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REVIEW OF STANDARD OPERATING PROCEDURES (SOPs)

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ABSTRACT

This study is a review of twenty-three Standard Operating Procedures (SOPs), submitted by participants of the National Marine Biological Analytical Quality Control scheme (NMBAQC). SOPs covering both field sampling methodology and laboratory analysis were reviewed. The aim of the study was to identify and report on examples of good practice and, in cases where inconsistencies were identified, to advise on means for future improvement, so as to promote harmonisation between laboratories.

The majority of SOPs broadly fulfilled the requirements of the task. However, the level of information provided was, on occasion, insufficient to ensure that tasks could be performed by an individual, not familiar with procedure, to an acceptable standard without external guidance. Differences between laboratory's SOPs relating to equipment dimensions, reagents and AQC procedures were also identified. Recommendations for future improvements are given.

1. INTRODUCTION

This review, covering both benthic field sampling methodology and laboratory analysis, was undertaken on behalf of the NMBAQC Committee as part of its remit to improve the quality of benthos data generated from sampling programmes in UK estuaries and coastal waters. The aim of the review was to identify and report on examples of good practice and, in cases where inconsistencies were identified, to advise on means for future improvement, so as to promote harmonisation in approaches between laboratories. SOPs were submitted voluntarily on the understanding that no reference would be made to individual laboratories.

A Standard Operating Procedure is 'a written procedure which describes how to perform certain routine laboratory tests or activities normally not specified in detail in study plans or test guidelines' (Good Laboratory Practice Regulations, 1997). SOPs are an integral part of any Quality Assurance programme and help to ensure that data collected by a laboratory are scientifically valid, comparable and adequate to meet the study objectives. This is particularly important when different laboratories are engaged in the input of data to the same study such as the UK National Marine Monitoring Programme (NMMP) (see Anon., 2001). In these instances it is vital that the process of sample collection and analysis are consistent across all participants. This need is addressed at a generic level by existing guidelines such as the NMMP 'Green Book' (Anon., 2001) and guidance by Rumohr (1999). The purpose of a SOP is to provide a detailed and practical expression of the implementation of such guidelines, usually in a laboratory-specific context. An absolute requirement that all laboratories carry out tasks in exactly the same way would be unrealistic, as procedures are often legitimately tailored to the needs of the specific laboratory and the environment in which the staff operate. Where approaches differ between laboratories it is essential to establish that the differences do not have adverse implications for the quality and comparability of data sets.

A total of twenty-three SOPs, covering five different tasks (sub-tidal sampling, inter-tidal sampling, trawl sampling, laboratory analysis of benthic macrofauna and particle size analysis) were received. SOPs were submitted from seven different laboratories participating in the NMBAQC scheme. Of these seven laboratories two were from the same parent organisation.

The outcome of detailed examination of all submitted SOPs was tabulated, allowing a direct comparison of each of the individual components. This information can be found in Appendices 1-5. A summary of the main findings can be found in the following sections (2-9) with italicised suggestions, where appropriate, for means of improvement. Concluding remarks, along with a list of the major recommendations arising from the review, are given in Section 10.

2. GENERAL CONSIDERATIONS FOR ALL SOPS

The review of SOPs highlighted significant variability in the structure of the documents, and in the level of detail provided. However, several examples of good practice were identified and these are outlined below. Further discussion on the characteristics of a good SOP can be found in Rees (1999).

2.1 Layout

The layout of SOPs varied enormously from unstructured accounts, through to a logical separation of each aspect of the task into different sections. A useful feature of many of the SOPs was the recording of the following information at the top of each page: procedure title, issue number, author, page number, total number of pages and procedure reference number.

The information outlined above allows users to ensure that they are working to the correct and most up-to-date procedure. The adoption of a standard structure (evident in the submissions of a number of laboratories) along the following lines is to be recommended:

- **Purpose and principle**

This section was present in the majority of SOPs and provides an overview of the task to be performed, and

the use of the resulting data. Supporting literature and other related SOPs were also referred to in this section. Some SOPs also stated any limitations of the procedure and what implications these may have for the final use of the data.

The inclusion of this section in an SOP is considered very important, as individuals who clearly understand the purpose of the task are more likely to perform it to a high standard.

- **Personnel**

The majority of SOPs stated both the number and level of experience of personnel required to carry out the task. In most cases, the stated requirement is that individuals should be either adequately trained in the procedure or required to work under the supervision of an appropriately trained person.

Clearly, insufficient training could be a significant, and unacceptable, source of error in the resulting data. In this section the number of individuals required for a task, and the level of training required, should be explicitly stated.

- **Equipment**

In some SOPs equipment is simply referred to in the text, whilst in others a check-list is provided. One procedure usefully stated that spares should be available in case of malfunction or loss.

The inclusion of an equipment list makes it easy for an individual to check that they have all the necessary equipment before embarking on fieldwork or initiating a laboratory procedure. The list should provide enough information about each of the items to avoid any confusion and to allow items to be replaced. The procedure should also give details of any servicing requirements.

Vessel

Vessel requirements were not dealt with by all the SOPs, perhaps because, for some laboratories, the same vessel is used on a regular basis. The following basic considerations were highlighted in two of the SOPs:

1) Does the vessel have a winch, able to lift the grab, and is there sufficient warp for the sampling location?

2) It should also be possible to monitor how much warp is let out so that the device can be gently lowered onto the seabed. This can be achieved using a meter wheel or markings on the warp in conjunction with a depth-sounder.

3) Is the vessel fitted with a gantry or derrick for lifting the grab over the side?

4) Is the vessel equipped with a positioning system and echo sounder?

5) Does the vessel have a deck wash hose, if on-board processing is to be carried out, and is there sufficient deck area for the equipment?

6) Is the vessel able to maintain its position on station using either the engine or an anchor? This is essential as any drift can cause toppling of the sampling device at the seabed.

It is important that the choice of vessel is given proper consideration in SOPs in case the normal vessel is not available (e.g. in the case of mechanical breakdown). Of particular importance when chartering a vessel the SOP should either stipulate the vessel requirements or, at least, refer to the appropriate laboratory's guidance in these matters. When selecting a vessel, in addition to the necessary safety considerations (see DETR, 1998), the questions detailed above should be asked.

- **Reagents**

The amount of information provided by different SOPs varies from a simple statement of the reagent required through to detailed instructions for their preparation, use, storage and any relevant safety issues.

It is recommended that SOPs either provide detailed information, or at least refer to the relevant 'Control of Substances Hazardous to Health' (COSHH) assessments. Relevant safety information, including reference to COSHH assessments can be provided here or under 'safety' below.

- **Safety**

This aspect is considered in a number of SOPs.

Attention to safe working practices is essential, not only for the obvious purpose of protecting personnel but also because of implications for the quality of the eventual data. For example, a swinging grab may clearly be a danger to personnel but may also result in damage to the equipment. Any damage to the grab may in turn affect the quality of samples taken. It is recommended that SOPs identify potential dangers and appropriate actions to minimise risks. They may also, where appropriate, give details on how to deal with accidents (e.g. formalin spillage). SOPs should refer to any other relevant safety information (COSHH assessment, Risk Assessment, Safety handbook, etc). SOPs should also list any protective clothing that is required whilst carrying out certain tasks.

- **Procedure**

In a number of SOPs the task is broken down into a logical series of stages.

Separation of the procedure into a series of stages facilitates ease of use, which should reduce the likelihood of mistakes being made. Each individual stage should provide sufficient information to avoid any confusion.

- **Quality control**

This important consideration is referred to in the majority of SOPs although there are differences in the specific approaches.

It is recommended that individual laboratories examine each stage of a procedure and, where there is the potential for variation in the quality of output, decide upon acceptable boundaries. Where a specific level of accuracy or precision is required then this should be stated and a Quality Control procedure established to ensure that standards are met. At the very least, the quality control procedure should document the standard attained. By highlighting the quality of the data it is possible to ensure that it is not used in an inappropriate way.

- **Figures and plates**

In one procedure a photograph provided a useful aid in the setting up of a complicated piece of equipment.

Photographs and diagrams can be very useful to clarify certain procedural steps and should be included where appropriate.

- **References/Bibliography**

This section, found in most of the SOPs, included references quoted in the text and/or useful additional literature, including taxonomic keys.

The inclusion of an up-to-date list of appropriate references, including taxonomic keys for laboratory work, is to be recommended.

- **Appendices**

In a number of SOPs the following information was usefully incorporated into this section: examples of datasheets; equations for any calculations; file formats for input of data to relevant software packages; chemical hazard sheets and instrument calibration forms.

The inclusion of relevant information in Appendices is recommended, as this will limit the necessity to access literature sources from elsewhere, and hence may save valuable time.

3. COMMON CONSIDERATIONS FOR FIELD SAMPLING SOPs

3.1 Reagents

3.1.1 Formalin

Safety information

Formalin is identified, in all except two of the SOPs, as being a toxin of 'medium hazard'. Two of the SOPs also state that it is carcinogenic and an irritant. In all these SOPs the wearing of eye protection and protective gloves is required and one SOP also requires personnel to wear wet weather gear whilst preparing dilutions of the substance. COSHH (Control of Substances Hazardous to Health) forms are referred to in only one SOP.

It is recommended that all SOPs include the information outlined above. It is also recommended that SOPs provide, or refer to, instructions on how to deal with an accident.

Preparation

The level of detail provided for the preparation of a working solution of formalin varies enormously between laboratories. A 10% buffered solution of formalin is normally prepared from 40% formaldehyde, Borax (used as a buffer) and seawater. Two SOPs state that extreme caution should be exercised when transferring neat chemical from one container to another and recommend the use of a funnel or a siphon.

It is recommended that explicit instructions for the preparation of the formalin solution be given in all SOPs.

Storage container

As recommended in a number of SOPs, a rigid polypropylene container provides a safe means for storing a solution of formalin at sea. The diluted solution should be dispensed from either an aspirator or a 'pressmatic' dispenser. One SOP rightly states that formalin should not be stored or dispensed inside the vessel.

All containers should be firmly secured and stored on the deck of the vessel.

Use

In most SOPs this usually involves the addition of a quantity of 10% formalin similar in volume to that of the sample, including any supernatant water, to achieve a final concentration of around 5%. However, in one SOP it is added in the ratio of 3:1 for muds. For this

reason sample containers should not be filled more than half-full of the sample and supernatant. If the container is overfilled then some of the supernatant liquor should be decanted off using a sieve to prevent the loss of any animals during this process. As an alternative, some SOPs allow the addition of a small volume of 40% formalin.

The addition of such a concentrated solution of formalin should not be encouraged as it is not advisable to deal with such concentrated solutions on a vessel, other than when preparing dilutions and only whilst observing the necessary safety precautions.

SOPs recommend between 72 hours and four days, as a minimum, before sample fixing has occurred. Two SOPs also recommend that samples are inverted or shaken to achieve thorough mixing of the fixative. Three laboratories state that polymerisation of formaldehyde occurs below 5°C, removing its fixative qualities.

Samples should be stored above 5°C.

3.1.2 Rose Bengal

Safety information
Rose Bengal is identified by half the SOPs as being an extremely hazardous carcinogen which should only be handled in a well-ventilated area or under fume extraction, whilst wearing gloves and eye protection.

All SOPs should include this safety information.

Preparation
In one procedure Rose Bengal, in its powder form, is added to the formaldehyde solution in the field. This operation is carried out in a well-ventilated area. In other SOPs a concentrated aqueous solution is made up in the laboratory, for addition to the fixative solution in the field. Alternatively it can be added, again in the laboratory, directly to the concentrated fixative solution. Where added the amount varied up to a maximum final concentration of $0.1g\ l^{-1}$.

A well-ventilated area does not guarantee that an individual will not come into contact with the chemical, particularly when in a powder form. It is therefore recommended that all SOPs insist upon the use of this chemical under fume extraction only. It must therefore be used in either of the other two ways described above. It is also recommended that some consensus is reached as to what constitutes an appropriate amount as too much may obscure certain features and make identifications difficult.

Storage
In the SOP where a concentrated solution of Rose Bengal is used in the field the solution is stored in a suitable container (e.g. 'safebreak' bottle with a pipette dispenser).

3.2 Safety

Protective clothing
It is recommended in most SOPs that personnel wear a hard hat, toecapped boots or shoes and preferably a life jacket or harness when operating the gear, particularly on a small vessel.

It is recommended that explicit instructions concerning the wearing of protective and safety gear be given in all SOPs.

3.3 Procedures

3.3.1 Position fixing

Some SOPs still refer to 'DECCA Navigator' which is now obsolete. This illustrates that some SOPs require updating! Most SOPs make use of a Global Positioning System (GPS), often with a differential signal (DGPS) which further increases the accuracy of position fixes. Visual transects (i.e. where stations can be referenced against land based structures) are still used in a number of SOPs and, to avoid any potential for confusion, they are recorded in one SOP with a photograph and description. One SOP gives details on the area around a station position within which sampling of that station can take place. A circle, of diameter 50 metres, known as a 'range ring' defines this area and the use of tailored software is presented as a visual navigational aid for vessel manoeuvring.

The ability of a ship to manoeuvre into a 50 m or smaller range ring may be a realistic objective for small vessels operating in estuaries but it may be less realistic for larger, less manoeuvrable vessels operating offshore and hence it is difficult to give specific guidance. Realistic operational limits for acceptable sampling should, however, be specified, and these may vary according to the objectives of different surveys.

3.3.2 Information recorded at each station

All but one of the SOPs give explicit instructions on the information to be recorded at each station. Information is either recorded on pre-printed sheets or in a logbook. The sheets contain prompts for all the information required and should minimise the possibility that information is not recorded.

It may also be useful to offer a list of options for recording certain variables (e.g. sediment type), to improve objectivity. An advantage of a sturdy book format is that all the information is contained within one unit; also, it is perhaps less likely to be damaged in the field situation than individual sheets.

3.3.3 Labels

The majority of SOPs correctly stated that labels should be applied to both the outside and the inside of any sample containers. The inside, waterproof, label should accompany the sample through each stage of processing.

3.3.4 Replication

The number of sample replicates required by the SOPs varied between 1 and 10.

SOPs must be flexible in this respect, because the number of replicates required will vary with the objectives of the survey.

3.3.5 Attempts

Only two SOPs deal with the possibility of failure when collecting a grab sample. Both SOPs state that three attempts should be made at the original station position. If this fails to provide a sample then further attempts should be made at progressive distance, until suitable sediment has been located. The co-ordinates of this new position should be noted.

Although the example above relates to grab sampling the possibility of failure to collect a suitable sample should be considered in all field-sampling SOPs. Consideration should be given to the use of an alternative sampling device, provided that it yields comparable information to the gear of first choice. Appropriate guidance is therefore essential.

4. SAMPLING FOR SUB-TIDAL SEDIMENTS

4.1 Equipment

4.1.1 Grab

Van-Veen/Day Grab
There is agreement on the dimensions of both the Day and Van Veen grab, in order to achieve a sampling area of 0.1 m^2. One laboratory evidently uses both types of grab but the procedure offers no guidance on the circumstances in which each should be used. Both types of grab are fitted with self-closing windows to allow the inspection of the sample surface following deployment, which is important both for determining the acceptability of a sample (see Section 4.3.2 – Sample rejection criteria) and for the extraction of any sub-samples.

Hand-held Van Veen Grab
Hand-held Van Veen grabs are deployed from small vessels in two of the SOPs in situations where it is impossible to deploy the larger 'parent' device (e.g. in shallow water not suitable for a larger vessel). All four laboratories using this device use a grab with a different surface area, ranging from 0.019 m^2 to 0.025 m^2. One laboratory uses two grabs of differing surface area.

There is clearly a need for standardisation in the size of this device. Attention should also be given to the serious implications for data comparability, arising from the collection of samples of different surface area.

Hamon Grab
A Hamon grab is used in one SOP for gravel substrates. This device consists of an articulated bucket, within a weighted frame, which scoops up a sample and then closes against a plate to prevent loss of material on retrieval.

The deployment of conventional grab sampling devices on gravelly substrates is frequently problematic, due to the tendency for the jaws to be jammed open by larger particles. The mode of operation of the Hamon grab much reduces this source of error. For this reason it is recommended that this device be used on gravel substrates, at least for biological sampling. The disadvantage of the Hamon grab is that it does not provide an undisturbed sediment surface on retrieval, such as may be required for the collection of sub-samples for later chemical analyses. Additional sampling using, for example, a Shipek grab, may be appropriate for this purpose

Weighting
Half of the SOPs call for the addition of weights to the Day grab to allow for optimal penetration of the sediment. Of the laboratories using a Van Veen Grab, none appeared to provide the facility for the addition of extra weights.

The weighting of a grab may be changed between stations in a survey so that the volume sampled can be kept as constant as possible. According to Rumohr (2000) the standard grab should weigh between 35-40 kg and there should be a facility to increase this up to 100 kg for use in coarser sediments. Any additional weights should be removed on very soft sediments to prevent the device from excessive sinking into the sediment on deployment. It is recommended that all grabs should have the facility for the addition of extra weights.

4.1.2 Corers

According to two of the reviewed SOPs, the Craib corer, unlike either the Van Veen or Day grab, does not produce a bow wave. For this reason it is used for the collection of samples which present an undisturbed sediment surface on retrieval. The Craib corer also tends to minimise distortion to the vertical structure of sediments, compared with grab samples. One SOP also

states that corers are more likely to sample deep dwelling fauna, although this will clearly depend on the depth of penetration. A 'Box corer' is used by another laboratory for the same reasons. One procedure also covers the use of hand-held cores for use by divers: either a Perspex 6 cm diameter core or a stainless steel core of 5 cm diameter, although no information is given about the circumstances in which each of these cores should be used.

The dimensions of the different coring devices vary, even within the same organisation. The Box corer referred to above samples an area of 0.025 m^2 whilst the Craib corer samples an area of 0.0028 m^2 or 0.0027 m^2.

Core dimensions need to be standardised so that results can be compared. There also needs to be further evaluation of the relative merits of the Box, Craib and other corers.

4.1.3 Hopper/Grab stand

Reference was made to this piece of equipment in four out of the six SOPs covering sub-tidal sampling.

The absolute dimensions of this piece of equipment are unimportant so long as it allows the efficient transfer of the whole sample into an appropriate collection vessel.

4.1.4 Sieve

Mesh size

All laboratories use both 1 mm and a 0.5 mm sieve meshes in survey work. Four of the SOPs give no guidance as to which size sieve should be used in which circumstances. The other two state that a 0.5 mm sieve should be used in estuaries and a 1 mm sieve at offshore sites.

Rees et al. (1990) state that a 1 mm mesh is suitable for wide spatial surveys and either a 0.5 mm or a 1 mm mesh should be employed for temporal trend analysis, depending on which is the most likely to retain the most adult fauna. Clearly the choice of mesh size will be dependent on the objectives of the survey. However, for any given survey repeated over time or contributing to a larger (inter-laboratory) programme, instructions need to be explicit, and strictly adhered to.

Square mesh sieves are used in all the SOPs reviewed.

The advantage of a square mesh sieve is that it has a larger percentage open area than the round mesh variety and is therefore less likely to become clogged during the sieving process. The disadvantage of the square mesh sieve is that the diagonal distance is larger than the stated mesh size. For this reason results obtained using square and round mesh sieves may not be comparable. According to Rumohr (2000), round sieves cause less damage to specimens. Despite this advantage it is recommended that square mesh sieves continue to be used as a reflection of established UK practice and because they are less likely to become clogged in the field situation.

Height

Only one procedure calls for a minimum height of the sieve surround (of 10 cm and preferably 15 cm). One procedure requires that the sieve should be placed inside another larger sieve of the same mesh size in case of any spillage.

The deeper the sieve the less likely that any sample will be lost through spillage, either through splashing or overfilling.

Size

The nature and size of sieve-mesh supports varied: from circular (20 cm diameter) to rectangular (45 cm x 70 cm). Three of the SOPs state that sieves must conform to BS410.

The sieve should have a mesh that can be easily cleaned and the sieve should be large enough to prevent spillage during the processing stage. However, the sieve should be of a manageable size, to facilitate the easy transfer of the sample into the storage container. As long as the sieve meets these criteria then the absolute dimensions and construction material are of little consequence. Sieves should be discarded at the first sign of damage to the mesh.

4.2 Procedure

4.2.1 Gear deployment

Only three of the SOPs provided guidance on deployment. Two of the three SOPs state that the deployment rate should be slowed as the device nears the bottom. The other procedure stated that the deployment rate should not exceed 1 m s^{-1}. Advice on the rate of retrieval ranges from raising gently at first to 'not exceeding 1 m s^{-1} initially, followed by 3 m s^{-1}'. Only one procedure states that the winch operator should pause to allow the device to bite.

It is recommended that instructions for the handling of the gear should be given, even where an experienced crew is routinely used in the collection of samples, as personnel and vessel used may change. Of particular importance is the rate at which the gear is deployed, as this will affect the bow wave that the gear produces. For this reason it is recommended that the deployment rate be slowed as the device nears the bottom. The winch operator must therefore be aware of both the water depth and the quantity of warp that has been let out. On making contact with the bottom the winch operator should pause before raising the device, slowly at first. This allows the jaws of a grab to fully close, or

for full penetration of a corer and the efficient operation of its closing mechanism. It is difficult to see how the rates of deployment and recovery stated in one of the SOPs (see above) and in the generic guidance available can be accurately adhered to at all times, particularly when a meter wheel is not available and/or the weather is rough, causing unpredictable vessel heave. However, every effort should be made to standardise practices so as to maximise sampler efficiency and therefore data quality.*

4.2.2 Sample collection

Particle Size Analysis (PSA)

There are differences amongst SOPs about whether sediment collected for PSA should be taken as a sub-sample of the material collected for benthos or from an entirely separate replicate sample.

It is recommended that PSA samples are taken as a sub-sample of the sediment collected for benthos. This allows a direct comparison with the benthos of that sample, with the proviso that the size of the sub-sample is sufficiently small so as not to significantly affect the outcome of later laboratory processing of the retained biological material.

Another procedure used a 5.9 cm diameter core for the collection of a 300 g sample.

There is some debate over what constitutes an appropriate amount of material required for particle size analysis (see PSA procedure). The use of a core of 5.9 cm diameter will lead to the removal of a significant proportion of the total surface area of a grab sample (0.1 m^2). The result of using devices of different dimensions to obtain sediment sub-samples is to risk prejudicing the comparability of data between laboratories. There is a clear need to decide on both the dimensions and depth of deployment of a suitable sub-sampling device, bearing in mind the distribution of organisms in the sediment.

A modified 50 ml syringe with a 3 cm diameter, deployed to a depth of 5cm, is usefully used in one procedure for the collection of sands and muds.

The NMMP 'Green Book' (Anon., 2001) states that samples should be taken from the surface down to a depth of 5 cm. Samples should be kept cool and frozen as soon as possible to prevent decomposition from affecting grain size.

Meiofauna

In one procedure meiofauna are collected from a Day grab, in the same way as the PSA sample, using a modified 50 ml syringe.

Day grabs can cause distortion of the sample and some of the surface material may be washed out if the grab fails to close properly. For these reasons collection of undisturbed cores using alternative sampling methods are preferable, but in weather conditions which make the use of a coring device difficult this method of collection provides an acceptable (if more variable) alternative means of sample collection.

Macrofauna

Sieving

The sieving method employed varies amongst laboratories. Most favour the breaking up of the sample using gentle hose pressure, in the receptacle used to collect it from the grab. Another laboratory uses a sprinkler placed beneath the sample. According to this procedure the technique helps prevent sieve clogging and has been shown to produce better quality (i.e. less damaged) samples of the fauna. It also removes the effect of sampler bias that can result from varying treatment regimes (e.g. hose pressure) employed by different individuals.

The use of the above sprinkler technique can be recommended, subject to wider testing of its efficiency. There may also be health and safety benefits, as individuals are not required to bend over a sieving table for long periods.

Fractionation

In one procedure, depending on the nature of the material, the sample can be stored in two fractions of 1-5 mm and >5 mm. This is helpful for samples with a significant coarse component.

This fractionation of the sample may assist with the preservation of the sample, in that the smaller and more delicate fauna are less likely to be damaged by some of the larger material. This practice is therefore to be recommended, especially in gravel areas which present a large range of particle sizes.

Puddling

This action, employed in a number of SOPs, helps further reduce the bulk of the sample. The sieve containing the sample should be gently moved up and down in water. One procedure recommends correctly that a side to side action should be avoided as this can damage the specimens through abrasion on the sieve.

Transfer of sample to container

Only one procedure gives details on how material should be transferred from the sieve to the final sample container.

During this stage in the process there is the potential for loss of sample material and therefore appropriate

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* *The proposed ISO guidelines state that the deployment rate should not exceed 0.2 m sec^{-1} between 5 and 10 m from the bottom.*

means to avoid this should be specified in all SOPs. The sample should be backwashed into the container using a funnel or other 'foolproof' device. The entire process should be carried out over a suitable container, such as a 'fish box' or a larger sieve, in case of any spillage. The sieve should be checked for any enmeshed fauna.

Hose pressure
This factor is given consideration in the majority of SOPs. Hose pressure should be adjusted as necessary to avoid damage to the fauna. One procedure recommends the use of a hand, placed over the hose outlet, to control the pressure if this cannot be achieved directly.

Avoiding excess hose pressure is particularly important in the initial stages of the process during which time most of the lighter fauna are dislodged from the sediment en route to the sieve mesh.

The following samples are collected by only two of the six laboratories and both these laboratories are from the same organisation:

Trace metals
In both SOPs where sediment sub-samples are collected for later analyses of trace metal concentrations, material is taken from the top 0-2cm of the sample, using a plastic scoop, and stored in a sealed polythene bag. In one procedure there is no mention of the type of spoon to be used.

A clean plastic spoon should be used to minimise the risk of contamination as trace metals present in stainless steel may contaminate the sample. Grab buckets should be constructed from stainless steel and the edges of the sampling device should be avoided when collecting these sub-samples.

Organic carbon and nitrogen
In both SOPs where sediment sub-samples are collected for later analyses of organic carbon and nitrogen, material is taken from the top 0-2 cm of the sample, using a plastic or metal scoop, and stored in a sealed polythene bag.

Organochlorine pesticides/hydrocarbons
In both SOPs where sediment sub-samples are collected for later analyses of organochlorine pesticides/hydrocarbons, material is taken from the top 0-2 cm of the sample, using a plastic or metal scoop, and stored in a hexane-rinsed aluminium container. In one SOP it is not clear what sort of scoop (plastic or metal) should be used for the collection of the different samples. Another difference is the use of a glass jar with an aluminium lid liner, both of which have been rinsed with hexane.

Advice provided by the NMMP 'Green Book' (Anon., 2001) states that samples for metals, organic chemicals and organic carbon should be taken from the top 1 cm of the sample, avoiding the edge of the grab bucket and the material from any anoxic layer. However the chosen sampling depth will ultimately depend on the objectives of the investigation.

Sub-samples collected for later analysis of organic contaminants can be stored in either glass or metallic containers. All samples should be frozen as soon as possible.

It is unclear in one SOP whether the sediment taken for later chemical analysis should be treated as a sub-sample and the remaining material processed for macrofauna. In view of the likely need for removal of a substantial amount of the surface layer, this action should be avoided, as it will almost certainly result in the loss of a significant proportion of the fauna present in the sample. It is therefore recommended that the advice given in another procedure is followed, namely that if samples for later chemical analysis are taken then the remaining material should be disposed of and a separate taken for macrobenthos. The NMMP 'Green Book' (Anon., 2001) should be referred to for additional advice to minimise the risks of sample contamination from extraneous sources.

4.3 Quality control

4.3.1 Equipment checks

All but one of the SOPs calls for the inspection, and in some cases testing, of the equipment prior to the vessel sailing. Any damaged equipment should be replaced.

4.3.2 Sample rejection criteria

The following criteria are used in the SOPs for the rejection of samples. In some of the SOPs not all of the criteria are included.

It is recommended that all laboratories adopt all of these measures:

Surface inspection
This criterion was included in only one of the SOPs and states that if the sample is incomplete or there is some evidence of 'washing out' then the sample should be rejected.

This is important in cases where sub-samples for later chemical analyses are required as these are commonly taken from the top 2 cm of the sample. It is equally important when collecting benthos samples as a large percentage of the fauna may inhabit the surface layer of sediment.

Acceptable depth/volume
All SOPs had an acceptance criterion based on depth and/or volume, as measured in a Day or Van Veen grab.

In two of the SOPs, the minimum acceptable depth was 7 cm in soft or muddy sediments and 5 cm in hard packed sands or coarse sediments. These depths, as measured at the deepest part of the grab, equate to volumes of 5 and 2.5 litres respectively. In another SOP, grab samples are rejected if the grab is less than a third full for muddy mixed sediments and less than a quarter full for hard packed marine sands. These latter criteria were also used for the hand held Van Veen grab. In another procedure a minimum acceptance depth was simply stated as 5 cm with no differentiation for hard and soft sediments.

Subjective estimates, for example, 'a third or a quarter full' are undesirable. It is therefore recommended that the sample depth is actually measured using a graduated plunger or ruler and the rejection criteria applied.

In one procedure where a Hamon grab was used then the minimum acceptance volume was 6.5 litres.

Pooling
This is the practice of amalgamating two samples, individually rejected due to insufficient volume, to give one acceptable sample, and was permitted in only one SOP.

This procedure is invalid, as faunal occurrences are expressed in terms of unit area, not volume. Volume is used as a practical measure of sampling efficiency. The relationship between volume and faunal content is more complex and more unpredictable than that of surface area: the two measures should not be confused.

Jaw closure
This criterion was identified in two of the SOPs and states that the sample should be rejected if the jaws of the grab are not fully closed, or if the supernatant water has drained out. This may indicate that there may have been some loss of sample material on retrieval of the device.

Uneven bite
This criterion, not included in any of the SOPs, is detailed in Rumohr (2000) and states that the sample should be rejected if the grab has taken an obviously uneven bite.

It is recommended that this criterion is included in all SOPs.

Sample clearly different from station replicates
Only one procedure refers to this criterion and states that if a sample is clearly different from the other replicates then it should be rejected.

This criterion is also recommended in Rumohr (2000) which states that the sample should be rejected and another more representative sample taken. However, the rejection of these samples should be noted in the survey log as they provide an indication of the patchinesss of the area. The appropriateness of this criterion in all circumstances will depend on the objectives of the particular study but, as a matter of principle, such bias in sampling approach is undesirable.

Washing gear
Only two SOPs refer to the practice of washing the gear and hopper used for the collection and processing of benthos samples. One SOP calls for a wash between each station, whilst it is only required by the other if sediment, particularly clay, has stuck to the device.

It is good practice to scrub sieves between samples as clogging of the sieve by small particles effectively reduces the size of the mesh. This may then have implications for the size of fauna retained, as well as the time taken for sieving. This problem is countered to a certain extent in the laboratory if the sample is again washed over a sieve mesh of the same size. However, fixed specimens may be more brittle than their live counterparts, resulting in further loss of material, and so sieve meshes in the field should be maintained in an 'unclogged' state at all times.

5. SAMPLING FOR INTERTIDAL SEDIMENTS

5.1 Equipment

5.1.1 Cores

The gear used in these types of survey varies enormously, even between laboratories within the same organisation. Generally two types of core are used, a 'Box core' with a surface area varying from 0.01m^2 to 0.0625 m^2 and a plastic cylindrical core, with a surface area varying from 0.00273 m^2 to 0.0176 m^2. Explanations as to the circumstances in which cores of different design/area are to be used are vague.

5.2 Safety

All but one of the SOPs refer to this important consideration, either directly or by referring the reader to a safety manual.

Sampling in the intertidal zone can be extremely hazardous (quick-sands, tides etc) and SOPs should highlight these dangers and/or refer to the relevant risk assessments/safety handbook.

5.3 Procedure

5.3.1 Sampling depth

Advice given in SOPs varies from no specification of depth to stipulation of different depths for different types of sediment. One SOP cites the appropriate

literature in order to justify the stated depth requirement of 15 cm. In another SOP it is recommended that individual laboratories ascertain the appropriate depth required through pilot studies, reference to the literature, or from previous work.

It is good practice to state the depth to which the device should ideally be inserted, as this will ensure that samples are comparable. The required depth must ultimately depend on the specific questions being addressed and the type of sediment being sampled. Where laboratories are contributing data to a wider study, then the same SOP should be followed, and explicitly referred to. Any deviation from the norm must be properly justified.

5.3.2 Sample collection

PSA
Different laboratories collect PSA samples in different ways. One laboratory uses a separate 5.9 cm diameter core deployed to a depth of 10 cm, whilst another takes a surface scrape of the top 2 cm.

Clearly these two methods may produce differing results, as the particle size distribution is not necessarily homogenous throughout the sample. If a sample has been obtained for later infaunal analysis it is important for the accompanying PSA sub-sample to be representative. Evidence from several sources indicates that the top five centimetres accounts for the majority of the fauna in terms of densities and diversities - but this is not 'foolproof' (e.g. benthic biomass may be significantly greater at depth than at the surface). If, however, the PSA data is to be related to the outcome of chemical analyses of sub-samples obtained from the top 2 cm, as is accepted practice, then it is appropriate to take the PSA sub-sample from the same surface area.

Benthos
Samples are either processed on site, if time allows, or back in the laboratory. One SOP states that, in the latter case, seawater should be used as freshwater will result in damage to specimens.

The following samples are collected by only two of the five laboratories who submitted SOPs and both these laboratories are from the same organisation:

Trace metals
In both SOPs where sediment sub-samples are collected for later analyses of trace metal concentrations, material is taken from the top 0-2 cm of the sample, using a plastic scoop, and stored in a sealed polythene bag. In one SOP there is no mention of the type of spoon to be used.

A clean plastic spoon should be used, to minimise the risk of contamination from extraneous sources.

Organic carbon and nitrogen
In both SOPs where sediment sub-samples are collected for later analyses of carbon and nitrogen content, material is taken from the top 0-2 cm of the sample, using a plastic or metal scoop, and stored in a sealed polythene bag.

Organochlorine pesticides/hydrocarbons
In both SOPs where sediment sub-samples are collected for later analyses of organochlorine pesticides/hydrocarbons, material is taken from the top 0-2 cm of the sample, using a plastic or metal scoop, and stored in a hexane-rinsed aluminium container. In one SOP it is not clear what sort of scoop (plastic or metal) should be used for the collection of the different samples. Another difference is the use of a glass jar with an aluminium lid liner, both of which have been rinsed with hexane.

Sediment cellulose
Where collected a clean metal or plastic spoon is used to collect a surface scrape of 1-2 cm (100-200 g).

There must be close collaboration between analytical chemists responsible for laboratory analysis and those engaged in field sampling, to ensure that samples collected are of appropriate quality and condition. All samples should also be frozen as soon as possible.

6. TRAWL SAMPLING

6.1 Principle and purpose

In one SOP trawl sampling is used to sample the epifauna, including fish. If a relatively large mesh size is used the technique can be useful for generating data in a relatively short period of time, as the material is often amenable to processing on deck. Trawls may also be suitable devices for surveys of marine litter. Trawl sampling is also used for obtaining fish, for the purpose of determining tissue concentrations of, for example, trace metals and organochlorine compounds.

6.2 Equipment

6.2.1 Trawls

Beam trawl
As the SOPs for trawl sampling have slightly different aims, it is not surprising that the type of gear used also varies. In one SOP use is made of a 2 m Agassiz trawl with a tickler chain and a mesh size of 72-74 mm, reduced to 13 mm in the codend. In another SOP a standard Lowestoft 2-metre beam trawl with a cod end mesh size of 3 mm is used. Details are given in Riley *et al*. (1986).

Obviously the mesh size used has implications for the size of material retained by the gear. There is a need for standardisation of equipment, especially with regard to beam width, net codend mesh size and the

mesh used for on-board processing. This is essential for the comparability of data collected by different laboratories.

Otter trawl
In one SOP, use is also made of an Otter trawl, presumably for the collection of fish.

6.3 Procedure

6.3.1 Tows

In one SOP the gear is towed over a fixed time period of 5-10 minutes. The positions of the start and end of tow are recorded to allow a calculation of the distance covered over the ground. This SOP also allows for the use of an odometer wheel for measuring the actual distance the gear has been in contact with the seabed. In the other SOP the trawl is towed over a fixed distance and in confined waters, buoys or some other form of marker are used to identify the position of the tow.

For beam trawl samples it is recommended that the start of the tow is recorded when the gear makes contact with the seabed and the winch is stopped. The end of the tow should be recorded when the winch is engaged to haul it back in. Start and end tow positions should be recorded, even in cases where towing over a fixed time interval is the primary goal.

In one SOP, presumably referring to sampling in estuaries, tows are conducted during low water slack as it states that this is when fish are likely to be concentrated in channels. The other SOP is intended for use further offshore.

The efficiency of the sampling gear will often be dependent on the different tidal and wind conditions that prevail at the time of sampling. Thus sample size and quality may vary from one sampling occasion to the next, irrespective of whether tows are conducted over fixed times or fixed distances. It is essential that information such as the state of the tide and weather conditions are recorded as they may have an effect on the catchability of the more mobile epifauna, particularly fish.

A sieve (5 mm mesh) is used in one of the SOPs to sieve the catch prior to further processing.

The presence of certain material, including large amounts of Flustra folicea, may block up the trawl mesh. As a result, the trawl may not consistently catch individuals smaller than the minimum trawl mesh size. For this reason it is recommended that all samples be passed over a sieve of at least the same minimum mesh size as the trawl. Any material passing through the sieve should be discarded.

An element of expert judgement is usually required regarding the acceptability of samples, and this also emphasises the need to recognise that the data generated are, at best, 'semi-quantitative' in nature. Further effort is required to improve the comparability of epifaunal data generated from trawl surveys

6.3.2 Sub-sampling

SOPs offer little or no advice concerning sub-sampling of trawl catches.

There is a clear need for more guidance on approaches to sub-sampling of trawl catches. As a general principle, it should be remembered that (as with grab samples) it is not possible to sub-sample for species occurances.

6.4 Quality control

Examination of the gear
Only one SOP calls for the inspection of the gear before and after tows. Any repairs should be carried out immediately and any damage noted in the field log. Surveys should be terminated if severe damage occurs to the gear.

It is recommended that all SOPs should include this measure. Nets should also be thoroughly washed down between samples, to minimise the risk of cross-contamination of samples.

Ensuring contact of the gear with the seabed
This consideration is only detailed in one SOP. It is achieved in three ways, firstly by visually monitoring the warp to ensure that it remains under tension and hence that bottom contact is being maintained. The second measure calls for the inspection of the beam trawl shoes which should be polished on the underside if the trawl has made contact with the seabed. Clearly, this cannot provide confirmation of the extent of bottom contact. The third measure includes the use of a meter wheel, which can be attached to the trawl to give a measure of the actual distance covered over the ground.

Trawling will always produce data that is at best semi-quantitative but adoption of these criteria can improve confidence in the collected data.

7. LABORATORY ANALYSIS OF MACROFAUNA

7.1 Equipment

7.1.1 Illuminated magnifier

All SOPs require samples to be sorted under some form of illumination and all but one also require some form of magnification. In most SOPs these two demands are catered for using a x1.5 illuminated magnifier. One

SOP states that very fine material should be sorted directly under a binocular viewer.

It is recommended that illumination and magnification be used for all sorting activity where there is a risk that fauna may be missed, particularly when there is any residual sediment.

7.1.2 Binocular viewer

This is used in all SOPs for the identification of fauna. Magnification ranges from x10 to x160. An eye piece graticule is usefully employed by one laboratory as an aid to measuring proportions and sizes.

7.1.3 High power microscope

The maximum magnification available used by the different laboratories ranged from x630 (one laboratory) up to x1000 (four laboratories).

7.2 Reagents

7.2.1 Preservative

Generally the preservative mixture comprises 70% ethanol or Industrial Methylated Spirits (IMS), 10% Glycerol and 20% water. In one SOP the formalin fixative is replaced with an alcohol-based preservative if samples are to be kept for more than a month before further processing. This will prevent the dissolution of any mollusc shells.

7.2.2 Polyvinyl lactophenol/Ammans's lactophenol

This reagent, used in only one of the SOPs, is highly toxic and the procedure states that it should only be used in a well-ventilated area, whilst wearing disposable gloves.

7.2.3 Sodium hypochlorite

This reagent, used in only one of the SOPs, is highly toxic and the procedure states that it should only be used in a well-ventilated area, whilst wearing disposable gloves and eye protection.

7.3 Procedure

7.3.1 Elutriation

This phase of the processing is not detailed in all the SOPs.

Care should be taken to minimise the risk of damage to specimens wherever possible. Elutriation has the potential to allow the smaller and more delicate animals to be removed from the bulk of the sample and, by doing so, to improve the speed and efficiency of the sorting process. It is recommended that elutriation should be carried out by all laboratories where samples contain significant amounts of residual coarse material.

One SOP employs a smaller sieve mesh size in the laboratory than that used during sample processing at sea.

Such a procedure may result in the retention of a range of smaller sized individuals than expected, in the event of incomplete sieving of the retained material at sea. However, a counter-argument would be that the effect of preservation is to make animals more brittle and hence more prone to fragmentation in the laboratory, resulting in loss of species which in their live state would have been retained. There is clearly a need for agreement on a standard approach to ensure comparability of results. The consensus based on this review is that the same mesh size should be used in the field and laboratory.

7.3.2 Sorting

For ease of subsequent sorting some laboratories carry out fractionation, whereby the entire sample is washed onto a stack of sieves and the material picked out from each fraction.

When carrying out fractionation great care must be taken to guard against loss of material and cross contamination.

Most SOPs advocate the use of a shallow white tray for sorting through the sample. Trays are marked into squares to allow for systematic sorting. A pipette and/or flexible forceps are used in one SOP for the removal of the most delicate organisms. In two SOPs the residue is sorted twice, either by the same or a different analyst. Sorting is carried out under an illuminated magnifier or, if the material is very fine, then under a binocular microscope.

To minimise the risk that individuals are overlooked during the sorting process it is recommended that trays are not overfilled with material (it should be possible to see the marked lines).

Sub-sampling
There were two techniques outlined in the SOPs for sub-sampling, using a tray or a Perspex quarteriser.

Following the outcome from the 1997 NMBAQC Humber workshop (Proudfoot et al., 1997) it is recommended that a quarteriser be adopted by those laboratories still using the marked tray method.

7.3.3 Specimen preparation techniques

In two SOPs, a number of techniques are outlined to aid in the identification of certain groups of animals.

The confident identification of certain faunal groups may be very difficult without their use and they should therefore be included in all SOPs.

They include the following:

Staining
Methyl blue is used for highlighting the internal structure of oligochaetes and for staining sponge spicules.

Clearing (Oligochaetes)
This technique involves immersion of the specimen for 15 minutes in each of the following: 100% IMS followed by 70% IMS, 30% IMS and, finally, water to remove the glycerol. The water is removed by blotting. The specimen is placed in Polyvinyl lactophenol or Ammans's lactophenol on a microscope slide. A coverslip is then placed on top and the edges sealed with a slide sealant. The slide is left for 48 hours to clear.

Clearing (Sponges)
Thin sections are cut perpendicular to the surface of the sponge using a razor. The section is then immersed in absolute alcohol for ten minutes to dry, and cleared in dried clove oil.

Removal of soft tissues
Soft tissues are removed by immersing the specimen in a watch-glass of hypochlorite solution so that the hard parts can be examined more easily. This technique is particularly useful for the opening of the valves of delicate bivalves to allow the examination of internal shell features.

7.3.4 Identification

Three of the SOPs provide an approved list of keys and relevant papers that should be used for identification.

These lists can then be updated as new material becomes available.

Very little information is provided in any of the SOPs concerning the criteria for exclusion of certain taxa from the identification process. What little guidance there is states that the following should not be included in quantitative datasets: meiofauna (nematodes), calanoid copepods, motile and colonial sessile epibenthic taxa and headless specimens. Only one SOP requires a distinction to be made between adults and juveniles.

Species name should conform to the latest version of the Species Directory by Howson, C.M. and Picton, B.E. (eds) (1997) and not Howson (1987) as reported in one SOP. Where a taxon is not included in Howson and Picton (op. cit), classification should follow the most recent available taxonomic publication.

7.3.5 Determination of biomass

All SOPs determine biomass as 'wet weight', which can then be converted to 'ash free dry weight', using conversion factors. These are usefully detailed in the appendix of one SOP.

Balance
Balances weigh to the nearest 0.1 mg or 0.01 mg, depending on the individual SOP.

In one procedure readings are taken after stability has been reached while, in another, after 30 seconds. Another SOP gives no information as to when the reading should be taken.

On practical grounds, it could be argued that measurement after a set time has elapsed is preferable, as stability can take a considerable time to reach with some specimens. There is clearly a need for further work towards standardisation of methodology.

Weighing container
The use of a water-filled, tared vessel, which stops further evaporation of preservative from the specimen, was the chosen method of two of the three laboratories carrying out determination of biomass.

Preservative used
Laboratories differ in their use of preservative for samples for biomass determination. Two laboratories use a preservative solution of 70% IMS, 20% water and 10% glycerol, one of which stipulates that samples must remain in this solution for a minimum of three months to allow for weight loss stabilisation, in accordance with Rees *et al.* (1990). Another laboratory uses a similar solution, but without the glycerol (70% IMS and 30% water) since, as it is non-volatile, it does not evaporate from the surface of specimens and can therefore affect the weights obtained. This problem can be countered to a certain extent by rinsing with water although this may not result in the removal of all the glycerol. A fourth laboratory avoids the use of a preservative mixture altogether until after the biomass has been measured, as placing the specimen in alcohol can, with time, cause a marked weight reduction.

Clearly whilst all these differing approaches are individually justified there is a clear need for standardisation if results are to be truly comparable between laboratories. It may be that if glycerol is not used then the potential for evaporative loss is increased and thus one source of error is exchanged for another. Avoiding the use of a preservative altogether can only be an option if biomass is to be measured within a short time frame, which may not be realistic for all laboratories. Further evaluation of these factors is recommended and best practice identified.

Rinsing
Both of the two SOPs using a preservative containing glycerol require the specimen to be rinsed twice in water, prior to blotting, to remove as much of the preservative as possible.

If using a glycerol-based preservative then this action should be encouraged.

Blotting
Either blue tissue paper or white blotting paper is used for this process. There is a consensus view that samples should not be squeezed but merely moved around until no wet patch is left on the absorbent paper.

As it is easier to see a wet mark on coloured tissue paper this material may be superior to white blotting paper.

Taxonomic level when measuring biomass
All three SOPs determined biomass but to varying taxonomic level.

Treatment of specific fauna
It is the practice of two laboratories to puncture and drain Echinodea and Echiurida so that fluid trapped inside the test, which would otherwise affect the weight, is allowed to escape.

SOPs differ in their approach to dealing with tube-dwelling taxa. Taxa are either removed from their tubes and weighed or, alternatively, weighed in their tubes and a conversion factor used to calculate an 'untubed' weight. This conversion factor is determined by weighing the tubes from a sub-sample of animals.

Weighing taxa in their tubes, followed by application of a conversion factor to determine untubed weight, can cut down on the time required for processing and is less likely to lead to damage of the fauna in question. However, the potential for error needs to be determined empirically before this can be recommended as an approach.

Only one SOP gives advice on dealing with polychaete fragments. The SOP in question allocates the total weight of the fragments across all identified polychaete species, in proportion to the amount of biomass represented by each (calculated from the biomass of specimens with heads).

The advantage of this approach is that it ensures that all live material within the sample is accounted for. However, an associated problem is that it arbitrarily increases the mean individual weight of each species, which may create difficulties in fine scale comparative exercises. It is recommended that consensus is reached on how to deal with fragments.

7.4 Quality control

The approach taken to control the quality of sample identification and enumeration varies between laboratories although there are some common practices.

7.4.1 Reference collection

Four of the five SOPs reviewed require the maintenance of reference collections. In two of these SOPs a separate collection is produced for each survey. One laboratory ensures that specimens, previously unrecorded by the laboratory, are externally validated.

It is strongly recommended that a separate reference collection be maintained for each new survey area. In this way it is easy to carry out a universal edit of the data if mistakes are found in the reference collection for that survey. The formation of a reference collection also allows the analyst to progress with the analysis of a sample when faced with a difficult specimen. The problem specimen can be put into the collection and referred to by a number, or a description. The identification of this specimen can be ascertained at a later stage, perhaps with the aid of a more experienced analyst. Also, by imposing 'ownership' of the identifications in a particular collection, which then join a common resource, there is more incentive for an individual to exercise greater care.

7.4.2 Sample re-analysis

Percentage of samples re-analysed
Of the laboratories which carry out a re-analysis of samples a random selection of 10% is the proportion chosen. In recognition of the time required for re-analyses one laboratory excludes very large samples (those contained in buckets).

The problem with this approach is that because the analyst will know that these large samples are not going to be checked then it is possible that the analysis of these samples may be conducted less thoroughly. It is therefore recommended that no samples should be excluded from the possibility of selection, and re-analysis of less than 10% of the samples is given consideration if time is a critical issue.

In one SOP samples are selected at random before the initial analyses. After completion of each sample the analyst checks with the quality manager to see if this sample is to be retained for subsequent re-analysis.

The problem with this approach is that if the percentage of samples to be re-analysed from a sample batch is known then the analyst can easily work out when all the check samples have been selected. It is then possible that any remaining samples to be processed may not receive the same care and attention,

as the 'driver' for thorough work has been removed. However, the advantage of this approach is that only sediment residues from samples identified for repeat analysis need to be kept. When the 10% of samples for re-analysis are identified after all samples have been processed then all sample sediment residues must be retained until the quality control checks have been carried out.

Sample sorting efficiency
This is measured by a re-sorting of the sieve residue (see above). Only one SOP requires the re-sorting to be carried out by an independent analyst.

Only one SOP requires a quality control measure to ensure that any sub-sampling is representative of the sample as a whole. In this practice sub-sampled animals are returned to the sorting tray along with the sieve residue and the sampling procedure is repeated by an independent analyst.

Quality control of sub-sampling procedures should be encouraged.

Analytical Quality Control (AQC) criteria
AQC criteria are found in all but one of the SOPs reviewed and are used to assess the standard of sample re-analysis.

These criteria serve not only to determine the standard achieved in analysis but also to invoke actions to raise the data quality to the required standard.

There were two approaches to the application of AQC criteria. In both approaches a repeat sample analysis is carried out. In the first approach, if a reanalysed sample fails one or more of the following criteria, specified by the NMBAQC Committee (see Unicomarine, 2001), then the whole batch is reanalysed, except in the case of (a) where only relevant specimens may be re-examined. If the variation is caused principally by inadequate sorting then resorting of the entire batch may be required.

a) where taxa contributing 5% or more of the total sample abundance have been misidentified; or

b) where the total number of taxa varies by more than 10% or 2 species (whichever is the greater);or

c) where the total abundances of the two samples vary by more than 10% or 10 individuals (whichever is the greater); or

d) where the Bray-Curtis similarity coefficient for the two sets of analyses is less than 0.90.

The advantage of this approach is that it encourages high standards of work and is to be recommended. However, occasionally, expert judgement will be required as to whether a complete reanalysis is warranted for relatively minor breaches of criterion for acceptability, relative to the objectives of particular surveys.

In the second approach acceptability is determined using only the Bray-Curtis similarity measure, based on the transformed data matrix for each of the two analyses (original and quality control check). The overall mean similarity for each of the sample checks is then calculated using the Bray-Curtis measure. The SOP states that a similarity of 90% or greater is sufficient to ensure the same groupings of survey stations. Thus, errors which would not have affected the subsequent interpretation of the data are considered acceptable.

The problem with this approach is that it ignores the possibility that the data may be of use in the future for another purpose. It is therefore recommended that Bray-Curtis is calculated using untransformed data. It should also be noted that different transformations allow different components of the data to be investigated (i.e. more severe transformations take more account of rarer species).

Reporting of results
Two of the three laboratories carrying out a 'repeat-analysis' QC procedure require that these results be detailed in any final report.

Detailing the results of AQC measures provides the reader with some notion of the confidence which may be placed in the reported findings. It also allows the laboratory to demonstrate its commitment to high quality work and to make sure that the data are used appropriately.

8. PARTICLE SIZE ANALYSIS

8.1 Principle and purpose

In environmental studies, particle size data are used principally for the interpretation of faunal data, and of the outcome of chemical analyses of sediment fractions.

Particle size is determined by two different methods, depending on the individual laboratory and the sediment size fraction involved. The larger particles are assessed using dry sieving and direct weighing whilst the 'fines', once separated from the coarse sediments by wet sieving, are measured using laser diffraction or pipette analysis. In one SOP a laser sizer is used to measure all particles less than 2 mm in size, thus avoiding the use of a sieve for the <2 mm size fraction.

8.2 Equipment

8.2.1 Sieves

The mesh sizes of sieves specified in the SOPs varies, with two SOPs specifying meshes at half-phi intervals whilst another specifies meshes at one-phi intervals.

8.2.2 Sieve shaker/pneumatic trough

These devices, used in two of the four SOPs, can help ensure that sieving is carried out to a consistent standard.

8.2.4 Laser sizer

There are at least two different manufacturers of laser sizers used in the SOPs reviewed.

Investigation is required into the comparability of results produced using different laser sizers.

8.3 Reagents

8.3.1 Hydrogen peroxide

This reagent is used for the removal of organic material from the sample. It is only used by one of the four laboratories carrying out this procedure.

8.3.2 Sodium hexametaphosphate

This reagent is used in only one of the SOPs to help separate the individual particles.

The benefits of use of this reagent should be further evaluated.

8.4 Procedure

8.4.1 Volume/weight of sample for analysis

The amount of sample used for the particle size analysis varies between laboratories: specifications include 110 g, 50 g and a variable amount not stipulated.

8.4.2 Homogenisation

SOPs differed in their approach to the homogenisation stage with two SOPs calling only for thorough mixing or stirring. In another SOP sediment is added to 200 ml of deionized water and 20 ml of sodium hexametaphosphate. The sample is mixed for 15 minutes, soaked overnight, then restirred for 10 to 15 minutes.

Standardisation of this step is advisable to remove a source of error that may be introduced by the different practices adopted.

8.4.3 Oven drying (prior to wet sieving)

In one SOP, prior to the wet sieving phase, the sample is first dried in an oven at 70°C.

Oven drying is likely to 'bake hard' any fines causing them to agglomerate. This can make the subsequent sieving process prone to error. It is therefore recommended that this drying stage be omitted.

8.4.4 Wet sieving

This process is used in all SOPs to obtain the fine fraction for later analyses. It can be made easier with the aid of a pneumatic trough. The sieve size chosen varies between laboratories (see discussion below).

8.4.5 Oven drying (after wet sieving)

If the fine fraction is not to be analysed further the coarse and fine fractions are oven-dried at a temperature of between 70°C to 105°C, depending on the SOP.

8.4.6 Dry sieving

A stack of sieves and a sieve shaker is used in all except one SOP for separating the dried coarse sediment into different size fractions.

8.4.7 Weighing

Each sediment fraction from the dry sieving is then weighed to the nearest 0.01 g in two SOPs and to the nearest 0.001 g in the other.

8.4.8 Freeze drying

The 'fine' fraction is freeze-dried in only one SOP prior to its analysis. The sample is frozen in a freezer at -10°C, then freeze dried at -40°C to -60°C until all the ice is removed.

8.4.9 Analysis of fine fraction

There were two techniques used to analyse the fine sediment fraction. Of the four SOPs reviewed the laser granulometer technique was used by three of the laboratories and pipette analysis by the fourth.

It is worth pointing out that a further technique using a Sedigraph® also exists. The definition of 'fines' varied between laboratories and hence certain particles could be measured using either a sieve of stated mesh size or one of the two techniques mentioned above, depending on which SOP is followed. For example, in one SOP particles <500 microns are analysed using the laser diffraction technique whilst another SOP uses the laser to analyse the <125 micron fraction. In a third SOP the laser is used to analyse particles from 2 mm –0.1

micron. In the fourth SOP the pipette analysis technique is used to analyse the <63 micron fraction. There are a number of issues to consider here. Firstly, for what particle size range should sieving be used, bearing in mind sieving efficiency and the potential for loss of some of the finest particles on the sieve. Secondly is it legitimate to compare samples which have been analysed in these different ways (different techniques and different particle sizes involved, different makes/models of equipment, and so on)?

8.5 Quality control

It is possible to carry out AQC on PSA samples although this is not routinely undertaken by all of the laboratories whose SOPs were reviewed. Actions include a re-analysis of the fine fraction, using some of the retained sediment and/or a checking of the performance of the laser sizer at regular intervals. A complete re-analysis, using spare sediment, is carried out on 10% of a batch of samples in one SOP. This check is carried out by another analyst and the percentage of the fine fraction should not vary between the two analyses by more than 10% of the total weight. If this value is exceeded then the reasons for failure are investigated, checking both methodology and calculations. The entire batch of samples may be reanalysed if necessary. If the procedure fails to identify a likely source of error then a third analysis can be carried out if sufficient sample remains. The two closest measurements are used to calculate the derived sediment parameters.

It is recommended that all SOPs include quality control measures for particle size analysis, at least for the analysis of NMBAQC samples (see Unicomarine 2001).

Ring Tests
Participation in Ring Tests and other intercalibration exercises is detailed in one SOP and should also be recommended.

Servicing, calibration and maintenance
Only one of the SOPs gives details for the servicing, calibration and maintenance of equipment.

The condition of instruments can be a source of error and all laboratories should have in place servicing, calibration and maintenance procedures.

9. CONCLUSIONS AND RECOMMENDATIONS

Most of the SOPs submitted broadly fulfilled the requirements of the task. However, the level of information provided was, on occasion, insufficient to ensure that tasks could be performed by an individual, not familiar with the procedure, to an acceptable standard without external guidance. For this reason it is recommended that a 'dry run' of all SOPs is carried out for the benefit of an individual, not familiar with the task, to expose any inadequacies. Comprehensive instructions reduce the possibility of any confusion and therefore sources of error arising.

The following recommendations are identified as being of most importance for improving both the quality and comparability of data produced by different laboratories:

SOP structure

- SOPs should contain all the necessary information to allow an individual, not familiar with the procedure, to carry out the task to the required standard.
- For ease of use, SOPs should be structured around the following sections: Purpose and Principle, Personnel, Equipment, Reagents, Safety, Procedure, Quality Control, Figures and plates, References/Bibliography and Appendices.

Sampling for subtidal sediments

- The dimensions of the following items of sub-tidal sampling equipment should be standardised: hand-held Van Veen grab, Craib and Box corers.
- Powdered Rose Bengal, if used, should be added to the concentrated formalin solution in the laboratory whilst under fume extraction. Alternatively a concentrated solution can be produced for use in the field.
- Grab samples of insufficient volume should not be pooled to produce an acceptable sample.
- Sample rejection criteria should be more widely adopted and strictly adhered to.
- There needs to be a standard protocol for the sub-sampling of sediment for PSA.

Sampling for intertidal sediments

- The dimensions of the following items of sub-tidal sampling equipment should be standardised: hand-held and Box corers.
- A standard protocol for the sub-sampling of sediment for PSA is required.

Trawl sampling

- Standardisation of trawl design and mesh sizes would allow much greater confidence to be placed in comparison of data between laboratories than at present.
- Trawl samples should be sieved on a mesh of the same size, or larger, than the cod-end mesh size.

- The state of the tide and weather conditions should be routinely recorded, as they are likely to have an important effect on the catchability of the more mobile epifauna, particularly fish.
- There is a clear need for more detailed guidance on the approaches to sub-sampling of trawl catches.

Laboratory analysis of macrofauna

- The recommendations for sub-sampling arising from the Humber Workshop Report (Proudfoot *et al*., 1997) need to be more widely adopted (e.g. use of a Perspex quarteriser in preference to other techniques).
- The effect on biomass measurements from the use of different solutions for the storage of samples requires investigation.
- A consensus view is required on the treatment of polychaete fragments in relation to biomass determination.
- All laboratories should adopt NMBAQC criteria for the quality control of macrobenthic infaunal identification and enumeration.

Particle Size Analysis

- There needs to be wider adoption of QC measure for Particle Size Analysis.
- Investigation into the following aspects of Particle Size Analysis is recommended:
    1) Evaluation of the use of sodium hexametaphosphate
    2) The Effect of oven drying on the consolidation of the 'fines' fraction
    3) Comparison of the different techniques for particle size analysis (see Section 8.4.9).

10. ACKNOWLEDGEMENTS

As the review was carried out without reference to named laboratories it is not possible to thank organisations or individuals by name but their co-operation is greatly appreciated, as without it a review of this nature would clearly not be possible.

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APPENDICES 1 TO 5

DETAILED EXAMINATION OF INDIVIDUAL SOPs

Appendix 1. Sampling for sub-tidal sediments

Laboratory		1	2
Procedure Title		Sampling of Sublittoral Sediments.	Sampling Procedure for Marine and Estuarine Subtidal Sediments.
Purpose and Principle		Yes	Yes
Personnel			
Experience		Trained individuals (scientists & crew) or trainee under supervision.	Trained individuals (scientists & crew) or trainee under supervision.
Number		Minimum of 2.	Minimum of 2.
Equipment			
Equipment list		Yes	Yes
Day Grab	Area	0.1 m^2	N/A
	Modifications	Flaps/windows for sub-sampling.	N/A
	Weighting of grab	No details given.	N/A
Long Arm Van Veen Grab	Area	0.1 m^2	0.1 m^2
	Weights	No details given.	55 kg (total weight)
	Arm length	No details given.	115 cm
	Modifications	No details given.	Doors
Hand-Held Van Veen	Area	0.02 m^2 and 0.025 m^2	0.019 m^2
	Sample processing	In the field or at the laboratory using seawater.	
Hamon Grab	Area	N/A	N/A
	Penetration depth	N/A	N/A
	Use	N/A	N/A
Craib Corer	Diameter & area	6 cm (28.3 cm^2)	5.9 cm (27.3 cm^2)
	Use	Useful for collecting undisturbed sediment profiles or sediment water interface samples (produces no bow wave unlike Van Veen).	When parameter to be measured changes with depth, surface layer required (produces no bow wave unlike Van Veen).
	Time allowed for penetration	Depends on the nature of the sediment.	2 mins
Box Corer	Diameter	N/A	N/A
	Use	N/A	N/A
Diver cores	Diameter	Perspex 6 cm diameter (28.27 cm^2), stainless steel 5 cm (19.63 cm^2).	N/A
	Penetration depth	15-20 cm	N/A
	Use	May be used in areas of patchy sediment.	N/A
Grab stand		Yes	No details given.

3	4	5	6
Grab Sampling for Benthic Invertebrates in Marine and Estuarine Sediments.	Sub-tidal Benthic Sample Collection.	Sampling for Marine Sub-tidal Soft Sediments/ Sampling for Marine Sub-tidal Gravel Sediments.	Collection, Processing and Preservation of Macroinvertebrate Samples from Estuarine and Coastal Subtidal Sediments for Community Assessment.
Yes	No	Yes	Yes
At least one individual must be experienced in the procedure	No details given.	At least one individual must be experienced in procedure.	Only trained/qualified individuals to operate the equipment.
2 in addition to the skipper.	No details given.	Minimum of 2 scientists and	One to operate the winch
Yes	No	Yes	No
0.1 m^2	0.1 m^2	0.1 m^2	0.1 m^2
Stainless or galvanised steel construction. Flaps/windows for sub-sampling. Jaws supported within an open framework. Facility to attach weights.	No details given.	Stainless steel buckets and flaps/windows for sub-sampling. Jaws supported within open framework. Facility to attach weights.	Stainless steel buckets. Stainless steel or galvanised frame. Flaps/windows for sub-sampling. Jaws supported within an open framework.
Adjusted to obtain optimal penetration of sediment.	No details given.	Adjusted to obtain optimal penetration of the sediment.	Adjusted to obtain optimal penetration of the sediment.
N/A	0.1 m^2	N/A	0.05 m^2 0.1 m^2
N/A	No details given.	N/A	See operating instructions.
N/A	Long armed.	N/A	See operating instructions.
N/A	No details given.	N/A	See operating instructions.
N/A	0.023 m^2	N/A	N/A
N/A	No details given.	N/A	N/A
N/A	N/A	0.1 m^2 (mini Hamon Grab) 0.25 m^2 (large Hamon Grab).	N/A
N/A	N/A	30 cm	N/A
N/A	N/A	Sampling of coarse sediments.	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	0.025 m^2
N/A	N/A	N/A	For collection of an undisturbed sample.
N/A	N/A	N/A	
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
Made from galvanised steel, allows jaws to be opened. Hopper catches all material and washings from the grab.	No details given.	Made from wood, allows jaws to be opened. Hopper catches all material and washings from the grab.	Yes

Appendix 1. continued: Sampling for sub-tidal sediments

Laboratory		1	2
Sieve table		Yes	Yes
Seive	Diameter	45 x 70 cm	18 inch
	Mesh size	1 mm or 0.5 mm BS410. No advice given as to which size should beused in which circumstances.	Brass 1 mm or 0.5 mm. No advice given as to which size should be used in which circumstances.
	Make	Wooden box	No details given.
	Mesh material	Stainless steel	No details given.
	Round or square mesh?	No details given	No details given.
	Height of surround	No details given	No details given.
	Sieving location Smaller grabs/cores may	In the field for 0.1 m^2 grabs. Laboratory (small vessel). be processed at the lab.	On board (large vessel).
	Vessel	No details given.	No details given.
Reagents			
Formalin	Strength of concentrate.	37-41% formaldehyde. pH 7.0 buffered	37-41% formaldehyde.
	Dilution	No details given.	10-fold
	Strength of working solution.	10% formaldehyde solution?	4% containing Rose Bengal.
	Buffer	Borax (Di-sodium tetraborate 10 hydrate). Added direct to sample 2 g/litre.	Borax
	Min Temp	No details given.	5 deg C
	Storage container	Pressmatic dispenser.	25 litre container.
	Ratio of fixative volume/ sample volume	Not clear.	1:1
	Fixing time	Must be undertaken within 24hrs.	Must be left for a minimum of 72 hrs.
Rose Bengal	Concentration	Not used.	0.1 g/litre
	Added to concentrated formaldehyde solution in the lab or to the working formaldehyde solution in the field?	No details given.	Concentrated formaldehyde solution in the lab.
n-Hexane		Used but not listed under reagents.	Yes
Safety			
Section highlighting safety matters		Yes	Yes
Vessel	Survey vessel	Department of Transport conditions for survey vessel.	See Safety manual.
	Small vessels	See Safety manual.	See Safety manual.
Diving		HSE conditions	N/A

3	4	5	6
Washing trough.	No details given.	Yes	Hopper.
20-30 cm	20 cm	30 cm	No details given.
0.5 mm or 1 mm	0.5 mm estuaries, 1 mm coastal/offshore.	0.5 mm, 1.0 mm, 2.0 mm (depending on objectives).	Estuaries 0.5 mm, 1 mm for intermediate and offshore sites.
No details given.	No details given.	Endecotts Lab. Test sieve (BS410).	No details given.
No details given.	No details given.	Stainless steel.	No details given.
No details given.	No details given.	Square.	No details given.
Minimum of 10 cm	No details given.	No details given.	No details given.
In the field.	On board the vessel.	On board the vessel.	On board the vessel.
Winch of = or >1 tonne capacity, sufficient warp to reach bottom, derrick or gantry, position fixing system, deck wash hose.	No details given.	Research vessels conform to BS EN ISO 90002. Consult laboratory guidelines for charter vessels.	Vessel must be able to maintain its position when sampling as vessel drift can cause toppling of sampling devices (Kingston, 1998).
40% formaldehyde, solution, pH 7.0 (40 g/2.5 litres).	No details given.	30% formaldehyde	see COSHH assessment.
4-fold	No details given.	3-fold	see COSHH assessment.
10% formaldehyde solution.	10% formosaline solution.	10% formaldehyde	5% formaldehydesol buffered with Borax.
Borax	Borax	Sodium tetraborate (Borax).	Disodium tetraborate.
5 deg C	No details given.	No details given.	No details given.
No details given.	No details given.	10 litre plastic drum.	Rigid polypropylene containers only. Storage on deck.
1:1 Container shouldn't be filled more than half full. Decant supernatant liquor if pot too full. Add 125 ml 40% formaldehyde solution where pot full.	No details given.	1:1	Muds 3:1, Sands 2:1, Organic muds require 10% formaldehyde solution.
No details given,	Must be left for a minimum of 72 hrs and shaken once.	No details given.	No details given.
0.05 g/litre no details given.	Normally added but biologist.	Added at discretion of biologist.	Added at discretion of
Concentrated solution prepared in the lab for use in the field.	Working solution in the field.	Working solution in the field	Added direct to sample?
N/A	N/A	Yes	N/A
Yes	No	Yes	Yes
Individuals must possess a sea survival certificate, befamiliar with the safety provisions of the boat. Visitors must be given appropriate safety instructions.	No details given.	Sea survival certificate. Familiarity with risk assessment for this type of work.	Health and Safety manual. Awareness with safety procedures and equipment.
No details given.	No details given.	See Safety manual.	No details given.
N/A	N/A	N/A	N/A

Appendix 1. continued: sampling for sub-tidal sediments

Laboratory		1	2
Identified dangers		None detailed.	None detailed.
Protective Clothing		Appropriate protective clothing including steel toe-capped boots & hard hats.	See Safety manual.
Formaldehyde solution	Danger (see COSHH)	Toxin - medium hazard.	Toxin - medium hazard.
	Protective clothing	Eye protection, disposable gloves.	Eye protection, disposable gloves.
	Handling of chemical	Dispense with 'pressmatic' dispenser in well ventilated area.	Handle in well ventilated area.
	Transporting samples	No details given.	No details given.
	Instructions for accident	Rinse immediately & seek medical advice.	No details given.
Rose Bengal	Danger	Not used.	Extremely hazardous carcinogen.
	Handling	N/A	Well ventilated area.
	Protective clothing	N/A	Eye protection, disposable gloves.
n-Hexane	Danger	No details given.	No details given.
	Handling	No details given.	No details given.
	Protective clothing	No details given.	No details given.
Procedure			
Position fixing	Differential GPS/GPS used?	GPS	No details given.
	DECCA	Yes	No details given.
	Visual transect/ bearing (operating from small vessels)	Yes	No details given.
	Range ring	50 m of sampling point	No details given.
	Computer based system for displaying station, positions and vessel location?	No details given.	No details given.

3	4	5	6
Use of gear in rough weather.	None detailed.	Use of gear in rough weather.	Winch and cables, hydraulic gantry, only qualified individuals to operate winch, obstructing line of sight of winch operator, placing fingers inside any mechanisms or interfering with warp - guide gear with flat of hands, using gear in rough weather (decision rests with vessel master), safe lifting practices, firm seds shouldn't be broken up by hand in case of sharps, secure moving parts when not in use. Health hazards associated with marine and estuarine waters, particularly with reference to sewage contamination.
Lifejacket to be worn on deck.	None detailed.	Life jacket to be worn on charter vessels and when gates open on research vessel.	Life jacket or safety harness, hardhat, steel toe-cap boots and gloves recommended when working with sampling gear.
Toxin - medium hazard.	Not mentioned.	Toxin - medium hazard.	Toxic, carcinogenic, irritant.
Eye protection, disposable gloves.	None detailed.	Eye protection, disposable gloves & wet gear whilst preparing dilutions of formaldehyde.	Eye protection, chemical resistant gloves.
Siphon to be used when transferring neat chemical from one container to another.	No details given.	Funnel to be used when transferring neat chemical from one container to another.	Dispense on deck, never in hold or survey cabin. Don't use in rough weather. Wash spillages overboard immediately and alert all crew members.
No details given.	No details given.	Samples must be transported in a compartment separate from driver.	Fixed samples stored in airtight containers. Samples must be transported in a compartment separate from driver.
No details given.	No details given.	No details given.	Individuals to be aware of precautions and clean up procedures before use (refer to COSHH assessments - copy on vessel and lab).
Extremely hazardous carcinogen.	Not mentioned.	Extremely hazardous carcinogen.	See COSHH form.
Under fume extraction only (neat chemical). Prepare working solution for use in field.	Not mentioned.	Under fume extraction only (neat chemical). Prepare working solution for use in field.	See COSHH form.
Disposable gloves.	Not mentioned.	Eye protection, disposable gloves.	see COSHH form.
N/A	N/A	No details given.	N/A
N/A	N/A	No details given.	N/A
N/A	N/A	No details given.	N/A
GPS	DGPS and hand-held GPS	DGPS	DGPS
Yes	Not used.	Not used.	Not used.
Not used.	Not used.	Not used.	Not used.
No details given.	No details given.	No details given.	No details given.
No details given.	No details given.	'Sextant'.	B303 - Qubit Trac C for plotting positions.

Appendix 1. continued: Sampling for sub-tidal sediments

Laboratory		1	2
Operation of gear	Instructions?	Poor	No details given.
	Deployment rate (affects bow wave)	No details given.	No details given.
	Rate of retrieval	No details given.	No details given.
	Warp	No details given.	No details given.
Replication		No details given.	1-5 for chemical sampling and 1-10 for faunal sampling.
Attempts		Samples of insufficient volume may be pooled.	No details given.
Info recorded at station		Odour, presence of animal tubes, sediment texture and type, vol and depth.	Station/replicate number, sample location, water depth, time of sampling, whether conditions, sea state and characterisitcs of surface sediment (odour, presence of tubes, volume, depth of discontinuity layer).
	Recording of data	Waterproof notebook.	No details given.
PSA Sample		Sample removed from the top of the grab.	5.9 cm core 300 g.
Meiofauna		N/A	No details given.
Macrofauna	Sieving	Sample placed directly onto box sieve, then washed.	No details given.
	Samples split into fractions?	No	No details given.
	Transfer of fauna to sample container.	Backwashing using funnel. Pots attach to funnel.	No details given.

3	4	5	6
Good	No details given	Good	Separate procedure.
Slow just before hitting seabed.	No details given.	'Slow' nearing bottom.	Not exceeding 1 m per sec.
Pause to allow grab to bite then retrieve gently at first.	No details given.	Slow initial retrieval to allow closing of jaws.	Ensure gear fully closed before being pulled free of seabed. Initial rate of retrieval not exceeding 1 m per sec then 3m/sec once clear of the bottom.
No details given.	No details given.	No details given.	Kept as plum as possible to ensure sampler set down and lifted vertically.
Normally 2-3 per station.	Normally 3.	No info.	1-10 depending on survey objectives.
3 attempts (except where evidence substrate is uneven and hard). Repeat at progressive distance until suitable sediment located. Note co-ordinates.	No details given	Minimum of 3 attempts. Further attempts at increasing distance. Note co-ordinates of new position.	No details given.
See sampling sheet.	St no., position, depth, depth of sample etc (record sheet for each station providing prompt).	Depth of sediment, sediment description, least abundant component first.	Plotter, colour, smell, depth of RPD, texture, presence of surface features, depth of sample (used to calculate vol), photograph.
Field sheets.	No details given.	Log book.	Recording sheet (including prompts).
No details given.	Small amount removed.	Day - allow surface water to drain and collect sample using a 50 ml modified plastic syringe (3 cm diameter) deployed to at least 5 cm depth. Hamon collect a 500 cm^3 sample using a plastic scoop.	See separate procedure for collection of particle size samples.
N/A	N/A	50 ml modified syringe (3 cm diameter) deployed to at least 5 cm, and the excess returned to bulk sample.	N/A
If considered worth while (many small and delicate animals present) wash the surface of the grabs contents and collect overspill in sieve held beneath hopper. A plastic bin should be placed beneath the sieve to catch any spillage.	No details given.	Gentle pressure in box. Hand to produce less powerful spray.	Sprinklers beneath the sieve helps prevent clogging, produces better quality samples, removes effect of sample bias? Large animals and stones picked out. Sieve should be placed inside another larger sieve in case of spillage.
No	No	In coarse sediments sample may be split into 1-5 mm and >5 mm fractions.	No
Use funnel to transfer animals from sieve to pot.	No details given.	Backwashing using funnel. Excess water decanted off. Carry out this process within fish box in case of spillage.	Backwashing using funnel. Scoops not to be used. Excess water decanted off.

Appendix 1. continued: Sampling for sub-tidal sediments

Laboratory		1	2
Collection of other samples	Trace metals	0-2 cm surface scrape type of scoop not stipulated, pot storage.	100-200 g from the top 1-2 cm using a plastic scoop, sealable polythene bag.
	Total Organic Carbon	0-2 cm surface scrape type of scoop not stipulated.	100-200 g from the top 1-2 cm using a plastic or metal scoop, sealable polythene bag.
	Trace Organochlorine	100-200 g 1-2 cm surface scrape, hexane rinsed metal scoop into hexane rinsed glass jar with aluminium lid liner.	100-200 g from the top 1-2 cm using a hexane rinsed metal scoop, hexane rinsed aluminium.
	Redox potential	Insert probe directly into sediment whilst in the grab.	5.9 cm diameter core pushed vertically through the sediment. Sample analysed according to separate procedure. Sediment retained for PSA sample.
	Sediment sulphides	Small subsamples analysed on site in the vessel laboratory.	No details given.
Quality Control			
Equipment inspection		Grabs, corers, cores, position fixing equiment, sieves and sampling positions.	Van Veen grab/Craib corer, plastic fish boxes, sieves.
Sample Rejection Criteria	Surface inspection	No details given.	No details given.
	Acceptable depth/vol of Sediment	Muddy sediments 7 cm (5 ltr), hard packed sands 5 cm (2.5 ltr). Pooling of individually unacceptable grabs (noted in log).	No details given.
	Jaws	No details given.	No details given.
	Similar replicates	No details given.	No details given.
Sieve check between samples		Emeshed animals removed.	No details given.
Hose pressure		Checked and adjusted as necessary.	No details given.
Washing equipment between stations		No details given.	No details given. if material stuck.
Labelling		Pre-labelled sample pot.	No details given.
Bibliography		Present.	No details given.

3	4	5	6
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
Position fixing equipment, deck-wash pump, winch, sampling equipment.	No details given	Check list of equipment, inspect for damage, stow safely.	Test grab before use to ensure when closed no material lost during retrieval. Sieves checked for damage.
No details given.	No details given.	Reject if washed out.	No details given.
5 cm (measured in grab) and not solely gravel particles >4 mm.	5 cm coarse substratum, 7 cm soft.	5 cm (measured in Day grab) 3 cm measured in bin (Hamon grab).	see OSPARCOM recommendations (<5 dm^3 in mud, <2.5 dm^3 hard packed sands).
Check seal before deployment.	No details given.	Check seal before deployment.	Reject if open.
No details given.	No details given.	No details given.	Samples selected as faunal replicates must be representative of the site and as identical as possible.
No details given.	No details given.	No details given.	No details given.
Gently as possible.	No details given.	Gentle, use hand to produce less forceful spray if pressure cannot be varied.	Not too high.
Sediment hopper and grab	No details given. deployment.	Wash grab with hose prior to	Wash equipment between samples.
Inside and out.	Inside and out.	Inside and out.	Inside and out.
Yes	No	Yes	Yes

Appendix 2. Sampling for intertidal sediments

Laboratory		1	2
Procedure Title		Sampling of Intertidal Sediments.	Sampling Procedure for Marine and Estuarine Intertidal Sediments.
Purpose and Principle		Yes	Yes
Personnel			
Experience		Trained individuals (see training records) or trainee under supervision of the senior scientist.	Trained individuals or trainee under supervision.
Number		No details given.	2
Equipment			
Equipment list		Yes	Yes
Box corer	Area	25 cm x 25 cm box corer (0.0625 m^2)	25 cm x 25 cm box corer (0.0625m^2)
	Insertion depth	10-20 cm	20 cm
Hand Corer	Area	0.0176 m^2 circular plastic	0.00273 m^2 circular plastic
	Diameter	15 cm	5.9 cm
	Insertion depth	10-20 cm	10 cm
Vessel		Used on flooding tide	Not used.
Sieve	Mesh size	1 mm/0.5 mm	1 mm/0.5 mm
	Diameter	45 cm	No details given.
	Height	No details given.	No details given.
Reagents			
Formaldehyde Solution	Strength of working solution.	Borax buffered 10% formaldehyde solution.	Borax buffered 4% formosaline containing Rose Bengal.
	Ratio of fixative volume/ sample volume.	No details given.	No details given.
	Dispensing container	No info.	25 l container.
	Shaking	Sample shaken to ensure mixing.	Sample shaken to ensure mixing of fixative.
	Formaldehyde added in field or lab.	Lab or on site if >24 hrs	Lab.
	Min. time for fixing/staining	No info.	72 hrs
Rose Bengal			
Concentration		Not used.	0.1 g/litre
Safety			
Field		Refer to safety procedures.	Refer to safety procedures manual.

3	4	5
Core Sampling of benthic invertebrates in intertidal and shallow-water subtidal marine and estuarine sediments.	Intertidal Sampling Procedures.	Collection, Processing and Preservation of Macroinvertebrate Samples from Estuarine and Coastal Intertidal Sediments for Community Assessment.
Yes	Yes	Yes
One individual to be experienced in procedure.	No details given.	No details given.
2	No details given.	2
Yes	No	Sampling devices only.
N/A	N/A	0.1 m^2 Intertidal Box corer (separate procedure for use).
N/A	N/A	To be determined by pilot study, refer to literature and/or previous work.
0.00196 m^2 or 0.00785 m^2 plastic.	0.01 m^2/0.0425 m^2 steel.	0.01 m^2
5 cm or 10 cm	No details given.	No details given.
10 cm (hard clay) and 15 cm (soft silt).	No details given.	No details given.
Not used.	Not used.	Hovercraft used when large distances involved.
1 mm/0.5 mm	1 mm/0.5 mm	0.5 - intertidal, 1 mm - coastal samples (must conform to BS 410).
No details given.	20 cm	No details given.
10 cm minimum, preferably 15 cm.	No details given.	No details given.
Borax buffered 10% formalinosaline solution.	10% formaldehyde solution.	Borax buffered 5% formaldehyde solution.
No details given.	No details given.	Muds 3:1, sandy samples 2:1, organic muds 10% formaldehyde sol?
No details given.	No details given.	Rigid polypropylene container.
No details given.	No details given.	No details given.
Field.	No details given.	Lab.
No details given.	No details give.	No details given.
0.05 g/litre	Normally added but no details given.	Used at discretion of biologist concerned.
Never alone on mudflats. Where possibility of sinking/in rough seas lifeline and harness to be used (sampler walks ahead. Refer to tide tables.	No details given.	Dangers highlighted (quick sand, sharps in sediment, rapid tidal fill, Health Hazards - protective clothing etc). Guidance for intertidal and boat work read before undertaking work.

Appendix 2. continued: Sampling for intertidal sediments

Laboratory		1	2
Formaldehyde Solution	Danger	Toxin of medium hazard.	Toxin of medium hazard.
	Handling	Well ventilated area.	Well ventilated area.
	Protective clothing	Eye protection, disposable gloves.	Eye protection, disposable gloves.
	Dispenser	Pressmatic' dispenser	No details given.
	Emergency guidance	Rinse with plenty of waterin case of contact with eye and seek medical advice.	No info.
Rose Bengal	Danger	Not used.	Extremely hazardous carcinogen.
	Handling	Not used.	Fume extraction.
	Protective clothing	Not used.	Disposable gloves.
Procedure			
Time of Collection		HW/LW	LW
Position fixing		Visual transect/bearings and distances from landmarks (1-50 m accuracy).	Visual transect/bearings and distances from landmarks (1-50 m accuracy).
Replication		1-10 depending on objectives.	1-10 depending on objectives.
Info recorded at station		Time of sampling, depth of sample, description of sediment, sieve mesh size used.	Notes?
Where data recorded		Recorded using pencil and water resistant notebook.	Field sampling notebook.
Collection of Benthos samples	Sieving carried out in laboratory or field?	On site if time permits. Sea water must be used in the laboratory.	Laboratory.
	Transfer fauna sieve to container.	Backwashing using funnel.	Sample transferred from core direct to sample bag.
Collection of other samples	PSA	100-200 g 1-2 cm surface scrape type of scoop not stipulated, pot storage.	Entire round core to 10 cm sealable polythene bag.
	Trace metals	100-200 g 1-2 cm surface scrape type of scoop not stipulated, pot storage.	100-200 g 1-2 cm surface scrape, plastic scoop, sealable polythene bag.
	Organic C & N	100-200g 1-2 cm surface scrape type of scoop not stipulated, pot storage.	100-200 g 1-2 cm surface scrape, plastic scoop or metal scoop sealable polythene bag.
	Organochlorine pesticides / hydrocarbons	100-200g 1-2cm surface scrape, hexane rinsed metal scoop into hexane rinsed glass container with aluminium lid liner.	100-200 g 1-2 cm surface scrape, hexane rinsed metal scoop into hexane rinsed aluminium container.
	Redox potential	Insert probe directly into sediment.	Refer to separate procedure.
	Sediment cellulose	N/A	100-200 g 1-2 cm surface scrape, plastic scoop or metal scoop sealable polythene bag.
	pH	Dig hole and measure Ph of interstitial water using a probe.	N/A

3	4	5
Toxin of medium hazard.	Not mentioned.	Toxic, carcinogenic, irritant.
Fume extraction. Siphon for transfer of neat chemical.	No details given.	Fume extraction - lab, well ventilated area.
Eye protection, disposable gloves, label re to.	No details given.	Eye protection, chemical resistant gloves.
No details given.	No details given.	Rigid polypropylene, no glass.
No info.	No details given.	Workers to be familiar with precautions and clean-up procedures.
Extremely hazardous carcinogen.	Not mentioned.	No details given.
Fume extraction.	No details given.	No details given.
Disposable gloves and refer to label.	No details given.	No details given.
LW	No details given.	LW
Marker posts/description in relation to landmarks.	Hand-held GPS.	With reference to fixed landmarks. Compilation of site directory recommended for each survey including grid reference, photos, etc. Tide heights calculated using tidal info. Start on falling tide.
25 samples (5 cm core) and 13 samples (10 cm core) but multiple replicates where community richer?	3 at high, mid low tide level giving transect down beach (5-7 with 0.0425 m^2).	5 for temporal trends. Increase number in coarse sediments 0.01 m^2 corer.
Position of sample sites, depth of water, depth and nature of sediment.	No details given.	Colour, smell, texture, redox discontinuity depth, presence of surface features, photograph.
Field sheets.	No details given.	Sampling record sheet (includes prompts).
On site/laboratory within 12 hrs.	Laboratory?	Laboratory as there is a lack of control over conditions and potential for cross contamination in the field. Samples should be fixed ASAP as tap water impairs specimen quality.
Backwash.	Backwashed over a tray to catch any spillage.	Backwashing using funnel. Emeshed fauna removed with forceps. Sample rejected if material spilt.
No details given.	No details given.	No details given.
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A
N/A	N/A	N/A

Appendix 2. continued: Sampling for intertidal sediments

Laboratory		1	2
Quality Control			
Equipment inspection (pre-survey checks)		All equipment checked prior to use.	Sieve checked before use.
Sieve check between samples	Sieves	Check for emeshed animals.	No details given.
Washing equipment between stations		Yes	No details given.
Analysis of sample	Depth	No details given.	No details given.
Labelling		Pots should be labelled?	Pots should be labelled?
References		Yes	No

3	4	5
Check all equipment for damage before use/check positions and charts taken on survey.	No details given.	Sieves disposed of at first sign of damage.
Yes	No details given.	Wash between samples to prevent clogging of the sieve.
No details given.	No details given.	Yes
No details given.	No details given.	Kept constant (marker on outside of sampling device).
Survey Area, station code, replicate number, data.	No details given.	Waterproof, site name, rep, date. Label container and lid. Label from field should remain with sample.
No	No	Yes

Appendix 3. Trawl sampling

Laboratory		1
Procedure Title		Sampling of Fish and Epifauna for Biological or Chemical Analysis.
Purpose and Principle		Water quality and environmental impact assessment. Tissues samples analysed for trace metals and organochlorine analyses.
	Limitations	Trawling efficiency affected by wind, tide, clogging of mesh by jellyfish, pebbles etc. Larger debris may damage gear.
Personnel		
Experience		Trained individuals only (scientists and crew).
Number		Minimum of 2.
Equipment		
Other equipment listed		Yes
Beam Trawl	Name	2 m modified Agassiz Trawl with tickler chain
	Cod end mesh	13 mm
	Mesh size	72-74mm
Otter Trawl	Name	Otter Trawl (23 m headline)
	Cod end	No details given.
	Mesh size	70-85 mm
Safety		
Fieldwork	Vessel	Department of Transport conditions for survey vessel.
	Protective Clothing	Appropriate protective clothing including steel toe-capped boots, hard hats, protective gloves.
	Identified dangers	Moving cables, winch drums, spiny fish, urchins, stinging jellyfish, sharp jagged debris, explosive devices, corrosive materials. Wash hands at end of sampling.
Procedure		
Position fixing	Differential GPS	Yes
	DECCA	Yes
	Visual transect/ bearing (operating from small vessels)	Yes
Information recorded	Distance towed	Pre-set tows.
	Positions recorded	If deviating from set positions.
	Tow time	Noted
	Tow speed	Variable
	State of tide	Low water slack in estuaries (fish concentrated in channels).
	Other info noted	Tide and weather.
Instructions for handling gear		Yes

2

Sampling and Analysis of the Epibenthos of Soft Substrates using Beam Trawls.

Epifauna sampling (distribution of these animals too sparse relative to a 0.1 m^2 grab). Assessment of marine litter and pollution status of an area. Generates data in short time period.

Capture efficiency varies with substrate type and weather conditions. Data is semi-quantitative and not suitable for detection of subtle numerical trends.

No details given.

No details given.

No

Lowestoft 2 m beam trawl (wooden beam, 3 tickler chains). Gear details are referred to in another document.

3 mm

No details given.

N/A

N/A

N/A

No details given.

No details given.

None.

Yes

Not used.

Not used.

0.5- 1 km (allows full census of animals).

When shooting is complete and when hauling has begun.

5-10 mins

<1 knot

Variable.

Weather conditions, wind speed and direction, sea state, start and end position, time of day, depth range, sample volume, presence of artefacts.

No

Appendix 3. continued: Trawl sampling

Laboratory		1
Sample Processing		
Sieve		No details given.
Identification	Colonials/encrusting animals	No details given.
	Problem specimens	No details given.
	Infauna	No details given.
	Residual material	No details given.
Sub-sampling		No details given.
Quality Control		
Maintenance of good bottom contact		Gear examined before and after each trawl. Repair carried out onsite. Interferences noted in sampling notes. Survey terminated with severe damage.
Pre survey checks		Positions and transects checked prior to survey.

2
5 mm mesh.
Recorded on relative abundance scale.
Preserved and returned to lab for further invest.
Noted but omitted from results.
Noted in log.
Divide catch on mesh or take measured vol, count and multiply up.
Warp remains under tension, examination of shoes, meter wheel?
No details given.

Appendix 4. Laboratory analysis of benthic macrofauna

Laboratory		1	2
Procedure Title		Determination of Benthic Macrofauna species abundance, biomass, tomato pips and other seeds.	Marine and Estuarine Fauna Analysis.
Purpose and Principle		Yes	Yes
Personnel			
Experience		Trained individuals or trainee under supervision.	No details given.
Number		No details given.	No details given.
Equipment			
Equipment list		Yes	Yes
Illuminated Magnifier		Or bench lamp.	No
Stereo magnifier		x10-40	x50
High Power microscope		x100-1000 with calibrated graticule.	x630
Sieve	Mesh	0.5 mm, 1.0 mm, 2 mm-8 mm	0.5 mm, 4mm (BS410)
	Diameter	No details given.	No details given.
Balance		0.01 mg - 1000 mg (fauna < 200 mg) and 0.01 g - 100 g (fauna>200 mg). Separate maintainance procedure.	N/A
Reagents			
Fixative	Strength of formaldehyde	4% buffered formaldehyde solution.	10% buffered formaldehyde solution.
	Minimum temperature permitted	<5 deg C polymerisation of formaldehyde occurs removing its fixative qualities	No details given.
	Length of time for fixation	No details given.	No details given.
Rose Bengal	Strength	5 ml of (0.01%) Rose Bengal	625 mg/25 ltr. concentrate added to a sample after washing for 20 minutes then washed out.
Preserving solution		70% Industrial Methylated Spirits (IMS), 10% glycerol, 20% water.	70%IMS, 10% glycerol, 20% water.
Polyvinyl lactophenol/ Ammans's lactophenol (clearing agents and slide mounts)	Used	Yes	No
Sodium Hypochlorite Solution (dissolves soft tissues from specimens, avoiding damage to calcareous parts)	Used	No	No
Safety			
Separate section		Yes	Yes
Formalin	Danger	Toxin of medium hazard.	See Hazard info sheet (appendix).
	Storage container	No details given.	See Hazard info sheet (appendix).
	Handling of chemical	Under fume extraction.	See Hazard info sheet (appendix).

3	4	5	6
Identification and Enumeration of Benthos.	Laboratory Analytical Procedures.	Analysis of Macrobenthic Infauna.	Procedure for the Determination of Biomass of Marine and Estuarine and Benthic Invertebrates.
Yes	No	Yes	Yes
No details given.	Experienced taxonomist.	Trained individuals or trainee under supervision.	No details given.
No details given.	Ideally one person.	No details given.	No details given.
Yes	No	Yes	N/A
x1.75	x1.5	Yes	N/A
x10-50 with calibrated graticule?	x80 (x160 with addition of x2 eye piece).	x10-40	N/A
x100 - 1000 with calibrated graticule	x1000	x100-1000	N/A
4 mm, 2 mm, 1 mm, 0.5 mm	0. 5 mm (estuarine), 1 mm (coastal)	2 mm, 1 mm, 0.5 mm (BS410)	N/A
No details given.	No details given.	20 cm	N/A
N/A	N/A	0.01 mg - 1000 mg	Weighing to 0.0001 g.
4% formaldehyde and mixing. Store for at least 4 days.	10% buffered formaldehyde solution.	4% buffered formaldehyde solution	N/A
<5 deg C polymerisation of formaldehyde occurs removing its fixative qualities.	10 deg C constant.	No details given.	N/A
If sample is to be stored for 1 month or more before further processing then fixative should be relaced with an IMS based preservative (min conc 50% meths - without glycerol if samples to be biomassed).	Min 72 hrs, shaking once during period.	No details given.	No details given.
Add to approx 0.01%.	Concentration not stated.	Added at discretion of the biologist.	N/A
70%IMS, 5% glycerol, 25% distilled water or 70% IMS, 30% distilled water if biomassing.	No details given.	Ethanol 70%, glycerol 10%, water 10%	70% IMS, 10% glycerol, 20% water.
Yes	No	No	N/A
Yes	Yes	No	N/A
Safety information included in text	No	Yes	No
Toxin of medium hazard.	No details given	Toxin of medium hazard.	N/A
No details given.	No details given.	50 ltr plastic aspirator.	N/A
Siphon used for transfering neat chemical from container to container.	No details given.	Funnel should be used when transferring the neat chemical from one container to another.	N/A

Appendix 4. continued: Laboratory analysis of benthic macrofauna

Laboratory		1	2
	Handling of fixed samples	Samples thoroughly washed under fume extraction before handing in the open.	Samples washed under fume extraction.
	Protective clothing	Eye protection, disposable gloves.	No details given.
	Instructions for accident	No details given.	Wash off skin thoroughly and see Hazard info sheet (appendix)
Industrial Methylated Spirits/ Ethanol	Danger	Toxic, highly flammable.	See Hazard info sheet (appendix).
Rose Bengal	Danger	Extremely hazardous carcinogen.	No info.
	Handling	Neat chemical handled under fume extraction.	No info.
	Protective clothing	Disposable gloves to be worn when handling a solution of substance.	No info.
Glycerol		No details given.	See Hazard info sheet (appendix)
Polyvinyl lactophenol/ Ammans's lactophenol	Danger	Highly toxic.	N/A
	Handling	Well ventilated area.	N/A
	Protective clothing	Disposable gloves.	N/A
Sodium Hypochlorite	Danger	Not used	N/A
Solution	Protective clothing	N/A	N/A
Procedure			
Elutriation		Yes	No
	Rinsing	Rinse thoroughly with water under fume extraction.	Rinse thoroughly with water under fume extraction.
	Sieve mesh size used when rinsing sample	Same mesh size or smaller than that on which sample originally retained.	No details given.
Fractionation (partition sample for ease of sorting)	Sieve sizes.	Sample may be fractioned using 2 or 3 sieves (8 mm, 2.8 mm, 1/0.5 mm).	Not carried out.
Sorting	Method	Shallow white tray.	Shallow white tray.
	Picking tools	Forceps or pipette.	No details given.
	Illumination/Magnification used for sorting?	Bench lamp or illuminated magnifier. If 0.5 mm sieve used then sorting under stereo microscope.	Low power stereo microscope when samples contain large amounts of fine detritus.
	Residue checked twice?	No	No
	Level of sorting	Family.	Lowest possible.
Sub-sampling	Amount required for subsample.	No advice.	1/4 for 50 individuals, 1/8 - 100, 1/16 - 200 (may include different species).

3	4	5	6
Samples thoroughly washed under fume extraction before handing in the open.	No details given.	Samples thoroughly washed under fume extraction before handing in the open.	N/A
Eye protection, disposable gloves ref to label.	No details given.	Eye protection, disposable gloves ref to label, clearly labelled	N/A
None	No details given.	No details.	N/A
No warning.	No details given.	No warning.	Highly flammable.
Extremely hazardous carcinogen.	No details given	Extremely hazardous carcinogen.	N/A
Fume extraction.	No details given.	Fume extraction.	N/A
Disposable gloves.	No details given.	Disposable gloves.	N/A
No details given.	No details given.	No details given.	Ref to COSHH.
No details given.	N/A	N/A	N/A
No details given.	N/A	N/A	N/A
No details given.	N/A	N/A	N/A
Hazardous?	No details given.	N/A	N/A
Eye protection and disposable gloves.	No details given.	N/A	N/A
Yes	Yes	Yes	N/A
Rinse thoroughly with water under fume extraction for a mininimum of in formalin or 5 mins if sample contained in an IMS based preservative.	Sample washed, no mention of fume extraction?	Carry out in a fume cupboard. 30 mins for a sample contained	N/A
Same mesh size or smaller than that on which sample originally retained.	Not clear.	Same mesh size as that on which sample originally retained.	N/A
4 mm, 2 mm, 1/0.5 mm. Careful inspection of sieves essential to prevent animals being lost or carried over to different sample.	Nest (smallest mesh 0.5 mm).	Carried out at sea 1-5 mm >5 mm, >64 mm.	N/A
Shallow white tray.	Shallow white tray.	Shallow white tray (marked into 16 equal rectangles). Amount of material on tray shouldn't obscure black lines.	N/A
Flexible forceps (avoid damage to delicate mollusc shells/ other small organisms).	No details given.	Watchmakers forceps.	N/A
Illumuinated magnifier. Small volumes can be sorted directly under stereomicroscope.	Illumuinated magnifier.	Illuminated magnifier.	N/A
No	By another analyst.	By same analyst.	N/A
No info.	Family.	Family.	N/A
4 1/16s for a quarter. Further subsample for v abundant species.	1/4 sample. Process repeated if still large nos.	1/4 minimum.	N/A

Appendix 4. continued: Laboratory analysis of benthic macrofauna

Laboratory		1	2
	Method	Tray	Tray?
	Large and rare animals	No details given.	Picked out in the normal way.
	Reported in any final report?	Yes	No
Sieve residue		Retained for QC.	Retained for QC.
Identification	Level	Lowest possible	Species or lowest possible if specimens damaged or taxonomic features undeveloped
	Animals not included in quantitative dataset	No details given.	No details given.
	Specimen preparation techniques 1. Staining	Not used.	Not used.
	Specimen preparation 2. Clearing	For the examination of Oligochaetes: immersion 100% IMS, 70%, 30%, water 15 minutes each to remove glycerol. Removal of water by blotting. Place in polyv lact or Amman's lactophenol on microscope slide. Seal slide with glyceel. Examine after 48 hrs to clear. Biomass should be determined before clearing.	Not used.
	Specimen preparation techniques 3. Removal of soft tissues	Not used.	Not used.
Biomass	Method	Wet weight (conversion factors allowing calculation of Ash Free Dry Weight). Conversion factor in Appendix.	N/A
	Weighing container	No details given.	N/A
	Rinsing with water	No details given	N/A

3	4	5	6
Tray	Perspex quarteriser.	In fume cupboard whole sample spread out onto tray. 1/4 or more. All material from 2 squares removed washed. Count number of individuals of species to be subsampled. Repeat process for another two rectangles. If no. of individuals in common = at least 90% no requirement to further subsample. Then process entire sample ignoring subsampled species.	N/A
Removed from the whole sample as they are unlikely to be sufficiently represented in any subsample.	Removed from the whole sample.	No info.	N/A
Yes	No	No	N/A
Retained for QC.	Not retained.	Retained for QC.	N/A
Lowest possible	Lowest possible (species) unless agreed.	Lowest possible	N/A
No details given.	Incomplete animals without anterior ends, motile and colonial sessile epibenthic taxa and meiofauna.	Headless specimens.	N/A
Methylene blue used to highlight certain features of polychaetes.	Not used.	Methyl blue to stain polychaetes.	N/A
For examination of oligochaetes and spicule formation of sponges. Oligochaetes: immersion 100% IMS, 70%, 30%, water 15 minutes each to remove glycerol. Removal of water by blotting. Place in polyvinyl actophenol or Amman's lactophenol on microscope slide. Seal slide with glyceel. Examine after 48hrs to clear. Sponges: cut thin sections, using a razor, perpendicular to the sponge, top to bottom. Immerse in absolute alcohol for 10 mins to dry. Section cleared in dryed clove oil.	Not used.	Not used.	N/A
Used to open juv bivalves destroying shell. without Specimen immersed in small vol of hypochlorite solution in a watch-glass.	Used to open juvenile bivalves without destroying shell.	Not used.	No details given.
N/A	N/A	Wet weight (conversion factors allowing calculation of AFDW). Conversion factor in Appendix.	Wet weight.(conversion factors allowing calculation of AFDW).
N/A	N/A	Tared vial of water on the balance.	Half filled water containing weighing boat/cruicible (water stops further evap of preservative from specimens).
N/A	N/A	No	Remove as much preservative as possible.

Appendix 4. continued: Laboratory analysis of benthic macrofauna

Laboratory		1	2
	Blotting	Blue tissue paper.	N/A
	Time before reading	30 secs	N/A
	Level of biomassing	Taxon	N/A
	Taxa containing water (Echinoidea, Echiurida)	Punctured and drained.	N/A
	Tube dwelling taxa	May be weighed in tube (conversion factor applied)	N/A
	Polychaete fragments	No advice.	N/A
	Storage liquid prior to biomassing.	Water	N/A
Quality Control *AQC Criteria*		If reanalysed samples fails 1 or more of following criteria then whole batch reanalysed except where only relevant specimens reanalysed or where error caused by inadequate sorting (resorting required): a) where taxa contributing 5% or more of the total sample abundance have been mis identified; or b) where the total number of taxa varies by more than 10% or 2 species (whichever is the greater); or c) where the total abundances of the two samples vary by more than 10% or 10 individuals (whichever is the greater); or d) where the Bray-Curtis or b) where the total analyses is less than 0.90.	10% difference (Bray Curtis) then repeat entire analysis. However errors which would not have affected subsequent interpretation of the data considered acceptable.
	Samples not included in reanalysis	None.	Bucket samples disposed of after sorting.
	Applying transformations to QA data	No	AQC data should be subjected to the same transformation as original data before analysis. Overall mean similarity for each of the sample checks calculated. A similarity of 90% or greater (<10% error) is sufficient to ensure that the same grouping of survey stations according to fauna type should result from original and repeated analysis.

3	4	5	6
N/A	N/A	Blotting paper.	Move around until no wet patch is left. Re-rinse then blot again.
N/A	N/A	Once stability reached.	No info.
N/A	N/A	Taxon	Usually family.
N/A	N/A	Punctured and drained	No info.
N/A	N/A	No details given.	Removed from tubes and rinsed in water.
N/A	N/A	Weight allocated across all identified species in proportion to the amount of biomass represented by each species of complete specimens.	No details given.
70% IMS, 30% Distilled water.	N/A	70% IMS, 10% glycerol, 20% water.	70% IMS, 10% glycerol, 20% water for min 3 months to allow for weight loss stabilisation (Rees *et al*, 1990).
See quality control procedure?	No criteria.	If reanalysed samples fails 1 or more of following criteria then whole batch reanalysed except where only relevant specimens reanalysed or where error caused by total number of taxa varies by more (resorting required): a) where taxa contributing 5% or more of the total sample abundance have been misidentified; or b) where the total number of taxa varies by more than 10% or 2 species (whichever is greater); or c) where the total abundances of the two samples vary by more than 10% or 10 individuals (whichever is the greater); or d) where the Bray-Curtis similarity coefficient for the two sets of analyses is less than 0.90.	N/A
See quality control procedure?	None.	None.	N/A
See quality control procedure?	N/A	No	N/A

Appendix 4. continued: Laboratory analysis of benthic macrofauna

Laboratory		1	2
Check Sorting		Yes	Yes
	% reanalysed	10% 1-14 - 1 sample reanalysed, 15-24 - 2 samples reanalysed,etc	10% from survey area randomly selected before processing begins (very large vol samples excluded from QC exercise)
Efficiency of sub-sampling checked?		No	Sub sampled animals returned to sorting tray along with sed residue. Sub-sampling procedure repeated by independent analyst.
Identification	Accuracy of fauna ID.	All fauna re-identified.	All fauna re-identified.
	Difficult ID involving 2 analysts	Discuss consider id to lower level or refer to outside help (museum).	External verification or identification to a lower taxonomic level.
	Procedure requires checking of species matrix checked to ensure all specimens found are within their known geographic range?	Yes	No
Accuracy of enumeration		Yes	Yes
Reporting of results		Results inc in final report	Results of AQC analysis should be presented and interp in same report as fauna data.
Biomass		None	None
Reference collection maintained?		Specimens previously unrecorded by lab can be sent for external validation.	No details given.
Approved list of taxonomic keys and literature?		Appendix approved list.	No

3	4	5	6
Yes	Yes	Yes	N/A
See quality control procedure?	100%	10%. Every tray check sorted by same analyst.	N/A
See quality control procedure?	No	No	N/A
See quality control procedure?	Cross checking by experienced analyst and external verification.	All fauna re-identified.	N/A
See quality control procedure?	External verification.	Individuals not encountered put in vial with id code for independent ID by another analyst. If confirmed sample goes into ref collection.	N/A
See quality control procedure?	No	No	N/A
See quality control procedure?	No	Yes	N/A
See quality control procedure?	No	No	N/A
See quality control procedure?	N/A	None	None
Maintained for each survey.	Yes	Maintained for each survey.	N/A
Appendix approved list.	Appendix approved list (Rees *et al* (1990).	No	N/A

Appendix 5. Particle Size Analysis

Laboratory		1
Procedure Title		Determination of Sediment Particle Size (by weight) of Marine and Estuarine Sediments.
Purpose and Principle		Yes
	Limit of detection	8 mm to 3.9 µm.
	Results reported	% dry weight based on Wentworth scale.
Personnel		
Experience		Trained individuals or trainee under supervision.
Equipment		
Equipment List		Yes
Laser Sizer		Laser granulometer.
Sieves		8 mm - 63 µm in half phi units.
Pneumatic trough		Yes
Mechanical sieve shaker		Yes
Filtration equipment for GFC Filtration		No
Reagents		
Hydrogen peroxide solution (removal of organic matter)	Strength used	6% w/v H_2O_2 GPR
Sodium Hexametaphosphate solution	Strength used	Not used.
Water		Tap-water.
Safety		
Hydrogen peroxide solution	Hazard	Mild oxidising agent.
Protective clothing		Lab coat.
Procedure		
Samples frozen prior to analysis?		Yes
Oven Drying (prior to homogenisation)?		No
Homogenisation		In plastic bag using spatula.
Removal of Organic Matter		If requested using Hydrogen peroxide
Vol/weight sediment used		100 ml of treated or 100 g of wet homogenised sediment.
Oven Drying (after homogenisation)	Temp	70 +/- 10 deg C
	Time	No details given
Total weight of sample measured		Yes (to 2 decimal places).
Wet Sieving (removal of fine fraction)	Sieve size	125 µm sieve inside pneumatic trough.
	Time	Until filtrate clear.
Oven dry (coarse fraction)	Temp	70 +/- 10 deg C (>125 µm).
	Time	No details given.
Sieve Shaker	Sieves	See Equipment section.
	Time	10 minutes.
Oven dry fine fraction (no further analysis of fine fraction)		No

2	3	4
Particle Size in Marine Sediments	No details given.	Standard Operating Procedures - Sediment Analysis
Yes	No details given.	No details given.
4 mm to 2.8 µm.	2 mm to 0.1 µm.	No details given.
% dry weight based on Wentworth scale.	No details given.	% dry weight based on Wentworth scale.
No details given.	No details given.	No details given.
Doesn't include all apparatus required.	No	No
Not used.	Malvern MasterSizer X laser diffraction particle size analyser.	Coulter LS 130 Laser-Sizer.
Stainless steel 4 mm - 63 µm in phi units.	Not used.	Stainless steel. Largest sieve determined by largest particle size down to 500 µm n half phi units.
No	N/A	No
Yes	N/A	Yes
Yes	No	No
Not used.	Not used.	Not used.
6.2 g/ltr	No details given.	Used.
Distilled or de-ionized.	No details given.	Distilled
N/A	No details given.	N/A
N/A	No details given.	N/A
Yes	No details given.	Yes
Yes (temperature not given).	No details given.	No
Add sediment to 200 ml deionized water & 20 ml Sodium Hexametaphosphate. Stir10-15 mins, soak overnight, re-stir 10-15 mins.	No details given.	Thoroughly mix half the sample using a plastic scoop. Take small scoops from whole sample.
No	No details given.	Not used.
50 g of oven dried sediment .	No details given.	Variable.
No	No details given.	No
N/A	No details given.	N/A
Yes (to 4 decimal places prior to homogenisation).	N/A	Yes (to 2 decimal places).
63 µm sieve, sample manually puddled.	N/A	500 µm sieve using a sieve shaker.
Until filtrate clear.	N/A	Until filtrate clear (minimum of 15 minutes).
105 deg C (>63 µm).	N/A	80 deg C +/- 5 deg C.
Overnight	N/A	12 hours.
See Equipment section.	N/A	See Equipment section.
15 minutes +/-30 secs.	N/A	10 minutes.
No	N/A	80 deg C +/-5 deg C.

Appendix 5. continued: Particle Size Analysis

Laboratory		1
Freeze dry fine fraction		No
Weighing		Separated coarse fractions weighed to 2dp. Brush used to ensure all material transferred.
Results	Coarse	Calculate % of each fraction from weights.
	Fines (Total weight)	Determined by subtraction of total coarse fraction weight.
Detailed analysis of fines (Laser)		Yes (only when <125 µm fraction >5%).
	Mixing	Add material to 1000ml measuring cylinder and mix.
	Sub-sample size	30ml
Detailed analysis of fines (Pipette Analysis)		N/A
	Mixing	N/A
	Sub-sample size	N/A
	Oven drying	N/A
	Time	N/A
Quality Control		
Re-analysis of samples	% reanalysed	10% by another analyst using retained spare sediment.
	Time allowed for re-analysis	2 weeks
	QC Criteria	% fine (<63 micron) should not vary between two analyses by >10% total weight. 3rd analyses if source of error not identified and 2 closest used to calculate derived sediment parameters.
Procedure for use, maintenance/service and calibration of laser sizer		Separate procedure.
	Sieves checked for cleanliness and damage before use	Yes
Appendix		File format for interactive statistics package. Equations for calculate of derived sediment parameters. Sediment nomenclature (explains sediment parameters).

2	3	4
N/A	N/A	Place sample in freezer at -10 deg C until frozen then freeze dry at -40 to -60 deg C until all ice removed.
Separated coarse fractions weighed to 4dp. Brush used to ensure all material transferred.	N/A	Coarse and fine fractions (when not processed further) transferred. weighed to 2dp. Brush used to ensure all material transferred.
Calculate % of each fraction from weights.	N/A	Calculate % of each fraction from weights.
Determined by subtraction of total coarse fraction weight.	N/A	Weighed directly.
No	Yes	Yes
N/A	No details given.	
N/A	No details given.	Determined by machine.
Yes	N/A	N/A
Add material to 1000ml measuring cylinder and mix.	N/A	N/A
20 ml during each time period at 20 cm below the surface.	N/A	N/A
105 +/- 5 deg C for 24 hrs.	N/A	N/A
Samples taken after specific time periods correspond to different sediment size classes.	N/A	N/A
None	No details given.	No details given.
No details given.	No details given.	No details given.
No details given.	No details given.	No details given.
No details given.	No details given.	No details given.
No details given.	No details given.	No details given.
No details given.	No details given.	No details given.