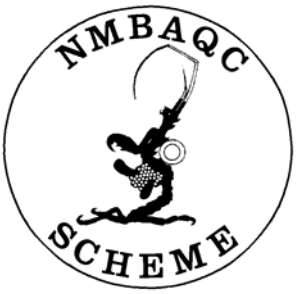

National Marine Biological Analytical Quality Control Scheme

Sorting Methods Questionnaire

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Report NMBAQCsortmeth
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1. Introduction

Standard operating procedures (SOPs) in marine biological sample collection and analysis were reviewed for the National Marine Biological Analytical Quality Control Scheme (NMBAQCS) by Cooper & Rees (2000). However, that report focussed primarily on sampling methods and safety and did not deal with all issues concerning the fundamental requirements of processing of macrobenthos samples.

Few agencies or other organisations that commission samples for analysis of macrobenthos give clear guidelines as to the required treatment of samples. Laboratories that carry out sample analysis generally develop their own in-house practices. The practices are often not explicitly written down but become established through tradition. As the agencies requiring data do not give clear guidelines and as they often subcontract their sample analysis to more than one laboratory, it is important to ascertain the consistency of practice between laboratories. Consistency is particularly important where data collected by different organisations are to be used for comparative purposes, as with the National Marine Monitoring Plan (NMMP).

2. Methods

On 20th October 2000, a questionnaire (Appendix 1) was sent to twenty participants of the NMBAQC Scheme. Reminders for outstanding questionnaires were circulated on 26th January 2001. The purpose was to evaluate the consistency of sample processing and, consequently, of data quality between different laboratories that carry out NMMP macrobenthos sample analysis. The questions were designed to highlight areas of likely discrepancy between different laboratory practices that had been noticed during examination of data sets submitted through the NMBAQC Scheme. The ordering of the questions on the questionnaire was random but here the most basic sample handling issues are dealt with first, followed by more detailed issues of specimen identification and enumeration. The questions from the questionnaire (Appendix 1) are quoted in the text below with question numbers in brackets.

2.1 Sample collection

There are many issues relating to the sampling process itself that are beyond the scope of this report. The design of the sampling grid, numbers of replicate samples, sampling type and methodology all have a great impact on the value of the final data set. They must be considered elsewhere. Some aspects of sampling, however, have a more direct impact on the nature of the samples themselves, as received for further analysis. The type and nature of the preservative have a great affect upon the quality of the samples and specimens contained within them. Factors include formaldehyde concentration and the addition of buffers such as borax. The nature of the sediment affects the effectiveness of preservation. The amount of water contained within sediment changes the concentration of added preservative. Coarse sediments with many empty shells need less buffer (for preventing the decomposition of mollusc shells) than soft muds. The degree and style of any processing (e.g. sieving) before preservation affects the condition of preserved biota. There is also a need for clear labelling of samples. These issues were considered by Cooper & Rees (2000).

One of the questions on the form (stated below) was concerned with the addition of stain to the samples. Stains are generally added at the same time as the preservative as part of the sample collection process.

> "Do you routinely use any form of staining in your sample processing? If so give details and reasons for use" (Q.7)

2.2 Initial sample processing

Most of this report is concerned with laboratory processing. Generally, samples for macrofaunal analyses arrive at the laboratory (which may or may not be directly connected to the organisation that originally collected the samples) contained in watertight containers with a volume of sediment and associated biota preserved in formaldehyde. The required remit is generally no more precise than e.g. extraction, identification and enumeration of macrofauna to the lowest taxonomic level possible. Instructions for biomass, reference collections and return of specimens and residues are often provided but there is much room for different interpretations with most of the other requirements. We asked laboratories to describe their methods for a hypothetical complex sample:

> "If your samples contained stones with *Pomatoceros* tubes, *Sabellaria* reefs, barnacles, hydroids and encrusting bryozoans attached, how would you proceed with the sorting?" (Q.5)

Samples with very large volumes of sediment are not generally searched in their entirety due to time (cost) restraints. It is therefore necessary to ask how different laboratories subsample such sediments:

> "If your samples contained several litres of 0.5-1mm and 1-4mm sediment fractions, how would you process these fractions?" (Q.6)

2.3 Extraction of fauna

Extraction of fauna may seem to be a simple requirement. However, the title has already assumed that plant material need not be extracted or recorded. Plants may be an important aspect of the biology within certain samples. Many laboratories also assume that only benthic animals need be extracted, some assume only macrofauna should be recorded and some assume that only infauna are required. The assumptions are not consistent and are rarely defined in protocols. In addition, the terms benthic, macrofauna and infauna are not clearly defined and interpretations have been known to vary between laboratories. The following questions were asked of participating laboratories. Some examples of problem taxa were provided (see Appendix 1).

> "Which of the following do you routinely extract and record:"
> "List any additional taxa that you would not record:" (Q. 4A & 4B)

In addition to macrofauna, some laboratories extract, or require extraction of, anthropogenic items or seeds. Protocols are usually more clear with such requirements but routines were investigated with the following question:

"List any additional materials (non-faunal) that you record" (Q.4C)

2.4 Recording of fauna

The issues considered so far concern only the basic processes of extracting animals from a sample. Greater discrepancies might be expected with the actual recording and identification. One of the simplest issues is how to record fragmented animals.

"What constitutes a countable individual for the following taxa:" (Q.2)

Identification involves many more sources of inconsistency and error than those connected with whether or not a particular identification is "correct". The usual requirement of "lowest taxonomic level possible" appears not to recognise the fact that different levels of identification are possible for different laboratories. Individual laboratories may have established traditions of identification levels for different taxa at different sizes but they may not be consistent between laboratories. Small individuals are often recorded as juveniles. We attempted to test the consistency of recording of juveniles in different taxa and the sizes at which they were considered to be juveniles:

"Please list all taxa that you separate into adults and juveniles" (Q.1)

Laboratory traditions concerned with taxa that are considered too difficult to identify to species were compared by the following question:

"List all taxa which you would normally identify at a higher taxonomic level than species:" (Q.3)

Finally, we asked for participating laboratories to provide any further comments that might be relevant to the study:

"If you have any further comments please use the reverse of this sheet". (Q.8)

3. Results

The questionnaire was sent to twenty laboratories that participate in the NMBAQC Scheme, including government organisations and independent consultancies. Twelve laboratories provided full returns, which would have included some from the same organisation. Another laboratory provided an additional response with comments loosely related to the questionnaire. A high proportion of other respondents had also misunderstood the purpose of the survey and the form layout.

3.1 Staining

Rose Bengal was routinely used by ten of the laboratories that responded to the questionnaire. The reason generally given was that it increased sorting efficiency or that it could be used to distinguish live-collected material from debris. Some specified light stain, in recognition of the problems that can be caused by the masking of

specimens and features in pink liquid. Methyl blue and Crystal violet were each mentioned by one laboratory for use in enhancing identification features. Two laboratories did not use stain.

3.2 Initial sample processing

3.2.1 Processing complex samples

The most thorough method of processing such a sample would be first to separate the stones and *Sabellaria* from the sediment. Obvious animals, including the *Pomatoceros* worms would then be removed from the stones for examination. Encrusting life would be examined while attached to the stones. *Sabellaria* reefs would be crushed to extract the worms and other associated fauna for counting. Old *Pomatoceros* tubes would be treated in the same way. The sediment would then be separated into light and heavy fractions for sorting under the microscope by separate size fractions.

Most (seven) laboratories mentioned separate examination of stones and picking off *Pomatoceros* etc. There was much variation in the recording of sessile taxa, though seven participants said that stones would be examined. That issue will be dealt with in more detail later. *Sabellaria* reefs were not specifically mentioned by all laboratories. Four laboratories said they would be crushed, while four said they would be broken up. Two laboratories noted the fact that other species (besides *Sabellaria*) would be present in the tubes. One laboratory suggested that the portions of reef would be sub-sampled by weight.

3.2.2 Processing large samples

Some form of decanting of light fractions (containing most of the fauna) would be necessary, here, and was mentioned by eight laboratories. Separation of the float into size fractions would also be useful and was also suggested by eight laboratories. One laboratory stated that separation of fractions was not done. Most workers would then need to save time by avoiding a thorough search of the heavy portions. However, two laboratories (one of which did not collect large volume samples) said that all would be fully sorted by fraction separation and searching manageable portions. Five laboratories mentioned quick sorting of handfuls in a tray by eye for molluscs. There was one suggestion of sub-sampling by weight. Successive extractions of lighter residue by stronger water jets until no more animals were found were mentioned by two laboratories.

3.3 Extraction of fauna

3.3.1 Taxa routinely extracted

The different approaches adopted by different laboratories with respect to which organisms to extract and record are summarised in Table 1. Six groups of sessile animals, seven groups of small invertebrates and invertebrate fragments were suggested in the questionnaire. Some participants stated that certain taxa would be recorded on data sheets but not included in the data sent for the NMMP. The results

show that there was very little agreement on which organisms to record and no two laboratories appeared to have the same protocols.

Of the sessile animals, hydroids and tunicates were each extracted by eight laboratories and ignored by four. Sponges, erect bryozoa and barnacles were each extracted by seven laboratories and ignored by five. Encrusting bryozoa were ignored by half of the laboratories to return the questionnaire. A few laboratories stated that they would count the colonial taxa (presumably colony counts) but most recorded them only as present. Solitary tunicates were counted by all that recorded them, while barnacles were counted by three laboratories (i.e. recorded only as present by four). Recording criteria varied from simple presence to the presence of various internal organs to attachment to the substratum. The taxonomic level for recording varied from species to phylum for many taxa.

Nematodes were extracted by nine laboratories and ignored by three. Insect larvae were recorded by eight of the laboratories and benthic copepods by six (half of the returns). Five laboratories extracted parasites, hard (podocopid) ostracods and pelagic copepods. Soft (myodocopid) ostracods were ignored by most but extracted by four laboratories. There was much variation between laboratories with respect to whether the above extracted taxa were counted or only recorded as present. Taxonomic levels for recording were similarly variable. Head presence was the usual recording criterion.

Invertebrate fragments were extracted by eight laboratories for biomass purposes but counted by none. They were generally assigned to species.

The following additional taxa were each listed as not recorded by one laboratory: anthozoa, decapoda, pelagic decapod larvae, foraminifera, periwinkles and anything deemed non-benthic. It is likely that some of these (e.g. pelagic larvae, foraminifera) would also have been ignored by other laboratories while others (e.g. decapoda, periwinkles) would be recorded by most.

3.3.2 Additional materials recorded

Anthropogenic materials and seeds were recorded by a minority of laboratories and a few others stated that they would record them if asked. There was little correlation between laboratories on materials to be recorded or on whether to count them or record as present (see Table 2). They were not generally weighed.

3.4 Recording of fauna

3.4.1 Countable individuals

Several taxa were listed for participants to suggest recording criteria. Most animals are recorded on the basis of the presence of a head but some taxa are problematic. Heads were still suggested by most laboratories for the problem groups but tails were sometimes used for maldanids and mysids. One participant said that separate counts would be made for heads and tails. There was further confusion with molluscs. Some said that whole animals would be needed or simply that there needed to be flesh in the shell. Siphons or hinges were used for bivalves. Gastropod counts could be based on

heads, opercula or shell apices. Ophiuroid counts were based on oral discs for some and upper discs for others. One laboratory said that the whole animal would be needed for amphipods. The results are summarised in Table 3.

3.4.2 Adults and juveniles

This question confused some, who took it to be a taxonomic issue. Most laboratories said that separation of juveniles was based on whether or not they could be identified. Several taxa were generally listed, with a note on the taxonomic level to which the juveniles would be identified. The results are summarised in Table 4. Different participants suggested different taxa and the size considered being juvenile varied. The only consistently separated taxon was that suggested as an example (*Nephtys*). Some participants gave lengths at which they would be considered juvenile and identified only to genus. The lengths varied from 0.5 to 3 cm. Subdermal eyespots were mentioned by two laboratories. The usual criterion for other taxa, too small to identify, would be expected to vary depending upon skill.

3.4.3 Identification at higher taxonomic levels than species

Table 5 shows the range of taxa that would not be identified to species by different laboratories. Nemertea and nematoda were mentioned by most but there was some variation in the taxonomic level to which they were recorded. Other groups were generally mentioned each by two or three laboratories, with much variation in the final identification level. The reason given was usually concerned with the difficulty of identifying certain groups.

3.4.4 Further comments

Additional comments were made by only one laboratory, recognising the problem of inconsistent recording policies between laboratories.

4. Conclusions

It is clear from the results of the questionnaire that there is little or no consistency in recording criteria between different laboratories participating in the NMBAQC Scheme. Recording consistency is important if data from different laboratories is to be compared, as is the case with NMMP data.

Some of the differences in practice, such as staining and different extraction procedures, would only be a problem if they affected the quality of sample sorting, which could be tested by quality control procedures. However, as NMBAQCS results show that sorting efficiency is often poor, it may be necessary to suggest a common approach.

Inconsistencies in recording policies are a more serious problem. Currently, sample quality control operates on the individual laboratories' procedures such that, for example, hydroids will not be recorded if the participant did not record them. Unfortunately, this means that results from different laboratories are not truly comparable. It is important that a standard approach be developed as soon as possible

so that maximum benefit can be derived from the data. Standardised extraction and recording procedures should be produced through the NMBAQC Scheme.

Differences in the taxonomic levels to which animals are identified also reduce the comparability of data. Current quality control procedures, again, do not highlight the problems as identifications to higher taxonomic levels are taken to be correct. Reduction of data to the lowest common denominator (i.e. highest taxonomic level) is a poor short-term solution to the use of the data that will not ensure maximum benefit. It would be difficult to standardise definitions for juveniles and required taxonomic levels for identification, as they would necessarily differ for different species and higher taxa. However, such a system is necessary for adequate quality control and some priority should be given to its development. It is suggested that representatives from the organisations involved in NMMP processing and individuals with relevant taxonomic expertise (museum staff, etc.) should be tasked with producing an NMMP extraction and recording protocol.

Development of the standard approaches suggested above should be applied firstly, and most urgently, to NMMP data. A comprehensive set of protocols for all laboratories processing the samples must be produced. Ideally, the same protocols should then be applied to all sampling, so that data from a variety of sources can be used in many ways.

5. References

Cooper, K., & Rees, H., 2000. Review of standard operating procedures (SOPs). NMBAQC. National Marine Biological Analytical Quality Control Scheme.

Table 1. Extraction and recording.

Taxa	Nos. of labs: Extracted		Counted		Biomass		Recording criteria	Nos. of labs	Taxonomic level	Nos. of labs
e.g. Hydroids	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*	*Polyps present*	-	*Species*	-
Hydroids	8	4	1	11	1	6	Polyps	4	Species	7
							Present	3	Order	1
							Substrate attachment + polyps	1		
Sponges	7	5	1	11		6	Present	4	Species	3
							Attached	1	Phylum	2
							Living colony	1	Genus	1
									Varies	1
Encrusting Bryozoans	6	6	1	11		7	Present	3	Species	7
							Zooid membranes	2	Phylum	1
							Polypides present	1		
							Animal in situ	1		
Erect Bryozoans	7	5	2	10	1	6	Zooid membranes	2	Species	7
							Present	2	Phylum	1
							Polypides present	1		
							Animal in situ	1		
							Number of colonies	1		
Solitary Tunicates	8	4	8	4	2	5	Present	3	Species	6
							Inards present	2	Genus/species	1
							Animal in situ	1	Class	1
Colonial Tunicates	8	4	2	10	1	6	Present	3	Species	6
							Inards present	2	Genus/species	1
							Animal in situ	1	Class	1
Barnacles	7	5	3	9	1	6	Present	4	Species	6
							Inards present	2	Barnacle sp	1
							Animal in situ	1		
Hard Ostracods (Podocopida - benthic)	5	7	3	9	2	3	Presence	2	Order	3
							Heads	1	Class	2
							Number	1		
Soft Ostracods (Myodocopida - pelagic)	4	8	4	8	2	2	Number	3	Order	1
							Presence	1	Class	1
									Species	1
Benthic Copepods	6	6	4	8	3	3	Presence	3	Order	2
							Heads	1	Species	1
							Number	1	Subclass	1
							Whole animal	1	Family	1
									Class	1
Pelagic Copepods	5	7	3	9	2	3	Presence	3	Order	2
							Number	1	Subclass	1
							Whole animal	1	Class	1
Nematodes	9	3	9	3	5	2	Number	2	Phylum	7
							Presence	2	3 species identified	1
							Heads	1		
							Whole animal	1		
Invertebrate fragments	8	4	0	12	7		Polychaete bits	1	Species	1
Aquatic Insect larvae	8	4	4	8	2	4	Present	3	Family	3
							Heads	1	phylum	1
							Number	1	Order	1
									Insect larvae	1
Parasites	5	7	3	9	2	3	Heads	2	Species	2
							Presence	2	Order	1

Additional taxa not recorded:

The following were listed as not recorded by one laboratory each

Anthozoa
Decapoda
Pelagic decapod larvae
Foraminifera
Periwinkles etc.; anything deemed non-benthic

Table 2. Additional materials (non-faunal) recorded.

	Number of laboratories								
Taxa	**Extracted**			**Counted**			**Recording criteria**		**Included in biomass**
e.g. Tomato pips	*Yes*	*No*	*If asked*	*Yes*	*No*	*If asked*	*Presence*	*Number*	*No*
Tomato pips	2	7	2	2	4	1	3	1	5
Raspberry pips	1	8	2	1	5	1	1	1	4
Kiwi pips	1	8	2	1	5	1	1	1	4
Anthoprogenic matter	3	6	2	2	4	0	5	0	5
Glass splatter	0	8	2	0	4	0	1	0	3
Metal splatter	0	8	2	0	4	0	1	0	3
Others									
Wood	0	1	0	0	1	0	1	0	1
Leaf litter	0	1	0	0	1	0	1	0	1
Coal	0	1	0	0	1	0	1	0	1

Table 3. Definition of a countable individual.

Taxon	Criteria for enumeration	Nos. of labs
Maldanidae	Head	8
	Head & some of body	1
	Heads or tails (genus dependant)	1
	Separate tail count	1
	Heads or tails (whichever most common)	1
Oweniidae	Occupied tube	1
MYSIDACEA	Head	5
	Antennules & telson (most of body)	2
	Carapace	1
	Separate tail count	1
	Eyes & rostrum	1
	Enough to identify	1
	Heads (tails for some spp.)	1
AMPHIPODA	Antennules & telson (most of body)	1
GASTROPODA	Head	4
	Animal present	3
	Shell & animal	2
	Whole animal	1
	Most of spire (esp. top)	1
	75% animal	1
	Aperture/operculum	1
PELECYPODA	Hinge presence	5
	Animal present	3
	Whole animal with hinge	2
	Siphons	1
	Tissue in complete shell	1
Ensis	Siphons	1
ECHINODERMATA	Oral disc	1
OPHIUROIDEA	Disc	1
	Oral area	1
ECHINOIDEA	Mouth	1
HOLOTHURIA	Oral area	1
Phoronis	Occupied tube	1
Others	Any other part	2

Table 4. Separation of adults and juveniles.

Taxa	Nos. of labs	Criteria for age division	Nos. of labs	Taxonomic level used for juveniles	Nos. of labs
Various / none specified	3	Too small to identify to species	3	Lowest possible	3
Others not specified	3				
juv/sp issue	1				
SIPUNCULA	1	Too small to identify to species	1	Genus	1
Harmothoe	1	Too small to identify to species	1	Genus	1
Nephtys	9	Too small to identify to species	3	Genus	9
		Presence of subdermal eyespots	2		
		3 cm	1		
		2 cm	1		
		1 cm	1		
		0.5 cm	1		
Nereididae	4	Small size	2	Family	2
		30 chaetigers	1	Genus	2
		Too small to identify to species	1		
Glyceridae	2	1 cm	1	Genus	2
		Too small to identify to species	1		
Eteone	1	Too small to identify to species	1	Genus	1
Eumida	2	Too small to identify to species	2	Genus	2
Lumbrineris	1	Too small to identify to species	1	Genus	1
Magelona	1	Too small to identify to species	1	Genus	1
Cirratulidae	1	Too small to identify to species	1	Family	1
Cirriformia	1	Presence of subdermal eyespots	1	Genus	1
Cirratulus	1	Single pair of eyes	1	Genus	1
Maldanidae	1	Too small to identify to species	1	Family	1
Ampharete	1	Too small to identify to species	1	Genus	1
Terebellidae	1	Too small to identify to species	1	Subfamily	1
Tubificidae	1	Too small to identify to species	1	Family	1
Corophium	2	Small size	1	Genus	2
		Too small to identify to species	1		
Ampelisca	1	Too small to identify to species	1	Genus	1
Bathyporeia	1	Too small to identify to species	1	Genus	1
Amphipods	1	Too small to identify to species	1	Genus or family	1
Gammaropsis	1	Too small to identify to species	1	Genus	1
Lembos	1	Too small to identify to species	1	Genus	1
Gnathia	1	Too small to identify to species	1	Genus	1
Diastylis	1	Too small to identify to species	1	Genus	1
Pagurus	1	Too small to identify to species	1	Genus	1
Portunidae/brachyurhyncha	2	5 mm.	1	Species if possible, or family	1
		Zoeae	1	Family	1
GASTROPODA	1	1 mm	1	Class	1
Philine	1	Too small to identify to species	1	Genus	1
Nucula	2	Too small to identify to species	2	Genus	2
Mytilidae	1	Too small to identify to species	1	Family	1
Mytilus	1	Too small to identify to species	1	Genus	1
Anomia	1	Too small to identify to species	1	Genus	1
Chlamys	1	Too small to identify to species	1	Genus	1
Cerastoderma / Acanthocardia	2	5 mm.	1	Species	1
		Small size	1	Superfamily	1
Parvicardium	2	Too small to identify to species	2	Genus	2
Mya spp.	2	5 mm.	1	Species	1
		Too small to identify to species	1	Genus	1
Dosinia	1	Too small to identify to species	1	Genus	1
Tellinacea	2	small size	1	Superfamily	1
		Too small to identify to species	1	Family	1
Abra	3	Too small to identify to species	3	Genus	3
Scrobicularia plana	1	5 mm.	1	Species	1
Spisula	1	Too small to identify to species	1	Genus	1
Thracia	3	Too small to identify to species	2	Genus	1
		5 mm.	1	Genus	2
Gari	1	5 mm.	1	Genus	1
Ensis	1	Too small to identify to species	1	Genus	1
PELECYPODA	2	1 mm	1	Class	1
		Too small to identify to species	1	Genus or family	1
Echinoidea	1	1 cm	1	Species if possible or genus	1
Amphiura	3	Too small to identify to species	3	Genus	3
Ophiura	2	Too small to identify to species	2	Genus	2

Table 5. Taxa normally identified at a higher taxonomic level than species.

Taxa	Nos. of labs	Taxonomic level	Nos. of labs	Explanation (if necessary)
Others (not defined)	2			
Meiofauna	1	Class	1	
PORIFERA	1	Phylum	1	Key features difficult to determine confidently
Anthozoa	1	Various	1	Key features difficult to determine confidently
Campanulariidae	1	Family	1	Key features difficult to determine confidently
Obelia	1	Genus	1	
TURBELLARIA	3	Phylum	1	
		Subphylum	2	
NEMATODA	8	Phylum	7	Key features difficult to determine confidently
		Genus	1	
NEMERTEA	11	Phylum	9	Key features difficult to determine confidently
		Order	1	
		Family/Genus	1	
Tubulanus	1	Genus	1	
SIPUNCULA	2	Phylum	2	
Golfingia	1	Genus	1	
Polynoidae	2	Genus	2	If damaged
Autolytus	2	Genus	2	Key features difficult to determine confidently
Syllidae	1	Various	1	Lack of experience
Syllis	1	Genus	1	Taxonomic confusion
Ophryotrocha	2	Genus	2	
Polydora	1	Genus	1	
Tharyx	1	Genus	1	Taxonomic confusion
Cossura	1	Genus	1	
Protodrilus	1	Genus	1	
Maldanidae	2	Genus	2	If tails missing
Amphartete	1	Genus	1	
Sabellidae	1	Family	1	If small
OLIGOCHAETA	3	Family	1	Specialised techniques; scattered literature
		Order	2	Clearing (COSHH)
Tubificidae	1	Family	1	
Enchytraeidae	3	Family	3	
Halicaridae	1	Family	1	
Lysianassidae	1	Family	1	
Aoridae	3	Family	3	morphological similarity
Isaeidae	1	Genus	1	morphological similarity
TANAIDACEA	2	Order	2	
Gnathia	2	Genus	2	Juveniles & females
DECAPODA	1	Genus	1	If no legs
CRUSTACEA	3	Various		Females sometimes indeterminable
				Larvae not done
OPISTHOBRANCHS	1	Genus	1	
GASTROPODS	1	Various	1	
Philine	1	Genus	1	
NUDIBRANCHIA	1	Order	1	
	1	Family	1	
BIVALVIA	1	Family	1	If small, key features not discernable
ECHINODERMS	1	Family	1	Experience
OPHIUROIDEA	1	Family	1	If small
TUNICATES	2	Genus	1	
		Subphylum	1	

Appendix 1. The sample sorting methods questionnaire sent to participants.

NMBAQCS Sorting Methods Questionnaire.

LabCode:

If your laboratory carries out NMMP sample analysis and your NMMP and non-NMMP sample analysis procedures differ please produce a copy of this questionnaire for both methods.

This questionnaire has been completed according to NMMP/regular/all (please delete appropriately) sample sorting procedures employed within this laboratory.

1.) Please list all taxa that you separate into adults and juveniles:

Taxa	Criteria for age division	Taxonomic level used for adults/juveniles
e.g. Nephtys	*Presence of subdermal eyespots*	*Genus*

2.) What constitutes a countable individual for the following taxa:

Taxa	Criteria for enumeration
e.g. Bivalves	*Hinge presence*
Bivalves Gastropods Maldanids Mysids *Please list others (if other than presence of head).....*	

3.) List all taxa which you would normally identify at a higher taxonomic level than species:

Taxa	Taxonomic level	Explanation (if necessary)
e.g. Autolytus	*Genus*	*Key features difficult to determine confidently*

4.A) Which of the following do you routinely extract and record:

Taxa	Extracted	Counted	Recording criteria	Taxonomic level	Included in biomass
e.g. Hydroids	*Yes*	*No*	*Polyps present*	*Species*	*No*
Hydroids					
Sponges					
Encrusting Bryozoans					
Erect Bryozoans					
Solitary Tunicates					
Colonial Tunicates					
Barnacles					
Hard Ostracods (Podocopida - benthic)					
Soft Ostracods (Myodocopida - pelagic)					
Benthic Copepods					
Pelagic Copepods					
Nematodes					
Invertebrate fragments					
Aquatic Insect larvae					
Parasites					

4.B) List any additional taxa that you would not record:

4.C) List any additional materials (non-faunal) that you record:

Taxa	Extracted	Counted	Recording criteria	Taxonomic level	Included in biomass
e.g. Tomato pips	*Yes*	*Yes*	*Presence*	*n/a*	*No*
Tomato pips					
Raspberry pips					
Kiwi pips					
Anthoprogenic matter					
Glass splatter					
Metal splatter					
Please list others……					

5.) If your samples contained stones with *Pomatoceros* tubes, *Sabellaria* reefs, barnacles, hydroids and encrusting bryozoans attached, how would you proceed with the sorting?

6.) If your samples contained several litres of 0.5-1mm and 1-4mm sediment fractions, how would you process these fractions?

7.) Do you routinely use any form of staining in your sample processing? If so give details and reasons for use.

8.) If you have any further comments please use the reverse of this sheet.

Thank you for taking the time to complete this questionnaire. Please return your completed copy as soon as possible to:

David Hall, Unicomarine Ltd., 7 Diamond Centre, Works Road, Letchworth, Hertfordshire, SG6 1LW
FAX: 01462-483103
E-mail: davidhall@unicomarine.com

Questionnaires will be collated and conclusions will be included in the NMBAQC Scheme Year Seven Annual Report.

Appendix III. NMMP Oligochaete SOP (provisional).

Unicomarine Ltd. Extraction/Recording/Biomass SOP for Macrobenthic Samples
NMMP Version 1.1 Oligochaeta

			Extracted*		Preservation		Recorded/Identification				Biomass			Key literature (not comprehensive)
Class	Family	Genus	All	In part	Alcohol	Dried	Enumeration	Present/absent	Tax. level**	Juv. separated	Weighed	Fragments incl.	Tubes/shells incl.	
Oligochaeta			☑		☑		☑		Varied	☑☒	☑	☑	n/a	Brinkhurst, 1971 & 1982, 1994 Oligochaete workhop notes, In-house tables & notes.
	Naididae		☑		☑		☑		Varied		☑	☑	n/a	
		Amphichaeta	☑		☑		☑		Species		☑	☑	n/a	
		Chaetogaster	☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971
		Nais	☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971, 1982
		Paranais	☑		☑		☑		Species		☑	☑	n/a	
		Stylaria	☑		☑		☑		Species		☑	☑	n/a	
		Uncinais	☑		☑		☑		Species		☑	☑	n/a	
	Tubificidae		☑		☑		☑		Varied (Family, except where stated below)		☑	☑	n/a	Brinkhurst 1971, 1982, In-house notes.
		Monopylephorus	☑		☑		☑		Family (except M.irroratus to species)		☑	☑	n/a	
		Limnodriloides	☑		☑		☑		Genus		☑	☑	n/a	
		Clitellio	☑		☑		☑		Species		☑	☑	n/a	
		Heterochaeta	☑		☑		☑		Species		☑	☑	n/a	
		Limnodrilus	☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971
		Tubifex	☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
		Tubificoides	☑		☑		☑		Species (except T.brownae, T.crenacoleus, T.diazi and T.pseudogaster, all as T.pseudogaster agg.)		☑	☑	n/a	
		Psammoryctides	☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Enchytraeidae		☑		☑		☑		Family (except Grania spp. to genus)		☑	☑	n/a	Brinkhurst 1982
		Grania	☑		☑		☑		Genus		☑	☑	n/a	
	Branchiobdellidae		☑		☑		☑		Genus		☑	☑	n/a	Brinkhurst 1971
	Aeolosomatidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Haplotaxidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Lumbriculidae		☑		☑		☑		Family		☑	☑	n/a	Brinkhurst 1971
	Dorydrilidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Glossoscolecidae		☑		☑		☑		Species		☑	☑	n/a	Brinkhurst 1971
	Lumbricidae		☑		☑		☑		Family (except Eiseniella tetraedra to species)		☑	☑	n/a	Brinkhurst 1982

*=some taxa will be counted in situ/subsampled if present in high numbers

**=minimum level required (good condition given)