NRA

STANDARD METHODOLOGIES

Assessment of Estuarine/Marine Water Quality using Benthic Macroinvertebrates

Final Draft
September 1993

ENVIRONMENT AGENCY

National Rivers Authority
Anglian Region

STANDARD METHODOLOGIES

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using benthic macroinvertebrates.

1. INTRODUCTION.

Under the Water Resources Act (1991) the NRA has the following, main, statutory duties in Pollution Control and Water Quality which are relevant to marine biology.

- to monitor the extent of pollution in controlled waters (Section 84).
- to produce a system of classification for controlled waters (Section 82).
- to achieve Water Quality Objectives in all controlled waters (Section 84).
- to conserve and enhance the amenity of inland and coastal water, and of land associated with such water (Section 16).
- to determine and issue consents for discharge of wastes to controlled waters (Schedule 10, Section 2, Paragraph 5).
  A requirement for biological monitoring of the impact of marine discharges can be included within the consent. The schedule states that "The conditions subject to which a consent may be given under this paragraph shall be such conditions as the Authority may think fit ..."
- to monitor effluents to demonstrate compliance with consents (Water Act 1991, Section 106).
- to prosecute for polluting water, such that it is injurious to fish, under Section 4 of the Salmon and Freshwater Fisheries Act.
- whenever possible to prevent pollution at source (Section 92).

In addition, there are a number of EC Directives: Dangerous Substances Directive [76/464/EEC], Environmental Assessment Directive [85/337/EEC], Urban Waste Water Treatment Directive [91/271/EEC], Shellfish Water Quality Directive [91/492/EEC], Nitrate Pollution Directive [91/676/EEC] and Habitats Directive [92/43/EEC]. There is also the

North Sea Action Plan for which work has been incorporated into the National Marine Monitoring Programme (NMMP). As part of the NMMP baseline, surveys of key estuarine and intermediate coastal water sites are carried out.

The principal aim of monitoring marine and estuarine waters is to protect the ecosystem rather than the water itself as a potential resource. The use of biological material for pollution monitoring of the estuarine/marine environments, in combination with chemical and sediment analysis, provides valuable information about environmental quality. Chemical analysis of saline waters can be difficult due to the many natural solutes it contains. In addition, pollutants may, be present at concentrations below the detection threshold, vary in their activity with respect to macroinvertebrates, be trapped in sediments, or discharged intermittently, thereby remaining chemically undetected, biological monitoring can overcome these problems, giving a time-integrated and directly relevant indication of environmental quality.

The benthic macroinvertebrate group of organisms is more suitable for this kind of monitoring than any other estuarine/marine group. Comprising mainly the infauna of uncompacted sediments, certain epifauna may also be included. Their advantage over micro- and meio- fauna is that as their life cycle is longer and they are comparatively immobile. Macroinvertebrates, therefore, provide a static record of conditions which have existed for some time preceding sampling and are, thus, also suitable for use in long-term surveys. They are of considerable importance in estuarine/marine food chains. Their size range, usually regarded as being 0.5 mm upwards, enhances collection, extraction and identification and accumulated background and comparative data which is available also increases their usefulness.

Biological assessment of coastal quality, is not without its drawbacks, particularly with respect to species identification in some of the major taxa (*e.g.* Polychaeta). However, this situation is steadily improving with the publication of more accurate and reliable keys.

Since water quality is not an absolute concept, the use of estuarine/marine benthic macroinvertebrates for its assessment is most effective if used in a comparative way.

2. DEFINITION.

Macroinvertebrates comprise many phyla and orders within the animal kingdom. The macroinvertebrates of primary interest are relatively sedentary in habit and live in the estuarine/marine bed as infauna rather than organisms found on the surface of the sediment (epifauna, although some epifauna are collected) or those in the water column (plankton). The

mesh aperture size of the sieving apparatus (*i.e.* 0.5/1 mm) restricts the organisms identified to those retained on the mesh.

3. PRINCIPLE.

One factor affecting the presence of macrofauna, in an estuarine/marine habitat, is environmental quality. Changes in environmental conditions affect species diversity and numbers of individuals. As pollution levels increase, the number of species tends to decline and the resulting lack of competition can lead to a proliferation of pollution tolerant species. Gross pollution, particularly toxic, usually results in a decline of all species. More subtle or chronic pollutions can be difficult to differentiate from natural phenomena. Many natural factors influence the distribution of estuarine/marine macroinvertebrates, such as geography, season, physical nature of the substrate, salinity regimes, currents and plant growth and biologists must use their experience to differentiate between the effects of environmental quality and natural factors influencing the fauna found at each location.

The method, thus, depends upon obtaining a representative sample of the benthic macroinvertebrate community, of the habitat under study, for comparison with other stations and/or with an expected "normal" community.

As the method is comparative it is extremely important that the techniques used are standardised so that data can be directly compared. Any differences can then only be attributable to environmental factors and not differences in the methodology. The methods described in this manual are Nationally agreed recommended **minimum** requirements.

4. CHEMICAL HAZARDS ASSOCIATED WITH COLLECTION AND ANALYSIS.

4:1. Inventory of chemicals.

i) Water.
Although dilution and dispersion are considerable within the marine/estuarine environments, where sewage or other discharges occur localised pollution may pose a significant health hazard. The risks associated with *Leptospirosis* and other water borne diseases must be appreciated (Appendix 1). Physical contact should be avoided by use of appropriate clothing including long PVC/rubber gauntlets and all wounds should be covered with waterproof dressing.

		Use	COSHH No.
ii)	Formaldehyde	Fixative/preservative for biological material	0106
ii)	Methylated spirit (IMS)	Preservative for biological samples (recommended for long term storage)	0157
iv)	Disodium tetraborate	Buffer for formal saline (500 gL^{-1}),if used	1107
v)	Glycerol	Added to IMS (1:20) to reduce evaporation	0167
vi)	Polyvinyl lactophenol	Clearing agent used to aid identification	0503
vii)	Ammans lactophenol	Alternative clearing agent (less toxic)	0576
viii)	Acetone	To remove permanent ink from containers	0009
ix)	Chroma - FNC	Formalin neutraliser for significant spills	

4:2. **COSHH assessments.**

For detailed information on each of the chemicals listed in section 4:1 and their handling refer to the relevant COSHH assessments and safety manuals, held in the laboratory.

5. PHYSICAL HAZARDS.

5:1. The sea-shore.

Never sample alone, always work in pairs or groups.

In some cases, such as working along the strand-line of a sandy shore, the hazards are minimal, but most types of sea-shore work must be considered as hazardous at best and some as dangerous. Rocky shores are always potentially hazardous because of their uneven surfaces, slippery weed cover and fissures, while exposed headlands, liable to violent wave action, are dangerous. Muddy shores are often hazardous, due to their thixotropic nature and also because they can be slippery, especially where extensive diatom growth occurs at the surface; sandy and muddy shores can be additionally hazardous because of rapid tidal fill, broken glass and other obstacles, and dangerous if very extensive.

5:1:1. Clothing.

Clothing should be suitable for the worst potential weather, and if inclement weather is at all likely, additional clothing should be carried. In colder weather a pair of long PVC/rubber gauntlets and some form of hat can prevent excessive heat-loss from the hands and head. In warm weather the possibility of sunburn should be borne in mind.

Some form of footwear **must** be worn, either rubber boots or waders. The use of chest waders should be avoided, but where they are necessary only trained staff, aware that the buoyancy of the waders may hold them in an inverted position if they are swept away, should wear them together with a life-jacket. The soles of any footwear should have adequate tread and/or studs, especially for work on rocky shores.

5:1:2. **Equipment.**

Each group should know the tide times or carry a set of local tide-tables, especially when working on shores liable to be cut off by the tide.

Each person should take adequate food and drink for the duration of the excursion. Always wash hands and forearms before eating or drinking even if gloves have been worn, bearing in mind the hazards mentioned in Section 4:1:i.

If possible, a portable marine band VHF radio should be carried. Working in hazardous and dangerous areas at darker times of the year is discouraged. A torch and spare batteries must be taken, ***N.B.*** International Distress Signals (Appendix 3). In hazardous areas, each group should carry a whistle, a watch and a first aid kit, plus flares and a length of rope. On extensive shores, each group should also carry a compass and maps.

All staff must wear life-jackets (with attached lights) when sampling or working on or near deep water, this includes water where the maximum depth exceeds knee height. If chest waders are used a life-jacket **must** be worn (see 5:1:1).

5:1:3. **Procedures.**

A procedure exists for recording the time that the biologist(s) leave for and are expected to return from fieldwork. Details of the route, locations and estimated times of departure and arrival should be recorded and left with a designated person. Any significant changes from the plan should be reported, *i.e.* delays of 1 hr or more, changes in the sites/area to be visited. When the work is completed the designated person should be told. If staff have not reported in by the expected time (+ 1 hr) then the designated person will set the emergency search procedure in motion. The procedure will involve search parties who will check the areas intended to be visited.

For each site and situation assessment of the hazards should be made and suitable precautions taken, such assessment should include prevailing weather and provision for abandoning work should conditions change.

On all shores all participants should be made aware of potential hazards and dangers, and how best to proceed. For example, it should be pointed out that on rocky shores fissures can occur beneath weed, so they should try their footing before putting weight down. Jumping from rock to rock is dangerous. Also, loose rocks can occur unexpectedly. On sandy and muddy shores, rapid tidal flow can cut off areas very quickly. Work should always be carried out on the ebb, working down the shore, and the return journey should begin, depending on the distance to be covered and the softness of the substrate, from one hour before to one hour after the predicted time of low water. If the substrate is particularly difficult or extensive then the aim should be to be clear of the shore before the tide turns.

The Leader should ascertain from charts or local experts where there are special dangers, such as thixotropic sediments (quicksands) liable to give way. If a participant sinks into such an area the following procedure applies:

i) Call for help, and if necessary summon additional help by whistle, torch or flare.
ii) Attempt to shuffle out; do **not** try to lift the feet.
iii) If this fails, lie down, spread out and make swimming movements to move out of the sinking areas.
iv) The affected person may be suffering from exposure and/or shock by this stage, so the party should take appropriate measures, *i.e.* keep warm and seek medical advice.

In some situations, such as steeply shelving beaches and/or beaches liable to unpredictable wave action, any member entering the water or required to stand beyond the water's edge, should be roped to a member on shore, who should also be firmly anchored.

If you see any strange-looking objects on shore which may be a cartridge, shell, mortar or canister of dangerous chemical, do not touch it but report the matter at once to the Police or Coastguard. If you see another person, not necessarily in your group, or any flare out to sea, act at once by giving or calling for assistance as appropriate, do **not**, however, take unjustified risks.

N.B. Refer to safety information held in the laboratory or see your Safety Officer for further details.

5:2. **The use of boats for subtidal sampling and access to some intertidal sites.**

Only a brief outline of boat policy is given here for guidance, further information is contained in the NRA Code of Practice - Marine Activities:- Part I: Use of sea-going vessels & Part

II: Use of small vessels (in draft at present time).

Working in boats must be regarded as a dangerous activity. Small boats carry up to 12 people, in addition to any crew members. Boats with motors, inboard or outboard, require different preparation and equipment from rowing boats.

Boat users should be qualified to RYA Level I as a minimum and all visitors should ideally have some boat handling experience, however, if the ratio of trained to untrained crew is 2:1 then this requirement may be relaxed. If a boat is hired with crew, the responsibility for safety rests with the operator, but the Leader should be satisfied that adequate precautions are being taken.

5:2:1. **Clothing and equipment.**

Where adverse weather conditions are likely to occur clothing must be effectively waterproof and anticipate the worst conditions likely to arise.

At sea life-jackets *e.g*, NRA approved twin chamber automatic inflation -Crewsaver "Seafire Solas", **not** buoyancy aids must be worn at all times by all persons on board, and the operation of the jackets must be explained before departure. This may be waived on larger, covered vessels. Life-jackets must be tested/serviced at least once a year.

At sea, charts must be carried of the area of operation and local information obtained, if possible, and pencilled onto the chart, of tidal conditions, races, rocks, wrecks, and other likely hazards.

When grab samples are being taken (section 7) care is required regarding the winch and winch cables, the moving gantry (the wearing of a hard hat is advisable) as well as the grab mechanism itself.

5:2:2. **Procedure.**

Before use, the Leader or most experienced person must judge if the boat is safe and adequate for the job, and is not overfilled, either by people or equipment. In some circumstances, such as using boats at sea or in large rivers, a Leader may need to insist that all members of the party must be competent swimmers.

When intending to use boats at sea, inform the Coastguard/Harbour Authority of the route plan, estimated arrival times and details of the work to be undertaken.

6. INTERTIDAL SAMPLING METHODS.

The methodology comprises two elements, dependent upon the sediment type.

1) The basic **Core** method applied at all sites.
2) The **Supplementary Sampling Method** to be applied at sites where coarser sediments prevail. The specific method used depends on the habitat type, *i.e.* estuarine or coastal.

6:1. Core method.

6:1:1. Equipment.

0.01 m^2 cylindrical stainless steel/perspex corer with 15 cm marker on external surface plus a bung of appropriate size.

6:1:2. Operation.

The corer is placed vertically on the surface and pushed straight down into the sediment until buried up to the 15 cm mark. The bung is inserted, to prevent the sample from falling out of the corer. To remove, the corer may need to be manoeuvred to break the sealing effect of the surrounding sediment. It may also be necessary to free the corer and sediment at the base by hand. The sediment core is transferred into a clean container, preferably a plastic bucket with a sealing lid or plastic bags with cable ties, an identification label should be included.

A minimum of **5** replicate samples are to be collected from each sample position, up to 20 m either side of the marker point (this distance depends on the size of the survey grid, for example where sites are 100 m apart usually 10% of the distance between sites is allowed) but not up or down the beach if any significant gradient exists. Each replicate is retained separately.

6:1:3. Site location.

The location of each sampling point is determined by fixed landmarks, *e.g.* marker beacons, and compass bearings. Anchored structures should not be used as reference points as they move considerably in relation to wind, tides and currents. Samples taken at different shore heights are levelled either from a fixed point or taken at known states of the tide. Samples should be taken at consistent tidal height ($\pm$ 0-2 m) throughout the survey. The deviation allowed will depend upon the slope of the shore.

All details of the location should be recorded accurately so that relocation can be precise. It is recommended that a site directory be compiled for each survey with full descriptions of each site including (where appropriate) grid references, compass bearings, latitude/longitude, landmarks (including site markers such as stakes and paint) and photographs plus a description of the sediment type and sample method used. If all information is accurate it should be possible to return to an intertidal site within ± 2 m on stable shores where markers have been left. For surveys carried out on mudbanks the level of accuracy will be less due to shifting of sediment between surveys and the lack of landmarks, but again provided that details have been accurately recorded a site should be relocated within the stated accuracy of the equipment being used, *e.g.* differential GPS within 20 m.

6:1:4. **Quality Assurance.**

- in order to reduce the risk of cross contamination between sites the corer must be washed between sample sites.
- all site details must be recorded on a standard form (Appendix 4), including site code, replicate number/code, date and observations. In addition, sampling method/equipment and type of ancillary data collected (see section 8) should also be noted.
- as mentioned in section 6:1:3 above the accuracy of site relocation will depend upon the condition of the survey area but it is recommended that relocation be within ± 2-20m. Exactly how this could be verified needs to be further investigated, but one possibility is that a photograph of the site is taken on each occasion for comparison with original information in the site directory. Such information can also be of use in interpreting data and to show long-term alterations at the site which may not otherwise be apparent.

6:2. **Sieving and Preservation.**

The reliability of field sieving is regarded as unproven, and so only laboratory sieving can be confidently recommended. Samples should, therefore, be returned to the laboratory intact and processed with minimal delay, preferably within 24 hr of sampling, longer (up to 2 days) if refrigerated. Samples are not to be frozen unless the freeze-thaw method is being used (see section 6:2:2; Barnett, 1980).

6:2:1. **Sieving.**

The samples are processed by washing the sample with tap water through a sieve (mesh size 0.5/1 mm) to remove sediment. Sandy sediment samples are best sieved by washing from above whilst over a bucket of water. By agitating the sample in the water below the heavier sediment particles drop through the mesh and are quickly separated from the lighter

organisms. Muddy cores are best processed by eroding the core with a water jet to loosen the material into the sieve. In order to minimise damage to specimens, it is advisable to gently wash or elutriate the soft/light part of the sample off initially, and keep it separate while the remainder is sieved.

6:2:2. **Freeze-thaw technique** (Barnett, 1980).

This technique is suitable for both intertidal and subtidal samples. It is used to process clay/stiff mud which is difficult to break up during normal sieving procedures.

The sample should be stored in formaldehyde solution for some days, in order to fix the organisms. Then the sample undergoes freezing and subsequent thawing. The sample is elutriated and, if necessary, the remaining sediment is treated with water-softener, *e.g.* Calgon, and thoroughly shaken. After 24 hr the remainder is sieved.

6:2:3. **Preservation.**

The remaining fraction is transferred into a labelled, screw-cap jar of appropriate size, ensuring that all organisms are removed from the sieve. The lid must have sufficient thread to ensure a vapour-tight seal. The material is fixed with formaldehyde solution with a final concentration of 5% formaldehyde, the quantity added will vary with sample type. For example, where there is a high organic sediment content a ratio of 1:3 or 4 (sample:formaldehyde solution) should be used, for sandy samples 2:1. Jars should be labelled on the outer surface of the jar (**not** the lid, as lids can be exchanged) with waterproof/spirit resistant (permanent) felt-pen. A second label should also be placed in the container. Details should include: site code, replicate number, sample date and operator's initials.

6:2:4. **Quality Assurance.**

In order to reduce error:

- care is required to avoid washing material over the sides of the sieve. A precaution against this is the use of a double sieve. The sample is washed through a sieve placed inside a larger one, therefore, should any organisms be washed over the side they will be retained by the second sieve.
- water pressure must not be too high as this can force organisms through the mesh or damage them. Other sources of specimen damage include vigorous shaking of the sieve and transferral of retained material from the sieve. This can best be reduced by correct training.

- each of the replicates from a single site should be sieved consecutively and the sieve thoroughly washed between sites to avoid cross-over of material and mislabelling of samples.
- the processor should record their initials and the date on the record sheet, plus other comments about the appearance of the sample.

6:3. **Supplementary Sampling Methods.**

Supplementary sampling is necessary on sands and coarser sediments as cores tend to under-sample the larger, more deeply-dwelling and rarer taxa present. Increasing the number of replicates is likely to lead to unnecessary effort on finer sediments and so, additional sampling is needed on coarser sediments. This supplementary sampling takes the form of additional core samples or, preferably, large box-cores, especially on beach sands of open coast locations.

In order to decide which method is to be used, both sediment type and habitat need to be classified.

6:3:1. **Sediment classification.**

To decide whether the site is sandy, any, or all of the following criteria can be used.

i) Historical records, if available, are important as sediments are liable to change and a single assessment can be misleading. This is particularly significant if the assessment is carried out some time before the survey or if the area is to be re-examined over a number of years. It is also of importance to appreciate that outfall construction and alterations to flood defence works can influence the sedimentary regime.

ii) Visual inspection from a distance.

iii) Visual and physical examination, carried out by walking on the site or survey area and taking test cores for visual assessment.

iv) Preliminary survey for particle size analysis only, median grain size can be assessed. As a rule of thumb coarse sediments are those which are retained by a 500 μm sieve.

6:3:2. **Habitat Classification.**

i) Estuaries: in estuarine situations sands are likely to be fine or prone to siltation, therefore, deployment of additional cores is most appropriate.

ii) Open coast: sandy beaches on open coasts are more likely to tend towards coarser sediments, therefore, the use of large box cores is more appropriate.

The distinction, between estuary and coast is somewhat arbitrary, but can be made on the basis of geography and/or salinity.

6:3:3. **Methods to be used.**

1) Estuaries: an additional **5** 0.01 m^2 cores (minimum) supplementary to the Core method to be taken and processed as above (section 6).

2) Open coast: an additional **3** (minimum), preferably **4**, 0.1 m^2 box-cores to be taken supplementary to the Core method.

The box-core samples are to be sieved to 1 mm (in the field if appropriate) and all subsequent processing as in the standard method.

6:3:4. **Site location.**

See section 6:1:3 above.

6:3:5. **Quality Assurance.**

- in order to reduce the risk of cross contamination between sites the corer/box-core must be washed between sample sites.
- all site details must be recorded on a standard sheet, including site code, replicate number/code, date and observations in addition, sampling method/equipment and type of ancillary data collected (see section 8) should be noted.
- as mentioned in section 6:1:3 above the accuracy of site relocation will depend upon the condition of the survey area but it is recommended that relocation must be within $\pm$ 2-20 m. Verification of this needs to be investigated but photographs of sites may be a useful option.

7. SUBTIDAL SAMPLING METHODS.

7:1. <u>Equipment.</u>

A 0.1 m^2 stainless steel van Veen or Day grab with lifting flaps to allow access to the sample surface for noting depth, texture, colour, smell and for subsampling for physico-chemical analysis.

Both the van Veen and Day grab are based on mechanical leverage, the Day grab is recommended because of its wider application. In addition, the pyramid-shaped frame supporting the mechanism, makes the grab robust and stable so that it is unlikely to tip over during use, especially in difficult sampling conditions. The van Veen grab is simpler and generally has no supporting frame (framed van Veen grabs are now available), it is, therefore lighter and suitable for use on soft sediments. However, it is prone to misfiring and tipping over.

Grabs are heavy and, therefore, a large boat with a winch and gantry is needed. In addition, a stand and a suitable container are required to receive the grab as well as a hopper to wash the sample in.

The area sampled is 0.1 m^2, while the depth of the sample, which is limited anyway (maximum 10-15 cm), varies depending upon the sediment and weight of grab.

The sieves required have a mesh size of either 0.5 mm (used for estuarine samples) or 1 mm (used for coastal samples). For initial processing a large (*ca.* 50 cm diameter) nylon sieve is used. A large metal sieve may also be used but they tend to be heavy and more difficult to clean.

7:2. **Operation.**

7:2:1. **Sampling.**

The grab is lowered slowly on a cable, by the winch, until it reaches the bottom. Two people should guide the grab over the back of the boat and help to retrieve it. The jaws are closed by lever action as the grab is lifted.

The number of replicate samples taken depends upon the site and application. In estuaries a minimum of **3** samples is required and where a time series of data is envisaged from the outset, **5** replicates are recommended. In coastal situations a minimum of **5** samples should be collected due to the wider species complex found in such locations.

A visual inspection of the sample is made and notes taken on sediment type, volume, red-ox *etc.*. Sub-samples are taken at this stage for the determination of physico-chemical parameters (see section 8) using a suitable implement and are transferred, with an appropriate label, into containers. The removal of subsamples has a negligible effect upon the number and species of macroinvertebrates found.

Grabs are then released into plastic trays (*ca.* 60 cm x 35 cm and 20 cm deep) prior to processing on board by washing with sea water on a sieve, mainly to reduce the bulk, making transport easier. A waterproof label should be included in the tray with the sample, so that identification of each grab is possible if several are waiting to be processed. Whilst quick and convenient it would be much better, scientifically, to wash the sample directly from the grab into the hopper to avoid loss of material. Often this is precluded by the demands of the survey but it is recommended that the losses associated with transfer of samples should be quantified.

7:2:2. **Grab processing.**

The mesh size of the sieve depends upon the habitat, 0.5 mm for estuarine and 1.0 mm for coastal. The use of a 1 mm sieve for the processing of coastal samples has been agreed Nationally as a satisfactory compromise between the time available for grab processing and any loss of material. It is unlikely that any associated reduction in abundance has a great effect upon the findings of the survey, however, loss of the smaller taxa may be significant. This is currently undergoing further evaluation (Wash survey data) and so it is recommended that the samples are sieved through both a 1 and 0.5 mm mesh sieve and that the 0.5 mm fraction be retained for reference. If the Wash data supports the use of a 0.5 mm sieve then the 0.5 mm fraction could be retained for sorting if necessary or subsampled while the 1 mm fraction is fully sorted. The choice would depend upon the survey requirements.

Samples should be sieved as gently as possible to reduce specimen damage. Sieve agitation and gentle water pressure are recommended and not scrubbing of mesh screens with a jet of water.

The sample can be broken up in the tray by gentle washing and gradually washed out into the hopper, in order to avoid overloading the sieve. Alternatively, the sample can be washed out of the tray into the hopper for dispersal and the gate, on the hopper, used to control the transfer of the sample onto the sieve. Once all of the sample has been washed through, the sieve is transferred to the hopper for further washing, if necessary. A second sieve should be placed at the hopper outlet in order to catch any material accidentally washed out of the sieve.

The reduced sample is transferred to a clean bucket/jar with care. Bulky samples can be removed using a plastic spatula. Any material left in the sieve can be washed into a smaller one and, either knocked out, or washed with a minimum of water, via a funnel, into the

container. Forceps and other instruments should be used to remove delicate or enmeshed specimens.

The sample is then fixed, using 6-8% formaldehyde solution in seawater to take account for slight dilution by seawater present in the sample. As with intertidal samples the quantity added will depend upon the type of sample *e.g.* organic sediment or sand, but must be sufficient to cover the sample. Each sample will already have a waterproof label detailing the site code, replicate number and date and this information should be repeated on the container in water/spirit resistant marker pen. The container, whether a bucket or a jar, should have lids or caps which give a good vapour-tight seal.

Samples are then returned to the laboratory for further processing, sorting and identification.

7:2:3. **Site location.**

Sites can be located (on Sea Vigil) using GPS with a probability that 98% of the position fix will be accurate to 100 m (2drms) or differential GPS (DeccaLink) to within 20 m (98% 2drms). Alternatively, where greater accuracy is required, Microfix can be used which locates to within a repeatable accuracy of ± 1 m. This is valuable for grid surveys where sampling stations may only be 50 or 100 m apart. Microfix can be quite costly if the system has to be rented, *ca.* £300 per day.

An important point to bear in mind is that position fixing is usually made in relation to the signal-receiver or measuring equipment, usually found towards the front of the vessel, and which can be a significant distance from the sampling gear, thus introducing a further source of error.

All details of the location should be recorded accurately so that relocation can be precise. It is recommended that a site directory be compiled for each survey with full descriptions of each site including (where appropriate) latitude/longitude, sextant readings, landmarks and photographs plus sediment descriptions.

7:2:4. **Quality Assurance.**

- checks are required to ensure that the quality of grab samples is consistent, *e.g.* stones can wedge the jaws slightly open, resulting in loss of sediment from the sample by washing-out on ascent; where there is a strong current the grab may not enter the sediment vertically; or in rough conditions under-sampling can occur, as the rise and fall

of the boat causes the grab to be snatched off the sea-bed. Such samples should be discounted.

Excluding exceptional sediment types or difficult substrates, such as clay reefs, acceptable volume or depth of the sediment to be sampled should be determined with reference to OSPARCOM recommendations which include rejection of samples of <5 L (7 cm depth) in muds and <2.5 L (5 cm depth) in hard packed sands. Attempts should be made to get as much sample as possible by using additional weighting. Acceptable grab volume for each site should be determined and recorded in the site directory.

If a grab is not satisfactory, further grabs should be made until sufficient replicates have been accumulated and a note made on the record sheet as to the total number of grabs required to acquire enough replicates.

- details about the site must be noted on a standard record sheet (Appendix 4), including the site code, date and comments on specific or unusual fauna.
- the replicate samples should be sieved consecutively.
- the sieve should be thoroughly scrubbed and washed between sample sites (taking special care to ensure nothing is stuck between the mesh and rim), in order to avoid cross-over of organisms.
- poor quality specimens and high variation in numbers occur as a result of rushed sieving and high hose pressure. Training is the best way to avoid this, as is the presence of sufficient staff (minimum of 3/4 if possible) to handle the grab samples correctly between sites.
- relocation of subtidal sites should be within the stated accuracy of the equipment being utilised provided that the details have been recorded correctly, *i.e.* 2-20 m (Microfix & differential GPS). Verification of the accuracy of relocation needs to be further investigated. Future use of Cubit systems will enable the combination of track plotter information with navigational data and provide a record of sample sites which could be used to confirm the accuracy of site relocation.

8. ANCILLARY DATA COLLECTION.

The need for physico-chemical data is widely acknowledged. Certain basic items should be included, depending on the survey requirements. The removal of subsamples for physico-chemical analysis has a minimal effect upon the number of individuals and taxa found.

The methods involved and appropriate AQC procedures for ancillary data are to be documented elsewhere, below is a list of likely determinands and the volume/weight required for each where appropriate.

i) Particle size analysis (PSA) - all surveys } same sample 100-
ii) Organic carbon (some) } 200 ml
iii) Metal analysis (some) - 200 ml (mud only)
iv) Bacteriological sample (some) - 30/40 ml (mud)
v) Depth and description of anoxic layer - all surveys.
vi) Sediment type recorded - all surveys.
vii) Salinity (estuaries) (some).

9. SORTING PROCEDURES.

Intertidal samples must be thoroughly washed with tap water in a fume cupboard/under a fume hood fitted with the correct filter to remove all traces of formaldehyde solution (0.5 mm sieve) prior to processing.

Subtidal samples will require further washing on receipt in the laboratory prior to sorting, in order to remove the formaldehyde solution and to reduce the volume still further. The formalin is tipped off through a small 0.5 mm sieve via a funnel into a waste, plastic-coated, glass winchester. It is recommended that waste formalin be recycled (procedures under review). The samples are then washed with tap water for at least 2-3 min, taking care to break up any lumps of sediment. This is carried out in a fume cupboard or under a fume hood. Once the formalin has been removed the sample can be processed in an ordinary sink where the sample is washed and elutriated.

9:1. Elutriation (Rees *et. al.* 1990).

For most purposes coarse elutriation can be recommended. The sample is agitated with freshwater and the supernatant poured into a fine mesh sieve until no more sample material is being removed. This gives a 'light' fraction of polychaetes and crustaceans and a residual 'heavy' fraction containing molluscs, echinoderms and other heavier species. Alternatively, sieving through a series of meshes may be used to allow easier sorting and to remove larger particles which may obscure smaller specimens.

9:2. **Sub-sampling** (NRA (Anglian) TiO_2 Report, 1989).

Where practical the whole sample should be sorted, but for some subtidal samples some form of subsampling is required. It should be noted that sub-sampling is not used very frequently. There are two reasons for subsampling:

1) large detrital volume;
2) large numbers of one particular taxa.

Intertidal samples are usually smaller in volume with fewer animals and are very rarely subsampled.

Two techniques can be used, depending upon the quantity and nature of the detritus.

1) Large volume samples, *e.g.* from around outfalls, and those containing woody/peaty fragments and resistant clay need more processing. Initially, samples are screened using a 2 mm/4 mm mesh sieve, and the material retained ('coarse fraction') is sorted completely by eye. The finer detritus (<2 mm/4 mm) is mixed with water to produce a known volume of slurry from which ten equal volume subsamples are siphoned off. Specimens are extracted and counted from one/two of these subsamples. Any material retained within the siphon tube and residue from the base of the mixing vessel ('residual fraction') is also sorted. For samples of exceptionally large volume the size of the subsample can be reduced to 1/20th.

2) Although containing small amounts of detritus, some samples yield high densities of organisms. All individuals of the species are extracted and counted from a proportion of the total sample (¼ or less) yielding at least 500 individuals. The whole sample must be sorted to ensure that all other taxa are extracted. The remaining fractions are retained for further analysis, if necessary.

The efficiency of subsampling for species acquisition has been tested using data from samples collected during the 1985 baseline survey for a new SCM outfall. On average, 77% of the taxa present were recorded from the combined data of the coarse, residual and 1/10th subsample and relatively few additional species were recovered from sorting of additional subsamples (based on subsamples taken using a 2 mm mesh and 1/10th subsample). Within normal time constraints, sorting of these three fractions provides acceptable results. Inaccuracies arising from subsampling may be reduced by examining replicate samples from the same site.

9:3. **Sorting.**

All material is transferred into petri dishes, containing water, for sorting. All sorting is to be carried out under a binocular microscope (magnification at least 6x) using a cold light source. Organisms are removed from the sample for identification and enumeration. For estuarine samples, which generally contain a large number of relatively few, easily identified, species (sometimes at high density) a one step sorting and identification/enumeration is most efficient. For marine, coastal samples a two stage sort and identification is the best choice.

9:4. **Quality Assurance.**

- the same individual should sort a sample.

9:5. **Analytical Quality Control**

The detritus from 5% of a survey's samples, chosen at random, should be re-sorted by an independent biologist (from another Area) to assess the extraction of taxa and individuals. Where several individuals have been involved in sorting, each person's output should be checked. Acceptable levels are to be 100% of taxa and 95% of all individuals, both from entire samples and subsamples. It is at the discretion of the biologist whether the overlook of a taxa represented by only one individual is of significance. Any problems should be pointed out to the individuals concerned and if there is a consistent oversight of a taxa then the sorting technique of the individual needs to be reviewed and altered. Concern regarding the results should occur if more than 20% (2/10) of the samples fail and it might be necessary to re-sort the whole survey.

10. IDENTIFICATION AND ENUMERATION.

Where possible all organisms should be identified to species using a stereo zoom microscope and/or compound microscope (magnification 6-80x) and with reference to recommended texts and keys (Appendix 5). The abundance of each taxa is recorded. Where partial specimens occur they are to be included in counts only if the head is present. Other partial specimens which can be identified can be noted down as being present. Juveniles should be recorded separately, since these may introduce seasonal bias to the data and account can, therefore, be made for this in analysis if they are counted separately.

10:1. **Quality Assurance.**

- in some cases, especially with taxa which are difficult to identify and where there may still be some uncertainty as to the species' characteristics, different workers may consistently make different choices as to species identification. There must be an agreed common laboratory/survey identification so that all taxa are named consistently. It is advantageous to have a Regional/Laboratory nominated/ recognised expert for difficult taxa so that problem identifications can be sorted out.
- a standard list of taxonomic references should be used and updated Appendix 5).
- a standard species list should be adopted, *e.g.* Marine Conservation Society list (in process of being revised).
- a validated reference collection should be maintained to enable the checking of identifications.
- it is essential to retain samples, both specimens and detritus, so that they can be rechecked should any subsequent query arise regarding identification. Sorted sub-samples should be kept separate. It is recommended that specimens and detritus be retained indefinitely.

10:2. **Analytical Quality Control.**

In order to assess the accuracy of identification and to pin-point any repeated mistakes 5% of survey samples should be sent to an independent biologist for checking (as above, these samples should be the same as those checked for % extraction). The accuracy of identification of individuals should be checked.

Discrepancies in enumeration are only likely to occur if there are damaged specimens, *i.e.* both heads and tails counted, or where separate species have been identified as a single species.

It is difficult to set acceptable limits for identification. The importance of incorrect identifications should either be left to the discretion of the senior biologist/quality control co-ordinator or a list of acceptable levels of identification for each species, *e.g.* family, genus or species, should be compiled. Misidentified species should be commented upon and a representative returned for reference where appropriate. Individual problems with identifications should be dealt with in the laboratory or by attendance at training courses.

11. DATA HANDLING AND ANALYSIS.

All data should be recorded in a methodical and precise manner and arranged in such a way as to make transfer to computer straightforward. Recording should be carried out on blank (no species listed) standard paperwork (Appendix 4).

All data should be transferred to computer for storage and analysis. A hierarchical coding system should be used which is suitable for archiving (when available), such a code is likely to be developed for WAMS, as is some form of validation so that incorrect entries are more likely to be queried or rejected. At the present time all data should be double checked by a second biologist at the time of transfer by comparing the original data sheets with the computer listing. The data should be analysed using a set suite of statistical methods (including multivariate and cluster analysis) and reports should include the results of these, in a standard form, as a minimum requirement. Simple graphs/maps are most desirable for summarising data. An indication of the error in the data would be useful and inclusion of, for example, mean abundance per replicate per site $\pm$ 95% confidence interval/standard error in an appendix might be appropriate.

A brief summary, in an appendix, of the results of AQC results should be included with each report to give an indication of the quality of the data.

The reporting form should be that of a standard 'paper' format (*i.e.* Introduction, Methodology, Data analysis and results, Discussion, Conclusions/Recommendations, References, Appendices, Name of Biologist and date) including a brief summary of the findings so that reports are accessible to other Sections/functions.

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APPENDICES

Appendix 1: Information regarding *Leptospirosis* (Weil's Disease) and Tetanus.
(Southern NRA Safety Code of Practice - Field Sampling Activities)

Leptospirosis letero is a listed Industrial Disease under the R.I.D.D.O.R. Regulations 1985. This strain of the disease can be contracted through contact with material/water which has been contaminated with urine from infected rats.
The infection commences with high temperature and general muscle and joint pains. Medical advice **must** be sought **immediately** as the symptoms are similar to influenza, pneumonia, tonsillitis, rheumatic fever or nephritis and later catarrh, jaundice or gall stones. Show the doctor the Leptospiral Jaundice card issued to all NRA employees at risk, in addition, a letter should be kept with your medical records informing medical staff of your occupation and the risk of Weil's disease.

Tetanus is a reportable disease under R.I.D.D.O.R. Regulations 1985. It is a disorder of the nervous system, causing rigidity and spasms of the muscles. It is caused by a bacillus which inhabits soil and road dust.

It can be fatal, causing death through spasms, the loss of limbs has also been known.

The onset of the disease generally follows a wound contaminated with soil, especially deep puncture wounds and lacerations.

Symptoms usually appear 4 to 5 days after injury but can be delayed for 3 or 4 weeks. The first signs are usually muscle stiffness near the wound followed by stiffness in the jaw muscles.

Tetanus can be prevented by immunisation and persons exposed to soil and road dust in their work should have effective immunisation. Initial immunisation is achieved by a course of three injections with a booster every 5 years for those at risk.

Precautions against infection.

After contacting sewage, water from a watercourse or cattle, wash hands and forearms with soap and water - even if gloves have been worn. It is especially important to do this prior to eating or drinking. If clothing or footwear becomes contaminated it should be thoroughly washed.

DO NOT WORK IN WATER OR SEWAGE WITH OPEN WOUNDS ON HANDS OR ARMS

Take care to wash and cleanse with antiseptic any cut, scratch or abrasion as soon as possible, whether caused at work or not. Keep any wound covered even when wearing gloves.

Appendix 2: Procedure for handling formaldehyde (under review).

Principle

Formaldehyde, in the form of a solution of Formalin is used as a fixative agent prior to the preservation of biological material, or to prevent subsequent microbial growth. Formalin is a hazardous material and requires careful handling, reference must be made to the COSHH assessment 0106. Formalin should only be used where absolutely necessary.

The stock solution normally purchased is a 37-41% solution of the gas formaldehyde in water. This may be known as "100% formalin", "40% formaldehyde solution" or "concentrated formalin". The working dilution for invertebrate fixation/preservation is usually a 1 in 10 dilution of this, giving 4% formaldehyde or 10% formalin.

Formalin is required for the fixation of marine/estuarine benthic invertebrate samples due to unavoidable delay in sorting the material.

Toxicity

Formalin is acutely toxic. There have been 13 deaths due to the ingestion of amounts estimated to be 100 ml (or a few drops in the case of a child).

Inhalation is the most likely hazard in the biology laboratory. The threshold for detecting an effect on the eyes has been claimed to be as low as 0.01 ppm, symptoms of mild throat irritation occur at about 0.5 ppm and it is intensely irritating to the eyes at about 4 ppm. Brief exposure to 50 ppm would cause very serious injury. There is some evidence that continued exposure can result in desensitisation to the irritant effect.

Splashes to the eye of 40% solution have resulted in permanent eye damage. Splashes of a 4% solution produce a strong irritant effect and visual disturbance for one day, after which the eye returned to normal.

Contact with the skin at concentrations greater than 2.5% may cause dermatitis. Skin sensitisation and allergic contact dermatitis can occur.

Carcinogenicity

There is no evidence to suggest that exposure to formaldehyde has produced cancer in humans, nor is there acceptable evidence for any adverse effects on the reproductive system. However, formalin has been shown to be carcinogenic in laboratory animals and so a possible risk of cancer caused by chronic inhalation exists. Precautions are required when using formaldehyde solution.

First Aid.
Standard Treatment:

Eyes	Irrigate thoroughly with water for at least 10 minutes. **OBTAIN MEDICAL ATTENTION.**
Lungs	Remove casualty from exposure, rest and keep warm. In severe case or if exposure has been great **OBTAIN MEDICAL ATTENTION.**
Skin	Drench the skin with plenty of water. Remove contaminated clothing and wash before re-use. Unless contact has been slight **OBTAIN MEDICAL ATTENTION.**
Mouth	Wash out mouth thoroughly and give water to drink. **OBTAIN MEDICAL ATTENTION. DO NOT INDUCE VOMITING.**

Exposure limits

Long and short term exposure limit is 2 ppm or 2.5 mg m^a. This is well below the threshold of mild irritation and it is safe to assume that if Formalin cannot be detected in the laboratory it is below the MEL. Routine checks for Formaldehyde should be carried out using a Drager gas detecting kit.

General precautions (see COSHH assessment 0106)

Clothing.
When dealing with >500 ml of formaldehyde (COSHH regulation) and also formalin a PVC apron, Grade 2C plastic goggles/visor and appropriate gloves *e.g.* black chemical resistant heavyweight Marigold gloves, **not** disposable vinyl gloves, **must** be worn.

In situations of high formaldehyde vapour the use of an appropriate respirator is recommended, *e.g.* 3M formaldehyde respirators which protect up to the OEL. In addition, goggles which seal around the face, rather than eye shields should be used in such situations.

Spillage.

In the event of a spill of more than 500 ml of 40% formalin the laboratory should be evacuated and assistance from the fire brigade requested. Use formalin neutraliser Chroma FNC for significant spillages. Smaller or more dilute spills can be handled.

All sources of ignition should be shut off and the area evacuated - do **not** re-enter until ventilation has been achieved. Wearing a face-shield or goggles and gloves the formalin can be mopped up with plenty of water and run to waste, diluting greatly with water. The area should be well ventilated to evaporate remaining liquid and to dispel vapour.

Under no circumstances should formalin be disposed of down general laboratory sinks not designated for the purpose.

Formalin must not come into contact with hydrochloric acid to avoid the formation of Bis-chloromethyl ether (BCME) a known carcinogen.

Handling procedure

Dilution of stock formaldehyde solution.

For tissue fixation formaldehyde soln. needs to be diluted to 4%.

For use in estuarine or marine samples a 10x dilution of 40% formaldehyde soln. using sea/estuarine water is made.

A large plastic aspirator with a tap is filled with seawater to *ca.* 20 L. A full 2.5 L winchester of formaldehyde soln. is then emptied into the container. Seawater is then added to the container to a volume of 25 L (volume measured previously in laboratory).

When handling formaldehyde soln. at 40% and 4% a **PVC apron (>500 ml), gloves & eye protection must be worn.**

Dilution of stock solution must be carried out on the open deck.

Protective clothing must be worn when handling 40% formaldehyde solution.

Any spills should be immediately hosed overboard.

Alternatively, the required volume of formaldehyde soln. can be taken on board in the aspirator and seawater this.

4% formaldehyde soln. is then added to sieved samples on the vessel.

Where samples can be quickly returned to the laboratory, 4% formaldehyde soln. is added to the sample in the laboratory following sieving.

Transport 40% formaldehyde soln. in 40% sealed bottles in suppliers polystyrene packaging. Containers added to must be clearly marked with hazard symbols.

Eye protection and gloves must be worn while adding 4% formaldehyde soln.

Addition must be carried out in the open air or if in a laboratory a fume cupboard must be used.

Transport of samples containing formalin

Samples should be carried in well sealed containers. Where possible they should be carried in a vehicle with a closed truck or cab or trailer. Where a closed van or car is used containers should be enclosed in a secondary container and it should not be possible to smell formalin vapour in the vehicle.

On board the condition of formalin containers, particularly aspirators/taps should be checked and all such containers should be secured and checked especially in rough conditions.

It is important that the containers plastic or the vehicle are clearly labelled. A TREM (transport emergency) card should be available and displayed.

Handling and storage of samples in the laboratory

Samples containing formalin should be suitably labelled and stored in a well ventilated store with an extractor fan.

Preliminary sieving should be carried out using a 0.5 mm sieve. Excess liquid should be tipped through a small 0.5 mm sieve via a funnel into a waste glass plastic-coated winchester or other suitable container, *e.g.* a well sealed sample bucket, to be recycled.

This **must** be carried out in a ventilated sink (fume cupboard).

Eye protection **must** be worn.

Samples are then washed with tap water to remove the all traces of formalin.

Examination of samples following fixation in formalin.

Provided washing (above) was adequate samples can be examined in the laboratory without risk. However, if there is any residual smell of formalin, or if the staff member is concerned, the samples should be examined in a fume cupboard.

After sorting and identification the picked specimens are stored in 70% IMS made up with 20% water, 10% glycerol. The sorted detritus is put back into formalin.

Appendix 3: Distress signals.

The International Distress Code at Sea:

1. Whistles and torches.

   Morse-code signal 'SOS' - three short blasts/flashes - three long - three short - pause - repeat.

2. **Red** flares or **orange** smoke.

3. Outstretched arms, raised and lowered slowly and repeatedly.

4. An oar with a cloth tied to it, waved slowly from side to side.

The International Distress Code on Land:

Six long flashes/blasts/shouts/waves in succession, and repeated at 1-minute intervals.

Appendix 4: Standard record sheets - attached.

1. Intertidal sampling record sheet
2. Subtidal station record sheet
3. Species list - routine intertidal
4. Species list - subtidal record sheet
5. Sub-sampled tidal benthos record sheet

INTERTIDAL SAMPLING RECORD SHEET

SITE NAME: SITE NO:

LOCATION: DATE:

Shore Position (I) ("CORE") Sample Point Code:

SAMPLE No.	CONTAINER CODE	COMMENTS (SIEVING OBSERVATIONS)	SIEVED BY: DATE:

Sediment samples: Metals PSA O-C H-C Other .....

FIELD OBSERVATIONS:

Sediment Type: Depth of Anoxic layer (cm):

General Comments:

Shore Position (II) or SSR (Core/Boxcore) Sample Point Code:

SAMPLE No.	CONTAINER CODE	COMMENTS (SIEVING OBSERVATIONS)	SIEVED BY: DATE:

Sediment samples: Metals PSA O-C H-C Other .....

FIELD OBSERVATIONS:

Sediment Type: Depth of Anoxic layer (cm):

General Comments:

SITE CHARACTERISTICS AND GENERAL OBSERVATIONS:

Visible pollution: Sampled by:

SUBTIDAL STATION RECORD SHEET

STATION NO: LOCATION:

DATE: POSITION FIX R: P:
(DGPS/Microfix)

TIME:

DEPTH:

SAMPLING DEVICE:

SAMPLE				Sediment Samples	
No.	Depth (cm)	SEDIMENT TYPE	COMMENTS	Particle size	Chemical Analysis

General comments:

Sampling Officer:

SPECIES LIST - ROUTINE INTERTIDAL

Station Code:	Site No. & Location:
Date:	Shore position:
Sediment description & comments	
1	
2	
3	
4	
5	

Date of sorting:								
Sorted by:								
No. of petri dishes:								
No. of taxa:								
No. of specimens:								
SPECIES	REPLICATE NUMBER							
	1	2	3	4	5	TOTAL	AVE	NO/m²

SPECIES LIST - SUBTIDAL RECORD SHEET

Station No: Date: Location:

	A	B	C	D	E	TOTAL
DATE OF SORTING NO. PETRI DISHES						
TOTAL NO. OF SPECIMENS						
TOTAL NO. OF SPECIES						

SPECIES	SPECIMEN NUMBERS					

SUB-SAMPLED TIDAL BENTHOS RECORD SHEET

STATION NO: DATE: LOCATION:

REPLICATE:

	HEAVY FRACTION	COARSE LIGHT FRACTION	FINE LIGHT FRACTION			TOTAL
			SUB-SAMPLE		RESIDUE	
DATE OF SORTING						
NO. OF PETRI DISHES						
DETRITAL VOLUME						
MULTIPLICATION FACTOR						
TOTAL NO. OF SPECIES						
TOTAL NUMBER OF SPECIMENS						
SPECIES	SUBTOTAL	SUBTOTAL	COUNT	SUBTOTAL	SUBTOTAL	

SPECIES	SUBTOTAL	SUBTOTAL	COUNT	SUBTOTAL	SUBTOTAL	TOTAL

COMMENTS:

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General:

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