NRA

A REPORT ON QUALITY ASSURANCE PROCEDURES FOR USE IN BIOLOGICAL SURVEYS OF MARINE/ESTUARINE BENTHOS

ENVIRONMENT AGENCY

National Rivers Authority
Anglian Region

NRA August 1993

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1. INTRODUCTION

The results of biological sampling are likely to be increasingly used and challenged by dischargers, Government Departments and other Agencies as the costs of compliance with Statutory Water Quality Objectives, the EC Bathing Water Directive and improving water quality are evaluated. Biological data are currently used by a number of outside organisations including those for the assessment of discharges and abstractions and for the assessment of conservation value. Where biological data are used in pollution prosecutions they must also be robust enough to stand up to legal scrutiny.

Confidence in the validity of the results will also be of increasing importance internally as the data are used to assist in a wide range of different functional activities.

It is likely that as Environmental Quality Standards are developed a RIVPACS-like predictive model (as used in freshwater benthic biology) will also evolve for marine and estuarine communities. It is vital that the baseline data produced for such projects are of a consistent standard.

The above can only be achieved by ensuring that the quality of the product, including its applicability and presentation, is assured.

In the near future NAMAS (the National Measurement Accreditation Service) accreditation will be sought for biology, in line with National and Regional policy. NAMAS require compliance with rules regarding management, staffing, facilities and, most relevant to this report, equipment and procedures. A standard methods manual must be produced and followed; internal quality control must operate and where this is not possible sample exchanges must occur; formal audits are required that are planned, documented and acted upon; staff training needs to be documented and the system must be reviewed on an annual basis. One water authority laboratory has gained NAMAS accreditation for the analysis of benthic macroinvertebrate samples and a number of others have obtained BS 5750 status for benthic sample collection.

Definitions of the terms used to refer to various aspects of the quality processes vary. In this report the term Quality Assurance refers to all of the aspects contributing to the quality of the data produced, including methodology, training and quality control procedures. Analytical Quality Control (AQC) is the process of monitoring data by reanalysing samples, ideally the results of these checks are examined statistically to assess whether performance is acceptable.

The use of Analytical Quality Control (AQC) is well developed in chemistry, approximately 5% of all samples processed in 1991 by Anglian NRA were for AQC purposes (NRA, 1991). The only NRA quality assurance schemes in biology are AQC for microbiology and an external audit of freshwater macroinvertebrate samples carried out by the Institute of Freshwater Ecology (IFE). There are few protocols for quality control of biological samples available because this aspect of biological work has never been fully addressed either at a Regional or National level.

This report covers the development of a Quality Assurance scheme for marine/estuarine benthic sampling and analysis. A standard methodologies manual has been produced and

is included in this document together with further notes upon the methods and associated errors. The manual also outlines Quality Assurance measures, including AQC procedures to be adopted for both sampling and analysis of marine/estuarine benthic samples.

2. STANDARD METHODOLOGIES

2:1. Summary

Benthic infauna (mainly macroinvertebrates) are used, in combination with chemical and physical parameters, to assess the impact of human activity upon the quality of the marine/estuarine environment. In order to detect the effects of pollution on the benthos other sources of variation need to be quantified or minimised.

Variability can be:
a) truly random, *i.e.* due to natural variations in the population distribution (marine/estuarine benthic macroinvertebrates tend to be contagiously distributed);
b) linked to specific factors, such as consistent overlook of a taxa by one worker; or
c) temporal, *e.g.* improvement with experience or a decline as the workload increases.

Variation comes from a number of sources relating to sampling and processing. The variation associated with sampling is due to operator differences, habitat/sediment variability and distribution of invertebrates within the habitat. During processing taxa can be missed, misidentified or recorded incorrectly.

For data to be comparable (both spatially and temporally) it is essential that samples are obtained and processed using a standard method. The basic requirement of Quality Assurance is to eliminate as much variability as possible by attention to methodology leaving only truly random variation to be assessed. To this end a standard methodologies manual has been compiled from a variety of sources.

Standard methods for intertidal and subtidal sampling have been agreed at a National level (NRA Marine Biology Sub-group). The purpose being to establish methodology which is scientifically valid and defensible so that data is widely acceptable. The methodology satisfies the requirements of all normal investigations likely to be carried out by the NRA as well as being practical with respect to manpower and equipment. There are a minimum of alternative methods in order to avoid inconsistency and remove the need for preliminary surveys to determine the method to be used. The recommended methodologies are based upon a consensus of opinion from all of the NRA Regions with modifications made using experience available within the NRA.

The more detailed descriptions were compiled by consultation with marine biologists in the Area laboratories in Lincoln (Northern), Brampton (Central) and Haddiscoe/Ipswich (Eastern).

The manual also incorporates Quailty Control procedures, which are inextricably linked with the method descriptions.

For completeness the manual also refers to hazards associated with the collection and processing of marine/estuarine benthic samples and suitable safety procedures (Sections 4 and 5). These are designed to be basic guidelines and should be read in conjunction with other safety information issued by the NRA.

The manual is intended for use as a laboratory reference, and will need to be reviewed annually and updated when necessary, *e.g.* as new technology/R&D becomes available. It is recommended that all staff involved in marine/estuarine benthic sampling should read the manual on an annual basis.

2:2. Methods manual - Appendix One

2:3. Notes on the methods manual

2:3:1. Sampling requirements

Sampling requirements depend upon the purpose of each survey, for example, in order to establish distribution of communities or occurrence of taxa over an area it is sufficient to take a single sample at numerous sites. Alternatively, should the requirement be to establish whether there is a difference between the benthic communities of sites A and B at least 2 replicates would be required at each location. Two replicates gives a measure of the variation encountered, while three is the minimum number on which any statistical manipulation can be carried out. The standardised methodologies to be adopted recognise that flexibility of approach is required while a minimum standard for the accuracy of data is obtained.

One aspect of Quality Assurance is the quantification of errors associated with each stage of the process. There are a few points which need to be made regarding the accuracy of the data generated using the standard methods.

To establish whether sufficient replicates have been taken to give an accurate estimate of species composition many replicates need to be obtained and a cumulation curve plotted (McIntyre *et al.*, 1984). Sufficient replication has occurred when the species-accretion curve levels out. It is, however, not possible to do this at all sites, the samples cannot be assessed as they are taken and so the calculation of a cumulation curve is impractical. In addition, the ideal number of sample replications is likely to be too great to handle in the time available. Therefore, this technique would probably only be used in preliminary work for base-line surveys or long-term studies.

A study carried out by Yorkshire NRA compared the efficiency of sampling intertidal benthic fauna of sandy coastal habitats with corers/0.5 mm sieve and box-cores/1 mm sieve. The results suggest that box-cores give a more complete species list than 0.01 m^2 cores, as they cover a larger area (0.1 m^2) and collect deeper dwelling and more sparse species (Fig 1a). In terms of species-accretion, 5 box-cores appears to be a reasonable number of replicates for most beaches (Fig 1b) with the asymptote of the cumulative species-sample curve being reached. On coarse coastal sites it appears that a combination of cores and box-cores, as in the standard methodology, gives a good estimate of both species composition and abundance. On exposed and impoverished beaches 2 or 3 may be sufficient.

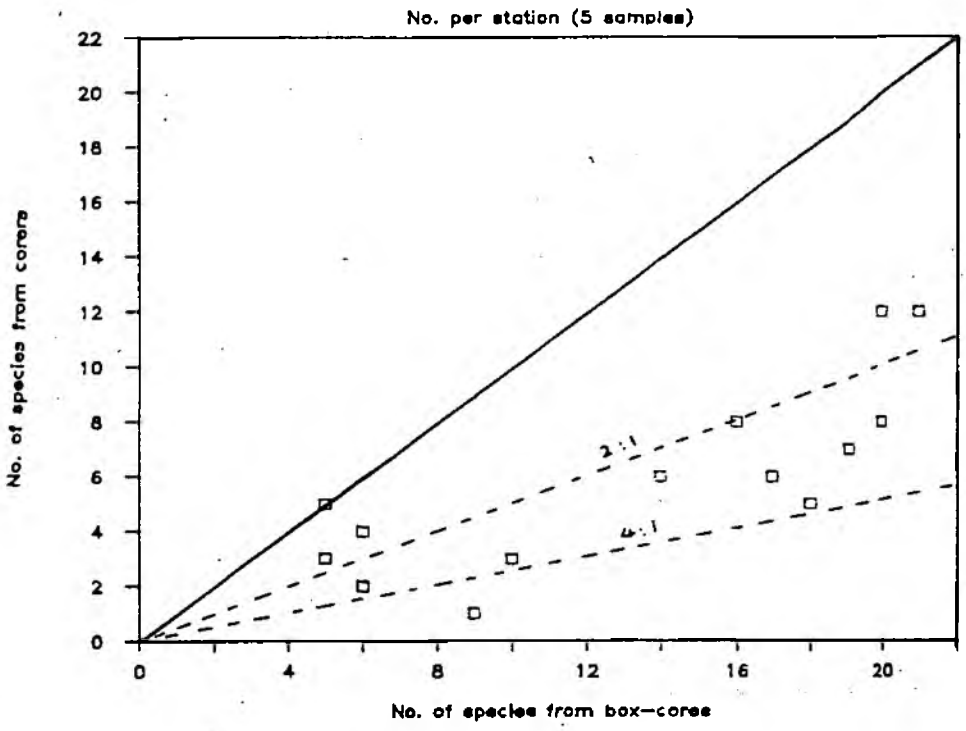
Subtidal grab data, presented by Rees (1979), suggest that 3 or 5 grab replicates at each site is almost certainly insufficient to give an accurate assessment of individual site species composition (Fig 2). In general, however, both 3 and 5 replicates, compared with greater replication, give a ranking of the sites which is reasonably accurate, *e.g.* Station 7 has low species diversity while Stations 205 and 204 are higher. This is ultimately based upon the gradient of the curve and, as this is dependent upon the order of the replicates, will not be consistent. In addition, in the data from five sites, 3 or 5 grabs yields the majority of the taxa obtained by up to 20 replicates (Table 1). Rees *et al.* (1990) state that three grab samples in soft silt might be expected to collect 60% of the species present in an area while five samples would usually yield over 70%. If a complete list of the species present is required then the number of grab replications would have to be large.

Regarding the abundance of each taxa, again the more replicates taken the more accurate any estimate of the number of individuals per unit area is likely to be. Most NRA work deals with total abundance pooled from all replicates, for use in multivariate and cluster analyses, and is not calculated as a mean $\pm$ error. Where, however, mean abundance per unit area is presented an estimate of accuracy should be given, for example, mean $\pm$ 2 standard errors. If an estimate of mean numbers of organisms is a requirement of a survey then larger sample replication may be required to give an acceptable margin of error, *e.g.* a standard error of 20% of the mean.

On intertidal coarse sediment the use of a core gives the best estimates of abundance for the smaller and more abundant species (Fig 3). The box-core tends to under estimate abundance, possibly due to the loss of small taxa by the use of 1 mm mesh size for processing. Therefore, a combination of box-core and core, as described in the standard method, is ideal for coarser sediments on exposed coastline.

Figure 1: **Accumulation of species - cores *vs.* box-cores (information supplied by Yorkshire NRA)**

a) Species acquisition



b) Accumulation of species with sample size

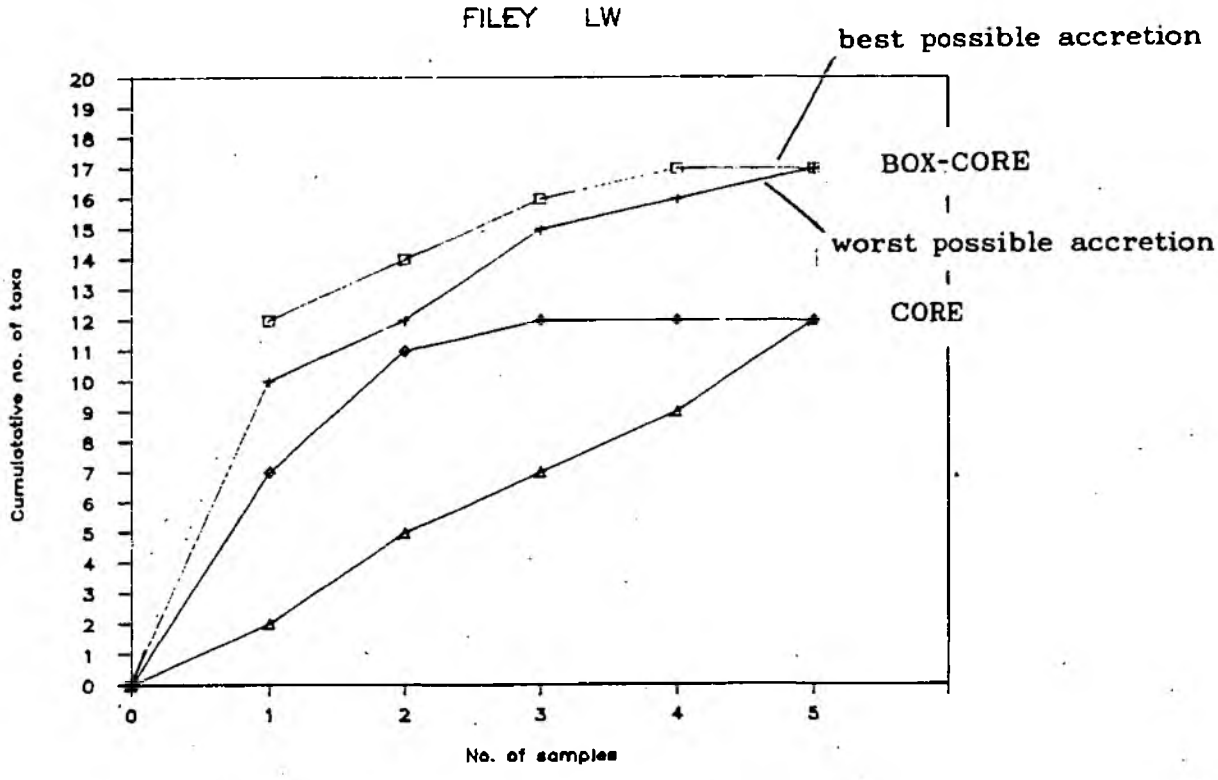


Figure 2: **Accumulation of species with sample size - 0.1 m² Day grabs (after Rees, 1979)**

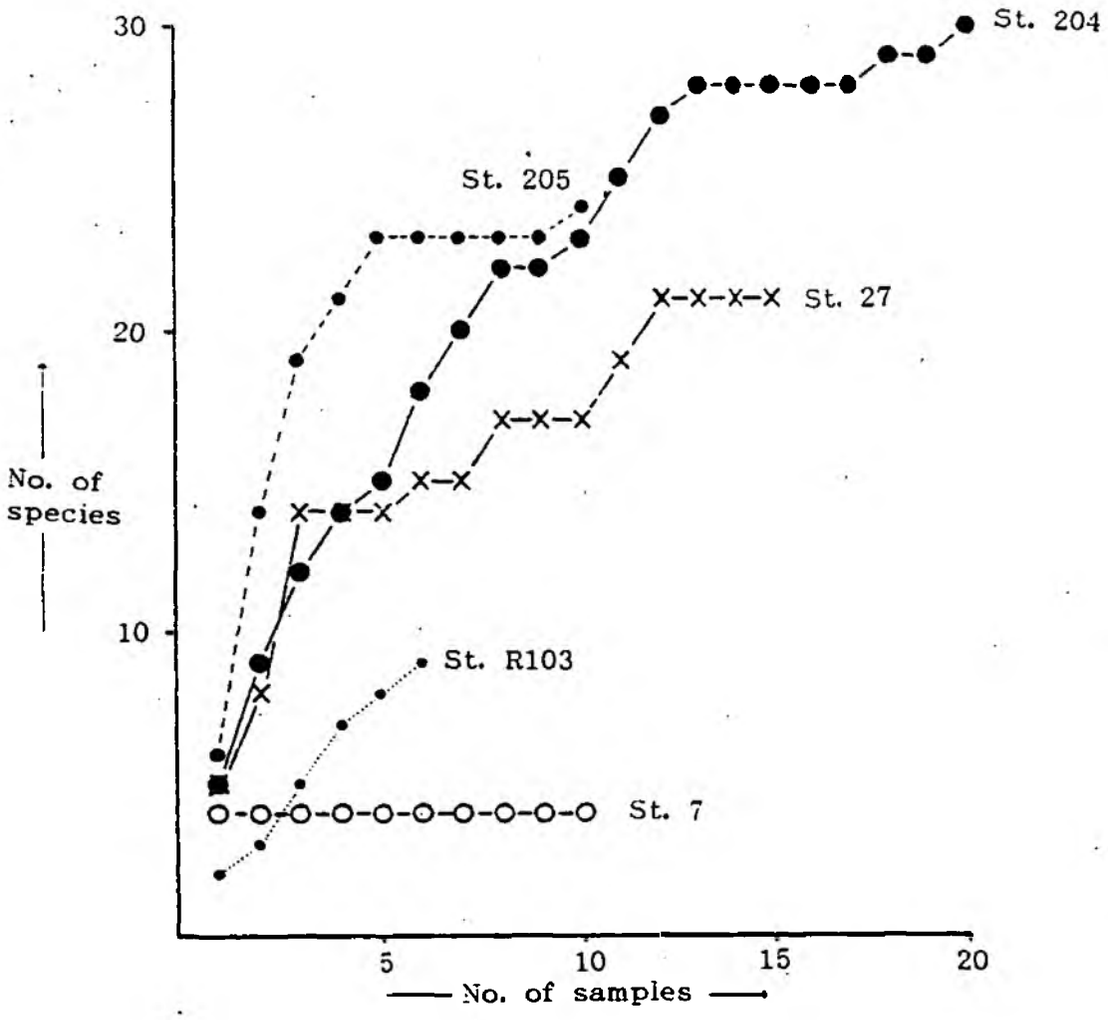
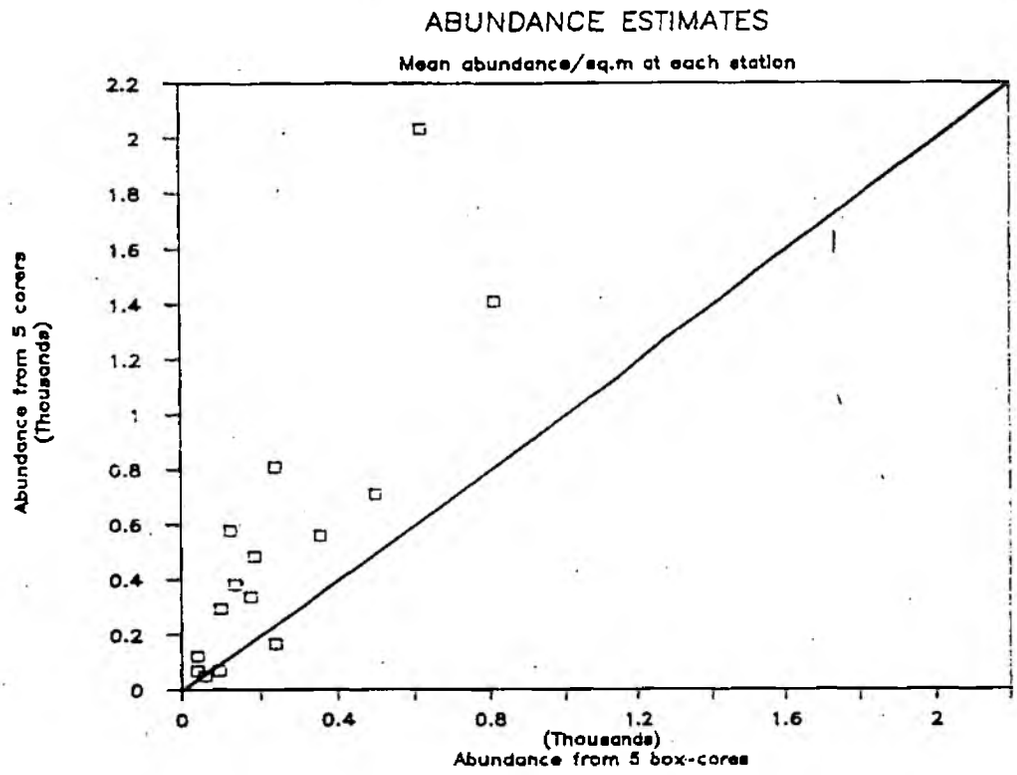


Table 1: **Taxa obtained in 3 and 5 grabs as a percentage of the total in all replicates taken at each site.**

Station	Percentage of total taxa			
	3 grabs		5 grabs	
7	100%	4/4	100%	4/4
27	64%	14/22	64%	14/22
103	63%	5/8	75%	6/8
204	40%	12/30	50%	15/30
205	79%	19/24	96%	23/24

Figure 3: **Comparison of cores (0.01m²) with respect to abundance estimates (information supplied by Yorkshire NRA)**



Rees (1979) has shown that increases in the precision of the estimate of the mean number of organisms per grab sample is minimal following analysis of 15 and 20 grabs compared with the first 5 (Table 2). Similar analysis carried out upon the data for a number of individual taxa (Table 3) shows that the accuracy of the mean can only be fixed within wide limits for fauna with low counts and marked aggregation. For fauna with higher counts (*e.g. Nephtys cirrosa* Station 27), as for total abundance, the precision of the mean number of individuals per 0.1 m^2 is only minimally improved by analysis of more than 5 grab samples (Table 3). It would seem, therefore, that to estimate abundance of all taxa present, within a stated confidence limit, would necessitate massive replication. In general, however, it is the dominant taxa which are of interest and reasonably accurate estimations of their abundance can be made from 5 grabs. In addition, 3-5 grab replicates gives a reasonable assessment of the relative abundance of the taxa present and so, for the majority of surveys, is adequate.

In conclusion, if quantitative data is required then a minimum of 3 grabs at selected sites is considered to be representative of major communities giving adequate quantification of commoner species but not the less common,nor will it establish the full range of rarer taxa. Five grab replicates are preferred at coastal sites, reflecting the more diverse communities encountered. Taking a limited number of replicates at each site is a practical necessity, however, providing each site is sampled with the same intensity and all samples are processed in the same way then the data produced will give a reasonable account of the community composition, will be comparable and differences between sites will be determined.

3. QUALITY ASSURANCE PROPOSALS

In addition to Quality Assurance and AQC procedures detailed within the method manual several others were investigated. These are discussed below, together with suggestions for the implementation of the recommended AQC programme.

3:1. Review of AQC in the Anglian Region

At the present time AQC checks operate in two laboratories (Northern and Central) on a fairly informal basis. In the past, work carried out by contractors has also been assessed.

In both laboratories 5% of each survey's samples are assessed to determine extraction efficiency and identification. Acceptable levels for extraction have been set at 100% of taxa and 95% of the total number of individuals. With respect to checks on species identification, this has primarily been useful as a learning process with representatives of problem/'new' species being returned for reference.

Table 2: Precision of abundance estimates with different sample size.

Station	Number of samples	Derived mean (y) + 95% confidence interval
7	3	4.1 (0 - 42.6)
	5	7.2 (1.7 - 24.2)
	10	8.4 (4.5 - 15.0)
27	3	14.9 (5.4 - 38.8)
	5	15.3 (10.6 - 21.8)
	15	15.6 (14.1 - 17.3)
204	3	27.8 (10.8 - 69.6)
	5	27.3 (19.6 - 37.9)
	20	25.8 (22.5 - 29.5)

The data have been normalised [$\log_{10}$ (x+1)] and, therefore, the derived mean (y) and confidence intervals have been calculated using the transformed data. (after Rees, 1979).

Table 3: Precision of abundance estimates for different taxa with increasing replication.

Taxon	Number of samples	Derived mean (y) + 95% confidence interval
Station 7: Capitellidae	3	0.8 (0 - 6.2)
	5	3.2 (0 - 18.1)
	10	5.17 (1.9 - 12.2)
Station 27: *Nephtys cirossa*	3	9.14 (5.8 - 14.1)
	5	9.84 (9.0 - 10.7)
	15	9.76 (7.4 - 12.8)
Station 205: Cirratulidae	3	3.25 (0 - 98.9)
	5	3.99 (0.6 - 14.9)
	10	3.95 (2.1 - 6.9)
Paraonidae	3	0.71 (0 - 2.0)
	5	0.82 (0 - 4.1)
	10	1.00 (0 - 3.6)

Data has been normalised [$\log_{10}$ (x+1)] and the derived mean (y) and confidence intervals calculated using transformed data.

(source of data Rees, 1979)

The same criteria are applied to new staff. Initially, samples are sorted under observation. After a short time new staff sort independently but all samples are checked until a consistent and acceptable standard has been achieved.

AQC has been carried out in the Central area by swapping samples within the laboratory. In the Northern area samples have been sent to external independent biologists who carry out the checks. At present analysis and application of the AQC information is being assessed. It has proved useful in the detection of individual problems such as 'species blindness' during sorting. On the whole very few problems have become apparent suggesting that the training and methods used at the present time are effective.

3:2. Internal AQC checks

A set quantity/number of samples are double-checked in the laboratory. If several people have been involved in sample processing then each individual's work is assessed. In addition, if the survey covers a number of sediment types then samples from each type should be checked.

Sorted detritus is re-sorted to determine the extraction efficiency of organisms. In addition, the accuracy of identification and enumeration of organisms is also assessed.

The procedure identifies problems with respect to sorting *e.g.* repeated over-look of certain taxa, under-counting and misidentification. It can be a valuable aid to training in that misidentifications can be corrected and the characteristics of newly defined species drawn to the attention of staff. The system can also be used to assess the quality of work produced by external contractors used for surveys.

Internal checks could be carried out within each laboratory by swapping samples or by exchanging samples between laboratories. Alternatively, the AQC checks could be carried out externally, minimising interference with on-going work and overcoming problems in laboratories where there is a single marine biologist. Samples are sent to external biologists for checking (as in Northern Area). This could be co-ordinated by one of the marine biologists or centrally from the Regional biology laboratory (NAMAS require the selection of a Quality Manager to oversee QA). The co-ordinator would also need to assess the results of any AQC in general terms and produce a performance report for each survey and/or annually.

Another option is to have the AQC service located in the Regional biology laboratory responsible for Quality Assurance. Extraction efficiency might then be carried out in-house but with a minimum of hinderance to the Area laboratories' work. Confirmation of identifications would probably then need to be performed by an external expert as it is not feasible to employ someone with sufficient expertise on a full-time basis. An important point to bear in mind is

that AQC checking is likely to be patchy and other duties for such a post need to be included, such as analysis of AQC and audit results, checking of data input, organisation of intercalibration exercises, ring sorts, seminars and other training.

The AQC process above prevents recurrence of errors, but one problem remains in that if a consistent error has occurred in the samples checked what is to be done with other data which may be biased by such error? To avoid such a dilemma it might be necessary to check one replicate from each sample site and if that fails then the remaining replicates are checked and corrected, therefore, errors do not enter the data set. The retrospective alteration of data is not generally acceptable, as this can lead to further biasing of the data. Only the results from those samples which fail the set criteria are corrected and not others which, although they have passed, are likely also to contain some errors.

A similar procedure operates for freshwater benthic samples in one of the NRA Regions. Samples are put into groups of five and one is chosen at random and re-sorted, if more than two extra scoring taxa are found then the remaining samples in that group are re-sorted. This has the advantage of giving instant feedback and although it adds up to *ca.* 30% to the workload it may mean that people work faster as they know that they are still able to attain the required standard by spending less time on each sample. In the same Region, for marine benthic samples, each sorter has one replicate per site double sorted to remove blind-spots and one representative of each taxa is sent to an external independent biologist for confirmation. If any identifications are wrong then the remaining replicates are checked and corrected. This method should not bias data as the only alterations which are made are the names given to taxa. It would be possible to operate a similar system and only check 5/10% of samples.

AQC procedures in manufacturing and chemistry/biochemistry utilise control charts to indicate whether the system is 'in control'. These show the results of small-scale repeated sampling. There are a number of types of control chart, such as the Shewart Chart and the Cumulative Sum (Cusum) Chart (Hector, 1986). They, basically, rely upon deviations from established/acceptable mean values. The system is 'in control' if the results fall within predefined limits, *e.g.* $\pm$ 3 standard deviations.

Audit data of river macroinvertebrates has been analysed using Shewart Charts (WRc, 1991). The charts used the number of additional taxa found in samples when audited and were based on a Poisson distribution with a mean of 1.72 (the average number of families found in samples from the best three Regions) $\pm$ 3 standard deviations. A similar approach has been taken in Welsh NRA using the number of added taxa. This system recommends the re-analysis of samples in batches of ten. The number of additional taxa are plotted on a control chart to check compliance with the following rules:

1. The upper acceptable limit is four missed taxa.

2. For 80% of the time the number of missed taxa must not exceed two.

3. For 90% of the time the number of missed taxa must not exceed three.

The number of allowed failures per batch has been calculated using the WRc ZEBRA model in order to reduce the risk of false alarms.

These systems work reasonably well when a range of 'error' is allowed but the criteria likely to be used regarding marine benthic macroinvertebrates will not be amenable to this type of treatment, *i.e.* 100% of taxa are to be extracted from the sample. In addition, control charts are most effective when constructed on a daily basis. Any AQC for marine/estuarine benthic samples will, by the nature of the work, occur at irregular intervals. It is, therefore, unlikely that control charts would be of much use.

The number/proportion of samples to be check sorted depends on the required repsonse time. This is based on the following elements:

1. The average run length (ARL) before a Type 1 risk, which is the risk of a false alarm, should be as long as possible;
and
2. the ARL before a Type 2 risk, which the risk of not detecting a change occurs, should be as short as possible.

Once the ARLs are set then the response time can be chosen, *e.g.* the ARL for detecting an 'out of control' situation may be 10 samples and if it is decided that such a situation needs to be identified in two weeks then 10 samples must be analysed every fortnight. The proportion analysed for AQC, therefore relies upon the ARL and the throughput of the laboratory, *i.e.* if 50 samples are analysed then the AQC batch is 20% if the number samples is 200 then the proportion is only 5%.

Until an AQC system has been in operation then the ARL cannot be known and so at the present time a fixed proportion of the samples must be checked and the system reviewed at a later stage to decide if it is adequate or adding an excessive amount to the work load.

3:2:1. Cost of AQC checks

Estimates of the extra time required to implement various AQC programmes (Tables 4-6) have been made. The figures are based upon the number of samples taken in the last two financial years and feedback from the areas as to the amount of time required to carry out sorting and identification. Obviously, a number of assumptions have had to be made but it is hoped that these figures give at least some indication of the resource implications of a full AQC programme.

It can be seen that there is likely to be an increase in the workload associated with AQC checks, with the percentage increase in the time spent sorting and identifying ranging from 5% to 29% overall.

Table 4: Cost of Internal AQC - Intertidal samples

Assume 5 replicates per site

	Year		Time to do analysis[a]		% Increase in workload
	90/91	91/92	90/91	91/92	
No. samples	667	672	1334_s 667_i 2001_{total}	1344_s 672_i 2016_{total}	
No. samples % extraction					
1 per site	133	134	133[b]	134[b]	
10%	67	67	67	67	
5%	33	34	33	34	
I.D. & counts					
1 per site	133	134	133[c]	134[c]	
10%	67	67	67	67	
5%	33	33	33	34	
Total no. AQC samples					
1 per site	133	134	266	268	13
10%	67	67	134	134	7
5%	33	33	66	68	3

a - assume 2 hrs to sort and 1 hr to identify

s - sort time
i - identification and enumeration time
total - time spent analysing intertidal samples

b - assume 1 hr to check % extraction
c - assume 1 hr to check identifications and counts

Table 5: Cost of Interal AQC - Subtidal samples

Assume 3 replicates per site

	Year		Time to do analysis[a]		% Increase in workload
	90/91	91/92	90/91	91/92	
No. samples	940	873	4700_s	4365_s	
			2820_i	2619_i	
No. samples			7520_{total}	6984_{total}	
% extraction					
1 per site	313	291	1565[b]	1455[b]	
10%	94	87	470	435	
5%	47	44	235	220	
I.D. & counts					
1 per site	313	291	939_c	873_c	
10%	94	87	282	261	
5%	47	44	141	132	
Total no. AQC samples					
1 per site	313	291	2504	2328	33
10%	94	87	752	696	10
5%	47	44	376	352	5

a - assume 5 hrs to sort and 3 hrs to identify & count

s - sort time
i - identification and enumeration time
total - time spent analysing intertidal samples

b - assume 5 hrs to check % extraction
c - assume 3 hrs to check identification and enumeration

Table 6: Cost of Internal AQC - All benthic samples

	Year 90/91	Year 91/92	% increase in workload	estimated cost (£)
Total no. samples	1607	1545		
Total time on analysis	9521	9000		
Total time for AQC				
1 per site	2770	2596	29	26,250
10%	886	830	9	8750
5%	442	420	5	4190

Costings assume an 8 hour day and 225 man days per year, an average wage of £14 K per annum (Grade 5) plus 25% for NI and superannuation and do not include building costs *etc.*.

3:2:2. AQC proposal

At the present time it is envisaged that an internal AQC check of 10% of samples should be the aim and that this could be best achieved by swapping samples between the Area laboratories thus introducing some degree of independence and overcoming the problem of single workers. The process needs to be co-ordinated. NAMAS require the appointment of an independent Quality Manager and it is likely that this post will be based at Regional HQ. In addition, deputy Quality Managers will be appointed in each laboratory to oversee the day to day running of the Quality Assurance programme. Technical managers and deputies will also be identified in each laboratory responsible for the practical aspects of the work. The likely structure is shown in Appendix 2.

Acceptable figures for extraction efficiency are to be 100% of taxa and 95% of specimens both from whole samples and subsamples. It is to be left to the discretion of the quality managers whether the overlook of a taxa represented by a single specimen is of significance. It is proposed that concern regarding results should occur if more than 20% of the samples checked fail. If this occurs it might be necessary to re-sort the whole survey, such a decision would be made by the biologist in charge of that particular survey.

Representatives of any unusual/'new' species should be pointed out and returned for reference. It is difficult to define suitable levels of accuracy for identifications. It might be necessary to draw up a comprehensive list of species and assign acceptable taxonomic levels of identification, *e.g.* family, genus or species, based upon the ease of identification and purpose of the survey. Alternatively, it would be up to the discretion of senior, experienced, staff to decide the importance of identification errors as they arise and the training/action required. The first option is recommended as this is the most suitable for a formal Quality Assurance system.

Enumeration of each taxa must be checked and it is recommended that counts should be within 10%. Discrepancies in counts are only likely to occur where there are damaged specimens *e.g.* heads and tails counted, or where species identification differs.

The results of AQC checks should be recorded on standard forms (*e.g.* Fig 4 and 5) which are to be retained for reference. The individual results of any AQC programme must be anonymous, *i.e.* only the individual concerned and the senior marine biologist/quality manager for that laboratory will see identified results. This is particularly important where samples are exchanged between laboratories for AQC checks. It will be the responsibility of the Quality Manager to assess the results of checks and pass all information back to the laboratories.

Figure 4

AQC Results 1993

Extraction Efficiency

Area _____ Survey _____

Sorter _____ Site/Sample code _____
Code only

AQC Species List		Original Species List	
Species	Count	Species	Count

Taxa missed:

Taxa
% extraction =

Counts
% extraction =

Figure 5

AQC Results 1993

Identification & Enumeration

Area _____ Survey _____

Sorter _____ Site/Sample code _____

Code only

AQC Species List		Original Species List	
Species	Count	Species	Count

Comments

PASS/FAIL

A summary of AQC results should be compiled on an annual basis and made available to the Regional Biologist, Environmental Managers and functions which utilise marine/estuarine biological data.

In most cases any consistent errors in both extraction and identification can be easily corrected by a review of techniques used and/or simple training in the laboratory. If it is thought necessary attendance at a specialist course may be appropriate (see section 5).

It is important that feedback occurs soon after each survey so that problems are rapidly identified and action can be taken as soon as possible. It may also be necessary to include some information regarding the outcome of AQC in reports, *e.g.* the number samples checked, % passing the set criteria, problem taxa. It is, therefore, recommended that the AQC checks are carried out for each survey prior to report writing and before sorting and identification of samples from another survey.

To implement the recommended internal checking of 10% of samples would require an extra 0.5 man years per annum (this assumes an 8 hour day and 225 man days per year). Based on an average wage of £14 K per annum (Grade 5) plus 25% for NI and superannuation but not including building costs *etc.* the extra cost for the Anglian Region would be approximately £8750 per year (Table 6).

3:3. External AQC

If a thorough internal AQC system operates the requirement for external AQC is minimised. However, it is not completely negated and is still a valuable aspect of the programme. It is envisaged that external AQC would be run annually, in a similar manner to the audit of freshwater benthic samples carried out by the Institute of Freshwater Ecology. It is likely that an audit of 5% of all samples would be sufficient, resulting in a maximum of ~ 80 samples being checked each year (based on the previous two financial years). Samples for audit should be selected at random but include all workers and surveys. The results of an audit should be recorded on standard forms (as above [3:2:2] and in Fig 4 and 5) and collated and analysed by the Quality Manager.

A number of organisations have been asked to quote the cost of carrying out an audit of 80 samples (*ca.* 35 intertidal and 45 subtidal), details of the institutes are outlined in Table 7. Costs for an external audit range from approximately £2500 up to £20 000.

It can be seen that external AQC is costly and as NAMAS does not stipulate an external audit it might not be an absolute requirement of the Quality Assurance system.

Table 7: Cost estimate for an Annual External Audit of 5% of samples taken.

Institute	Contact	Comments
Field Studies Council Research Centre Fort Popton, Angle Pembroke Dyfed SA71 5AD Wales Tel:(0646) 641404 Fax:(0646) 641425	Mr David Levell Senior Research Officer	FSCRC have taken part in ring sorts and are working towards BS5750 accreditation. Cost: £10250 - £14750
Institute of Estuarine & Coastal Studies University of Hull Hull HU6 7RX Tel:(0482) 465503 Fax:(0482) 466340	Debbie Meakin Biology project manager	Participate in intercalibration exercises organised by the Marine Pollution Monitoring Management Group Cost: £17195
Seas Ltd. Scottish Environmental Advisory Services Dunstaffnage Marine Laboratory Oban Argyll PA34 4AD Scotland Tel:(0631) 62244 DML Fax:(0631) 65518	Dr Tom Pearson	Sorting & identify benthic samples for survey and research projects (UK & Scandinavia). All sorting undertaken by fully qualified technical biologists with a minimum of 2 years experience. All identification carried out by fully qualified chartered biologists with a minimum of 4 years experience in marine invertebrate taxonomy. Cost: £2340 - £3000 + VAT
Dove Marine Laboratory Cullercoats Tyne and Wear NE30 4PZ Tel:(091) 2524850 Fax:(091) 2521054	Dr C.L.J. Frid	Cost: £17750 - £20000 + VAT

3:4. Ring sorts

This is calibration by circulation of samples carried out on a routine basis. Ring sorts can be carried out both within a laboratory and between laboratories.

Ring sorts assess the accuracy of identification only. It can be very beneficial with respect to training with immediate feedback upon procedures and identification.

Ring sorts are a relatively quick and easy method of checking inter/intra-laboratory variation regarding identification. It is an ideal opportunity to discuss and agree on common laboratory identifications (Methods Manual, Section 10:1).

It is proposed that informal intra-laboratory ring sorts be undertaken during each survey and carried out simply by passing sorted samples around for identification and enumeration. Results are to be discussed within the laboratory. Inter-laboratory sorts can be arranged in a similar way. The frequency of inter-laboratory ring sorts should be at least twice yearly and intra-laboratory ring sorts should occur at least quarterly and preferably during each survey.

Ring sorts are viewed by NAMAS as being more valuable in terms of information obtained and instant feedback than external AQC especially when carried out between a large number of laboratories. Ring sorts are, therefore, recommended.

3:5. Intercalibration exercise

When using similar sampling methods statistically significant differences in results can occur. For example, Ankar *et al.* (1979) compared the sampling and processing techniques of two laboratories and differences between results were attributed to the grab design (two types of van Veen grab were used), sieving technique and possibly to the methods used to sort and preserve the samples. Even when standard methods are used worker variability needs to be accounted for.

Although an intercalibration exercise is time consuming it has the benefit of giving increased confidence in results obtained if it is successful. If the exercise shows marked differences in the results between the areas then the reasons for these need to be identified and corrected, *e.g.* a training day organised to ensure that all workers are using identical equipment and procedures. The intercalibration should be carried out following standard methods.

The exercise should be organised so as to minimise inconvenience and increases in workload. One possibility is to incorporate the exercise into survey work, however, this would give unfair advantage to one area and would probably cause too much disruption of the survey.

It should be sufficient to sample from 3 sites for both inter- and sub- tidal benthos. This will create 15 core replicates (if sites are all on fine sediment) and 9 grabs. The inclusion of some coarse sediment intertidal sites should be considered to enable the use of the supplementary sampling procedure (SSR). This would necessitate additional sites, *e.g.* 2/3 coarse sediment coastal/estuarine sites, the choice of the SSR required should be left up to the biologists.

To minimise costs it would be ideal to carry out all the grab sampling on a single day. This requires the sites to be easily accessible, with a minimum of travel time to/from and between the sites. It would be difficult to get all three areas out on one day and it might be necessary to continue on the next day. The exercise needs to be conducted at a time of year when the weather is likely to present few problems and which also avoids other survey work. In the past two years there have been no benthic field samples taken in May and this may prove to be suitable, provided that Sea Vigil and all staff are available.

Regarding intertidal sampling, ideally the sites should be accessed from land to avoid the complications of hiring and handling boats. Due to the nature of the sampling it will be necessary to spread the exercise over at least three days so that the marks created by sampling are removed by the tide.

The location of the sites requires careful consideration, they need to represent 'real' sites and contain a variety of fauna. In addition, they must be convenient for all areas to reach without too great a travelling time, *i.e.* somewhere central to all the areas such as the Wash or north coast of Norfolk. Site locations will be given in a manner similar to that of a true survey *i.e.* grid reference, compass bearings, longitude/latitude or landmarks. Location of subtidal sites will be less Area dependent as this is carried out by Sea Vigil crew.

One danger of intercalibration exercises is that any differences in the results from the different working groups may be due to site depletion due to repeated sampling. As relocation of sample points is unlikely to be absolutely accurate this should, however, not prove to be too big a problem. In addition, for intertidal sampling, the time between samples (at least one tide cycle) should allow for some 'recovery' to occur.

The samples will be processed and analysed as in the standard procedure and a **brief** report of findings made. Obviously with only a few sites the use of multivariate analysis will be of limited value. It probably would be more appropriate to present the data as number of taxa, abundance and use simple diversity indices which would be fairly readily compared and should give an indication of the compatibility of the data generated. This, together with raw data will then need to be compared by an independent biologist (*e.g.* AQC co-ordinator) to ascertain whether any serious differences in the data or interpretation exist. It would be hoped that the results of any intercalibration exercise would be produced with a minimum of delay, thus preventing any significant differences in data produced by the areas from continuing to occur.

An estimate of the time (working days) required for an intercalibration exercise, based upon average times taken to process samples, is shown below:

Subtidal:
Three sites, 3 replicate grabs per site giving a total of **9** samples.

Field work -	**1** day (including travelling and boat work)
Processing (in lab) -	**3** days
Analysis -	**9** days (8 hrs per sample)
Data analysis & reporting -	**3** days
TOTAL: *ca.*	**16** days

Intertidal: 'CORE'
Fine sediment, three sites, 5 replicates per site giving at total of **15** samples.

Field work -	**1** day
Processing (in lab) -	**2** days (*ca.* 1 hr per replicate)
Analysis -	**5.6** days (3 hrs per sample)
Data analysis & reporting -	**3** days
TOTAL: *ca.*	**12** days

Intertidal: 'SSR'
Coarse sediment, two sites, 5 cores + 5 cores (site 1) and 5 core + 3 box-cores (site 2), giving a total of **18** samples.

Field work -	**1** day (could combine with intertidal sampling above)
Processing (in lab) -	**2** days
Analysis -	**6.75** days (3 hrs per sample)
Data analysis & reporting -	**3** days
TOTAL: *ca.*	**13** days

If all intertidal sampling is carried out on a single day then the total time taken to carry out the calibration exercise would be approximately **40 days** (0.17 of a man year costing *ca.* £3000 in wages, based on a Grade 5 £14 K plus 25% for NI and superannuation) for each area. The total cost of this intercalibration exercise would be £10,120 (including the cost of Sea Vigil for two 8 hour days at a recharge cost of £70 per hour) plus the cost of sample containers and formaldehyde solution.

It would probably be more manageable to run a separate exercise for each type of sampling to avoid creating too great a work load concentrated into a short space of time.

Any inter-lab calibration is likely to be run as a one-off exercise or repeated at interval of five years due to the quantity of extra work involved.

3:6. Miscellaneous quality assurance processes

3:6:1. Double sorting

After samples have been sorted the residue is checked by a second person, any fauna found are removed and pooled with those found in the initial sort for identification and enumeration (Nixon *et al.*, 1992).

Double sorting has been shown to increase the number of species found in three replicates by *ca.* 13%. The extra taxa recovered were mostly polychaetes and crustaceans (Coleman, 1980).

One problem with this procedure is that it is not suitable for use in laboratories where there is only one marine biologist. In many cases a form of double sorting occurs anyway with a sorter checking over the sample until they are satisfied that nothing further remains. Secondly, double sorting by a second biologist demands a large, possibly unacceptable, increase in the workload. Based on the number of samples taken within the Anglian Region in the last two financial years, and assuming that it is reasonable to spend about half the amount of time it takes to do an initial sort, it would add up to about 2900 hours (1.6 man years) of extra work a year. Such an increase needs to be justified and it would be best to assess any benefits of double sorting perhaps as part of an inter-calibration exercise.

3:6:2. Sample duplication and splitting

A recent WRc report (Nixon *et al.*, 1992)) suggested the following as means of assessing the quality of marine/estuarine benthic sampling.

i) an external audit of duplicate samples, *e.g.* 10% for each survey, to obtain information on sample homogeneity and method precision, and

ii) an external audit of split samples, *e.g.* 10%, to gain an indication of analytical repeatability.

This is really a continuation of research into the errors involved in the process and need not be a regular occurrence. Findings could be incorporated into procedures as they develop.

These proposals have been criticised on a number of points. Firstly, duplicating 10% of samples would in some surveys only give one extra sample which would not give much information about method precision and would, probably, only indicate just how patchy a community is, strengthening the case for greater replication (see section 2:3:1 above). In addition, split samples would not necessarily give information about analytical repeatability as it too will be clouded by patchiness.

Basically, large sample heterogeneity will confuse any pre-defined variation leading to unnecessary criticisms.

3:7. Data transfer

The checking of data input does not appear to have been addressed previously in biology. It is important that having ensured a minimum of error throughout the process that the transfer of data from paper to computer is also accurate.

This can be carried out as an internal/external check of a set percentage of data. If an AQC service were set up in the Regional biology lab, data could be pooled enabling easy access to check data input with a minimum of interference with normal laboratory work.

An alternative is the standard AQC procedure of data validation, whereby each item is keyed in twice by independent operators and the data point is only accepted if both values agree. Double entry is probably not acceptable as it is time consuming and would require software modifications. It would, therefore, seem that the only way to assess this is to compare the paper and computer records of a set percentage of data. At the present time data is always double-checked when transferred, simply by reading out the paper record and comparing it with that on the screen.

3:8. Final comments

The Quality Assurance programme discussed above is a combination of standard methods and stringent checking of samples and data at all stages. All of these activities should be integral to the process of taking, processing and analysing marine/estuarine benthos and not something added on as an afterthought.

It is proposed that internal AQC and possibly external audits should operate and be acted upon with respect to training, both in the laboratory and by attending courses. Should a substantial proportion of the samples (*e.g.* 20%) fail the set criteria then consideration must be given to the possibility that the whole survey needs to be re-sorted.

In addition, ring sorts should be carried out at regular intervals (twice yearly for inter-lab and quarterly or one per survey within the lab). At least one full intercalibration exercise should be completed as a baseline for all subsequent work. Consideration should be given to repeating the exercise, say, every 5 years. Intercalibration exercises would provide a good opportunity to assess the cost and benefit (extraction efficiency) of double sorting different types of sample. Other research could also be carried out, such as the assessment of losses associated with transferring samples from the grab to plastic containers prior to sieving on board, as discussed in section 7:2:1 of the Methods manual.

All of the above will add to the work load of the laboratories, although it is hoped that disruption will be minimal. The resource implications of a thorough AQC scheme have been outlined. Some sources suggest that 20% of analytical time is being devoted to AQC procedures in the Water Industry (Elliott *et al.*, 1992). In addition, for NAMAS accreditation a thorough system is necessary and such a commitment is required.

4. TRAINING RECOMMENDATIONS

Due to the practical nature of benthic sampling and assessment the majority of training is undertaken within the laboratories as and when required, therefore, most of the following already occurs in the Region.

4:1. New staff

4:1:1. General comments

The laboratory manual should contain sufficient detail for new staff to obtain a clear idea of the procedures used and it is recommended that the manual be read prior to carrying out any work. This should save time and enable useful survey work to be carried out in conjunction with training. Training videos for basic skills may also be of use. It must be stressed, however, that there is no substitute for hands-on experience.

It is important that all training is relevant to the staff and that the techniques are to be used regularly and relatively soon after initial training otherwise time is wasted in reiterating the methods. This is particularly important for certain techniques, *e.g.* Day grabs, which for safety reasons must be operated correctly. At all times safety aspects of the procedures should be pointed out, *e.g.* the use of protective clothing.

During training it should be stressed that if there are any problems/doubts, either regarding a technique or identification, advice should **always** be sought.

Training must only be carried out by personnel with sufficient experience. All training should be recorded with the technique, date and trainer noted (Fig 6). A file listing all the training completed should be created for each member of staff as a permanent record.

4:1:2. Sampling procedures

The operation of sampling equipment (cores and grabs) ought to be demonstrated in the field by an experienced member of staff and then

Figure 6: Example of staff training record sheet

NAME: _____

JOB TITLE: _____

Date	Procedure explained	Name, Title & Signature of Trainer

carried out under supervision to ensure that it is being done correctly and that replicates are consistent.

4:1:3. Sieving of samples

As for sampling, sieving of samples should be demonstrated and subsequently carried out under supervision. A training video, especially for on-board sieving of grab samples, may be useful to demonstrate the best way to handle samples in order to minimise damage to the organisms, for example, hose pressure should not be too high as this forces organisms through the mesh. Such a video has been proposed by Northumbria Region NRA as an effective way to demonstrate Nationally agreed methods, the cost has yet to be assessed.

4:1:4. Sample sorting and analysis

Initially, sample sorting should be supervised until a basic level of skill is attained, the time spent doing this will depend upon the level of expertise which the new staff member already possesses. A teaching head on microscopes would be useful, as this ensures that the trainee sees the correct organism or feature being discussed. After this time, all samples should be checked for extraction efficiency and identification. This procedure stops when the desired level of accuracy is reached (criteria as for AQC, section 3:2:2 above). Staff should be introduced to recommended taxonomic literature and be made aware of common laboratory identifications (Methods manual, Section 9:2:1).

4:2. Safety training

Basic training should occur which complements the guidelines laid down in the manual. For example, a training day to include basic first aid, water safety (swimming in boots/waders *etc.*), use of tide tables, and map and compass navigation. First aid and boat handling courses can be arranged by Personnel if there is sufficient demand.

4:3. On-going training

As mentioned above, there is no substitute for experience in this type of work, however, there are benefits to be gained from continued training.

One recommendation is that all staff should read the methods and safety manuals annually and make a record that this has been completed.

In addition, with respect to identification, the classification of marine benthic invertebrates is not fixed and species continue to be identified and re-described. It is, therefore, important that staff are aware of such changes. External training courses are vital for this and for the development of Regional expertise in certain taxa (Appendix 3).

External workshops on computing packages and data analysis may also be of benefit, *e.g.* PRIMER workshop at Plymouth Marine Laboratory; the PRIMER package is currently being assessed both in the Region and Nationally.

Much information is given in external courses and this should be disseminated to **all** staff involved in marine/estuarine benthic sampling. This could be in the form of a **brief** report circulated to the Area laboratories and, also, the Regional Biology Laboratory and Personnel. Alternatively/in addition, informal seminars could be held and information passed on to staff who were unable to attend the course.

More tailored training, *e.g.* regarding local species or Regional developments in R&D, could be achieved by holding day workshops/seminars in the Area/Regional Biology Laboratories, this would give the opportunity for specific problems to be addressed and for more members of staff to attend.

Contacts willing to hold workshops/courses are listed in Appendix 3.

4:4. UnicoMarine Image Database - "UNICORN"

4:4:1. General information about the system

The system uses Windows 3.1 and is, therefore, easy to operate, being mainly mouse-driven with very few keyboard functions.

The database contains species information in the form of text (general descriptions and occurrence) plus a set of images, including a general picture of the species plus others highlighting diagnostic features. There is also provision to enter other information such as, the typical substrate type and depth of occurrence for each species. The images are

compiled by capture from a video camera (there is the possibility of image enhancement using the image analysis package at the Regional Biology Laboratory).

The database can be accessed at a number of 'taxonomic' levels, from phylum down to species. Groups of organisms can also be accessed which are not necessarily taxonomic groupings but 'user friendly', *e.g.* 'scale worms', not a single family but readily recognised as 'scale worms'.

Up to four images from a number of species can be called up and displayed on one screen to enable easy comparison of characteristics.

The database also includes a **Key guide** which is a list of references held by UnicoMarine and could be tailored to suit individual laboratories or Regions and to include the location of texts. This list can be searched using key words, title or author details.

There is a **glossary** which, again, can be searched using key words and gives the definitions of terms used in keys plus an image of that feature.

Finally, there is provision for updating the system with a **edit facility**. This enables additions and alterations to existing species, including images, and also the inclusion of new species to the database.

The estimated cost of the system is *ca.* £1500 for suitable hardware (100 megabyte hard disk) plus about £2000 for the software, depending upon the content. The software could be supplied simply as a database system or as an 'encyclopedia' of British marine benthic species.

4:4:2. Comments.

The "UNICORN" system has been seen by a number of people throughout the Region. The database is good as a teaching aid, with photographic images used rather than line drawings and would be useful for training. For general use the system needs to be used in conjunction with recommended keys in the same way as a reference collection.

The database would need to be tailored to include regional fauna and so the simple software package might be sufficient. The process of compiling the text for each species found in the Region and to create relevant images would take quite some time and effort. The database would not, therefore, be suitable for immediate use.

The images can be printed out, given a suitable colour printer but the picture quality is not as good as photographic reproduction and the requirement for illustrations of fauna in reports is small and so this cannot be regarded as particularly relevant to any decision made.

In the Region at present there are a number of experienced marine biologists able to deal with identification of marine benthos whose expertise can be utilised in training new staff and for identification of difficult taxa. It is, therefore, unlikely that the database would be much used in the laboratories due to the expertise already present and, bearing in mind the capital outlay (*ca.* £3500 per laboratory), may not be very cost effective. It might be possible to have the database as a central service, based in the Regional Biology Laboratory but this defeats the object of the package as it should be available on a day to day basis. A second alternative is to have the database networked (if possible), therefore, only one software package would be required (higher initial cost for a network copy). There is sufficient hardware already present in the Regional Biology Lab to run it and each Area lab has PCs through which to access the system.

5. RECOMMENDATIONS

- The methods detailed in the manual should be regarded as the **minimum** requirement for all marine/estuarine work carried out within the Region.

- Consideration should be given to the use of a double sieve to avoid the loss of specimens.

- The use of sieves with a 1 mm mesh size for processing coastal grab samples is undergoing assessment (Wash data). It is, therefore, recommended that samples be sieved through both 1 and 0.5 mm sieves and that the 0.5 mm fraction be retained for sorting if necessary. If the Wash data supports the use of a 0.5 mm mesh size due to loss of important information by the use of 1 mm sieve one option is to subsample the 0.5 mm fraction rather than sort it completely. Alternatively, it could be retained for reference if it is felt that more information is required for a particular site.

- A site directory should be compiled for each survey to include a full description of each sample site with landmarks, compass bearings, grid references, longitude/latitude, and photographs where appropriate plus sediment description and sampling method used core, supplementary sampling or grab (note down acceptable volume).

- It is recommended that sites should be relocated within the accuracy of the equipment used, that is $\pm$ 2-20 m for both inter- and subtidal sites. Verification of the accuracy of relocation needs to be assessed, at the present time photographs for comparison with the directory seems to be the most suitable option for intertidal surveys. In the future the use of Cubit systems will enable assessment of the relocation of subtidal sites.

- Suitable grab sample volumes should follow the OSPARCOM recommendations except on difficult sediments (clay reefs). For each site acceptable volumes should be recorded in the site directory.

- It is recommended that a verified reference collection be maintained.

- It is recommended that internal AQC should operate, with, to begin with, 10% of all samples checked for both extraction efficiency and accuracy of identification and enumeration. The samples should be selected at random but cover all the individuals involved in sorting and identification. The AQC should be carried out by swapping samples between Area laboratories.

- Acceptable extraction efficiency is to be 100% of taxa and 95% of all individuals. If 20% or more of checked samples fail then serious consideration **must** be given to resorting the

whole survey. If a taxa is repeatedly overlooked then the sorting technique of the individual should be reviewed.

- The counts of each species should be within 10%. Discrepancies are only likely to occur if damaged specimens are present.

- For identification it is recommended that a list of species plus acceptable taxonomic levels could be compiled. Training in the laboratory should be sufficient to sort out any problems, but attendance at a specialist course may also be appropriate.

- Consideration of an external audit of 5% of samples on an annual basis should occur.

- An intercalibration exercise for both inter- and sub- tidal benthic sampling should be run. The benefits of double sorting could be assessed during the exercise. In addition, the losses associated with the transfer of material might also be evaluated. Intercalibration exercises should be repeated at least every five years.

- Both intra- and inter- laboratory ring sorts should be organised on a regular basis. A minimum of two per year is recommended, it should be possible to have more frequent in-lab ring sort (quarterly/one for each survey). These provide an ideal opportunity to agree upon a common laboratory and Regional identifications.

- A training record must be kept for each worker with details of the techniques plus date and trainer (this is a requirement for NAMAS accreditation).

- The "UNICORN" image database is particularly good for training and also useful for every day identifications, however, bearing in mind the experience of the marine biologists in the Region it would probably not be cost effective unless networked.

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APPENDIX 1

STANDARD METHODOLOGIES

Assessment of estuarine/marine water quality
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. <u>Supplementary Sampling Methods.</u>

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 an 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 $\pm$ 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.

References

Baker, J.M. and Wolff, W.J. 1987. *Biological surveys of estuaries and coasts. Estuarine and Brackish Water Sciences Association Handbook.* Cambridge University Press, 449 pp.

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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:

Appendix 5: TAXONOMIC REFERENCES

General:

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Hartley, J.P. (1981) The family Paraonidae in British Waters: a new species and new records with a key to species. *J.M.B.A. UK.* **61**:133-149.

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In addition a number of unpublished Workshop keys:

Sedentary Polychaetes:-

Capitellidae: ESCA (1990) Notes, includes a British species list.
Cirratulidae: George, J.D. Key to British Cirratulidae. Workshops key.
Magelonidae: ASYM/ECSA (1990) Workshop key.
Spionidae: ASYM/ECSA (1990) Workshop key.
Sabellidae: Knight-Jones, P. ECSA Workshop.

Errant Polychaetes:-

Aphiodididae, Polynoidae, Polyodontidae & Sigalonidae: ECSA (1990) Workshop key - includes amendments to Tebble and Chambers (1982) and Chambers (1985).
Glyceridae & Ganiadidae: EBWSA (1985) Workshop.
Hesionidae: EBWSA (1990) Workshop.
Nephtydidae: ECSA (1990) Workshop.
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Tunicata:

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Picton, B.E. 1985. *Ascidians of the British Isles. A colour guide.* Marine Conservation Society. (useful photographs)

Linnean Society Synopses of the British Fauna (New Series):

Volumes 1-28 inclusive available from the Linnean Society of London, Burlington House, Piccadilly, London, W1V 0LQ.

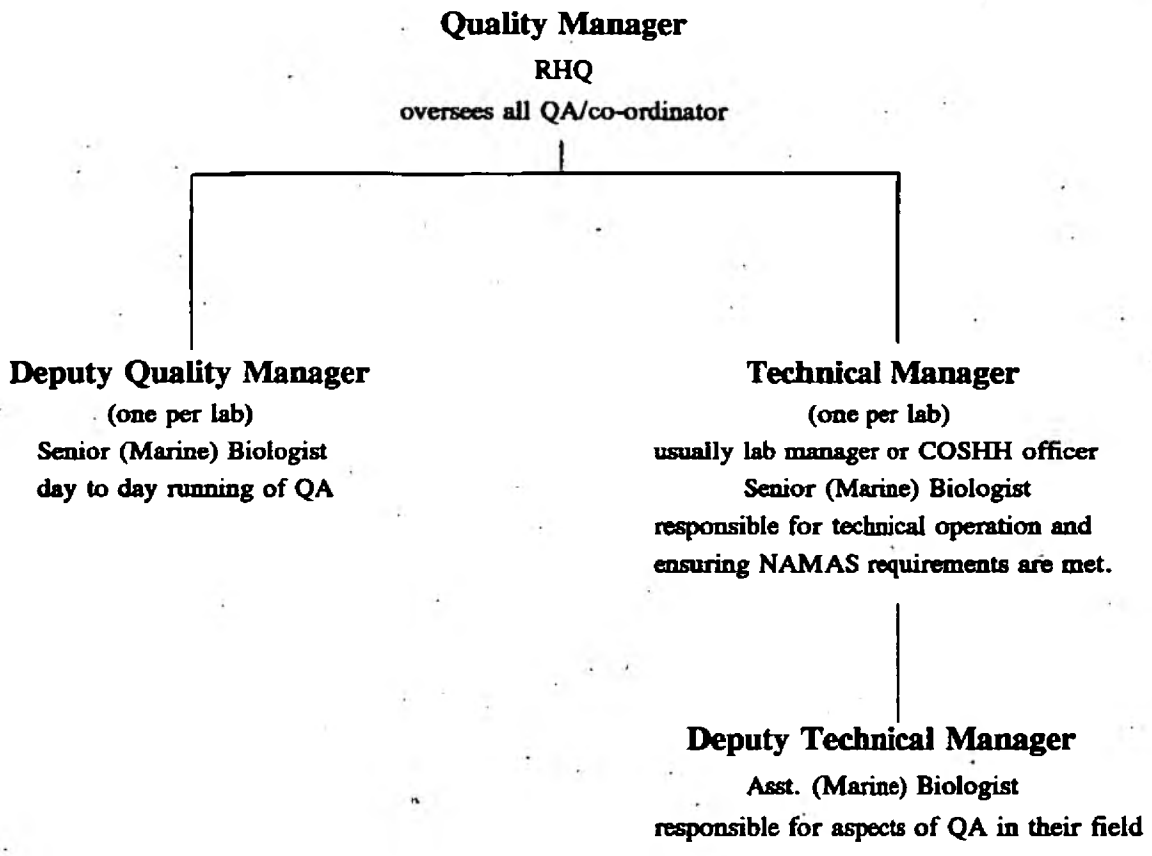
Volumes 29-41 & 43 may be obtained from E.J.Brill, Publishing Company, Leiden, The Netherlands.

Volumes 42 and 44 onwards are obtainable from Universal Book Services, Warmonderweg 80, 2341 KZ Oestgeest, The Netherlands.

All volumes in print are available from Natural History Book Service, Totnes, Devon, TQ9 5XN.

APPENDIX 2

Proposed organisation of a Quality Assurance team



APPENDIX 3

TRAINING

External Courses

Examples:

Polychaete identification course held at Cardiff Museum cost of 5 day course *ca.* £240 per person (Nov.1992), organised Nationally (Martin Attrill, Thames NRA).

Also workshops run by the Estuarine and Coastal Sciences Association (ECSA; formerly the Estuarine and Brackish-Water Sciences Association, EBSA).

Internal courses/workshops

The following are general details of institutes and individuals who are prepared to hold training courses/workshops for marine/estuarine benthic identification.

Contact: Dr Peter Garwood
Identichaet
8 Lesbury Road
Heaton
Newcastle upon Tyne
NE6 5LB

Tel: 091 2650567

Approx. cost £120 per day to hold a tailored workshop in the Region, 8-10 people to allow sufficient time with each.

Contact: Natural History Museum
Training Office
Cromwell Road
London
SW7 5BD

Tel: 071 9389123
Fax: 071 9388754