Marine invertebrate sample processing procedures

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Introduction

Surveys of the marine environment usually generate numerous samples of marine organisms and supporting data. The most appropriate final destination for some or all of that material may be a natural history museum. The main reasons for depositing specimens and data in a museum are to obtain authoritative specimen identifications; and to make specimens available to other researchers by depositing them in a permanent collection. The latter reason is the more important both for immediate quality control purposes (repeat identifications of specimens by other authorities is possible if desired) and to support future taxonomic research projects with increasingly comprehensive study materials. (Museums, and for botanical specimens, herbaria, are required by legislation to maintain collections in perpetuity. Collections in other institutions typically are discarded or deteriorate as priorities change over time.)

New collections, especially large collections, impose a significant burden on resources at museums. Samples should be processed and documented so that they arrive in good condition and can be incorporated efficiently into museum collections and databases. Samples in poor condition or which are poorly documented will usually be rejected by museums. The purpose of this document is to provide guidelines for processing samples of marine specimens to standards that will maximise the taxonomic reliability of the data collected, and to ensure that those samples are acceptable to natural history museums.

Organisations and individuals who undertake marine environmental surveys each have different ways of processing samples (saving time and money is often a significant issue). This document makes recommendations for adopting particular set of procedures. Many organisations and individuals will need to change their practices, so it needs to be emphasised that the following recommendations are not based on some arbitrary preference or arcane and ancient museum process. Instead, the procedures and standards outlined here are chosen to ensure that specimens and data can immediately form a part of the emerging national and international network of museums which maintains, studies and provides access to biodiversity data.

The immediate purpose of this document is to provide input to a draft manual *Australia and New Zealand Monitoring Manual for Marine Pests*. However, the content is applicable to marine surveying and collecting generally.

Scope

The following topics are covered:

- Consultation (early contact with museums and taxonomists).
- Sample processing (separating organisms from substrate in the field and laboratory).
- Fixing and preserving samples (killing and narcotising, fixing, preserving).
- Documenting samples (station numbering, labelling, data formats).
- Sorting (dividing samples into major and minor taxonomic groups 'taxa' in the laboratory).
- Identification and taxonomic verification (finding appropriate taxonomic tools, use of voucher collections and making contact with relevant taxonomists, costs and budgeting).
- Transferring samples (packing and mailing, safety and legal issues, contacting museums and collection managers, costs and budgeting).
- Other resources (relevant scientific and natural history societies).

The following topics are not covered:

- Sample collecting methods (this document assumes that the sample is already in the boat/bucket).
- Treatment and handling of micro- and macroalgae and fish. Meiofauna and plankton are also not covered, although a couple of relevant sources are referred to by way of introduction.
- Software is not mentioned or reviewed (only relevant data standards are covered).
- No thorough listing of taxonomic works and identification tools has been attempted, since that is a vast topic. However a few comprehensive works are listed in Table 2 in the section *Identification and taxonomic verification*; full citations are in the References section (the emphasis is on recent and comprehensive titles). Table 2 is more complete and relevant to Australia than to New Zealand, but should not be considered comprehensive.

Consultation

Preamble

Museums and taxonomists have their own projects and priorities; resources are inevitably limited and committed months or even years in advance. An initial contact is necessary if a study hopes to involve taxonomists in museums or other institutions, or if it is hoped to deposit specimens in a museum. Appropriate contacts are provided below (see *Identification and taxonomic verification* and *Transferring samples*) and making these initial contacts during the planning stages will avoid many problems.

Further development of the procedures raised in this document, and their adoption by museums, will have wider implications for resourcing and priorities. Any significant new sampling initiative, such as national port surveys for introduced species, will have equally wide ramifications. This document does not pretend to be an undertaking, implicit or otherwise, that museums will automatically rearrange their priorities to address new projects, whether large or small. However museums do recognise their obligations to be the best possible provider of biodiversity skills and data. The appropriate forum for discussions of this nature is the Council of Heads of

Australian Faunal Collections (CHAFC), a body formed from representatives of every Australian museum, and CSIRO Divisions, that hold significant faunal collections. CHAFC meets at least annually and one of the contacts listed below in Table 3 will be able to help to initiate those discussions with CHAFC representatives.

Strategic planning of collections

Museums cannot store all possible specimens and must make strategic choices when deciding what specimens to keep. Each museum will have their own collection management strategy, and initial discussions with curators and collection managers are the best means of deciding if a given study will generate material that will be desirable to other scientists in perpetuity. Factors that will be important include: Are other samples from the region in question already in a museum collection? Is the region of particular environmental significance? Do the taxa being collected integrate with research interests of museum staff? How would multiple duplicate samples (*eg* from repeated years of sampling) be dealt with? These and other issues will be discussed between museum staff and the initiator of a given survey.

Time and other resources as well as who will bear the real financial costs need to be considered and will be an important part of initial consultation.

Sample processing

Preamble

Damaged or incomplete specimens are very often unidentifiable, and large numbers of unidentifiable specimens will typically reduce the value of a study. Therefore the principle goal of sample processing in the field and laboratory should be to protect specimens from damage. This is done by separating specimens from damaging substrates, and where possible from each other.

Labelling and sample treatment

Efficient labelling of samples relies on inserting a label with a single unique station number (see 'Documenting samples' below). Museum standard label paper has a high-rag content and is resistant to damage (museums themselves may be able to help find a supplier – see contacts listed in Table 3). Other papers will disintegrate, although some protection can be obtained by sealing labels within small snap-top plastic bags before placing them in a sample. Conduct trials if in doubt. Plastic labels, labels written on ordinary bond paper, and labels written on outside of jars or on lids are unacceptable.

Station numbers can be preprinted (offset printing with indelible ink; laser printed labels are unsuitable since toner will abrade unless special precautions are taken). Alternatively, labels can be written in pencil as required, and this is often easier than searching through a batch of preprinted labels. Repeating station data on both sides of the label is a helpful backup. Samples in portions should be labelled "part x of y" and stored so that for convenience all can be located and all material combined into a single set of samples during lab sorting. Position the label so it can be read without opening plastic bags and jars, which should be transparent.

Size fractions and sieves

Sample processing requires repeated washing of material over sieves, either to remove mud and other fine material, or to replace seawater with a fixative or preservative

solution. A choice of sieve mesh size must therefore be made and applied consistently throughout all sample processing. Quantitative benthic infaunal studies typically use sieves of 0.5 mm (= 500 μ m) mesh aperture and that is the standard usually adopted when results will be compared with other studies nationally and internationally. It may be appropriate to use coarser sieves with samples collected with coarser gear, especially if only local objectives must be met. Meiofauna and microalgae and plankton samples will require treatment with finer mesh sieves in the range 50-200 μ m (refer to Hulings & Gray (1971), Deibel (2001) and similar handbooks).

Elutriation

Elutriation is a method of separating light material (in this case, mostly organisms) from heavy material (mostly sediment) by momentarily suspending light material in an agitated volume of water and pouring that suspension through a sieve before the organisms resettle on the sediment. Elutriation is like gold panning in reverse (here it is the lighter fraction that typically contains much of the 'gold').

Elutriation is done by agitating a volume of unsorted sample in a suitable container that is about half filled with sea water (if the container is full the sample will spill during agitation). A rectangular box with proportions similar to a fish-box works best since it provides a corner for pouring and a long axis along which the sample 'sloshes' easily. However, even a bucket will suffice. The water supply should be seawater (a low pressure deck hose is ideal) but should be filtered to exclude planktonic organisms from the sample. Repeated filling, agitation and pouring will result in successively 'cleaner' suspension, at which point the sample portion retained on the sieve can be transferred gently to a container for fixing. The heavy fraction remaining in the container will still contain specimens, including some that will not be in the light fraction (large molluses and other heavy organisms). Therefore, both portions of the elutriated sample must be kept and treated in the laboratory. Samples in portions should be labelled "part x of y" and stored so that for convenience all can be located and all material combined into a single set of samples during lab sorting.

Omnivorous animals will eat or damage other organisms in the sample, and should be separated when they are noticed. If multiple specimens are placed live in the same container then subsequent attempts to disentangle the preserved specimens will usually result in loss or damage to appendages. Wherever possible, separate organisms that produce slime or mucus (*eg* sponges) and objects such as bryozoan, coral or large molluscs to prevent damage to delicate organisms. Organisms which require different fixing and preserving treatment (see Table 1 and following section) will of course also need to be separated immediately. Some of these problems can be partially alleviated if samples are fixed immediately.

Elutriation and other separation of incompatible parts of the sample will protect many fragile specimens from damage and should be employed routinely. The additional time taken in the field will be repaid many times over during identification.

Fixing and preserving samples

Preamble

Specimens of some taxa can be identifed irrespective of the method of preservation used. However, some taxa are unidentifiable unless specific methods are used. The

goals and organisms targeted in a given study will determine which method or methods will have to be used. A comprehensive treatment of fixatives and preservatives for invertebrate animals can be found in Lincoln & Sheals (1979); only commonly used techniques are covered here.

Safety

Formalin is toxic and carcinogenic – it kills specimens and coagulates their protein and will do the same to you if used carelessly. Ethanol is less dangerous if used sensibly, but all fixatives and preservatives should be assumed to be harmful until proven otherwise. All employers are required by law to provide a safe workplace. Provision of Material Safety Data Sheets (see <u>http://www.msds.com.au/</u>) is mandatory, as is adoption of approved handling procedures. Contact the WorkCover Authority in your state for further details. The Victorian WorkCover Authority provides links to WorkCover Authorities in other Australian states, as well as international sites including New Zealand at

http://www.workcover.vic.gov.au/dir090/vwa/home.nsf/pages/related+sites.

Killing and narcotising

Fixative will kill all specimens but some will contract and die in unidentifiable state. Narcotisation ('relaxing') anaesthetises specimens which can then be fixed without distortion. Narcotisation is almost mandatory for many soft-bodied groups such as ascidians and anemones. Magnesium sulphate or magnesium chloride added over a period of an hour or so as a 25% solution, and propylene phenoxytol works well (and more quickly) up a final concentration of 1% in seawater. Menthol crystals added to seawater is also successful for many taxa, *eg* ascidians. Freezing a specimen in a small volume of seawater often works well and avoids playing with chemicals. Preferred methods of anaesthetising specimens are multitudinous and those with an interest in specific taxa will evolve their own methods, with Lincoln & Sheals (1979) as the starting point.

Fixation

Fixation is a process which coagulates and stabilises protein in specimens so that they do not distort or deteriorate during preservation, study and storage. Fixative is applied as soon as possible after collection and elutriation, and after narcotising if appropriate.

Formalin is purchased as a solution of 40% formaldehyde in water; this is equivalent to 100% formalin and is diluted 1:9 with seawater to achieve 10% formalin. Fishes and other vertebrates are preserved in 10% formalin. Invertebrates typically require only 4% formalin, but 10% formalin is convenient to have available since the volume of the sample in seawater must be considered in estimating final concentrations (*eg* 600 ml of sample in seawater plus 400 ml 20% formalin will result in a sample fixed in 4% formalin). Formalin is an acid which will quickly dissolve delicate calcareous structures (this is why echinoderms and many other taxa are fixed in ethanol). Mixing formalin in seawater is a partially effective buffer against acidity; addition of a small quantity of sodium tetraborate ('borax') crystals is better (a level teaspoonful of borax per litre of 10% formalin seems to be adequate).

Preservation

Preservation is storage of specimens in a fluid in which they are protected as much as possible from deterioration. Fixation and preservation are often confused because

some solutions can be used as both fixative and preservative. Fixative fluid (often formalin) is washed from the sample in the laboratory and replaced by preservative - 70% ethanol unless otherwise specified. Ethanol is available in a 95% solution; dilution to 70% should be done with filtered tap water, or distilled water if use of tap water causes a precipitate.

Table 1 – Preferred fixation and preservation methods for major groups of
marine invertebrates (Also to be made available and updated via the AMIT web site:
http://researchdata.museum.vic.gov.au/amit/.)*

Taxon	Fixation	Preservation	Comments
Annelida	4% formalin	70% ethanol	Leeches and some polychaete
(Leeches,			families are easier to identify if
Oligochaetes,			anaesthetised, but this is generally
Polychaetes)			impractical in large benthic studies.
Crustacea	4% formalin	70% ethanol	
Brachiopoda	4% formalin	70% ethanol	
Bryozoa	70% ethanol	70% ethanol	
(=Ectoprocta)			
Cnidaria	70% ethanol	70% ethanol	Formalin will dissolve spicules and
Octocorallia			render many octocorals
			unidentifiable.
Cnidaria	4% formalin	4% formalin	
Scyphozoa			
Cnidaria (others)	4% formalin	70% ethanol	
Ctenophora	4% formalin	4% formalin	
Echinodermata	70% ethanol	70% ethanol	Formalin will render many
			echinoderms unidentifiable,
			especially holothurians.
Echiura	4% formalin	70% ethanol	Narcotise (freezing or propylene
			phenoxytol or MgCl ₂) if at all
D	40/ 0 1:	2 00/ 11 1	possible
Entoprocts	4% formalin	70% ethanol	
Mollusca	4% formalin	70% ethanol	Narcotise (freezing or propylene
Opisthobranchia			phenoxytol or MgCl ₂) if at all
(=nudibranchs)			possible; photographs recording
N (11	40/ 6 1	700/ /1 1	colour in life are also very useful
Mollusca	4% formalin	70% ethanol	
Nemertea			Probably unidentifiable unless
			narcotised (freezing or propylene
DI 'I	40/ 6 1	700/ /1 1	phenoxytol or MgCl ₂)
Phoronida	4% formalin	70% ethanol	
Platyhelminthes	4% formalin	70% ethanol	Fix living specimens on frozen 4%
			formalin [see safety notes above] or
			narcotise (freezing or propylene
			phenoxytol or $MgCl_2$). Otherwise
D :C	700/ 1 1	700/ /1 1	probably unidentifiable.
Porifera	70% ethanol	70% ethanol	Formalin will render most sponges
D	700/ - 1 1	700/ 41 1	unidentifiable
Pycnogonida	70% ethanol	70% ethanol	
Sipuncula	4% formalin	70% ethanol	Very difficult to identify unless first
			narcotised (freezing or propylene
			phenoxytol or $MgCl_2$)

Tunicata	4% formalin	70% ethanol		
All others	4% formalin	70% ethanol	"default method"	

*The recommendations in Table 1 are a compromise between what is ideal and what is practical when treating large unsorted samples of benthic invertebrates. Specific methods, especially narcotisation procedures, are preferred when possible for various taxa. Further information may found through the sources listed in Table 4.

Samples for DNA and other biochemical analyses

[The following section has been contributed by Paula Cisternas, Museum Victoria. Another relevant document, available online, is O'Meally & Livingstone (2002).]

Knowledge of systematics of many marine taxa has greatly benefited from the generation and analysis of molecular data, in particular DNA sequence data. Molecular data can provide a rapid, sensitive and efficient method to identify individuals from different species and to resolve phylogenetic relationships of related taxa. Although there are no strict guidelines or textbooks that deal with the processing of marine invertebrate tissues for DNA sequencing, this section provides a number of general guidelines that can be followed for collection, preservation and processing of genetic material.

It is more effective to collect specimens and later remove tissue for extractions (availability of the entire specimen is crucial for later confirmation of identification and can provide additional sources of tissues to be trialled for extractions). Tissue such as muscle and gonads are preferred as they do not contain as many sources of enzymes that can degrade DNA/RNA. Gut and digestive tract tissues have high levels of enzymes and of foreign materials and are thus not ideal sources of DNA/RNA. Tissue that will be used to produce genetic data should be collected and stored in sterile containers/vials. The amount of tissue should be at least enough to be able to extract DNA several times (in case of degradation). Three or four pinhead-sized tissue samples per specimen are sufficient for one extraction, although larger samples are usually easier to handle if the size of the specimen permits. For RNA, it is better to collect several samples as the quality of the tissue can vary greatly and it is not always reliable.

Extraction of DNA is easier and more reliable than RNA, which tends to degrade quickly due to both endogenous and exogenous sources of enzymes. For this reason, material for RNA work should be always fixed in liquid nitrogen (snap frozen) and preserved at -80°C in DNase and RNase-free vials, until use.

Several methods are available for preservation of DNA. The most common method is to fix tissue in 70-95% alcohol (99.9-100% AR grade). Absolute alcohol is to be avoided, as it will dehydrate the tissue, break down cells and degrade DNA. The concentration of alcohol can be varied if needed, 80% appears to be as good as higher alcohol solutions and in some instances better than 95% because water buffers the dehydration effect of the alcohol on softer more fragile tissues. Salt buffer can be a great alternative where access/ transport of large quantities of alcohol (or samples in alcohol) are limiting factors. Salt buffer has the added bonus of being a salt-saturated solution which is more appropriate for material collected from sea water. Salt buffer solution, and samples in salt buffer, can be stored for long periods of time at room temperature. However, it does contain hazardous chemicals in diluted form so

Material Safety Data Sheets and procedures will need to be developed. A recipe for salt buffer is contained in Sambrook *et al.* (1989).

Numbers of samples (individuals from a population, species, congeners, etc.) collected should be adequate for the kind of analyses and procedure in mind and this can vary depending on the question being addressed (see Chapter 2 in Hillis *et al.*, (2001)). Hillis *et al.*, (2001) is also an excellent source of information for beginners and as an introduction to all aspects of the field including choosing appropriate techniques, design of a genetics project and so forth.

For standard methods on total DNA/ RNA extractions see Sambrook *et al.* (1989). However, the more laborious phenol/chloroform+ CTAB extraction protocol yields larger amounts of DNA and has the added bonus of removing calcium carbonate (calcareous tissues) and a large proportion of the mucus (polysaccharides) often produced by marine invertebrates. Similarly, the guanidinium thiocyanate phenol chloroform protocol (sounds highly toxic and it is) is often the most efficient at yielding high quantities and cleaner preparations of RNA than most others available. Extraction kits often work well with most tissues however, if contaminants such as skeletal tissue and mucus are likely to be abundant in the starting tissue, then a CTAB clean up step can be used to remove extra contaminants.

It may be possible to extract DNA from dry specimens or even specimens fixed in formalin or other fixatives. However, it is much harder to obtain enough non-degraded DNA from such material. A number of protocols are available for use with difficult material, but they are mostly techniques applied to plant and ancient DNA materials rather than marine invertebrate specimens. Such protocols include Chelex and the salting out methods (refer to Sambrook *et al.* (1989) for recipes).

Detailed instructions and background information on all the relevant methods are found in Sambrook *et al.* (1989), which although old remains the the most comprehensive source of protocols, recipes and information for molecular biologists. A more recent source of protocols is Ausubel *et al.*, (2003) although this is aimed at people working primarily with mice and other vertebrates. The Simple Fool's Guide to PCR (Palumbi, *et al.* 1991) is great for getting starting on PCR and universal primers for a whole range of taxa across most phyla. This book also gives some pointers on general methods associated with DNA extraction and amplification.

Documenting samples

Preamble

It is typical and natural for a researcher to label and document samples with only immediate needs in mind. However, samples and data that are intended for museums must be evaluated in a much wider context.

Museum databases and the internet

The advent of digital technologies and the internet has provided the means of uniting biodiversity data in museums and making those data accessible through a few major portals. The following organisations and web sites effectively comprise a federation of museum databases where single queries can retrieve answers of vast scope. Users of these data can be confident that individual data points can be verified by using the unique registration code to locate the relevant specimen in a collection. These goals

are now realised through OZCAM (Online Zoological Collections of Australian Museums - <u>http://www.ozcam.gov.au</u>). OZCAM has provided online access to a distributed query network to faunal collections in Australian museums since its launch in June 2003. OZCAM is an initiative of the Council of Heads of Australian Faunal Collections (CHAFC). OZCAM will, in turn, become a part of a similar initiative of global scope: GBIF (Global Biodiversity Information Facility; <u>http://www.gbif.org/</u>). The mission of GBIF is to make the world's primary data on biodiversity freely and universally available via the Internet. OZCAM data will be available to GBIF through the Australian Biodiversity Information Facility (ABIF <u>http://www.deh.gov.au/biodiversity/digir/index.html</u>) and data relating to marine taxa will also be made available via the Oceans Biogeographic Information System (OBIS) regional node, currently being developed by the National Oceans Office (NOO) and CSIRO Marine Research in Hobart. (Australian botanical data are made available by a similar initiative: Australia's Virtual Herbarium (AVH), http://www.chah.gov.au/avh/index.html).

Data formats

Achieving unified databases as described above has required the development of standard data formats. OZCAM data providers have adopted Darwin Core as the standard specimen-level schema. There are several versions of Darwin Core – OZCAM uses an amalgam of Darwin Core 1.2, plus OBIS extensions (Darwin Core 1.3) plus some fields from the proposed Darwin Core 2.0, plus some unique fields (such as a flag field for denoting "Port Survey" material) for exchange of data. The AMIT web site (http://researchdata.museum.vic.gov.au/AMIT/) provides a representation of Darwin Core as used by OZCAM in spreadsheet format; this spreadsheet is the preferred format for provision of data to Australian museums. Other international standards for biodiversity data are listed at http://www.gbif.org/links/standards.

Station numbering

One highly relevant implication of unified databases and the underlying data formats is a simpler approach to station numbering. Station numbering schemes favoured by ecologists typically involve a multi-part code in which different parts of the code denote location, sample method, date and depth. Multi-part station numbering schemes with multiple meanings are an attempt to create a mini-database on a label; they make sense to their originator but to no-one else. They are cumbersome to use and their complexity means that they are often transcribed in a way that creates errors, which are discovered when eventually labels are matched against supporting spreadsheets.

Simple station numbering schemes are greatly preferred. Station numbers only have to be unique; clarity and brevity are the next most important criteria. These goals can be achieved with a simple meaningful prefix followed by an incrementing integer *eg* BSS 129. Operators must rely on field notebooks for other data, and the Darwin Core spreadsheet (OZCAM version) referred to above should be used to generate a book of station forms for use in the field. Station prefixes may occasionally be found to be already in use in museum databases; the new stations can be made unique by adding letters or digits to the prefix and this is easily done by database managers in museums at the time of data import.

Surveys which are conducted from established oceanographic research vessels will find that the ship operator maintains their own long-standing log and station numbering scheme (often an alpha-numeric or string-numeric combination quite similar to that suggested above). Since these data are widely used and well supported, and since they are usually provided to participants in digital versions at the conclusion of a cruise, there is much to be gained from adopting "native" schemes of ship operator if available.

Positions should be recorded with global positioning system (GPS) units if at all possible. Use the GPS to give waypoints station codes at the time of collection. It is recommended that data are saved in the native format of the software supplied, and are also exported as comma-limited file (.csv) suitable for import to Excel spreadsheet, and as an ESRI shape file (.shp) suitable for ArcGIS[®] and related software. Positions recorded as decimal degrees are preferred by OZCAM data providers but degrees and decimal minutes, and northings/eastings, can also be converted.

Museum registration and references

When it is necessary for a publication or study to refer to a particular specimen in a museum collection, cite the museum (or other institution) name in full, and a registration number. This will allow collection managers to locate the specimen. These details are also accessible via query to the OZCAM web site - http://www.ozcam.gov.au.

Sorting

Preamble

The primary goal for initial sorting of the sample must be to achieve reproducible results. In other words, the sample must be sorted into taxonomic groupings that can be recognised by all sorters without error, or at least by making consistent errors which are much more easily repaired when discovered.

Organising samples

Organisations with an ecological or environmental focus typically group specimens from a single sample or station, even after sorting and identification. This allows some saving since a single locality label can be applied to all vials from a sample. However, this practice works against making consistent identifications since different specimens of the same taxon are widely spread around the storage area. It is therefore important to move from organising material in station lots to taxonomic lots as soon as possible, preferably at the start of sorting. By doing this, comparison between specimens during sorting and identification is easily done, and errors are detected and more easily fixed.

All members of the sorting team will need to adopt a system for labelling specimens. Specimen lots must be recorded on spreadsheet or database that will allow generation of required outputs, *eg* data matrices for pattern analysis, and station lists and species lists.

Organising material into taxonomic lots is essential before transferring to museums. Material that is sorted and arranged in station lots will be expensive for museums to label and process or will simply be rejected.

Recombining samples

Some organisations attempt to separate, identify and record species or other taxa present in a sample but then recombine all material into one container. This practice is justified on the grounds of saving time, money and resources, even though the savings are far less than would be required to resort and re-identify taxa in the sample. A more significant consequence is that the data are effectively unverifiable since re-examination of the sample by any other person would first require that they provide the time, money and resources required to sort the sample again. This almost never happens.

It is possible that there are some small studies which are of such ephemeral interest that recombining of samples could be justified. However, it should be recognised at the outset that the burden of resorting material is such that there is little practical difference between recombining samples and discarding them. Recombining of samples should never occur in any study which is publicly funded or where the standards of quality assurance and quality control apply. All components of a sample must be retained as separate labelled lots, maintained and stored in a way that allows rapid access. Budgets, tender and contract documents will need to reflect this commitment.

Identification and taxonomic verification

Preamble

Taxonomic knowledge of the marine fauna of Australia and New Zealand is very incomplete. Much of the primary literature describing the fauna is not easily found or used; it is published in journals in many languages and requires access to large libraries. Taxonomists are attempting to remedy this situation, and for a few taxa there are now comprehensive guides that will enable the identification of most species collected (Poore 2004; R.S. Wilson, *et al.* 2003), but these remain the exception.

The organisms themselves also present a major challenge. Most of the organisms in most marine samples are invertebrates a few mm in size and which require skilled and time-consuming dissection to reveal the structures of taxonomic importance. Furthermore, species richness in Australian region is high by world standards (Gray, *et al.* 1997) and a significant fraction of the fauna remains undescribed. For many taxa, study of specimens in museum collections is the only "identification guide".

Taxonomists train themselves for many years to overcome these challenges, to learn about systematics and the distribution of biodiversity on a global scale, and to write original papers and identification guides that will assist others. Acquisition of appropriate taxonomic skills by inexperienced would-be identifiers requires a major commitment from the would-be identifier, and from the experienced taxonomists who devote time to training. Nor can any one identifier be expected to work efficiently and accurately across all taxa that will be collected (crustaceans, polychaetes, molluscs, echinoderms etc). Once a suitable identification team is assembled or trained, the identification work itself is time-consuming. Taxonomic work is invariably the major part of any study, and greatly exceeds the time spent collecting samples and doing final analyses.

In summary: identifying the fauna in marine samples is not a trivial task. In ecological studies, taxonomic work is the major task. Where detection of marine

pests is the goal, taxonomic work is almost the *only* task. Unless suitable expertise is made available to a study, identification errors will be numerous the value of the study will be questionable. The remainder of this section suggests strategies and procedures to minimise this very real taxonomic impediment.

Strategies

Appropriate strategies to achieve accurate identifications and access to taxonomic expertise will depend on the size of the study. Small projects are more easily accommodated by taxonomists employed elsewhere, whereas large projects will need to hire their own staff with appropriate skills, or who will need to be trained. Such staff can be considered as 'parataxonomists'.

Sorting and identification of species in a sample involves two distinct processes:

- 1. recognising species units and distinguishing them accurately (recognising "look-alikes")
- 2. identifying those species units (giving each species a genus and species name, or best level possible, which provides access to other published information about distribution and ecology)

Failure to keep these processes separate is common and is a major source of error, especially among inexperienced workers. If "look-alikes" are recognised accurately then a small voucher collection (see below) can be assembled and a taxonomist can provide names which can be applied with some confidence to all other material. However, if "look-alikes" confuse several species then all material will need to be resorted. It will be easiest to avoid error and resorting of samples if an experienced taxonomist is involved with training and/or quality control of identifications early in the sorting process.

Finding appropriate taxonomic expertise and tools

A comprehensive listing of the taxonomic literature is far beyond the scope of this document. However, a compact listing of local expertise and identification tools for the Australian region has been attempted in Table 2.

Table 2 – An introduction to taxonomic tools and sources for marine

invertebrate identification in Australia (also to be made available and updated via
the AMIT web site: <u>http://researchdata.museum.vic.gov.au/amit/</u>). No assumptions
should be made about the availability of people named in this table to assist with
identifications.

Taxon	Australian & New Zealand expertise ^{1,2}	Major identification tools and other sources ⁴
Annelida:	None	See Australian Biological Resources
Hirudinea		Study (1994-2005) for checklist last
(Leeches)		updated by Govedich in 2002.
Annelida:	None	None for marine taxa. See Australian
Oligochaeta		Biological Resources Study (1994-2005)
-		for checklist for Order Tubificida only
		last updated by Pinder in 2003.

Taxon	Australian & New Zealand expertise ^{1,2}	Major identification tools and other sources ⁴
Annelida: Polychaeta	C.J.Glasby (MAGNT); P.A.Hutchings (AM);	Wilson et.al. (2003) should allow identification of most Australian species.
	H.Paxton ³ (MQU); G.Read (NIWA);	See Australian Biological Resources Study (1994-2005) for checklist last
	G.Rouse (SAM); R.S.Wilson (MV)	updated by Hutchings & Johnson in 2003. For New Zealand, valuable resources are Read (2004a; 2004b).
Brachiopoda	J.Richardson ³ (MV)	See Australian Biological Resources Study (1994-2005) for checklist last updated by Middelfart & Reid in 2001. Richardson (1997) contains a key to about half of all species known from southern Australia.
Bryozoa (=Ectoprocta)	P.Bock ³ (MV); P.Cook ³ (MV); Peter Arnold (MTQ); D.Gordon (NIWA)	Very diverse, and very poorly known in Australia. There is no comprehensive reference. Bock (1982) includes only a few of the most common species. For New Zealand, a valuable resource is Gordon (2004).
Crustacea: Amphipoda	P.Berents (AM); J.K.Lowry (AM); G.C.B.Poore (MV); W.Zeidler ³ (SAM)	Barnard and Karaman (1991) allow identification of families and genera. Keys to families, and to Australian species for some families, can be found in Lowry & Springthorpe (2001).
Crustacea: Isopoda	N.L.Bruce (NIWA); S.Keable (AM); G.C.B.Poore (MV); G.D.F.Wilson (AM)	Keys to families can be found in Keable <i>et al.</i> (2002).
Crustacea (other Peracarida)	G.C.B.Poore (MV)	Keys to Tanaidacea families can be found in Larsen (2002). Keys to Mysidacea families can be found in Meland (2002).
Crustacea: Cirripedia	D.S.Jones (WAM)	Underwood (1977) treats part of the fauna but also contains errors and is out of print. The following publications by D.S.Jones are the most relevant resources: (Jones 1987, 1990a, b, 1991, 1992a, b, 1993, 1998; Jones, <i>et al.</i> 1990; Jones & Morgan 2002)
Crustacea: Copepoda	G.Walker-Smith (TMAG)	Keys to families of Calanoida can be found in Bradford-Grieve (2002)
Crustacea: Decapoda	S.Ahyong (AM); P.J.F.Davie (QM); G.C.B.Poore (MV); J.Yaldwyn (TP)	Poore (2004) provides a comprehensive treatment of the fauna of southern Australia. Keys to families of Stomatopoda can be found in Ahyong & Lowry (2001) and for Anomura families in McLaughlin <i>et al.</i> (2002)
Crustacea: Leptostraca	G.Walker-Smith (TMAG)	See publications of Walker-Smith (1998; 2000; 2001).
Cephalochordata	B.J.Richardson (UWS)	See Australian Biological Resources Study (1994-2005) for checklist last updated by Richardson in 1997.

Taxon	Australian & New Zealand expertise ^{1,2}	Major identification tools and other sources ⁴
Cnidaria: Anthozoa	None	Some of the temperate fauna is covered by in part in <i>The Marine Invertebrates of</i> <i>South Australia Part 1</i> (Thomas & Shepherd 1982)
Cnidaria: Hydrozoa	J.Watson ³ (MV)	Parts of the fauna are covered by Watson and colleagues (Vervoort & Watson 2003; Watson 1982; 2000)
Cnidaria: Octocorallia	P.Alderslade (MAGNT)	Fabricius & Alderslade (2001) is a guide tropical genera only; also see Alderslade (1998). Some of the temperate fauna is covered by in part in <i>The Marine</i> <i>Invertebrates of South Australia Part 1</i> (Grasshoff 1982a, b; Utinomi & Shepherd 1982; Verseveldt 1982)
Cnidaria: Scleractinia	C.Wallace (MTQ); J.E.N Veron ³ (AIMS)	For the tropical fauna see Wallace (1999), Veron (1996; 2000; 2003) and Veron & Stafford-Smith (Veron & Stafford-Smith 2002). Temperate fauna treated by Shepherd & Veron (1982)
Cnidaria Scyphozoa	LA.Gershwin (JCU)	Some of the temperate fauna is covered in Southcott (1982)
Ctenophora	LA.Gershwin (JCU)	None
Echinodermata: Asteroidea	T.O'Hara (MV); M.O'Loughlin ³ (MV)	Much of the tropical fauna is covered by Clark & Rowe (1971). Zeidler & Shepherd (1982) is useful for the temperate fauna.
Echinodermata: Crinoidea	G.Rouse (SAM)	Much of the tropical fauna is covered by Clark & Rowe (1971). Shepherd <i>et al.</i> (1982) is useful for the temperate fauna.
Echinodermata: Echinoidea	K.McNamara (WAM); T.O'Hara (MV)	Much of the tropical fauna is covered by Clark & Rowe (1971). Baker (1982b) is useful for the temperate fauna. Also see Miskelly (2003).
Echinodermata: Holothuroidea	T.O'Hara (MV); M.O'Loughlin ³ (MV); Maria Byrne (SU)	Much of the tropical fauna is covered by Clark & Rowe (1971). Rowe (1982) is useful for the temperate fauna.
Echinodermata: Ophiuroidea	T.O'Hara (MV)	Much of the tropical fauna is covered by Clark & Rowe (1971). Baker (1982a) is useful for the temperate fauna.
Echiura	None	See publications of Edmonds and colleagues (Edmonds 1987, 2000a; Stephen & Edmonds 1972)
Entoprocts	None	Introductions to the fauna and the literature have been published by Wasson (Wasson 2002; Wasson & Shepherd 1997)
Hemichordata	C.Burdon-Jones ³ (SAM)	See Burdon-Jones (1998) and Australian Biological Resources Study (1994-2005) for checklist last updated by Burdon- Jones in 1997.

Taxon	Australian & New Zealand expertise ^{1,2}	Major identification tools and other sources ⁴
Mollusca: Aplacophora	None	See Scheltema (1989; 1999)
Mollusca: Amphineura	K.Gowlett-Holmes (CSIRO)	No single comprehensive guide; a variety of publications are relevant in part (Ludbrook & Gowlett-Holmes 1989; Macpherson & Gabriel 1962; Ponder, <i>et</i> <i>al.</i> 2000)
Mollusca: Bivalvia	S.Boyd ³ (MV); T.Darragh ³ (MV); R.Willan (MAGNT); J.M. Healy (UQ)	No single comprehensive guide; a variety of publications are relevant in part (Lamprell & Healy 1992, 1998a; Macpherson & Gabriel 1962; Ponder, <i>et</i> <i>al.</i> 2000)
Mollusca: Cephalopoda	M.Norman (MV)	See Norman & Reid (2000)
Mollusca: Gastropoda: Prosobranchia	B.J.Smith (QVMAG); T.Darragh ³ (MV); W.Ponder (AM); F.Wells (WAFISH)	No single comprehensive guide; a variety of publications are relevant in part (Ludbrook & Gowlett-Holmes 1989; Ponder, <i>et al.</i> 2000; B.R. Wilson & Gillett 1971)
Mollusca : Gastropoda: Opisthobranchia	R.Burn ³ (MV); W.Rudman (AM); R.Willan (MAGNT)	No single comprehensive guide. About one-quarter of all temperate species are treated by Burn (1989), and many tropical species are illustrated by Coleman (2001)
Mollusca : Gastropoda: Pulmonata	B.J.Smith (QVMAG)	No single comprehensive guide; a variety of publications are relevant in part (Ludbrook & Gowlett-Holmes 1989; Macpherson & Gabriel 1962; Ponder, <i>et</i> <i>al.</i> 2000)
Mollusca: Scaphopoda	J.M. Healy (UQ)	See Lamprell & Healy (1998b; 2001)
Nemertea	None	A difficult and diverse group, poorly known in Australia; for an introduction see Gibson (1997) and references cited therein.
Phoronida	None	The small fauna is adequately covered by Emig and colleagues (Emig, <i>et al.</i> 1977; Emig & Roldan 1992; Shepherd 1997)
Platyhelminthes	L.R.G.Cannon (QM); L.Newman (AKM); L.Winsor (JCU)	A difficult and diverse group. The temperate fauna, especially, is poorly known in Australia (Cannon 1986; Newman & Cannon 2003, 2005).
Porifera	J.N.A. Hooper (QM); B. Alvarez de Glasby (MAGNT); L.J.Goudie, (MV); J.Fromont (WAM)	A difficult and diverse group, poorly known in Australia.
Pycnogonida	D.Staples (MV)	See Staples (1997)
Sipuncula	None	See publications of Edmonds and colleagues (Edmonds 1980, 2000b; Stephen & Edmonds 1972)
Tunicata	P.Mather ³ (QM) [publishes as P.Kott]	See Kott (1985; 1990a; 1990b; 1992a; 1992b)

¹ Institution abbreviations: AU-Auckland University, Auckland; AIMS-Australian Institute of Marine Science, Townsville; AKM-Auckland Museum, Auckland; AM-Australian Museum, Sydney; CALM-Department of Conservation and Land Management, Perth; CSIRO-Commonwealth Scientific and Industrial Research Organisation Marine Research, Hobart; JCU-James Cook University, Townsville; MAGNT-Museum and Art Gallery of the Northern Territory, Darwin; MV-Museum Victoria, Melbourne; MTQ-Museum of Tropical Queensland, Townsville; MQU-Macquarie University, Sydney; NIWA-National Institute of Water and Atmospheric Research, Wellington; QVMAG-Queen Victoria Museum and Art Gallery, Launceston; QM-Queensland Museum, Brisbane; SAM-South Australian Museum, Adelaide; SU-Sydney University, Sydney; TMAG-Tasmanian Museum and Art Gallery, Hobart; TP- Museum of New Zealand Te Papa Tongarewa, Wellington; UQ-University of Queensland; UWS-University of Western Sydney Hawkesbury; WAM-Western Australian Museum, Perth; WAFISH-Department of Fisheries, Western Australia, Perth.
² A few taxonomists based in New Zealand have also been included, but NZ expertise is not yet fully surveyed here. Even for Australia Table 2 cannot be considered comprehensive.
³ Retired or honorary.

⁴ Full citation in References.

Voucher collections

Vouchers collections are collections of one or preferably a few representatives of each species which are set aside to form reference specimens against which new material is compared. Building, documenting and maintaining a voucher collection is an important and valuable process, unless it becomes a substitute for applying similar standards to all samples in a study.

To be useful, a voucher collection will need to be documented with illustrations and descriptions of key characters, reference to published keys and descriptions, differences from similar taxa, other notes helpful to identifiers ("antennae often lost"). Index cards were once useful for this purpose, but computer-based media are more efficient for these data, including drawings which should be digitised or digital images.

A voucher collection should only be considered representative of all other identifications after a quality control process has been completed. An appropriate quality control process is yet to be developed but could include the following components: training, early checks of "look-alikes", random identification checks of voucher and non-voucher specimens; ability to produce identification notes or other documents demonstrating on what criteria identifications were made.

To assist this quality control of identifications, it is proposed that AMIT facilitate production of a document which describes for target species the procedures that any worker will need to have undertaken before a given species name can be applied to a specimen. For example, the current 'target species list' includes 7 species of spionid polychaetes in the *Polydora*-group (both introductions and cryptogenic). These cannot be identified unless prostomial and branchial features are examined and described using dissecting microscope and chaetae of segment 5 and position of hooded-hook chaetae on subsequent segments are examined with compound miscroscope. For other species, of course, different criteria will apply.

Local expertise and the 'target species' approach

In the context of surveys for introduced species, known and suspected introductions to Australian ports have been assembled into a list which is targeted for particular attention in future surveys. Species which are not yet known to be introduced are or can also be listed if they are seen to constitute a significant risk based on introductions elsewhere in the world. However, the target species approach is not a sufficient method for surveys since new introductions are unlikely to be listed since the species 'pool' from which future introductions will occur is a large part of the fauna of ports and harbours world-wide - many thousands of species. In Australia, and elsewhere, new introductions have been frequently discovered by a different means: skilled and observant taxonomists, amateur naturalists and community groups who know their local area and fauna well and notice "something new". A very recent example (yet to be fully investigated) is the appearance in Westernport and Port Phillip Bay of a nudibranch that is close to Ercolania boodleae from Japan and Hong Kong. The first known Australian records are Flinders Pier 2002 and Port Phillip Bay 2005. Specimens were noticed and photographed by amateur photographers and the tentative identification is by Robert Burn, opisthobranch expert and Honorary Associate at Museum Victoria. See

<u>http://researchdata.museum.vic.gov.au/marine/nudi_gallery/index.htm</u> for a photograph and further details of the community photographic atlas project. Similar groups, both formal and informal, operate throughout Australia and New Zealand. Fostering and supporting this kind of local expertise is likely to be a cost-efficient and highly effective means of early detection of new introductions.

Transferring samples

Preamble

It is tedious but important to emphasise some practical issues regarding transfer and transport of scientific specimens.

Packing and mailing

Formalin, and ethanol exceeding 24%, are Dangerous Goods and must be packed and transported responsibly and legally (*ie* by someone with an appropriate packing qualification). Formalin is Corrosive Liquid, Hazard Class 8; ethanol exceeding 24% is Flammable Liquid, Hazard Class 3. Sending scientific specimens in ethanol exceeding 24% through mail services or carrying the same in luggage on an airplane is illegal and is a criminal offence however small the volume and however secure the packing. (However, feel free to continue to carry 2 litres of 45% ethanol in the form of, eg, fine Scotch whisky in unprotected glass bottles on all your international flights.) IATA regulations document these standards and contradictions in great detail. Collection managers in each museum (see Table 3) may be able to recommend approved dangerous goods packers and couriers. Civil Aviation Safety Authority (http://www.casa.gov.au/) can provide contact details for training providers in each state. Undergoing dangerous goods packaging training for staff within an organisation is far more cost-effective than hiring a qualified packer. Surface transport of dangerous goods within Australia involves a fairly modest surcharge but overseas air shipment is prohibitively expensive and now constitutes a major obstacle to exchange of scientific loans between overseas museums.

Temporary transfer of specimens to from 70% ethanol to 20% ethanol for transport purposes (and back to 70% on arrival) solves the above problems. Most invertebrates

will not deteriorate if previously well-fixed and held in 20% for a week or two. However, this method is impractical when numerous samples or minute specimens are involved. DNA tissue can be removed from 95% ethanol and transported in vials empty of fluid. Samples in 20% ethanol must be carefully labelled or mail authorities will assume they are Dangerous Goods. A further complication is that under quarantine legislation (in Australia, administered by AQIS), specimens have to be preserved in 70% ethanol, 10% formalin or 2% gluteraldehyde before they can be imported. It will usually be necessary to supply AQIS with a letter from the sender stating the specimens had originally been fixed in an approved way but had been transferred to 20% ethanol for shipping.

Material that has been fixed in formalin can be transported damp without liquid if it is in sealed containers. This can greatly reduce weight for transport. However, replace preservative as soon as practicable. Delicate specimens and alcohol specimens must have some liquid around them when transported, but the volume can be reduced. Alcohol specimens must remain moist with a little liquid in a well-sealed container.

Museums collection manager contacts

Preferred contacts for the most relevant curators or collection managers in Australian museums are provided in Table 3.

Museum	Contact details
Australian Museum,	Dr Penny Berents; email: pennyb@austmus.gov.au; web:
Sydney (AM)	http://www.amonline.net.au/invertebrates/index.htm
Museum and Art	Dr Chris Glasby; email: <u>chris.glasby@nt.gov.au;</u> web:
Gallery of the	http://www.dcdsca.nt.gov.au/dcdsca/intranet.nsf/pages/magnt_nat
Northern Territory,	uralscience
Darwin (MAGNT)	
Museum Victoria,	Chris Rowley; email: <u>crowley@museum.vic.gov.au</u> ; web:
Melbourne (MV)	http://www.museum.vic.gov.au/collections/sciences/marine.asp
Queen Victoria	Dr Brian Smith; email: <u>Brian.Smith@qvmag.tas.gov.au</u> ; web:
Museum and Art	http://www.qvmag.tas.gov.au/zoology.html
Gallery, Launceston	
(QVMAG)	
Queensland	Dr Peter Davie; email: <u>PeterD@qm.qld.gov.au</u> ; web:
Museum, Brisbane	http://www.qmuseum.qld.gov.au/organisation/biodiversity.asp
(QM)	
South Australian	Thierry Laperousaz; email: <u>laperousaz.thierry@saugov.sa.gov.au</u> ;
Museum, Adelaide (SAM)	web: <u>http://www.samuseum.sa.gov.au/orig/invert.htm</u>
Tasmanian Museum	Dr Genefor Walker-Smith; email: Genefor.Walker-
and Art Gallery,	<u>Smith@tmag.tas.gov.au;</u> web:
Hobart (TMAG)	http://147.109.254.132/Collections1.html
Western Australian	Melissa Titelius; email: <u>melissa.titelius@museum.wa.gov.au</u> ; web:
Museum, Perth	http://www.museum.wa.gov.au/default.asp?et=2&ei=85&styleShe
(WAM)	et=perth.css

Table 3 – Contacts for marine invertebrate curators/collection managers in
Australia (also available and updated via the AMIT web site:
http://researchdata.museum.vic.gov.au/amit/)

Other resources

Preamble

The issues raised in this document are not new, although I am unaware of any other single source which covers the same subject matter. However, many scientific and natural history societies encompass much relevant expertise. The web sites and listserver archives of the societies listed in Table 4 are valuable sources of additional information and provide the best means of contacting biologists with expertise in particular taxa.

Society/listserver	URL	comments
All organisms	http://tolweb.org/tree/	Tree of life project
Cnidaria	http://www.ucihs.uci.edu/bioch	A forum for cnidarian (coelenterate)
Newsgroup	em/steele/newsgroup.htm	biologists including taxonomists.
Hexacorallians of	http://hercules.kgs.ku.edu/hexa	Catalogue of species, bibliography etc
the world	coral/anemone2/index.cfm	for sea anemones, corals and their
		allies.
Crustacean Society	http://www.vims.edu/tcs/	A forum for crustacean biologists
		including taxonomists.
World of copepods	http://www.nmnh.si.edu/iz/cop	Bibliography and lists of taxa and of
	epod/	researchers on copepods and
		branchiura.
Echinoderm-l	http://nic.museum/archives/ech	A forum for echinoderm biologists
	inoderm-1.html	including taxonomists.
International	http://www.nhm.ac.uk/hosted_	A forum for bryozoan (Ectoprocta)
Bryozoology	<u>sites/iba/</u>	biologists including taxonomists.
Association		
Annelid resources	http://www.annelida.net/	A forum for polychaete biologists
		including taxonomists.
Leech publications	http://research.amnh.org/~sidd	Publications of Mark Siddall [Leech
	<u>all/pubs.html</u>	(Hirudinea) web pages by Siddall and
		Burreson are no longer online]
Malacalogical	http://www.amonline.net.au/in	A forum for Australian mollusc
Society of	vertebrates/mal/malsoc/	biologists including taxonomists.
Australia		Includes links to international mollusc
		societies.
Nemertes forum	http://nemertes.si.edu/mod/foru	A forum for nemertean biologists
	<u>m/</u>	including taxonomists.
Porifera list	http://www.jiscmail.ac.uk/lists/	A forum for sponge (Porifera)
	porifera.html	biologists including taxonomists.
Society for the	http://www.spnhc.org/	A multidisciplinary organization
Preservation of		composed of individuals (including
Natural History		many museum professionals) who are
Specimens		interested in development and
(SPNHC)		preservation of natural history
		collections.

Table 4 – Professional links and societies (also available and updated via the AMIT
web site: <u>http://researchdata.museum.vic.gov.au/amit/</u>)

Society/listserver	URL	comments
Southern California	http://www.scamit.org/	Promotes the study of marine
Association of		invertebrate taxonomy in southern
Marine		California and developing a regionally
Invertebrate		standardized taxonomy. Connects
Taxonomists		taxonomists with ecologists and
(SCAMIT)		consultants in southern California
		through newsletters and meetings.
TAXACOM	http://biodiversity.bio.uno.edu/	Listserver for taxonomists.
	mail_archives/taxacom/	
Taxonomic	http://www.tdwg.org/	A forum for biological data projects,
Databases Working		develops and promotes the use of data
Group (TDWG)		standards and
		facilitates exchange of taxonomic data.
Tunicata list	http://www.jiscmail.ac.uk/lists/	A forum for tunicate (ascidian)
	tunicata.html	biologists including taxonomists.

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