ECA Regional Monitoring Blueprint

Specifications and Operating Procedures

Processing Requirements Protocol (PRP) for the analysis of macrobenthos samples: Eastern Channel Association Projects

Document Information

This document has been prepared jointly by Emu Ltd and Unicomarine Ltd to ensure that a standardised set of benthic sample analysis procedures are applied during analysis work that will be carried out on benthic samples taken during the ECA regional monitoring programme.

This SOP is a draft version that is under discussion. Further issues of this SOP will be circulated once a final version is complete. This SOP has been circulated with Blueprint Version v071005.

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1. Introduction

This document represents the first draft of a standardised specification for the processing of macrofaunal samples. It is specifically designed to ensure comparability between data produced by laboratories working on samples from the eastern English Channel for the Eastern Channel Association (ECA). It is also apparent that no specification currently exists detailing processing requirements to a level sufficient to allow inter-laboratory comparability without extensive data truncation. For this reason, it is hoped that the present document will serve as a starting point for data comparability at a national level in the future. This processing requirements protocol (PRP) is distinct from a standard operating procedure (SOP) in that it details only elements essential to the processing of the samples; an SOP includes procedures recommended within a particular laboratory, which could be done differently elsewhere while producing the same results. For example, counting all polychaete heads would be specified in the PRP, while separating polychaetes into family pots before identification would be an optional recommendation in the SOP of some laboratories.

Currently, organisations requiring macrofaunal sample analysis provide broad specifications that are interpreted differently by different laboratories, such that the only reliable means of ensuring data comparability has been to use a single laboratory. Different laboratories have different working methods and skills and different taxa are identified to varying levels of accuracy by each laboratory. Standardisation of the process is possible between people within a large laboratory but only through either constant communication or a very precise protocol. Sample processing specifications are generally vague and do not allow comparison between the work of different laboratories. There has long been a need for more precise specifications. The opportunity is taken here to begin the development of documents for the current project that will eventually prove useful on a national level. We would propose that the products become public domain and form a basis of later national standards, possibly through the National Marine Biological Quality Control (NMBAQC) Scheme.

For the Eastern Channel samples, there is a need for clear processing requirements and coordination between the organisations that will work on the samples, now and in the future. For the current stage of the work the main contractor to the ECA (Emu Ltd) will oversee the project, while the initial development of protocols will be through Unicomarine Ltd. The most urgent requirement is for a concise document to detail the nature of the data to be produced from the samples by each laboratory. This is the first draft of the processing requirements protocol (PRP) document. The aim is to ensure that data are comparable between laboratories.

The following headings detail the stages in macrofaunal sample processing. They are followed by specifications for the current project, with reference to tables or other documents, as necessary.

2. Benthic Laboratory Team Structure

The overall analysis project should be managed by a Primary Contract Manager (PCM). Each laboratory involved in analysis should appoint a Project Manager (PM) to assume responsibility for the conduct of the macrofaunal analysis. Each Project Manager should appoint a team to conduct the analysis, quality control and data management of the project. All procedures should be documented; all personnel involved in the project should be named and their work detailed. All documentation should be retained and made available to the PCM, as necessary. Each laboratory should work to its own standard operating procedure (SOP), approved (by the PCM), and available for inspection by other laboratories. Any procedures different to those listed in this document, or noted as requiring agreement, must be discussed with the PCM and all authors of this document (the Channel PRP working group) before proceeding.

3. The Nature of the Samples

The assumed starting point is that each sample is in a clearly labelled watertight container (or group of containers clearly identified as representing a single sample). Each sample is assumed to be complete (without loss of material prior to containment) and adequately preserved. Specifications for fieldwork and sample collection will be detailed elsewhere.

4. Sample Logging

Each laboratory should produce and maintain a written log of all samples connected with the project within its premises. The log should be available electronically and sent to the PCM for confirmation prior to sample analysis. The log should then form the basis of a Sample Progress Form (SPF).

5. Washing and Sieving

The name of the person sieving each sample should be recorded in the SPF. The laboratory SOP must specify how personnel are supervised while sieving or how their suitability to work alone is ensured.

Samples containing formalin should be processed outside on a sorting table or in a fume cupboard. All sieves used for washing must be certified as suitable. The sieves should be cleaned thoroughly before use with each sample to avoid contamination from other samples. Each sample must be sieved over a standard 1 mm mesh. No material, liquid or sediment, must be lost from the sample prior to extraction unless it passes through the sieve. Coarser sieves may be used to subdivide a sample into manageable fractions but must be placed above a retaining container or another sieve, of 1 mm or finer, whenever they contain a portion of the sample. Samples should be sieved at 1 mm until no particulate material passes through the sieve.

Samples should be divided into a light and a heavy fraction during sieving. The light fraction should include all material that can be poured off the sample after agitation in water. It is also recommended that coarse, heavy material is divided into sieve-size fractions for ease of sorting. All fractions must be clearly labelled at all times and no container that may contain biological material should be left without adequate preservation for more than 24 hours.

6. Sorting

The name of the person sorting each sample should be recorded in the SPF. The laboratory SOP must specify how quality control is ensured during sorting. All QC procedures should be documented and the form of documentation approved by the PCM.

Exceptions to the requirements in this paragraph are listed below but must be agreed with the PCM. All biological material deemed to have been alive at the time of sample collection must be removed from each sample; where there is doubt as to whether material may have been alive at the time of sample collection, it should be extracted. It is advised that abundant and easily identifiable taxa are counted and that taxa are separated into major taxonomic groups during extraction. It will be necessary to break tubes, bored shells and soft rock to extract cryptic fauna. All biological material must be preserved in 70% industrial methylated spirits (IMS).

- **Taxa occurring in very high numbers may be subsampled or counted in situ (see below).**
- **Sessile organisms deemed to have been small enough to pass through a 1 mm sieve had they been loose may be ignored (agree taxa with PRP working group).**
- **Certain sessile calcareous organisms, such as coralline algae, encrusting bryozoa or barnacles, may be preserved in a dried state.**

Residues from which all biological material has been removed must be retained until completion of in-house QA. As no external QA of sorting is proposed, residues may be discarded after this.

6.1. Subsampling and *In situ* Counts

If the total count of any taxon in the light fraction of a sample is liable to exceed 200, a one quarter subsample of the light fraction may be prepared for counting that taxon. If the total count of any taxon in the heavy fraction of a sample is liable to exceed 100, that taxon may be counted in situ. Less abundant taxa must be counted in full, from the whole sample. Subsampling of one quarter of the entire light fraction (all taxa) of a sample may be permitted if the settled volume of such material in water exceeds 200 ml. Smaller subsamples may be used with the express agreement of all laboratories involved. All samples requiring subsampling must be agreed with the PCM and details of the procedure used must also be agreed, summarised in the SPF and detailed in the SOP. Subsample residues should be stored in a separate container to the main sample.

7. Identification

The name of the person identifying each sample should be recorded in the SPF. The involvement of more than one person per sample should also be documented. The laboratory SOP must specify how quality control is ensured during identification. All QC procedures should be documented and the form of documentation approved by the PCM.

All organisms removed from each sample must be identified to the most accurate taxonomic level practicable, usually species. Nomenclature must follow the most recent published or NMBAQC standard literature (see below). The practicable level for identification differs between taxa and laboratories but will be standardised as far as possible by the methods detailed below.

After identification, the identifier must separate the material into a reference collection (see below) and taxonomic groups, as required for biomass. All non-countable portions of animals must be added to the separate containers at this stage.

7.1. Literature

A preliminary version of the NMBAQC standard identification literature list will be circulated. Each laboratory will obtain any published literature considered essential (after discussion with the PRP working group). Unpublished literature deemed necessary will be circulated by the PRP working group. The working group must be notified of any literature used by any involved laboratory that is not on the literature list; it will then be added, if deemed important.

7.2. Exchange of Data Sets

Prior to identification, each laboratory must circulate a data matrix, with a complete taxon list in taxonomic order, which they have produced from a survey of a similar habitat to the East Channel samples. The PRP working group will then discuss differences in recording style and reach a conclusion on a standard name for each taxon.

7.3. Taxonomic Discrimination Protocol (TDP)

Identifications must be to the species level unless otherwise stated in a taxonomic discrimination protocol (TDP), to be produced in preliminary form prior to identification. Any taxon recorded at a higher taxonomic level by any laboratory or for which adults are separated from juveniles must be agreed by the PRP working group and added to the TDP. The first draft will detail, to the class (or possibly order) level, which taxa would be quantified in what way and at which taxonomic level they should be identified.

Project specifications often ask that animals be identified to species, where possible, and this would be the vague statement for the main groups in the first draft. Later versions will detail what is to be considered possible/practicable at more precise taxonomic levels.

7.4. QC of dentifications

Each laboratory must follow its own in-house quality control procedure for the samples. In additional, there will be an exchange of reference collections between laboratories, with the aim that each laboratory sees and agrees upon every taxon recorded in the project as a whole. The entire reference collection from each laboratory must be circulated at the end of analysis. Difficult specimens should also be circulated in small numbers before this, by agreement. Reference collections should then be returned to each analysing laboratory for storage.

7.5. Enumeration

Enumeration would normally be carried out during identification, by the identifier. All taxa that occur as discrete individuals should be counted by heads, if they have distinct heads, or by hinge lines for bivalves or mouths for echinoderms. Exceptions may be noted in the TDP, by agreement. Taxa that occur as discrete individuals but for which only non-countable portions are present in a sample should be recorded as 'Present'. Plants and colonial taxa should be recorded on an abundance scale based on the SACFOR scale. The PRP working group should be notified where there is doubt as to how to record a particular taxon and details will be added to the TDP.

All identifications and enumerations should be recorded on an individual Sample Data Form (SDF).

8. Biomass

The name of the person weighing each sample should be recorded in the SPF. The laboratory SOP must specify how personnel are supervised for biomass or how their suitability to work alone is ensured.

Blotted wet weight biomass is required by major taxonomic groups for each sample. The major groups are to be divided as follows:

- **Oligochaeta,**
- **Polychaeta,**
- **Crustacea (excluding barnacles),**
- **Mollusca,**
- **Echinodermata,**
- **Others (excluding non-countable and sessile – attached – taxa).**

NB: sessile taxa are not weighed

Animals from each group should be removed from IMS with forceps (or sieved out, if necessary) and placed on absorbent paper. They should then be gently dried (blotting with tissue is recommended) until no free surface moisture is apparent and weighed to an accuracy of grams to 3 decimal places. Care must be taken to avoid damage to the specimens; particular care must be taken with reference collection material, which would be treated separately from the main part of the sample. All animals should be weighed intact, including the shells of molluscs and tests of echinoderms and the tubes of small polychaetes, where removal would cause excessive damage or time loss. Larger animals should, however, be removed from their tubes. Details of taxa to be removed from tubes for biomass will be specified in the TDP. The biomass data collected from the reference collection material must be added to the relevant major group and recorded on a form (e.g. SDF). Biomass data should be supplied to the PCM as blotted wet weight.

9. Storage

The processed sample will comprise three parts, as listed in the following headings. All stored material should include internal labels clearly written or printed with an alcohol-resistant ink and detailing all relevant sample information.

9.1. Residue

Sediment from which all biological material has been extracted may be discarded, following in house QC, and a record of disposal made on the SPF. Sediment containing animals counted in situ or subsamples with non-extracted animals should be retained in IMS or formaldehyde solution. All residue containers to be retained should have external labels detailing the nature and concentration of the preservatives contained, as well as sample/subsampling details; the latter information should also be on the internal labels. Subsample residues should be stored in a separate container to the main sample.

9.2. Extracted fauna

All extracted fauna should be retained. Some of the material should be separated as a reference collection (see below). The remainder should be stored in watertight containers, clearly labelled with sample and survey details, separated into the taxonomic groups used for biomass (with the addition of a container of non-weighed taxa). With the exception of certain sessile calcareous organisms, which may be dried (see above), fauna should be stored in 70% IMS.

9.3. Reference collection

An example of each of the taxa recorded by each laboratory must be retained in a separate container, as a reference collection. Each reference container should include all of the specimens and identifiable portions of that taxon from its sample (exceptions may be made by agreement if a proportion of specimens is needed for another purpose). A reasonable effort should be made to ensure that those specimens selected for reference are among the most suitable for that purpose (in terms of condition, size range and numbers of individuals in the reference pot). Multiple reference lots should be made for rare or taxonomically difficult taxa. Reference lots must be clearly labelled and preserved as for the extracted fauna. A record must be kept (e.g. on the SDF) of which specimens have been removed from the sample for reference. Reference collections should be maintained by each analysing laboratory, after completion of QC.

10. Data management

All information documented during processing must be written by hand on a series of laboratory forms and retained for later inspection, if necessary. The nature of the forms would follow each laboratory's SOP but should include, as a minimum:

- **Sample Progress Forms (SPF) with analysis details and QC ,**
- **Sample Data Forms (SDF) with taxa, counts, biomass figures and reference collection selections.**

The information from the forms should be transcribed electronically and supplied to the PCM, on completion of the project.

The name of the person entering data into an electronic form for each sample should be recorded in the SPF. The laboratory SOP must specify how quality control is ensured during data entry. All QC procedures should be documented and the form of documentation approved by the PCM.

11. Development of the PRP

Any issues noted by laboratories as requiring specification or clarification but not included in this PRP should immediately be brought to the attention of the PRP working group. An amended version will then be circulated.

12. Abbreviations

NMBAQC	National Marine Biological Analytical Quality Control
PCM	Primary Contract Manager
PM	Project Manager
PRP	Processing Requirements Protocol
QC	Quality Control
SDF	Sample Data Form
SOP	Standard Operating Procedure
SPF	Sample Progress Form
TDP	Taxonomic Discrimination Protocol

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