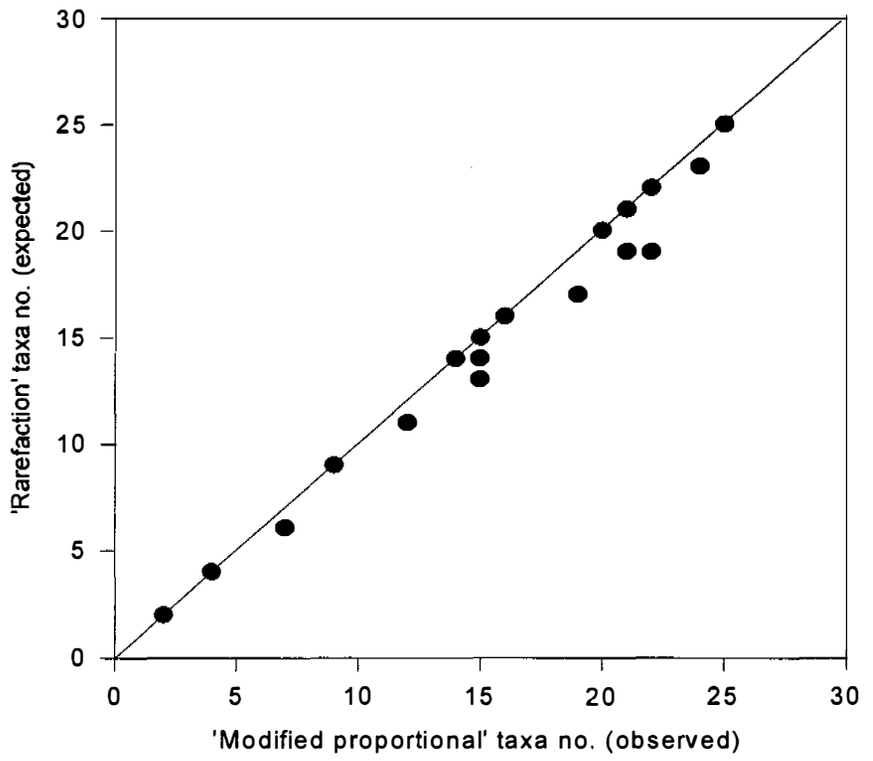

Figure 1. Relationship between expected taxa number using rarefaction formula of Heck et al. (1975) and modified proportional scaling method from this study. Diagonal line is a plot of 1:1 relationship.

There was a very slight tendency only for the proportional scaling method to overestimate taxa number, though the 95% CIs for the slope and Y-intercept for the regression relationship contained, respectively, the values 1 and 0 (indicating the regression equation was not significantly different from a 1:1 relationship). As described below, poor taxa recovery was a feature of live-sorted samples and hence the results of this method comparison would indicate that this problem was slightly underestimated in the QA/QC assessments where proportional scaling was employed.

3.1.2 Other evaluations of WSE derivation, calculation and precision

Some measure of the consistency (precision) with which the WSE was derived (including residue subsampling and processing) and calculated for the external QA/QC assessments was sought from a small study in which invertebrates from two live-sorted samples, and successive subsamples of the respective large residues, were identified and enumerated. Both residues were subsampled to small fixed proportions and processed in their entirety. Summary characteristics of the live-sort (LS) and entire residue components of the two samples are provided in Table 2.

Table 2. Invertebrate compositional characteristics of 2 live-sorted samples.

Sample	Sample component	No. of taxa	No. of animals
1	Live-sort	21	222
	Residue	33	6229
2	Live-sort	18	196
	Residue	37	8961

WSE data were computed for 3 randomly-selected subsamples from each residue, as well as for the entire residue of each sample (with WSE data scaled-down to the same abundance as that of the respective LS samples). QA/QC assessment criteria were applied to all WSE and respective LS data, with results shown in Table 3. As can be seen from Table 3, QA/QC results are similar whether WSE data are derived from entire residues or from small subsamples of the residues.

Table 3. QA/QC assessment criteria calculated for different subsample components of residues.

Sample	Fraction of residue sorted (%)	QA/QC Assessment criterion		
		LS/WSE	Dissimilarity (pres/abs)	Dissimilarity (abundance)
1	5.5	1.16	0.26	0.49
	5.5	1.11	0.20	0.54
	5.5	1.31	0.29	0.48
	100	1.11	0.25	0.48
2	5.25	1.13	0.24	0.58
	5.25	1.13	0.35	0.64
	5.25	1.06	0.36	0.64
	100	1.00	0.25	0.61

3.1.3 Internal checks of data quality

Internal checks of the consistency of results derived from external audits, ie residue subsampling and sorting and WSE estimation, were performed occasionally through the study. In these cases, a second subsample of the agency residue was taken and the two external audit samples then compared with one another. Thus, agency-QAQC1 result could be compared with QAQC1-QAQC2 result. Such pairs of results for 3 ACT agency samples are shown in Table 4. (Different fractions of residue between sample pairs are taken to account for the removal of residue from the first QAQC sample.) Similar low variation in results between the two QAQC samples (Table 4) was encountered in these checks as was reported for the evaluation above (section 3.1.2).

Table 4. QA/QC assessment criteria calculated for (i) agency-QAQC subsample 1, and (ii) duplicate QAQC subsamples, for ACT agency residues.

Sample code	Comparison	Fraction of residue sorted (%)	QA/QC Assessment criterion		
			LS/WSE	Dissim (p/a)	Dissim (abund)
ACT144	Agency-QAQC 1	5.0	0.87	0.328	0.133
	QAQC 1 - QAQC 2	5.5	0.94	0.094	0.112
ACT152	Agency-QAQC 1	5.5	0.93	0.515	0.229
	QAQC 1 - QAQC 2	6.0	1.21	0.197	0.165
ACT170	Agency-QAQC 1	5.0	0.83	0.306	0.263
	QAQC 1 - QAQC 2	5.5	0.95	0.174	0.176

Collectively, the results of sections 3.1.2 and 3.1.3 indicate that the systematic and consistent application of methods applied to all MRHI samples in this study give rise to WSE values that adequately reflect community composition and structure for a given sample size.

3.2 Applicability of results from subsampling R&D in deriving QA/QC acceptance criteria

In a related R&D project, the efficiency (precision) of four subsampling devices in deriving subsamples across 3 common samples was evaluated. Results showed similar precision amongst the devices for each of the samples, as determined by pairwise replicate (5 reps per sample and device) comparisons for various assessment criteria (Storey & Humphrey 1997a). Data from pairwise comparison of the replicate subsamples derived from the samples can be used to define upper benchmark thresholds (= best possible result) for QA/QC acceptance criteria of agency laboratory subsampling and sorting, and field live-sorting. To this end, mean taxa number ratios and (Bray-Curtis) dissimilarity values amongst all possible pairwise comparisons for the 4 devices and 3 samples are provided in Table 5. (Raw data are presented in Storey and Humphrey (1997a).)

Table 5. Mean amongst pairwise replicate comparisons and across 3 samples for various assessment criteria, derived from assessment of precision of 4 subsampling devices. Mean of minimum and maximum values shown in parentheses. (For taxa number ratio, greater of the two taxa scores always used as denominator.)

Assessment criterion		
Taxa ratio	Dissimilarity (pres/abs)	Dissimilarity (rel abund)
0.89 (0.79-1.00)	0.12 (0.03 - 0.21)	0.13 (0.08 - 0.17)

3.3 Results of QA/QC assessment for different agencies, habitat and according to operator experience

Sorted and identified residue and corresponding live-sort components from 95 live-sort agency samples, representing 5 habitats, together with 40 subsampling agency samples and residues, representing 6 habitats, have been used in an assessment of the efficiency of agency sample processing procedures. Results of QA/QC analyses are discussed below in terms of community structure (relative abundance) and composition (presence/absence) data. Differences amongst agencies and habitat and according to operator experience are presented under these two community summary types.

Note that analyses comparing LS-WSE similarity in the tables and figures below and in Appendix 3 employ the Bray-Curtis dissimilarity measure (the complement of the similarity criteria described in section 2.2.1 above).

3.3.1 Community structure (relative abundance)

As described in sections 2.2.1 and 2.2.2, taxa 'relative abundance' criteria for QA/QC assessment were based upon comparison of LS-WSE (live-sort data) or of 2 independently derived subsamples (lab subsampling data) using the Bray-Curtis (dis)similarity index. Results of all QA/QC assessments are presented in Table A3 (Appendix 3). Results are discussed according to agency, habitat and operator experience, as follows:

Amongst agencies

Summary results for dissimilarity measures amongst agencies are shown in Figure 2 with raw data provided in Table A3. With analyses conducted using dissimilarity measures, 36% of live-sort samples failed to meet the acceptance criterion (dissim < 50%), whilst 15% of samples from the lab subsampled data did not meet the criterion (dissim < 30%) (Table A3).

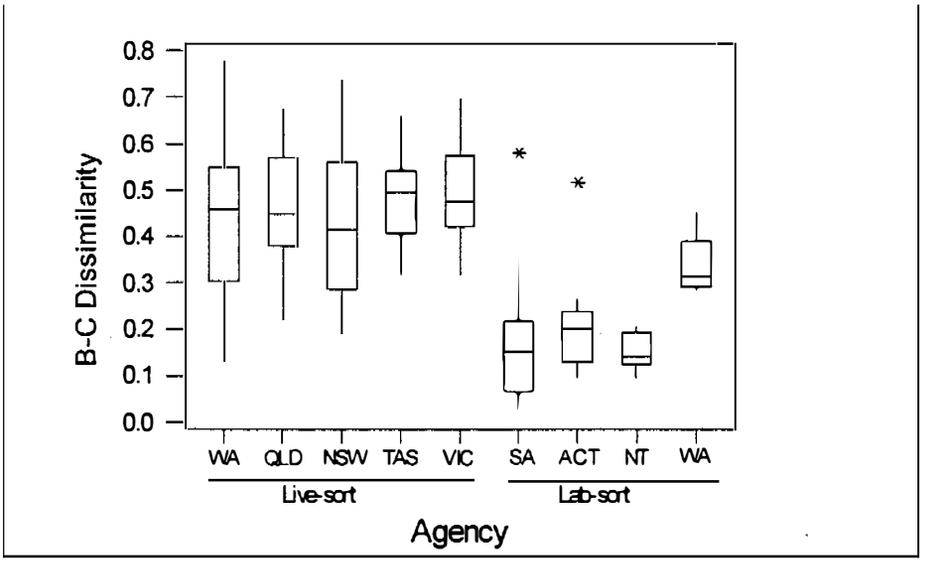

Figure 2. Boxplot showing comparison of LS-WSE (live-sort relative abundance data) or of 2 independently derived subsamples (lab subsampling rel abundance data) for the different agencies using the Bray-Curtis dissimilarity index.

Forty percent of live-sorted samples which did not meet the acceptance criteria contained < 100 animals indicating that with low sample size, representation of community structure of samples was poor. One sub-agency for WA (University of WA) used a systematic 'field subsampling' method for sorting, processing the sample in consecutive defined portions and with the aid of jeweller's visors. With these samples, only 12.5% failed to meet the dissimilarity criterion (0.5) compared with the overall average for live-sort agencies overall of 36%. This success rate is high and indicates the methods used were very efficient at in deriving data representative of community structure.

The majority of the lab subsamples which did not meet the acceptance criteria were from WA and SA (Table A3). The WA agency used a method which was highly efficient in recovering a very broad range of taxa with lesser regard to recovery of relative abundance data (see methods, Appendix 5); thus results differed greatly to the QA/QC method which simply recovered all animals from one small subsample. Both lab samples from SA which did not meet the criteria contained < 100 animals in the subsample. The final lab sample which did

not meet the criterion was from the ACT; the error appeared to be the result of mis-labelling with the wrong sample being sent to ***eriss*** (R Norris, pers comm).

In summary, only through careful subsampling and sorting was it possible to recover relative/rank abundance data in sample processing procedures. Such recovery was met in most lab subsampled data but less successfully in live-sorted data where generally, these procedures were not adhered to (Figure 1). The protocol of Davies (1994) does not mention the need for recovery of rank abundance data.

Habitat

Amongst live-sort agencies and with analyses conducted using dissimilarity measures, 12.5, 34, 43 and 66% of samples failed to meet the acceptance criterion (dissim < 0.5) for channel, riffle, edge/macrophyte and pool habitat respectively (Table A3). The distribution of these dissimilarity values for different habitat is shown in Table 6. Thus, reasonable recovery of relative abundance data was achieved only in habitats generally free of fine silt and detritus (riffle and channel) - associated material that would otherwise obscure animals and hinder live sorting.

Table 6. Assessment of MRHI agency sample processing procedures according to criteria based upon relative abundance.

Method/Habitat	Sample Size	Distribution of Dissimilarity Values (%)			
		<0.2	0.2-0.3	0.3-0.4	>0.4
COMPARISON OF HABITATS ACROSS AGENCIES					
Laboratory subsampling (all 4 agencies)	39	59	26	10	5
Live-sorting (all 5 agencies)					
Riffle	34	6	9	27	58
Edge/Macrophyte	43	2	9	7	82
Pool	7	0	0	15	85
Channel	11	9	27	27	37

Operator experience

No obvious differences were found amongst operators of varying levels of live-sorting experience in recovery of relative abundance data (Fig 3). However, there is a possible tendency in the data to suggest that the most inexperienced operators achieved more favourable results in this respect than the other operators. It is possible that lack of knowledge of the fauna by inexperienced operators tends to result in recovery of taxa in proportion to their visual occurrence. (Uncertainty about the identity of taxa might lead operators to include 'everything just to be certain'.) More experienced operators may be either more complacent in this respect or may be more concerned in recovering a broad range of taxa than rank abundance data.

3.3.2 Presence/absence data

As described in section 2.2.1, taxa 'presence/absence' criteria for QA/QC assessment are based upon comparison of LS-WSE (live-sort data) or of 2 independently derived subsamples (lab subsampling data) using the Bray-Curtis (dis)similarity index and LS/WSE taxa number ratio. As described above (section 2.2.1), ideally results would be assessed against objectives of both obtaining representative samples and maximising taxa number. Only for the LS/WSE taxa number ratio has it been possible at this stage to assess results against the objective of maximising taxa number. Results of all QA/QC assessments are presented in Table A3

(Appendix 3). Results are discussed according to agency, habitat and operator experience, as follows:

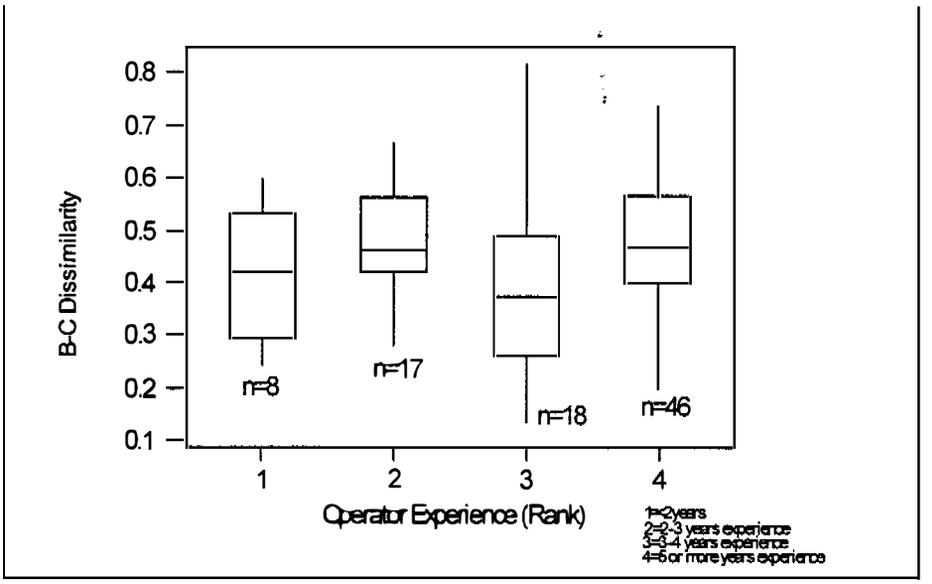

Figure 3. Boxplot showing comparison of LS-WSE (relative abundance data) for operators of different levels of live-sorting experience using the Bray-Curtis dissimilarity index.

Amongst agencies

Summary results for dissimilarity measures amongst agencies are shown in Figure 4 with raw data provided in Table A3. Analyses conducted using dissimilarity measures (- for this report assessed only according to the objective of representativeness -) show that 41% of live-sort samples and 12% of lab subsampled data failed to meet the acceptance criterion (dissim < 35%) (Table A3). For live-sort agencies, NSW and WA agencies performed most poorly in LS-WSE comparisons of p/a data and QLD the best (Figure 3 and Table 10). Poor results in lab subsampled data were often associated with low agency sample size (N < 100 animals) (Table A3), a factor expanded upon in correspondence to relevant agencies (Appendix 4) and in section 4.1.

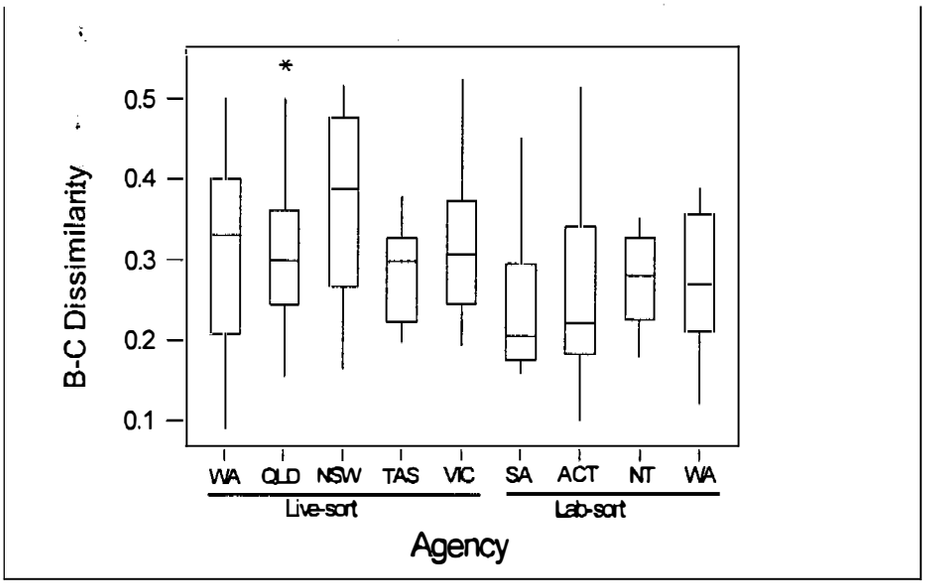

Figure 4. Boxplot showing comparison of LS-WSE (live-sort pres/abs data) or 2 independently derived subsamples (lab subsampling pres/abs data) for the different agencies using the Bray-Curtis dissimilarity index.

For live-sort agencies, a 'poor' result based upon dissimilarity measures can reflect either taxa missed during sorting, or taxa sorted that are in addition to those found in the residue. Because of the latter possibility (ie maximisation of taxa recovery), the dissimilarity measure calculated in this report is a less accurate gauge of agency performance than the LS/WSE taxa number ratio. (In fact, results from the LS/WSE taxa number ratio analysis show that the 'high' dissimilarities associated with LS-WSE comparisons are mainly the result of missed taxa, see section 3.5.1.)

Results of LS/WSE taxa number ratios are shown in Figure 5 with raw data provided in Table A3. (For these comparisons, WSE data were scaled to the same sample size as the agency live-sort or lab subsample size.) Results are considered with respect to performance against the objectives of 'representative' subsampling and/or sorting, and sorting to 'maximise taxa recovery'.

Recovery of representative samples. The LS/WSE taxa number ratio showed that 19% of live-sorted and 5% of lab subsampled data failed the acceptance criterion (ratio < 0.8) (Table A3). These results underestimate the problem of poor taxa recovery in the sense that WSE data are presented here only in terms of standardisation to sample size of the agency sample (ie not WSE_{100}). NSW and TAS performed the most poorly of the live-sort agencies (Fig 5). Poor results in lab subsampled data were associated with low agency sample size (N < 100 animals) (Table A3).

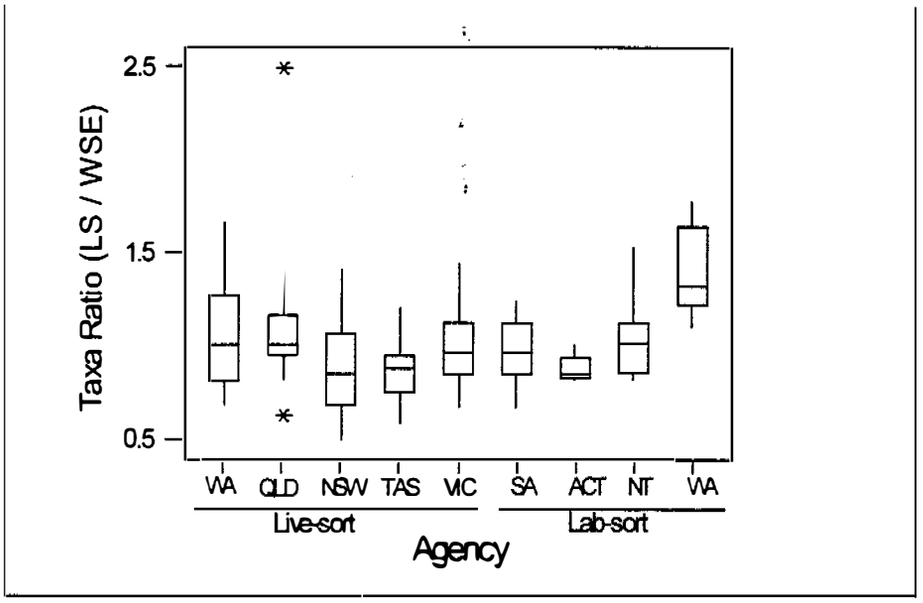

Figure 5. Boxplot showing comparison of LS-WSE (live-sort/whole sample estimate ratio) or of 2 independently-derived subsamples (lab subsampling 'LS'/WSE ratio data) for the different agencies.

Maximisation of taxa recovery. For live-sort agencies, the LS/WSE taxa number ratio was less than 1 for 51% of samples whilst for the WA lab subsampling agency and all other lab subsampling agencies, the ratio was less than 1 for 0 and 57% of samples respectively (Table A3). Thus, if the objective of live-sorting is to maximise taxa recovery (in which case LS/WSE would be expected to exceed unity) then in about 50% of cases this condition was not met. Lab subsampled data achieve results consistent with an objective of recovery of representative samples (ie LS/WSE $\approx$ 1). The WA laboratory subsampling agency out-performed all other agencies in its ability to recover a broad range of taxa (Fig 5). The consistently high LS/WSE ratio reflects the efficiency of WA subsampling and sorting techniques in meeting this objective (see Appendix 5).

Habitat

The distribution of p/a dissimilarity and taxa number ratio values for different habitat is shown in Table 7.

Table 7. Assessment of MRHI agency sample processing procedures according to criteria based upon presence/absence data.

Method/Habitat/ Agency	Sample Size	Distribution of Dissimilarity Values (%)				Live-Sort: WSE Ratio (% of samples lying within)		
		<0.2	0.2-0.3	0.3-0.4	>0.4	±10%	±10-20%	≥±20-30%
COMPARISON OF HABITATS ACROSS AGENCIES								
Laboratory subsampling (all agencies)	39	23	46	26	5	38	38	24
Live-sorting (all agencies)								
Riffle	34	18	41	26	15	36	32	32
Edge/Macrophyte	43	0	31	44	25	29	27	44
Pool	7	13	29	29	29	43	14	43
Channel	11	46	18	18	18	18	36	46

Amongst live-sort agencies and with analyses conducted using dissimilarity measures, 20, 35, 51 and 50% of samples achieved a dissimilarity measure less than 0.35 for channel, riffle,

edge/macrophyte and pool habitat respectively (Table A3). Using LS/WSE taxa number ratio, 20, 17, 11 and 16% of live-sorted samples achieved a ratio < 0.8 for channel, riffle, edge/macrophyte and pool habitat respectively (Table A3), a more even distribution of errors amongst habitat. As was also observed for relative abundance data, there was reasonable recovery of presence/absence data for habitats generally free of fine silt and detritus (riffle and channel).

Operator experience

No obvious differences were found amongst operators of different levels of live-sorting experience in recovery of presence-absence data as assessed by dissimilarity values (Figure 6). Lack of obvious trends in the data is possibly associated with the fact that LS-WSE assessments were based only upon the objective of recovery of representative samples in live-sorting (as per lab subsampling - see above). Patterns with LS/WSE taxa number ratio and operator experience, however, were more obvious (Figure 7). Using this assessment criterion, it appears that the least experienced operators were more consistently underrepresenting taxa, with lowest ratios found amongst these sorters (Figure 7). Reduced rates of taxa recovery may be associated with the slower rate of sorting (ie lower sample size) found for these operators (section 3.5.2).

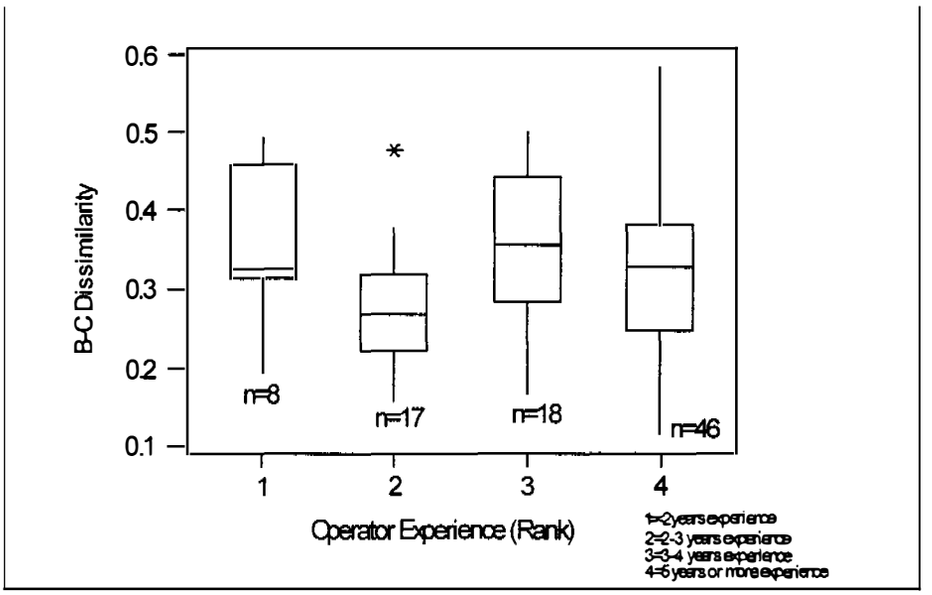

Figure 6. Boxplot showing comparison of LS-WSE (presence/absence data) for operators of different levels of live-sorting experience using the Bray-Curtis dissimilarity index.

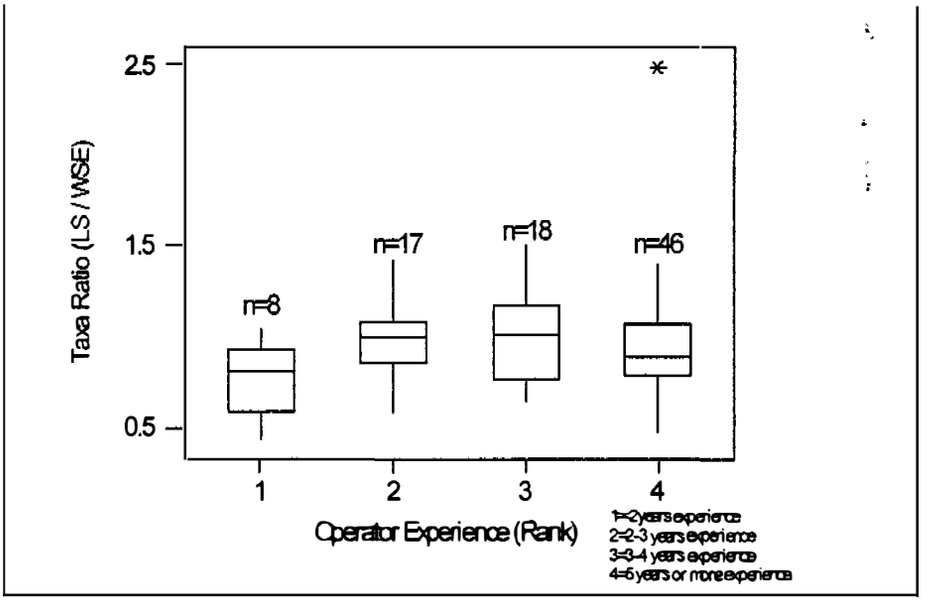

Figure 7. Boxplot showing comparison of LS-WSE (presence/absence data) for operators of different levels of live-sorting experience using the LS/WSE taxa number ratio.

3.4 Potential problems with live-sort and lab subsampled data

In the latter part of 1996, a tabulated list of factors contributing to difficulty in achieving aims of live-sorting was distributed to agency staff as well as to MRHI technical experts; this list in a revised form is reproduced in Table 8. Since distributing this list, it has been possible to analyse data further so that a number of key concerns, particularly for live-sort data, may be narrowed down and where possible, critically evaluated for the effect they may have in compromising data quality.

Table 8. Factors contributing to difficulty in achieving aims of live-sorting where objective is collection of (a) broad range of taxa, (b) representative sample. (Incidences of occurrence amongst QA/QC samples)

AGENCY		FACTOR									
		[1]Missed common taxa		[2]Mean no. of missed common taxa	[3]Over collecting specific taxa		[4]Small sample size		[5]Poor taxa recovery		[6]Range in LS/WSE ratio
		n	%		n	%	n	%	n	%	
QLD	(n=22)	19	86	1.8	6	27	4	18	1	5	0.75-2.3
NSW	(n=19)	18	94	3.4	1	5	6	32	5	26	0.42-1.42
VIC	(n=17)	17	100	3.2	4	24	4	24	2	12	0.56-1.38
TAS	(n=15)	14	93	2.5	0	0	1	6	5	33	0.58-1.09
WA LS	(n=23)	9	39	0.56	1	4	11	48	5	22	0.63-1.66
SA	(n=13)	0	0	0	8	61	4	30	2	13	0.58-1.22
ACT	(n=10)	8	80	1.9	4	40	0	0	0	0	0.82-1.01
NT	(n=10)	7	70	1.3	5	50	0	0	0	0	0.82-1.53
WA LAB	(n=10)	3	30	0.8	0	0	2	33	0	0	1.11-1.77
MEAN			66	1.7		21		23		15	

[1-5] Concern for (a) and (b);

[1] No. of samples containing missed common taxon, ie taxon which was present in >50% of Live-sort (LS) or 'Whole sample estimate (WSE)' samples;

[2] Mean number of missed common taxa (as defined for 1) per sample

[3] No. of samples for which abundance value for any LS taxon ≥ 100;

[4] No. of samples for which total no. of animals in LS ≤ 100;

[5] No. of samples for which taxa number ratio LS/WSE < 0.8;

[6] Range in LS/WSE ratio across all samples.

Two main factors identified in Table 8 appear to have the potential to create difficulties for MRHI model development, or otherwise to reduce the sensitivity of the model insofar as accurately diagnosing the status of water quality at a site. These factors are: (i) under-representation of taxa; and (ii) a broad range in taxa recovery observed amongst operators. (It should be noted that these concerns focus solely upon recovery of presence/absence data; the viability of relative abundance data as a basis for modelling is discussed elsewhere (section 4.2.2). These factors are dealt with here in turn whilst some brief assessment of the significance of these factors in contributing to error in model development/outcomes is discussed in section 4.1.3.

3.5.1 Underrepresentation of taxa in live-sort samples

A potential source of error in samples processed for MRHI is underrepresentation (poor recovery) of taxa - see issue 5 of Table 8. This error is almost solely confined to live-sorted samples (Table 8). *[It is important to note that these results underestimate the problem of poor taxa recovery in that WSE data are presented here only in terms of standardisation to sample size of the agency sample, ie not WSE_{100} (see section 3.3.2)].* Factors contributing to poor taxa recovery in live-sorted samples include, (i) low live-sort sample size, (ii) operator inexperience, and (iii) missed taxa occurring commonly in samples and across sites. These factors are described as follows:

1 Low sample size

The percentage of agency samples characterised by poor taxa recovery, in relation to abundances of animals in agency-processed samples and total sample abundance, is listed in Table 9. Averaged across LS agencies, approximately 20% of samples are characterised by poor taxa recovery, with over 50% of the low taxa samples from WA, VIC and NSW associated with low LS sample size (< 100 animals). On average, 50% of the LS agency samples characterised by poor taxa recovery are also associated with low overall sample abundance (< 1000 animals) (Table 9). These observations are elaborated upon below.

Table 9. Agency samples characterised by poor taxa recovery, in relation to abundances of animals in agency-processed samples and total samples.

AGENCY	Features of agency samples			
	Percent of samples with LS/WSE taxa number ratio < 0.8	Percent of col 1 samples with low LS sample size (< 100 animals)	Percent of col 1 samples with overall low abundance (< 1000 animals)	Number of samples examined
WA (Live-sort)	22	62	62	23
TAS	33	0	20	15
VIC	12	50	0	17
NSW	26	55	55	19
QLD	5	0	100	22
SA	15	100	50	13
WA (Lab-sort)	0	0	0	6
NT	0	0	0	10
ACT	0	0	0	10

For live-sort sample size < 300 animals, significant positive regression relationships were found between taxa recovery and LS sample size for combined live-sort agency data. These relationships are shown in Figure 8. Two regression equations were derived, each using different values of WSE for live-sort sample size < 100, namely (i) WSE scaled to the same sample size as the live-sort sample, and (ii) WSE scaled to 100 animals (WSE_{100}). The derived regression equations for (i) and (ii) respectively are:

(i) $LS/WSE_N = 0.856 + 0.000687LS$ ($R^2 = 0.03$, $P = 0.05$),

(ii) $LS/WSE_{100} = 0.749 + 0.00124LS$ ($R^2 = 0.14$, $P < 0.001$),

where LS/WSE = live-sort/whole-sample estimate taxa number ratio and LS = live-sort sample size. (The two LS/WSE_N values > 1.5 shown in Figure 8 were considered outliers and were not included in the regression analysis.)

Thus, low taxa number ratios (< 1) typically accompany a small LS sample size (Fig 8), a feature that is highlighted in WSE data normalised to 100 animals for LS sample size < 100 animals. This result indicates that there is currently insufficient sorting effort for recovery of taxa under these conditions. Both regression lines meet the taxa number ratio value of 1 at a live-sort sample size of about 200 animals (Fig 8). Corroboration of this value as a recommended sample size target for future live sorting under the MRHI is provided in section 4.2.2.

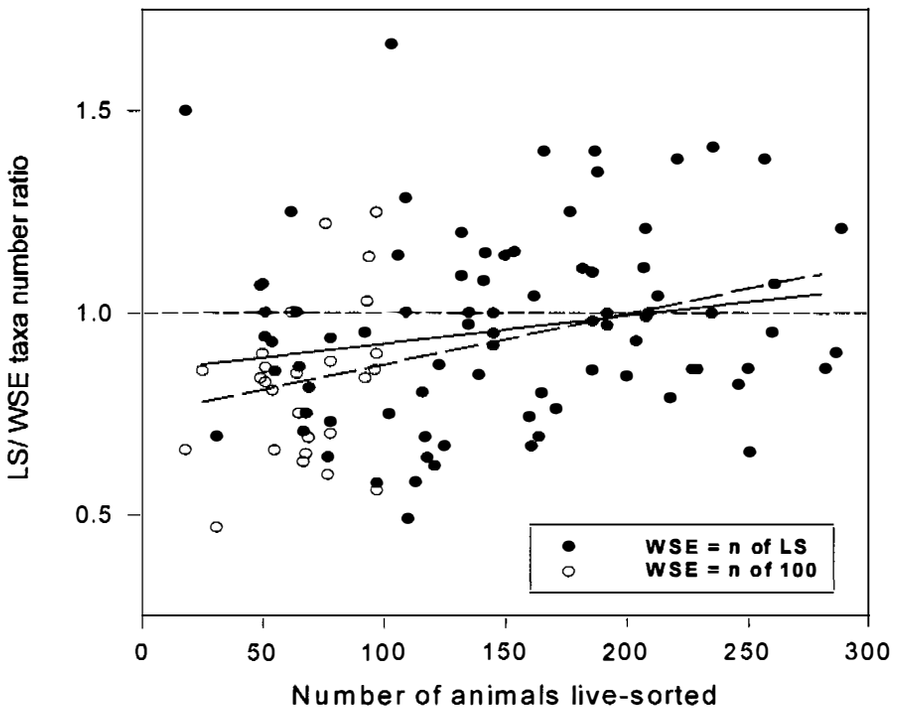

Figure 8. Relationships between live-sort taxa recovery (as measured by the LS/WSE taxa number ratio) and total number of animals live-sorted. Unbroken line is regression relationship using WSE scaled to the same sample size as the live-sort sample for live-sort sample size < 100, whilst broken line is regression relationship using WSE scaled to 100 animals (WSE_{100}) for live-sort sample size < 100.

Figure 9 shows a positive relationship between live-sort sample size and the estimate of the total number of animals in the sample (LS and residue components). The data indicate that a factor contributing to low LS sample size is 'low' overall abundance of animals in the sample. As discussed in section 4.2.2, this is evidence that the time allocated to live-sorting (30 mins) is often too short.

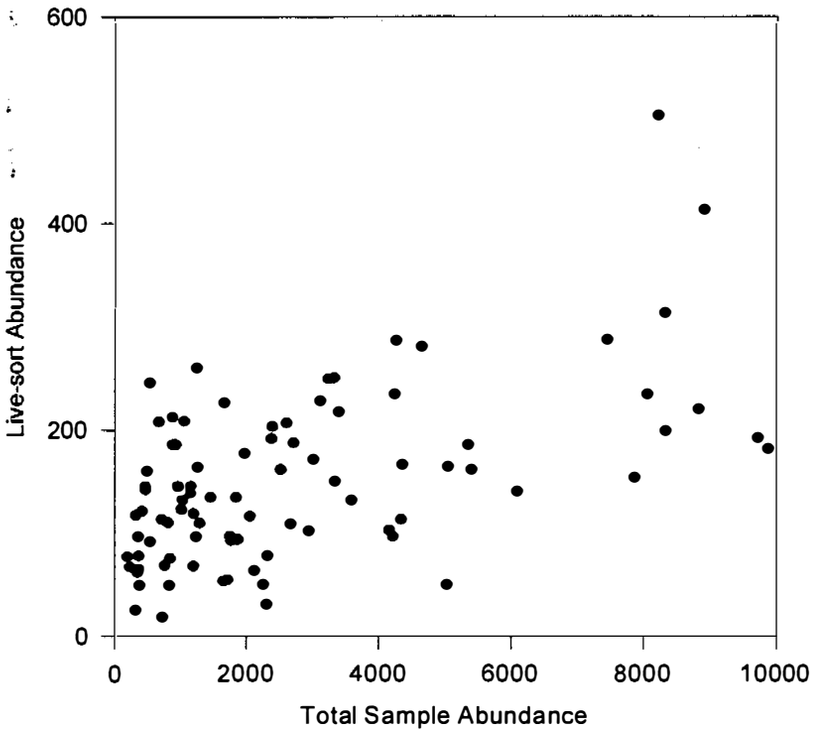

Figure 9. Relationship between number of animals live sorted and total sample abundance amongst all agency samples. (4 outliers omitted)

2 Operator inexperience

A second factor contributing to poor taxa recovery in live-sorted samples was operator inexperience. As described in section 3.3.2, least experienced operators were more consistently achieving poorer taxa recovery rates than more experienced operators, with lowest LS/WSE taxa number ratios found amongst these sorters (Figure 7).

3 Missed taxa occurring commonly in samples and across sites

A feature of live-sorted data was the frequent absence of taxa occurring commonly in samples and across sites. This was a major factor contributing to poor taxa recovery in live-sorted samples and an analysis of this issue can provide information about *how* poor taxa recovery was manifested.

In a previous milestone report, we noted that in taxa lists pertaining to live-sort data, small and/or cryptic taxa were often missing, these taxa being easily overlooked using the live-sort method of sample processing. For MRHI, recovery of taxa that have a frequency of occurrence in a group of >50% is particularly important as these taxa are used in modelling and represent taxa 'expected' at a site. To quantify the extent to which these key taxa were being missed from live-sort samples, the taxa were firstly identified as those occurring in more than 50% of samples from any of the agencies, for either the LS or corresponding WSE component. (This is not the same as taxa occurring in 50% of samples from an agency classification group, a factor which would lead to some underestimation of actual taxa affected in the present analysis.) For each of these taxa and for each agency, the percentage occurrence amongst all samples for which the taxon was found in both LS and corresponding WSE components was recorded. Results of this analysis are shown in Figure 10 with raw data provided in Appendix 6.

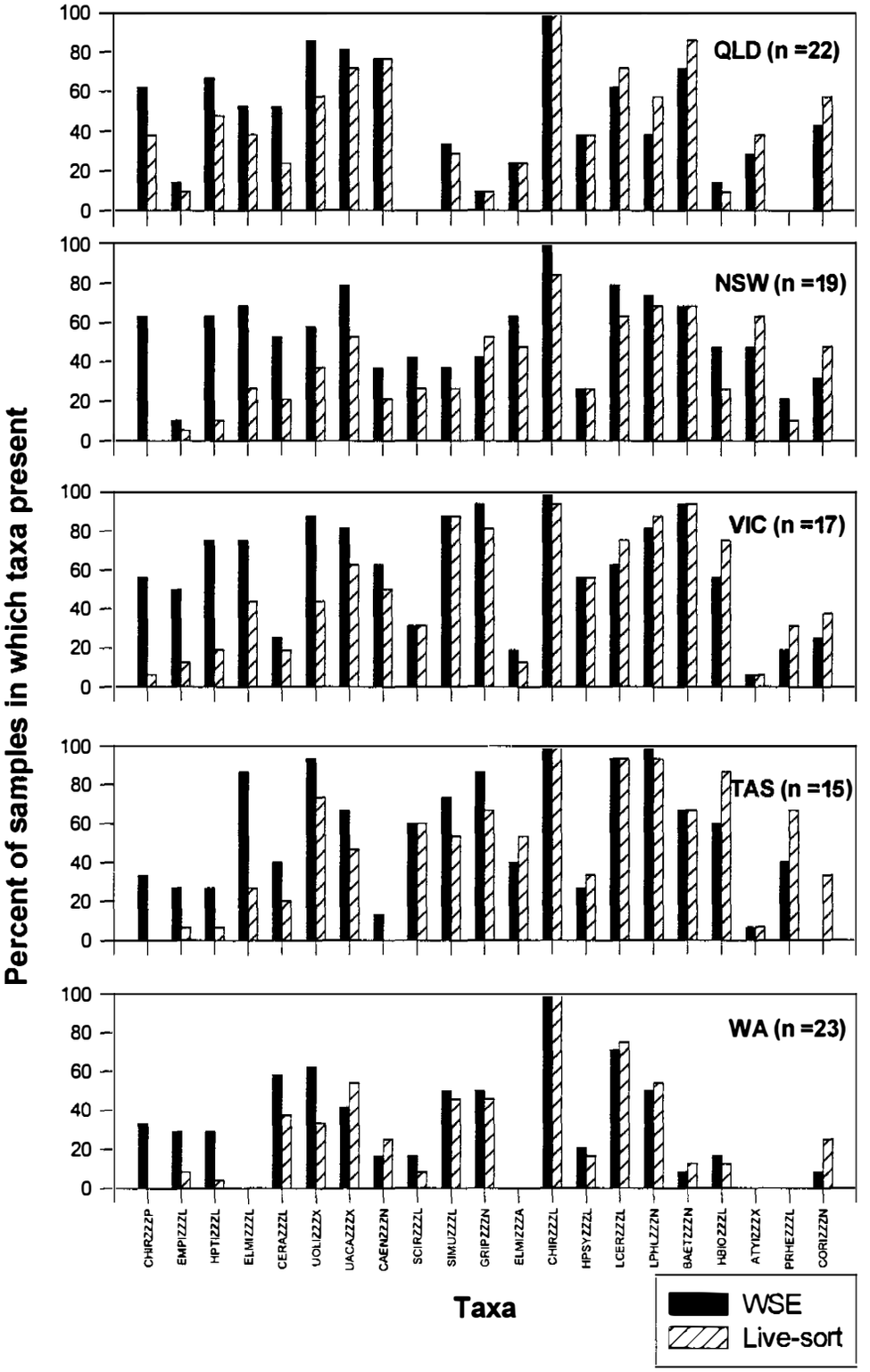

Figure 10. Taxa occurring commonly across MRHI samples and their percentage occurrence in both LS and corresponding WSE components of agency samples. Codes to taxa provided in Appendix 2.

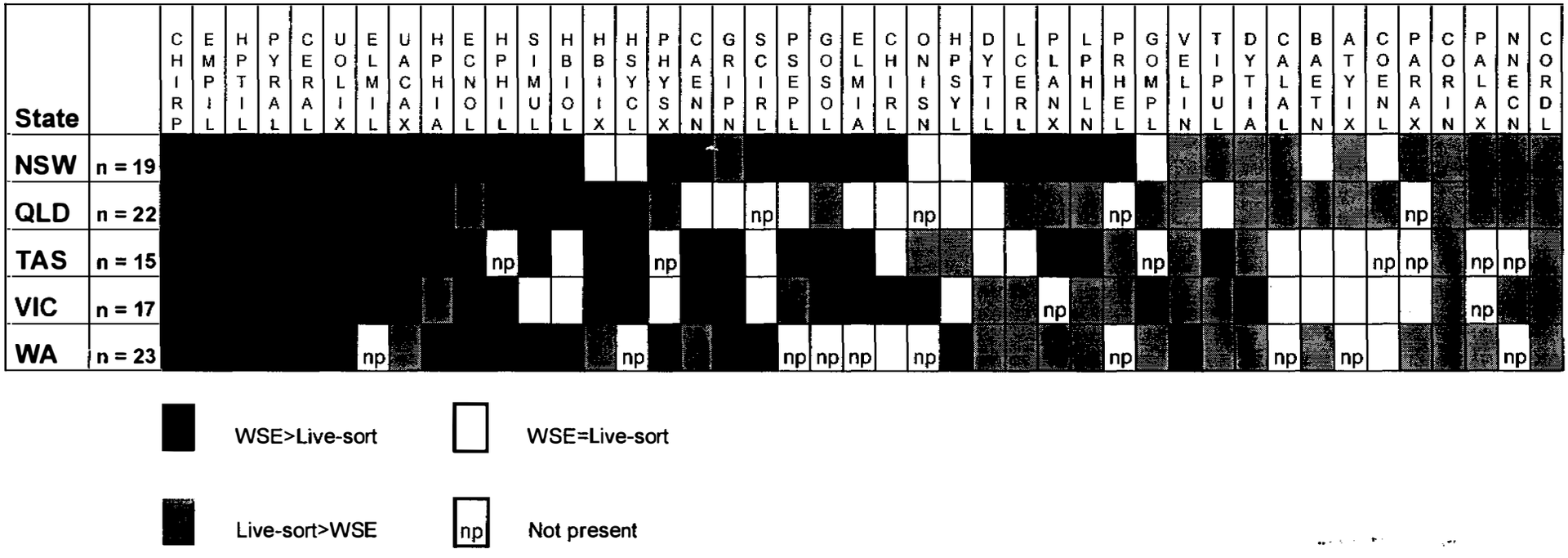

Figure 11. Taxa occurring commonly across MRHI samples and their percentage occurrence in both LS and corresponding WSE components of agency samples. Codes to taxa provided in Appendix 2.

In Figure 10, taxa have been ranked, from left to right, from greatest deficit to greatest surplus in occurrence in LS component compared to occurrence in corresponding WSE, when data were averaged across all live-sort agencies. Comparisons between occurrences of taxa present in the LS and corresponding WSE components show that similar taxa were either missed or better represented (in comparison to WSE occurrence) across all agencies and operators (Figure 10, Table A6). This is portrayed more graphically in Figure 11 where the same information has been coded (shaded) differently for 'deficit' or 'surplus' taxa. Importantly, the results show there is consistency amongst all agencies and their staff in the biases in taxa recovery (Figs 10 & 11, Table A6).

These results confirm the earlier-held belief (Humphrey & Thurtell 1997), that small and/or cryptic taxa are often overlooked during the live-sort process, regardless of agency or operator. Thus, chironomid pupae and other small cryptic Diptera taxa such as ceratopogonids and empidids were frequently missed during live sorting (Fig 10 and Table A6). This is also the case with the cryptic elmid larvae, hydroptilids (caddis-flies which build purse-like cases) and oligochaetes. Surprisingly, the fast moving water mites (Acarina) are also frequently missed during live sorting (Fig 10 and Table A6).

The data of Figure 10 and Table A6 do not reveal an additional problem of live sorting in the potential for low recovery of chironomid subfamilies. (Chironomidae are resolved to subfamily level for the MRHI.) Whilst chironomid subfamily data are unavailable for WSE samples, an indirect approach to assessing the extent of this problem was to determine the frequency of low abundance of chironomids in LS samples; we regarded low chironomid numbers in a sample (say < 10) as indicating a likelihood of a missed subfamily. For this analysis, the abundances of chironomid larvae in LS and corresponding WSE components (where WSE abundances were > 10) were recorded for all live-sort agencies. The frequency of samples containing chironomids with abundance of less than 10 individuals is shown in Table 10. A high proportion of live-sort samples (23-58%) contained chironomid larvae with less than 10 individuals, with a high likelihood, therefore, of missed subfamilies within these samples.

Table 10. Percent of Live-sort samples with chironomid abundance <10.

	Agency				
	NSW	QLD	TAS	VIC	WA
%	58	23	26	58	35
N samples	19	22	15	17	23

The results of Figure 10 and Table A6 demonstrate that there is also an assemblage of taxa that is better represented in live-sort data than WSE (= lab subsampled and sorted) data. Thus, the large, but less abundant taxa, such as the odonates, shrimp and adult beetles are often missed during the subsampling process (Fig 10, Table A6). In effect, these results show the extent to which lab subsampling and sorting are biased in taxa recovery. In practice, this possibility is minimised for most MRHI agencies processing samples in the laboratory because an additional search of the entire sample for taxa missed during the subsampling process is usually carried out (Appendix 5).

In itself, biases in taxa recovery are not necessarily a problem for classification and model development. The key observation is that the biases are consistent within and amongst agencies. Live-sorting is efficient, but lab subsampling and sorting less efficient, at recovering the large, conspicuous and rare taxa. Live-sorting is inefficient, however, at

retrieving a group of small and cryptic taxa. The potential problem in the bias of live sorting, is that the taxa that are commonly missed in live-sort data are those that occur reasonably commonly both in samples and across sites (often occurring in >50% of samples for a given agency). As described above, such common taxa which are likely to have a high probability of occurrence at a site, are important for MRHI classification and modelling. The taxa that are commonly missed, moreover, include many for which in Australia at least, have been shown to be informative in impact assessment studies and which are known to be sensitive to some types of water quality stress (Chessman 1995).

Conversely, the taxa that live-sorting is particularly effective at recovering are those that are uncommon in samples but also not particularly common across samples/sites (Fig 10, Table A6). These taxa would not be expected to be as influential in models. Thus, even if an additional screening of the sample was not undertaken by lab subsampling agencies for these taxa, the loss of these data is likely to be of little consequence for model development and sensitivity.

3.5.2 A broad range in taxa recovery observed amongst operators

Implicit in the findings of this study and in concurrence with the results of others, is thattime-constrained sorting, unlike proportional or fixed sample size sorting, is prone to varying rates of taxa recovery depending upon the efficiency of the operator. Some superficial evidence for this is contained in Table 7, showing the range in LS/WSE taxa number ratio values from amongst the samples examined within each agency. These values are quite broad and though there are a number of reasons for this, operator experience is certainly a contributing factor. Thus, the highest QLD LS/WSE value of 2.3 was obtained for a sample sorted by the agency team leader, a very experienced stream biologist (Table 8). Moreover, we have shown elsewhere that least experienced operators generally have the poorest rates of taxa recovery (section 3.3, Fig 7).

Metzeling and Chessman (1996) conducted a number of controlled and balanced field and laboratory trials to compare the performance of operators of different experience in sampling, sample processing and identification. In several important respects the design of the study was confounded in being unable to isolate sources of operator error. Moreover, because residues were not processed, only *relative* error rates could be derived for sorting efficiency. Within these constraints, nevertheless, the authors did show that novice operators recovered fewer taxa (2-4 per sample) than expert operators (Metzeling & Chessman 1996). Further, B Chessman, cited in Growns et al (1997), found that experienced personnel were able to sort more than twice as many animals as inexperienced personnel in 30 min time trials (unpubl. data). Extrapolating this finding to our results, a small sample size obtained by novices would represent fewer taxa recovered in the 30 min sorting period (Fig 8).

These findings lend support to the need to prescribe a fixed sample size for retrieval of invertebrates in the live sorting process (section 4.2). This need extends also to lab subsampling and sorting; for the SA agency, employing a proportional subsampling method, low taxa recovery (LS/WSE < 0.8) was always associated with low sample size (< 100 animals subsampled and sorted).

4 Discussion and conclusions

4.1 Assessment of the quality of State/Territory data

4.1.1 Agencies using a field live-sorting method

In summary and when viewed collectively, data derived from live-sorting do not appear to meet either objective of 'representativeness' (taxa recovery as per lab subsampling) or maximising taxa recovery, as assessed by external QA/QC (see section 4.2.2, 'Sample size'). Rather, live-sort data reflect the following two features:

1. Generally poor recovery of a suite of small/cryptic taxa occurring commonly in samples and across sites.
2. Variable taxa recovery rates depending upon the efficiency and/or experience of different operators sorting to a time limit (confirmed also by Metzeling and Chessman (1996) and Growns et al (1997)).

No further analysis of the types and extent of errors arising in agency live-sort data to that provided in the main body of the report is provided here. Instead, the possible consequences of these 'problems' to successful model development and sensitivity are reviewed in section 4.1.3 below. Future objectives of live-sorting and recommendations for improving data quality are also discussed separately below.

With regard to the results of individual agencies, it is interesting to note that the performance of different agencies did not appear to correlate with the order of perceived experience of these agencies (VIC on average having the most experience and QLD the least of any of the agencies with biological sampling of this type). The QLD agency performed the best on virtually all criteria used to assess performance. It would be worthwhile examining the type of training the QLD staff received for live sorting at the commencement of the program; elements of the training program might be identified that could be usefully applied more widely in the future.

4.1.2 Agencies using a laboratory subsampling and sorting method

Quite variable results were found for the four lab subsampling and sorting agencies, ACT, SA, northern WA and NT, partly the result of the different sample processing procedures used. Fixed-count subsampling and sorting was employed in the NT and ACT, proportional subsampling in SA and a mix of proportional and time-based subsampling and/or sorting in WA that aimed to maximise taxa recovery (see protocol descriptions in Appendix 5). The ACT was the only agency not to screen the sample coarsely to recover additional large, rare taxa. ACT, SA and NT agencies generally produced data representative of community structure of the sample. However, poor results were derived for two SA samples where sample size was < 100 animals (a consequence of proportional subsampling), whilst there was some breakdown in internal QA/QC checks for the ACT agency where the lowest quality results of any of the lab subsample agencies were obtained. (In the ACT mislabelling problems as well as staff inexperience [missed common taxa] were evident in the results, see also Appendix 4.) The methods of the WA agency aimed to maximise taxa recovery and to this end the approach was very effective.

An important virtue of lab subsampling and sorting methods lies in the ability to revisit samples for cases where a common generic error has arisen (ie providing samples have been stored for a sufficient period of time). The SA agency, for example, has processed additional

residue for samples where N fell below 100 animals (P Goonan, pers comm). The extent to which other agencies have redressed referred problems is unknown.

Lab subsampled and sorted data contained a number of errors (Appendix 4) though in almost all cases, data pertaining to taxa commonly occurring in samples and across sites were recovered. Whilst models developed using lab subsample agency data will by no means be error free, recovery of data for common taxa in the data sets should lead to more robust and sensitive models than for data sets for which these taxa have been frequently missed in sample processing. Additional R&D is required to determine the possible consequences of lab subsample errors to successful model development and sensitivity. The WA MRHI model contains data from across the state, combining lab subsampled (northern WA) and field live sorted (south-west WA) data. Taxa recovery for lab subsampled data well exceeded that effort for field live-sorted data (Table A3). The overall consequences of this imbalance to model development and sensitivity should also be studies. Future objectives of lab subsampling and sorting and recommendations for improving data quality are also discussed separately below.

4.1.3 Possible implications of live-sort errors for development of agency models

Metzeling and Chessman (1996) found that inexperienced operators recovered fewer taxa in sample processing (field live sorting and lab identification) than experienced operators. From this they deduced that if predictive models were applied using novice test data, they would be much more likely to judge both undisturbed and disturbed sites as impaired (ie Type I error), regardless of whether the models were developed using data collected by experts or novices. In the light of our results, this position would extend beyond the level of operator inexperience to other reasonably common situations resulting in poor taxa recovery, of missed common taxa and small sample size. In reality, our results indicate that current (live-sort) models are based upon *fewer* taxa than are actually present at reference sites. Additional taxa in test site data recovered under new protocols and/or by more experienced staff (or the same staff that had become more experienced) would be inconsequential in site assessments that were based upon current models. Rather, improvement in the procedures for taxa recovery in ongoing MRHI studies would only stand to benefit monitoring programs if the quality of data for reference sites are also improved through a re-sampling, data replacement and re-modelling program.

As described in the Final Report (Humphrey et al 1997a) preliminary investigations have been undertaken to determine the consequences to model development and sensitivity, of errors arising in live-sorting. The approaches simulated and introduced sample processing errors into an ACT agency UPGMA classification and/or model that was relatively 'error-free' (ie derived from a lab subsampled and sorted data set). Two types of simulated sample processing error were introduced into the agency model to replace the original data in their entirety, namely that which preserved in a systematic manner, the pattern of error (Storey & Humphrey 1997b) and that which represented actual error (biases in taxa recovery) (Humphrey et al in draft) as observed in agency data.

In the first 'whole-model' simulation, the pattern of community structure and preservation of dissimilarity and taxa number ratio between 'live-sort' and 'whole sample estimate' data were superimposed upon the original agency data set. No model could be successfully derived using the 'error' data (Storey & Humphrey 1997b). This approach was limited in not superimposing the nature of the errors, ie types of taxa typically missed or overrepresented in live-sort data. This deficiency was redressed in the second simulation in which taxa in the same original ACT data set (for which data on taxa commonly-occurring across sites were

well represented) were altered to match the bias observed in live-sort data (Humphrey et al in draft). Two sets of live-sort data were used in the simulations: that of NSW, one of the poorer performing agencies, and that of the average bias observed across eastern states, QLD, NSW, VIC and TAS. The average agency bias was not as severe as that for the single agency. For each of the single agency and average agency data, 3 separate simulations and classifications were run.

For the classifications derived after error rates for the single agency were applied to the ACT data, dissimilarity cut-offs for the groups were found to be higher in the altered data indicating introduction of errors. Even so, in 2 of the 3 classifications, some preservation of the original classification was evident, even if there was a loss of one or 2 groups from the new classifications; only in one classification was there evidence of breakdown or "chaining" in classification structure.

In the eastern states classifications, however, there was less evidence of preservation of group structure and all exhibited 'chaining'. Interspersion of the original group sites was also more evident in the eastern states classifications. This is contrary to expectations, ie the better data produced more poorly-defined classifications. No diagnosis of the classifications has been conducted as yet to indicate why this result might have occurred suffice it to say that it appears to represent a weakness in cluster analysis as a basis for group definition. Moreover, the level of taxonomic resolution used for MRHI, family-level p/a, may be sufficiently coarse as to place any structure present in classifications finely balanced. This might be exacerbated in data sets from small geographical regions, such as the ACT, where group definition based upon family-level p/a data could be expected to be quite subtle and vulnerable to introduction of even small errors.

Regardless, all of the classifications produced in the study appeared to contain greater inherent variability (as assessed by higher dissimilarity cut-offs for the groups) and the potential, therefore, to adversely affect the sensitivity of models. Further R&D is required to determine the consequences to model development, sensitivity and outputs, of data of the type found in this QA/QC program. Leaving aside the issue of the consequences of error rates, and as stated earlier in this report, the absence of taxa occurring commonly across sites from live-sort data sets represents lost information, particularly as most of the specified taxa are regarded as being sensitive to particular types of pollution (Chessman 1995).

4.2 Recommendations for future sample processing procedures

Growns et al (1997) state the following:

> *"It has been argued that random subsampling has the advantage of avoiding operator bias in selective subsampling,* ['selective subsampling' = live sorting, selecting a maximum of 10 individuals of a given taxon] *such as a tendency to favour picking of larger or more conspicuous taxa (R.H. Norris, University of Canberra, personal communication). However, Chessman's (1995) procedure specifically states that small, inconspicuous taxa should be searched for, and the limit of 10 individuals of any type of organism means that large taxa do not dominate the samples and taxon lists from studies using the method contain many small and cryptic species ..."*

We take issue with some of the statements made by Growns et al (1997). In theirs (Growns et al 1997) and related studies (Chessman 1995, Metzeling & Chessman 1996), implicit and explicit assumptions are made about the ready detection and recovery from samples of small and encased animals because of their movement whilst alive. Our results show that even when advice to this effect is provided in protocols, at a national level a generally poor success

rate has been achieved by MRHI staff in recovering such small and cryptic taxa. Surprisingly, none of the researchers cited above developing RBA methods for Australian conditions appears to have examined residues to determine the nature and extent of possible biases in the live-sort method.

Whilst it is possible that small teams of very experienced biologists can achieve results of the quality purported by the aforementioned authors, we believe there are a number of important revisions and training steps required of any of the existing and recommended protocols (Davies 1994, Chessman 1995, Growns et al 1997) before they can be applied further in national biological monitoring programs. Without detailed prescriptive protocols and extensive training, and given the reality of mixed-experience staff amongst MRHI agencies, approaches canvassed by Chessman (1995) and Growns et al (1997) simply ask too much of human capability.

4.2.1 Re-stated objectives of agency sample processing procedures

The original objective for live-sorting (sensu Davies 1994) should be restated and adhered to; maximising taxa recovery and emphasising recovery of presence/absence data. This objective should also apply to lab subsampling and sorting by supplementing sample processing with a search for large, rare taxa. The advantage of sample processing objectives targeted at maximising taxa recovery lies mainly in provision of data for conservation and biodiversity studies.

4.2.2 Agencies using a field live-sorting method

Over the period November 1996 to February 1997, the commissioned QA/QC team for MRHI sample processing procedures (***eriss*** and UWA) recommended changes to future live sorting and forwarded to agencies and technical experts for MRHI, draft protocols for live sorting. At a MRHI meeting held in Canberra in February (1997), the recommendations and proposed changes to the LS protocol were essentially endorsed though there was some concern expressed by some that the level of scrutiny and detail being proposed were driving the approach away from the essence of rapid assessment. Nevertheless, from this meeting it was decided to implement some of the recommended changes to the protocol immediately and implement others as results of additional R&D came to hand. The rationale behind the protocol and the circulated draft protocol are provided in Attachment 3 to the Final Report. These issues are restated here with corroborative evidence for one or two of the recommendations made thereafter. Thus:

1. To be accountable, the MRHI requires clearly-defined objectives with agreed QA/QC assessment criteria. To this end, the original objective for live-sorting (sensu Davies 1994) needed to be re-stated, maximising taxa recovery and emphasising recovery of presence/absence data (see section 4.2.1).
2. Aspects of the original protocol under which conspicuous taxa, including 'large rares', were successfully recovered, would be retained;
3. A portion of the sample would be 'subsampled' in the field and carefully sorted using visual aids for small common and/or cryptic taxa;
4. Rules would apply to sample size (ie fixed-count sorting of ~200 animals, stopping at one hour if this target is not met), including a minimum number of chironomids;
5. Mesh size would be increased from 250 to 500 μm.
6. For recovery of small common/cryptic taxa:

(i) A training element would be implemented to increase awareness amongst staff of commonly-missed taxa in their region, what they looked like and how/where to find them in samples; and

(ii) Staff would consciously seek these taxa through mental notes and written checklists appended to data sheets.

7. With a new live sorting objective, QA/QC assessment would need to be revised to apply to the new data. A list of primary and secondary criteria were proposed (see section 4.2.3).

With respect to some of the issues raised above:

1 Relative/rank abundance

The rigour required to fully recovery relative abundance data in any revised live-sort protocol has been widely viewed as excessive and likely to defeat the ethos of 'rapid assessment'. In any case, temporal (interannual) variability of stream macroinvertebrate communities over most of Australia is too high for relative/rank abundance to be recovered in long-term data sets, negating the advantage in accruing such data (Humphrey et al 1997b). Nevertheless, in field 'subsampling' to recover small/cryptic taxa, proposed techniques may coincidentally lead to recovery of rank or relative abundance of taxa.

2 Sample size

We agree with Growns et al (1997) that time-based sorting suffers from the variable efficiencies of different operators in recovery of animals. A number of recent studies conclude that a fixed-count approach to sample processing is more effective than proportional sample processing (Barbour & Gerritsen 1996, Walsh 1997). Regardless of different operator efficiencies, for samples of total organism abundance below a particular threshold, time-based sorting becomes a form of proportional subsampling and sorting. (Above this threshold, sample size per unit time is more constant as the only constraint to taxa recovery is the rate at which an operator can remove animals - see Fig 9.) Even if a significant proportion of live-sort samples reflects a condition at sites of generally low invertebrate abundance (Fig 9), 'proportional' sample processing of this type has been shown to be not a particularly effective way of representing this result. Thus, Walsh (1997) showed that 'fixed-count' sampling/subsampling procedures more faithfully represented the similarity in community structure between treatments than did proportional sampling/subsampling.

Chessman (1995) and Growns et al (1997) recommended a fixed sample size of 100 animals for live sorting in south-eastern Australia. If the objective of live sorting is to recover a broad range of taxa, then we believe the target sample size/taxa number in live sorting should be such that the LS/WSE taxa number ratio is ≥ 1. Only 50% of agency samples processed in this study met this criterion. As demonstrated below, a sample size of 100 animals would be too small to maximise taxa recovery in this manner. A target of 100 animals may well be a suitable one for small, highly skilled teams of the type tested under controlled field conditions by Growns et al (1997), but it does not appear to be applicable to MRHI agencies whose staff vary widely in levels of skill and experience, and who operate under a variety of field situations and conditions. (See Attachment 3 to the Final Report for a description of potential problems with the other live-sort protocols developed in Australia.)

In section 3.5.1/1 evidence was provided from a plot of the LS/WSE taxa number ratio vs number of animals live-sorted (Fig 8) that a target sample size for future live sorting by MRHI agencies should approach at least 200 animals (ie the figure at which LS/WSE reached a value of 1).

A further albeit coarse guide to the sample size that might be appropriate for future live sorting was obtained by constructing taxa accretion curves from WSE data. Two scatter plots of 'taxa number in WSE' vs 'number of animals in WSE' were derived, one each for eastern Australia and south-western Australia, similar taxa richness being assumed within each of these broad geographical regions. (This assumption would need to be tested for more detailed investigations of this type.) For each MRHI sample, two data points were derived, namely taxa number vs WSE abundance for: (i) combined subsampled residue and live-sort component data prior to scaling down to sample size of the live-sort component (column E of the macro report sheet, see Appendix 2); and (ii) WSE derived for sample size of the live-sort component (col G of macro report sheet, Appendix 2). The scatter plots are shown, with samples coded for different habitat, in Figures 12 and 13 for eastern Australia and south-west WA respectively.

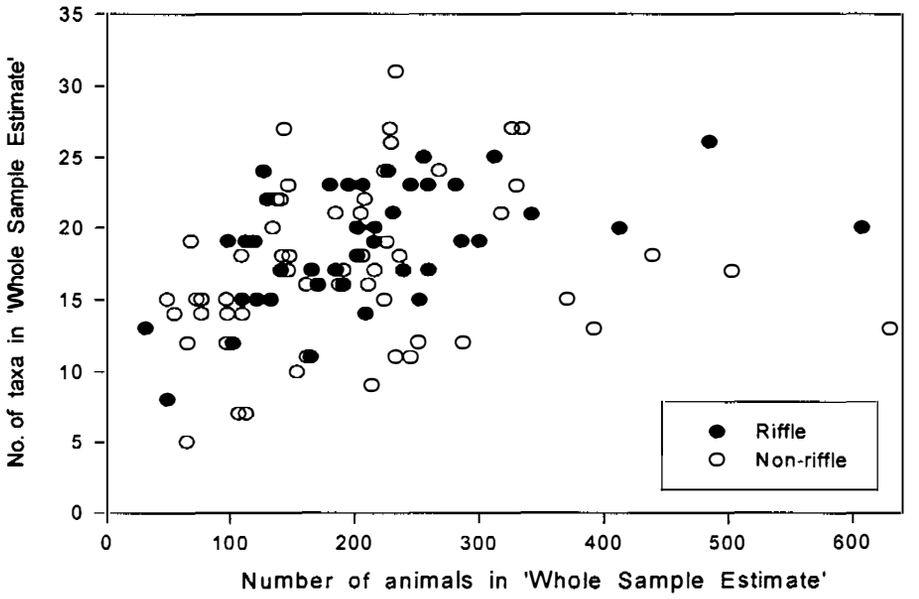

Figure 12. Relationship between number of taxa vs number of invertebrates recorded in the whole sample estimate for samples from all eastern state live-sort agencies, according to different habitat.

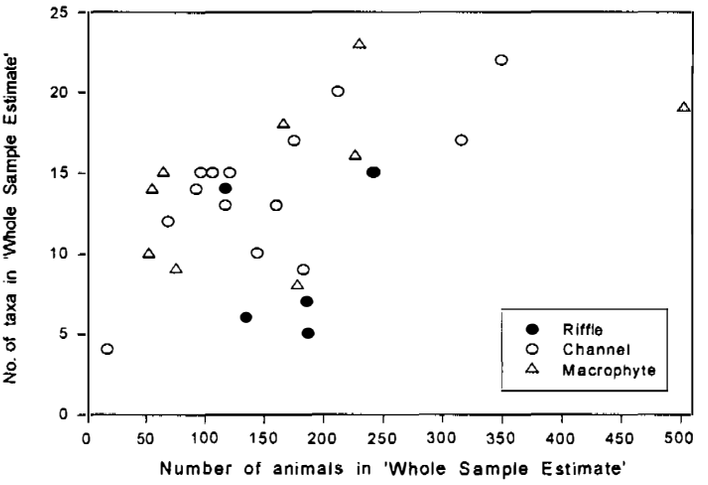

Figure 13. Relationship between number of taxa vs number of invertebrates recorded in the whole sample estimate for samples from all south-west Australian live-sort agencies, according to different habitat.

The results indicate a plateauing of taxa number for generally all habitats after a sample size of at least 200 animals has been reached, for both eastern and south-western Australia. Thus, these results are similar to the sample size estimate suggested by the approach of LS/WSE to unity, as discussed above (see Figure 8). Thus, for relatively 'fail-safe' recovery of a broad range of taxa in live sorting, it is recommended that a fixed sample size of 200 animals be targeted by agency staff, or sorting ceases at one hour if this target cannot be reached in this time.

3 Mesh size

A move to increase mesh size from 250 to 500 μm was made in response to the belief that sampling and sample processing using nets of the smaller mesh led to collection of many small animals difficult to see with the naked eye. This factor was exacerbated by clogging of mesh and introduction of much fine detritus and silt so that many of the organisms sampled were obscured. An additional problem arose during laboratory identification of organisms because many organisms between the range 250 and 500 μm were too small to be identified accurately. A small study conducted in the upper South Alligator River (NT) to determine whether or not there were differences in taxa recovered as a consequence of sampling using 250 vs 500 μm mesh showed negligible differences (Humphrey et al 1997b). (Before a final decision is made on this issue, further R&D will be conducted to corroborate these results for other regions.)

4.2.3 Agencies using a laboratory subsampling and sorting method

In draft revisions of sample processing protocols forwarded to, and discussed with, agencies and technical experts for MRHI (Nov 1996 - Feb 1997), some recommended changes leading to standardisation of laboratory subsampling and sorting protocols were made. These included (i) an emphasis on maximising taxa recovery (including 'large rares') through a coarse-screen search of the entire sample, and (ii) fixed-count subsampling. The rationale for these changes is provided above (sections 4.2.1 and 4.2.2) and in Attachment 3 to the Final Report. Most lab sample processing agencies now adopt a protocol that incorporates these features.

4.2.4 Future QA/QC assessment criteria to apply to sample processing procedures

In properly conducted QA/QC programs, an assurance is provided that an assessment criterion, averaged over all analysts within a laboratory, remains at or above an acceptable limit, with unacceptable quality being detected promptly to allow remedial action. An excellent model for such QA/QC, as applied to the national biological monitoring program conducted in the UK, is provided in van Dijk (1994). The UK approach to QA/QC is a statistical one adapted from quality control of industrial processes (see Storey & Humphrey (1996) for details of the approach).

Formal QA/QC procedures, involving statistical responsiveness to control charts and cusums, were not applied in this external QA/QC program because (i) at the onset there was no clear objective to sample processing that live-sort agencies were adhering to, and (ii) assessment criteria and acceptance thresholds developed early in the study had no empirical basis (Storey & Humphrey 1996). There is now information available to redress these deficiencies - see section 4.2.1 for sample processing objectives, and Storey & Humphrey (1997b,c) for thresholds to apply to assessment criteria. QA/QC criteria to adopt in the assessment of future MRHI agency sample processing procedures (field live-sorting and laboratory subsampling and sorting) would focus on maximisation of taxa recovery (presence-absence data). A number of primary and secondary QA/QC assessment criteria and acceptance thresholds are proposed:

Possible primary and secondary QA/QC assessment criteria and acceptance thresholds to apply to live-sorted samples

Primary criteria

- The dissimilarity measure (p/a) calculated between taxa in the live-sort component that are common to those in the WSE, and all WSE taxa, should be ≤ 0.3;

i.e. taxa A-L

	TAXA																	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
L-S	X		X	X			X	X		X		X	X	X	X	X	X	X
WSE	X	X	X	X	X	X	X	X	X	X	X	X						

- The number of taxa present in the live-sort component must be greater than that in the corresponding WSE.

Secondary criteria

- The number of chironomid larvae must exceed 20 individuals (target 30) wherever the corresponding number in the WSE also exceeds this value.
- The abundance of individual taxa should not exceed 50 animals.
- Sample size should be:

  (i) within 200 ± 10% wherever total number of animals in the sample is estimated to exceed 1000 animals, unless sorting has proceeded for one hour;

  (ii) >100 wherever total N lies between 500 - 1000 animals, unless sorting has proceeded for one hour.

Possible primary and secondary QA/QC assessment criteria and acceptance thresholds to apply to lab subsampled and sorted samples

Primary criteria

- The dissimilarity measure (p/a) calculated between taxa in the agency subsampled fraction (less additional taxa collected from coarse fraction) and taxa recovered from an additional same-sized subsample from the residue, should be ≤ 0.3;
- The number of taxa present in the agency subsampled fraction combined with those collected from the coarse fraction of the sample, must exceed that in an additional same-sized subsample from the residue.

Secondary criteria

- Sample size should be:

  (i) within 200 ± 10% wherever total number of animals in the sample is estimated to exceed 1000 animals;

  (ii) >100 wherever total N lies between 500 - 1000 animals, unless sorting has proceeded for two hours.

Acceptance threshold values that would apply to the primary assessment criteria of both lab subsampled/sorted and field live-sorted data would need to be bounded by confidence intervals derived from the sizes of non-operator error encountered in lab subsampling and sorting (see section 3.2).

Acknowledgments

We are most grateful to Tony Mount of the ***eriss*** Information Technology section for writing the Paradox for Windows macros used in assessing quality of agency results. We thank also the staff of the various state and territory agencies for providing samples and data upon which to make the assessments, Andrew Storey (UWA) and Ken Thomas (Environment Australia) for their insights into the many important issues raised in the report and to Max Finlayson for editorial comment on an earlier draft. This project was jointly funded by the Commonwealth Environment, and Primary Industry and Energy Departments through the Land and Water Resources Research and Development Corporation.

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Table A1. Details of agency samples requested and processed for external QA/QC

Table A1. Details of agency samples requested and processed for external QA/QC. '% habitat requested' refers to % of 'site x no. of habitats sampled per site', '% Subs' refers to size of residue subsample taken for external audit.

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
TAS	3	D14 North Esk	Edge	yes	10	14-Feb-96	29-Jun-96
		A18 Staneley	Riffle	yes	30	26-Feb-96	29-Jun-96
		Dmon4 Great Forester	Riffle	yes	30	27-Feb-96	29-Jun-96
		Bmon4 Emu	Edge	yes	30	22-Feb-96	29-Jun-96
		B7 Keith	Edge	yes	20	02-Jul-96	29-Jun-96
		C15 Quamby	Riffle	yes	10	20-Jun-96	29-Jun-96
		Cmon4 Meander/Birralee	Riffle	yes	10	19-Jun-96	29-Jun-96
		D13 Nile	Riffle	yes	30	16-Feb-96	29-Jun-96
		B19 Leven/Blackmarsh	Edge	yes	10	13-Feb-96	29-Jun-96
		% habitat requested		**5**			
	4	Amon2 Little Henty	Edge	yes			
		Bmon2 Wilsons Ck	Riffle	yes			
		Cmon2 Western Ck	Edge	yes			
		Dmon1 Sth Esk	Edge	yes	30	04-Mar-97	04-Mar-97
		A12 Ewart Ck	Riffle	yes			
		A15 Farrell Rt	Edge	yes			
		A15 Farrell RT	Riffle	yes	10	06-Mar-97	06-Mar-97
		B10 Black Ck	Edge	yes	10	03-Mar-97	03-Mar-97
		B10 Black Ck	Riffle	yes	10	07-Mar-97	07-Mar-97
		B16 Flowerdale	Riffle	yes			
		C3 Lobster Rt	Riffle	yes			
		C13 Meander	Edge	yes			
		D2 Nth George/Tasman Hwy	Edge	yes	10	28-Feb-97	28-Feb-97
		D23 Little Forester	Riffle	yes	10	06-Mar-97	06-Mar-97
		% habitat requested		**8**			
VIC	3	003809 Moleside Ck	Riffle	yes	10	19-Apr-96	May-96
EPA*		032900 Yackandandah Ck	Edge	yes	10	24-Apr-96	May-96
		033300 Kiewa R @ Bonegilla	Riffle	yes	20	18-Apr-96	May-96
		037100 Victoria R	Riffle	yes	5	17-Apr-96	May-96
		036400 Mitta Mitta R	Edge	yes	10	22-Apr-96	May-96
		00501O Shannassy R	Edge	yes	10	16-Apr-96	May-96
		297100 Cement Ck	Riffle	yes	20	26-Apr-96	May-96
		% habitat requested		**3**			
EPA**	4	003701 Eumerella R	Edge	no			
		032800 Running Ck	Riffle	yes			
		003811Glenelg R @ Dartmoor	Edge	yes			
		005012 Armstrong Ck	Edge	yes			
		003436 Woady Yaloak	Edge	yes			
		033600 West Kiewa R	Riffle	yes			
		033600 West Kiewa R	Edge	yes			
		003819 Grange Burn Ck	Riffle	yes			
		004982 Yarra R @ Peninsula	Edge	yes			
		004982 Yarra R @ Peninsula	Riffle	yes			
		% habitat requested		**13**			

Table A1. (Contin.)

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
VIC	3	408202 Avoca R @ Ampitheatre	Pool	yes			
Water		942668 Wonnangatta R	Pool	yes			
Eco		224998 Wonnangatta R	Riffle	yes	20	02-Jan-97	20-Jan-97
		232211 Moorabool R	Riffle	yes			
		223997 Tambo R	Riffle	yes	5	30-Dec-96	20-Jan-97
		223998 Timbarra R @ Timbarra	Riffle	yes			
		231213 Lerderberg R	Riffle	yes			
		231230 Parwan Ck @ Rowsley	Pool	yes			
		230202 Jackson Ck @ Sunbury	Riffle	yes			
		230209 Barringo Ck	Pool	yes			
		230205 Maribyrong R @ Bulla	Pool	yes			
		404996 Ryans Ck	Riffle	yes	10	10-Jan-97	20-Jan-97
		404214 Broken Ck	Pool	yes	20	31-Dec-96	20-Jan-97
		% habitat requested		**6**			
	4	224206 Wonnangatta R	Riffle	yes	5	08-Jan-97	20-Jan-97
		223214 Tambo R us Smith Ck	Edge	yes	5	08-Jan-97	20-Jan-97
		404998 Moonee Ck	Riffle	yes	5	30-Dec-96	20-Jan-97
		231998 Werribee R	Riffle	yes			
		224995 Wentworth R	Pool	yes	5	07-Jan-97	20-Jan-97
		224995 Wentworth R	Riffle	yes			
		223210 Nicholson R @ Deptford	Pool	yes			
		232211 Moorabool R	Riffle	yes	5	16-Dec-96	20-Jan-97
		230209 Barringo Ck @ Barringo	Pool	yes	5	09-Jan-97	20-Jan-97
		230209 Barringo Ck @ Barringo	Riffle	yes			
		% habitat requested		**7**			
NSW	3	Hast 22	Edge	yes	30	20-Aug-96	26-Aug-96
		Mann06	Riffle	yes	30	21-Nov-96	21-Nov-96
		Hunt04	Riffle	yes			
		Shoa24	Edge	yes	100	22-Aug-96	26-Aug
		Clyd21	Riffle	yes	10	27-Nov-96	23-Dec-96
		Hawk08	Edge	yes	100	26-Aug-96	27-Aug-96
		Towa05	Riffle	yes	20	22-Nov-96	23-Dec-96
		Snow05	Riffle	yes	10	19-Sep-96	29-Oct-96
		Lach01	Macro	yes	10	08-Aug-96	23-Dec-96
		Bega07	Riffle	yes			
		Murr11	Edge	yes	30	19-Aug-96	22-Aug-96
		Murr15	Edge	yes	50	27-Aug-96	27-Aug-96
		Murr22	Riffle	yes			
		Clar19	Edge	yes	30	21-Aug-96	21-Aug-96
		Clar30	Riffle	yes			
		Macq10	Macro	yes	20	08-Feb-96	01-Mar-96
		Rich01	Pool	yes	100	08-Feb-96	04-Mar-96
		Rich01	Edge	yes	30	07-Feb-96	04-Mar-96
		Bidg10	Edge	yes	20	18-Sep-96	23-Dec-96
		Lach19	Edge	yes	100	29-Aug-96	
		% habitat requested		**4**			
	4	Clar03	Edge	yes			
		Bell15	Edge	yes			
		Hast18	Edge	yes			
		Hunt10	Riffle	yes			
		Shoa05	Riffle	yes			
		Bega04	Riffle	yes			

Table A1. (Contin.)

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
NSW	4	Snow08	Riffle	yes	10	29-Nov-96	23-Dec-96
		Murr27	Riffle	yes	10	02-Nov-96	23-Dec-96
		Murr27	Edge	yes			
		Gwyd10	Macro	yes			
		Clar10	Riffle	yes			
		Macl06	Riffle	yes			
		Mann10	Edge	yes			
		Hawk01	Edge	yes			
		Clyd18	Edge	yes			
		Towa04	Edge	yes			
		Towa04	Riffle	yes	20	12-Dec-96	23-Dec-96
		Murrm5	Edge	yes			
		Lach09	Edge	yes			
		Darl02	Edge	yes			
		Parr03	Edge	yes			
		Snow06	Macro	yes			
		Snow06	Riffle	yes			
		Towa10	Edge	yes			
		Macq10	Macro	yes			
		% habitat requested		**5**			
ACT	3	Peppers Ck	Edge	yes	10	29-Apr-97	29-Apr-97
		Yass R	Edge	yes	25	29-Apr-97	29-Apr-97
		Murrumbidgee R	Edge	yes	5.7	30-Apr-97	30-Apr-97
		Alum Ck	Edge	yes	4.8	30-Apr-97	30-Apr-97
		Mullion Ck	Edge	yes	24	01-May-97	01-May-97
		Tea Tree Ck	Riffle	yes	4.8	01-May-97	01-May-97
		Brindabella Ck	Riffle	yes	6	02-May-97	02-May-97
		Cooma Ck	Riffle	yes	3	06-May-97	06-May-97
		Peppercorn Ck	Riffle	yes	3	06-May-97	06-May-97
		Murrumbidgee R	Riffle	yes	4.8	07-May-97	07-May-97
		% habitat requested		**5**			
	4	Blue Bull Ck 36co	Riffle	No			
		Oaks Ck 23br	Edge	No			
		Kybeyan R 40co	Riffle	No			
		Numeralla R 38co	Edge	No			
		Murrumbidgee 8c	Riffle	No			
		Murrumbidgee 631	Edge	No			
		% habitat requested		**5**			
QLD	3	Palmer R @ Drumduff Rd	Riffle	No			
		Rifle Ck @ Font Hills	Pool	No			
		Hann R @ Cape York Rd	Riffle	No			
		E. Normanby @ Dev Rd	Bank	No			
		E. Normanby @ Dev Rd	Pool	No			
		Normanby R @ 12 Mile Hole	Macro	No			
		Peets Ck @ Causeway	Riffle	Yes	5	20-Jan-97	20-Jan-97
		Babinda Ck @ Babinda	Pool	No			
		Babinda Ck @ Babinda	Macro	No			
		Sth Johnstone R @ Corsi's	Riffle	Yes	5	23-Jan-97	23-Jan-97
		Taylors Ck @ Warraker	Bank	Yes			
		Nth Johnstone R @ Malanda	Pool	No			
		Ithaca Ck @ Clarks Track	Macro	Yes			
		Gowie Ck @ Abergowrie	Pool	Yes			

Table A1. (Contin.)

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
QLD	3	Millstream Ck @ Diversion Weir	Macro	Yes			
		Flinders R @ Walkers Bend	Bank	No			
		Corella R @ Lake Corella	Riffle	Yes			
		Corella R @ Lake Corella	Pool	Yes			
		Porcupine Ck @ Emu Plains	Macro	Yes			
		Wyandotte Ck @ Wyandotte	Riffle	Yes	5	23-Jan-97	23-Jan-97
		Burdekin R @ Blue Range	Bank	Yes			
		Pelican Ck @ Kerale	Pool	Yes			
		Chinaman Ck @ Hydrosite	Macro	Yes			
		Gregory R @ Collingvale	Riffle	No			
		Middle Lethe Brook	Bank	Yes	10	06-Mar-96	16-May-96
		Gregory R @ Collingvale	Pool	Yes			
		Middle Lethe Brook	Macro	No			
		O'Connell R @ Caping Siding	Bank	Yes			
		St Helens @ Brothwells	Pool	Yes			
		Cattle Ck @ Williams Rd	Macro	Yes			
		Funnel Ck @ Main Rd	Pool	Yes	5	01-Apr-96	20-May-96
		Dawson R @ Taroom	Macro	No			
		Burnett R @ Mt Lawless	Riffle	Yes	10	19-Mar-96	20-May-96
		Burnett R @ Mt Lawless	Pool	Yes	5	19-Mar-96	20-May-96
		Burnett R @ Eidsvold	Bank	No			
		Kolan R @ Bucca Xing	Macro	Yes	5	09-Apr-96	14-May-96
		Amamoor Ck @ Zachariah	Riffle	Yes	10	11-Apr-96	10-May-96
		Mary R @ Fishermans Pocket	Bank	Yes	10	11-Apr-96	13-May-96
		Mary R @ Home Park	Pool	Yes	100	10-Apr-96	14-May-96
		Obi Obi Ck @ Alpin Rd	Macro	Yes	5	26-Mar-96	16-May-96
		Coochin Ck @ Bruce Hwy	Macro	Yes	5	11-Apr-96	09-May-96
		Logan R @ Rathdowney	Riffle	Yes	10	01-Apr-96	20-May-96
		Logan R @ Yarrahappini	Bank	Yes	10	11-Apr-96	15-May-96
		Brisbane R @ Savages Xing	Riffle	Yes	10	14-Mar-96	20-May-96
		Brisbane R @ Xing 26	Bank	No			
		Brisbane R @ Xing 26	Macro	Yes	10	13-Mar-96	20-May-96
		Coomera Ck @ Tuckers Lane	Riffle	No			
		Tallebudgera Ck	Bank	Yes	50	21-Mar-96	13-May-96
		Coopers Ck @ Boolloo Boolloo	Pool	No			
		Warrego R @ Wyandra	Bank	Yes	5	22-Mar-96	16-May-96
		Macintyre R @ Goondiwindi	Macro	Yes	10	26-Mar-96	15-May-96
		Condamine R @ Chinchilla	Pool	Yes	5	26-Mar-96	14-May-96
		Condamine R @ Chinchilla	Bank	Yes	10	12-Mar-96	07-May-96
		% habitat requested		7			
	4	Rifle Ck	Bank	yes			
		Mitchell R	Macro	yes			
		East Normanby R	Pool	yes			
		Normanby R	Bank	yes			
		Fishery Fall Ck	Bank	yes			
		Nth Johnstone	Bank	yes			
		East Barrata Ck	Pool	yes			
		Broken R	Pool	yes			
		Broken R	Macro	yes			
		Proserpine R	Macro	yes			
		Boulder Ck	Riffle	yes			
		Pioneer R	Bank	yes			
		Carnarvon Ck	Riffle	yes			
		Baffle Ck	Bank	yes			

Table A1. (Contin.)

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
QLD	4	Baffle Ck	Pool	yes			
		Sth Maroochy R	Bank	yes			
		Coochin Ck	Pool	yes			
		Condamine R	Riffle	yes			
		Warrego R	Pool	yes			
		Warrill Ck	Pool	yes			
		Albert R	Pool	yes			
		Currumbin Ck	Pool	yes			
		Mitchell R	Pool	yes			
		Palmer R	Riffle	yes			
		Hann R	Riffle	yes			
		Gowrie Ck	Riffle	yes			
		Gowrie Ck	Bank	yes			
		Proserpine R	Riffle	yes			
		O'Connell R	Macro	yes			
		Finch Hatton Ck	Riffle	yes			
		Crinum Ck	Macro	yes			
		Dawson R	Bank	yes			
		Burnett R	Bank	yes			
		MacIntyre R	Bank	yes			
		Dumaresq R	Riffle	yes			
		Brisbane R	Bank	yes			
		Tallebudgera Ck	Riffle	yes			
		Coomera Ck	Macro	yes			
		% habitat requested		**10**			
SA	3	Yankalilla Ck @ Main South Rd	Edge	yes	10	01-Aug-96	01-Aug-96
		Scotts Ck @ Scotts Bottom	Riffle	yes	10	29-Jul-96	31-Jul-96
		Sturt R @ Sturt Rd	Macro	yes	10	22-Jul-96	31-Jul-96
		Gawler R @ Gawler Junction	Pool	yes	10	22-Jul-96	31-Jul-96
		Light R @ Kapunda Bridge	Macro	yes	10	17-Jul-96	31-Jul-96
		Hill R nr Andrews	Pool	yes	10	31-Jul-96	31-Jul-96
		Middle R @ Western R d	Pool	yes	10	16-Jul-96	31-Jul-96
		Rocky R nr NP Headquarters	Riffle	yes	10	01-Aug-96	01-Aug-96
		Burra Ck @ World's End	Pool	yes	10	30-Jul-96	31-Jul-96
		Truro Ck	Edge	yes	10	31-Jul-96	31-Jul-96
		Mt. Barker Ck us Mt. Barker Springs	Riffle	yes	10	24-Jul-96	31-Jul-96
		Mt. Barker Ck us Mt. Barker Springs	Macro	yes	10	23-Jul-96	31-Jul-96
		Drain M @ Penola-Robe Rd	Edge	yes	10	19-Jul-96	31-Jul-96
		% habitat requested		**5**			
	4	Myponga R	Macro	yes			
		First Ck @ Waterfall Gully	Riffle	yes			
		Torrens R @ Windsor Grove	Macro	yes			
		North Parra R @ Rowland Flat	Riffle	yes			
		Willochra Ck S of Partacoona	Edge	yes			
		Oratunga Ck @ Third Spring	Pool	yes			
		Todd R @ Koppio	Edge	yes			
		Todd R @ Koppio	Riffle	yes			
		Wilson R S of Penneshaw	Edge	yes			
		Artimore Ck @ Nildottie Springs	Edge	yes			
		Artimore Ck @ Nildottie Springs	Macro	yes			
		Morambo Ck @ The Gap	Pool	yes			

Table A1. (Contin.)

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
QLD	4	Baffle Ck	Pool	yes			
		Sth Maroochy R	Bank	yes			
		Coochin Ck	Pool	yes			
		Condamine R	Riffle	yes			
		Warrego R	Pool	yes			
		Warrill Ck	Pool	yes			
		Albert R	Pool	yes			
		Currumbin Ck	Pool	yes			
		Mitchell R	Pool	yes			
		Palmer R	Riffle	yes			
		Hann R	Riffle	yes			
		Gowrie Ck	Riffle	yes			
		Gowrie Ck	Bank	yes			
		Proserpine R	Riffle	yes			
		O'Connell R	Macro	yes			
		Finch Hatton Ck	Riffle	yes			
		Crinum Ck	Macro	yes			
		Dawson R	Bank	yes			
		Burnett R	Bank	yes			
		MacIntyre R	Bank	yes			
		Dumaresq R	Riffle	yes			
		Brisbane R	Bank	yes			
		Tallebudgera Ck	Riffle	yes			
		Coomera Ck	Macro	yes			
		% habitat requested		**10**			
SA	3	Yankalilla Ck @ Main South Rd	Edge	yes	10	01-Aug-96	01-Aug-96
		Scotts Ck @ Scotts Bottom	Riffle	yes	10	29-Jul-96	31-Jul-96
		Sturt R @ Sturt Rd	Macro	yes	10	22-Jul-96	31-Jul-96
		Gawler R @ Gawler Junction	Pool	yes	10	22-Jul-96	31-Jul-96
		Light R @ Kapunda Bridge	Macro	yes	10	17-Jul-96	31-Jul-96
		Hill R nr Andrews	Pool	yes	10	31-Jul-96	31-Jul-96
		Middle R @ Western R d	Pool	yes	10	16-Jul-96	31-Jul-96
		Rocky R nr NP Headquarters	Riffle	yes	10	01-Aug-96	01-Aug-96
		Burra Ck @ World's End	Pool	yes	10	30-Jul-96	31-Jul-96
		Truro Ck	Edge	yes	10	31-Jul-96	31-Jul-96
		Mt. Barker Ck us Mt. Barker Springs	Riffle	yes	10	24-Jul-96	31-Jul-96
		Mt. Barker Ck us Mt. Barker Springs	Macro	yes	10	23-Jul-96	31-Jul-96
		Drain M @ Penola-Robe Rd	Edge	yes	10	19-Jul-96	31-Jul-96
		% habitat requested		**5**			
	4	Myponga R	Macro	yes			
		First Ck @ Waterfall Gully	Riffle	yes			
		Torrens R @ Windsor Grove	Macro	yes			
		North Parra R @ Rowland Flat	Riffle	yes			
		Willochra Ck S of Partacoona	Edge	yes			
		Oratunga Ck @ Third Spring	Pool	yes			
		Todd R @ Koppio	Edge	yes			
		Todd R @ Koppio	Riffle	yes			
		Wilson R S of Penneshaw	Edge	yes			
		Artimore Ck @ Nildottie Springs	Edge	yes			
		Artimore Ck @ Nildottie Springs	Macro	yes			
		Morambo Ck @ The Gap	Pool	yes			

Table A1. (Contin.)

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
SA	4	Eight Mile Ck	Riffle	yes			
		Eight Mile Ck	Macro	yes			
		% habitat requested		**5**			
WA	1	MUR06	Channel	Yes	20	05-Jul-96	09-Oct-96
Murdoch		MUR17	Riffle	Yes	20	10-Jul-96	09-Oct-96
Uni		MUR24	Macro	Yes	20	15-Jul-96	09-Oct-96
		MUR24	Channel	Yes	10	07-Jun-96	09-Oct-96
		MUR30	Channel	Yes	20	11-Jul-96	09-Oct-96
		MUR37	Macro	Yes	10	11-Jul-96	09-Oct-96
		MUR40	Riffle	Yes	10	05-Jul-96	09-Oct-96
		MURM2	Channel	Yes	20	04-Jul-96	09-Oct-96
		% habitat requested		**10**			
CaLM	3	CLM18	Riffle	Yes	25	23-Apr-97	28-Apr-97
		CLM30	Channel	Yes	10	23-Apr-97	24-Apr-97
		CLM35	Pool	Yes	5	24-Apr-97	24-Apr-97
		CLM43	Macro	Yes	5	24-Apr-97	24-Apr-97
		CLM46	Channel	Yes	5	24-Apr-97	24-Apr-97
		CLMM3	Riffle	Yes	2	28-Apr-97	24-Apr-97
		% habitat requested		**4**			
Uni WA		UWA2	Channel	Yes	40	09-Oct-96	09-Oct-96
		UWA3	Channel	Yes	50	15-Oct-96	15-Oct-96
		UWA9	Macro	Yes			
		UWA13	Macro	Yes	10	10-Oct-96	10-Oct-96
		UWA17	Channel	Yes	100	14-Oct-96	14-Oct-96
		UWA23	Channel	Yes	50	10-Oct-96	10-Oct-96
		UWA24	Organic	Yes			
		UWA27	Macro	Yes	100	30-Apr-96	10-Oct-96
		UWA29	Macro	Yes			
		UWA34	Macro	Yes	100	10-Oct-96	10-Oct-96
		UWA37	Riffle	Yes	10	10-Oct-96	10-Oct-96
		UWA41	Channel	Yes			
		% habitat requested		**10**			
Edith	4	ECU06	Channel	Yes	10	08-Apr-97	18-Apr-97
Cowan		ECU12	Channel	Yes			
Uni		ECU12	Macro	Yes	10	18-Apr-97	18-Apr-97
		ECU12	Pool	Yes	100	17-Apr-97	18-Apr-97
		ECU18	Channel	Yes			
		ECU18	Macro	Yes	5	11-Apr-97	18-Apr-97
		ECU21	Channel	Yes			
		ECU30	Pool	Yes			
		ECU30	Channel	Yes			
		ECU37	Pool	Yes	10	17-Apr-97	18-Apr-97
		ECU37	Channel	Yes			
		ECU37	Riffle	Yes			
		ECU42	Channel	Yes	25	14-Apr-97	18-Apr-97
		ECU44	Channel	Yes	50	09-Apr-97	18-Apr-97
		ECU46	Channel	Yes			
		ECUM5	Channel	Yes			
		ECUM6	Channel	Yes			
		% habitat requested		**25**			

Table A1. (Contin.)

State	Round	Site	Habitat	Received	% Subs	Sorted & ID	Completed
NT	3	VC02	Sand	Yes	5	09-May-97	27-May-97
		DA01	Sand	Yes	5	12-May-97	27-May-97
		GY02	Sand	Yes	15	12-May-97	27-May-97
		DA06	Edge	Yes	9	15-May-97	15-May-97
		DA06	Sand	Yes	70	16-May-97	16-May-97
		EA05	Edge	Yes	9	19-May-97	19-May-97
		AD03	Edge	Yes	9	19-May-97	19-May-97
		GY02	Edge	Yes	5	20-May-97	20-May-97
		KP03	Edge	Yes	3	28-May-97	28-May-97
		KP03	Sand	Yes	4	29-May-97	29-May-97
		% habitat requested		**4**			

* Envelopes not received in time for some catchments

** Only 5 catchments sampled in round 4 (78 habitats)

QA/QC methodology applied to the assessment of the effectiveness of agency sample processing procedures for MRHI

The following report describes an approach in which agency sample processing procedures are assessed against a criterion that the agency-processed samples are representative of the whole sample in terms of community composition and structure. The ***eriss*** QA/QC Report attached to this Appendix, compares the representativeness of the agency-sorted samples against that of a 'whole sample estimate', the latter derived from agency-sorted fractions and residues.

The steps in the ***eriss*** QA/QC Reports used to assess the representativeness of the agency-sorted samples were adapted from similar assessments performed by Davies et al (1997). Calculation of the steps involved were performed using a Paradox for Windows (1994) macro; these steps are described as follows:

Live-sorted samples

The broad procedures involved in the audits, according to table columns in the QA/QC report, are:

(i) a taxa list with relative abundances of organisms for 2 fractions of the sample, live-sort (LS) component (col **A**) and a subsample of the preserved residue (col **B**) is compiled, the latter with total no. of organisms $\geq$ the LS component, but always with minimum sample size of 100 organisms;

(ii) a 'subsample' of organisms from LS component (cols **C & D**), equivalent in proportion to the subsampled residue, is added to the residue fraction;

(iii) the new combined LS and residual fraction (**E**) is scaled down and rounded to the sample size of the LS component for LS sample size >100 (**F & G**), or to 100 for LS < 100 (**I & J**), this fraction then being termed 'whole sample estimate' (WSE); and

(iv) WSE and LP fractions are compared for presence/absence data by way of taxa number (LP/WSE) and taxa composition (Bray-Curtis dissimilarity) and for relative abundance data by way of Bray-Curtis dissimilarity.

Proportional scaling is used between cols A & D, E & G and E & J. The rounding required within these three steps follows particular rules: (i) scaled taxa abundances > 1 are rounded to the nearest whole number; (ii) taxa fractions (< 1) that have the same value are summed then, on the basis of the cumulative sum, randomly assigned a value '1' to the number of corresponding taxa that match the nearest whole number of the summed fractions; and (iii) random assignments to such rare taxa are allocated from highest to lowest value of the summed fractions. A worked example of these calculations is provided in Attachment 1.

Random allocations of summed fractional occurrences to rare taxa have flow-on effects through the calculations of WSE taxa lists such that different results may occur depending upon the taxa allocation. Hence, the macro programmed to compute the steps towards calculation of WSE, performs 10 iterations, taxa number and B-C dissimilarity for presence-absence and relative abundance data being averaged over the 10 iterations.

As MRHI models are only based upon presence/absence data, relevant assessment criteria for agencies will be LS/WSE (analogous to the RIVPACS 'observed/expected' ratio) and B-C dissimilarity for presence/absence data. WSE and assessment criteria are calculated separately for cases in which LS <100 animals and LS >100 animals. Acceptance/rejection thresholds for the assessment criteria are being determined. Advice on acceptability or otherwise of agency data is provided in the cover letter.

Laboratory subsampling and sorting of preserved samples

The QA/QC procedure adopted for assessing the representativeness of laboratory subsampled and sorted (preserved) samples typically uses as its basis, subsampling of another fraction of equivalent size from the residue, sorting and identifying organisms from the second fraction, then comparing community composition and structure of the first (agency) and second (externally-derived) fractions. This is the principle used for external QA/QC of MRHI samples except for one important factor - the second fraction is calculated as an *independent* estimate of community composition and structure as opposed to one whose result is *dependent* upon the outcome of initial subsampling for the first fraction. Thus, rather than direct comparison of the two fractions, the data are analysed by way of the same macro used to compare LS and WSE data from live-sorting (i.e. 'subsampling' of the first fraction, addition of this fraction to the second subsample data, then scaling to equivalent sample number). This assessment procedure for subsampled data has been used so that evaluation of LS and subsampled data are directly comparable (i.e. the two fractions being compared, agency and external audit components, are independent estimates of the original sample). (A more stringent approach could be adopted in future 'agency'/'external QA/QC' subsample comparisons in that column E could be scaled to the sample size of column B, the QA/QC subsample, instead of column A, the agency subsample.)

Explanation of calculations and assessment criteria used in evaluation of agency subsampling and sorting procedures is the same as that described above for agency live-sorting procedures. For laboratory subsampling and sorting, however, the larger of the subsampled fractions (agency or ***eriss***) is always entered in column B of the macro. The column to which the agency component has been entered (A or B) has been highlighted.

Worked example of proportional scaling and rounding procedures

Table 1. Procedure used in proportional scaling and rounding of taxa abundances in QA/QC calculations: hypothetical example of calculations used to derive a 10% 'subsample' of agency live-sort component (columns A to C) with subsequent rounding (col D).

Taxon	A (original live-sort)	C (10% 'subsample' of live-sort)
a	10	1
b	7	0.7
c	8	0.8
d	9	0.9
e	4	0.4
f	5	0.5
g	6	0.6
h	5	0.5
i	5	0.5
j	4	0.4
k	4	0.4
l	4	0.4
m	4	0.4
n	3	0.3
o	3	0.3
p	3	0.3
q	2	0.2
r	2	0.2
s	1	0.1
t	1	0.1
u	1	0.1
v	1	0.1
w	1	0.1
x	1	0.1
Total of fractions (<1)		**8.4**

For new scaled taxa abundances < 1.0 (col C):

Cum. summed fractions for original taxa abundance value of '1': 0.1 x 6 values = 0.6 animals
Cum. summed fractions for original taxa abundance value of '2': 0.2 x 2 values = 0.4
Cum. summed fractions for original taxa abundance value of '3': 0.3 x 3 values = 0.9
Cum. summed fractions for original taxa abundance value of '4': 0.4 x 5 values = 2.0
Cum. summed fractions for original taxa abundance value of '5': 0.5 x 3 values = 1.5
Cum. summed fractions for original taxa abundance value of '6': 0.6 x 1 values = 0.6
Cum. summed fractions for original taxa abundance value of '7': 0.7 x 1 values = 0.7
Cum. summed fractions for original taxa abundance value of '8': 0.8 x 1 values = 0.8
Cum. summed fractions for original taxa abundance value of '9': 0.9 x 1 values = 0.9

Re-assignment of taxa abundance values:

Original taxa	Cumulative summed	Order of re-assigned taxa	No. of animals randomly re-assigned
10	0.6	8	0
2	0.4	(<0.5; no re-assignment)	0
3	0.9	3	1 assigned amongst 3 positions
4	2	1	2 (1,1) assigned amongst 5positions
5	1.5	2	2 (1,1) assigned amongst 3 positions
6	0.6	7	0
7	0.7	6	1 assigned to 1 position
8	0.8	5	1 assigned to 1 position
9	0.9	4	1 assigned to 1 position
Total	8.4		8 (rounded from 8.4)

Possible outcome for column D after taxa re-allocations (1 outcome from 10 iterations)

Taxa	A (original live-sort)	C (10% 'subsample' of live-sort)	D (new rounded 10% subsample)
a	10	1	1
b	7	0.7	1
c	8	0.8	1
d	9	0.9	1
e	4	0.4	
f	5	0.5	
g	6	0.6	
h	5	0.5	1
i	5	0.5	1
j	4	0.4	1
k	4	0.4	
l	4	0.4	
m	4	0.4	1
n	3	0.3	1
o	3	0.3	
p	3	0.3	
q	2	0.2	
r	2	0.2	
s	1	0.1	
t	1	0.1	
u	1	0.1	
v	1	0.1	
w	1	0.1	
x	1	0.1	
Total	-	9.4	9

References

Davies PE, Mitchell N & Barmuta LA 1996. *The impact of historical mining operations at Mount Lyell on the water quality and biological health of the King and Queen River catchments, Western Tasmania.* Mount Lyell Remediation and Demonstration Program. Supervising Scientist Report 118. Supervising Scientist, Canberra.

Paradox for Windows 1994. Borland International Inc, Version 5.0, Scotts Valley, CA, USA.

Taxon Codes

Code	Family	Stage
AESHZZZL	AESHNIDAE	L
ANCYZZZX	ANCYLIDAE	X
APERZZZN	AUSTROPERLIDAE	N
APTEZZZL	AMPHIPTERYGIDAE	L
ATHEZZZL	ATHERIDAE	L
ATRIZZZL	ATRIPLECTIDIDAE	L
ATYIZZZX	ATYIDAE	X
BAETZZZN	BAETIDAE	N
BLEPZZZL	BLEPHARICERIDAE	L
BRENZZZL	BRENTIDAE	L
BRENZZZA	BRENTIDAE	A
CAENZZZN	CAENIDAE	N
CALAZZZL	CALAMOCERATIDAE	L
CCIDZZZL	CALOCIDAE	L
CDALZZZL	CORYDALIDAE	L
CERAZZZL	CERATOPOGONIDAE	L
CERAZZZP	CERATOPOGONIDAE	P
CHIRZZZL	CHIRONOMIDAE	L
CHIRZZZP	CHIRONOMIDAE	P
CHLOZZZL	CHLOROLESTIDAE	L
CHRYZZZL	CHRYSOMELIDAE	L
CHRYZZZA	CHRYSOMELIDAE	A
COENZZZL	COENAGRIONIDAE	L
COLOZZZN	COLOBURISCIDAE	N
CONOZZZL	CONOESUCIDAE	L
CORBZZZX	CORBICULIDAE	X
CORDZZZL	CORDULIIDAE	L
CORIZZZA	CORIXIDAE	A
CORIZZZN	CORIXIDAE	N
CULIZZZL	CULICIDAE	L
CURCZZZL	CURCULIONIDAE	L
DIXIZZZL	DIXIDAE	L
DUGEZZZX	DUGESIIDAE	X
DYTIZZZL	DYTISCIDAE	L
DYTIZZZA	DYTISCIDAE	A
ECNOZZZL	ECNOMIDAE	L
ELMIZZZL	ELMIDAE	L
ELMIZZZA	ELMIDAE	A
EMPIZZZL	EMPIDIDAE	L
EPHEZZZN	EPHEMERELLIDAE	N
EPHYZZZL	EPHYDRIDAE	L
EUSTZZZN	EUSTHENIIDAE	N
GELAZZZA	GELASTOCORIDAE	A
GELAZZZN	GELASTOCORIDAE	N
GERRZZZA	GERRIDAE	A
GERRZZZN	GERRIDAE	N
GIPHZZZX	GLOSSIPHONIIDAE	X
GOMPZZZL	GOMPHIDAE	L
GOSOZZZL	GLOSSOSOMATIDAE	L
GRIPZZZN	GRIPOPTERYGIDAE	N
GYRIZZZN	GYRINIDAE	N
HALIZZZL	HALIPLIDAE	L
HALIZZZA	HALIPLIDAE	A
HBIIZZZX	HYDROBIIDAE	X

Taxon Codes

(Contin.)

Code	Family	Stage
SISYZZZL	SISYRIDAE	L
SPHAZZZX	SPHAERIIDAE	X
STAPZZZL	STAPHYLINIDAE	L
STAPZZZA	STAPHYLINIDAE	A
STRAZZZL	STRATIOMYIDAE	L
TABAZZZL	TABANIDAE	L
TASIZZZL	TASIMIIDAE	L
TEMNZZZX	TEMNOCEPHALIDEA	X
THAUZZZL	THAUMALEIDAE	L
THIAZZZX	THIARIDAE	X
TIPUZZZL	TIPULIDAE	L
UACAZZZX	UNID. ACARINA	X
UAMPZZZX	UNID. AMPHIPODA	X
UANIZZZL	UNID. ANISOPTERA	L
UDIPZZZP	UNID. DIPTERA	P
UEMEZZZL	UNID. EPHEMEROPTERA	L
UHIRZZZX	UNID. HIRUDINEA	X
UISOZZZX	UNID. ISOPODA	X
UNEMZZZX	UNID. NEMATODA	X
UOLIZZZX	UNID. OLIGOCHAETA	X
UPLEZZZL	UNID. PLECOPTERA	L
UTRIZZZL	UNID. TRICHOPTERA	L
UTURZZZX	UNID. TURBURELLIA	X
UZYGZZZL	UNID. ZYGOPTERA	L
VELIZZZN	VELIIDAE	N

LIFE STAGE:

L = LARVAE

N = NYMPH

X = LIFE STAGE NOT IDENTIFIED

A = ADULT

P = PUPAE

Table A3. Comparison of agency live-sorted (LS) component and whole sample estimate (WSE), using Bray-Curtis dissimilarity measures (presence/absence (p/a) and relative abundance data) and taxa number ratio.

State/ Habitat	N (live-sorted)	QA/QC ASSessment Criteria		
		Dissimilarity (p/a)	LS/WSE taxa number ratio (%)	Dissimilarity (rel. abund.)
QLD				
Edge 1	141	0.3241	113	0.4737
Edge 2	781	0.2116	101	0.3673
Edge 3	150	0.3918	114	0.3796
Edge 4	145	0.3428	62	0.4243
Edge 5	504	0.3134	82	0.5436
Edge 6	97	0.5850	90	0.5280
Pool 1	114	0.5240	250	0.6338
Pool 2	235	0.2857	100	0.6026
Pool 3	145	0.2303	100	0.4438
Pool 4	154	0.3602	115	0.5447
Riffle 1	208	0.1565	121	0.2705
Riffle 2	204	0.3698	103	0.3129
Riffle 3	414	0.3140	95	0.3842
Riffle 4	218	0.2586	95	0.5376
Riffle 5	192	0.2756	100	0.4522
Riffle 6	188	0.2011	134	0.4182
Riffle 7	51	0.1556	86	0.4113
Macro 1	289	0.2537	134	0.2152
Macro 2	221	0.4102	141	0.2718
Macro 3	106	0.2824	114	0.6676
Macro 4	78	0.4325	101	0.6340
Macro 5	64	0.4768	100	0.6608
NSW				
Edge 1	49	0.4206	120	0.6525
Edge 2	209	0.3080	106	0.4366
Edge 3	142	0.3315	115	0.7456
Edge 4	110	0.4214	49	0.2361
Edge 5	246	0.4337	82	0.2756
Edge 6	65	0.5640	92	0.4273
Edge 7	208	0.2841	99	0.3248
Edge 8	164	0.4304	68	0.1975
Edge 9	55	0.5440	85	0.6327
Edge 10	77	0.2767	64	0.2935
Pool 1	121	0.5051	62	0.4122
Riffle 1	186	0.3258	109	0.3017
Riffle 2	97	0.4952	56	0.4146
Riffle 3	165	0.3980	80	0.5987
Riffle 4	192	0.1934	96	0.5029
Riffle 5	116	0.4652	80	0.4010
Riffle 6	69	0.4447	81	0.5132
Riffle 7	261	0.1654	107	0.1892
Macro 1	236	0.3775	142	0.5831

Table A3. (Contin.)

State/ Habitat	N (live-sorted)	QA/QC Assessment Criteria		
		Dissimilarity (p/a)	LS/WSE taxa number ratio (%)	Dissimilarity (rel. abund.)
TAS				
Edge 1	145	0.3358	89	0.4991
Edge 2	132	0.3685	121	0.4570
Edge 3	109	0.3522	114	0.6665
Edge 4	171	0.3354	79	0.5875
Edge 5	161	0.2966	66	0.5428
Edge 6	251	0.3248	65	0.5278
Edge 7	186	0.2223	85	0.4958
Riffle 1	250	0.2867	92	0.3146
Riffle 2	282	0.2191	88	0.4639
Riffle 3	287	0.2089	95	0.4078
Riffle 4	102	0.2857	75	0.3382
Riffle 5	113	0.2785	58	0.4612
Riffle 6	200	0.2024	84	0.3800
Riffle 7	260	0.2320	95	0.5296
Riffle 8	93	0.3778	102	0.5936
VIC				
Edge 1	162	0.3872	106	0.5724
Edge 2	135	0.2325	97	0.4807
Edge 3	94	0.2554	117	0.4616
Edge 4	227	0.3060	87	0.4255
Edge 5	315	0.3784	85	0.4009
Edge 6	186	0.2692	109	0.5799
Edge 7	97	0.5387	125	0.4598
Edge 8	139	0.3105	82	0.4328
Riffle 1	257	0.3797	145	0.6754
Riffle 2	31	0.4736	69	0.7248
Riffle 3	123	0.2116	90	0.5364
Riffle 4	50	0.2679	107	0.4790
Riffle 5	608	0.2378	105	0.4194
Riffle 6	132	0.3675	119	0.4828
Riffle 7	697	0.1924	86	0.3177
Riffle 8	125	0.3569	67	0.5886
Riffle 9	229	0.2731	85	0.3557
WA				
Channel 1	213	0.1135	105	0.2553
Channel 2	96	0.2015	87	0.3330
Channel 3	117	0.1749	70	0.3242
Channel 4	160	0.1813	78	0.2649
Channel 5	18	0.5000	150	0.8151
Channel 6	68	0.3142	81	0.2923
Channel 7	92	0.3332	105	0.4258
Channel 8	182	0.4444	111	0.1315
Channel 9	207	0.1667	111	0.3671
Channel 10	62	0.2222	100	0.5802
Channel 11	78	0.6371	70	0.2643

Table A3. (Contin.)

State/ Habitat	N (live-sorted)	QA/QC Assessment Criteria		
		Dissimilarity (p/a)	LS/WSE taxa number ratio (%)	Dissimilarity (rel. abund.)
WA				
Macro 1	177	0.3658	129	0.4147
Macro 2	67	0.3971	75	0.5406
Macro 3	76	0.3020	123	0.4731
Macro 4	54	0.4485	95	0.4824
Macro 5	51	0.3184	101	0.5945
Macro 6	109	0.2383	128	0.5366
Macro7	103	0.4000	166	0.5512
Riffle 1	135	0.3636	100	0.4593
Riffle 2	118	0.3201	68	0.3029
Riffle 3	187	0.5000	140	0.1444
Pool 1	25	0.1846	85	0.6726
Pool 2	166	0.3478	140	0.4941
SA (LAB)				
Edge 1	631	0.1625	83	0.1621
Edge 2	56	0.1810	120	0.3564
Pool 1	693	0.2703	100	0.0205
Pool 2	240	0.1614	88	0.1483
Pool 3	74	0.1644	125	0.2216
Pool 4	29	0.3804	67	0.3819
Riffle 1	96	0.2031	95	0.1708
Riffle 2	456	0.2457	106	0.0585
Riffle 3	5032	0.1976	97	0.0437
Macro 1	1981	0.3141	118	0.0724
Macro 2	67	0.3542	72	0.2140
Macro 3	1091	0.2674	88	0.0895
Macro 4	292	0.2828	108	0.2169
ACT (Lab)				
Edge 1	211	0.3740	101	0.5186
Edge 2	199	0.2231	82	0.2220
Edge 3	190	0.5154	92	0.2294
Edge 4	260	0.3063	83	0.2627
Edge 5	208	0.1810	83	0.1934
Riffle 1	204	0.2194	100	0.1547
Riffle 2	234	0.0998	92	0.0927
Riffle 3	208	0.3280	86	0.1329
Riffle 4	200	0.2188	83	0.1086
Riffle 5	206	0.1838	85	0.2068
NT (Lab)				
Edge 1	245	0.3403	82	0.1454
Edge 2	198	0.2233	109	0.1354
Edge 3	150	0.2706	109	0.1310
Edge 4	169	0.1796	103	0.2030
Edge 5	162	0.2527	86	0.2054

Table A3. (Contin.)

State/ Habitat	N (live-sorted)	QA/QC Assessment Criteria		
		Dissimilarity (p/a)	LS/WSE taxa number ratio (%)	Dissimilarity (rel. abund.)
NT (Lab)				
Sand 1	341	0.2258	153	0.1085
Sand 2	218	0.3510	100	0.1284
Sand 3	210	0.2948	92	0.1881
Sand 4	113	0.2902	85	0.0925
Sand 5	315	0.3231	125	0.1768
WA (Lab)				
Riffle 1	145	0.2800	177	0.2828
Riffle 2	214	0.1208	126	0.3679
Macro 1	89	0.3874	133	0.2963
Pool 1	136	0.2418	111	0.2952
Channel 1	90	0.3446	160	0.4511
Channel 2	128	0.2575	132	0.3294

APPENDIX 4

Correspondence to agencies re results of external QA/QC assessments

Letter 1: Correspondence to all live-sort agencies (31 October 1996)

Letter 2: Correspondence to SA agency (lab subsampling and sorting) (20 November 1996)

Letter 3: Correspondence to VIC agency (live-sorting) (23 January 1997)

Letter 4: Correspondence to NSW agency (live-sorting) (27 February 1997)

Letter 5: Correspondence to TAS agency (live-sorting) (27 February 1997)

Letter 6: Corresepondence to WA agency (lab subsampling and sorting) (6 May 1997)

Letter 7: Correspondence to ACT agency (lab subsampling and sorting) (16 May 1997)

Letter 8: Correspondence to NT agency (lab subsampling and sorting) (29 May 1997)

REF

31 October, 1996

cc.

Dear

As you are aware, ***eriss*** has been conducting an external QA/QC audit of MRHI agency sorting procedures for samples collected during rounds 3 & 4. This has entailed an assessment of the representativeness of taxa composition and relative abundances of agency sorted samples (live-sorted or laboratory preserved and subsampled) against that of corresponding residues.

Results of QA/QC audits were presented at the Canberra workshop in October. In our collective presentations (Lisa Thurtell, Chris Humphrey and Andrew Storey [UWA]), we expressed concern at the high proportion of *live-sorted* samples with seemingly 'high' errors - see Table 1 for a summarised breakdown of these results. The full implications of these errors for successful modelling are still unknown and this important issue is the subject of continuing R&D. In a preliminary "worst-case scenario" simulation presented in Canberra, high live-sort errors appeared to have drastic consequences for model development. Whilst these results should be viewed cautiously at this stage until further work is carried out, the MRHI Technical Advisory Committee nevertheless agreed that there were already steps that could be taken by agencies to screen live-sorted data with a view to possible removal of poor quality samples from inclusion in model development.

Attached are data sheets (together with explanations) from analysis of the QA/QC samples examined from your agency. We offer the following comments in interpreting data and in possible screening for exclusion of these and other data with common generic errors in your development of predictive models:

- Firstly, you should be aware of course that the goal of this external QA/QC was not accuracy of agency or external auditor identifications; such (taxonomic) QA/QC is the subject of a separate project being conducted by MDFRC and ***eriss.*** Hence, there may well be instances (minor we hope!) where in the attached data sheets you disagree strongly with the identity of a particular taxon. Possible misidentification at our end is not an issue for those samples in which we have examined both live-sorted and residue components; in these cases we are confident the error is *consistent* in the two portions - the key issue for this QA/QC audit. However, where only data sheets for live-sorted components were received (NSW round 3, VIC rd 3, WA both rds), it is possible that discrepancies might arise. Please notify us of possible problems in this regard; the error should be easy enough to check and rectify. (Note that we do not anticipate significant changes to QA/QC results as a result of agency vs external auditor discrepancies.)
- The QA/QC audits were consistent with the taxonomic groups selected for MRHI study for all taxa other than Chironomidae. We did not identify chironomids to subfamilies as this would have slowed down the audits considerably. The overall effect of this decision is to give a slightly more conservative result to the QA/QC outputs.

Guidance for exclusion of data from models:

- From separate analyses which we will forward at a later date, it is likely that we will recommend as part of a revised live-sort protocol, a minimum sample size of 200 animals. The exception is the south-west of WA, where the critical sample size will probably be near 100 due to the depauperate nature of the fauna. For collections to date, it is clear that samples with fewer than 100 animals live-sorted (~50 animals in SW WA) under-estimate taxa number significantly. (In the QA/QC results, these will typically have a Live Sort (LS)/Whole Sample Estimate (WSE) ratio <0.8.) Agency judgement on naturally-depauperate sites and habitats will also be required in this assessment.
- Samples where 'common' taxa are missed (see attached Table 1) are likely to present problems in classification and modelling; our experience suggests that UPGMA may be particularly sensitive to this type of error.
- Regardless of sample size (total no. of animals live-sorted), QA/QC data with a LS/WSE ratio <0.75 and Bray-Curtis dissimilarity (pres/abs) >0.4 constitute to our way of thinking, large errors which could potentially compromise the resolution and sensitivity of agency models. We suggest you examine these results to determine their cause.

Perhaps the best advice we can offer is to identify live-sort samples with the types of errors described above and give serious consideration to their removal from further analysis. Of course, without residues, judgement on which taxa can be considered 'common' and missed in live-sorting will need to be assessed against other samples collected in the bioregion and habitat of concern. Even so, alarm bells should be ringing if taxa such as Chironomidae and Oligochaeta are missing from live-sorted samples. Even chironomid larvae whose combined LS abundance value is not much less than 10 run a serious risk of omission of a common subfamily. Personnel associated with these errors should be identified and all his/her past (and future) samples checked to determine whether the error is consistently re-appearing. (To this end, there is clear advantage in agencies implementing their own internal QA/QC program to assess operator efficiency for sample sorting.) Specific comment and suggestions for additional training of agency staff in live-sorting procedures will probably be forwarded from Peter Davies at a later stage.

Regards

The QA/QC team (hippos)

REF

20 November, 1996

Chris Madden
Australian Water Quality Centre
Private Mail Bag 3
SALISBURY SA 5108

cc.

Dear Chris

As you are aware, ***eriss*** has been conducting an external QA/QC audit of MRHI agency sorting procedures for samples collected during rounds 3 & 4. This has entailed an assessment of the representativeness of taxa composition and relative abundances of agency sorted samples (live-sorted or laboratory preserved and subsampled) against that of corresponding residues.

Attached are data sheets (together with explanations) from analysis of the QA/QC samples examined from your agency. We offer the following comments in interpreting data, particularly insofar as discrepancies between your (agency) results and those of ***eriss*** are concerned:

- Firstly, you should be aware of course that the goal of this external QA/QC was not accuracy of agency or external auditor identifications; such (taxonomic) QA/QC is the subject of a separate project being conducted by MDFRC and ***eriss.*** Hence, there may well be instances (minor we hope!) where in the attached data sheets you disagree strongly with the identity of a particular taxon. Possible misidentification at our end is not an issue for those samples in which we have examined both live-sorted and residue components; in these cases we are confident the error is *consistent* in the two portions - the key issue for this QA/QC audit. However, where only data sheets for agency-sorted components were received - the case for SA samples - it is possible that discrepancies might arise. Please notify us of possible problems in this regard; the error should be easy enough to check and rectify. (Note that we do not anticipate significant changes to QA/QC results as a result of agency vs external auditor discrepancies.)
- The QA/QC audits were consistent with the taxonomic groups selected for MRHI study for all taxa other than Chironomidae. We did not identify chironomids to subfamilies as this would have slowed down the audits considerably. The overall effect of this decision is to give a slightly more conservative result to the QA/QC outputs.

Guidance for identifying data that may be problematic in MRHI model development:

- From separate analyses which we will forward at a later date, it is likely that we will recommend as part of revised sorting protocols (laboratory subsampling and field live-sorting), a minimum sample size of 200 animals. The exception is the south-west of WA, where the critical sample size will probably be near 100 due to the depauperate nature of the fauna. For collections to date, it is clear that samples with fewer than 100 animals sorted (~50 animals in SW WA) under-estimate taxa number significantly. There is debate in the literature as to whether samples should be subsampled to a fixed count or fixed proportion (see Barbour & Gerritsen, 1996; Courtemanch, 1996; Vinson & Hawkins,

1996). There are merits in either approach. However and as noted above, we will be recommending that future sorting be carried out using a fixed count method (as per assessment of aforementioned literature) rather than the fixed proportion approach of your agency. For now, we strongly advise that you return to residues where sample size is <100 and sort addition material so that N approaches 200 animals. (Note the generally poor QA/QC results at very low N.)

Apart from small sample size, no other serious discrepancy has arisen between SA agency results and external QA/QC results that would indicate poor quality agency data. However, there is a matter of minor concern that is worth pointing out. The ***eriss*** subsample of the residue (11% of the forwarded residue compensating for the 10% already removed) was consistently smaller in sample size than the SA component (9 out of 11 cases - see attached Report sheets). We are confident about our (***eriss***) subsampled proportions, these being derived from a modified Marchant multi-cell (100) subsampler. One possible explanation for the discrepancy is sample deterioration to the extent that some material from most of the samples reaching ***eriss*** may no longer have been recognisable as intact invertebrates. We doubt this possibility, however, and wonder whether the discrepancy is more closely linked to your method of estimating proportional area represented in each of the vials of the SA subsampler, as elaborated upon below.

In our own subsampling trials at ***eriss*** in which we evaluated the performance of different agency subsampling devices, difficulties were encountered in estimating vial subsampling area of the SA device. We established that an areal determination - calculating the total internal area of the device and subtracting from this interstitial area - could overestimate vial subsampling area. Apart from correct estimation of internal vial area (based on the top lip) the main problem we determined was that detritus and invertebrates falling upon or *near* the points of contact of the vials were more likely to fall *within* a vial than outside of it. This could lead to over-estimation of material falling inside vials.

An approach we pursued to correctly estimate internal vial area was one based on gravimetry: a sample is placed in the device and treated as per routine subsampling. Material falling into vs outside the vials is collected separately and the 2 fractions of the sample dried overnight at 80°C. Dry weight of the 2 fractions may then be used to estimate sample area. We obtained equivocal results using this method, possibly because of the nature of the organic residue used in the trials. We do recommend, however, that you conduct your own R&D to define more accurately the area subsampled in the vials of your subsampler. The gravimetric method using a range of different residue types could be worth pursuing.

Regards for now,

The QA/QC team (hippos)

References

Barbour, M.T. & Gerritsen, J. (1996) Subsampling of benthic samples: a defense of the fixed-count method. **J. N. Am. Benthol. Soc.** 15(3):386-391.

Courtemanch, D.L. (1996) Commentary on the subsampling procedures used for rapid bioassessments. **J. N. Am. Benthol. Soc.** 15(3):381-385.

Vinson, M.R. & Hawkins, C.P. (1996) Effects of sampling area and subsampling procedures on comparisons of taxa richness among streams. **J. N. Am. Benthol. Soc.** 15(3):392-399.

REF

27 February, 1997

Eren Turak
NSW EPA
Locked Bag 1502
BANKSTOWN NSW 2200

Dear Eren

Please find enclosed an additional 11 reports for NSW live-sort samples I have recently completed for QA/QC assessment. Guidance on the interpretation of these data sheets was provided in previous correspondence to you. However, the calculations used to derive a Whole Sample Estimate (WSE) rounded to 100 animals (columns I and J) were incorrect in the data sheets provided in previous correspondence; this affected those live-sorted samples with live-sort N < 100. As a consequence, we have recalculated QA/QC endpoints pertinent to those NSW samples, namely CLAR19 and RICH01, and data sheets for these sites have also been provided.

In comparing your live-sort results to those of the 'Whole Sample Estimate' and also to those results of other agencies using the live-sort procedure, there are a couple of comments to make. The enclosed data sheets exemplify all of the problems that apparently Chris Humphrey raised at the MRHI meeting in Canberra in early February. Thus: low live-sort sample size (and consequent high error rates) and missed taxa occurring commonly in samples and amongst sites, especially Corbiculidae, Chironomidae (pupae), Empididae, Hydroptilidae, Ceratopogonidae, Oligochaeta, Elmidae (larvae), Hydrophilidae and Simuliidae (larvae). In addition, there are instances of low live-sort abundance (<10 animals) for chironomid larvae, with the likelihood therefore, of missed subfamilies.

Training will play a essential part in redressing these concerns. The training program, which is mentioned in the new autumn sampling contracts, will be crucial prior to the upcoming sampling round.

Regards

Lisa Thurtell, ***eriss***

REF

27 February, 1997

David Oldmeadow
Dept. of Primary Industries
Land and Water Resources Division
GPO Box 192B
HOBART TAS 7001

Dear David

How are you? Please find enclosed an additional 6 reports for theTasmanian live-sort samples I have recently completed for QA/QC assessment. Guidance on the interpretation of these data sheets was provided in previous correspondence to you.

Round 4 presence/absence dissimilarity values were very close to round 3 results, decreasing from a mean value in round 3 of 0.28 to a mean value in round 4 of 0.27. Two of the 6 samples had a very low representation of taxa (LS/WSE < 0.7), while chironomid larvae abundances in 3 out of 6 samples were less than 10 (increasing the chances of missed subfamilies).

Training will play a essential part in redressing these concerns. The training program, which is mentioned in the new autumn sampling contracts, will be crucial prior to the upcoming sampling round.

Regards

Lisa Thurtell, ***eriss***

REF JK-02-13

6 May, 1997

Mick Smith
Department of Conservation and Land Management
PO Box 51
Wanneroo WA 6065

cc.

Dear Mick

Please find attached 6 QA/QC reports assessing your agency's subsampling and sorting techniques for MRH. The assessment procedure adopted for preserved samples involved subsampling another fraction from the residue which was sorted and identified, then compared to the community composition and structure of the agency's original fraction. The data were then analysed by way of the same macro used to compare Live-sort and Whole Sample Estimate data.

In terms of meeting the MRHI Bioassessment Manual stated aim of capturing and collecting the broadest range of biota at a site, CLM has been particularly effective. CLM samples consistently contained a greater number of taxa than the QA/QC subsample, with all common taxa well-represented. This led to increased dissimilarity values for both community composition and structure but, with the exception of sample CLM46, values were still lower than most live-sort comparisons. All presence/absence dissimilarity values remained below 0.4. Values above 0.4 could be indicative of large errors which may compromise the resolution and sensitivity of agency models.

In revised protocols for MRHI sorting procedures, sorting to a *fixed* sample number will be recommended, Certainly for live-sorted data N<100 has led to serious problems of taxa underrepresentation. Whilst this has not occurred in the case of your data, it is likely that there is a threshold N about which taxa recovery has most 'efficiently' been attained. Using your samples, data sets and distinctive sorting methods, it would be worthwhile pursuing the matter. You should contact Chris Humphrey for further information about this.

For any other information regarding the assessment procedure please contact me on (08) 8979 9731.

Yours sincerely

Lisa Thurtell

REF JK-02-13

16 May, 1997

Ken Thomas
CRC Freshwater Ecology
University of Canberra
PO Box 1
Belconnen ACT 2616

cc.

Dear Ken

Please find attached 10 QA/QC reports assessing your agency's subsampling and sorting procedures for MRHI. The assessment procedure adopted for preserved samples involved subsampling another fraction from the residue equivalent in size to the agency sample, sorting and identifying the new fraction, then comparing the community composition and structure of the QA/QC fraction to that of the original agency fraction. The data for the two fractions were analysed by way of a computer macro that compared community composition and structure viz taxa number ratio and (Bray-Curtis) dissimilarity. Details of the calculations used in the macro and taxa codes etc are attached.

In terms of meeting the MRHI Bioassessment Manual's stated aim of capturing and collecting the broadest range of biota at a site, the CRC has not been as effective as other preserved-sample agencies. Thus, QA/QC subsamples consistently contained a greater number of taxa than the agency subsample, with particularly large discrepancies occurring between the agency and QA/QC subsample for sites 117 and 166. Taxa occurring commonly in the sample were usually well-represented in both agency and QA/QC subsamples. Exceptions to this were the mites and, to a lesser degree, hydroptilids, both taxa being omitted from a number of agency subsamples. Given that both taxa probably occur commonly across sites, this should be of concern to the CRC. Of further concern are samples ACT170 and ACT117 where 32 gripopterygids and 45 hydropyschids respectively, were found in the QA/QC sample but none in the agency sample. In contrast, for ACT117 29 hydrobiids were found in the agency subsample but were absent from the QA/QC subsample (and, quite possibly, also absent from the rest of the residue which I cursorily examined).

Following on from above, it is worth noting that other preserved-sample agencies are now using the recommended approach of screening the entire sample to retrieve conspicuous rare taxa with low probability of occurrence in subsamples. Such an approach does not appear to have been adopted by the CRC.

Dissimilarity values for both community composition and structure were generally quite low, with the exception of samples ACT117 and ACT152 where presence/absence dissimilarity values were close to or above 0.4. We suspect the error inherent in these samples could be sufficiently large as to be unacceptable for inclusion in agency models.

In the case of ACT152, the large p/a dissimilarity value may be the result of uneven distribution of the sample in the box subsampler which can occur when large amounts of sand or filamentous algae are present. It is clear that the fault lies with the agency sample. Thus, when a second QA/QC subsample was taken and compared to the first QA/QC subsample,

much lower p/a and relative abundance dissimilarities were derived - see attached result sheet for ACT1522.

Because of other discrepancies which arose between agency and QA/QC subsamples, additional 'secondary' QA/QC subsamples were processed and data compared with those of the first QA/QC subsample. This was performed for samples ACT170 and ACT144 and was carried out mainly as a check and verification of our external QA/QC procedures. Both results show much closer agreement between the two QA/QC subsamples than between QA/QC and agency subsample - see report sheets ACT 1442 and 1702. These results clearly demonstrate subsampling and sorting problems within your agency.

The result for ACT117 is of greater concern because of the huge difference in total numbers from the two subsamples, agency and external QA/QC, both of which were similar-sized fractions of the original sample (assuming the CRC has reported the subsample fraction correctly). As mentioned above, this sample was also noteworthy for its absence of Hydrobiidae from the QA/QC subsample and, conversely, absence of Hydropsychidae from the agency subsample. The large differences in the two subsamples suggest errors occurring either in agency subsampling and sorting and/or labelling of the residue component.

If agency QA/QC protocols are in place at the CRC, there is evidence from this assessment that they are not being adhered to.

For any other information regarding the assessment procedure please contact me on (08) 8979 9731.

Yours sincerely

Lisa Thurtell

REF JK-02-13

29 May, 1997

Jane Suggit
Dept of Land, Planning and Environment
PO Box 1096
DARWIN NT 0801

cc.

Dear Jane

Please find attached 10 QA/QC reports assessing your agency's subsampling and sorting procedures for MRHI. The assessment procedure adopted for preserved samples involved subsampling another fraction from the residue equivalent in size to the agency sample, sorting and identifying the new fraction, then comparing the community composition and structure of the QA/QC fraction to that of the original agency fraction. The data for the two fractions were analysed by way of a computer macro that compared community composition and structure viz taxa number ratio and (Bray-Curtis) dissimilarity. Details of the calculations used in the macro and taxa codes etc are attached.

In terms of meeting the MRHI Bioassessment Manual's stated aim of capturing and collecting the broadest range of biota at a site, your agency has been generally effective. On most occasions, the agency subsample contained a similar or greater number of taxa than the QA/QC subsample. The exception was for site MR-DA-06 (sand), containing only 113 animals in the agency subsample, where a larger number of rare taxa was recovered in the QA/QC sample. Across all samples, taxa commonly occurring in the preserved sample were well-represented in both agency and QA/QC subsamples.

There is a suggestion (only) from the results, that the agency sorting operator may not be recognising elmid beetles, these animals appearing more often in QA/QC samples than in agency subsamples.

Dissimilarity values for relative abundance were all well below 0.3. A dissimilarity based on presence/absence above a value of 0.3 is a concern for those samples where QA/QC taxa number exceeds agency taxa number (as determined from the LS/WSE ratio). This was generally only a problem for site MR-DA-06 (sand). The discrepancy between the agency and QA/QC result for this sample may be explained by the small number of animals subsampled and identified by the agency. To ensure a good recovery of rare taxa, a minimum number of 200 animals should be taken from the sample (see below). In the case of site MR-VC-02 (sand), where a high p/a dissimilarity was also reported, the agency and QA/QC subsamples each contained rare taxa which were not common to both samples. This result appears to be an artefact of low taxa richness and the presence of a proportionately large number of rare taxa in the sample. (Thus, there is no fault with your processing procedures for this sample.)

The number of animals in the agency subsample for site MR-AD-03 was well in excess of that present in similar subsample fractions taken for both the QA/QC sample and a second agency subsample provided by you. This resulted in a greater range of taxa recovered in the

agency subsample compared with that recovered in the QA/QC subsample and the second agency sample (see report sheets for MR-AD-03 and MR-AD-032 *), though it is possible that pre- or post-screening of the first agency subsample also contributed to this result. Regardless, the result clearly suggests an uneven distribution of animals through the agency subsamples and warrants an investigation of your subsampling procedures.

In revised protocols for MRHI sorting procedures, sorting to a *fixed* sample number will be recommended. Certainly for live-sorted data N<100 has led to serious problems of taxa underrepresentation. Whilst this has not occurred in the case of your data, it is likely that there is a threshold N about which taxa recovery has most 'efficiently' been attained. Using your samples, data sets and sorting methods, it would be worthwhile pursuing the matter. You should contact Chris Humphrey for further information about this.

For any other information regarding the assessment procedure please contact me on (08) 8979 9731.

Yours sincerely

Lisa Thurtell

* MR-AD-03 report sheet = first agency subsample vs QA/QC subsample;
MR-AD-032 report sheet = second agency subsample vs QA/QC subsample.

Agency laboratory subsampling methods

Agency 1: SA (Australian Water Quality Centre)

Agency 2: WA (Conservation and Land Management)

Agency 3: ACT (CRC for Freshwater Ecology)

Agency 4: NT (Dept. of Land, Planning and Environment)

Agency 1 - SA (AWQC) laboratory subsampling method

The sample is prepared for subsampling by rinsing through a 250μm sieve to remove fine sediment. Subsample vials (precise number varies between 102-104) are then fitted into a plastic box. The vials are held into place by a grilled brace while the sample is added to the subsampler and water added to the height of the brace. If the sample contains a large amount of coarse debris which will not pass through the brace then more water is added to ensure all debris is covered by water.

The subsampler lid is fitted and the contents are mixed by lifting and tilting the subsampler from side to side. The lid is removed and brace taken out and any animals/algae/detritus left on the brace is washed back into the subsampler. After the suspended matter in the subsampler has settled, 13 randomly chosen containers (10% of the vials) are removed and all macroinvertebrates counted and identified.

After the subsample has been processed, the remaining sample in the subsampler is removed and examined in a sorting tray for any taxa not present in the subsample or for large specimens which may confirm uncertain identifications from the subsample.

When identifying these samples, the abundances from the 10% subsample are recognised as a fraction of the whole sample. The rare taxa which did not occur in the subsample are given a single occurrence value.

Changes to the subsampling method were recommended in the QA/QC report to SA (see Appendix 4). Recommendations included a minimum number of animals to be removed from the sample. Thus, if <200 animals were found in 10% of the sample, additional subsamples would be taken until 200 animals were recovered. SA have now adopted this protocol.

Agency 2 - WA (C&LM) laboratory subsampling method

The sample processing protocol for preserved samples involved the division of the sample into two parts by emptying the sample into a stack of sieves (2 mm, 500 μm, 250 μm). The 2 mm fraction was searched by eye and large invertebrates removed. Following this, the contents of the 2 mm fraction were transferred to a petri dish and examined under a dissecting microscope for 15 minutes. The 500 μm and 250 μm fractions were combined into a petri dish and also examined under a dissecting microscope for 15 minutes. Animals were removed, with the aim of collecting as many different taxa as possible.

If samples contained a large amount of detritus they were subsampled. The 2 mm fraction was rarely subsampled but the combined 500 μm and 250 μm fractions were often subsampled. This subsampling process involved dividing the sample into two homogenous parts using a dividing jug. At times it was necessary to divide the fractions further so that only a quarter or an eight of the material was sorted.

Workers estimated the abundance of each order of invertebrate collected while sorting. These estimates were on a log scale where scores of 1-3 were allocated to each family. A score of 1 represented 1-10 animals, 2 represented 11-100 animals and 3 represented >101 animals. Estimates were made of the total numbers in the entire sample, not just the fraction that was sorted. After the animals had been identified, estimates of family-level abundances were made. Conversion from order to family was completed by calculating the ratio that each family contributed to the total abundance of that order. The same three log categories were used for this procedure as with the previous procedure.

The protocol employed for laboratory sorting was based on a series of invertebrate family accretion curves derived at the commencement of the project. These were derived by sorting samples separately for successive 5 minute intervals. Invertebrates in each 'time portion' were then identified and the cumulative number of families present was plotted the against time. These curves showed that the majority of families were collected after 10 minutes. The sorting period was extended to 15 minutes to ensure all common families were collected.

Agency 3 - ACT (CRCFE) laboratory subsampling method

The sample is rinsed through a 250 μm sieve to remove any fine silt. The sample is placed into a box subsampler which is a large square box divided into 100 smaller squares of even size. The subsampler is rocked from side to side and from front to back until the sample is evenly distributed in the subsampler. A number of squares are then randomly chosen and the contents of each square pumped out (using a vacuum pump) and examined until 200 animals are found. All the pooled subsamples initially selected must be analysed even if the total number of animals exceeds 200.

Subsamples are to be sorted under a stereo microscope using low power. No search sorting is included, ie no rare or large individuals are preferentially selected.

Changes to subsampling protocols were recommended in the QA/QC report to the ACT (see Appendix 4) including the need for tighter internal QA/QC procedures. It was also recommended that large, rare taxa which may be missed during subsampling also be sought and removed though advice has since been received from the ACT that this aspect of the subsampling protocol has not changed.

Agency 4 - NT (DLPE) laboratory subsampling method

The subsampling device and protocol is similar to that adopted in SA. The sample is emptied into a 250 μm sieve and washed to remove fine silt. If a large amount of sand and coarse inorganic matter is present in the sample, it may be necessary to elutriate animals from the sample before placing them into the subsampler.

The subsampler consists of vials packed into a container and covered with a grilled wire spacer. The sample is transferred to the subsampler and water added to a specified level (approximately two times vial height). Downward pressure is applied to the wire spacer to keep vials in place. A plastic sheet is then placed between the lid of the container and the wire spacer before closing the subsampler securely.

The subsampler is turned upside down and rocked from side to side and from front and back, six times. Keeping the contents of the box moving, the subsampler is turned upright, the lid and plastic sheet removed and suspended material allowed to settle.

The wire spacer is then removed and placed in a tray with a small amount of water. Any macroinvertebrates left on the spacer are removed and placed in a vial labelled "extras". The vials in the box are divided into 14 columns and 6 rows. Twenty 20 vials are removed using a table of randomly paired numbers (column: row).

The contents of each selected vial are emptied into a sorting tray and sorted under a microscope at 10X magnification. Vials are to be completely sorted until at least 200 animals have been collected. No longer than 3 hours should be spent sorting a single sample.

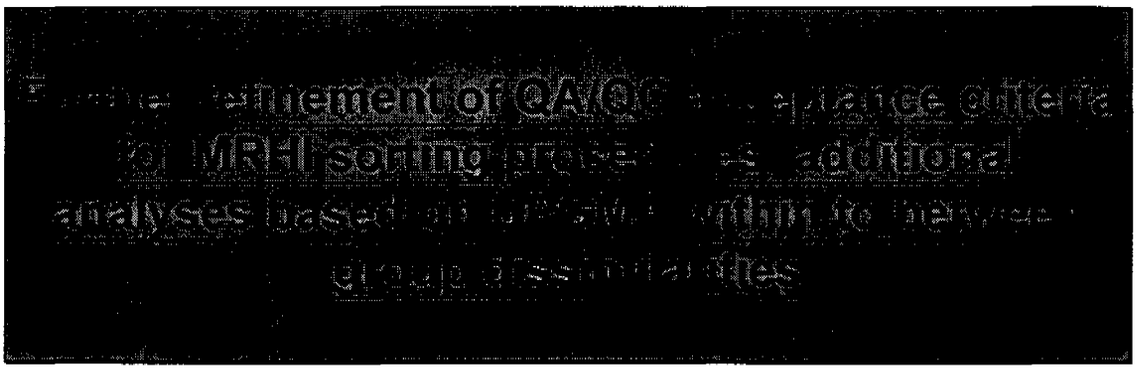
The remaining unsorted fraction is placed into a large sorting tray and examined under a Magi lamp or by eye to remove large rare taxa. These animals are placed in the "extras" vial.

When identifying the samples, the 200 animals will be identified first and their abundances recognised as a fraction of the whole sample. The "extras" are then identified and only families which have not previously occurred will be counted. These animals are given a single occurrence value.

Table A6. Occurrence of taxa in both live-sort (LS) and corresponding Whole Sample Estimate (WSE) components of agency samples as a percentage of total number of agency samples. Codes to taxa provided in Appendix 2.

TAXON	STATE									
	VIC		NSW		QLD		WA		TAS	
	WSE	LS	WSE	LS	WSE	LS	WSE	LS	WSE	LS
CHIRZZZP	56.2	6.2	63.1	0	61.9	38	33.3	0	33.3	0
EMPIZZZL	50	12.5	10.5	5.2	14.2	9.5	26.6	6.6	26.6	6.6
HPTIZZZL	75	18.7	63.1	10.5	66.66	47.6	26.6	6.6	26.6	6.6
ELMIZZZL	75	43.7	68.4	26.3	52.3	38	86.6	26.6	86.6	26.6
CERAZZZL	25	18.7	52.6	21	52	23.8	40	20	40	20
UOLIZZZX	87.5	43.7	57.8	36.8	85.7	57.1	93.3	73.3	93.3	73.3
UACAZZZX	81.2	62.5	78.9	52.6	80.9	71.4	66.6	46.6	66.6	46.6
CAENZZZN	62.5	50	36.8	21	76.1	76.1	13.3	0	13.3	0
SCIRZZZL	31.2	31.2	42.1	26.3	0	0	60	60	60	60
SIMUZZZL	87.5	87.5	36.8	26.3	33.3	28.5	73.3	53.3	73.3	53.3
GRIPZZZN	93.7	81.2	42.1	52.6	9.5	9.5	86.6	66.6	86.6	66.6
ELMIZZZA	18.7	12.5	63.1	47.3	23.8	23.8	40	53.3	40	53.3
CHIRZZZL	100	93.7	100	84.2	100	100	100	100	100	100
HPSYZZZL	56.2	56.2	26.3	26.3	38	38	26.6	33.3	26.6	33.3
LCERZZZL	62.5	75	78.9	63.1	61.9	71.4	93.3	93.3	93.3	93.3
LPHLZZZN	81.2	87.5	73.6	68.4	38	57.1	100	93.3	100	93.3
BAETZZZN	93.7	93.7	68.4	68.4	71.4	85.7	66.6	66.6	66.6	66.6
HBIOZZZL	56.2	75	47.3	26.3	14.2	9.5	60	86.6	60	86.6
ATYIZZZX	6.2	6.2	47.3	63.1	28.5	38	6.6	6.6	6.6	6.6
PRHEZZZL	18.7	31.2	21	10.5	0	0	40	66.6	40	66.6
CORIZZZN	25	37.5	31.5	47.6	42.8	57.1	0	33.3	0	33.3
SPHAZZZX/ CORBZZZX	26	0	15.8	5.2	40.9	27.2	0	0	40	0
CORDZZZL	5.9	23.5	5.2	10.5	9	27.2	8.7	43.5	0	6.6
AESHZZZL	5.9	29.4	0	10.5	0	4.5	21.7	52.2	6.6	6.6
COENZZZL	5.9	5.9	10.5	10.5	18.2	40.9	8.7	8.7	0	0
GOMPZZZL	17.6	17.6	15.8	21.0	4.5	27.2	8.7	8.7	0	0
ONISZZZN	11.8	11.8	0	5.2	0	0	0	0	26.6	26.6
N samples	17		19		22		23		15	

ATTACHMENT 2



by

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December 1997

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Introduction

External QA/QC checks of sample processing by S/T agencies have shown that live sorting of samples results in frequent and often large errors in the data (Humphrey & Thurtell, 1997). As part of the process of defining QA/QC acceptance criteria for these errors, Storey & Humphrey (1996) used a random selection of errors, representative of the types of errors to be found in all MRHI data sets collected using the live-sort protocol, to superimpose onto an existing MRHI data set. Modelling and subsequent testing using these 'error' data demonstrated the potential effects of sorting errors on the construction and performance of MRHI models. Issues such as a breakdown in the biological structure of the classification resulting in poor discriminant analysis, reference sites appearing as impacted due to the effects of errors, impacted test sites appearing as more severely impacted due to errors, impacted test sites appearing as unimpacted, and classification of samples to incorrect groups in the classification were all identified.

Results of these analyses were used to define live sort:whole sample estimate (WSE) dissimilarity values to be used as thresholds for QA/QC acceptance criteria (Storey & Humphrey, 1996). Subsequently, the authors have investigated further the issue of QA/QC acceptance thresholds and have developed an additional objective method upon which thresholds may be determined, using the unaltered existing MRHI UPGMA classifications. The rationale and results of this subsequent approach are reported herein.

Methods

An additional (or alternative) method for defining thresholds for error rates is to calculate 'within' and 'between' group mean dissimilarities for the UPGMA classification upon which the existing original MRHI model (*viz.* the ACT edge model) was based (Figure 1). In the first instance, the mean dissimilarity for all pairwise comparisons within each of the groups (n=6) identified in the 'original' ACT model were determined. Then, the mean dissimilarity for all pairwise comparisons between each of the groups was calculated. The difference between the means of these values was taken as the threshold. The number of pairwise dissimilarities between 'error' (i.e. samples with representative live sort errors superimposed) and 'original' samples above and below this threshold was determined, the former indicating error sufficient to be interpreted as an impact, and the latter as unimpacted.

Results

Determination of within (range from 0.32 to 0.38; mean of 0.358) and between-group (range from 0.39 to 0.53; mean of 0.461) mean dissimilarities for each of the six groups in the 'original' ACT edge model demonstrated no overlap in dissimilarity values (Table 1, Figure 2). As a conservative threshold, the mid-point between the upper 95% confidence interval of the mean within group and the lower 95% confidence interval of the mean between group dissimilarity was taken as the threshold (= 0.409; Figure 2). It is assumed that a pairwise dissimilarity between an 'error' and an 'original' sample below this threshold indicates the 'error' sample will stay in the same classification group as its original (i.e. is unimpacted), whilst a pairwise dissimilarity above this threshold indicates the sample will classify to another group (i.e. is impacted). Using this threshold and assumption, 68 of the 96 'error' samples were assessed as unimpacted, whilst 28 were impacted.

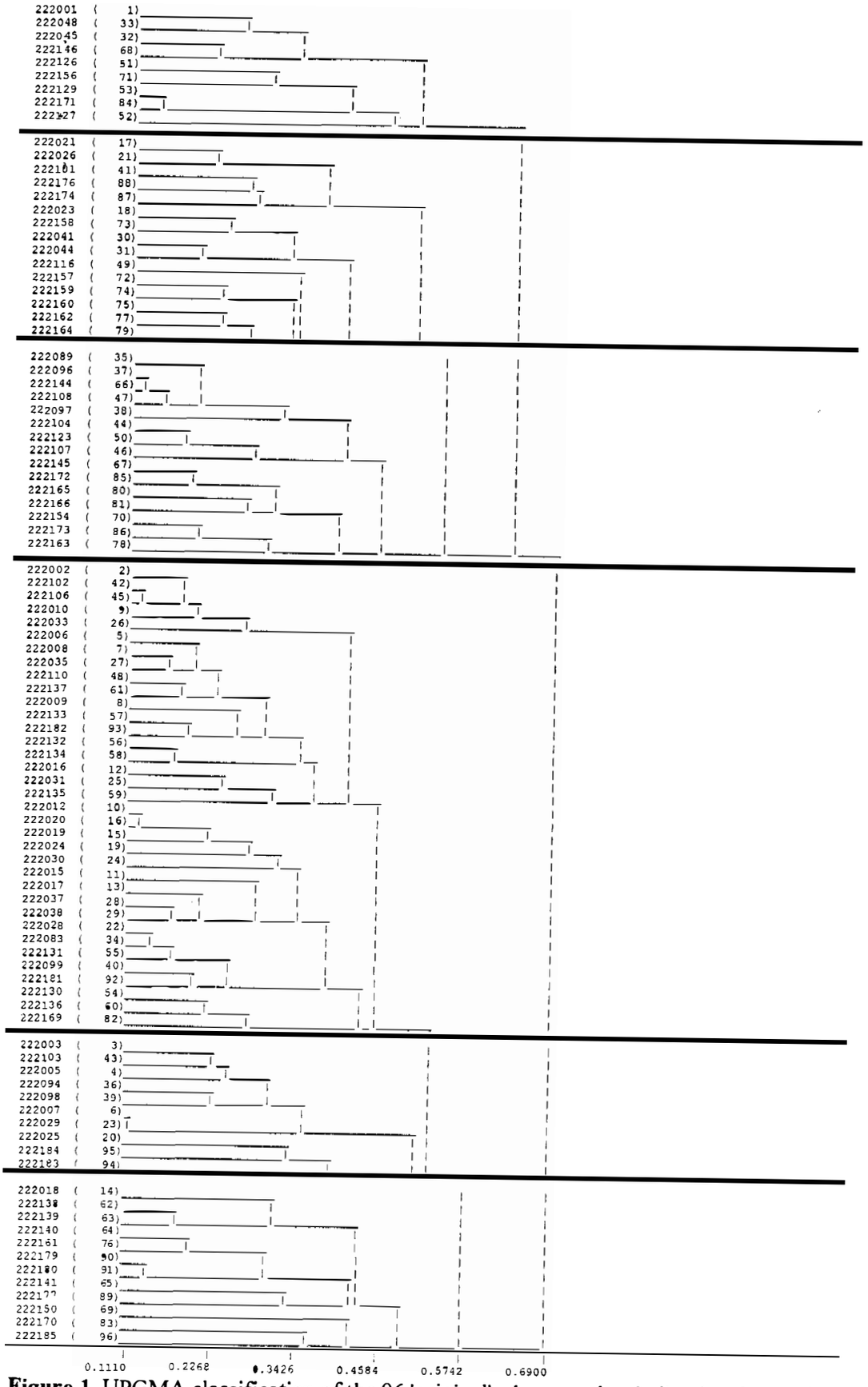

Figure 1. UPGMA classification of the 96 'original' edge samples, indicating 6 groupings used to construct the 'original' edge model.

A less conservative approach in setting the threshold would be to take the upper 95% confidence interval of the mean within group dissimilarity (0.377) on the logic that any site with a pairwise dissimilarity greater than this value would classify to another group. Using this threshold and assumption, 62 of the 96 'error' samples were assessed as unimpacted, whilst 34 were assessed as impacted.

Table 1. Mean within and between group Bray-Curtis dissimilarities for each pairwise combination of the 6 groups as used in the 'original' ACT edge model (see Figure 1).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	0.3866					
Group 2	0.4684	0.3638				
Group 3	0.4813	0.4107	0.3332			
Group 4	0.4857	0.4239	0.4558	0.3243		
Group 5	0.5110	0.4344	0.4818	0.3976	0.3564	
Group 6	0.5322	0.4514	0.4809	0.4106	0.4906	0.3810

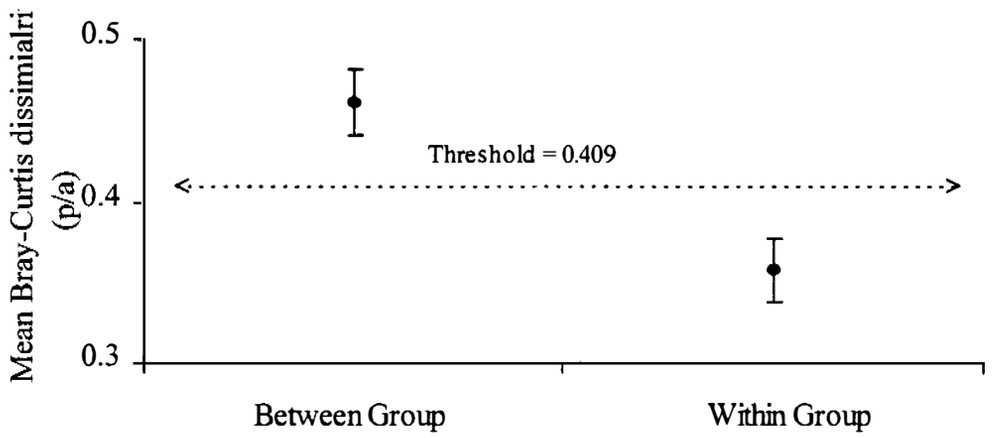

Figure 2. Mean (± 95% CI) within and between-group Bray-Curtis dissimilarities for the 6 groups used in the 'original' ACT edge model (see Figure 1), indicating a possible threshold for QA/QC acceptance criteria of approximately 0.41.

Discussion

Storey & Humphrey (1996) reported Bray-Curtis pairwise dissimilarity values for use as QA/QC acceptance criteria for live-sort errors, of approx. 0.35 (threshold between unimpacted (Band A) and impacted sites (Bands B, C & D)), and 0.38 (for reclassification of 'error' samples into the original UPGMA classification) (see Storey & Humphrey, 1996 for details). Additional thresholds identified by the latest analysis are of the same order as those reported by Storey & Humphrey (*op. cit.*). The conservative approach of splitting the difference between the upper and lower confidence intervals of the within and between group means (0.41) was slightly higher than the original values, whilst the less conservative approach of taking the upper 95% confidence interval of the within group mean dissimilarity (0.38) was comparable to the above estimates. The salient point is that all approaches are giving highly comparable results as to QA/QC acceptance criteria.

A rapid means of determining the mean within-group pairwise dissimilarity from a UPGMA classification is to take the dissimilarity value at which the group in question 'joins' to the rest of the dendrogram (i.e. Group 6 joins the other groups in Figure 1 at a dissimilarity of 0.574).

However, this may only be done when the dissimilarity scale on the dendrogram is scaled 0 to 1, and this only occurs when the Beta value used in the calculation for the classification is set to zero (Dan Faith, pers comm.). The standard approach for developing MRHI model classifications is to perform a UPGMA classification using the default settings in PATN, which is a Beta of –0.1. Occasionally a more negative Beta may be used (i.e. –0.2) to help better define groups. As a result, the dissimilarity scale on the dendrogram is seldom 0 to 1 (see Figure 1). Unfortunately, re-calculating the UPGMA classification with a new Beta value is not an option as in most cases this will change the structure of the classification dendrogram (e.g. it may change the structure and number of groups). Therefore, within to between group mean dissimilarities must be calculated manually. However, this approach appears to give a quick and accurate estimate of QA/QC acceptance criteria and could be readily applied to other agency data sets and classifications.

References

Humphrey C & Thurtell L 1997. External QA/QC of MRHI agency subsampling and sorting procedures. *In* Development and implementation of QA/QC protocols for sample processing components of the MRHI agency bioassessment program by C Humphrey, A Storey & L Thurtell, Final Report to Land and Water Resources Research and Development Corporation (Reference No. ARR2), December 1997.

Storey AW & Humphrey CL 1996. Refinement of QA/QC acceptance criteria for MRHI sorting procedures and a preliminary assessment of the effect of live-sort errors on the robustness of MRHI models: A conservative analysis through data simulation. Attachment No. 3 to December 1996 Milestone Report to LWRRDC Project Reference No. ARR2.

ATTACHMENT 3

Proposed revised protocol for MRHI sample processing procedures and rationale, as distributed to agency staff and technical experts in January 1997

Revision of Sample-Processing Protocols for MRHI

Chris Humphrey & Andrew Storey

A. Live-sort protocol

(i) Objectives of live-sorting in data recovery

Whilst we feel that the best approach is to recover relative or rank abundance data, as this automatically recovers common taxa (*viz.* as currently provided by laboratory subsampling), we acknowledge the concerns of others about too much rigour and deviation from the RBA approach/ethic. We (and others) also foresee problems and limitations in use of relative/rank abundance for many situations in Australia (mainly as a result of temporal variability). As a consequence, we now view the objective for MRHI live-sorting as providing as complete as possible a listing of taxa (families) present within a habitat at a site - ensuring in the process that common taxa are recovered. Thus presence/absence data are sought of common taxa - always recovered in laboratory subsampling - as well as additional large, rare taxa that would normally be underestimated in, or missed from, unmodified laboratory subsampling procedures.

However, whilst QA/QC assessment procedures can be devised for this objective (see below), we anticipate difficulties in developing a suitable protocol to recover such data without setting in place procedures that coincidentally lead to recovery of rank or relative abundance of at least a portion of the sample. For example, there seems on the surface, intuitive appeal to the original Chessman live-sort prototype of selecting a maximum number of individuals of a taxon - up to 10 - for recovery of presence/absence data. However, we foresee problems in implementing such a procedure. It seems to us that only very experienced personnel would have the ability to distinguish in the field 20-25 families of invertebrates let alone be aware of the cumulative tally of individuals of a particular taxon being gathered. Simply, agencies will never have at their disposal staff of the calibre required to implement such a protocol. (Consideration must be given here to realistic estimates of staff turnover in agencies.) Such a (Chessman) sorting procedure, moreover, does not overcome the problem of missed common taxa as observed both for experienced and relatively novice agency staff. The protocol must be relatively 'fail-safe' so that experienced and reasonably inexperienced sorters can achieve a 'good' and comparable result. Indeed, this is essential if the protocol is ever to be adopted safely, in terms of data quality, by community groups such as Waterwatch.

(ii) Key elements of a revised live-sort protocol

Principle

An important basic principle of the recently-circulated draft proposal should remain, namely, careful successive sorting of sufficient small randomly-selected aliquots (i.e. subsampling and sorting). Only in this manner will small and/or cryptic common taxa be reliably and consistently recovered. To this end, we insist on the use of vision visors/benchtop anglepoise magnifying glass to assist in sorting of these taxa. For presence/absence data, however, a sorting procedure that sets a limit upon the number of individuals of the most common taxa might be implemented. Examination of any list of taxa present at a site together with relative abundance information shows that numerical abundance is dominated by usually just several taxa - a truism of lognormal distributions of benthic invertebrates. Whilst we suggested above that placing limits on the maximum number of individuals of *each* taxon for sorting would not be feasible, we do believe that sorters, both experienced and inexperienced, could quickly recognise the few very dominant

taxa and hence be in a position to limit the numbers of animals of these taxa sorted (to say no less than 10 individuals per taxon). In this manner, the sorting is not 'bogged down' with recovery of the common taxa, with the concomitant loss/under-representation of less abundant taxa. A minimum sample size for chironomids would need to be decided upon separately, recognising that adequate numbers of animals for recovery of subfamilies is required.

Sample size

From results of our QA/QC assessments, time-based live sorting is generally ***not*** reflecting the abundances of organisms at a site, contrary to the view of some respondees. For the QA/QC data gathered to date, we observe a poor relationship between number of animals sorted and total number of animals in the sample (live-sort + residue components) - see Figure 1. Rather, sample size it appears, is dictated mainly by both a natural limit to the numbers of animals that can be sorted in 30 mins and also the efficiency of individual sorters (possibly also reflecting the ease with which animals may be recovered from a sample due to differing levels of detritus/algae/mud etc).

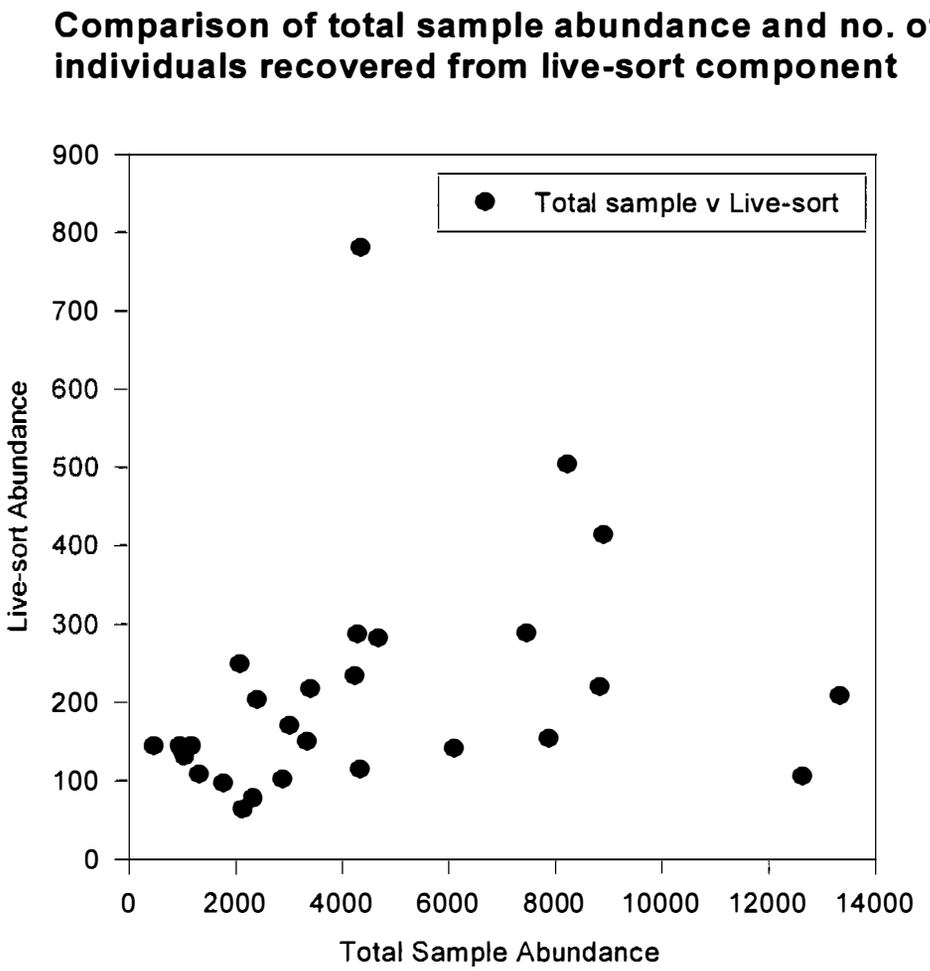

Figure 1: Relationship between the number of animals live-sorted and number of animals present in the entire agency sample. (Data for QLD, NSW, VIC & TAS combined.)

A couple of respondees highlighted the value in information derived from a low sample size in reflecting some level of disturbance at a site. Indeed for this reason, there may be insufficient animals in a sample to achieve a pre-set minimum sample size; a revised protocol and QA/QC program can accommodate this. Nevertheless, we are convinced of the need to move from time-based sorting to, wherever circumstances permit, sorting to a pre-set number. In the circulated protocol, we listed a number of recent papers from the Journal of NABS with pros and cons for proportional vs fixed-count (sub)sampling. We found the argument for fixed sample size persuasive: Barbour and Gerritsen (1996) compared the sensitivity of the two approaches (fixed-count vs proportional subsampling) in detecting a known disturbance gradient of macroinvertebrate communities in 9 Florida lakes. The fixed-count approach performed the best in discriminating amongst the lakes and hence was the more sensitive of the approaches.

From a plot of no. of animals live-sorted vs no. of taxa recovered from QA/QC live-sort data, taxa no. appears to plateau off at about 200 animals for eastern Australian states (Fig. 2) and 100 animals for southwestern WA (Fig. 3). It is recommended that this sample size apply initially to future live-sorting. For live-sort samples with N less than this figure, agencies would be required to stipulate the cause, e.g. 'muddy' sample or disturbed site. Further, an upper time limit could be stipulated - e.g. no more than 1 hr if less than 200 animals can be found, and unless the whole sample has been picked clean. For QA/QC assessment of these low N samples, unless some explanation was provided from the sorter, a penalty could naturally be imposed wherever taxa no. was below expectations *and* the total sample size as estimated from the residue is say >1000 animals. A penalty might also be imposed for large N - if only from the perspective that sorting excess animals represents time wasted in the field and in later laboratory identification. A range in sample size would need to be specified (e.g. 100 or 200 +/- 20% as recommended by Barbour and Gerritsen (1996)).

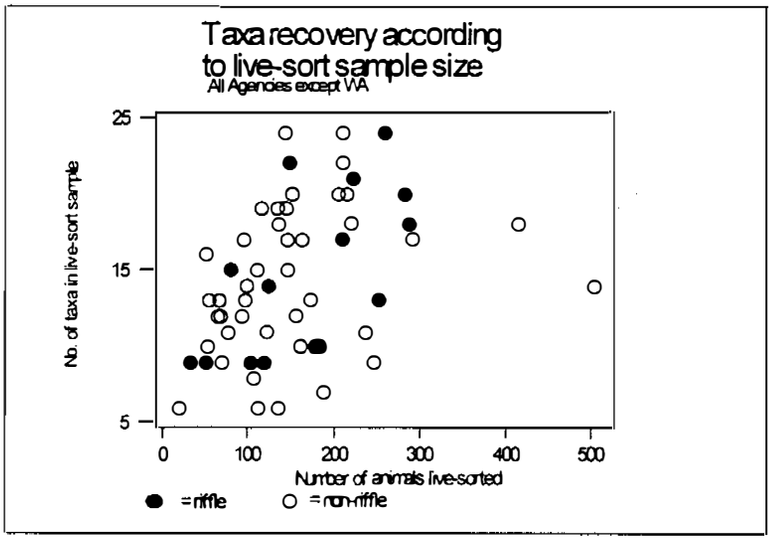

Figure 2: Relationship between number of animals live-sorted and number of taxa (families) recovered in agency live-sort samples. (All agencies except southwest WA.)

We are aware that refinement of the sample size issue will be required and that this is best achieved empirically through data gathered from further sampling rounds of the program. Thus,

the sample sizes indicated above are based on results of live-sorting carried out to date where numbers of individuals of a taxon collected were not necessarily restricted. With sole emphasis now on recovery of presence/absence data, with some attendant restriction placed on the numbers of individuals of the very common taxa sorted, it is very likely that a plateau in taxa number will be achieved at a lower N. Note, however, that if adequate recovery of taxa is being achieved at a lower sample size, a penalty would not be incurred in QA/QC assessments of such samples because such assessment is based on *expected* taxa number for the level of sorting effort prescribed.

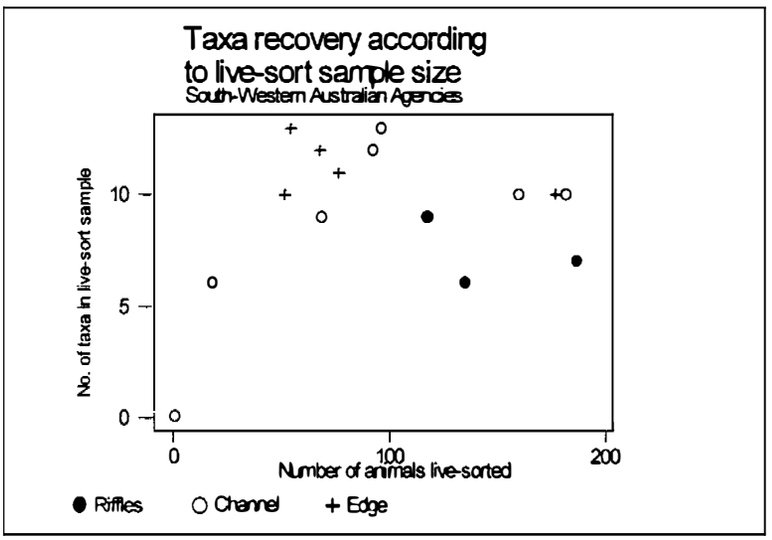

Figure 3: Relationship between number of animals live-sorted and number of taxa (families) recovered in agency live-sort samples. (Southwest WA agencies.)

We recommend, arbitrarily for now, that half the effort in live-sorting be devoted to 'subsampling' (recovering small cryptics/ 'commons') and half to conventional live-sorting (recovering large 'rares'). This implies that about 100 animals (50 for southwest WA) are recovered in each type of sorting procedure if the sample so permits.

Mesh size

Most respondees were either indifferent or thought we should bite the bullet and move up a notch with mesh dimensions (from 250 to 500μm). Southwest WA might be an exception - as it is with sample size - but with good biogeographical rationale so this shouldn't be a great concern. Richard Norris' comments on this issue are particularly pertinent: he has conducted the necessary R&D, results of which appear to allay concerns about compatibility of data sets. Thus, there is good evidence that small mesh size is contributing to the live-sort errors (and also to problems in identification of animals) and if this is the case then this should be redressed. Note, however, that before a final decision is made on this issue, further R&D will be conducted to corroborate results from the ACT.

Summary

What we are advocating in the above are the following elements:

- Re-statement of the original objective for live-sorting (sensu Davies 1994), but with recovery of presence/absence data only;
- Retain aspects of the original protocol in which conspicuous taxa, including large rares, are recovered;
- A portion of the sample is subsampled and carefully sorted using visual aids for small common and/or cryptic taxa;
- Rules apply to sample size, including minimum no. of chironomids;
- Training element after which staff emerge with renewed appreciation of the need to recover small commons and 'rare' taxa generally (because analysis associated with modelling decides on the issue of rarity, and not their respective abundance in an individual sample, it is important that as many taxa as possible are recovered), knowledge of the appearance (identity) and behaviour of small commons and cryptics, and a mental checklist of these taxa for scoring as sorting progresses.

Aspects of the revised protocol involving training will be raised for discussion at the Canberra meeting in early February.

There are two QA/QC assessment criteria that would be applied to data gathered under the revised protocol: (i) 'common' taxa present in a 'whole sample estimate' (WSE, calculated from residue and corresponding live-sort components) must also be present in the live-sorted sample (dissimilarity measure based on p/a data - criteria value yet to be determined); and (ii) number of taxa present in the live-sort component must be greater than that of the corresponding WSE. Note that for success in meeting criterion (ii), agencies will have to 'lift their game' considerably. This is because, up to now, only a little over 50% of samples received for external QA/QC assessment have managed to score a live-sort/WSE taxa no. fraction better than 1. This is despite the objective as stated in Davies (1994) of the need to collect a 'broad range of taxa'. It is only in applying more rigour (= time spent in the field in sorting) that the re-stated objective will be met.

B. Laboratory subsampling and sorting protocol

We noted in a number of responses the uncertainty about the adequacy of laboratory subsampling. Apart from some (good) suggestions about pre-sorting the coarse fraction for large rares before subsampling of the finer fraction (which SA and WA currently do), we see no need for further improvement here - and certainly no justification to reduce accuracy! Our own R&D on the adequacy of different subsampling devices (a final report on this project will be submitted to LWRRDC in mid 1997) shows that the data derived from subsampling by devices currently utilised are, consistently, very precise and have an associated low error. This is supported by results of external QA/QC of the one agency so far assessed using a laboratory subsampling protocol (SA). Moreover, this approach consistently recovers ***all*** common taxa - a major shortfall in the live-sort technique.

It can be argued that for monitoring of water quality and detection of change *per se*, the recovery of rare taxa is unimportant. However, it is restipulated from above that rarity in terms of MRHI modelling is determined from level of occurrence across all samples in a dataset, and not by level of abundance within a sample. Also, because a spinoff and selling point of the MRHI is information about the biodiversity and conservation status of the biota of Australia's fresh waters, an important objective for both live-sorting *and* laboratory subsampling and sorting would appear to be recovery of 'a broad range of taxa'. Such a common objective might also mean, ultimately, that presence/absence data derived from either sorting procedure (lab and field) can be combined if this was desired. For such compatibility of approaches, it would be necessary for all agencies

preserving samples for later subsampling and sorting in the laboratory to pre-sort samples for conspicuous rare taxa. We recommend that all agencies subsampling and sorting in the laboratory adopt such an approach.

A revised protocol for the live sorting of benthic macroinvertebrate samples collected under the MRHI

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Background

The following protocol for live sorting samples of benthic macroinvertebrates collected for the MRHI is a re-revision of that circulated in November 1996. Based on comments received, the protocol has been re-revised to incorporate and accommodate the suggestions and criticisms.

Objective

The overall objective of the live sort protocol is to provide presence/absence data for as complete as possible a listing of taxa (families) present within a habitat at a site - ensuring in the process that all common taxa are recovered. Operators will attempt to live sort approximately 200 animals (100 for south-western WA) (± 20%) from each sample as part of this objective.

Revised live sort protocol

As before, the revised protocol is presented with a number of different options. These are designed to cover different situations that may arise depending on the content/structure of the sample to the sorted (i.e. high/low detritus content, presence of filamentous algae etc). Other options are presented for open discussion as to which is the most readily and economically applicable.

Sample collection

A sample is collected from a pre-defined habitat and reach as described in the River Bioassessment Manual (Davies, 1994).

Sample washing

Whilst still in the D-frame pond net, the sample is 'washed' in the stream to remove fine detritus and/or sediment, with the objective of making the sample "cleaner" and so easier to process. There are various methods by which this may be achieved. The selected method will probably rely on operator preference.

Method A: The operator holds the pond net in the horizontal (i.e. net handle parallel to the waters surface), and agitates the contents of the net with one hand whilst the majority of the head of the net is submerged in the water.

Method B: If the net is very full of material, the D-frame of the net head may be held in both hands and agitated in the water.

Method C: The net is grasped in one hand between the bulk of the sample and the D-frame to seal the whole sample within the lower portion of the net and then it is vigorously shaken in the water or washed by baling water over it.

Method D: The sample is transferred to a large, robust, deep-sided 250 μm sieve and the sample agitated in the water.

In situations where the mouth of the net is left open, care must be taken not to submerge the whole net as taxa either may be lost or introduced. Although vigorous agitation will remove more of the fine material, excessive agitation will damage specimens in the sample - so a compromise is required. Operators will be able to determine the required degree of agitation through experience (i.e. at what level of agitation specimens appear damaged when subsequently being live sorted).

Sample pre-screening

The sample then must be pre-screened to separate coarse material (e.g. sticks, leaves, root mats, algae, plant fragments etc), containing large 'rare' taxa, from the finer material and smaller taxa. Pre-screening should be used in all situations as it will also assist in separating large rare taxa in samples dominated by fine material. In these situations a slightly finer mesh than recommended below (i.e. 5 mm) could be utilised.

To pre-screen a sample the operator transfers the sample from the D-frame net into a coarse-mesh sieve or a wire basket (of the type used to fry fish and chips) with approx. 1 cm mesh. This is performed whilst the sieve/basket is held over a bin/bucket to avoid spillage and loss. The sieve/basket needs to be robust, deep and preferably have two sturdy handles. The sieve/basket then is submerged into the bin/bucket containing water (pre-filtered through at least a 250μm mesh net to avoid introducing additional taxa that might contaminate the sample) and agitated vigorously until most finer material (< 1 cm > 250 μm) has been washed from the sieve/basket. For large samples this may be conducted in stages. The coarse fraction, in the sieve/basket, and the fine fraction, in the bin/bucket, are retained separately for subsequent processing.

Coarse fraction processing

Once the sample has been pre-screened, the coarse material should be sorted. This stage is designed to maintain compatibility with existing live sort data, which demonstrated a bias by operators towards the large and rare component of the sample, as well as fulfilling the objective of collecting a 'broad range of taxa'. Agencies must remember that rarity in terms of MRHI modelling is determined from level of occurrence across all samples in a dataset, and not by level of abundance within a sample. Therefore, a taxon with one individual in a sample may be as

equally important in terms of developing a model for MRHI as the most numerically dominant taxa in the sample, if the former occurs in all samples in the data set.

The objective of this stage is to collect approximately 100 specimens from the coarse fraction - maximising the number of different taxa. Sorting should continue until this target is achieved or until no new taxa are being recovered (i.e. specimens that are morphologically distinct and readily identifiable as new taxa are no longer recovered after an additional 5 minutes of sorting), or until the whole coarse fraction is sorted, whichever occurs the soonest. If the whole coarse fraction has been sorted, then all large and rare taxa will automatically have been collected. If the target is achieved and unsorted material remains, prior to discarding the unsorted material it is recommended that it is quickly scanned for additional obvious taxa. All collected animals should be preserved in 70% alcohol (with 2% glycerine).

To sort the coarse fraction, the material should be removed from the pre-screening sieve/basket and placed in a sorting tray, either *in toto* or in portions and large and rare taxa removed. It is important that the volume of material placed in the tray must be small enough so that when dispersed across the tray, the bottom of the tray is still readily visible (we are considering supplying several photographs so that operators know what is acceptable and unacceptable). This is to minimise the possibility of missing taxa hidden within the detritus. During sorting, it is essential that large leaves, twigs, rolled-up bark etc should be "combed" with wet fingers and the fragments inspected before being discarded. Specimens adhering to wet fingers then may be picked-off with forceps or washed-off into the tray or storage vial.

Fine fraction processing

The objective of sorting the fine fraction is to collect approximately 100 specimens from the fraction - maximising the number of different taxa obtained. Sorting should continue until the target of 100 specimens is achieved or until no new taxa are being recovered (i.e. specimens that are morphologically distinct and readily identifiable as new taxa are no longer recovered after an additional 5 minutes of sorting), or until the fine fraction has been sorted in its entirety or sorting has taken a maximum of 60 minutes, whichever occurs the soonest. If the whole of the fine fraction has been sorted within this time, then representatives of all taxa automatically should have been collected. Usually the fine fraction will be dominated by several taxa. It is recommended that a maximum of 10 individuals of any morphologically distinct dominant taxa is removed from the sample. This avoids an operator picking only the common, dominant taxa during live sorting, and missing additional, less abundant and obvious taxa. For samples containing Chironomidae, it is recommended that at least 50 individuals are selected (NB this is a conservative number which will not be achievable in some situations and needs to be refined following further R&D). This is to maximise the probability of recovering as many subfamilies as possible.

Sorting should be performed in a standard plastic white tray. As for sorting of the coarse fraction, the volume of material placed in the tray must be small enough so

that when dispersed across the tray, the bottom of the tray is still readily visible (we are considering supplying several photographs so that operators know what is acceptable and unacceptable). The purpose of this is to avoid missing taxa hiding within the detritus. Additional pre-filtered, clean water may be added to the tray to assist dispersal of the material.

As for the coarse fraction, retained animals should be preserved in 70% alcohol (with 2% glycerine), and forceps, pipettes, teaspoons etc should be used, as deemed appropriate for catching large or small specimens. A benchtop anglepoise magnifying glass (x ~ 3 magnification) or jewellers vision visors (x 2.5 magnification) **must** be used throughout the fine fraction sorting exercise so that operators are able to recover small and cryptic taxa. These taxa have been regularly missed and under-represented in previous data sets - usually because of an operators inability to see them with the naked eye. It is suggested that the sorting of the contents of a tray be conducted in stages, firstly removing larger, more mobile animals that tend to disturb the sample and distract the operator, then changing search image and using the visual aids to target the remaining smaller and less mobile animals. Operators may use a handheld talleycounter to record the total number of individuals removed from the sample.

In some situations (i.e. riffle or pool rock habitats) the fine fraction may be relatively small and may be sorted in its entirety. However, in the majority of situations it is likely that the fine fraction will be too large to be sorted completely and so an unbiased, 'representative' aliquot of the fine fraction needs to be derived to avoid operators depositing the whole fine fraction into the sorting tray. An aliquot may be derived by one of two methods, both of which have advantages and disadvantages.

Method A: The fine fraction is retained in the pre-screening bin/bucket and, if required, pre-filtered water is added so that the sample may be easily circulated and mixed in the bin/bucket. In the case of an extremely large sample, either a larger bucket should be used (e.g. 20 L), or the sample split between two buckets. If the latter is carried-out, then the following steps should be alternated between each bucket.

The sample is vigorously stirred and mixed in the bucket and an aliquot of the sample removed immediately from the bucket before settling occurs. This may be achieved either by pouring a portion of the sample directly from the bucket, or by removing an aliquot, using an appropriate low-cost container, such as a 500 mL plastic icecream container. The aim of this exercise is to select a portion that represents a subsample of the whole sample, therefore, the volume of the aliquot is not critical. If 100 animals are not obtained from the first aliquot, then a second is removed and sorted and so on. The advantage of this approach is that it is fast, but the disadvantage is that it introduces biases such as a.) heavy taxa (gastropods, decapods, cased caddis etc) remain at the bottom of the bucket because it is difficult to suspend and evenly distribute such organisms by vigorous stirring, and b.) the fauna in the aliquot is dominated by buoyant/efficient-swimming taxa taken from the

surface of the bucket (i.e. corixids). If this method is used, it is suggested that once sorting is complete, and if there is part of the fine fraction remaining in the bin/bucket, the remaining material is decanted off to leave the heavy material at the bottom of the bucket and this material is sorted, looking specifically for additional heavy/cased taxa.

Method B: The preferred and methodologically more rigorous approach, is to pour the contents of the sieve/bucket into a robust and simple subsampler, such as a jug splitter and derive aliquots by splitting the sample into 1/4s or 1/8s. An aliquot then is randomly selected. If an additional aliquot is required to attain the target of 100 specimens - then a second 1/4 or 1/8 is randomly selected. This approach will avoid the possible biases detailed above that may occur when taking aliquots directly from a bucket, however, the splitting exercise is more time consuming and technically more difficult to perform in the field.

Numerically depauperate samples

In the situation where the whole sample (coarse and fine fractions) is sorted in its entirety and the predetermined number of 200 animals (100 for sw WA) has not been attained, then an additional sample must not be taken from the site. Assuming the site has been properly sampled, the low abundance of animals will be a reflection of the conditions at that site (i.e. it is polluted, has a sandy substrate or is a very small stream). This fact will be recorded on the data sheets.

Also, situations will arise where samples contain few animals but have large quantities of organic and inorganic material. Processing of these samples will be slow and tedious and the target of 200 animals will probably not be attained. In these situations the operator needs to assess the situation and it is recommended that sorting is terminated once no new taxa (i.e. specimens that are morphologically distinct and readily identifiable as new taxa), have been recovered after a further 5 minutes of sorting effort. Comments to this effect should be recorded on the data sheet.

Filamentous algae

Some samples contain large quantities of filamentous algae which form dense mats which often containing a diversity of taxa (e.g. chironomids, trichoptera etc). These mats are very difficult to sort effectively. A recommended approach is to remove the filamentous algae from the pre-screening sieve/basket and place it into a tray. Disperse the material as best as possible and then physically cut the mat into 1/4s or 1/8s, and then select one fraction of the mat and sort in detail. The number of specimens of macroinvertebrate taken from the portion of the algal mat will be combined with those from the fine fraction to meet the set target.

Sorting trays

Standard white plastic sorting trays are to be used for sorting coarse and fine fractions. However, it is recommended that the trays are grided to provide operators

with a reference point. This is easily achieved by scoring the tray with a sharp implement and then drawing over the scores with a black permanent marker; the scoring helps retain the marked grid lines. A tray grided into 6, 8, 9 or 10 equal quadrats is recommended (at each operators preference).