Proceedings of the Humber Benthic Field Methods Workshop, Hull University 1997. Collection and Processing of macrobenthic samples from soft sediments; a best practice review

R&D Technical Report E1-135/TR

institute of estuarine & coastal studies

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Proceedings of the Humber Benthic Field Methods Workshop, Hull University 1997. Collection and Processing of macrobenthic samples from soft sediments; a best practice review.

R&D Technical Report E1-135/TR

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Statement of Use
This report is intended to provide best practise guidance to adopt for the sampling of macroinvertebrates in marine soft sediments. The report should be used to guide internal and external biologists. The report is also intended as furtherance to the pursuit of quality assurance for the National Marine Biology AQC Scheme

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The organising committee would like to thank the crews of the two survey vessels used, *Water Guardian* and *Sea Vigil* for making all the participants welcome onboard and ensuring the exercises ran smoothly. Particular thanks go to the crew of *Sea Vigil* who drew the short straw with the intercomparsion exercise, in particular Steve McCreedy who made extra efforts to ensure the exercises ran smoothly, to Nick Cutts for his patience in editing the video, to Krystal Hemmingway and Nick for ensuring both the workshop and the following up meeting ran smoothly and finally to Brian Barnett for his support in the intertidal and laboratory exercise.

The committee would also like to thank Mike Robertson (SOAEFD) and Dave Limpenny (CEFAS) for all their assistance during the workshop particularly with the gear demonstration exercise without whose help this aspect of the workshop would not have been possible.

The support of the Environment Agency through the provision of survey vessels and funding for follow up analyses is duly acknowledged without whose financial support the workshop would not have been possible.

The chair would also like to thank John Orr for supporting the project at its inception and throughout the intervening years between the workshop and the final publication of the proceedings.

National Marine Biology AQC Committee and the Estuarine and Coastal Sciences Association are also acknowledged for providing financial support and a forum for progressing the development of field quality assurance for macrobenthic sampling.

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EXECUTIVE SUMMARY

During the course of its activities, the NMBAQC scheme identified a number of problem areas, which required further investigation. In particular the workshop focused on:

- inter-laboratory comparisons of field sample processing from soft sediments both in the shallow subtidal and intertidal environment;
- benthic equipment demonstrations;
- laboratory wet weight biomass determination;
- laboratory sub-sampling.

The workshop aimed to address these areas and investigate sources of error by reviewing best practice from as wide a range of UK laboratories as possible with a view to producing further guidance on best practice and providing further foundations for setting standards for all to follow.

Aims of the Workshop

The following specific aims were set for the workshop:

- to assess the effects of equipment and worker bias during the shipboard processing of macrobenthic sediment samples;
- to review best practice for intertidal sediment sampling;
- to demonstrate a wide range of benthic sampling and processing equipment;
- to review laboratory sub-sampling techniques;
- and to review laboratory wet weight biomass determination.

During the preparation of the proceedings two additional studies were used to further develop some of the key issues highlighted during workshop. Industrial Research and Technology Unit (IRTU) undertook a further evaluation of the Wilson auto siever assessed during this workshop and Aquatic Environmental Services (AES) undertook a biomass comparison study using data collected as part of a discharge impact assessment. These studies are reviewed alongside the workshop proceedings in the appropriate sections.

Sediment physico-chemical field sampling was not reviewed at the main workshop although it was discussed at the follow up and the summary discussions are presented in the final section of these proceedings.

The effects of equipment and worker bias during the shipboard processing of macrobenthic sediment samples

The workshop illustrated the importance of quality assurance of field sampling for macrobenthic fauna. It is recommended that field quality assurance be given equal

weighting to laboratory quality assurance and that future quality assurance developments should be targeted in this area.

A best practice protocol was agreed at the workshop and should be implemented for all macrobenthic sampling activities involving the use of 0.1m² grabs for the sampling of marine macrobenthos.

The design principles of the methodology of the Wilson auto-siever should be further tested and more widely applied. Further comparative testing is recommended in fully marine waters exhibiting a higher diversity.

The principles of the Wilson auto-siever should be considered for application to all future macrobenthic monitoring programmes due to the significant advantages described in the workshop proceedings when compared to the more traditional manual methods. However, a change of methods may prevent the method being used with temporal monitoring programmes already in progress due to the significantly lower numbers of individuals being retained by the auto-siever in some sediments when compared to more manual methods, unless a period of concurrent sampling is undertaken to cross calibrate the two methods.

Best practice for intertidal sediment sampling

A range of intertidal techniques were demonstrated for field sampling of macrobenthos. A standard operating procedure such as that of the Environment Agency's was generally recommended as best practice.

Demonstrating a wide range of benthic sampling and processing equipment

The workshop participants agreed that the gear demonstrations were valuable exercises since the majority of participants had never seen many of the samplers and processing methods before. It is recommended that any future workshops consider the inclusion of such demonstrations to provide an insight into the range of potential equipment available to sample marine benthos.

Review laboratory sub-sampling techniques

A protocol should be the developed for the application of sub-sampling (this should include the nature of the equipment, pre-handling, size of sub-sample and occasions on which it is required).

Performance limits should be set for sub-sampling techniques e.g. all estimates should be within 20% of the known number, the mean of estimates should be within 10% of known number and all estimates should be within 20% of mean.

A Quarteriser should be adopted as best practice although the exercise in the workshop requires to be repeated with different sieve residue types.

Any adopted sub-sampling technique should be thoroughly tested on the samples to which it is to be applied.

Review laboratory wet weight biomass determination

It was not possible to attain consistent wet weight biomass between workers at the workshop or in the example case study when weighing individual species. Individual species wet weight analyses, particularly in temporal trend monitoring, should not be undertaken as a routine measurement.

For total wet weight biomass, in order to attain consistency and minimise bias between workers, a strict and prescriptive protocol is required. An example of best practice is highlighted in the workshop report from the Aquatic Environment Services case study.

Review of physico-chemical sampling

The discussion at the follow up seminar for physico-chemical sampling was designed to provide some guidance and information for biologists undertaking surveys rather than a detailed indication of physico-chemical sampling in marine areas. It was noted that samples taken to answer chemical or sedimentological questions might not be compatible with biologically based surveys. It was emphasised that all aspects, biological, chemical and physical, as well as all sources of variability, methodological, field and analytical, require to be quantified and/or minimised in benthic studies.

1. PREFACE – The Need for a Review of Best Practice

Foreword from the Workshop Chairman

For the last 10 years a large part of my work up until the present day working for the Environment Agency for England and Wales (formerly the National Rivers Authority) has related to benthic monitoring, particularly soft sediments. My career has developed from being responsible for field and laboratory processing of macrobenthic samples through to the management of large projects involving the assessment of macrobenthos and more recently to the role of Marine Quality Assurance Scientist for the Environment Agency.

During this short career I have encountered a wide range of procedures for field and laboratory processing of macrobenthic samples, some good, some novel and some very poor. I have also consequently encountered a wide range in the quality of the data produced. Poor quality data has often undermined the serious consideration of marine biological data in the decision making process for environmental management, for example for assessing the impact of a marine development such as an estuarine barrage, or monitoring the impact of a point source discharge of organic effluent to determine the need for effluent treatment. Marine biologists are increasingly being asked to form an opinion on the state of the local or broadscale environmental quality using marine biological data. Quality control and quality assurance should play a fundamental role in ensuring that surveys and analyses of the marine benthos are as accurate and cost-effective as possible. The workshop reported here primarily aimed to consider field methodological consistency, quality assurance and control in marine benthic surveys.

1.1 Introduction

The study of the marine benthos forms a central part of marine environmental impact assessments and quality determination. This is primarily because of the ability of the sediments and their fauna to integrate over time environmental changes. Similarly, the marine macrobenthos has been extensively studied, has a history of techniques and surveys covering the past century and has a well-defined taxonomy. This component has a central part in defining and testing for compliance with Environmental Quality Objectives (Elliott, 1996). Furthermore this has produced many studies which examine the spatial and temporal changes in the benthos as the result both of natural variability and anthropogenic variability. The essence of these surveys and analysis is the taking of representative samples of bed sediment, removing the excess sediment through sieving or other means, extracting the fauna from the sieve residue, counting and identifying the fauna to the desired (or achievable) taxonomic level, and obtaining other biological information such as biomass. Such an analysis is labour-intensive and so each of these stages has the potential to introduce error and thus has methods which require to be standardised.

The importance of the above factors, dictates that increasingly marine biologists are being asked to demonstrate the quality of their data through the adoption of analytical quality control procedures and quality assurance so that environmental managers can have confidence in decisions in those cases where marine biological data is relied upon (Elliott 1993; Moore & Elliott 1992). This pressure will increase in the future as a result of a change in emphasis in European legislation towards the detection of change

in systems against predefined reference conditions and compliance with ecological quality objectives, for example the Water Framework Directive. Similarly, the increasing desire to combine data from different laboratories, areas and even countries, such as with the UK National Marine Monitoring Plan and the Quality Status reports produced by OSPAR, requires a consistent and valid dataset.

In marine benthic studies, quality control of biological data is at an early stage of development when compared with its freshwater counterparts or with chemical analyses where considerable effort is expended upon quality assurance activities. The UK National Marine Biological Analytical Quality Control (NMBAQC) Scheme aims to address this deficiency and hence the workshop and this report are contributions to the pursuit of quality in marine biological data. Analytical Quality Control and Quality Assurance (AQC/QA) are the definition of methods and approaches which firstly allow the definition of the methods in use and secondly ensure that those methods are as good as possible and that they produced data comparable to the industry standard (Elliott 1993). It has been noted previously that AQC/QA in marine benthic studies can be achieved through standardising methods, both in the field and laboratory, having an agreed taxonomic list and literature, using experts to check identifications, and the use of intercalibration and intercomparison exercises to ensure methods which are cost-effective and fit for purpose (Elliott, 1993, Rees et al 1990).

It is of note that any changes in benthic communities determined during surveys can be the result of a combination of factors and sources of inherent bias and variability. The natural spatial and temporal variability, termed natural *field variability*, will operate at all scales from cm to global and subtidal-cycle to decadal respectively. The detection of this is often the main reason for the survey being carried out. Superimposed on this is the variability due to different methods and equipment being used (the *methodological variability*) and then in turn the variability due to different workers processing the samples (the *analytical variability*). The latter operates both in the field and laboratory. The inherent sources of variability additional to the main questions being answered are termed noise as compared to the signal (e.g. anthropogenic change) being detected. It is axiomatic that in order to detect valid changes and trends in the ecosystem, the noise has to be eliminated or at least quantified and thus the signal maximised. Hence the need for standardised methods and the elimination, or at least quantification, of the worker-introduced variability.

1.2 The Workshop

The following proceedings are an account of the Benthic Techniques Best Practice Review Workshop held during the 17-21st of March 1997 on the Humber Estuary. The workshop primarily focused on the collection and processing of macrobenthic samples from soft sediments and included field and laboratory exercises. A one-day follow up meeting on September 18th was held at the University of Hull to discuss the key results from the workshop.

The committee of the UK National Marine Biology Analytical Quality Control Scheme (NMBAQC) was set up under the auspices of the UK Marine Pollution Monitoring and Management Group in 1994. Up until 1997 the NMBAQC Scheme focussed primarily on the laboratory identification of the macroinvertebrates extracted from sediment residues following field or laboratory processing. It was recognised that the treatment

of field samples may contribute significantly to the quality of samples and may be equally important in determining the quality of the final data produced from macrobenthic sampling.

During the course of its activities, the NMBAQC scheme identified a number of problem areas that required further investigation. In particular the workshop focused on:

- interlaboratory comparisons of field sample processing from soft sediments both in the shallow subtidal and intertidal environment;
- benthic equipment demonstrations;
- laboratory wet weight biomass determination;
- laboratory sub-sampling.

The workshop aimed to address these areas and investigate sources of error by reviewing best practice from as wide a range of UK laboratories as possible with a view to producing further guidance on best practice and providing further foundations for setting standards for all to follow.

1.3 Aims of the Workshop

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During the preparation of the proceedings two additional studies were used to further develop some of the key issues highlighted during workshop. The Industrial Science Centre / Industrial Research and Technology Unit (ISC / IRTU) undertook a further evaluation of the Wilson auto-siever assessed during this workshop and Aquatic Environment Services (AES) undertook a biomass comparison study using data collected as part of a discharge impact assessment. These studies are reviewed alongside the workshop proceedings in the appropriate sections.

Sediment physico-chemical field sampling was not reviewed at the main workshop although it was discussed at the follow up and the summary discussions are presented in the final section of these proceedings.

2. FIELD METHODS

2.1 Subtidal Intercomparison

2.1.1 Introduction

The aim of the intercomparison exercise was to assess the effects of equipment and worker bias introduced into macrobenthic data as a result of shipboard sample collection and processing.

A number of potential sources of error and bias have been identified in the literature associated with macrobenthic sampling and processing. Studies investigating these errors and biases are rare and the significance of these errors and biases has not been demonstrated. The key steps in the process are deployment and recovery of the sampler, transfer of the sample from the sampler to the hopper, container, sieve (depending on processing method used), reduction of the bulk sediment sample to a more manageable form through sieving (to facilitate laboratory sorting and identification of the macroinvertebrates). Each step of the process conceivably has the potential to add significant error or bias into the process. Potential sources of error and bias have been identified as follows:

Gear, Deployment and Recovery

- sampler dimensions;
- sampler weight;
- speed of deployment (leading to "downdraft" effects);
- sampler leakage on recovery (lack of watertight seals);
- removal of sub-samples for physico-chemical analyses (with consequent removal of macroinvertebrates);
- sampling from a drifting vessel as opposed to being anchored.

Sample Transfer

- transfer of sample from sampler to hopper, container or sieve;
- sample residue in the sampler.

Sample Processing

- pre-treatment of the sample;
- elutriation and transfer of the elutriate to the sieve;
- hose pressure both in pre-treatment (to break down cohesive sediment) and reduction of sediment material on the sieve;
- sieve dimensions and mesh size, shape and quality
- sieve cleaning between samples and sites;
- transfer of sample residue to container;
- addition of preservative.

The latter stage of the process is the most likely to lead to the most significant errors and bias both in the macroinvertebrates eventually retained and the quality of the resulting specimens requiring laboratory identification.

An in depth study of each individual step of the process would require resources beyond the scope of the current workshop. Hence, the workshop was confined to making an assessment of the combined effects of all of the above processes on the final results. Each of the participating laboratories (L) were invited to compare the different ways in which each laboratory collected and processed macrobenthic samples in what was commonly accepted as a standard requirement for macrobenthic monitoring i.e. collect and process 5 x 0.1m² macrobenthic grab samples using their own grab and sample processing method. The list of participating laboratories is given in Appendix 1.

Following the outcome of the exercise, the findings would be assessed using a variety of statistical techniques and compared to field observations of techniques. The findings could then be compared to existing guidance with a view to deriving a common standard for shipboard sample collection and processing. In addition, the latter stage of sample processing was timed from the point at which workers began to reduce the bulk sediment sample to the final transfer of the sieved residue to a sample container. All processing techniques being equal the timing would allow some assessment of best practice in terms of efficiency.

2.1.2 Methods

2.1.2.1 Field

The logistics of organising such an intercomparison exercise posed a number of constraints, both physical and in time. Survey vessel time was limited to five days. The use of the survey vessel *Sea Vigil* posed certain constraints in terms of available deck space for the storage of sampling equipment although despite this, 11 laboratories were accommodated during the course of the workshop.

Sample sites were selected from benthic monitoring data previously collected in the lower Humber estuary. Two different sediment regimes were sampled to allow as wide-ranging a comparison as possible. Laboratories were briefed to collect 5 x 0.1m² grab samples in their normal way of sampling using a 0.5mm mesh sieve to process the samples.

Detailed questionnaires were completed on grab and sieve design and dimensions. Processing methods were described in detail and preservation techniques noted and each laboratory was videoed to support the field descriptions. All field observations normally recorded by the participants during routine sampling were recorded such as sample depth and description and in addition, processing times were recorded. At the end of the field exercise participants were given the opportunity to comment on how comparable the exercise was to their normal working practice.

The position of each grab sample was recorded with a positional accuracy of <5m using the onboard Differential Global Positioning System (DGPS). Using Differential GPS, all samples at the two sites were taken +/- 20m of the reference position in order to reduce bias introduced as a result of inherent spatial variability often associated with

macroinvertebrate sampling. The vessel position fixing made use of a gyro-compass which allowed the degree of lay-back to be accounted for when logging positions of the sampler at the stern of the vessel in relation to the GPS antennae.

Sample residues were preserved using the same formaldehyde in seawater solution (4%) and stored at the laboratory to await processing.

2.1.2.2 Laboratory Methods

A single laboratory, the Institute of Estuarine and Coastal Studies (IECS) undertook the laboratory sorting and identification of all 110 samples in order to eliminate worker variability that would otherwise have compounded the field and field-methodological variability. The samples from each laboratory were sieved and sorted by one member of the laboratory's staff to provide consistency. Prior to and after sieving a log was kept of the sedimentary characteristics and approximate percentage composition of each sample.

Prior to taxonomic analysis, each sample was washed through a 20cm diameter, 300cm stainless steel sieve to remove the preservative. The residue in the sieve was gently washed into a white melamine tray and fresh water was added, the contents then gently agitated and the light fraction decanted to another tray. This procedure, depending on the volume of light fraction, was repeated a number of times. The trays containing the light and heavy fractions were examined as a mono-layer under water, both by eye under a fluorescent bench light and 1.5x illuminated magnifier.

To assess the variable condition of the invertebrates removed from samples processed by different laboratories, an index of individual specimen condition was established by IECS. This index utilised a simple grading from 1-5, where 1 was assessed as being of poor quality and indicated fragmentation of even large polychaetes and disintegration of bivalve shells (very fragmented) and 5 being of extremely good condition featuring no visible damage to specimens (unfragmented). The grades in between relate to the degree of fragmentation, although, it must be emphasised that this was a qualitative assessment.

The sample scores from each of the sites (sandy and muddy) were totalled separately. This produced a grade for each site and when the two scores were summed, an overall grade was established for each laboratory *e.g.* Laboratory 11 scored 3.8 for site 1 and 3 for site 2, giving an average overall grade of 3.4.

The sites were assessed separately to allow for the difference in substratum, site 2 being of a much coarser nature. In addition, the major faunal component of site 1 was cirratulids, with the relative ease of fragmentation of this group of animals being well documented. To account for this, the samples as a whole were assessed with equal emphasis placed on other invertebrates present (for example, *Nephtys sp., Macoma, Spiophanes* etc.).

The fauna derived from the sample was stored by taxonomic group in appropriately labelled containers containing a solution of 70% Ethanol in freshwater, ensuring that the sample pot was labelled with its own specific code number (*e.*g.

HUMB/W/SH/Lab2.1.1). Each tray was re-checked by another member of IECS staff for any remaining organisms.

Where the sample contained a high degree of organic material or large abundance of invertebrates, sub-sampling was carried out. As no protocol for the separation of the sample for sub-sampling purposes was originally envisaged it was agreed after consultation with the workshop organisers that the laboratory's standard methodology should be employed. This involved the sectioning of the white melamine trays into 8 number 10.5cm x 7.5cm squares (78.75cm^2), each approximately 12.5% of the total sample. The sample material was placed into the white tray ensuring an equal coverage, a square(s) was randomly selected and all the material was removed from within the squares and placed into another white tray containing freshwater. The procedure described above for non sub-sampled samples was then carried out.

The remaining, non sub-sampled material was investigated and all large invertebrates (for example, *Nephtys, Nereis, Macoma* etc.*)* were removed and enumerated within the sample as a whole. The residue material from the sub-sampling procedure was replaced in the original sample container and the percentage volume of material retained marked clearly on the container. The material from the sub-sample was placed into a separate container and the percentage volume of the total sample marked on the container.

Identification of the specimens was undertaken by one member of IECS staff to ensure consistency throughout the identification process. An Olympus SZ30 zoom microscope with 10x and 20x eyepieces was used, giving maximum magnification of up to 80x. An additional 2x objective was occasionally used to increase the potential magnification to 160x. Compound microscopes were used for further magnification, up to 1000x, to identify and enumerate oligochaetes. During identification, all individuals were initially separated into families, with part animals being assigned to families where possible. The macrofaunal animals were then identified to species level using standard taxonomic keys, low and high power stereoscopic microscopes and dissection, when necessary, for identification.

Certain species were grouped together (*e.g. Aphelochaeta/Tharyx spp.)* due to the high abundance levels found and time constraints of the analysis period. Some species names given in text are not as recorded in the MCS Marine Species Directory (*e.g. Ampharate acutifrons)* but are as recommended by the external taxonomic auditor. All fauna has been retained by IECS under the standard codes, (*e.g.* HUMB/W/SH/ Lab2.1.1) and stored in the laboratory. A reference collection was also compiled. The taxonomic literature used for identification is essentially that as given in Rees *et al* (1990).

The laboratory procedures are based on those employed by the FRPB (SEPA East). Quality control procedures follow Rees *et al* (1990 and 1993) and Elliott (1994). All procedures are based on standard best laboratory practice. These practises include an independent check-sorting of all samples, regular cross checking of identified organisms and the external verification of taxonomic identification. In addition, a specimen collection is maintained and up to date taxonomic literature is stored in the laboratory and all staff involved in the sorting and identification are experienced marine or aquatic biologists. The IECS laboratory is a member of the National Marine Biological

Analytical Quality Control Scheme which entails a regular external auditing of test samples.

In addition to the above general practices, 10% of the samples were chosen randomly by the Environment Agency, North East Region and submitted for external audit and AQC by Dr Peter Garwood (Identichaete Limited). Little or no action was required as a result of this external audit.

Statistical Methods

Following laboratory analyses the final quantitative dataset was subjected to a number of statistical analyses to explore the differences between the laboratories. The biological primary community parameters species richness (S) and total abundance (A), and the secondary or derived variables Shannon Weiner diversity (H') and Pielou's evenness (J) were calculated for each replicate and for each laboratory (replicates combined) for both stations. Differences between laboratories for these parameters were carried out using ANOVA and the non-parametric Kruskal Wallis test. It is important to note that PRIMER, the statistical package used to generate the univariate statistics, defaults to Log Base e for univariate measures. Log Base 2 should be used and was used in this study (Rees et al, 1990)

Multivariate analysis was carried out using the PRIMER package (PML, 1994). Cluster analysis based on the Bray Curtis similarity coefficient, using group averaging after 4^{th} root transformation of the data, was carried out on the replicate and summed data. The routine ANOSIM was used to assess differences between laboratories.

Comparison of the macroinvertebrate data to environmental parameters was confined to the sediment particle size (PS). The ordination technique Multi-Dimensional Scaling (MDS) was undertaken for the macroinvertebrate replicate, summed and PS samples. The sedimentary parameters were superimposed onto the MDS plots of the PS samples and the BIO-ENV procedure of PRIMER was used to identify any correlations between the macroinvertebrate data and the environmental data.

2.1.3 Results

2.1.3.1 Field

The location of the sampling sites are shown in Figure 1 and the positions of the replicates at each site are given in Figures 10 and 15 later in the proceedings. Notes taken for each sample including position and a description of the sediment are given in Appendix 2 and notes on the sampling methods used by each laboratory are given in Table 1. For both sites the position of the replicates are in an area of 50m x 50m.

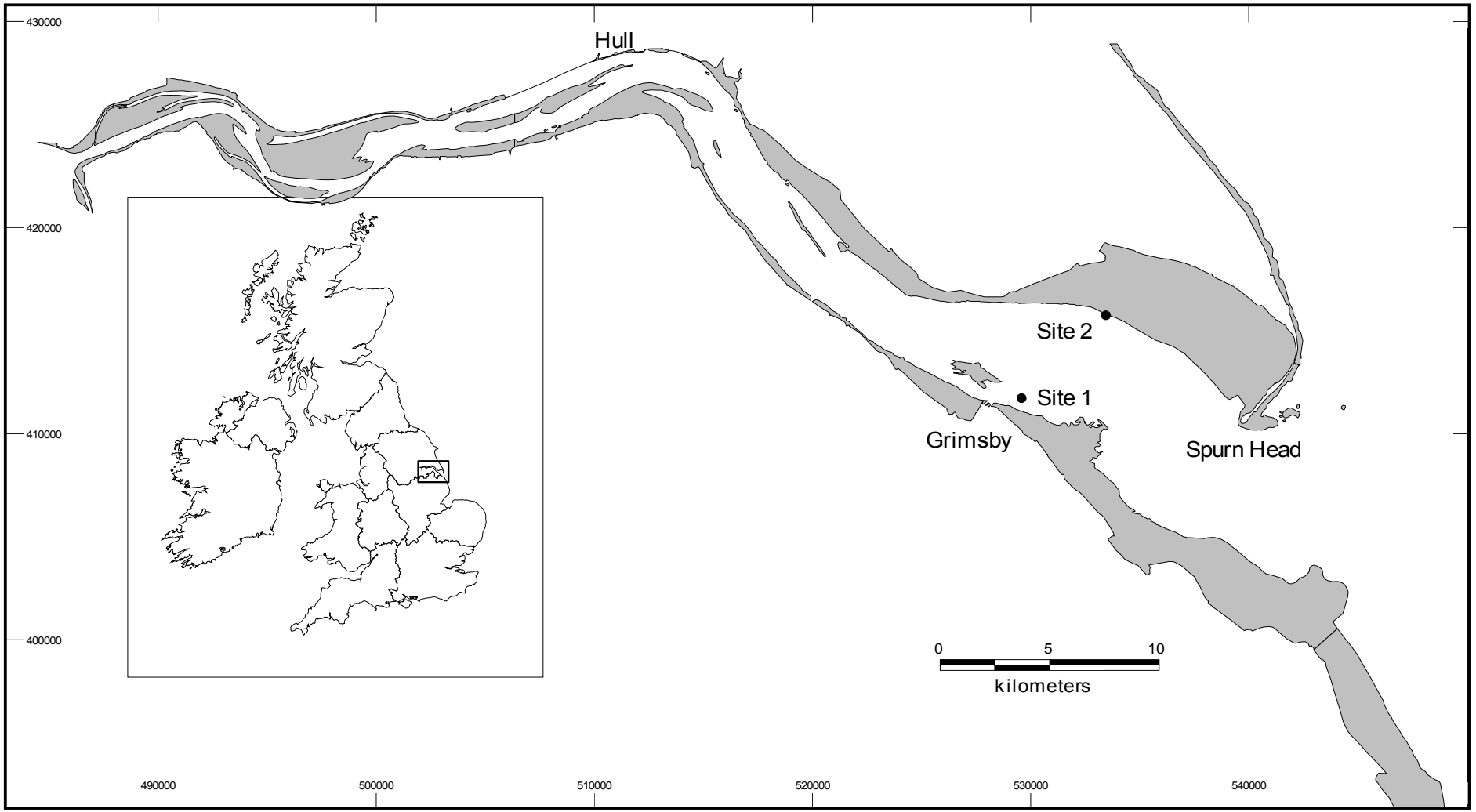


Figure 1: Map showing site positions in the Humber Estuary.

Table 1:

	Lab	1	2	3	4	5	6	7	8	9	10	11
Grab	Name	Day	Day	Day	Van Day	Day	Van Veen	Day *1	Day *1	Day	Van Day *2	Day *3
	Design	traditional with pressure plates	traditional with pressure plates	traditional with pressure plates	Catch trigger no pressure plates	traditional with pressure plates	long and short arm			traditional with pressure plates	Catch trigger no pressure plates	
	Manufacturer	In-house and local	??	??	Spartel	Ocean Scientific	Duncan Associates			somewhere in Wales	Spartel	
	Sampling area	1159	952	1062	1001	982	1024			1040	1001	
Dimensions	Depth	21.8	15.5	16.5	15.5	#	#	-	-	18.5	15.5	-
	Width at mid-point	16.5	14	14	13.5	#	#	-	-	14.2	13.5	-
Sample cut-off	Mud	Depends- not usually a problem	Half full	5	5	5	4 litres	??		5	5	-
	Sand	Approx. 7cms	One third full	5	5	5	4 litres	??		5	5	-
Weight in use	(kg)	??	Approx. 200	Approx. 200	140	#	25-70	-	-	Approx. 200	Approx. 200 (depends on sed type)	-
Sieves	Mesh size	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	1mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm
	Mesh type	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS
	Shape	Round	Square	Rectangular	Round	Round	Round	Round	Round	Square	Round	Round
	Dimensions	45	70x7	??	45	30	Cylinder 30	45	45	50x50	45	45
	Manufacturer	Endecott	Local	Local	Endecott	Endecott	Norwegian Aqua Plan	Endecott	Endecott	In-house	Endecott	Endecott
Processing sequence	G/H/S							♦			♦	Washing machine
	G/S		♦									
	G/Sr/H/S	♦				♦	♦					
	G/Sr/S											
	G/H/C/S				♦				♦	♦		
	G/C/S			♦								
Processing method	Hose on sieve	No	Deck hose	Deck hose	No	??	Some deck hose	Small hose	No	No	No	No
	Pre-treatment		No	Yes in coffin	No	??	No	Break up by hand	No	No	No	Washing machine
	Coarse elut.			♦		??						
	Med. elut.	♦				??	♦				♦	
	Gentle elut.				♦	??			♦			
Transfer to pot	Back wash			♦		??			♦		♦	♦
	Front wash	♦	♦	♦	♦	??		♦		♦		
Preservative	Onboard	♦	♦	♦	♦	♦	♦			♦	♦	♦
	<24hrs											
	<48hrs											
Formaldehyde	Conc. formal.	10%	40%	10%	10%	10%	40%	40%		10%	20%	10%
	In pot		??1:1	1:2	Approx. 4%	Approx. 7-10%	Approx. 4%	4-6%		Appprox. 4%	Approx. 10%	Approx. 4%
	Stain	None	RB	RB	Yes	RB	RB	??		RB	None	
	Buffer			Borax	Calcium carbonate	Sodium acetate	Borax	??		??	Manufacturer buffered	

KEY 1* used lab 1 grab for both sites
2* used own grab for Site 1, lab 1 grab for Site 2
3* used autosiever, lab 10 grab for Site 1, lab 1 grab for Site 2
standard round 45cm, 0.5mm Endecott sieve
?? Not recorded
SS stainless steel

Table 1 Participant *Laboratory Ship Procedures*

The replicates taken by each laboratory are generally not clustered together in discrete groups but are relatively well spread across the sampling area.

Grabs

Holme & McIntyre (1984) indicates the types of grab and core samplers and the differences between them. In the workshop, a total of 8 different grab samplers were used ranging from the traditionally designed 0.1m² Day grab (see Figure 2 below) through to what was described at the workshop as a van Day. The van Day was essentially a short-armed van Veen grab in a Day grab frame. Laboratory 6 used a 0.1m² van Veen sampler. There were at least 5 different manufacturers of the grabs used in the workshop. The majority of the grabs had some facility to add additional weights if required in more consolidated sediments. The range of depth of the samplers was 15.5 – 21.8cm with all but one of the grabs at the lower end of this range. The sampling area varied considerably considering the stated sampling area of 0.1m². The sampling area ranged from 952cm² to 1159cm², a difference of 20.7%.

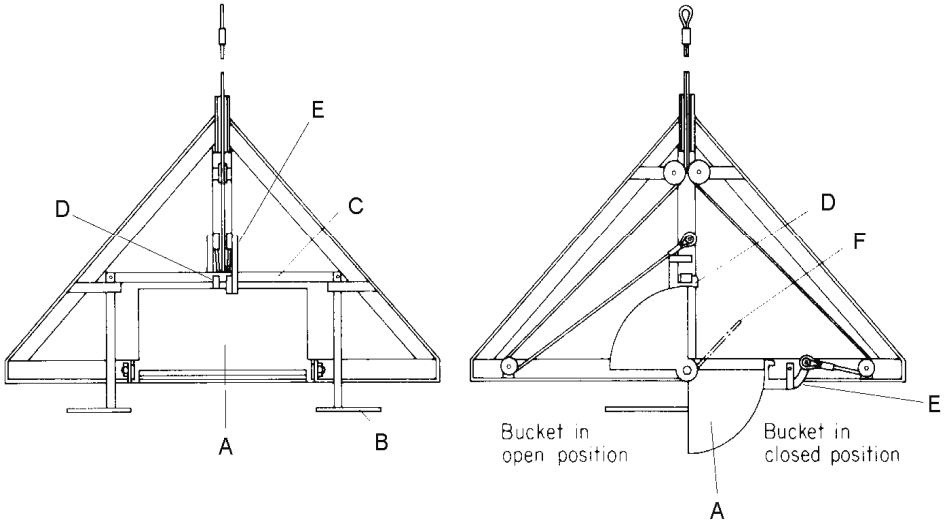


Figure 2: Schematic of the Day grab used during the workshop (after Holme and McIntyre, 1984)

L7, 8 and 11 all used the L1 grab and L10 used this grab at site 2 due to a failure of the L10 sampler at site 2. The grab associated with L1 was that which belonged to the survey vessel and was therefore more freely available during the workshop.

In general the grabs had the facility to add up to 200kg. In practice this was not required at the two sites although in some cases the depth of penetration at site 2 may have benefited from additional weight.

There was a large variation in shipboard procedures (see Table 1) with the sample being transferred from the grab either to a hopper or directly to the sieve.

Sediment Descriptors

Each laboratory was asked to describe the nature of the sediment in each grab sample. The descriptions are recorded in Appendix 2. It is clear that even at Site 1, which was relatively homogeneous in nature, there is no consistency in sediment descriptors. At Site 1 these ranged from silty mud to sandy mud and a variety of other descriptors in between. Descriptions were made more variable at Site 2 by the presence of a thin fluid layer of mud on the surface of silty sand, which also contained shell fragments.

Processing method and sequence

As well as a range of grabs, a variety of hoppers and containers were utilised for sample processing. Processing ranged from emptying the contents of the grab sampler onto the sieve directly, and then using the deck hose to wash the sample on the sieve, through to the use of a variety of containers and hoppers to gradually breakdown the sample using either the deck hose, small low pressure hose or by hand. For those using containers and hoppers, elutriate was generally decanted either directly into a sieve or down a chute to a sieve. A new automated method of sample processing (Wilson Auto-siever) was also tested by L11, which involved no worker processing and relied solely on under washing of the sieve using a spray connected to the deck hose. With this method the sample was emptied directly onto the sieve (see Figure 3 below for the principal of the method).

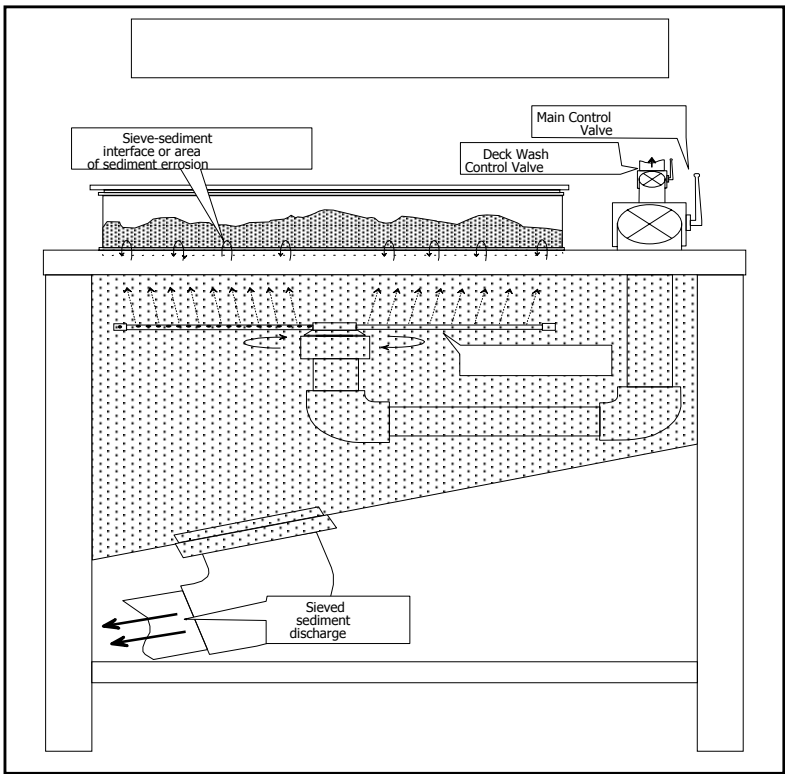


Figure 3: Schematic of the principle of operation of the Wilson auto-siever

In some cases the grab was not rinsed prior to deployment although all laboratories indicated that this would occur between stations.

Sample Processing time

The time taken by each laboratory to process the samples was measured and is presented in Figure 4 below. L6 was not included since L6 used a 1mm sieve as opposed to the 0.5mm used by the other participants.

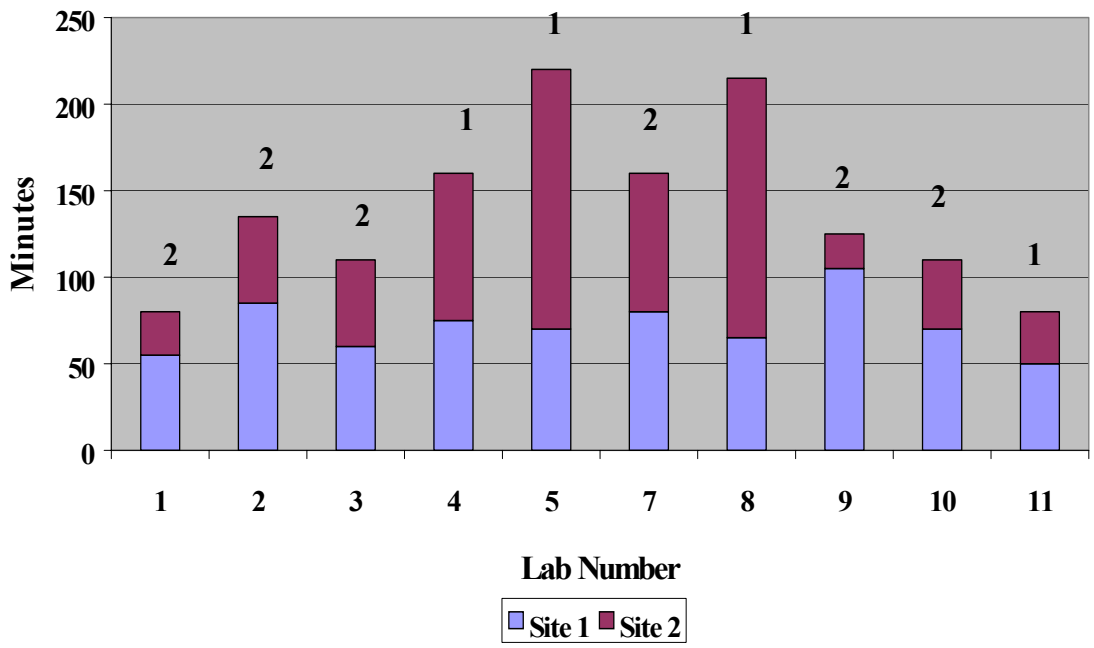


Figure 4: Mean sample processing times

Note: The number of workers involved in processing the sample is denoted above each column on the bar chart.

Figure 4 shows that site 1 took longer on average to process than site 2 for most laboratories. This is consistent with the consolidated nature of the mud substratum encountered at site 1, which had a high silt/clay content. Site 2 was much sandier and was relatively easier to process.

The time taken for processing varied significantly between the laboratories. L1 and L11 processed the samples in the least time. It was notable that L1 workers had experience of the sites being sampled prior to the workshop and L11 used only 1 worker but was able to process the sample in the same amount of time as L1.

Sieves

Sieves ranged in size from 45cm diameter round sieves to larger box sieves such as that used by laboratory 2 (70x7cm). All meshes were stainless steel square mesh with the exception of L6, who used a 1mm punched, round holed, stainless steel sieve. The

sieves that were manufactured by Endecott Ltd. were reportedly manufactured to BS510 standard.

Residue transfer

This was undertaken via upper or lower washing of the sample residue into either the corner or an edge of the sieve, and either a container of water, bottle wash or the low-pressure hose was used to flush the sample into the container. Laboratory 10 made use of a smaller sieve to decant off some of the overlying water in the sample container. This reduced the volume of water retained and hence the amount of preservative required.

In general, sieve cleaning between samples was not undertaken routinely by any of the laboratories although sieves were usually inspected for enmeshed macroinvertebrates following transfer of the sieve residue to a sample container. Only 2 of the 11 laboratories participating cleaned the their sieve between sites. When questioned after the exercise all laboratories indicated that sieve cleaning between sites was routine practice.

Laboratory

The quantitative species matrix derived from the laboratory analyses is presented in Appendix 3 along with the primary and derived statistics of number species (S), individuals (I) and diversity H' (Shannon Weiner) and E (Evenness) indices.

2.1.3.2 Laboratory Condition Index

Table 3 shows the individual specimen condition index for each replicate sample. The majority of laboratories scored higher grades for site 1 than site 2 and the highest overall mean score of 3.4 was achieved by L11 using the Wilson Auto-siever and also L5.

Table 2: Condition index scores for sites 1 and 2

lab	1a	1b	1c	1d	1e	Mean	2a	2b	2c	2d	2e	Mean	Overall mean
1	1	3	1	2	2	1.8	3	3	3	2	3	2.8	2.3
2	4	4	4	3	4	3.8	3	1	2	2	2	2	2.9
3	4	3	3	3.5	4	3.5	3	3	3	3	3	3	3.25
4	3	3	3	4	4	3.4	3	3	3	3	3	3	3.2
5	4	4	4	4	3	3.8	3	3	3	3	3	3	3.4
6	4	3	3	3	3	3.2	3	3	3	4	3	3.2	3.2
7	3	3	4	4	2	3.2	3	3	2	2	3	2.6	2.9
8	5	4	3	3	5	4	3	3	2	2	3	2.6	3.3
9	3	2	2	3	3	2.6	3	3	3	3	3	3	2.8
10	2	3	3	4	4	3.2	2	2	2	3	2	2.2	2.7
11	4	4	4	3	4	3.8	3	3	3	3	3	3	3.4

The mean index scores for each site are illustrated in Figure 5a and b and the results after analysis by ANOVA in Tables 3a and b. The ANOVA show that whilst there was a significant difference between the index scores at both sites many of the laboratories show no significant difference. The results of Tukey's multiple comparison test showed that at site 1 only L1 had a significantly lower score than L2, 3, 4, 5 and 8. At site 2 L2 had a significantly lower condition index than L3, 4, 5, 6 and 9. However, this was due in part to several of these laboratories having identical index scores for each replicate. Generally most of the laboratories showed no significant difference between each other.

Table 3a: Results of ANOVA for condition index at site 1

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SITE1	Between Groups	20.300	10	2.030	4.701	.000
	Within Groups	19.000	44	.432		
	Total	39.300	54			

Table 3b: Results of ANOVA for condition index at site 2

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SITE2	Between Groups	7.127	10	.713	4.612	.000
	Within Groups	6.800	44	.155		
	Total	13.927	54			

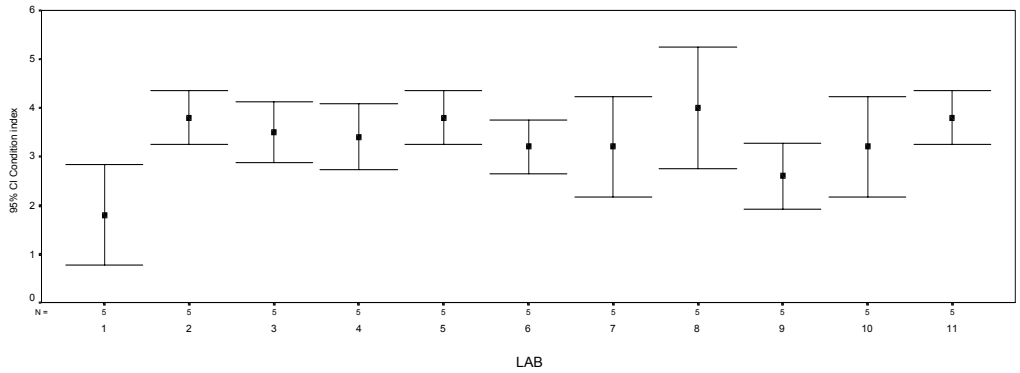


Figure 5a: Means and 95% confidence limits for condition index scores at site 1

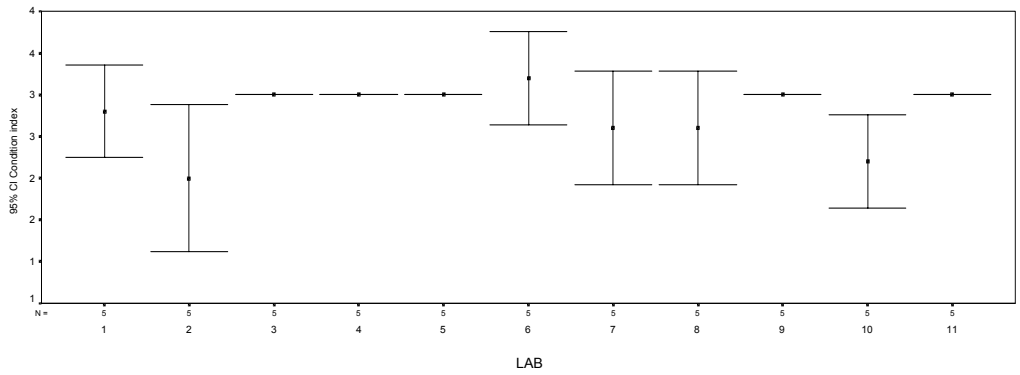


Figure 5b: Means and 95% confidence limits for condition index scores at site 2

2.1.3.3 Particle Size

Table 4 shows the results of particle size analysis from the samples at each station. Station one had a fairly uniform substratum comprised of sandy silt with silt levels ranging from 72.46% to 87.21% and median grain sizes ranging from 10.85 µm to 16.34 µm. Station two had a coarser more varied substratum range from sandy silts to gravelly silty sands with median grain sizes ranging from 12.11 µm to 191.73 µm.

Table 4a: Sedimentary Parameters for Site 1

Lab	SD	Skew	% Gravel	% Sand	% Silt	Median grain size	Mean grain size
1	1.66	-0.4	0	14.9	85.1	12.3	12.7
2	1.5	-0.4	0	13.6	86.4	11.8	12.1
3	1.6	-0.2	0	17.7	82.3	14.0	13.8
4	1.6	-0.3	0	17.5	82.5	13.1	13.4
5	1.9	-0.3	0	27.5	72.5	17.7	18.3
6	1.8	-0.4	0	24.3	75.8	15.2	16.3
7	1.6	-0.4	0	18.0	82.0	13.3	13.9
8	1.7	-0.4	0	20.0	80.1	13.6	14.4
9	1.7	-0.3	0	23.0	77.0	15.4	15.9
10	1.5	-0.4	0	12.8	87.2	10.9	11.5
11	1.7	-0.3	0	22.0	78.0	15.3	15.7

Table 4b: Sedimentary Parameters for Site 2

Lab	SD	Skew	% Gravel	% Sand	% Silt	Median grain size	Mean grain size
1	1.8	-0.7	0	19.2	80.8	12.1	14.0
2	1.9	-0.6	0	23.4	76.6	13.8	16.3
3	2.6	-0.3	3.2	47.9	48.9	50.2	41.7
4	2.2	-0.7	0.8	28.7	70.5	16.1	20.7
5	2.4	0.2	7.1	72.2	20.7	166.2	119.1
6	1.8	-0.7	0	19.2	80.8	12.6	14.5
7	1.7	-0.7	0	16.2	83.8	11.9	13.1
8	3.3	0.0	20.8	47.4	31.8	191.7	152.3
9	2.9	0.0	7.7	56.2	36.1	158.8	85.6
10	2.8	-0.5	4.5	39.5	56.0	28.1	39.7
11	2.1	-1.3	0	20.6	79.4	13.2	16.4

2.1.3.4 Statistical Analyses of the Dataset

The biological parameters for each replicate and each laboratory are presented in Appendix 3.

Table 5a and b show the summed replicate data and indicate the degree of variation between laboratories.

At Site 1 species richness ranged from 24 (L9) to 30 (L7 and 8), abundance ranged from 950 (L6) to 8690 (L7). Diversity ranged from 0.876 (L1) to 1.5 (L6) and evenness ranged from 0.2 (L1) to 0.3 (L6). The most noticeable difference between laboratories was levels of abundance with L6 having a very low abundance although this was the only laboratory to use a 1mm sieve.

Table 5a: Summed replicate univariate statistics for Site 1

Lab	S	A	H'	J
1	26	5280	0.9	0.2
2	25	2860	1.1	0.2
3	26	5200	0.9	0.2
4	25	6050	0.9	0.2
5	28	6770	1.2	0.2
6	29	950	1.5	0.3
7	30	8690	0.8	0.2
8	30	6640	1.3	0.3
9	24	2530	1.2	0.3
10	29	6970	1.3	0.3
11	34	2850	1.3	0.2

At Site 2 there were also some notable differences between laboratories but the pattern was different to Site 1. Species richness ranged from 22 (L11 and 6) to 29 (L10) whilst abundance ranges from 369 (L11) to 1500 (L9). Diversity ranged from 1.9 (L6) to 3.1 (L11) and evenness ranged from 0.4 (L7) to 0.7 (L11). At this station the mesh size of L6 appeared to be less important.

Table 5b: Summed replicate univariate statistics for Site 1

Laboratory	S	A	H'	J
1	28	1130	2.9	0.6
2	27	1070	2.6	0.5
3	26	709	2.5	0.5
4	26	766	3.0	0.6
5	24	604	3.0	0.7
6	22	673	1.9	0.4
7	28	1480	2.0	0.4
8	20	760	2.5	0.6
9	27	1500	1.9	0.4
10	29	1230	3.0	0.6
11	22	369	3.1	0.7

The means of the biologically parameters are shown in Figure 6a-d (Site 1) and Figure 7a-d (Site 2) along with the 95% confidence intervals. In order to test statistically the difference between the laboratories, analysis of variance and its non-parametric counterpart the Kruskall-Wallis test (used when the Levene test revealed a significant difference in variance) were used to test for differences in the means. This was followed by the Tukey LSD (least significant difference) and Tamhane multiple comparison tests to indicate which laboratories data were responsible for the significant difference. The results are given in Tables 7a-c and 8a-c.

Figure 6a-d: Means for biological parameters with 95% confidence limits for laboratories at Site 1:

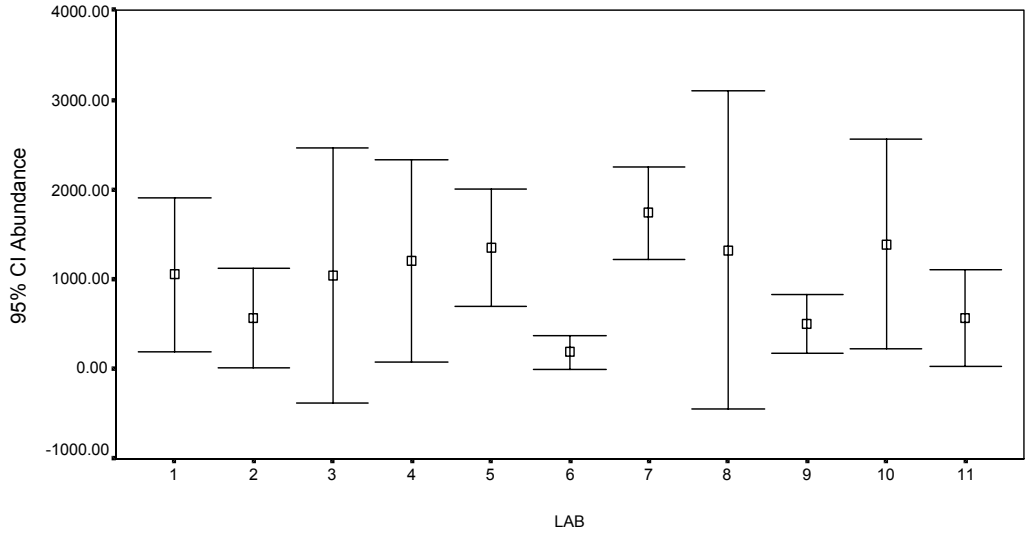


Figure 6a:

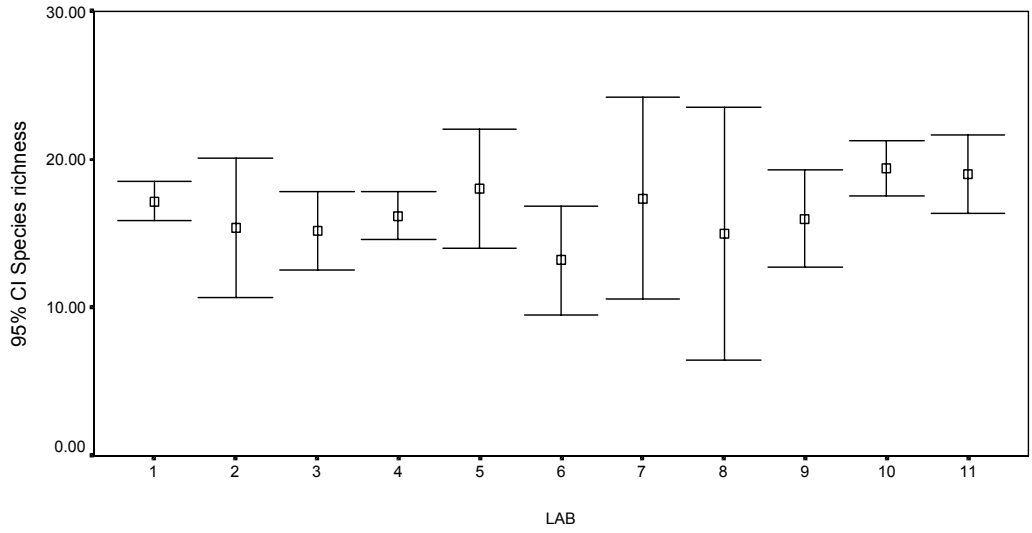


Figure 6b:

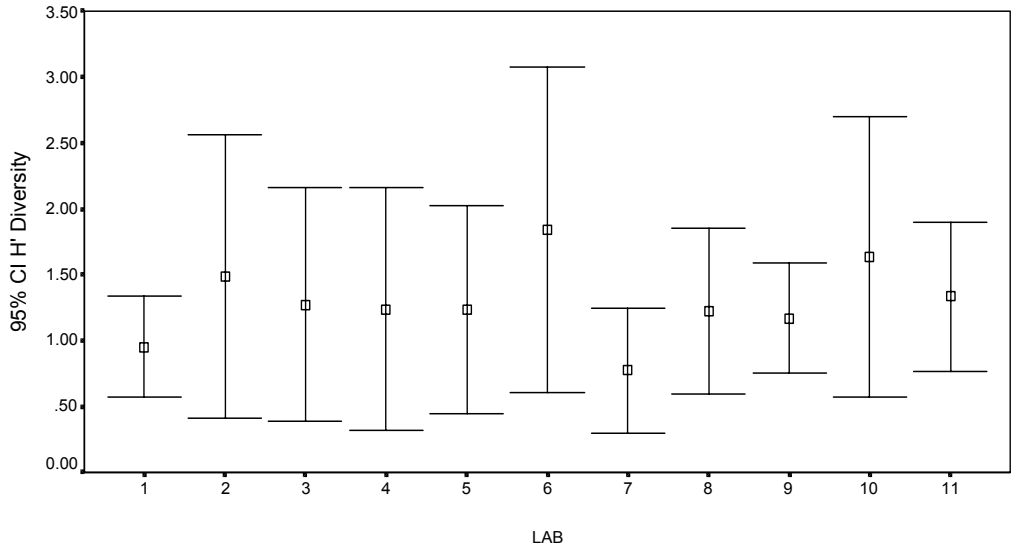


Figure 6c:

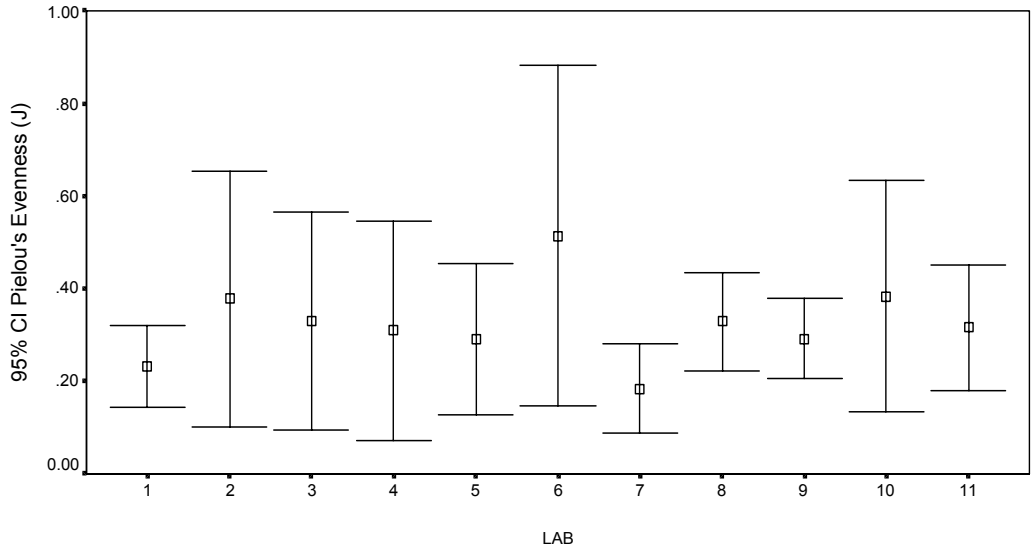


Figure 6d:

Table 6a-c: Results of ANOVA and Kruskall Wallis tests for Station 1:

Table 6a:

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
A	4.108	10	44	.000
H	1.631	10	44	.129
J	3.470	10	44	.002
S	2.812	10	44	.009

Table 6b:

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
H	Between Groups	4.340	10	.434	1.006	.453
	Within Groups	18.976	44	.431		
	Total	23.316	54			

Table 6c:

Test Statistics [a,b]

	A	J	S
Chi-Square	18.930	10.401	17.938
df	10	10	10
Asymp. Sig.	.041	.406	.056

a. Kruskal Wallis Test

b. Grouping Variable: LAB

At station 1, significant differences between laboratories at the 95% level were only found with abundance. The Tamhane multiple comparison test showed that there were differences between L6 and 7 and L7 and 9.

Figure 7a-d: Means for biological parameters with 95% confidence limits for laboratories at Site 2:

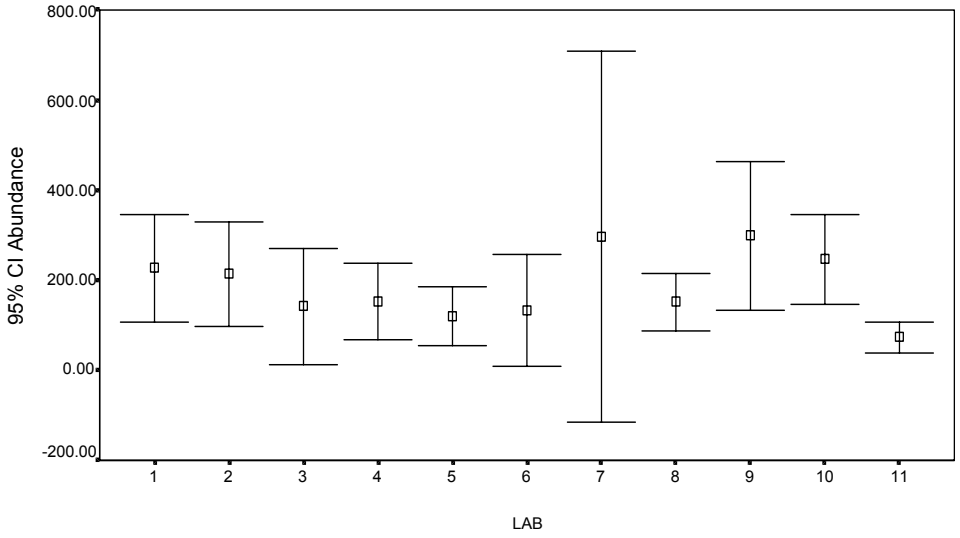


Figure 7a:

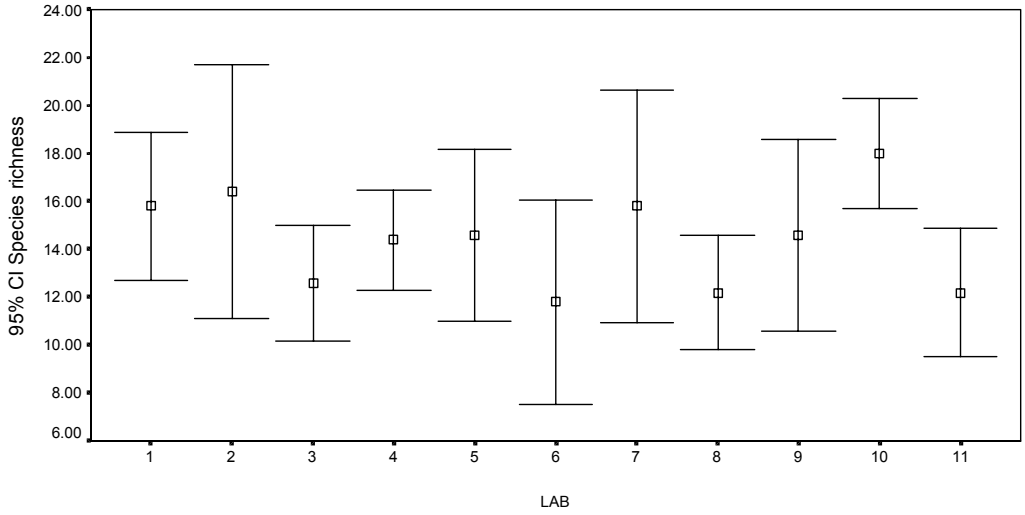


Figure 7b:

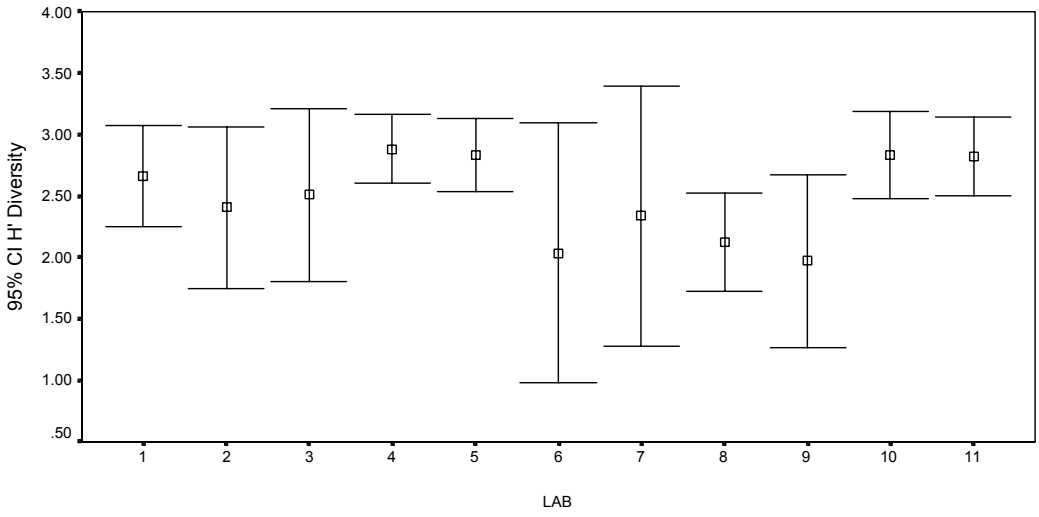


Figure 7c:

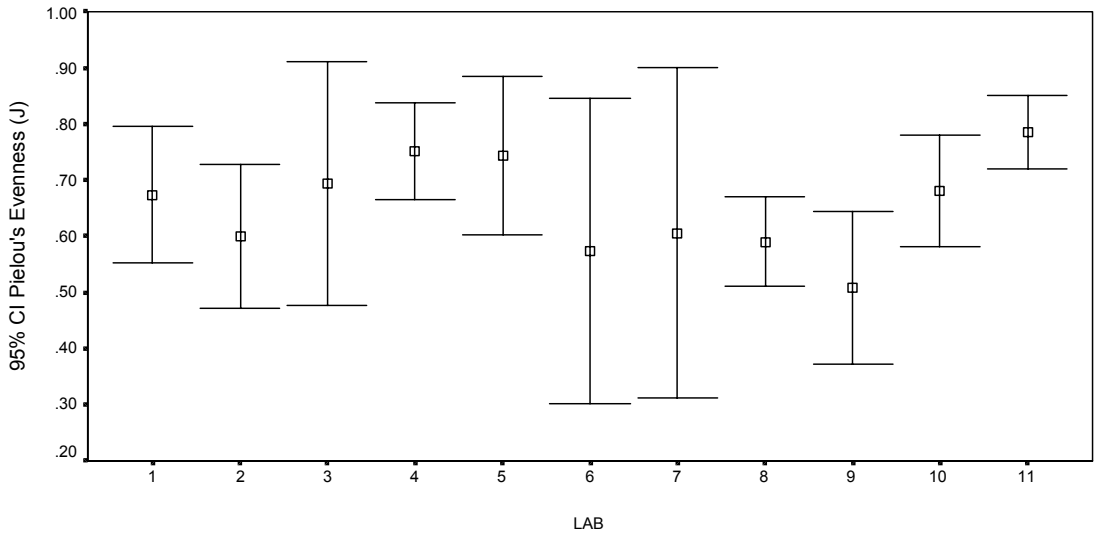


Figure 7d:

Table 7a-c: Results of ANOVA and Kruskal Wallis tests for Station 2.

Table 7a:

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
A	3.382	10	44	.002
H	2.389	10	44	.023
J	1.942	10	44	.065
S	1.512	10	44	.167

Table 7b:

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
J	Between Groups	.373	10	3.731E-02	2.080	.047
	Within Groups	.789	44	1.794E-02		
	Total	1.163	54			
S	Between Groups	203.200	10	20.320	2.526	.017
	Within Groups	354.000	44	8.045		
	Total	557.200	54			

Table 7c:

Test Statistics [a,b]

	A	H
Chi-Square	19.736	17.315
df	10	10
Asymp. Sig.	.032	.068

a. Kruskal Wallis Test

b. Grouping Variable: LAB

At Site 2 the parameters abundance and species richness showed significant differences and evenness was just significant. For species richness Tukeys test identified L6 and 10 as different and Figure 7b also shows that L8 and 10 and L10 and 11 are also significantly different. For abundance, the Tamhane test identified no laboratories as different although Figure 7a shows possible differences between L9 and 11 and L10 and 11.

It appears that whilst the parameters do differ somewhat between laboratories these differences are often fairly small and only rarely differ significantly, only L6, 7, 9, 10 and 11 appear to show any statistically significant differences.

Multivariate Analyses

Site 1

The results of cluster and MDS analysis on the replicate data are shown in Figure 8 and 9 respectively. The results of MDS agree well with the cluster analysis although the stress level is relatively high. Two outliers, L8e and L6e have been removed to improve the appearance of the ordination. These two samples were somewhat different from the others in containing very few animals.

With the exception of L8e and L6e, the remaining samples displayed similarities of between 55% to 85%. The majority of the samples exhibited similarities of over 75%.

Figure 8. Results of cluster analysis for station 1.

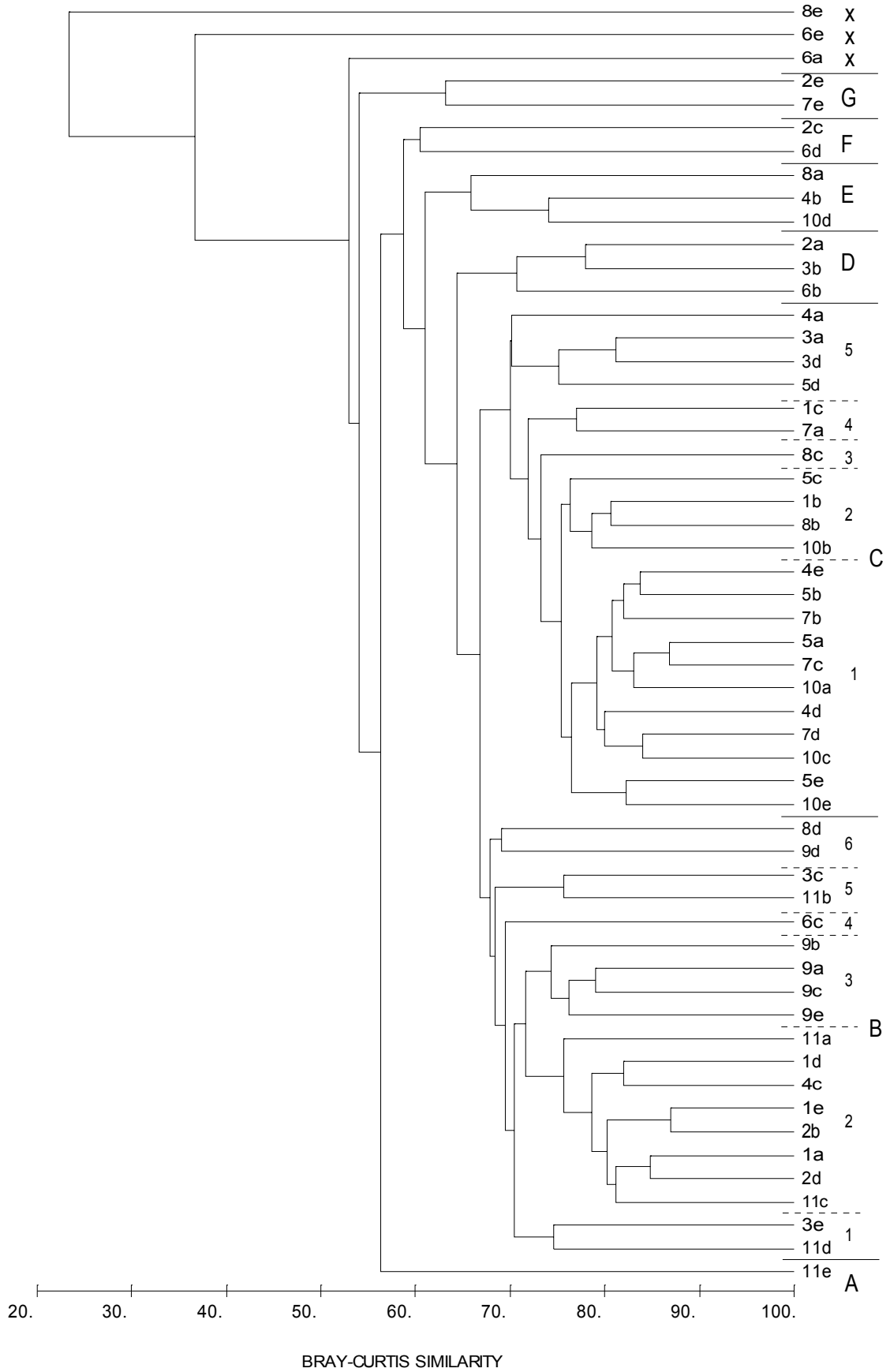
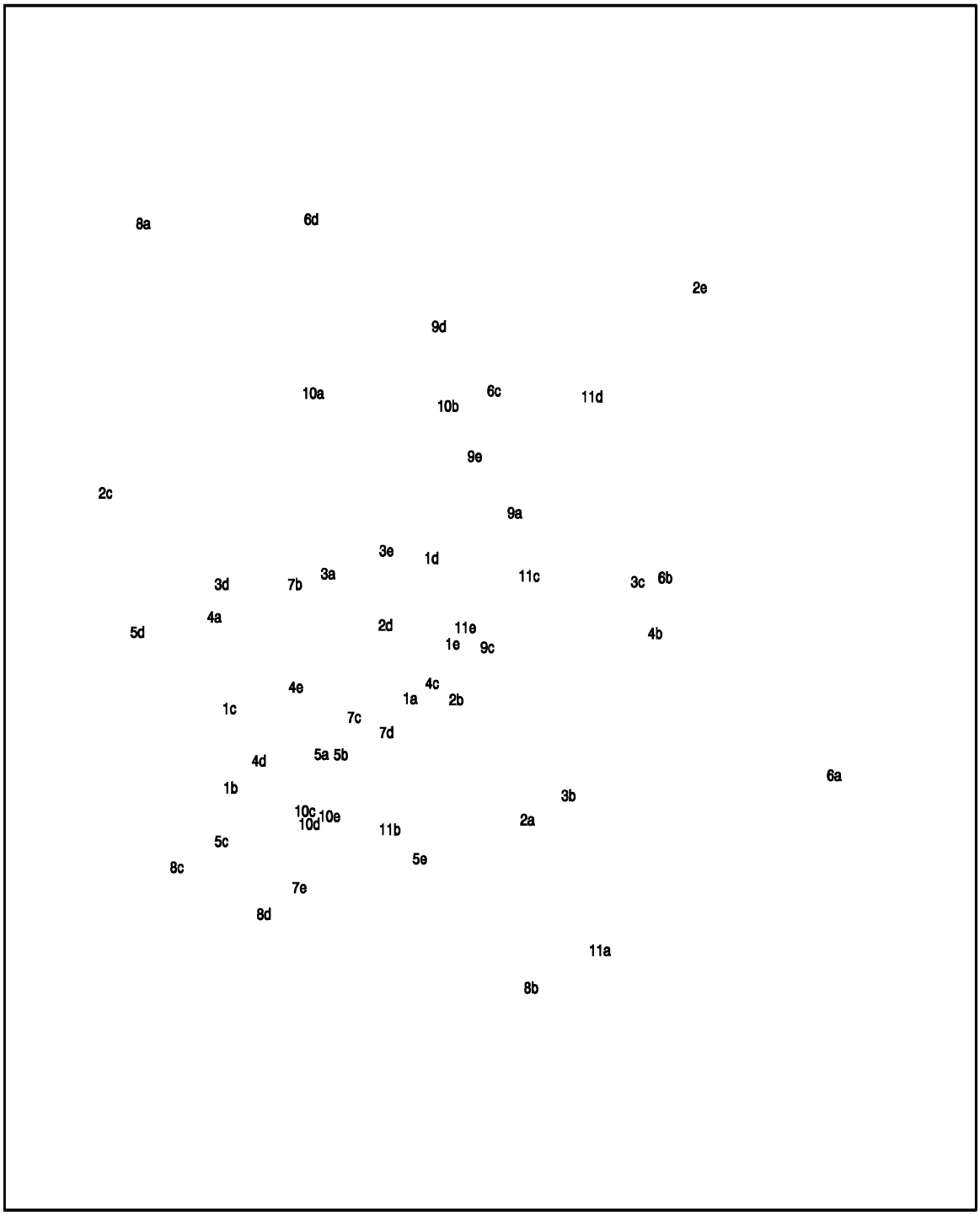


Figure 9. MDS results Station 1 (edited). Stress = .24



There were no clear groupings in the replicates according to laboratory, but samples have been divided into six groups, two of which have been subdivided further, leaving four samples on their own. Several of the replicates from L6 were amongst those that exhibited the lowest similarities to the other samples.

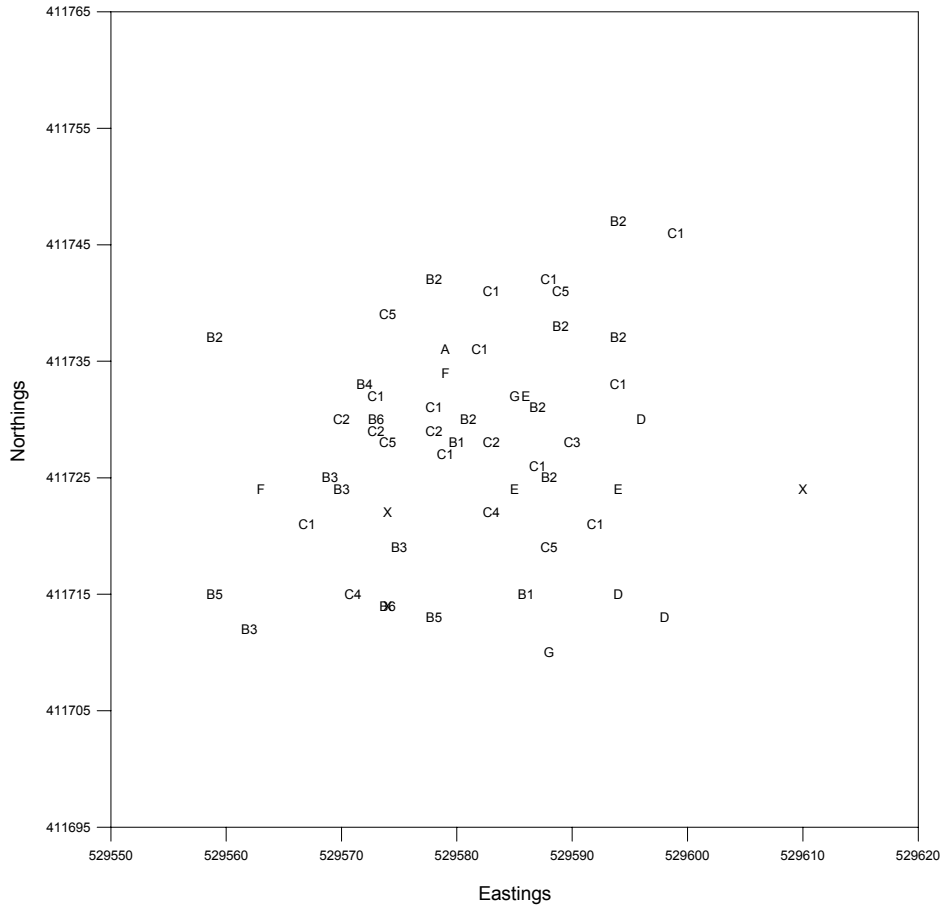
The groupings show that, whilst the replicates from some laboratories are similar (for example L9), the variation between the replicates taken by the same laboratory was relatively high relative to the other laboratories.

This may be explained by the sample position at the site. In order to see whether the groupings were due purely to sample position the cluster groups were superimposed onto the position of each replicate in Figure 10. This figure shows that there was no clear correlation between sample location and the group to which it belongs. Given the positioning of the replicates this is not surprising as a replicate from one laboratory was often closer to a replicate from another laboratory than to the remaining replicates from its own laboratory (see Figure 10).

Summed data cluster analysis on the data from Site 1 (Figure 11) showed that laboratories displayed similarities of over 70%. The top five dominant species and the biological parameters for each laboratory have also been displayed on the plot. It is apparent that there are large-scale differences between the laboratories. L6 is somewhat separate from the rest and this was the only laboratory to use 1mm mesh sieve. The lower abundances are therefore accounted for by this difference in methodology. L11 and 9 were also slightly separate although it is obvious that these differences were fairly small, as the dominant species in each case were very similar with dominance by *Aphelochaeta/Tharyx* spp. and *Pygospio elegans*. This is to be expected considering that each laboratory in theory sampled the same population. The MDS plot (Figure 12) also confirms these patterns.

The results of the ANOSIM test for Site 1 are presented alongside the field observations in Table 8a. ANOSIM has revealed significant differences between the laboratories, particularly L9, 10 and 11. These differences were explored further using the program SIMPER. SIMPER highlights the dominant species contributing to the differences between laboratories in terms of the Bray Curtis similarity index. The results for SIMPER are shown in Appendix 4. The majority of differences can be accounted for by differences in numbers of the small polychaetes and oligochaetes, particularly *Aphelochaeta/Tharyx* spp. With L9 and11 recording relatively lower abundances compared to the other laboratories such as L1, 3 and 5. No clear trend occurs between the methods used and those laboratories that are significantly different.

Figure 10. Station 1 sample groups.



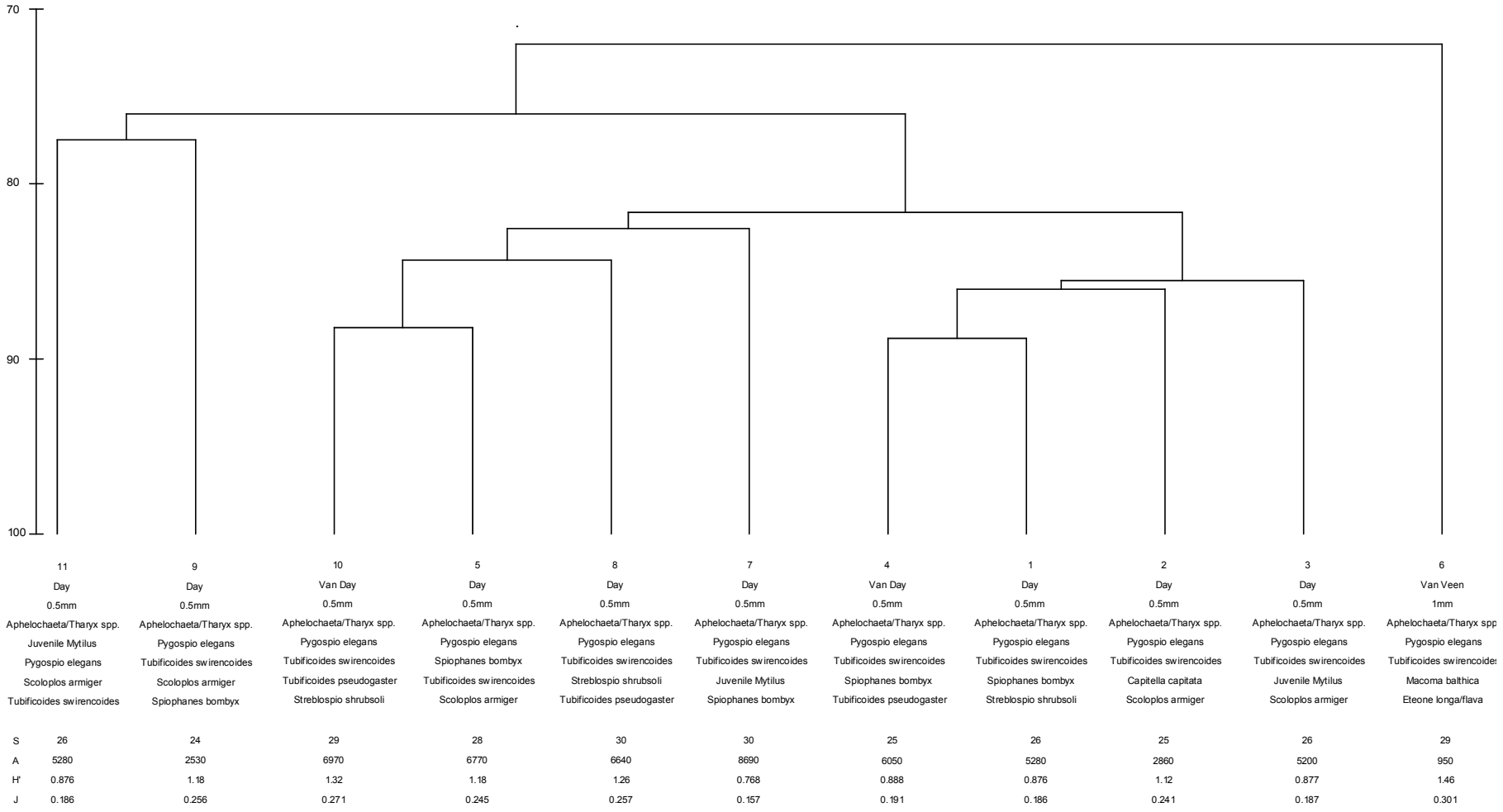


Figure 11: Results of Cluster Analysis for Station 1 (Summed)

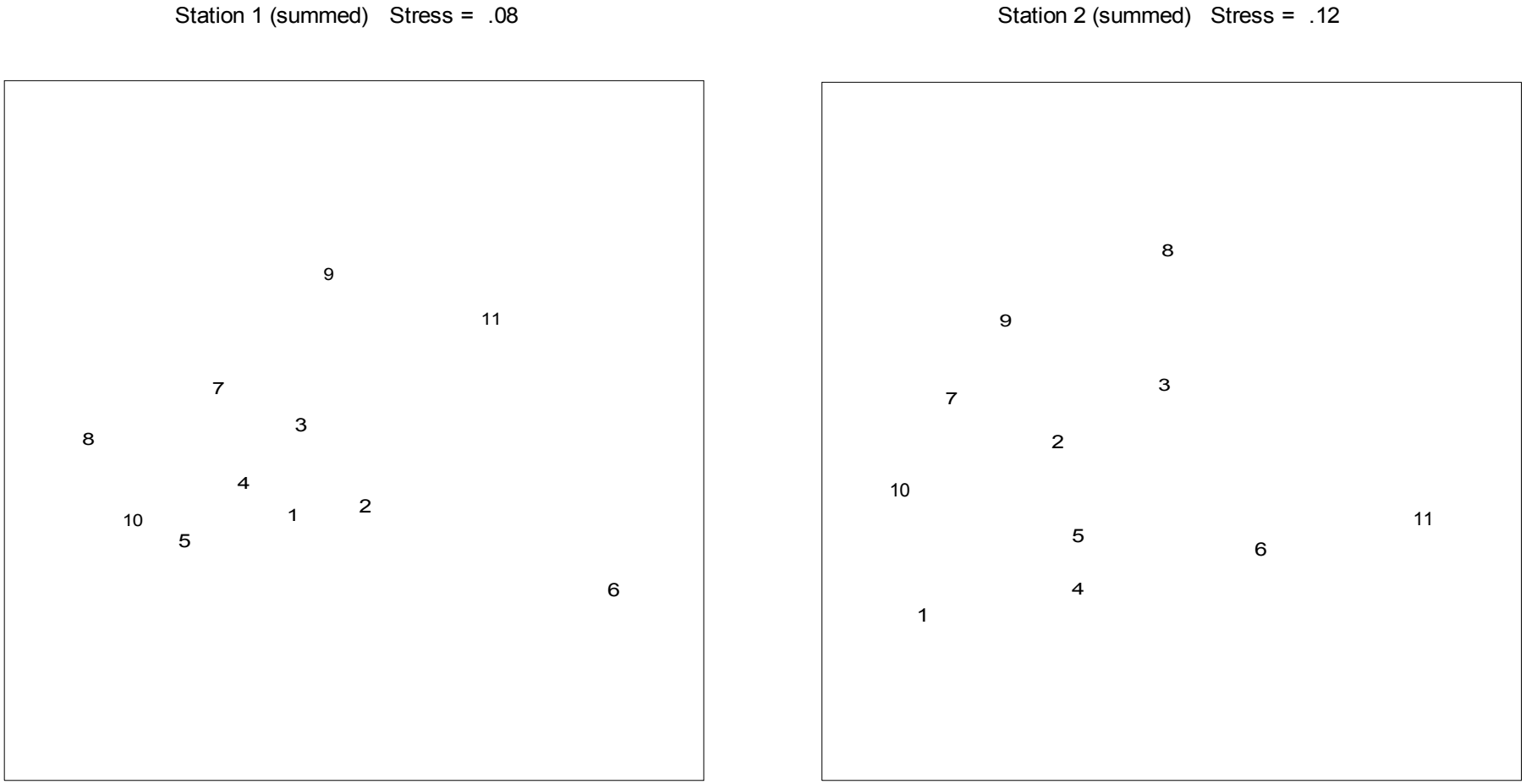


Figure 12. MDS results for summed data.

Table 8a: Anosim results showing statistical significance between labs and comparison of field techniques at Site 1

Grab area (cm²)	1159	952	1062	1001	952	1024	1159	1159	1040	1001	1001
Sieves	#	Sq 70x70	Sq. 70x70	#	#	1mm	#	#	Sq 50x50	#	#
Process Sequence	Sr/H/S	S	C/S	H/C/S	Sr/H/S	Sr/H/S	H/S	H/C/S	H/C/S	H/S	Autosiever
Process Method	MEl	DkHs	DkHs CsEl	GenEl	DkHs	DkHs MEl	SmHs	GenEl	MEl	MEl	Autosiever
Time process (mins)	55	85	60	75	70	-	80	65	105	70	50
Condition Index	1.8	3.8	3.5	3.4	3.8	3.2	3.2	4.0	2.6	3.2	3.8
Lab	1	2	3	4	5	6	7	8	9	10	11
1		-	-	-	-	**	-	-	***	**	*
2			-	-	**	-	-	-	***	**	-
3				-	-	-	-	-	**	**	-
4					-	-	-	**	-	-	*
5						***	-	-	***	-	**
6							**	-	**	***	-
7								-	**	-	-
8									**	-	*
9										***	**
10											**

Table 8b: Anosim results showing statistical significance between labs and comparison of field techniques at Site 2

Grab area (cm²)	1159	952	1062	1001	952	1024	1159	1159	1040	1159	1159
Sieves	#	Sq 70x70	Sq. 70x70	#	#	1mm	#	#	Sq 50x50	#	#
Process Sequence	Sr/H/S	S	C/S	H/C/S	Sr/H/S	Sr/H/S	H/S	H/C/S	H/C/S	H/S	Autosiever
Process Method	MEl	DkHs	DkHs CsEl	GenEl		DkHs MEl	SmHs	GenEl	MEl	MEl	Autosiever
Time process (mins)	25	50	50	85	150	-	80	150	20	40	30
Condition Index	2.3	2.9	3.25	3.2	3.4	3.2	2.9	3.3	2.8	2.7	3.4
Lab	1	2	3	4	5	6	7	8	9	10	11
1		-	**	-	-	***	-	***	**	-	***
2			-	-	-	-	-	**	-	*	**
3				-	-	-	-	-	-	-	***
4					-	*	-	**	**	-	**
5						-	-	***	**	**	***
6							-	***	*	***	-
7								*	-	**	***
8									**	***	**
9										**	***
10											***

Key: *p </= 0.10, **p </= 0.05, *** p</= 0.01 Sr = Sorting table, H = Hopper S = Sieve, CsEl = Coarse Elutriation, MEl = Medium Elutriation, GenEl = Gentle Elutriation, DkHs = Deck Hose, SmHs = Small Hose
N.B. # = the use of a standard round 45cm, 0.5mm Endecott sieve

Station 2

Figure 13 shows the results of cluster analysis on the replicate data for Site 2. As with Site 1 the samples are fairly similar grouped at between 60% and 85% with the majority grouped at over 80%. Seven groups have been formed, two of which have been subdivided. As with Site 1, at Site 2 there are no clear grouping of laboratories and this is confirmed in the results of the MDS ordination (Figure 14) showing moderately high stress levels. Two of the groups showing the lowest similarities to the other samples (groups D and E), again included several replicates from L6 and also L11 although overall similarities were quite high.

The sample groups have been superimposed onto the sample position in Figure 15 and whilst some of the groupings relate to their location, most do not.

Figure 16 represents the cluster analysis for the summed data and shows similar results. Again similarities are high (>70%) and vary only by between 10-15%. L6 and 11 again are slightly separate from the rest, but as before these differences are relatively small. The effect of the 1mm sieve was less noticeable here, and the dominant species were somewhat more variable than at Site 1. *Spiophanes bombyx* was noticeably the most common species.

The results of the summed analyses support the results of the univariate analyses in that the laboratories are shown to be fairly similar. The laboratories which do differ somewhat in the multivariate analysis were also those shown to differ significantly in the univariate analysis.

The results of the ANOSIM test for Site 2 are presented alongside the field observations in Table 8b. ANOSIM has revealed significant differences between the laboratories, particularly L8, 9, 10, and 11. The results for SIMPER are shown in Appendix 4. Again the majority of differences between the laboratories can be accounted for by differences in the abundance of small polychaetes and oligochaetes, particularly *Spiophanes bombyx*. L8 has consistently higher numbers of *Spiophanes* whereas L 11 consistently lower.

Relating the multivariate analyses to particle size

In order to indicate whether any differences between the laboratories were due to a change in substratum, cluster analysis and MDS were repeated using the particle size (PS) samples. This was followed by the Bio-env procedure to correlate the biological patterns to those in the environmental data. Figure 17 shows the results of cluster analysis for station 1.

There is some separation of laboratories based on the abundance of animals as the laboratories with highest abundances are in the centre of the plot. There is, however, no apparent trend in the environmental data, this is expected given the homogeneity of the sediment. The MDS plot (Figure 18) gives similar results to the cluster analysis and in Figure 19 the values of the environmental variables have been superimposed onto individual laboratories and these also show no clear trend.

Figure 13. Results of cluster analysis for station 2.

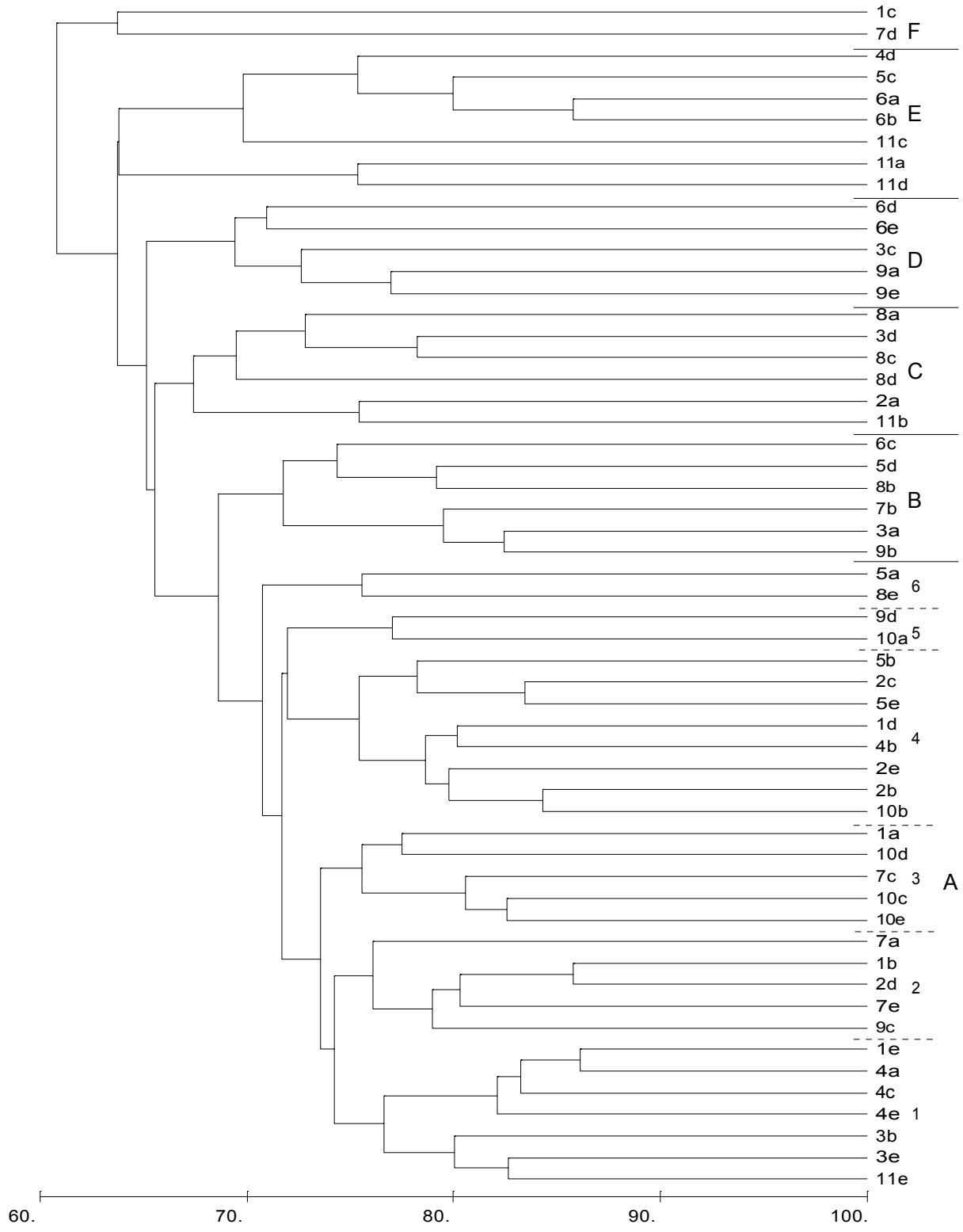


Figure 14. MDS results Station 2. Stress = .25

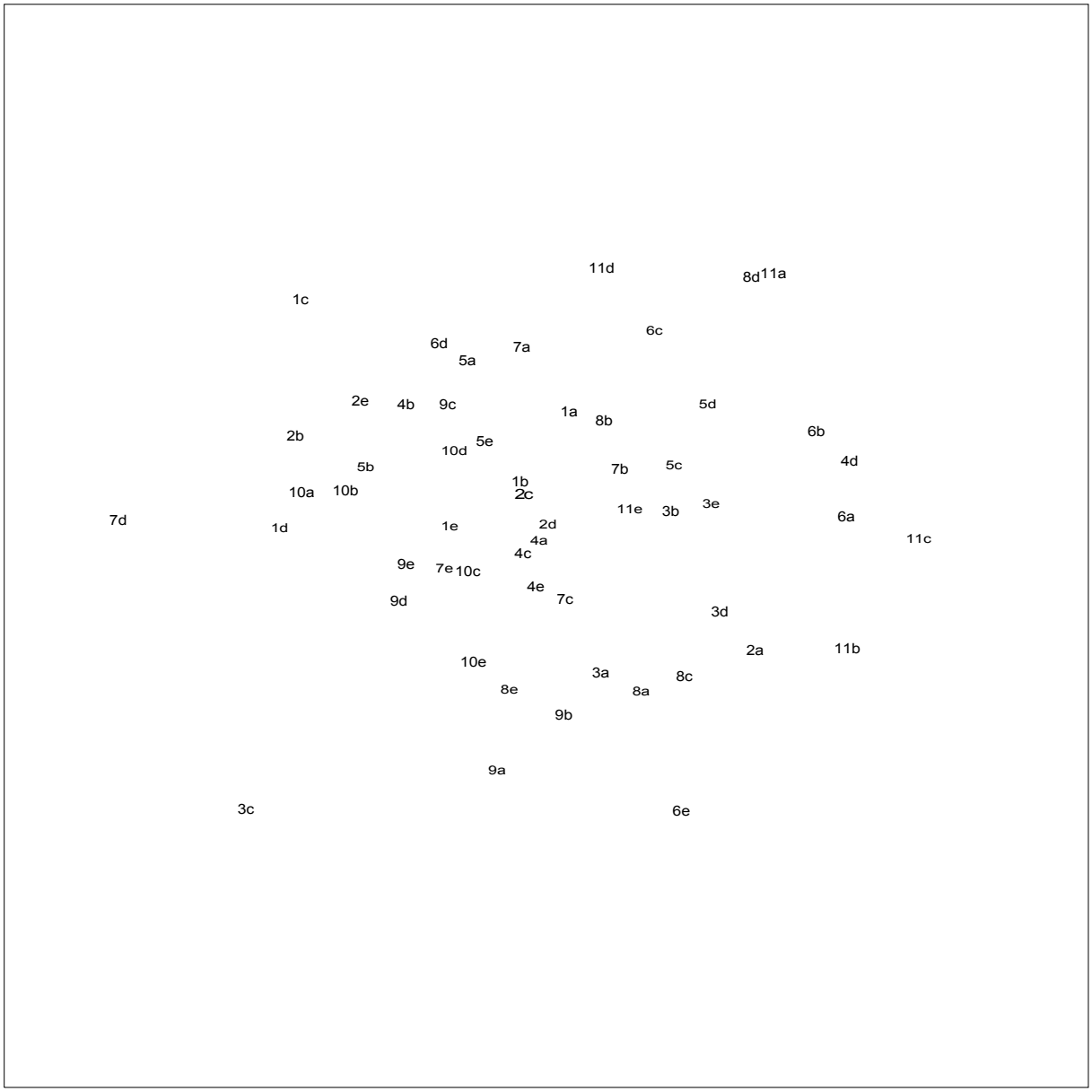


Figure 15. Station 2 sample groups.

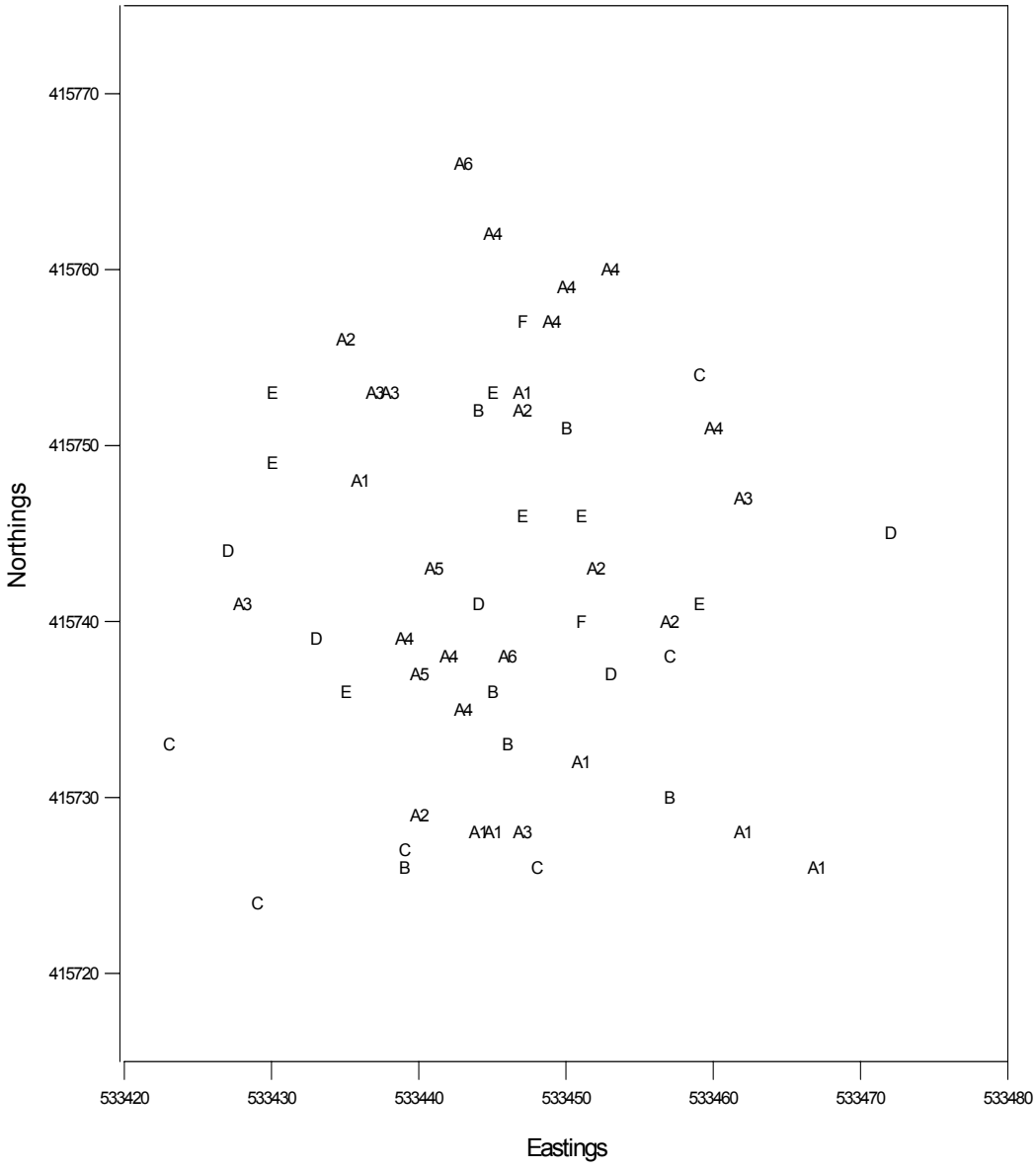
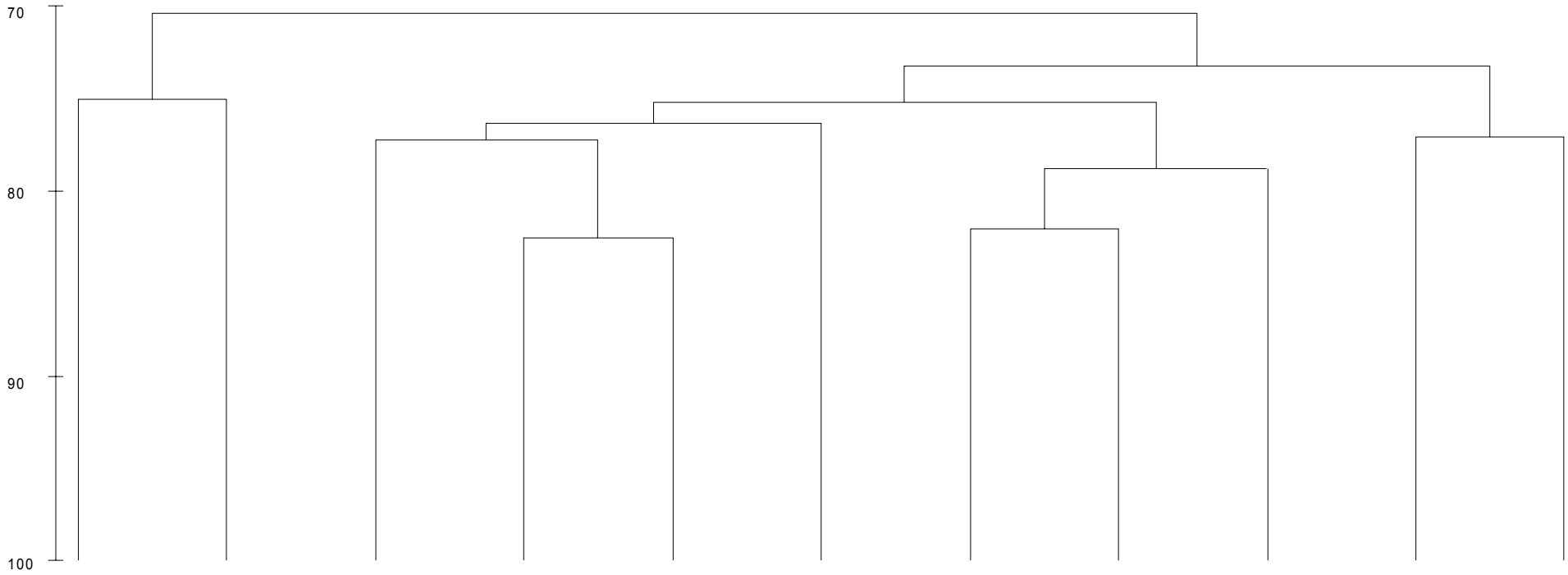
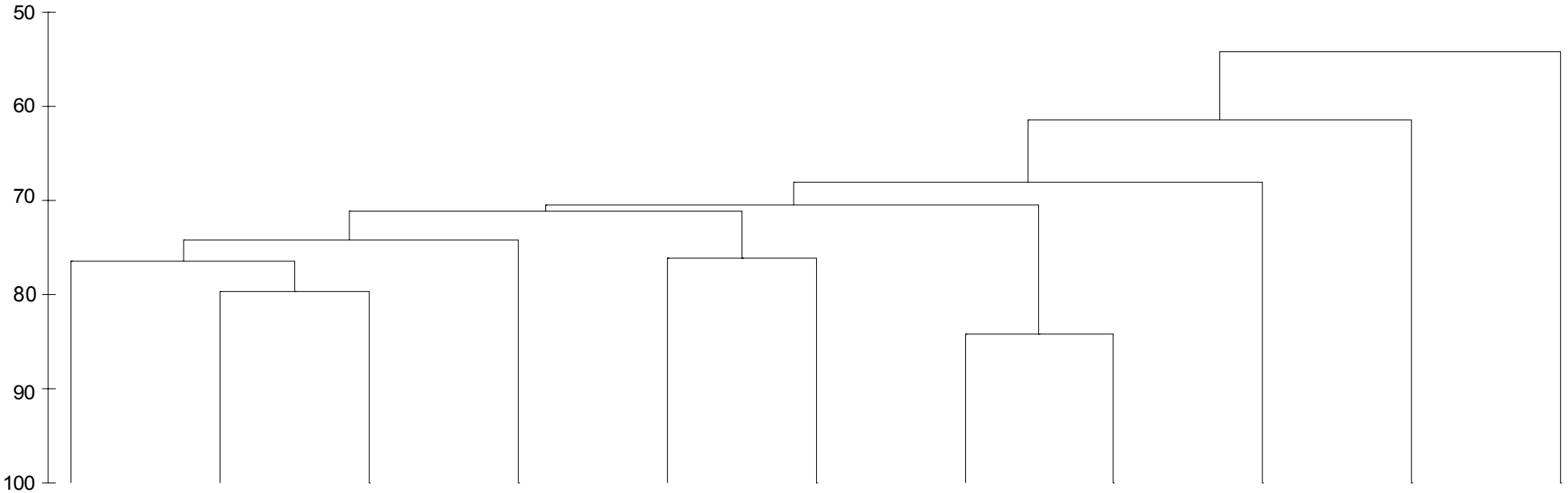


Figure 16. Results for cluster analysis for Station 2 (summed).



	11	6	10	5	4	1	9	2	7	8	3
	Day	Van Veen	Van Day	Day	Van Day	Day	Day	Day	Day	Day	Day
	0.5mm	1mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm
	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx
	Pygospio elegans	Nephtys hombergii	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Pygospio elegans	Aphelochaeta/Tharyx spp.	Scoloplos armiger	Scoloplos armiger	Aphelochaeta/Tharyx spp.	Pygospio elegans	Pygospio elegans
	Nephtys hombergii	Phyllodoce mucosa	Aricidea minuta	Scoloplos armiger	Aphelochaeta/Tharyx spp.	Scoloplos armiger	Aricidea minuta	Aphelochaeta/Tharyx spp.	Pygospio elegans	Aphelochaeta/Tharyx spp.	Scoloplos armiger
	Aphelochaeta/Tharyx spp.	Scoloplos armiger	Pygospio elegans	Pygospio elegans	Scoloplos armiger	Pygospio elegans	Aphelochaeta/Tharyx spp.	Pygospio elegans	Scoloplos armiger	Nephtys hombergii	Aphelochaeta/Tharyx spp
	Phyllodoce mucosa	Aphelochaeta/Tharyx spp.	Scoloplos armiger	Aricidea minuta	Aricidea minuta	Aricidea minuta	Nephtys hombergii	Aricidea minuta	Nephtys hombergii	Scoloplos armiger	Aricidea minuta
S	22	22	29	24	26	28	27	27	28	20	26
A	369	673	1230	604	766	1130	1500	1070	1480	760	709
H'	3.12	1.85	2.95	3.01	3.04	2.91	1.91	2.6	1.96	2.47	2.53
J	0.699	0.415	0.608	0.656	0.646	0.606	0.402	0.547	0.407	0.571	0.538

Figure 17. Results of cluster analysis for Station 1 (PSA samples).



	11	3	1	9	7	4	10	5	2	8	6
	Day	Day	Day	Day	Day	Van Day	Van Day	Day	Day	Day	Van Veen
	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	1mm
	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.
	Tubificoides swirencoides	Pygospio elegans	Pygospio elegans	Pygospio elegans	Pygospio elegans	Pygospio elegans	Pygospio elegans	Pygospio elegans	Pygospio elegans	Pygospio elegans	Pygospio elegans
	Scoloplos armiger	Spiophanes bombyx	Tubificoides swirencoides	Tubificoides swirencoides	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Mediomastus fragilis	Tubificoides swirencoides	Tubificoides swirencoides	Macoma balthica
	Juvenile Mytilus	Eteone longa/flava	Spiophanes bombyx	Macoma balthica	Tubificoides swirencoides	Tubificoides swirencoides	Tubificoides pseudogaster	Tubificoides pseudogaster	Tubificoides pseudogaster	Streblospio shrubsoli	Scoloplos armiger
	Eteone longa/flava	Tubificoides pseudogaster	Streblospio shrubsoli	Scoloplos armiger	Mediomastus fragilis	Nephtys hombergii	Streblospio shrubsoli	Scoloplos armiger	Scoloplos armiger	Tubificoides pseudogaster	Tubificoides swirencoides
SD	1.73	1.57	1.56	1.72	1.63	1.6	1.5	1.87	1.5	1.7	1.79
Skew	-0.34	-0.24	-0.44	-0.29	-0.42	-0.34	-0.44	-0.3	-0.37	-0.43	-0.36
% Gravel	0	0	0	0	0	0	0	0	0	0	0
% Sand	21.99	17.72	14.94	23.01	18.02	17.5	12.79	27.54	13.59	19.9	24.25
% Silt	78.01	82.28	85.06	76.99	81.98	82.5	87.21	72.46	86.41	80.1	75.75
Median μm	15.31	13.95	12.3	15.41	13.311	13.09	10.85	17.73	11.81	13.57	15.21
Mean μm	15.71	13.76	12.66	15.85	13.86	13.44	11.47	18.34	12.06	14.44	16.34
S	18	17	18	20	15	15	18	18	19	13	15
A	414	980	750	403	2140	2340	2540	1300	281	355	51
H'	1.04	0.76	1.15	1.71	0.514	0.345	1.01	1.13	2.24	1.31	3.2
J	0.249	0.186	0.276	0.397	0.131	0.0882	0.243	0.271	0.528	0.353	0.819

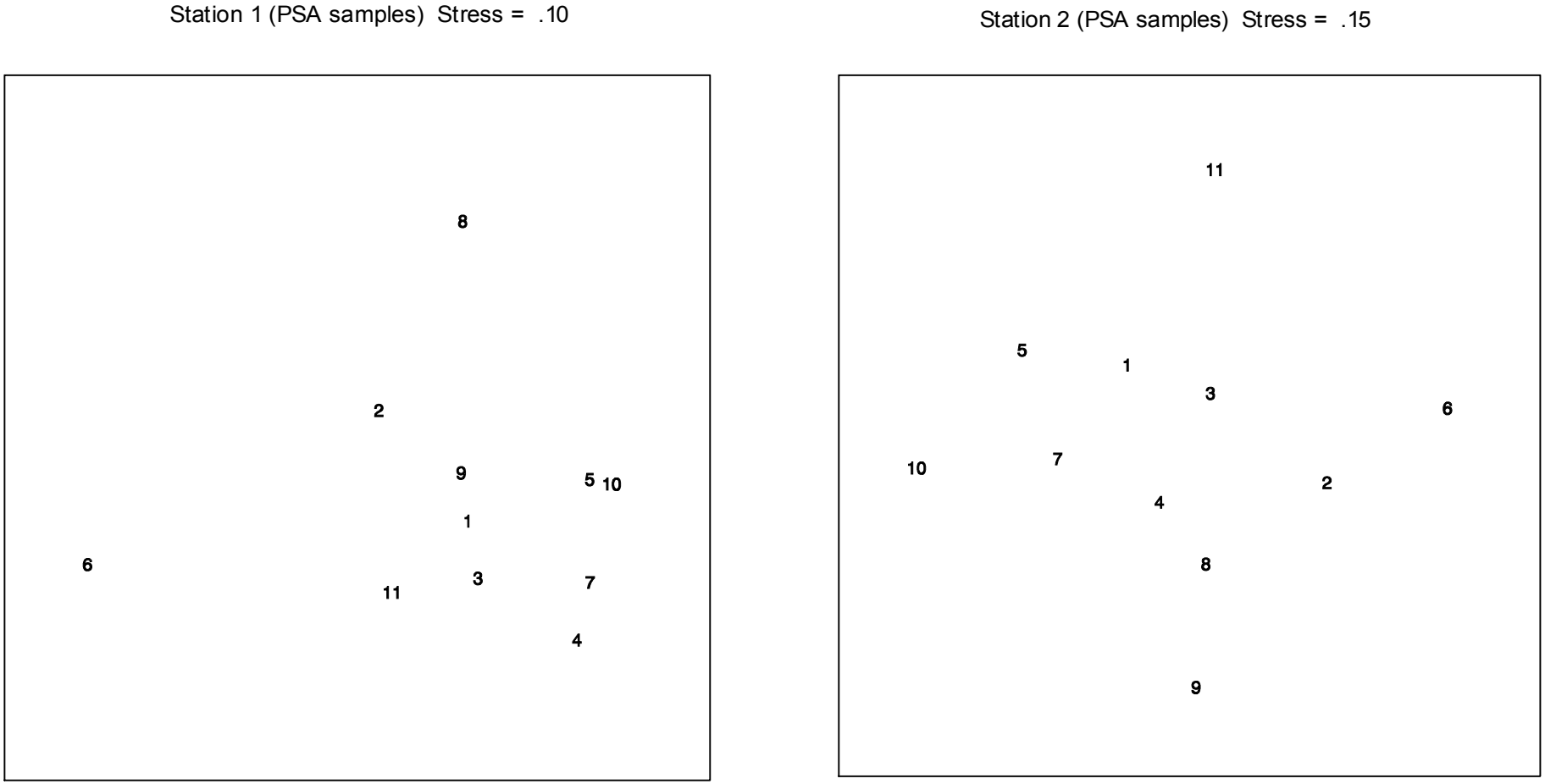
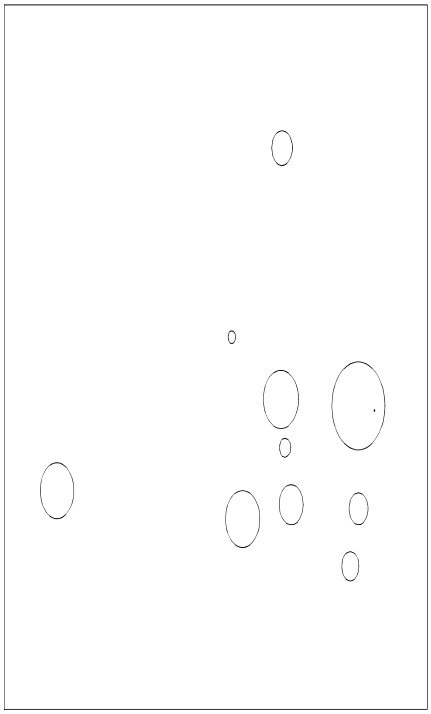


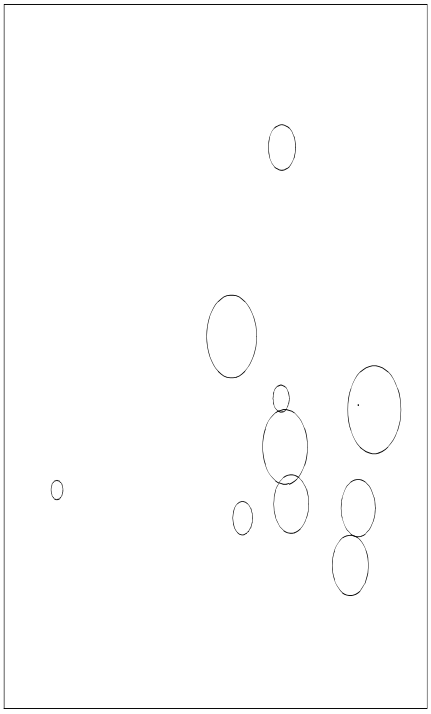
Figure 18. MDS results for PSA samples.

Figure 19. MDS plots with sedimentary parameters.

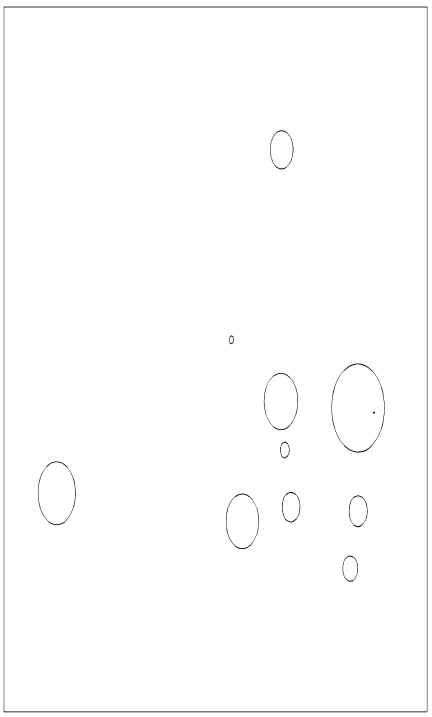
Median grain size (Station 1)



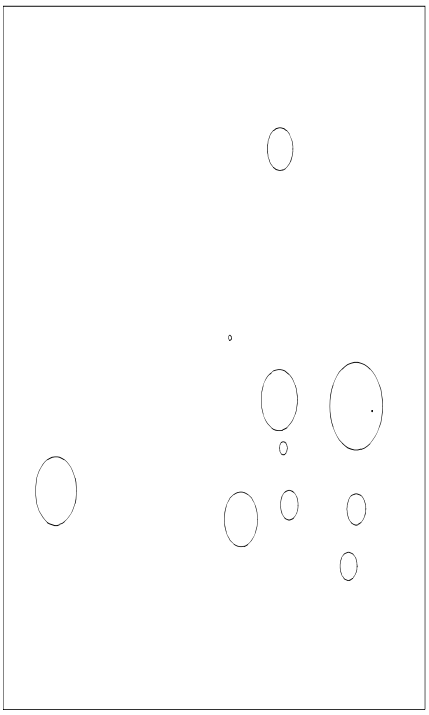
% Silt (Station 1)



Mean grain size (Station 1)



% Sand (Station 1)



At Site 2 the results of summed cluster analysis (Figure 20) show a similar trend to Site 1 with no clear trend in the environmental data. The MDS results (Figure 18 and 19) again confirm the results of the cluster analysis with no clear pattern evident in the environmental data.

The results of BIO-ENV showed that the correlations between the environmental data and the MDS results were very low with no correlation above 0.02.

2.1.4 Discussion

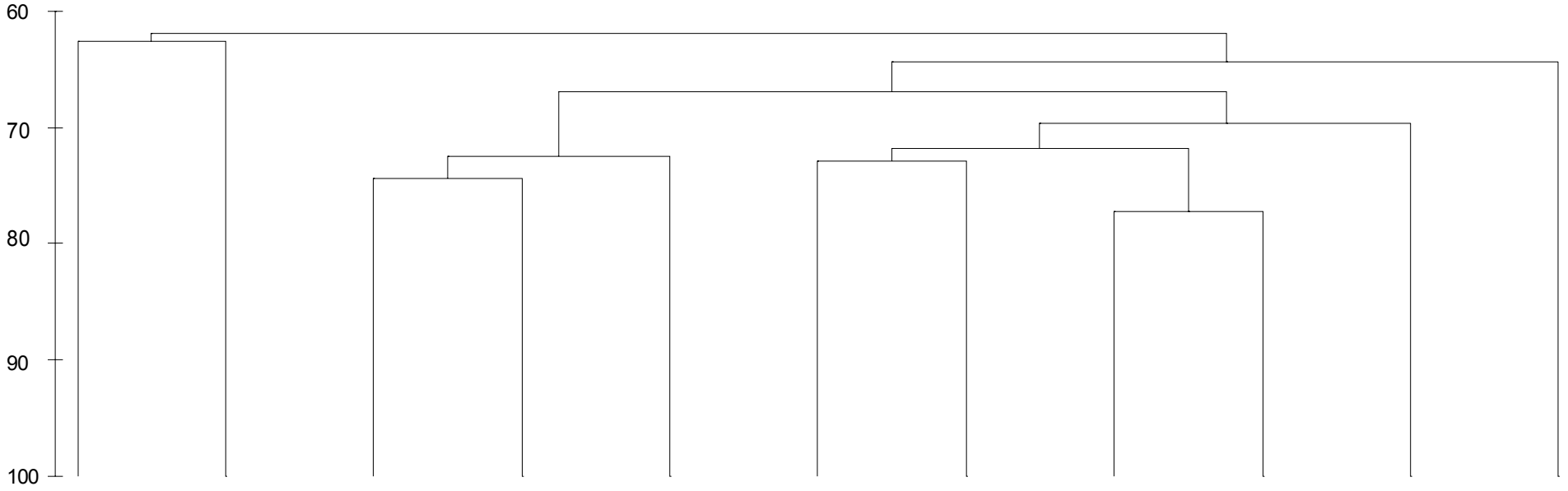
The aim of this study was to assess the consequences of using varied field survey techniques in the analysis of macrobenthic communities. Eleven different laboratories each used their own methodology to sample two stations in the Humber estuary. The methodologies employed by the laboratories differed in varying degrees from utilising different equipment through to differences in methods of processing the samples. The two stations chosen varied in substratum type. Site 1 had a relatively homogenous sandy silt substratum compared to Site 2, which had a heterogeneous substratum of silty, gravelly sands.

Each laboratory took five replicates at each station within an area of 50m x 50m. It was apparent that the positions of the samples taken by each laboratory were variable and there was a large degree of overlap between laboratories i.e. the laboratories did not sample discrete areas at each station but took random samples from one area. In any area of seabed there will be small scale fluctuations in substratum and community type and this study was not designed to separate natural fluctuations from those due to sampling methodology.

Any differences in sampling methodology may affect the results of a survey. Variations in grab design for example may lead to a change in the area or volume of sediment sampled and this will obviously have an effect on the number of individuals captured and even the type of fauna sampled (e.g. organisms living at depth in the sediment). Sieve size will also affect the number of species and individuals captured although, as shown here, this may vary depending on the type of substratum. Other less obvious variations in methodology may also be important, for example the way in which the fauna are separated from the sediment may effect the condition of the animals (which may in turn lead to problems in identification and hence alter the number of species recorded) and also determine how may animals are retained on the sieve.

The simplest way of analysing differences between laboratories is to use univariate statistical techniques to analyse the biological parameters which would be most affected by differences in methodology. However, univariate parameters such as diversity indices involve a loss of information on the community structure so multivariate analyses were also employed. Multivariate techniques are usually used to derive similarities between sites (or in this case laboratories) and to determine the dominant macroinvertebrate communities in an area. By assuming that other variables such as substrata type were fairly constant, the analysis of similarities between laboratories derived from multivariate analysis provides a useful guide as to how much the changes in methodology affect the results of a macrobenthic survey.

Figure 20. Results of cluster analysis for Station 2 (PSA samples).



	11	6	10	5	7	8	2	4	1	3	9
	Day	Van Veen	Van Day	Day	Day	Day	Day	Van Day	Day	Day	Day
	0.5mm	1mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm
	Pygospio elegans	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Pygospio elegans	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx	Spiophanes bombyx
	Spiophanes bombyx	Nephtys hombergii	Aricidea minuta	Scoloplos armiger	Pygospio elegans	Spiophanes bombyx	Pygospio elegans	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.	Pygospio elegans	Aricidea minuta
	Aphelochaeta/Tharyx spp.	Scoloplos armiger	Streptosyllis websterii	Pygospio elegans	Aphelochaeta/Tharyx spp.	Scoloplos armiger	Aphelochaeta/Tharyx spp.	Scoloplos armiger	Pygospio elegans	Scoloplos armiger	Scoloplos armiger
	Phyllodoce mucosa	Phyllodoce mucosa	Phyllodoce mucosa	Aphelochaeta/Tharyx spp.	Nephtys hombergii	Phyllodoce mucosa	Nephtys hombergii	Pygospio elegans	Mediomastus fragilis	Aphelochaeta/Tharyx spp.	Aphelochaeta/Tharyx spp.
	Nephtys hombergii	Pygospio elegans	Aphelochaeta/Tharyx spp.	Macoma balthica	Scoloplos armiger	Nephtys hombergii	Aricidea minuta	Aricidea minuta	Nephtys hombergii	Nephtys hombergii	Phyllodoce mucosa
SD	2.13	1.82	2.76	2.39	1.68	3.33	1.94	2.22	1.81	2.56	2.86
Skew	-1.26	-0.67	-0.51	0.24	-0.65	0.03	-0.57	-0.67	-0.66	-0.26	0.01
% Gravel	0	0	4.5	7.1	0	20.8	0	0.8	0	3.2	7.7
% Sand	20.61	19.17	39.47	72.23	16.2	47.39	23.36	28.69	19.19	47.88	56.17
% Silt	79.39	80.83	56.03	20.67	83.8	31.81	76.64	70.51	80.81	48.92	36.13
Median µm	13.24	12.63	28.09	166.18	11.85	191.73	13.76	16.08	12.11	50.21	158.81
Mean µm	16.42	14.47	39.73	119.1	13.06	152.34	16.27	20.69	14.03	41.69	85.58
S	12	9	20	16	16	12	12	14	15	12	10
A	106	69	267	150	94	182	86	141	137	176	381
H'	2.62	1.77	2.59	2.93	2.88	2.17	2.41	3.01	2.95	2.49	1.41
J	0.731	0.56	0.6	0.732	0.721	0.605	0.673	0.791	0.755	0.695	0.426

The graphical results of univariate analysis showed that whilst there did appear to be a significant degree of variability in the biological parameters, many of the laboratories were shown to be statistically similar using none parametric statistics. At Site 1 only abundance was shown to differ significantly whilst at Site 2, abundance, species richness and evenness were shown to differ significantly. However, the actual number of laboratories shown to differ was low. Only laboratories 6, 7, 9, 10 and 11 differed significantly from each other whilst the remaining laboratories were similar. The ANOSIM test confirmed this pattern. The species generally responsible for the differences between the laboratories were small polychaetes and oligochaetes as well as relative abundances of these groups, particularly *Aphelochaetae* at Site 1 and *Spiophanes* at Site 2. These differences can be considered biologically not significant due to the inherent patchiness that these two species exhibit in their spatial distribution on the sea bed.

At Site 1 the effect of the 1mm sieve used by L6 reduced the number of animals sampled, although due to the inherent variability of the samples only a few laboratories were shown to differ significantly from L6 with regard to abundance. At Site 2 the effect of the larger sieve size is less obvious which in part may be due to the coarser sediment at this site. This indicates that the size of the pore spaces of the sieve in relation to the sieve residue, i.e. the particles retained on the mesh, has a greater influence in some substrata than the absolute size of the mesh. It is possible also that the sieve mesh became blocked more easily with the large particle size at Site 2.

The multivariate analysis confirmed the results of the univariate analysis and showed that there were no major differences between the laboratories. Similarities between replicates and laboratories were generally high (>70%) and varied only by 10 -15%. Given that the laboratories effectively sampled the same population this is not unexpected. Any differences between laboratories were similar to those found in the univariate analysis with L6 and 11 consistently showing some separation from the rest. The dominant species at the sites showed little variation between laboratories, particularly at Site 1. The position of the samples and the nature of substratum also showed little effect overall on the observed patterns.

The results indicate that whilst many of the laboratories appeared to give similar results there were some differences evident, particularly with respect to the number of individuals sampled. It was apparent that the nature of substrata within a site and the spatial distribution of the samples did not greatly affect the results so any observed differences between the laboratories would be due either to differences in methodology or natural variability.

Since the differences in the statistics are not generally significant either statistically or biologically, other considerations become important particularly in relation to the efficiency of the process. Sample processing in the field can be very costly and can be particularly strenuous. The process nearly always involves at least two staff (at the workshop those laboratories which only employed one worker in the field would under normal circumstances have had two workers). Ship time may be affected although often there is usually sufficient time between sites to process samples or samples are can be stockpiled and processed later.

However, any method that reduces the time consuming process of processing may offer considerable benefits. The Wilson auto-siever (L11) was one such method. The results of the auto-siever prove that the technique is as effective for processing samples with one worker as the most experienced team (L1) with two workers from the local laboratory, familiar with the study sites. Furthermore the condition of the samples was significantly better than the rapid processing of L1. L1 showed significantly poorer sample condition compared to the other laboratories. It can only be inferred that laboratory identification time was increased as a consequence of the poor quality of the specimens, although laboratory processing time was not measured. As a consequence of this the vigorous sieving technique of L1 could be reviewed in light of the performance of other methods to improve the condition of samples from this laboratory.

2.1.5 Conclusions of the Humber Workshop Intercomparison Exercise

- The exercise highlighted the fact that there were common elements to all laboratories but each laboratory had its own set of practices. It was difficult to quantify the effect of each minor variation in methods and it appears likely that many of the differences cancel out the difference between laboratories.

- However, there were some elements of poor practice. For example, the majority of laboratories made no effort to clean their sieves between sites thus introducing the possibility of macroinvertebrates being transferred from one site to another.

- While practices used by a laboratory will ensure internal consistency, this is often lost between laboratories due to unquantified and subjective approaches. or example, sediment descriptors used by the participants were highly subjective and in some cases inconsistent with the sediment type.

- The equipment differences are perhaps the easiest aspects on which to standardise. For example, despite the view that the grabs were similar, in fact the sampling area of the grab samplers used in the exercise varied by as much as 20% with the largest area being 0.115m². This has large repercussions in calculating abundances per unit area.

    - The design of the exercise indicated that even with relatively homogenous sediments, it is difficult to separate worker and gear variability from field variability. However, it is of note that this could be achieved only with an experimental approach, i.e. the creation of a set field area over which the grabs could be used.

    - Despite these features, it is perhaps reassuring that the variations in sampling methodology for the two sites sampled in the Humber estuary generally had relatively little effect on resulting data either statistically or biologically.

    - However, differing field substrata may provide a different result during a similar exercise.

2.1.6 Review of Subtidal Field Method Guidelines

In order to put the findings of the workshop into context, available guidelines were reviewed before recommendations were made. Various methods for sampling equipment and sample collection and processing have been recommended in a variety of literature sources (Baker & Wolff 198x; Holme and MacIntyre, 1984). More recently attempts have been made to standardise the methods associated with shipboard collection and processing in the form of two guidance documents, Marine Pollution Monitoring and Management Group (hereafter called the "Yellow Book") (Rees, et al, 1985) and the International Council for the Exploration of the Seas (hereafter called the "Green Benthic Methods Book") (Rumohr et al, 1990). The key procedures highlighted in these guidance notes outlined below.

2.1.6.1 'Yellow Book' Methods: Sampling Equipment and Sample Collection:

The use of a 0.1m2 Day grab in which the top of grab has lifting flaps for surface access during sample collection. The jaws should shut tight to prevent the loss of fine material and this should be tested before use. There should be the facility to add weights as necessary to obtain better penetration. The operators should estimate and note the sample volume on retrieval and they should discard quantities <51 (7cm depth) in muds and <2.51 (5cm depth) in hard packed sands. The surface features, texture, colour and smell should be noted and sub-sampling for other analyses should not normally be allowed from benthic sample taken. However, it is of note that this has the potential to increase discrepancies between the faunal and environmental results obtained.

2.1.6.2 'Yellow Book' Methods: Sample Processing

There should be gentle sieving to minimise specimen damage and the latter will be minimised if the sample from the grab is deposited into a hopper. Sieving will be facilitated by gentle 'puddling' with seawater in the hopper prior to sieving. Suitable instruments e.g. forceps, should be used for removing delicate or enmeshed specimens from mesh. The samples should be fixed in buffered 10% saline formalin (4% formaldehyde) and a vital stain such as Rose Bengal should be added prior to sorting.

2.1.6.3 'Green Benthic Methods Book' Methods (ICES): Sampling Equipment:

This recommends the use of a $0.1m^2$ Van Veen grab, weighted to 35-40kg for mud/muddy sands and 70-100kg for sands. In order to reduce a bow-wave (shock wave) preceding the grab during deployment there should be large (minimum 60% area) windows on the upper side of a metal mesh 0.5 x 0.5mm. The design of the grab should be to prevent elevation during closure and there should be easy-open windows for inspection and subsampling prior to emptying. Winch operation should ensure gentle deployment and closure of grab and the warp should be kept vertical during setting down and retrieval in order for the grab bite to be perpendicular to the bed surface and so that material is not lost from the surface. The exact sampling area and volume or digging depth is to be measured during or prior to sampling or calculated from the dimensions of the grab. The sediment surface colour, depth, colour and smell (especially H_2S) should be noted during sampling together with the sediment description (concretions, bioturbation, etc.).

2.1.6.4 'Green Benthic Methods Book' Methods (ICES): Sample Processing (I):

Given that successive analysis is only as valid as the weakest part of the sampling, care should be taken to ensure no spilling of the sample once the grab is on board, especially during rough sea conditions. In order to ensure no transfer of material between samples, the grab should be rinsed thoroughly between samples. Similarly, each grab should be sieved, stored and documented separately. The volume of each sample should be measured and recorded by grading the container or by the use of a ruler. The grab should be emptied into a suitable container (hopper) and transferred gradually to the sieve as a sediment-water suspension. Hopper-attached sprinklers or a hand-operated shower should be used to disaggregate and suspend the sample. It may be necessary that very stiff clay will have to be gently broken by hand in water in the hopper or container.

2.1.6.5 'Green Book' Methods (ICES): Sample Processing (ii):

The sieve should be cleaned after each portion of the sample has been sieved in order to avoid clogging and ensure consistent sieving. In order to avoid damage to fragile organisms, the sample should not to be sieved with a direct jet of water against mesh. Similarly, fragile animals should be picked by hand during sieving and large stones and shells should be removed to avoid damage to the animals. Following sieving, all material should be gently washed off the sieve by backwashing into a suitable container and the use of spoons and other tools should be avoided. The review recommends that when a 0.5mm sieve is used, then the 0.5mm and 1mm fractions should be kept separate in processing. Following sieving, buffered 4% formaldehyde solution should be used together with vital staining to increase sorting accuracy.

As a further development of the above, European (ICES) Methods later recommended the use of van Veen grabs and suggested weights for various sediment types.

The following three sections were notes taken from general discussion and consensus at a follow up meeting from the workshop. Some of the consensus was not supported by the results of the intercomparison exercise although this may reflect the nature of the methods that adopted practice has evolved as being that which is most practicable under field conditions.

2.1.6.6 UKNMBAQC Methods (Proposed): Sampling Equipment:

Comments/General Agreements:

(i) The actual weights used on a grab may not need to be specified. It would be satisfactory to simply state that an adequate weight should be added to the grab in order to get sufficient penetration into the sediment (dependent upon sediment type).
(ii) Standardising sampling methods really depends upon the sediment type being worked. It is not possible to standardise one grab that will be suitable for all sediments.
(iii) PTFE flaps should be placed on the grab's upper side, covering a minimum of 60% of the upper surface, these should be freely opening on descent and easily closing on ascent. There can be problems with various types.

(iv) There should be easy opening windows that are required for inspection and sub-sampling prior to emptying the container.

(v) The grabs should be emptied into large buckets or a hopper and splashing should be controlled when the sample drops into the container. The bucket / hopper should be sufficiently large to ensure that there is no overspill.

2.1.6.7 UKNMBAQC Methods (Proposed): Sample Collection:

Comments/General Agreements:

(i) Given the need for accurate on-board recording, it is worthwhile having a third person on-board the vessel rather than just having 2 operators. Similarly, given the survey vessel costs, the additional hands can be important when there are time constraints on the survey.

(ii) The winch speed and thus the speed with which the grab descends and ascends is important for the quality of the sample by preventing the creation of a bow-wave during descent and the loss of material through winnowing on ascent.

(iii) The wire should be kept vertical for vertical setting down and lifting and to ensure that a corner bite only is not taken.

(iv) The depth of sample should be recorded using a ruler in the centre of the grab as accurately as possible.

(v) In order to ensure that the faunal and sediment data are concurrent and thus can be sued as such in the data analysis, a sediment (PSA) subsample should be taken from a faunal sample unless other samples are also needed. In the latter case then a separate sample should be used to prevent the loss of material from the faunal sample. However, there is a difference of opinion over this point whit some laboratories being concerned about the possible loss of faunal specimens. In general the consensus was to avoid removing any sub-samples for physico-chemical purposes from the faunal grabs.

(vi) The characteristics of the sediment should be noted in a consistent and previously agreed manner. This can be important for assessing general changes in the sediment and a useful confirmation of sediment type for the results of particle size analyses.

2.1.6.8 UKNMBAQC Methods (Proposed): Sample processing (i):

Comments/General Agreements:

(i) When measuring the volume of each grab, it may be possible to estimate the volume of grab rather than use a graduated container. It is of note that the estimates of grab volume proved to be somewhat subjective during the field comparisons. The measurement of sample depth at the centre of the grab using a ruler was agreed as best practice since grab volume could always be estimated from the depth.

(ii) There was a general consensus that samples should be emptied from the grab into a hopper rather than directly onto a sieve. This would prevent damage and general wear and tear on the sieve as well as discouraging processing the sample directly on the sieve.

2.1.6.9 UKNMBAQC Methods (Proposed): Sample processing (ii):

Comments/General Agreements:

(i) Samples should not be sieved with a direct jet of water against sieve mesh. It is of note that the most vigorous method of sieving gave the poorest quality specimens during the workshop. Hence a balance needs to be struck between the vigour of sample processing and the time taken to sieve the sample although too vigorous sieving will obviously waste the sampling effort if the samples are in too poor condition for identification. If samples are in poor condition then these will generally extend the amount of laboratory processing time, since identification will take longer. Samples processed by the auto-siever were shown to be in the best condition at the workshop and this was generally agreed as best practice.

(ii) Avoid the use of spoons or other tools for getting the bulk of the sieve residue into containers, most workers used their fingers but took care to wash them over the sieve or sample container.

(iii) Label both the outside and inside buckets. It is good practice to use a label with tags rather than the station number so that there is no bias introduced by the workers analysing the samples, especially in analysing replicates.

(iv) The use of stains to aid sorting was not agreed upon, as workers were equally comfortable with staining/not staining as an aid to sorting. It was agreed that providing that a laboratory passed routine analytical quality control checks then the addition of stain was a matter of preference.

2.1.6.10 Overall Recommendations from the Intercomparison Exercise

The workshop illustrated the importance of quality assurance of field sampling for macrobenthic fauna. It is recommended that field quality assurance be given equal weighting to laboratory quality assurance and that future quality assurance developments should be targeted in this area.

A best practice protocol was agreed at the workshop and should be implemented for all macrobenthic sampling activities involving the use of 0.1m² grabs for the sampling of marine macrobenthos.

The design principles of the methodology of the Wilson auto-siever should be further tested and more widely applied. Further comparative testing is recommended in fully marine waters exhibiting a higher diversity.

The principles of the Wilson auto-sieving work station should be considered for application to all future macrobenthic monitoring programmes due to the significant advantages described in the workshop proceedings when compared to the more traditional manual methods. However, a change of methods may prevent the method being used with temporal monitoring programmes already in progress due to the significantly lower numbers of individuals being retained by the auto-siever in some

sediments when compared to more manual methods, unless a period of concurrent sampling is undertaken to cross calibrate the two methods.

2.2 Field Trial of the Wilson Autosiever - A Case Study

2.2.1 Introduction

IRTU has been monitoring the North Channel Sewage Sludge Disposal Ground Site since 1987. A total of 10 sites, (F1 to F10) have been sampled annually in triplicate. During 1997 IRTU utilised the Wilson Auto-siever (see Figure 3) during the laborious rate limiting pre-sieving of macrofauna. Samples were sieved on a 0.5mm stainless steel square mesh sieve. The 10 sites cover a wide range of depths (20m – 85m) and consist of a heterogeneous sea bed matrix ranging from muddy fine sand to coarse shell gravel). Previous studies have shown the communities at the 10 sites to be generally stable with site discrimination predominately determined by sediment type

2.2.2 Results

Figure 21 shows a temporal Analysis of Variance (ANOVAR) of the samples collected during 1995 with 1997 and demonstrates no significant difference in any of the primary community variables as basic univariate statistics such as numbers of individuals or species, or their derived indices such as Shannon Weiner, Evenness etc.

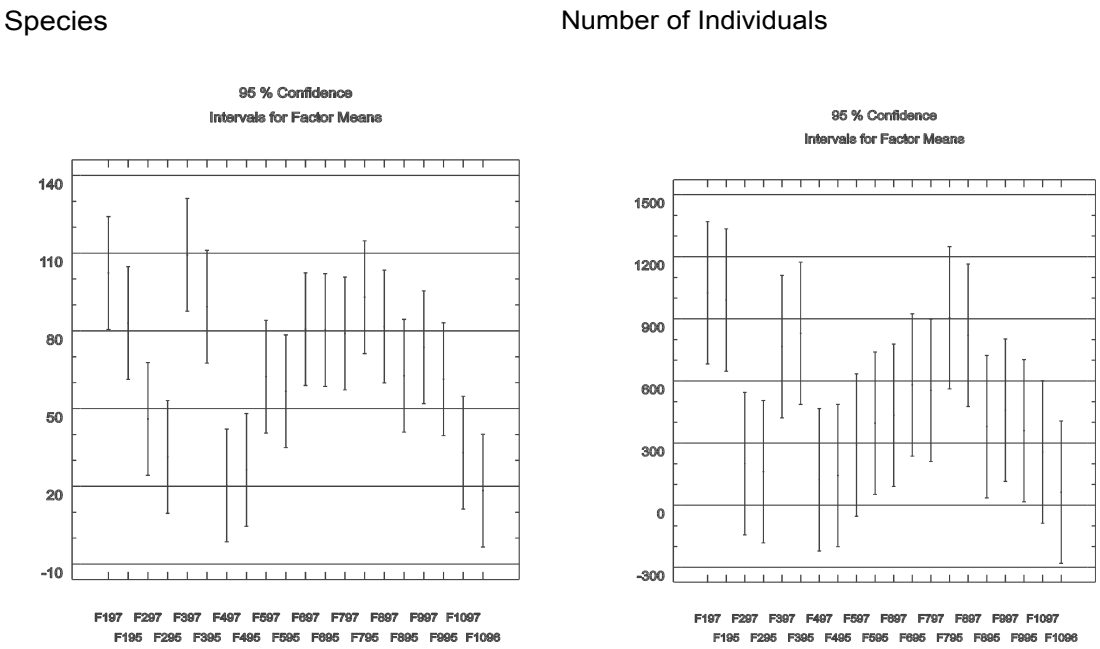


Figure 21: Comparison of univariate statistics for the 1995, 1997 North Channel Sewage Sludge Disposal Grounds, Northern Ireland

It would appear from the data that more species were retained using the auto-siever method except at site F4 (generally an impoverished variable site due to mobile sediments) while the numbers of individuals tend to vary less.

This would support the general observation that species retained by the auto-siever method are generally less damaged and hence easier to identify.

2.2.3 Discussion

A slight difference in the number of species was observed with enhanced numbers of species being found with the auto-siever. An experienced identifier assessed the species responsible for these differences and discovered that for some genera the auto-siever permitted the preservation of delicate diagnostic features allowing separation to species where previously genus may have been the lowest taxonomic level possible. It is probable that this arises as the mesh is constantly agitated from below resulting in less damage to the resulting specimens prior to preservation (i.e. no downward water pressure exerted on specimens enmeshed on the sieve resulting in specimen damage). This finding is supported by the laboratory study of the condition of the specimens in the Humber workshop where Laboratory 1 processed the samples most efficiently in terms of time (see Figure 4), although Laboratory 11, which used the auto-siever, showed that the highest condition samples were procured from the auto-siever with the poorest from Laboratory 1(see Table 2). It should be noted here that the more manual method of Laboratory 5 also attained good condition although took as much as three times longer to process the samples than the auto-siever (see Table 3 and Figure 4).

The main advantages over the traditional methodology are the reduction in the number of workers involved in the sample processing potentially freeing up staff resources to perform other functions. This is supported by the fieldwork observations in the case study and the Humber Workshop.

The flushing of the sieve from below had the additional potential benefit of reducing the level of mesh blockage thus maintaining a mesh aperture of 0.5mm and reducing the numbers of meiofaunal taxa retained. However, this was shown in both studies not to be a statistically significant factor.

Health and Safety issues associated with benthic sample processing are also important to consider in the context of the auto-siever since the need for manual handling of the sample is greatly reduced. This reduces the risk of back injury from continuous and repetitive strain (many benthic workers complain of back injury arising from continuous benthic sample processing). It also reduces the risk of injury from sharp elements contained within the sample with the auto-siever negating the need for direct handling of the sample commonly used to breakdown cohesive mud.

A major difference between the more manual methods and the auto-siever is that the auto-siever requires a high volume, constant water supply. The auto-siever can be self-contained with its own portable pump and generator making the equipment easily transportable from one vessel to another without the need to adapt the survey boat hose arrangements.

2.2.4 Conclusions

The Wilson auto-siever primarily works by gently washing the underside of the sieve with the beneficial consequence that the sieve does not block with fine sediments. With more manual methods this type of blockage was commonly removed by puddling or by applying direct hose pressure to the mesh surface which introduced the possibility of damaging delicate specimens caught on the mesh. The auto-siever increases the

likelihood of procuring intact specimens for later identification and potentially, as was shown in the case study, enhances the recorded diversity of the sample.

The auto-siever improves efficiency in terms of time to process samples in the field with a reduction in the need for two workers to one to process the sample with no statistically significant effect on the resulting data in both the case study and the Humber workshop.

The auto-siever has the potential to improve efficiency in terms of time spent analysing the samples in the laboratory inferred from the good condition of the specimens in both the case study and Humber workshop. However, this was not directly measured.

The auto-siever has the potential to wash through juvenile and meiofaunal invertebrates reducing the likelihood of skewed data sets due to inefficient sieving technique. It is widely accepted that in some sediments, the retained particles are acting as the mesh rather than the sieve and hence smaller animals are retained that would otherwise be the case.

The auto-siever reduces the ability to introduce early variability into the data set while automating what is generally acknowledged to be a laborious process often performed by the least experienced members of the sampling team.

2.2.5 Recommendations

It is recommended that the principles of the methodology of the Wilson auto-siever be further tested and more widely applied.

Whilst the Humber workshop has concluded that the sample processing methods utilised in the field did not lead to statistically significant differences in the resulting data in the estuarine environment of the Humber, it was shown that in the Northern Ireland Case study some enhancement of diversity was found due to taxonomic features being preserved in a fully marine community type. Further comparative testing is recommended in fully marine waters exhibiting a high diversity.

The principles of the Wilson auto-siever should be considered over the more traditional manual methods for application to all future macrobenthic monitoring programmes, due to the significant advantages described above. However, it is emphasised that such a change of methods may prevent the method being used with temporal monitoring programmes already in progress.

2.3 Intertidal Demonstration

2.3.1 Introduction

The aim of this exercise was for the participants to demonstrate their normal intertidal sampling procedures for soft sediments. The practical demonstrations were then reviewed and a best practice guide devised.

2.3.2 Methods

The field demonstrations took part at two sites in the Humber estuary: one soft silty mud, the other muddy sand. Participants brought a selection of coring devices and demonstrated their use at both locations.

As well as the field demonstrations, questionnaires (40) were sent to laboratories asking a suite of questions relating to sampling equipment, methods of deployment, actual sampling methodologies, what time of year sampling normally took place, etc. Given that only 12 questionnaires were returned, it has been assumed that those laboratories that did not return a questionnaire do not routinely undertake intertidal sediment sampling.

2.3.3 Results

A summary table showing the response to the questionnaire is outlined in Table 9.

Table 9: Summary of Questionnaire returns

	Number	Proportion
Questionnaires posted	40	
Questionnaires returned	12	30%
Regularity of intertidal sampling:		
Routinely/regularly	2	20%
Occasionally	7	58%
(Virtually) Never	3	25%
Respondents who consider that they undertake insufficient sampling to contribute to a consensus of methods	4	33%
Respondents contributing to a consensus of methods	8	67%

The observations during the field demonstrations and the results from the questionnaires were used to derive the following general points:

(i) Equipment

A variety of devices were used, predominantly round corers, but also box core and dug quadrat. Corers were generally inserted to a sediment depth of between 15 and 20cm. The overall length of corer ranged from 30 to in one case 200cm. Bungs were used predominantly for core retention, but plastic caps were also used.

(ii) Sampling and transport of sample

To facilitate sample extrusion from the core a variety of approaches were adopted from wiggling, plunging, shaking and blowing. In general the type and nature of the substratum dictated the method that was used.

Samples were then put into containers that were usually buckets, but also polythene bags. The reasons for choice of containers were weight and number of samples, area of sampling site, client requirements and access to water for on-site sieving.

(iii) Labelling

In general the sample container was labelled by permanent marker pen and internal label, although some laboratories used pre-printed adhesive labels. The majority of laboratories state that the internal label stayed with the sample throughout its lifetime as part of a quality assurance procedure. Other precautions used to avoid lost labels or mislabelling include keeping field logs.

(iv) Sieves and sample processing

Most participants in the workshop preferred to sieve the sample primarily in the field, but some used a combination of laboratory and field processing depending on the location and ease of access to water. Most laboratories thought that the location of the sample site provided an "adequately controlled environment". Seawater was primarily used to sieve the samples and circular sieves were used the most. In many cases, small mud cores would be returned to the laboratory for processing where as larger sand samples would be sieved in the field.

The diameter of the sieves used ranged from 20 - 50cm. Most sieves were manufactured from stainless steel, but brass and nylon were also used. Mesh size ranged from 0.5mm to 2.0mm in some cases, although this will be dependent on the survey objectives, with smaller mesh being used for finer sediments and perhaps estuarine samples which are expected to be dominated by oligochaetes. Square mesh was regarded as a common standard.

Most laboratories used different procedures for sieving mud and sand. Washing was the main procedure used, but also puddling, and the majority of laboratories used a combination of these procedures for mud, and the same for sand. Fluidising the sample while it is in the container before pouring it through the sieve was common to ease sieving and protect organisms from fragmentation.

Saline formalin was used predominantly as a fixative, with the ratio to seawater varying from 1:3 to 1:7 and the solution was buffered to prevent acidification affecting shelled organisms. Most laboratories added a vital stain such as Rose Bengal as standard procedure at this stage to aid sorting.

The above review and intertidal field demonstrations were used to refine the Environment Agency's standard operating procedure for intertidal sampling and equipment operating instruction for hand coring. These procedures are reproduced in full in Appendix 5.

A range of intertidal techniques were demonstrated for field sampling of macrobenthos. A standard operating procedure such as that of the Environment Agency's was generally recommended as best practice.

2.4 Equipment Demonstration

2.4.1 Introduction

The gear demonstration exercise onboard the survey vessel *Water Guardian* was aimed at participants experiencing the use of equipment which they otherwise might not have come across.

A variety of equipment was demonstrated, namely:

Bowers and Connelly Multiple Corer
0.05m² van Veen grab
0.1m² Day grab
Haps corer (0.0143 m^2)
Box corer (0.025 m^2)
Shipek grab
Hamon grab
Wilson Auto-siever
The Buchanan Hopper

Schematic diagrams and pictures of some of the equipment together with their operating instructions are included in Appendix 5 for reference.

There was some discussion at the follow up workshop regarding the length of time required to obtain and process a core sample compared to a grab. However, ICES generally recommend the box corer in preference to a grab sampler. In practice the grab tends to be more cost-effective due to sample turn-around time whereas the box core reduces sampling bias. Further research should focus on an optimal design for a benthic sampler that combines both features. The Shipek grab and Hamon grabs were also demonstrated and tend to be used for gravelly substrata. The multicorer and Craib corer are effective samplers for meiofauna and sediment studies and they produce an undisturbed sample essential for chemical determinands such as depth profiled redox measurements.

The Wilson Auto-siever and the Buchanan Hopper are designed for processing macrobenthic samples onboard survey vessels. A description and the principles of operation for the auto-siever are outlined in Figure 3. The Buchanan Hopper was designed by the Dove Marine Laboratory and is essentially a tilting cradle, which allows a benthic sample to be gradually broken down into a sediment / water suspension. The elutriate is then washed down a chute to an awaiting sieve. The Buchanan Hopper facilitates greater control of sample processing negating the need for any washing directly on the sieve. It also prevents delicate organisms being fragmented by hose pressure on the sieve mesh.

The workshop participants agreed that the gear demonstrations were valuable exercises since the majority of participants had never seen many of the samplers and processing methods before. It is recommended that any future workshops consider the inclusion of such demonstrations to provide an insight into the range of potential equipment available to sample marine benthos.

3 LABORATORY METHODS

3.1 Sub-Sampling Exercise

3.1.1 Introduction

The NMBAQC scheme has set a target standard of an error of +/- 10% for the number of individuals extracted in a macrobenthic sample. One of the aims of the sub-sampling exercise was to assess whether or not this target could be met when applying this standard to samples that had been sub-sampled. This exercise also assessed the variety of sub-sampling techniques currently in use.

A questionnaire requesting details of sub-sampling techniques currently being used was circulated to all participants. Based on the replies, six sub-sampling methods were demonstrated at the workshop, usually by staff from those laboratories routinely employing the technique. After the demonstration, each method was used to split previously prepared samples containing 1000 *Polydora* mixed with *ca*.400ml of peat. Unicomarine Limited prepared twenty-four samples so that there were four samples for each of the sub-sampling methods. As there was insufficient time to sort the sub-samples during the workshop, they were analysed subsequently by a single laboratory.

A report was produced in December 1997, giving details of the results of the analysis of four sub-samples from each method. Following the presentation of the results at the Workshop Results meeting in Hull in February 1998, additional sub-samples produced by each method were analysed.

3.1.2 Methods

The sub-sampling methods varied in complexity and also in the size of sub-samples produced. Each procedure was timed and the time involved in sorting the sub-samples was also recorded. This study was designed to allow a comparison of both the accuracy of each method and also the time required to prepare and analyse the samples.

The apparatus used for each method is shown in Figure 22. Participants took whatever sub-sample proportion they felt appropriate, the majority deciding on eighths. Most of the methods produced two 1/8 sub-samples from each sample, but only one of each pair was analysed at the workshop. Subsequently all sub-samples were analysed to lead to a greater statistical analysis of the resulting data.

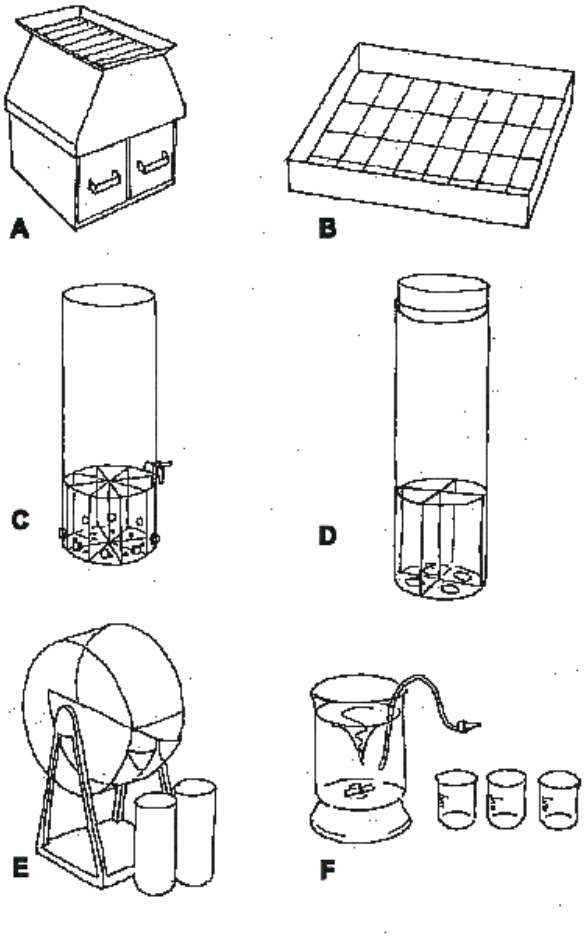


Figure 22: Schematics of the various sub-sampling methods demonstrated at the workshop.

3.1.2.1 Marked Tray

The samples were spread evenly across the surface of a white deep-sided tray marked with 32 rectangles (Figure 22B). With the aid of an illuminated bench magnifier, the material in two randomly selected rectangles was extracted using forceps and pipette to give two 1/32 sub-samples from each sample. These were kept separate, but the data combined to give 1/16 sub-samples from each sample.

3.1.2.2 Riffle Box

The Riffle Box (Figure 22A) contains a number of slots directed alternately into two receiving containers to split the sample in half. Smaller fractions can be obtained by repeating the process. The process was repeated twice to produce two 1/8 sub-samples from each sample.

3.1.2.3 Quarteriser

The samples were placed in a Perspex cylinder sectioned for approximately a third of its length into four equal compartments (Figure 22D). Water was added and the cylinder inverted a number of times to thoroughly mix the sample, which was then left to settle into

the four chambers splitting the sample into four quarters. Smaller fractions can be obtained by repeating the process. Two 1/8 sub-samples were produced from each sample.

3.1.2.4 Aerated Column

The samples were placed in an aerated column, which was approximately 1m in height and made from opaque plastic tubing (Figure 22C). The samples were allowed to settle eight equal chambers. The water above the settlement chambers was then decanted and the sub-samples drained from each chamber. The sub-sampler is modified from Gage (1982). Two 1/8 sub-samples were produced from each sample. In addition, the drained water was also sieved and this fraction retained for analysis.

3.1.2.5 Fulsom Splitter

The samples were placed into the Splitter (Figure 22E), which was rocked gently on its axis to mix them thoroughly. The Splitter was then turned so that the samples were split into two fractions; each being directed into separate receiving containers. Further splits were achieved by repeating this process. Two 1/8 sub-samples were produced from each sample.

3.1.2.5 Magnetic Stirrer - Siphon (Envirnoment Agency, Anglian)

This is probably the most complex of the systems demonstrated, and was developed principally to process samples with a sediment of peat which contain large populations of *Polydora*. The samples were placed in a large beaker, and water added (Figure 22F). These were then mixed using a magnetic stirrer and decanted into a number of measuring cylinders using a siphon. The volume in each cylinder and the volume of the remaining heavy material in the beaker were measured. A number of the sub-samples were analysed, and the total population estimated from the siphoned sub-samples and the remaining heavy fraction. Two sub-samples were produced for each sample. The proportion of each sub-sample varied (see Table 10). In addition, the heavy fraction was also kept separate and analysed.

All the techniques (with the exception of the Marked Tray and Magnetic Stirrer/Siphon) produced two 1/8 sub-samples from each sample, but only one was analysed initially and the results were presented at the Hull discussion of workshop results in February 1998. Additional sub-samples from the Riffle Box, Quarteriser, Aerated Column and Fulsom Splitter were available from the initial exercise. A further 1/8 sub-sample for each of these methods was analysed as before.

The Marked Tray and Magnetic Stirrer/Siphon techniques did not provide additional material, necessitating the sub-sampling process to be repeated on newly prepared *Polydora*/peat samples. The Magnetic Stirrer/Siphon procedure was repeated by the EA Lincoln Laboratory on four samples prepared by Unicomarine Limited. The resulting sub-samples were returned for analysis. Sub-samples approximating to 1/10 were provided for each sample, along with the heavy and the residual fractions. Two 1/10 sub-samples were analysed from each sample (which it was suggested would be the usual approach of the Laboratory. employing the technique), compared with only one previously. The results were recorded for both individual (1/10) and combined (1/5) sub-samples.

The Marked Tray method was repeated at Unicomarine. To allow for a more accurate comparison of the different sub-sampling methods, two additional 1/32 fractions were extracted from each sample, providing sub-samples totalling 1/8. Data were presented for both 1/16 and 1/8 proportions.

3.1.3 Results

The results of the sub-sampling exercise are summarised in Table 10.

Table 10: Summary of sub-sampling data

Method	No. of Sub-samples	Size of Sub-sample	Mean No. of *Polydora* extrapolated to complete sample (+/-SD)	Minimum	Maximum	Mean Processing/ Analysis Time (minutes)
Riffle Box	8	1/8	873 ± 149	768	1192	140
Marked Tray 1/16	8	1/16	1108 ± 240	672	1536	83
Marked Tray 1/8	4	1/8	1176 ± 254	968	1544	151
Aerated Column	8	1/8	870 ± 147	674	1104	177
Quarteriser	8	1/8	982 ± 92	872	1176	99
Fulsom Splitter	8	1/8	896 ± 131	704	1128	110
Siphon 1/5	4	1/5	936 ± 66	874	1030	209
Siphon 1/10 or 1/11	8	1/10 or 1/11	899 ± 75	747	994	189

None of the methods investigated consistently produce average estimates within 10% of the actual number of Polydora present in the samples (Figure 23), and only 41% of all estimates were within this margin. However, the majority of estimates (77%) were within 20% of the known number. Only estimates produced by the Siphon (1/5) and Quarteriser were consistently within 20% of the known number of *Polydora*. Of the 13 estimates with errors >20%, five were produced by the Riffle Box, and three from the Aerated Column.

The smallest ranges of estimates were produced by the Siphon technique (156 for 1/5, and 247 for 1/10), while the largest were associated with the Marked Tray (864 for 1/16, and 576 for 1/8).

Two thirds of all estimates were less than 1000. At least 75% of all Siphon, Riffle Box, Aerated Column and Fulsom Splitter sub-samples underestimated the number of *Polydora*. The Siphon (1/10) consistently produced underestimates. Conversely, the Marked Tray technique predominantly overestimates numbers.

The majority of mean estimates of *Polydora* numbers were less than 1000, with only the Marked Tray technique producing means greater then the actual number (Figure 24).

Only the means for the Quarteriser (982) and the Siphon (1/5) (936) were within 10%, although all the means were within 20%. There is a significant difference within the means (ANOVA: p=0.006, f=2.207, df= 7,48), although theses differences are only between the mean for Marked Tray (1/8) and those for the Aerated Column and Riffle Box (Tukey test for unequal sample sizes: $q_{0.05,\,48,\,8} = 4.521$).

The mean number of *Polydora* produced by sub-sampling altered significantly from the known number ($\chi^2 = 46.49$, $p<0.001$). Analysis of data from each technique indicates that only the Siphon (1/5) had no significant effect on the estimated numbers of *Polydora* ($\chi^2 = 7.82$, $0.05<p<0.10$).

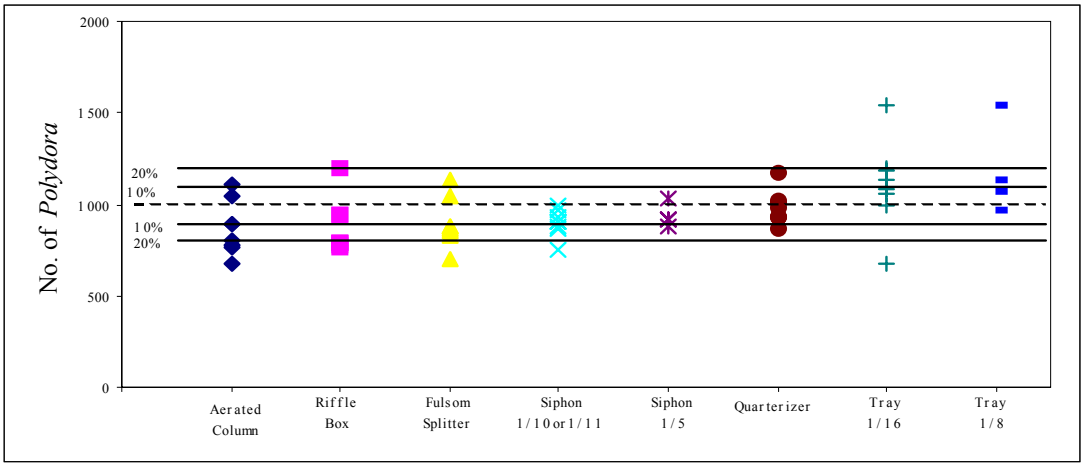


Figure 23: Estimated number of Polydora from each sub-sample.

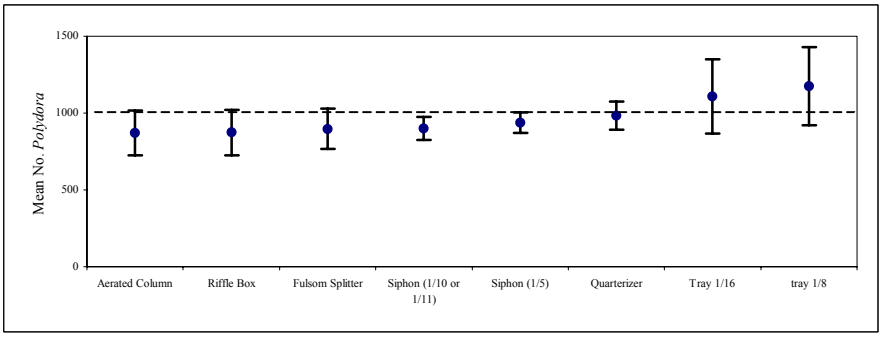


Figure 24: Mean number of Polydora ± SD

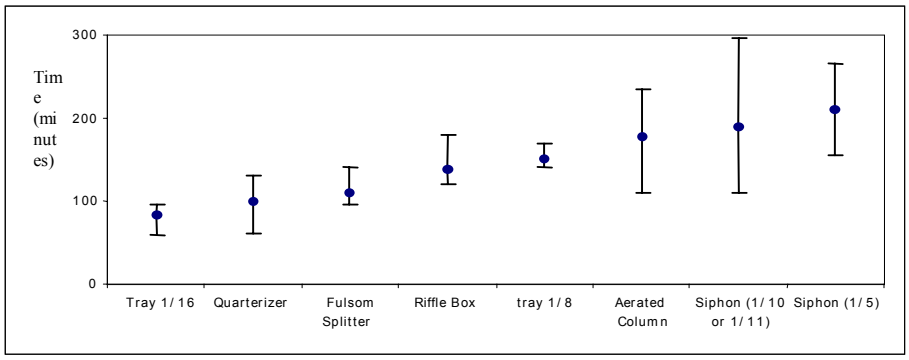


Figure 25: Mean time taken to process and analyse samples produced by each sub-sampling procedure. Vertical lines represent range in processing times.

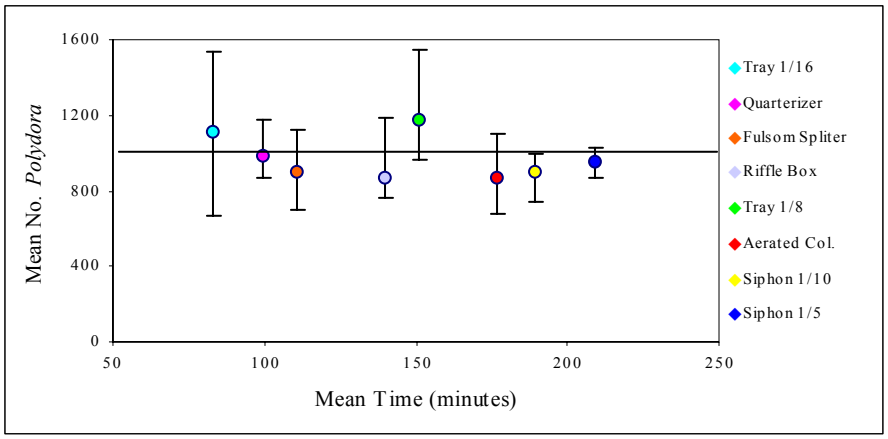


Figure 26: Mean processing and analysis time and mean number of Polydora for each sub-sampling procedure. Vertical lines represent range in estimates of Polydora numbers.

There is a marked difference in the mean times taken to process and analyse the sub-samples produced by each technique (Figure 25). On average, the Marked Tray (1/16) is the fastest (83 minutes), and the Siphon (1/5) is the most time consuming (209 minutes), although there is considerable variation within each technique. It is of note that the more rapid techniques provided estimates with the greatest variation (Figure 26).

3.1.4 Discussion

The workshop exercise achieved its aims of assessing different sub-sampling methods although consideration should be given to the behaviour of the "made up" residue compared to normal estuarine sediment. The sediment of peat created problems for some of the sub-samplers. The prepared sample, which will be lighter than most sieve residues, tended to block the tubing of the Siphon system, and small amounts of material tended to float and cause problems with some of the sub-samplers, particularly the Aerated Column.

This could have been avoided by floating off the lighter peat before the counted *Polydora* were added. However, benthic material is naturally varied and perhaps suitable techniques should be able to cope with this type of problem.

It was often difficult to estimate the time taken to prepare the sub-samples. The Aerated Column process, for example, was time consuming, but it is likely that the operator would normally undertake other work during the time it took for the sample to settle. The Magnetic Stirrer and the Aerated Column took the longest time to process and analyse. Both techniques produced material to be sorted in addition to the created sub-samples (heavy residual fraction from the Magnetic Stirrer, and a 'floaters' fraction from the Aerated Column).

The Marked Tray method was the least time consuming but also the least precise; the population estimates ranged from 672-1200. However, it is of note that the volume of the sub-sample was half that provided by other techniques (1/16 compared with 1/8).

Samples from the Fulsom Splitter, Quarteriser and the Riffle Box all took a similar time to prepare and analyse. These devices each provided sub-samples of 1/8. The Fulsom Splitter and Quarteriser both produced relatively accurate estimates of population size ranging from 848 - 1128 for the Fulsom Splitter and 936 - 1024 for the Quarteriser. However, all four samples from the Riffle Box consistently under-estimated the population (range = 768 - 792). Most of the methods tended to under estimate the population and it seems likely material was being lost during the sub-sampling process. However, it could be that an insufficient number of replicates were examined and by chance those analysed underestimated the population. All the techniques (with the exception of the marked tray) produced two *ca*. 1/8 sub-samples from each sample, but only one was analysed. It may be worthwhile analysing the additional replicates. The high population estimate from the additional Riffle Box replicate indicates that analysis of the additional replicates would be useful.

Protocols need to be developed to determine both when it is acceptable to sub-sample and how to approach the analysis of the rare taxa and perhaps large organisms occurring at low densities in sub-samples. It is possible that the approach adopted with zooplankton, in which there are high numbers of copepods and lower densities of other fauna, would be of benefit. In this case, the sample is scanned to extract the obvious and larger forms and then subsampled for the smaller and abundant forms. Consideration would also need to be given to the proportion of sub-sample that should be taken. Some laboratories using the methods demonstrated did have a set protocol to determine the sub-sample size, which was based on the number of animals found in a certain fraction. Additional protocols also need to agree the volume of the sub-samples should be recorded. Corrections for volume were not made for this study, but the original material is all preserved and volume corrections could be made if required.

The exercise indicated that some of the simpler, less time consuming methods (Fulsom Splitter and the Quarteriser) seemed more accurate than the more complex systems. The techniques that tended to underestimate the population (Aerated Column and Riffle Box) would need further testing before they could be recommended as a standard method.

3.1.5 Conclusions

The most accurate sub-sampling techniques appear to be the Quarterizer and Siphon methods, with the majority of estimates being within 10% of the actual number of *Polydora* in the samples, although the Siphon technique does tend to produce underestimates. Both methods are also relatively precise, producing estimates predominantly within 10% of their means.

The Aerated Column and Riffle Box techniques can be considered as imprecise and inaccurate, as their estimates vary appreciably from the known population of polychaetes. The Marked Tray can also be considered imprecise.

As the Aerated Column and Riffle Box produce significantly different means from the Marked Tray, these sub-sampling techniques should not be employed on the same projects. Furthermore, considering that these three methods were considered as the most imprecise and inaccurate in the present study, their continued use must be carefully considered.

It is apparent that all the sub-sampling methods studied have an effect on the numbers *Polydora* estimated, although, increasing the sample size from 1/10 to 1/5 for the Siphon technique appears to lessen this effect. For both the Quarterizer and 1/10 Siphon, the χ^2 result is raised above the critical level by only one particularly inaccurate replicate. Furthermore, as only four replicates were examined from the 1/5 Siphon, half the number of those analysed from the Quarterizer and 1/10 Siphon techniques, it is unclear whether these methods influence the number of polychaetes any more than the 1/5 Siphon.

The above discussions indicate that scientifically, the best sub-sampling techniques are the Siphon and Quarterizer. However, when considering the time taken to process samples, the Quarterizer is on average twice as rapid as the Siphon technique. This may not appear significant on a small-scale survey, but will become more important where an appreciable number of samples require sub-sampling.

3.1.6 Recommendations

- There should be the development of a protocol for the application of sub-sampling (this should include the nature of the equipment, pre-handling, size of sub-sample and occasions on which it is required).

- Performance limits should be set for sub-sampling techniques e.g. all estimates should be within 20% of the known number, the mean of estimates should be within 10% of known number and all estimates should be within 20% of mean.

- A Quarteriser should be adopted as best practice although the exercise performed here requires to be repeated with different sieve residue types.

- Any adopted sub-sampling technique should be thoroughly tested on the samples to which it is to be applied.

3.2 Biomass Exercise – Workshop Laboratory Exercise and North East Application of the NMBAQC Biomass Standard

3.2.1 Introduction

3.2.1.1 Workshop

Biomass can be determined according to several methods: wet weight by tissue blotting, oven drying, ash-free dry weight, and calorific value. The first of these methods is the most often utilised as a rapid indication of the fresh (live) weight of the organisms. However, by the nature of the method, the data generated are variable. The National Marine Biology Analytical Quality Control scheme has set a total biomass target that the result should be within +/- 20% of the actual value. Results to date illustrated that it was not possible to achieve this standard and the workshop aimed to assess potential sources of error and bias in the method.

During the workshop, participants were asked to record the blotted wet weights of specimens using whatever technique they felt was appropriate and, where possible, the technique they used for their own samples. Their results and descriptions of techniques used were recorded and the notes from the exercise are reproduced in Appendix 6. This was a relatively small exercise compared to the others undertaken at the workshop and it was hoped that the main benefit would be to record the different methods used by participants rather than provide sufficient data for a detailed comparison of techniques. However, a subsequent study undertaken by one of the participants as part of an environmental impact assessment gave a more in depth inter-laboratory comparison and is also discussed here.

3.2.1.2 North East Application of the NMBAQC Biomass Standard

As part of the Comprehensive Studies undertaken under the auspices of the EU Urban Waste Water Treatment Directive, benthic community investigations were required in the vicinity of long sea outfalls to determine the effects of sewage discharges at sea. In the North East the water company, Northumbrian Water Limited were required by the Environment Agency to undertake the studies to a high quality standard. The National Marine Biology Analytical Quality Control standards were applied to the laboratory analyses of the samples collected as part of the studies (see Appendix 6). There were a number of different contractors undertaking the analysis and all samples required a 10% audit. Wet weight biomass was required to species level and in addition, the primary contractor AES LTD undertook a 10% audit on approximately 300 samples.

3.2.2 Methods

3.2.2.1 Workshop Exercise

Prior to the workshop the following specimens were placed in vials with 70% IMS by the organiser.

A. 4x *Macoma balthica*
B. 4 x *Nephtys hombergii*
C. 1 x Oligochaete

D. 4 x *Lanice conchilega*
E. 4 x *Bathyporeia*

During the workshop 12 of the workshop participants were involved in the biomass exercise, 10 provided biomass data for all the specimens.

3.2.2.2 North East Application of the NMBAQC Biomass Standard

Non-destructive measurement of biomass was required in order that auditing could later be carried out on the species and numbers of individuals. The initial instruction given to the laboratories was simply that blotted wet weighing of the extracted material was to be carried out. Biomass estimations were to be made from the blotted weights using the Ash Free Dry Weight Method of Eleftheriou and Basford (1989) employing conversion factors derived in-house during a previous survey.

In practice, it became evident that interpretation of "blotting" could be subject to significant variation. Although the analysts had been instructed not to pierce shells and tests, the vigour with which some analysts undertook blotting was significantly greater than others. Despite being returned to IMS after weighing, some annelids retained the appearance of having been squeezed dry. This phenomenon manifested in the failure of the biomass audits for Laboratory 3. This prompted a more prescriptive biomassing protocol.

The full text of the biomassing protocol is presented in Appendix 8 and here in summary as follows:

Achieve a uniform degree of blotting, by, wherever possible, simply laying them upon a sheet of absorbent tissue and moving them around until they leave no wet marks.

Minimise evaporation of preservative by:

Allowing the minimum time to elapse between completion of blotting and transfer to the balance.

Enclosing the balance pan and sample immediately and note the weight after allowing a fixed time period to elapse (that time should be sufficient for the balance mechanism to stabilise despite evaporation continually occurring; in practice this may be 30 seconds).

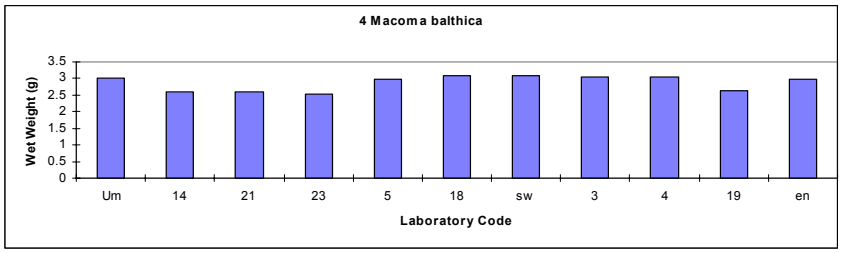
The total biomass target of ± 20% was required.

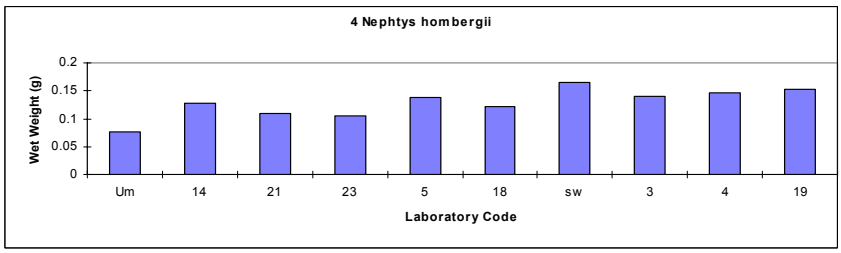
3.2.3 Results and Discussion

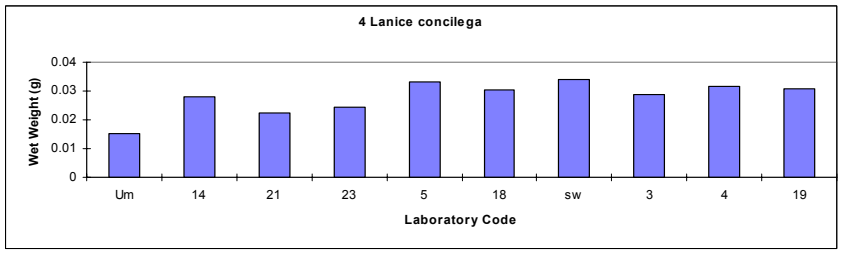
The results of the workshop exercise are presented in Table 11 and shown graphically in Figure 27. There was little difference in the biomass recorded for the *Macoma* sample. The weights of the two polychaete samples made by the demonstrator were lower than those made by the participants. With the *Bathyporeia* and oligochaete sample, only one participant recorded a lower weight than that recorded by the demonstrator.

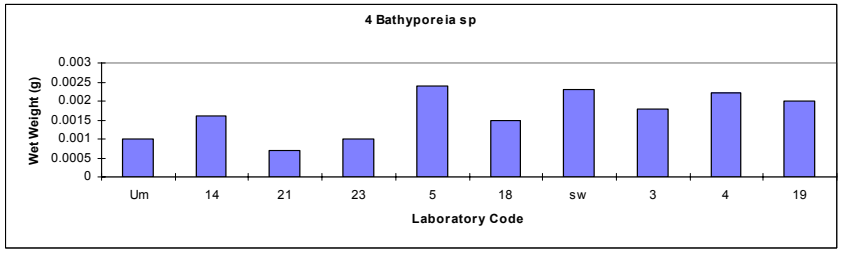
Table 11: Wet weight biomass (g) of each specimen recorded by each participant

Specimens	Taxa	Lab. Code Um	14	21	23	5	18
4	*Macoma*	3.0261	2.6157	2.5895	2.5360	2.9746	3.0760
4	*Nephtys*	0.0763	0.1281	0.1100	0.1053	0.1387	0.1224
1	Oligochaete	0.0004	0.0008	0.0003	0.0005	0.0007	0.0006
4	*Lanice*	0.0152	0.0279	0.0224	0.0246	0.0333	0.0303
4	*Bathyporeia*	0.0010	0.0016	0.0007	0.0010	0.0024	0.0015
		sw	3	4	19	en	
4	*Macoma*	3.0695	3.0534	3.0492	2.6189	2.9708	
4	*Nephtys*	0.1646	0.1400	0.1462	0.1529		
1	Oligochaete	0.0010	0.0005	0.0010	0.0006		
4	*Lanice*	0.0342	0.0290	0.0318	0.0310		
4	*Bathyporeia*	0.0023	0.0018	0.0022	0.0020		









Figures 27: Wet weight biomass (g) of each specimen recorded by each participant

The results of the biomass audits for total biomass are presented in Appendix 8 for the North East study. The species biomass data are shown in Figures 28a-c. The results presented in Figure 28 show that even after prescriptive protocols were adhered to significant differences for individual taxa still occurred.

3.2.4 Discussion

During the workshop there were several differences in the approach to sample weighing. One participant weighed specimens by placing a vial of water on the balance, taring the balance and then adding the specimen. This avoided evaporation, and the usual problem of a gradual reduction in weight as specimens dry out on the balance, but it would still be important to standardise on the amount of drying carried out before the specimen was transferred to the weighing vial. Some participants used weighing boats, and others placed the specimens on filter paper and used a new piece for each weighing. In the NE example the practice was to blot the organism and then weigh on the balance for a set period (30 secs).

At the workshop the main differences in biomass estimates were with the soft-bodied animals. This is perhaps not surprising as bivalve shells can easily be dried thoroughly. Several participants pointed out that they would normally open bivalves or pierce the shell in order to dry the tissue more thoroughly. This was not done during the exercise, as it would have made it difficult for other participants to use the material, hence intervalvular water may have been retained.

It was noted during the exercise that there was great variation in the approach to drying soft-bodied specimens. The demonstrator's approach was to press specimens firmly between tissue paper in order to remove much of the IMS, particularly with polychaetes where liquid could be released from the gut by firm pressing. The demonstrator maintained that using this technique the biomass results tend to be more consistent and the specimens are not damaged, but regain their shape when placed back into IMS. However, most participants dried the specimens much more gently and consequently their weights for all specimens other than *Macoma* tend to be greater than those taken by the demonstrator.

The North East study illustrated similar problems to the Humber Workshop with the greatest differences between workers arising from the degree to which specimens were blotted on paper. However, for total wet weight biomass of a sample it was found that application of the strict protocol by the laboratories resolved most of the total biomass discrepancies once it was put into practice. Once the protocol was adopted only one further failure of the biomass analysis criterion occurred. The failure was remedied by revisiting the samples and repeating the biomass measurements. This case study clearly illustrated the need to specify a strict protocol for wet weight biomass analyses.

The workshop biomass techniques and results were discussed briefly at the Workshop results meeting in Hull in September. The various approaches to drying were discussed, and there was concern by several participants that drying by pressing too firmly between tissue might remove body fluids, which would not be desirable since it would distort the measurement of live weight, producing data between true wet weight and oven dry weight.

Figure 28 a-c. Comparison of auditor wet weight species biomass vs laboratory wet weight biomass.

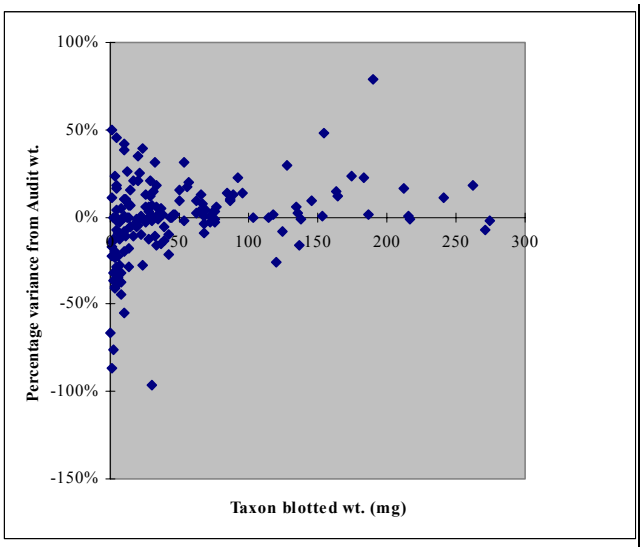


Figure 28a: Laboratory1

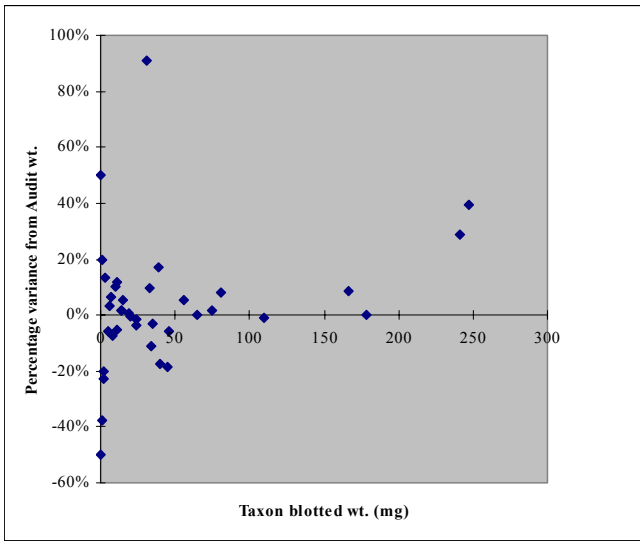


Figure 28b: Laboratory 2

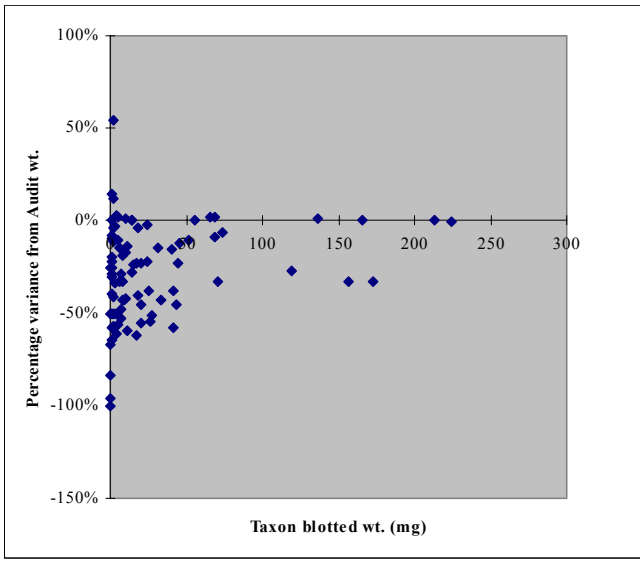


Figure 28c: Laboratory 3

The preservation of biota by freezing and thawing in formalin or in alcohol will also distort the biomass. This requires to be taken into account and where possible the weight change needs to be stabilised before weighing takes place.

3.2.5 Conclusions

Consistent total wet weight biomass measurements are achievable between workers to within +/-20% providing a strict protocol is defined and adhered to.

The protocol for wet weight biomassing requires to be prescriptively standardised to minimise variability, for example by defining the blotting and weighing period after ensuring the weight has been stabilised due to fixing and preservation.

It is not possible to attain consistent individual species wet weights between workers even with a prescriptive and standardised protocol.

3.2.6 Recommendations

It was not possible to attain consistent wet weight biomass between workers at the workshop or in the example case study when weighing individual species. Individual species wet weight analyses, particularly in temporal trend monitoring, should not be undertaken as a routine measurement.

In order to attain consistency and minimise bias between workers, a strict and prescriptive protocol is required for total wet weight biomass. An example of best practice is highlighted in the workshop report from the Aquatic Environment Services case study.

4 PHYSICO-CHEMICAL SAMPLING

The sampling for physico-chemical variables was not dealt with at the field workshop but was discussed in detail at the follow up seminar. The Environment Agency suggested standard sampling methodologies for physical and chemical parameters (from its own procedures) are outlined below in table 12 and these were suggested as best practice.

Table 12: Environment Agency physico-chemical sampling best practice

	Metals	**Organics**	**PSA**	**Organic carbon**	**Redox**
Grab/Core	s/s	s/s	-	-	Undisturbed core
Scoop	Polythene	s/s	-		n/a
Depth	1cm	1cm	1 or 5 cm	1cm	Appropriate depth intervals (minimum 10 cm)
Container	Polythene (not metal)	Glass not plastic	Poly bag (or freezable container)	?	n/a
Storage	Frozen	Frozen	Frozen	Frozen	n/a (analysis *in situ* or in laboratory
Analysis	<63µm	<63µm	Whole, Laser granulometer	Whole, CHN analyser	Every 0.5 to 4cm, then every 1cm

s/s - stainless steel

The majority of scientists use stainless steel grabs although galvanised grabs are used as an alternative. While the latter is suitable for biological samples, chemists have always recommended the use of stainless steel and so, for chemical determinands, the use of stainless steel grab is regarded as best practice.

Given that there is an inherent variability irrespective of which method is used then it is necessary quantify the sources of variability, both field and analytical. Field replication can be achieved by the use of the sampler in different sites within the area studied, i.e. by taking several grab samples and removing one subsample from each grab. This is preferable to taking several subsamples from a single grab as the latter will not represent field variability nor provide an adequate representation of the physico-chemical variable data against which to compare and interpret the faunal data. In contrast, analytical variability will be determined by taking several subsamples from a homogenised sample taken in the field. In particular, laboratory reference material should be used to determine the analytical variability.

If particle size is the only parameter being measured then a subsample can be taken from the faunal grab. Many workers consider it preferable that the particle size sample

is taken from a faunal grab in order that a valid interpretation can be made between the faunal and environmental data. However, the disadvantage to this is the loss of material from the faunal grab and thus the potential distortion of faunal data.

The particle size information is required to interpret both the faunal data as well as the other contaminant data, e.g. for the metals and organics. It should be noted that contaminant levels may be greatest in the surface detrital layers or, through diagenesis, in strata reflecting the contaminants' chemical behaviour. In contrast, the biota will occur throughout the upper substratum layers. Hence any sampling of physico-chemical determinands which produces data integrated throughout a depth greater than that occupied by the fauna will hamper valid interpretation.

The depth of the physico-chemical sample collected needs to relate to these considerations. If only the top layer is sampled and the sediment has an overlying layer of fluid mud over sand underneath, this will be reflected in the community structure but may not be detected just by analysing the very surface layers for the physico-chemical variables. It is important to be aware of sediment layering and take account of this in sediment sampling and the use of sediment descriptors in the field. Corers can be used to observing sediment profiles *in situ* to assess layering.

Depth*:*

This should cover the depth dominantly occupied by the fauna and thus be a minimum of 5cm for the particle size analysis to be of biological relevance. Impact assessments routinely sample the upper 1cm depth for metals and organics although samples at other depths could be taken depending on the nature of the study, for example to detect historical contamination. The majority of laboratories take a 1cm depth.

Grab/Core, samplers and containers:

A stainless steel grab bucket will reduce contamination although sediment should be taken from the centre of the grab to avoid contamination at the sides. Stainless steel or polythene core liners can be used depending on the contaminants being sampled. Decontaminant-washed stainless steel scoops should be used for sampling organics and polythene scoops for metals.

Polythene or glass containers should be used for holding samples for metal analyses and glass (not plastic) ones for organics. Contaminant-washed aluminium foil can be used for preventing plastic lids and seals from coming into contact with the sediment sample. Samples can be stored by deep freezing prior to analysis. Recommendations of the size-fraction of sediment analysed differs between workers and according to reasons for the analysis. The degree of contamination of the silt and clay fraction, material <63μm, may reflect recent inputs although some laboratories sample material <100μm.

Redox:

A depth profile of redox potential denotes the aerobic-anaerobic balance in the sediment, the result of organic enrichment or poor permeability and thus the degree of oxygenation from surface waters. It will reflect the nature of the organic tolerant populations and thus the response to organic pollution. A platinum electrode, either with an inbuilt reference electrode or a separate reference electrode, pushed into the centre of a grab sample will give some indication of the redox potential. However, it is more accurate to take an undisturbed core and mount this in such a way that the probe can be gently pushed into the sediment. In this way the redox potential at each stratum in the sediment, say 0.5mm levels, can be measured thus giving a profile. The redox profile should then be used to identify the Redox Potential Discontinuity (RPD as the 0mV depth) and the Redox level at 4cm (Eh_4) is also used as an indication of the chemical status in the depths most occupied by the infauna (Pearson & Rosenberg 1978). A period in which the probe equilibrates at each level will be required to produce consistent data.

4.1 Summary:

The discussion here of the physico-chemical determinands is designed merely to provide some guidance and information for biologists undertaking surveys rather than a detailed indication of physico-chemical sampling in marine areas. However, it is of note that samples taken to answer chemical or sedimentological questions may not be compatible with biologically-based surveys. It is emphasised that all aspects, biological, chemical and physical, as well as all sources of variability, methodological, field and analytical, require to be quantified and/or minimised.

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APPENDIX 1

Laboratories participating in the subtidal intercomparison exercise

Key to labs:

1 Environment Agency Anglian Region
2 Scottish Environment Protection Agency West
3 Zeneca Limited
4 Environment Agency Southern Region
5 Centre for Environment, Fisheries and Aquatic Science
6 SEAS Limited (using a 1mm mesh)
7 Environment Agency South West Region (used lab 1 grab for both sites)
8 Industrial Research and Technology Unit (used lab 1 grab for both sites)
9 Aquatic Environment Services Limited
10 Environment Agency North East Region (own grab used for Site 1, lab 1 grab for Site 2)
11 Institute of Estuarine and Coastal Studies (using the autosiever, lab 10 grab used for Site 1, lab 1 for Site 2)

N.B. Codes pertain to the subtidal intercomparison exercise only.

APPENDIX 2

		Lab	Station	Replicate	Northings	Eastings	Sample volume	Process Time	Description
#	Anglian	1	1	1	529559	411737	4/5ths	11	2cm soft mud with sand, firm dark mud below
		1	1	2	529583	411728	4/5ths	11	"
		1	1	3	529583	411722	3/4s	11	" + 1 Carcinus maenas
		1	1	4	529587	411731	3/4s	11	"
		1	1	5	529581	411730	3/4s	11	"
#	Anglian	1	2	1	533457	415730	1/3rd	5	Layers of sand and mud
		1	2	2	533467	415726	1/3rd	5	Sandy, little mud, lots of cockle shells
		1	2	3	533472	415745	1/3rd	5	Sandy, little mud, lots of cockle shells
		1	2	4	533457	415738	1/3rd	5	Sandy, little mud, lots of cockle shells
		1	2	5	533462	415728	1/3rd	5	Sandy, little mud, lots of cockle shells
#	SEPA West	2	1	1	529596	411730	Full	17	Cohesive mud, thin sand on top
		2	1	2	529594	411747	Full	17	Cohesive mud, thin sand on top
		2	1	3	529563	411724	Full	17	Cohesive mud, thin sand on top
		2	1	4	529589	411738	Full	17	Cohesive mud, thin sand on top
		2	1	5	529585	411732	Full	17	Cohesive mud, thin sand on top
#	SEPA West	2	2	1	533459	415754	Half	10	Clay with some sand
		2	2	2	533449	415757	3/4s	10	Shelly sand on clay
		2	2	3	533442	415738	Half	10	Muddy sand with shells
		2	2	4	533457	415740	Half	10	Muddy sand with shells
		2	2	5	533443	415735	Half	10	Muddy sand with a top layer of glutinous mud
	Zeneca	3	1	1	529574	411728	15cm	12	Mud
		3	1	2	529594	411715	15cm	12	Mud
		3	1	3	529578	411713	9.5cm	12	Mud on sand
		3	1	4	529589	411741	14.0cm	12	Mud
		3	1	5	529586	411715	11.0cm	12	Mud
	Zeneca	3	2	1	533438	415753	8.0cm	10	Sandy mud
		3	2	2	533435	415756	8.6cm	10	Sandy mud
		3	2	3	533447	415757	10,0cm	10	Sandy mud
		3	2	4	533450	415759	9.0cm	10	Sandy mud
		3	2	5	533447	415753	11.5cm	10	Sandy mud
#	Southern Agency	4	2	1	533451	415732	1/3rd	17	Loose soft clay on surface, broken shell ,clay and some sand below
		4	2	2	533460	415751	half	17	Loose soft clay at surface with sand below and broken shell
		4	2	3	533445	415728	half-1/3rd	17	Loose soft clay at surface with sand below and broken shell
		4	2	4	533445	415753	half	17	Loose soft clay on surface over compact clay with few broken shells
		4	2	5	533444	415728	half	17	Loose soft clay on surface over compact clay with few broken shells
#	Southern Agency	4	1	1	529588	411719	2/3rds	15	Soft mud, clayey, oxic to 1.5cm
		4	1	2	529585	411724	2/3rds	15	Some compacted surface, otherwise as for 4/1/1
		4	1	3	529578	411742	3/4s	15	"
		4	1	4	529588	411742	3/4s	15	Loose clay with some sand
		4	1	5	529599	411746	2/3rds	15	"
#	CEFAS	5	2	1	533443	415766	6	30	Fluid mud over shelly sandy mud, sheel stuck in jaws some washed out
		5	2	2	533445	415762	6	30	Shelly sandy mud
		5	2	3	533451	415746	5.5	30	Fluid mud over shelly sandy mud
		5	2	4	533444	415752	9	30	Veneer of fine sand over shelly mud
		5	2	5	533453	415760	9	30	Fluid mud over darkened sandy mud

#	CEFAS	5	1	1	529578	411731	12.5	14	Slightly fluid mud over sandy mud, blackened at depth
		5	1	2	529583	411741	12.5	14	"
		5	1	3	529570	411730	12.5	14	"
		5	1	4	529574	411739	11.5	14	"
		5	1	5	529582	411736	8	14	Slightly sandy mud over solid clay
	SEAS	6	1	1	529574	411722	10l	21	Thick black clay
		6	1	2	529598	411713	9.5l	21	"
		6	1	3	529572	411733	9.0l	21	"
		6	1	4	529579	411734	9.0l	21	"
		6	1	5	529610	411724	9.0l	21	"
	SEAS	6	2	1	533430	415749	4.0l		Mud and clay, fluid layer
		6	2	2	533447	415746	7.0l		Sand, mud clay, fluid layer
		6	2	3	533445	#####	6.0l		Sand with shells, dark streaks, fluid layer
		6	2	4	533453	415737	5.0l		Fine sand on top going down more compact, sand mud below, fluid layer
		6	2	5	533433	415739	6.5l		"
#	SW Agency	7	2	1	533447	415752	2/3s	16	1cm very soft brown mud (v. thin veneer of sand) over thickish grey mud/ sand mix - drained one side
		7	2	2	533450	415751	half	16	As above but more shells on surface
		7	2	3	533462	415747	<half	16	Muddy sand over slighlty muddy sandy gravelly mix
		7	2	4	533451	415740	<1/3rd	16	1cm of very soft over 3cms of muddy gravel over 1cm of muddy sand
		7	2	5	533440	415729	1/3rd	16	As above
#	SW Agency	7	1	1	529594	411724	3/4s	16	1cm of very soft brown mud over firmer grey mud
		7	1	2	529578	411729	3/4s	16	"
		7	1	3	529590	411728	3/4s	16	"
		7	1	4	529574	411714	3/4s	16	" firmer mud
		7	1	5	529574	411714	3/4s	16	"
#	ISC/IRTU	8	2	1	533429	415724	3/4s	30	Shelly mud on top of sand
		8	2	2	533439	415726	1/3rd	30	Shelly mud on top of sand
		8	2	3	533439	415727	half	30	Top layer of fine mud over sand, shelly
		8	2	4	533423	415733	1/3rd	30	Soft brown mud over darker grey sand
		8	2	5	533446	415738	1/3rd	30	"
#	ISC/IRTU	8	1	1	529571	411715	3/4s	13	Thick claggy mud, grey mud covered with a brown layer
		8	1	2	529587	411726	3/4s	13	"
		8	1	3	529594	411733	3/4s	13	As above but less brown surface
		8	1	4	529592	411721	3/4s	13	Layer of brown surface, black at bottom
		8	1	5	529588	411710	3/4s	13	"
	AES	9	1	1	529570	411724	12.0cm	21	Silty mud
		9	1	2	529562	411712	11.5cm	21	Silty mud
		9	1	3	529569	411725	12.5cm	21	Silty mud
		9	1	4	529573	411730	11.0cm	21	Silty mud
		9	1	5	529575	411719	9.0cm	21	Silty mud
	AES	9	2	1	533427	415744	7.0cm	4	Shelly sand
		9	2	2	533446	415733	6.0cm	4	Shelly sand
		9	2	3	533452	415743	7.0cm	4	Shelly sand
		9	2	4	533440	415737	5.0cm	4	Shelly sand
		9	2	5	533444	415741	7.0cm	4	Shelly sand
#	NE Agency	10	1	1	529573	411732	13.0cm	14	Clay - mud, <1cm RPD ,anoxic mud
		10	1	2	529573	411729	13.0cm	14	Clay - mud, <1cm RPD ,anoxic mud
		10	1	3	529567	411721	10.5cm	14	Clay - mud, <1cm RPD ,anoxic mud
		10	1	4	529586	411732	12.5cm	14	Clay - mud, <1cm RPD ,anoxic mud
		10	1	5	529579	411727	9.0cm	14	Clay - mud, <1cm RPD ,anoxic mud

#	NE Agency	10	2	1	533441	415743	7.0cm	8	Thin fine mud over shelly sand
		10	2	2	533439	415739	6.5cm	8	Thin fine mud over shelly sand
		10	2	3	533447	415728	5.5cm	8	Thin fine mud over shelly sand
		10	2	4	533437	415753	8.5cm	8	Thin fine mud over shelly sand
		10	2	5	533428	415741	9.5cm	8	Thin fine mud over shelly sand
	IECS	11	1	1	529588	411725	12.0cm	10	Silty mud
		11	1	2	529559	411715	10.5cm	10	Silty mud with small amount of sand
		11	1	3	529594	411737	11.5cm	10	Silty mud
		11	1	4	529580	411728	10.0cm	10	Silty sand (lg lump of wire removed some sediment from grab)
		11	1	5	529579	411736	9.5cm	10	Silty sand
	IECS	11	2	1	533430	415753	6.5cm	6	Mud over sand shell
		11	2	2	533448	415726	8.0cm	6	Mud under sand silt
		11	2	3	533459	415741	6.5cm	6	Sand/shell/clay
		11	2	4	533435	415736	6.5cm	6	Sand/shell/clay
		11	2	5	533436	415748	6.5cm	6	Shells/ sandy mud

APPENDIX 3a

	1a	1b	1c	1d	1e	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	4d	4e	5a	5b	5c	5d	5e	6a	6b	6c	6d	6e	7a	7b	7c	7d	7e	8a	8b	8c	8d	8e	9a	9b	9c	9d	9e	10a	10b	10c	10d	10e	11a	11b	11c	11d	11e
Platyhelminthes indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	8	0	7	1	0	0	0	0	0
Phoronis sp. indet (muelleri)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gattyana cirrosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pholoe sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Eteone longa/flava	5	7	3	2	4	0	2	1	7	2	6	1	4	6	1	4	2	3	2	6	1	1	7	3	4	0	1	0	5	2	4	4	1	5	5	1	3	2	1	0	2	1	1	6	4	1	4	4	1	0	6	2	1	4	8
Phyllodoce mucosa	3	0	1	0	1	0	3	0	3	0	0	0	1	0	0	1	2	0	1	2	1	1	2	0	2	0	0	0	0	0	2	1	1	4	0	1	0	0	1	0	3	1	1	0	1	5	1	5	5	1	2	3	0	2	7
Glycera alba	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Juvenile Glycera sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaerodoridae sp indet	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Nereis virens	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1	2	0	0	1	0	0	1	0	1	1	1	0	0
Nereis longissima	1	1	4	3	0	0	0	0	2	1	2	0	0	2	1	0	0	4	2	1	2	0	1	1	0	1	0	2	0	1	0	1	2	5	2	0	1	4	2	0	2	1	1	2	1	0	1	2	0	3	0	1	2	2	2
Nephtys caeca	2	0	0	2	1	0	0	0	1	0	1	0	0	1	1	1	1	0	1	1	0	0	0	0	3	0	0	2	0	0	0	1	0	0	0	0	0	1	2	0	0	1	1	0	0	1	1	0	0	0	2	2	1	0	0
Nephtys hombergii	1	2	0	4	2	2	6	0	3	0	3	0	0	3	0	5	0	0	0	7	0	5	0	0	0	0	1	1	3	0	4	1	2	0	0	2	3	0	0	0	2	1	3	2	0	0	2	0	0	0	1	3	2	0	1
Nephtys longosetosa	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	2	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Juvenile Nephtys sp. indet	0	12	0	1	1	1	1	0	0	0	1	2	0	6	0	0	0	3	10	1	8	6	5	2	1	0	1	0	1	0	0	2	4	0	4	0	8	0	10	1	3	1	4	4	0	1	6	0	0	2	0	0	0	5	0
Scoloplos armiger	4	16	5	1	2	9	5	7	2	1	0	11	12	9	16	4	3	8	0	16	20	9	20	0	47	3	0	2	1	0	2	11	12	4	0	0	28	8	12	0	5	3	5	2	5	11	10	16	4	2	8	4	2	2	10
Aricidea minuta	5	4	0	1	4	3	3	1	0	0	2	0	0	0	0	4	0	1	2	16	8	4	8	0	17	2	1	0	0	0	0	2	4	10	0	0	32	20	0	0	3	0	0	0	0	8	8	16	0	12	2	0	1	0	0
Polydora ciliata	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pygospio elegans	40	80	8	18	11	50	50	27	29	15	46	30	10	75	32	36	44	74	102	48	44	116	160	34	26	10	20	41	15	4	46	56	32	76	8	53	104	80	100	0	43	9	46	84	37	160	76	72	34	70	4	17	51	41	10
Spio martinensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spiophanes bombyx	12	24	16	14	3	1	2	4	10	0	8	1	6	15	6	8	1	11	14	16	12	26	44	12	24	0	1	1	2	1	14	16	6	16	0	0	20	8	10	0	4	1	2	8	3	64	20	16	0	6	3	3	3	2	0
Streblospio shrubsoli	9	4	24	2	3	1	4	0	1	0	0	7	4	0	0	0	2	7	10	0	4	8	0	0	7	0	4	2	1	0	6	8	6	12	0	5	60	32	2	0	1	0	0	0	0	24	48	40	15	12	0	1	1	1	0
Aphelochaeta/Tharyx spp.	629	###	###	637	219	148	334	922	975	35	882	126	177	###	574	###	64	317	###	###	###	###	###	###	347	14	165	227	332	7	###	###	###	###	###	267	###	###	360	0	291	253	446	812	279	###	###	###	67	504	360	148	318	###	317
Capitella capitata	0	0	8	0	2	1	2	32	2	3	4	3	0	9	6	4	2	0	12	6	8	2	0	4	3	0	0	1	2	1	6	4	6	8	4	1	16	4	0	0	3	2	0	0	2	8	4	4	2	2	0	0	1	1	1
Mediomastus fragilis	3	4	8	1	2	3	1	0	1	1	4	0	0	3	2	4	2	6	12	17	32	20	12	0	7	1	1	4	0	0	10	4	14	16	0	1	24	40	2	0	1	3	2	0	1	24	0	16	4	16	2	0	1	5	0
Myriochele oculata	0	0	1	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sabellaria spinulosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Amparete acutifrons	1	0	2	0	1	3	0	0	3	0	1	1	0	3	0	1	0	1	2	3	1	1	1	1	2	1	0	0	1	0	10	2	0	2	0	0	0	1	1	0	0	0	0	0	0	1	0	2	2	0	1	0	1
Juvenile Ampharete sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	2	0	0	2	1	0	0	0	1	0	0	0	3	0	
Lanice conchilega	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tubificoides benedii	1	4	0	0	0	1	3	0	0	0	0	2	1	0	2	4	1	0	0	0	0	6	4	6	3	2	1	0	0	0	0	2	2	0	0	2	8	0	0	0	3	0	0	0	0	4	4	7	4	0	0	5	1	2	
Tubificoides pseudogaster	7	4	24	4	2	10	4	2	2	0	6	1	0	6	14	0	0	2	26	16	28	6	48	8	5	2	0	3	0	0	0	24	10	18	0	4	44	28	0	0	0	0	0	0	0	48	32	52	5	6	2	0	2	11	4
Tubificoides swirencoides	22	44	16	8	11	42	9	1	26	7	6	53	34	6	4	8	7	19	12	32	16	8	48	22	23	3	18	3	2	0	14	30	32	44	4	16	148	112	30	27	24	6	8	20	7	16	68	80	18	18	9	2	2	7	3
Tubificidae sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Harpacticoid copepod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Praunus inermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Bathypoeia pilosa	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corophium volutator	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Eurydice pulchra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0			
Carcinus maenas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Achelia echinata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
Pycnogonum littorale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Juvenile Retusa obtusa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Juvenile Mytilus	1	0	2	2	1	1	1	0	4	4	2	2	6	0	44	0	5	1	0	3	12	4	0	0	15	1	2	0	0	0	6	18	6	0	32	0	0	8	10	1	3	0	10	2	2	2	1	2	26	7	7	11	12	27	106
Juvenile Caridae sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Macoma balthica	4	0	0	4	1	1	1	0	4	1	5	1	5	6	4	0	0	2	2	0	0	0	4	2	6	8	3	1	0	0	2	6	16	8	0	0	0	4	6	1	6	0	2	4	5	4	3	1	1	3	1	2	4	4	5
Abra alba	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
Juvenile Abra sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	8	2	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0
Petricola pholadiformis	0	4	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	3	1	0	0	0	1	0	0	2	2	0	1	0	0	2	0	0	0	0	0	1	1	1	1	1	0	0	0	1	3
Mya truncata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	4	0	0	0	0	2	0	0	0	0	0	1	1	1	0	0	4
Juvenile Hiatella arctica	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Juvenile bivalvia sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ophiura ophiura	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 3b

	1a	1b	1c	1d	1e	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	4d	4e	5a	5b	5c	5d	5e	6a	6b	6c	6d	6e	7a	7b	7c	7d	7e	8a	8b	8c	8d	8e	9a	9b	9c	9d	9e	10a	10b	10c	10d	10e	11a	11b	11c	11d	11e
Nemertea sp. indet	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platyhelminthes indet	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	3	2	0	0	0	0	0	0
Sipuncula sp indet	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eteone longa/flava	0	2	0	2	1	1	1	0	1	4	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	2	1	0	1	0	0	1	1	1	0	0	1	0	0	1	2	0	1	0	2	0	1	1	0	2	0	1	2
Phyllodoce mucosa	2	1	5	4	6	0	11	9	5	5	7	13	9	1	4	5	2	6	1	11	4	5	3	4	6	2	8	6	4	12	3	2	6	11	1	6	3	4	1	5	11	6	1	11	7	11	15	9	5	8	6	6	1	3	5
Glycera alba	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaerodoropsis minuta	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4	2	2	2	1	0	0	2	0	2	2	2	0	1	0	1	0	2	0	0	0	0	0
Streptosyllis websterii	0	0	6	6	4	0	1	0	0	2	0	0	0	0	0	0	8	0	0	0	3	4	0	0	1	0	0	0	0	0	1	0	0	2	0	0	1	0	0	0	0	0	1	0	0	16	3	0	0	0	0	0	0	0	0
Autolytus sp. (edwardsi)	0	0	0	12	0	0	6	0	0	4	0	0	0	1	0	0	2	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	2	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Nereis longissima	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nephtys caeca	0	3	3	2	0	0	3	1	1	1	0	0	1	0	0	0	2	0	1	0	0	3	1	0	1	1	1	0	1	1	1	1	0	4	1	0	0	0	0	0	1	0	1	0	3	1	1	0	1	0	0	0	0	0	0
Nephtys hombergii	8	3	1	8	12	8	6	9	10	6	9	6	3	6	5	6	7	6	5	6	3	3	3	12	2	13	5	8	7	13	8	7	15	1	9	6	11	14	9	8	7	5	8	9	9	6	8	9	9	11	6	9	6	11	10
Nephtys longosetosa	0	0	2	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Juvenile Nephtys sp. indet	1	4	1	0	3	1	1	2	1	1	0	0	2	4	2	4	0	2	0	4	0	2	0	0	2	0	0	1	3	0	0	0	8	4	0	3	3	6	0	0	10	0	2	0	4	3	3	5	2	5	0	1	2	4	0
Scoloplos armiger	5	15	21	28	38	2	41	10	12	31	16	8	13	6	3	16	30	6	2	6	21	12	7	3	7	3	2	4	10	9	5	15	8	13	21	8	1	9	16	1	17	14	12	26	19	8	36	8	16	9	4	1	6	6	3
Aricidea minuta	5	4	6	38	12	3	21	15	13	8	8	8	22	3	1	12	10	8	7	5	4	16	3	8	13	0	0	1	5	2	5	11	6	10	4	2	2	2	2	4	22	7	2	18	18	27	25	12	11	13	0	0	0	0	2
Polydora ciliata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pygospio elegans	27	6	6	34	14	20	3	4	7	31	40	20	0	10	8	16	6	38	6	57	14	3	9	7	14	2	5	10	0	0	15	4	32	0	20	92	4	4	2	16	6	9	7	2	4	4	18	21	23	21	42	8	2	6	5
Spio martinensis	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Spiophanes bombyx	39	66	160	118	140	38	149	207	97	88	74	24	222	51	12	46	68	74	12	85	62	69	21	14	84	44	19	24	169	222	38	68	56	790	86	50	48	144	60	96	294	89	79	300	298	149	190	60	48	96	27	20	11	30	38
Streblospio shrubsoli	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	3	1	0	1	0	0	0	0
Spionidae sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Dispio sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scalibregma inflatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aphelochaeta/Tharyx spp.	28	8	6	46	30	9	32	16	9	24	16	3	16	5	5	22	28	20	2	27	13	40	15	5	11	1	2	7	4	6	10	7	30	12	36	6	11	8	0	38	12	7	14	16	14	10	37	40	23	63	8	4	2	4	16
Capitella capitata	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0
Mediomastus fragilis	11	4	4	38	4	1	9	2	1	3	0	3	0	0	2	4	2	10	1	3	4	3	3	0	2	2	1	0	0	0	1	0	8	6	4	4	0	0	0	6	0	0	2	4	8	7	10	14	2	9	0	0	1	2	1
Sabellaria spinulosa	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Juvenile Sabellaria sp. indet	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		
Lagis koreni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pectinariidae sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amparete acutifrons	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	3	0	0	0
Lanice conchilega	0	0	0	2	1	0	0	0	0	0	0	0	9	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0	0
Tubificoides benedii	4	4	2	4	6	0	3	2	1	3	0	1	0	0	1	6	6	8	0	1	2	1	0	1	2	0	0	1	1	0	1	0	0	2	2	0	1	0	2	2	0	0	0	2	6	0	3	3	10	2	0	1	0	0	4	1
Tubificoides pseudogaster	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Tubificoides swirencoides	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubificidae sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Enchytraeidae sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Harpacticoid copepod	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Harpinnia antennaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphipod indet	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corophium volutator	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eurydice pulchra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tanaissus liljeborgi	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
Crangon crangon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0
Crangon allmani	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Juvenile Carcinus maenas	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Isotomidae sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phoronis muelleri	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrobia ulvae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Retusa obtusa	1	0	8	0	0	0	13	1	0	3	1	0	0	0	0	0	1	0	0	0	2	0	1	2	1	0	5	1	2	0	1	1	4	6	0	0	2	0	0	0	0	2	11	6	8	7	4	3	1	0	4	0	0	1	0
Juvenile Mytilus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Cerastoderma edule	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ensis sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macoma balthica	2	4	5	5	1	1	13	1	3	4	1	2	1	1	2	1	5	4	2	5	11	8	1	2	1	1	1	2	7	1	0	4	2	4	3	0	5	4	1	4	0	2	10	5	3	7	8	1	1	2	3	1	4	7	3
Abra alba	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ensis sp. indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Petricola pholadiformis	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	3	0	0	2	0	
Juvenile Mya truncata	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0		

APPENDIX 4

Site 1

```
            SIMILARITY PERCENTAGES (SIMPER)
            ===============================


SOURCE DATA FILE : A:\SITE1.PM1

SITE1

NUMBER OF SPECIES (ROWS) IN DATA SET =  53
NUMBER OF COLUMNS IN DATA SET        =  55

NO SPECIES REDUCTION

SPECIES NAME FILE : A:\SITE1.TXT


GROUP   SIZE   COLUMN NUMBERS
-----   ----   --------------
   1      5    1-5
   2      5    6-10
   3      5    11-15
   4      5    16-20
   5      5    21-25
   6      5    26-30
   7      5    31-35
   8      5    36-40
   9      5    41-45
  10      5    46-50
  11      5    51-55

DOUBLE SQUARE-ROOT TRANSFORMATION
BRAY-CURTIS SIMILARITY

Value for percentage cutoff =   90.0


AVERAGE DISSIMILARITY BETWEEN GROUPS  5 &  2 =  35.36

               GROUP  5   GROUP  2
               ========   ========
SPECIES    NO  AV ABUN    AV ABUN     AV TERM   RATIO  PERCENT    CUM
%
-------------------------------------------------------------------------
---------------------
Aphel spp.  23 1133.80     482.80        2.98    1.24    8.44     8.44
Spiop bomb  21   23.60       3.40        1.96    1.47    5.55    13.99
Medio frag  25   14.20       1.20        1.95    1.83    5.51    19.50
Juven Neph  15    4.40        .40        1.81    1.66    5.11    24.60
Tubif bene  31    3.80        .80        1.64    1.32    4.65    29.25
Arici minu  17    7.40       1.40        1.64    1.41    4.64    33.89
Tubif pseu  32   19.00       3.60        1.60    1.15    4.52    38.41
Scolo armi  16   19.20       4.80        1.58    1.63    4.48    42.89
Juven Myti  44    6.20       2.00        1.53    1.40    4.33    47.22
Streb shru  22    3.80       1.20        1.45    1.24    4.10    51.32
```

AVERAGE DISSIMILARITY BETWEEN GROUPS 6 & 1 = 42.81

SPECIES	NO	GROUP 6 AV ABUN	GROUP 1 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	149.00	941.00	5.11	1.51	11.94	11.94
Tubif pseu	32	1.00	8.20	2.40	1.48	5.62	17.56
Spiop bomb	21	1.00	13.80	2.22	2.01	5.18	22.74
Tubif swir	33	5.20	20.20	2.15	1.19	5.01	27.75
Streb shru	22	1.40	8.40	2.01	1.20	4.69	32.44
Arici minu	17	.60	2.80	1.82	1.33	4.25	36.69
Scolo armi	16	1.20	5.60	1.73	1.18	4.04	40.73
Macom balt	46	2.40	1.80	1.60	1.17	3.75	44.48
Medio frag	25	1.20	3.60	1.59	1.16	3.72	48.20
Capit capi	24	.80	2.00	1.54	1.27	3.60	51.79

AVERAGE DISSIMILARITY BETWEEN GROUPS 6 & 5 = 48.10

SPECIES	NO	GROUP 6 AV ABUN	GROUP 5 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	149.00	1133.80	5.48	1.69	11.39	11.39
Tubif pseu	32	1.00	19.00	2.89	1.78	6.01	17.41
Spiop bomb	21	1.00	23.60	2.60	2.70	5.40	22.81
Scolo armi	16	1.20	19.20	2.55	1.71	5.30	28.11
Medio frag	25	1.20	14.20	2.38	1.57	4.95	33.06
Arici minu	17	.60	7.40	2.15	1.59	4.46	37.53
Tubif swir	33	5.20	23.40	2.09	1.22	4.34	41.87
Juven Neph	15	.40	4.40	2.02	1.65	4.20	46.07
Juven Myti	44	.60	6.20	1.88	1.29	3.91	49.99

AVERAGE DISSIMILARITY BETWEEN GROUPS 7 & 6 = 47.75

SPECIES	NO	GROUP 7 AV ABUN	GROUP 6 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	1580.40	149.00	6.95	1.87	14.56	14.56
Juven Myti	44	12.40	.60	2.76	1.34	5.77	20.33
Tubif pseu	32	10.40	1.00	2.15	1.35	4.50	24.84
Tubif swir	33	24.80	5.20	2.13	1.26	4.45	29.29
Medio frag	25	8.80	1.20	2.09	1.47	4.38	33.67
Spiop bomb	21	10.40	1.00	2.05	2.07	4.29	37.96
Streb shru	22	6.40	1.40	1.87	1.34	3.92	41.88
Macom balt	46	6.40	2.40	1.81	1.21	3.78	45.66
Capit capi	24	5.60	.80	1.80	1.67	3.78	49.44

AVERAGE DISSIMILARITY BETWEEN GROUPS 8 & 4 = 43.78

SPECIES	NO	GROUP 8 AV ABUN	GROUP 4 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	1087.80	1069.00	5.39	.94	12.32	12.32
Tubif pseu	32	15.20	8.80	2.15	1.19	4.92	17.24
Pygos eleg	19	67.40	60.80	2.14	.66	4.89	22.13
Arici minu	17	10.40	4.60	2.13	1.48	4.86	26.99
Streb shru	22	19.80	3.80	2.07	1.29	4.74	31.73
Spiop bomb	21	7.60	10.00	2.01	1.00	4.59	36.33
Scolo armi	16	9.60	6.20	2.00	1.10	4.57	40.89
Medio frag	25	13.40	8.20	1.80	1.10	4.11	45.00
Capit capi	24	4.20	4.80	1.73	1.12	3.95	48.95

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 1 = 32.86

SPECIES	NO	GROUP 9 AV ABUN	GROUP 1 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Tubif pseu	32	.00	8.20	2.98	4.53	9.07	9.07
Streb shru	22	.20	8.40	2.64	2.42	8.03	17.10
Aphel spp.	23	416.20	941.00	2.18	1.55	6.64	23.75
Arici minu	17	.60	2.80	1.80	1.49	5.49	29.24
Juven Neph	15	2.40	2.80	1.40	1.38	4.27	33.51
Capit capi	24	1.40	2.00	1.39	1.17	4.22	37.72
Macom balt	46	3.40	1.80	1.34	1.13	4.06	41.79
Juven Amph	29	1.00	.00	1.26	1.19	3.83	45.62

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 2 = 35.32

SPECIES	NO	GROUP 9 AV ABUN	GROUP 2 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	416.20	482.80	2.43	1.41	6.89	6.89
Tubif pseu	32	.00	3.60	2.25	1.84	6.38	13.27
Juven Neph	15	2.40	.40	1.68	1.35	4.75	18.03
Capit capi	24	1.40	8.00	1.63	.99	4.62	22.65
Phyll muco	6	1.20	1.20	1.50	1.38	4.25	26.89
Arici minu	17	.60	1.40	1.46	1.14	4.13	31.03
Nerei long	11	1.40	.60	1.42	1.36	4.03	35.05
Petri phol	49	.00	.80	1.42	1.16	4.01	39.07
Nepht homb	13	1.60	2.20	1.40	1.12	3.97	43.03
Juven Amph	29	1.00	.00	1.39	1.18	3.95	46.98
Macom balt	46	3.40	1.40	1.35	1.19	3.83	50.81

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 3 = 32.74

		GROUP 9	GROUP 3				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	416.20	923.00	2.49	1.46	7.59	7.59
Tubif pseu	32	.00	5.40	2.36	1.74	7.22	14.81
Juven Myti	44	3.40	10.80	1.58	1.08	4.81	19.62
Capit capi	24	1.40	4.40	1.52	1.21	4.65	24.27
Nepht homb	13	1.60	1.20	1.47	1.25	4.48	28.76
Phyll muco	6	1.20	.20	1.45	1.48	4.44	33.20
Tubif bene	31	.60	1.00	1.40	1.18	4.26	37.46
Juven Neph	15	2.40	1.80	1.40	1.18	4.26	41.73
Streb shru	22	.20	2.20	1.37	.90	4.19	45.91
Juven Amph	29	1.00	.00	1.33	1.19	4.07	49.98
Scolo armi	16	4.00	9.60	1.29	1.62	3.95	53.93

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 5 = 37.72

		GROUP 9	GROUP 5				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Tubif pseu	32	.00	19.00	3.38	4.43	8.97	8.97
Aphel spp.	23	416.20	1133.80	2.43	1.83	6.44	15.42
Arici minu	17	.60	7.40	2.06	1.61	5.47	20.89
Medio frag	25	1.40	14.20	1.86	1.88	4.93	25.82
Tubif bene	31	.60	3.80	1.83	1.46	4.84	30.66
Ampar acut	28	.00	1.20	1.82	10.50	4.82	35.48
Scolo armi	16	4.00	19.20	1.56	1.91	4.15	39.63
Streb shru	22	.20	3.80	1.56	1.23	4.13	43.76
Juven Myti	44	3.40	6.20	1.52	1.32	4.03	47.79
Nepht homb	13	1.60	1.00	1.50	1.66	3.98	51.78

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 6 = 42.93

		GROUP 9	GROUP 6				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	416.20	149.00	3.65	1.12	8.50	8.50
Phyll muco	6	1.20	.00	2.07	1.87	4.81	13.31
Juven Myti	44	3.40	.60	2.01	1.28	4.68	17.99
Tubif swir	33	13.00	5.20	1.99	1.06	4.64	22.62
Juven Neph	15	2.40	.40	1.92	1.34	4.47	27.09
Macom balt	46	3.40	2.40	1.86	1.15	4.33	31.42
Scolo armi	16	4.00	1.20	1.79	1.10	4.17	35.60
Pygos eleg	19	43.80	18.00	1.60	1.33	3.72	39.31
Streb shru	22	.20	1.40	1.57	1.15	3.65	42.96
Juven Amph	29	1.00	.20	1.51	1.13	3.52	46.49
Eteon long	5	2.80	1.60	1.49	1.21	3.48	49.96
Nepht homb	13	1.60	1.00	1.47	1.06	3.42	53.39

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 7 = 34.71

SPECIES	NO	GROUP 9 AV ABUN	GROUP 7 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	416.20	1580.40	3.41	2.16	9.82	9.82
Streb shru	22	.20	6.40	2.09	1.79	6.01	15.83
Tubif pseu	32	.00	10.40	1.97	1.19	5.67	21.50
Juven Myti	44	3.40	12.40	1.68	1.21	4.84	26.34
Medio frag	25	1.40	8.80	1.61	1.62	4.65	30.99
Ampar acut	28	.00	2.80	1.43	1.09	4.12	35.11
Capit capi	24	1.40	5.60	1.42	1.25	4.08	39.19
Arici minu	17	.60	3.20	1.41	1.14	4.05	43.24
Spiop bomb	21	3.60	10.40	1.38	1.41	3.97	47.21
Macom balt	46	3.40	6.40	1.30	1.01	3.75	50.96
Juven Amph	29	1.00	.40	1.16	1.10	3.34	54.30

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 8 = 45.70

SPECIES	NO	GROUP 9 AV ABUN	GROUP 8 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	416.20	1087.80	4.93	.89	10.79	10.79
Streb shru	22	.20	19.80	2.55	1.63	5.59	16.37
Pygos eleg	19	43.80	67.40	2.48	.79	5.42	21.79
Tubif pseu	32	.00	15.20	2.16	1.18	4.72	26.51
Scolo armi	16	4.00	9.60	2.12	1.26	4.63	31.14
Spiop bomb	21	3.60	7.60	2.01	1.25	4.41	35.55
Medio frag	25	1.40	13.40	1.78	1.22	3.90	39.45
Capit capi	24	1.40	4.20	1.61	1.14	3.52	42.98
Juven Myti	44	3.40	3.80	1.61	1.30	3.52	46.50
Arici minu	17	.60	10.40	1.59	.94	3.47	49.97

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 1 = 29.71

SPECIES	NO	GROUP 10 AV ABUN	GROUP 1 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	1130.20	941.00	2.24	1.30	7.53	7.53
Capit capi	24	4.00	2.00	1.44	1.58	4.86	12.39
Tubif bene	31	3.80	1.00	1.43	1.31	4.81	17.20
Petri phol	49	1.00	.80	1.41	3.44	4.74	21.94
Platy inde	1	3.20	.00	1.39	1.13	4.68	26.62
Nepht homb	13	.40	1.80	1.34	1.46	4.49	31.11
Arici minu	17	8.80	2.80	1.29	1.34	4.34	35.45
Pygos eleg	19	82.40	31.40	1.27	1.59	4.28	39.73
Spiop bomb	21	21.20	13.80	1.23	1.05	4.15	43.88
Medio frag	25	12.00	3.60	1.17	1.84	3.94	47.82
Juven Neph	15	1.80	2.80	1.17	1.23	3.93	51.75

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 2 = 34.44

SPECIES	NO	GROUP 10 AV ABUN	GROUP 2 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	1130.20	482.80	3.15	1.46	9.15	9.15
Streb shru	22	27.80	1.20	2.74	2.05	7.95	17.10
Tubif pseu	32	28.60	3.60	1.88	1.34	5.46	22.56
Spiop bomb	21	21.20	3.40	1.87	1.42	5.43	27.98
Medio frag	25	12.00	1.20	1.74	1.73	5.06	33.05
Arici minu	17	8.80	1.40	1.74	1.53	5.05	38.10
Tubif bene	31	3.80	.80	1.59	1.34	4.61	42.71
Phyll muco	6	3.40	1.20	1.56	1.44	4.53	47.24

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 3 = 34.53

SPECIES	NO	GROUP 10 AV ABUN	GROUP 3 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	1130.20	923.00	2.91	1.57	8.43	8.43
Streb shru	22	27.80	2.20	2.63	2.13	7.60	16.04
Arici minu	17	8.80	.40	2.13	1.73	6.16	22.20
Phyll muco	6	3.40	.20	1.80	2.28	5.21	27.41
Medio frag	25	12.00	1.80	1.78	1.38	5.15	32.55
Tubif pseu	32	28.60	5.40	1.71	1.25	4.95	37.50
Petri phol	49	1.00	.00	1.66	11.23	4.81	42.31
Platy inde	1	3.20	.00	1.46	1.12	4.22	46.54
Spiop bomb	21	21.20	7.20	1.38	1.28	3.99	50.52
Tubif bene	31	3.80	1.00	1.26	1.39	3.64	54.16

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 6 = 48.79

SPECIES	NO	GROUP 10 AV ABUN	GROUP 6 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	1130.20	149.00	4.90	1.69	10.03	10.03
Tubif pseu	32	28.60	1.00	3.16	1.97	6.48	16.51
Streb shru	22	27.80	1.40	2.97	1.98	6.08	22.59
Phyll muco	6	3.40	.00	2.51	4.08	5.14	27.72
Tubif swir	33	40.00	5.20	2.43	1.36	4.98	32.71
Spiop bomb	21	21.20	1.00	2.27	1.98	4.66	37.36
Arici minu	17	8.80	.60	2.25	1.66	4.61	41.98
Medio frag	25	12.00	1.20	2.17	1.51	4.46	46.43
Juven Myti	44	7.60	.60	2.07	1.34	4.25	50.68
Pygos eleg	19	82.40	18.00	1.93	1.78	3.96	54.64

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 9 = 38.17

		GROUP 10	GROUP 9				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Tubif pseu	32	28.60	.00	3.61	4.62	9.46	9.46
Streb shru	22	27.80	.20	3.47	3.86	9.09	18.54
Aphel spp.	23	1130.20	416.20	2.70	2.09	7.08	25.62
Arici minu	17	8.80	.60	2.16	1.65	5.66	31.28
Tubif bene	31	3.80	.60	1.77	1.48	4.63	35.91
Petri phol	49	1.00	.00	1.70	12.00	4.45	40.36
Medio frag	25	12.00	1.40	1.65	1.79	4.32	44.68
Spiop bomb	21	21.20	3.60	1.58	1.75	4.13	48.81
Platy inde	1	3.20	.00	1.49	1.13	3.90	52.71

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 1 = 32.96

		GROUP 11	GROUP 1				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	470.20	941.00	2.27	1.53	6.89	6.89
Juven Myti	44	32.60	1.20	2.21	1.94	6.71	13.60
Streb shru	22	.60	8.40	1.71	1.58	5.18	18.78
Arici minu	17	.60	2.80	1.44	1.40	4.37	23.15
Spiop bomb	21	2.20	13.80	1.43	1.47	4.35	27.49
Juven Neph	15	1.00	2.80	1.40	1.17	4.25	31.75
Mya t trun	50	1.20	.00	1.23	1.18	3.73	35.48
Capit capi	24	.60	2.00	1.22	1.23	3.71	39.19
Medio frag	25	1.60	3.60	1.22	1.18	3.71	42.90
Tubif bene	31	1.60	1.00	1.22	1.13	3.70	46.59
Phyll muco	6	2.80	1.00	1.21	1.23	3.67	50.26

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 4 = 36.38

		GROUP 11	GROUP 4				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	470.20	1069.00	2.84	1.52	7.80	7.80
Juven Myti	44	32.60	1.80	2.42	1.71	6.66	14.46
Tubif pseu	32	3.80	8.80	1.68	1.38	4.62	19.08
Medio frag	25	1.60	8.20	1.62	1.37	4.45	23.54
Macom balt	46	3.20	.80	1.56	1.46	4.29	27.82
Capit capi	24	.60	4.80	1.53	1.44	4.20	32.02
Arici minu	17	.60	4.60	1.53	1.34	4.20	36.22
Nepht homb	13	1.40	2.40	1.47	1.58	4.04	40.25
Streb shru	22	.60	3.80	1.42	1.44	3.91	44.17
Juven Neph	15	1.00	2.80	1.42	1.14	3.89	48.06
Spiop bomb	21	2.20	10.00	1.29	1.59	3.56	51.62

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 5 = 37.35

SPECIES	NO	GROUP 11 AV ABUN	GROUP 5 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	470.20	1133.80	2.37	1.47	6.34	6.34
Juven Myti	44	32.60	6.20	1.96	1.19	5.24	11.58
Juven Neph	15	1.00	4.40	1.93	2.08	5.17	16.75
Medio frag	25	1.60	14.20	1.90	1.58	5.09	21.85
Spiop bomb	21	2.20	23.60	1.80	2.12	4.83	26.68
Arici minu	17	.60	7.40	1.76	1.67	4.70	31.38
Tubif pseu	32	3.80	19.00	1.47	1.18	3.94	35.33
Scolo armi	16	5.20	19.20	1.42	1.61	3.81	39.14
Nepht homb	13	1.40	1.00	1.41	1.65	3.77	42.91
Pygos eleg	19	24.60	76.00	1.36	1.41	3.63	46.53
Streb shru	22	.60	3.80	1.35	1.54	3.61	50.15

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 8 = 48.60

SPECIES	NO	GROUP 11 AV ABUN	GROUP 8 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	470.20	1087.80	4.58	.96	9.42	9.42
Juven Myti	44	32.60	3.80	2.47	1.37	5.09	14.51
Pygos eleg	19	24.60	67.40	2.47	1.12	5.08	19.59
Tubif swir	33	4.60	66.60	2.24	2.39	4.61	24.20
Streb shru	22	.60	19.80	2.10	1.63	4.31	28.51
Tubif pseu	32	3.80	15.20	1.99	1.40	4.09	32.61
Scolo armi	16	5.20	9.60	1.95	1.18	4.02	36.62
Spiop bomb	21	2.20	7.60	1.83	1.33	3.76	40.38
Medio frag	25	1.60	13.40	1.81	1.34	3.72	44.10
Arici minu	17	.60	10.40	1.67	1.17	3.44	47.54
Juven Neph	15	1.00	3.80	1.66	1.19	3.41	50.95

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 9 = 33.06

SPECIES	NO	GROUP 11 AV ABUN	GROUP 9 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Tubif pseu	32	3.80	.00	2.08	1.86	6.28	6.28
Juven Myti	44	32.60	3.40	2.06	1.30	6.23	12.51
Juven Neph	15	1.00	2.40	1.76	1.54	5.33	17.84
Aphel spp.	23	470.20	416.20	1.37	1.20	4.16	22.00
Tubif bene	31	1.60	.60	1.34	1.12	4.07	26.06
Mya t trun	50	1.20	.40	1.29	1.18	3.89	29.95
Juven Amph	29	.60	1.00	1.27	1.17	3.84	33.79
Ampar acut	28	.80	.00	1.22	1.17	3.69	37.48
Capit capi	24	.60	1.40	1.20	1.18	3.62	41.11
Medio frag	25	1.60	1.40	1.17	1.13	3.54	44.65
Nepht caec	12	1.00	.40	1.16	1.11	3.52	48.16
Pygos eleg	19	24.60	43.80	1.16	1.32	3.49	51.66

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 10 = 36.44

SPECIES	NO	GROUP 11 AV ABUN	GROUP 10 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Aphel spp.	23	470.20	1130.20	2.63	1.78	7.20	7.20
Streb shru	22	.60	27.80	2.59	2.98	7.10	14.30
Arici minu	17	.60	8.80	1.85	1.76	5.08	19.38
Medio frag	25	1.60	12.00	1.74	1.53	4.78	24.16
Tubif pseu	32	3.80	28.60	1.74	1.36	4.76	28.92
Spiop bomb	21	2.20	21.20	1.71	1.70	4.69	33.61
Tubif swir	33	4.60	40.00	1.56	2.00	4.28	37.89
Pygos eleg	19	24.60	82.40	1.41	1.61	3.88	41.77
Platy inde	1	.00	3.20	1.40	1.12	3.84	45.61
Nepht homb	13	1.40	.40	1.29	1.46	3.55	49.17
Capit capi	24	.60	4.00	1.27	1.38	3.49	52.66

Site 2

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            SIMILARITY PERCENTAGES (SIMPER)
            ===============================


SOURCE DATA FILE : A:\SITE2.PM1

SITE2

NUMBER OF SPECIES (ROWS) IN DATA SET =  59
NUMBER OF COLUMNS IN DATA SET        =  55

NO SPECIES REDUCTION

SPECIES NAME FILE : A:\SITE2.TXT


GROUP   SIZE   COLUMN NUMBERS
-----   ----   --------------
   1      5    1-5
   2      5    6-10
   3      5    11-15
   4      5    16-20
   5      5    21-25
   6      5    26-30
   7      5    31-35
   8      5    36-40
   9      5    41-45
  10      5    46-50
  11      5    51-55

DOUBLE SQUARE-ROOT TRANSFORMATION
BRAY-CURTIS SIMILARITY

Value for percentage cutoff =   90.0


AVERAGE DISSIMILARITY BETWEEN GROUPS  3 &  1 =  34.79

               GROUP  3   GROUP  1
               ========   ========
SPECIES    NO  AV ABUN    AV ABUN     AV TERM   RATIO  PERCENT    CUM
%
-------------------------------------------------------------------------
---------------------
Medio frag  27    1.00      12.20        2.68    1.66    7.71     7.71
Tubif bene  34     .40       4.00        2.26    1.93    6.49    14.20
Strep webs   8     .00       3.20        1.96    1.19    5.62    19.82
Spiop bomb  20   76.60     104.60        1.74    1.62    5.01    24.83
Nepht caec  11     .20       1.60        1.63    1.17    4.69    29.52
Pygos eleg  18   15.60      17.40        1.54    1.12    4.42    33.93
Eteon long   4     .20       1.00        1.47    1.13    4.23    38.17
Juven Neph  14    1.60       1.80        1.42    1.13    4.09    42.26
Lanic conc  33    1.80        .60        1.32     .94    3.78    46.04
Retus obtu  51     .20       1.80        1.28     .89    3.67    49.71
Aphel spp.  25    9.00      23.60        1.28    1.53    3.67    53.38


AVERAGE DISSIMILARITY BETWEEN GROUPS  6 &  1 =  37.13
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		GROUP 6	GROUP 1				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Medio frag	27	.60	12.20	2.93	2.00	7.88	7.88
Pygos eleg	18	3.40	17.40	2.58	1.40	6.95	14.84
Arici minu	16	1.60	13.00	2.54	1.35	6.85	21.69
Tubif bene	34	.40	4.00	2.52	1.74	6.78	28.47
Strep webs	8	.00	3.20	2.05	1.19	5.53	34.00
Juven Neph	14	.80	1.80	1.82	1.21	4.91	38.91
Aphel spp.	25	4.00	23.60	1.81	1.54	4.89	43.80
Spiop bomb	20	95.60	104.60	1.80	1.68	4.84	48.64
Retus obtu	51	1.60	1.80	1.75	1.17	4.72	53.36

AVERAGE DISSIMILARITY BETWEEN GROUPS 6 & 4 = 35.18

		GROUP 6	GROUP 4				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Pygos eleg	18	3.40	24.60	3.11	1.45	8.83	8.83
Arici minu	16	1.60	8.40	2.62	1.35	7.45	16.28
Tubif bene	34	.40	4.20	2.42	1.41	6.87	23.15
Medio frag	27	.60	4.00	2.28	1.63	6.49	29.64
Spiop bomb	20	95.60	57.00	1.98	1.50	5.63	35.28
Juven Neph	14	.80	2.00	1.89	1.13	5.38	40.66
Aphel spp.	25	4.00	19.80	1.89	2.01	5.37	46.03
Retus obtu	51	1.60	.20	1.80	1.09	5.10	51.13
Nepht caec	11	.80	.60	1.55	1.22	4.39	55.52

AVERAGE DISSIMILARITY BETWEEN GROUPS 8 & 1 = 34.31

		GROUP 8	GROUP 1				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Medio frag	27	2.00	12.20	2.67	1.42	7.78	7.78
Isoto sp.	48	2.40	.00	2.39	1.83	6.97	14.75
Strep webs	8	.20	3.20	1.90	1.26	5.53	20.28
Aphel spp.	25	12.60	23.60	1.88	.98	5.47	25.75
Nepht caec	11	.00	1.60	1.70	1.17	4.96	30.71
Tubif bene	34	1.00	4.00	1.66	1.24	4.84	35.54
Pygos eleg	18	23.60	17.40	1.52	1.50	4.43	39.98
Juven Neph	14	2.40	1.80	1.52	1.18	4.43	44.40
Sphae minu	7	1.00	.00	1.49	1.19	4.34	48.74
Scolo armi	15	7.00	21.40	1.49	1.60	4.34	53.08

AVERAGE DISSIMILARITY BETWEEN GROUPS 8 & 2 = 32.90

		GROUP 8	GROUP 2				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Isoto sp.	48	2.40	.00	2.47	1.77	7.52	7.52
Medio frag	27	2.00	3.20	2.10	1.82	6.39	13.91
Nepht caec	11	.00	1.20	1.92	1.90	5.83	19.73
Retus obtu	51	.40	3.40	1.75	1.17	5.32	25.05
Aphel spp.	25	12.60	18.00	1.69	.92	5.14	30.19
Juven Neph	14	2.40	1.20	1.50	1.63	4.56	34.75
Pygos eleg	18	23.60	13.00	1.47	1.21	4.46	39.21
Eteon long	4	.40	1.40	1.47	1.19	4.46	43.66
Sphae minu	7	1.00	.20	1.46	1.13	4.43	48.09
Scolo armi	15	7.00	19.20	1.41	1.58	4.28	52.38

AVERAGE DISSIMILARITY BETWEEN GROUPS 8 & 4 = 32.84

		GROUP 8	GROUP 4				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Isoto sp.	48	2.40	.00	2.60	1.82	7.90	7.90
Medio frag	27	2.00	4.00	2.32	1.53	7.05	14.95
Aphel spp.	25	12.60	19.80	2.08	1.13	6.33	21.28
Tubif bene	34	1.00	4.20	1.94	1.35	5.91	27.20
Pygos eleg	18	23.60	24.60	1.81	1.43	5.52	32.72
Juven Neph	14	2.40	2.00	1.77	1.06	5.39	38.11
Sphae minu	7	1.00	.00	1.61	1.18	4.90	43.01
Eteon long	4	.40	.60	1.27	1.00	3.88	46.89
Scolo armi	15	7.00	12.00	1.24	1.49	3.78	50.67
Spiop bomb	20	79.60	57.00	1.19	1.05	3.63	54.30

AVERAGE DISSIMILARITY BETWEEN GROUPS 8 & 5 = 34.09

		GROUP 8	GROUP 5				
SPECIES	NO	AV ABUN	AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Isoto sp.	48	2.40	.00	2.66	1.81	7.80	7.80
Medio frag	27	2.00	2.40	2.06	1.26	6.03	13.83
Retus obtu	51	.40	1.20	1.97	1.46	5.79	19.62
Juven Neph	14	2.40	.80	1.91	1.12	5.60	25.22
Aphel spp.	25	12.60	16.80	1.83	.93	5.37	30.59
Strep webs	8	.20	1.60	1.68	1.17	4.94	35.53
Sphae minu	7	1.00	.00	1.65	1.18	4.83	40.36
Nepht caec	11	.00	1.00	1.64	1.18	4.81	45.17
Pygos eleg	18	23.60	9.40	1.45	1.23	4.26	49.43
Spiop bomb	20	79.60	50.00	1.42	1.21	4.16	53.59

AVERAGE DISSIMILARITY BETWEEN GROUPS 8 & 6 = 36.03

SPECIES	NO	GROUP 8 AV ABUN	GROUP 6 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Isoto sp.	48	2.40	.00	3.02	1.79	8.37	8.37
Pygos eleg	18	23.60	3.40	2.97	1.23	8.24	16.62
Nepht caec	11	.00	.80	2.37	1.89	6.59	23.20
Juven Neph	14	2.40	.80	2.18	1.12	6.05	29.26
Aphel spp.	25	12.60	4.00	2.07	1.30	5.74	35.00
Spiop bomb	20	79.60	95.60	2.01	1.77	5.59	40.59
Retus obtu	51	.40	1.60	1.99	1.09	5.51	46.10
Medio frag	27	2.00	.60	1.96	1.10	5.44	51.54
Sphae minu	7	1.00	.00	1.86	1.18	5.15	56.70

AVERAGE DISSIMILARITY BETWEEN GROUPS 8 & 7 = 34.11

SPECIES	NO	GROUP 8 AV ABUN	GROUP 7 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Isoto sp.	48	2.40	.00	2.49	1.80	7.31	7.31
Retus obtu	51	.40	2.40	2.07	1.59	6.06	13.37
Nepht caec	11	.00	1.40	2.06	1.92	6.05	19.42
Medio frag	27	2.00	3.80	2.06	1.26	6.04	25.45
Pygos eleg	18	23.60	14.20	2.01	1.45	5.90	31.35
Juven Neph	14	2.40	2.40	1.92	1.11	5.64	36.99
Aphel spp.	25	12.60	19.00	1.77	.91	5.19	42.18
Autol sp.	9	.00	1.00	1.56	1.18	4.58	46.76
Spiop bomb	20	79.60	207.60	1.56	.94	4.58	51.33

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 1 = 31.19

SPECIES	NO	GROUP 9 AV ABUN	GROUP 1 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Retus obtu	51	5.40	1.80	1.99	1.42	6.37	6.37
Tubif bene	34	1.60	4.00	1.93	1.42	6.19	12.56
Medio frag	27	2.80	12.20	1.93	1.16	6.18	18.73
Strep webs	8	.20	3.20	1.71	1.24	5.49	24.22
Spiop bomb	20	212.00	104.60	1.61	1.52	5.16	29.39
Juven Neph	14	3.20	1.80	1.48	1.29	4.75	34.14
Sphae minu	7	1.20	.00	1.44	1.19	4.61	38.74
Nepht caec	11	1.00	1.60	1.32	1.15	4.24	42.98
Eteon long	4	.80	1.00	1.16	1.05	3.72	46.70
Lanic conc	33	.40	.60	1.00	.89	3.21	49.91
Macom balt	55	4.00	3.40	1.00	.93	3.21	53.12

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 4 = 32.61

SPECIES	NO	GROUP 9 AV ABUN	GROUP 4 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Retus obtu	51	5.40	.20	2.45	1.69	7.50	7.50
Spiop bomb	20	212.00	57.00	2.39	1.35	7.34	14.84
Tubif bene	34	1.60	4.20	1.97	1.28	6.03	20.87
Juven Neph	14	3.20	2.00	1.68	1.09	5.16	26.04
Medio frag	27	2.80	4.00	1.62	1.17	4.96	31.00
Sphae minu	7	1.20	.00	1.54	1.19	4.73	35.73
Nepht caec	11	1.00	.60	1.32	1.12	4.06	39.78
Pygos eleg	18	5.60	24.60	1.30	1.28	3.99	43.77
Eteon long	4	.80	.60	1.15	1.00	3.53	47.31
Macom balt	55	4.00	3.40	1.08	.92	3.30	50.61
Scolo armi	15	17.60	12.00	1.00	1.42	3.08	53.69

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 5 = 31.78

SPECIES	NO	GROUP 9 AV ABUN	GROUP 5 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Spiop bomb	20	212.00	50.00	2.70	1.46	8.48	8.48
Juven Neph	14	3.20	.80	1.80	1.09	5.67	14.15
Tubif bene	34	1.60	1.20	1.64	1.40	5.17	19.32
Retus obtu	51	5.40	1.20	1.61	1.47	5.05	24.38
Medio frag	27	2.80	2.40	1.59	1.08	5.01	29.38
Sphae minu	7	1.20	.00	1.57	1.18	4.95	34.34
Strep webs	8	.20	1.60	1.51	1.17	4.74	39.08
Eteon long	4	.80	.00	1.39	1.18	4.39	43.46
Platy inde	2	.40	1.20	1.31	.88	4.13	47.59
Nepht caec	11	1.00	1.00	1.30	1.08	4.10	51.69

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 6 = 34.75

SPECIES	NO	GROUP 9 AV ABUN	GROUP 6 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	13.40	1.60	2.93	1.42	8.44	8.44
Spiop bomb	20	212.00	95.60	2.70	1.53	7.78	16.22
Retus obtu	51	5.40	1.60	2.11	1.24	6.08	22.30
Juven Neph	14	3.20	.80	2.05	1.10	5.90	28.20
Medio frag	27	2.80	.60	1.89	1.23	5.43	33.63
Pygos eleg	18	5.60	3.40	1.85	1.16	5.31	38.94
Sphae minu	7	1.20	.00	1.75	1.18	5.05	43.99
Tubif bene	34	1.60	.40	1.53	1.03	4.40	48.39
Eteon long	4	.80	.60	1.46	1.08	4.19	52.58

AVERAGE DISSIMILARITY BETWEEN GROUPS 9 & 8 = 33.15

SPECIES	NO	GROUP 9 AV ABUN	GROUP 8 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Retus obtu	51	5.40	.40	2.57	1.62	7.74	7.74
Isoto sp.	48	.00	2.40	2.50	1.84	7.53	15.27
Spiop bomb	20	212.00	79.60	2.07	1.56	6.24	21.51
Medio frag	27	2.80	2.00	1.88	1.11	5.67	27.18
Juven Neph	14	3.20	2.40	1.86	1.12	5.62	32.80
Tubif bene	34	1.60	1.00	1.64	1.17	4.96	37.76
Nepht caec	11	1.00	.00	1.57	1.17	4.74	42.50
Aphel spp.	25	12.60	12.60	1.55	.87	4.69	47.19
Arici minu	16	13.40	2.40	1.48	1.62	4.46	51.64

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 2 = 27.87

SPECIES	NO	GROUP 10 AV ABUN	GROUP 2 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Streb shru	21	1.60	.00	2.11	5.20	7.57	7.57
Retus obtu	51	3.00	3.40	1.50	1.24	5.38	12.95
Strep webs	8	3.80	.60	1.37	1.00	4.93	17.88
Platy inde	2	1.20	.00	1.35	1.15	4.86	22.74
Sphae minu	7	.80	.20	1.15	1.10	4.13	26.87
Tubif bene	34	3.60	1.80	1.14	.95	4.09	30.96
Autol sp.	9	.20	2.00	1.09	.94	3.92	34.88
Petri phol	58	.00	.60	1.06	1.19	3.80	38.68
Phyll muco	5	9.60	6.00	1.05	.71	3.75	42.43
Eteon long	4	.80	1.40	1.03	1.04	3.68	46.11
Nepht caec	11	.60	1.20	.97	.96	3.49	49.60
Spiop bomb	20	108.60	115.80	.96	1.32	3.45	53.05

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 5 = 29.50

SPECIES	NO	GROUP 10 AV ABUN	GROUP 5 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Streb shru	21	1.60	.40	1.87	2.09	6.35	6.35
Juven Neph	14	3.60	.80	1.87	1.40	6.34	12.70
Strep webs	8	3.80	1.60	1.63	1.19	5.52	18.21
Spiop bomb	20	108.60	50.00	1.43	1.32	4.83	23.05
Medio frag	27	8.40	2.40	1.36	1.06	4.62	27.67
Platy inde	2	1.20	1.20	1.35	1.10	4.59	32.26
Sphae minu	7	.80	.00	1.32	1.17	4.46	36.72
Eteon long	4	.80	.00	1.29	1.19	4.38	41.11
Tubif bene	34	3.60	1.20	1.25	1.09	4.23	45.33
Retus obtu	51	3.00	1.20	1.15	1.19	3.90	49.23
Aphel spp.	25	34.60	16.80	1.11	1.39	3.76	52.99

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 6 = 37.72

SPECIES	NO	GROUP 10 AV ABUN	GROUP 6 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	17.60	1.60	2.95	1.76	7.81	7.81
Medio frag	27	8.40	.60	2.64	2.10	6.99	14.80
Pygos eleg	18	17.40	3.40	2.52	1.49	6.69	21.49
Aphel spp.	25	34.60	4.00	2.28	2.03	6.05	27.54
Juven Neph	14	3.60	.80	2.14	1.48	5.68	33.22
Streb shru	21	1.60	.20	2.07	2.01	5.49	38.72
Tubif bene	34	3.60	.40	2.01	1.38	5.34	44.06
Spiop bomb	20	108.60	95.60	1.69	1.67	4.48	48.53
Retus obtu	51	3.00	1.60	1.61	1.16	4.26	52.79

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 7 = 29.93

SPECIES	NO	GROUP 10 AV ABUN	GROUP 7 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Juven Neph	14	3.60	2.40	1.82	1.48	6.08	6.08
Streb shru	21	1.60	.40	1.74	2.11	5.81	11.89
Spiop bomb	20	108.60	207.60	1.44	1.16	4.83	16.72
Tubif bene	34	3.60	1.00	1.42	1.14	4.74	21.46
Strep webs	8	3.80	.60	1.40	1.04	4.68	26.14
Platy inde	2	1.20	.00	1.36	1.16	4.56	30.70
Medio frag	27	8.40	3.80	1.25	.96	4.19	34.89
Pygos eleg	18	17.40	14.20	1.21	1.01	4.03	38.92
Autol sp.	9	.20	1.00	1.20	1.15	4.02	42.95
Retus obtu	51	3.00	2.40	1.14	1.11	3.80	46.75
Lanic conc	33	.40	2.00	1.06	.95	3.53	50.28

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 8 = 34.62

SPECIES	NO	GROUP 10 AV ABUN	GROUP 8 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Medio frag	27	8.40	2.00	2.43	1.49	7.00	7.00
Streb shru	21	1.60	.00	2.36	6.17	6.82	13.82
Isoto sp.	48	.00	2.40	2.23	1.86	6.45	20.27
Aphel spp.	25	34.60	12.60	2.08	1.06	6.01	26.28
Retus obtu	51	3.00	.40	1.99	1.56	5.74	32.02
Arici minu	16	17.60	2.40	1.67	3.86	4.82	36.83
Tubif bene	34	3.60	1.00	1.53	1.17	4.43	41.26
Platy inde	2	1.20	.00	1.52	1.17	4.38	45.64
Strep webs	8	3.80	.20	1.44	.91	4.17	49.80
Pygos eleg	18	17.40	23.60	1.43	1.61	4.12	53.93

AVERAGE DISSIMILARITY BETWEEN GROUPS 10 & 9 = 29.50

SPECIES	NO	GROUP 10 AV ABUN	GROUP 9 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Streb shru	21	1.60	.00	2.13	6.28	7.23	7.23
Medio frag	27	8.40	2.80	1.72	1.17	5.83	13.06
Tubif bene	34	3.60	1.60	1.68	1.28	5.70	18.76
Spiop bomb	20	108.60	212.00	1.47	1.52	4.99	23.76
Juven Neph	14	3.60	3.20	1.34	1.17	4.55	28.30
Retus obtu	51	3.00	5.40	1.33	1.12	4.52	32.82
Platy inde	2	1.20	.40	1.30	1.12	4.41	37.23
Strep webs	8	3.80	.20	1.30	.89	4.41	41.65
Sphae minu	7	.80	1.20	1.13	1.08	3.83	45.48
Macom balt	55	3.80	4.00	1.05	1.10	3.57	49.05
Nepht caec	11	.60	1.00	1.05	1.04	3.57	52.61

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 1 = 36.68

SPECIES	NO	GROUP 11 AV ABUN	GROUP 1 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	13.00	3.57	2.58	9.73	9.73
Medio frag	27	.80	12.20	2.53	1.61	6.89	16.62
Spiop bomb	20	25.20	104.60	2.15	1.99	5.87	22.48
Strep webs	8	.00	3.20	2.03	1.19	5.53	28.01
Tubif bene	34	1.20	4.00	1.83	1.21	4.99	33.00
Nepht caec	11	.00	1.60	1.77	1.17	4.81	37.82
Scolo armi	15	4.00	21.40	1.68	1.82	4.59	42.41
Retus obtu	51	1.00	1.80	1.54	1.02	4.19	46.60
Aphel spp.	25	6.80	23.60	1.49	1.56	4.05	50.65

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 2 = 34.53

SPECIES	NO	GROUP 11 AV ABUN	GROUP 2 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	12.00	3.77	2.75	10.93	10.93
Spiop bomb	20	25.20	115.80	2.37	1.82	6.86	17.79
Nepht caec	11	.00	1.20	1.99	1.90	5.76	23.55
Retus obtu	51	1.00	3.40	1.82	1.18	5.28	28.82
Tubif bene	34	1.20	1.80	1.54	1.13	4.46	33.28
Medio frag	27	.80	3.20	1.51	1.14	4.37	37.65
Scolo armi	15	4.00	19.20	1.51	1.90	4.36	42.01
Petri phol	58	1.00	.60	1.48	1.16	4.29	46.30
Eteon long	4	1.00	1.40	1.40	1.10	4.06	50.36

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 3 = 34.90

SPECIES	NO	GROUP 11 AV ABUN	GROUP 3 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	8.40	3.72	2.46	10.65	10.65
Pygos eleg	18	12.60	15.60	2.06	1.39	5.92	16.57
Juven Neph	14	1.40	1.60	1.84	1.10	5.26	21.83
Spiop bomb	20	25.20	76.60	1.83	1.30	5.24	27.07
Eteon long	4	1.00	.20	1.80	1.12	5.17	32.23
Medio frag	27	.80	1.00	1.77	1.17	5.08	37.32
Tubif bene	34	1.20	.40	1.66	1.10	4.75	42.06
Retus obtu	51	1.00	.20	1.41	.87	4.05	46.11
Petri phol	58	1.00	.00	1.31	.79	3.75	49.86
Amphi inde	41	.00	.60	1.19	.80	3.40	53.26

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 4 = 33.90

SPECIES	NO	GROUP 11 AV ABUN	GROUP 4 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	8.40	3.74	2.76	11.03	11.03
Tubif bene	34	1.20	4.20	2.06	1.27	6.07	17.10
Medio frag	27	.80	4.00	1.85	1.23	5.46	22.56
Juven Neph	14	1.40	2.00	1.75	1.13	5.16	27.73
Aphel spp.	25	6.80	19.80	1.55	1.86	4.57	32.29
Pygos eleg	18	12.60	24.60	1.55	1.26	4.57	36.86
Spiop bomb	20	25.20	57.00	1.52	1.95	4.49	41.35
Eteon long	4	1.00	.60	1.46	1.06	4.32	45.67
Retus obtu	51	1.00	.20	1.28	.87	3.78	49.45
Petri phol	58	1.00	.00	1.20	.79	3.55	53.00

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 5 = 35.27

SPECIES	NO	GROUP 11 AV ABUN	GROUP 5 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	8.80	3.73	2.56	10.58	10.58
Retus obtu	51	1.00	1.20	1.89	1.31	5.36	15.95
Strep webs	8	.00	1.60	1.80	1.17	5.11	21.05
Eteon long	4	1.00	.00	1.77	1.15	5.02	26.08
Medio frag	27	.80	2.40	1.77	1.22	5.02	31.10
Juven Neph	14	1.40	.80	1.74	1.09	4.94	36.05
Nepht caec	11	.00	1.00	1.71	1.18	4.85	40.89
Tubif bene	34	1.20	1.20	1.53	1.09	4.34	45.23
Platy inde	2	.00	1.20	1.44	.79	4.08	49.31
Spiop bomb	20	25.20	50.00	1.34	1.73	3.80	53.11

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 7 = 39.60

SPECIES	NO	GROUP 11 AV ABUN	GROUP 7 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	7.20	3.41	2.48	8.60	8.60
Sphae minu	7	.00	2.00	2.86	6.35	7.21	15.81
Spiop bomb	20	25.20	207.60	2.45	1.18	6.18	21.99
Nepht caec	11	.00	1.40	2.14	1.92	5.41	27.41
Retus obtu	51	1.00	2.40	1.92	1.32	4.85	32.26
Juven Neph	14	1.40	2.40	1.90	1.22	4.79	37.05
Medio frag	27	.80	3.80	1.87	1.33	4.72	41.77
Pygos eleg	18	12.60	14.20	1.82	1.56	4.58	46.35
Autol sp.	9	.00	1.00	1.62	1.18	4.10	50.45

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 8 = 34.77

SPECIES	NO	GROUP 11 AV ABUN	GROUP 8 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Isoto sp.	48	.00	2.40	2.97	1.82	8.53	8.53
Arici minu	16	.40	2.40	2.80	1.99	8.06	16.59
Spiop bomb	20	25.20	79.60	2.07	1.73	5.96	22.55
Medio frag	27	.80	2.00	2.03	1.32	5.82	28.38
Juven Neph	14	1.40	2.40	2.02	1.18	5.80	34.17
Aphel spp.	25	6.80	12.60	1.96	1.11	5.63	39.80
Sphae minu	7	.00	1.00	1.83	1.19	5.26	45.06
Pygos eleg	18	12.60	23.60	1.78	1.12	5.12	50.18

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 9 = 37.49

SPECIES	NO	GROUP 11 AV ABUN	GROUP 9 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	13.40	3.96	2.41	10.55	10.55
Spiop bomb	20	25.20	212.00	3.76	2.23	10.03	20.59
Retus obtu	51	1.00	5.40	2.41	1.43	6.44	27.03
Juven Neph	14	1.40	3.20	1.89	1.19	5.04	32.07
Medio frag	27	.80	2.80	1.80	1.30	4.82	36.89
Sphae minu	7	.00	1.20	1.73	1.19	4.62	41.50
Tubif bene	34	1.20	1.60	1.72	1.20	4.60	46.10
Scolo armi	15	4.00	17.60	1.66	2.41	4.43	50.53
Nepht caec	11	.00	1.00	1.63	1.17	4.36	54.89

AVERAGE DISSIMILARITY BETWEEN GROUPS 11 & 10 = 38.21

SPECIES	NO	GROUP 11 AV ABUN	GROUP 10 AV ABUN	AV TERM	RATIO	PERCENT	CUM %
Arici minu	16	.40	17.60	3.92	3.49	10.25	10.25
Medio frag	27	.80	8.40	2.26	1.67	5.92	16.17
Spiop bomb	20	25.20	108.60	2.07	2.02	5.41	21.58
Streb shru	21	.20	1.60	1.98	2.05	5.19	26.77
Aphel spp.	25	6.80	34.60	1.87	1.84	4.89	31.65
Retus obtu	51	1.00	3.00	1.83	1.32	4.80	36.45
Tubif bene	34	1.20	3.60	1.69	1.23	4.41	40.86
Platy inde	2	.00	1.20	1.57	1.16	4.11	44.97
Juven Neph	14	1.40	3.60	1.50	1.15	3.93	48.90
Sphae minu	7	.00	.80	1.44	1.17	3.76	52.66

APPENDIX 5

STANDARD OPERATING INSTRUCTION FOR THE 0.1m² DAY GRAB.

The following is an operating instruction for the 0.1m² Day Grab adopted by the Environment Agency of England and Wales:

1. GENERAL DESCRIPTION

The Day grab (Figure 1) is a remote sampler primarily used to obtain quantitative macrofaunal samples from coastal and estuarine soft sediments. Due to the weight of the grab a boat equipped with a winch and gantry is required for deployment and retrieval. A minimum of three workers are also required, two to man the grab, and one to operate the winch.

The grab consists of two hinged jaw buckets mounted within a supporting pyramid-shaped frame. This design makes the grab more robust and stable so that it is unlikely to upend during use, especially in poor weather conditions. The jaw buckets are fabricated from stainless steel, while the frame may also be constructed from stainless steel or galvanised mild steel. The shape of the jaw buckets is a quarter cylinder, so that when they close they form a large semi-cylindrical bucket.

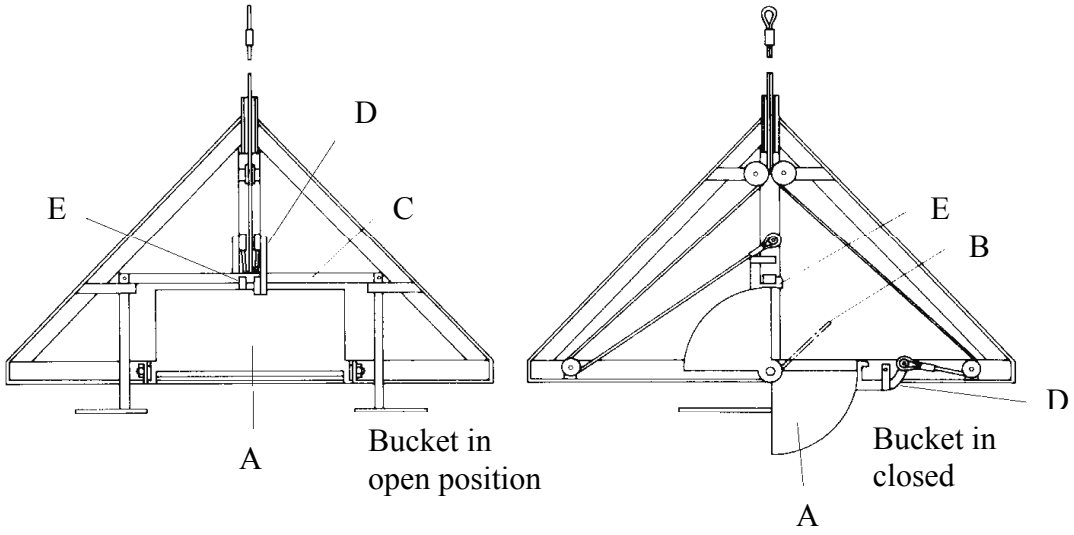

Figure 1: Day Grab. (see text for explanation of annotations)

The grab is lowered in a cocked (open) position. The jaws are held open by a transverse beam connected to two flat plates on the bottom of the sampler. When cocked the surface area of the jaw buckets is $0.1m^2$. As the grab settles on the bottom the plates push the retaining bar up, releasing the jaws, which bite into the sediment. The warp is attached to arms on each jaw bucket, so that the closure of the jaws is completed by mechanical leverage against the grab when hauling commences. The weight of the grab can be altered with lead weights attached to the frame so that efficient penetration can be achieved on a variety of sediments. The upper sides of each jaw bucket are fitted

with lids that are closed throughout deployment. On retrieval these lids can be opened to allow access to the sample. This enables sub-samples to be taken of surface sediments or undisturbed cores, and for observations of the sediment/water interface to be made.

2. OPERATING PROCEDURE

2.1 A total of three workers are required to operate the grab; one controlling the winch, and two working at the grab.

2.2 When inboard the grab must always be positioned on its stand.

2.3 Before deployment, the grab must be cocked, i.e. the jaw buckets (A) are set in the open position (see Figure 1). Before cocking, the lids (B) on the buckets are fastened shut. The two workers on the grab stand either side of the sampler and cock the jaws together. The transverse retaining beam (C) is lifted with one hand, while with the other hand the jaws are pushed open using the bars (D) attached to the buckets. When the buckets are fully opened the retaining beam is dropped into position so that it retains the catches (E) on the buckets and the grab remains cocked.

2.4 The grab is lifted above the level of the transom by the winch and then directed outboard by the hydraulic gantry, while the two workers manually control any swinging. Once clear of the stern, lowering can commence.

2.5 On retrieval, the grab will be lifted above the level of the transom, and brought inboard and lowered onto the stand, all the while being manually guided by two workers.

2.6 Once the grab is securely positioned on the stand and the hydraulics for the winch have been disengaged, the lids can be opened and the sample examined and any sub-samples collected. Any surface water can be drained off by the two workers levering the jaws open very slightly using the bars on the buckets. Alternatively, water can be siphoned off with a length of tubing to minimise loss of flocculent surficial sediments.

2.7 Once all inspection and sub-sampling is completed the grab can be emptied by fully opening the buckets. It must be ensured that there is sufficient slack on the warp to allow full opening of the grab. If the sample does not readily come out, it can be encouraged by vigorously banging the buckets against the retaining beam.

2.8 During transit, or when not in use, the jaws should be secured in the cocked position using the retaining bolts (D).

3. SAFETY

3.1 Suitable personal protection equipment should be worn:

(i) Twin chambered lifejackets must be worn by all staff working on the afterdeck of the survey vessel, alternatively a safety harness may be worn.

(ii) Hardhats must be worn when working with grab.

(iii) Steel toe-cap boots to be worn by all workers involved in operating the grab.

(iv) The wearing of protective gloves is recommended.

3.2 All personnel operating sampling equipment should have received training/instruction from a suitably experienced person in how to operate the gear correctly.

3.3 While the winch is in operation all workers on the afterdeck are responsible for being aware of where the cables are. Stepping over moving cables should be avoided wherever possible.

3.4 All workers on the afterdeck are responsible for being aware of the hydraulic gantry, and must keep away from moving parts when in use.

3.5 Only suitably qualified individuals should operate the winch. All other workers must be aware of which drum is being used, and keep clear of the winch and cables when in operation.

3.6 When the grab is being deployed and retrieved the line of sight of the winch operator must not be obstructed.

3.7 Hands and fingers should never be placed within the frame or open jaws of the grab, or interfere with the warp. When man handling the grab, especially when cocked, workers should guide the sampler with the flat of their hands placed on the outside of the frame.

3.8 If on retrieval the grab has not fired correctly it should be placed on the its stand and re-cocked. Care must be taken when guiding a mis-fired grab as the jaws could close without warning resulting in the grab suddenly dropping 30-40cm.

3.9 Between sites the grab should be positioned securely in its stand with the jaws in the closed position.

3.10 When the grab is not in use, or being transported, the jaws should secured in the cocked position with the retaining bolts fastened through the transverse beam.

3.11 The use of benthic sampling gear in rough weather is extremely dangerous. The decision of whether sampling proceeds is down to the Master of the vessel, as the safety of all on board is his responsibility.

3.12 Only suitably fit and able-bodied individuals should handle heavy sampling gear or lift or move samples. All workers must be suitably trained in lifting techniques and should refer to relevant Health and Safety Manuals.

3.13 Prior to undertaking work on any survey vessel all workers should refer to the Boatwork Code of Practice relevant to the vessel and should be aware of all safety procedures and equipment applicable to the vessel.

HAMON GRAB UTILISATION

Equipment

Hamon Grab(see *Figure 1*)

The grab consists of a square frame forming a stable support for a scoop. On reaching the seabed tension in the wire is released causing the release hook to be released. This allows the lifting arm to rotate through 90° driving the scoop through the sediment. At the end of its movement, the scoop mouth opening locates into a rubber covered steel plate, sealing the scoop completely, and preventing any wash out. The device samples an area of about $0.25m^{2.}$, and penetrates 30cm into the seabed.

Weights can be attached to the grab giving it greater weight with which to penetrate the sediment. Weighting of the grab should be adjusted in order to obtain optimum penetration of the sediment. The grab should not be overfilled as this could lead to loss of material on retrieval.

Grab stand

This structure is made from metal and supports the grab before and after sampling. The stand allows enough space beneath the grab for a box to be placed for sample collection.

Large plastic boxes

Suitable watertight boxes, small enough to be placed under the grab stand hopper but with sufficient capacity to contain the collected sediment and washings before sieving.

Sieve table

30cm diameter Laboratory Test Sieves certified to BS410 (0.5mm, 1.0mm and 2.0mm stainless steel meshes). Choice of sieve will depend on the objectives of the investigation.

Preparation of Equipment

Position the grab and stand beneath the derrick or gantry and attach the wire of the day grab to the boat's winch using a shackle and swivel. Check that the weights are securely fastened by means of split pins.

Set the Hamon grab by pulling the lever arm to horizontal and then insert a retaining bar beneath the release hook trigger plate to prevent the mechanism triggering on board.

Wash the grab through with the deck hose prior to deployment.

Place a clean plastic box under the grab stand hopper.

Deployment and Recovery

When the boat is at the station and has come to a stop raise the grab, remove the retaining bar from beneath the release hook trigger plate and then lower it to the bottom. On approaching the bottom the wire should be let out more slowly to avoid the 'bow wave' produced by the device which could wash away surface material. Once the Hamon grab has reached the sea-bed the wire will go slack and the winch can be stopped. Pause for a moment to allow the scoop of the grab to bite into the sediment, then raise the grab, slowly for the first 5 metres of wire, to maximise the grabs sampling efficiency. When the grab reaches the surface it should be swung onboard as soon as possible as the device presents a danger on a rolling vessel. Lower the grab onto the supportive frame. Enough winch cable should be released to enable the grab to be emptied.

In choppy weather the bows of the vessel should be facing into the sea thus minimising the roll of the vessel and hence swing of the grab during deployment and recovery.

Collection of Samples

Should the scoop of the grab fail to close against the stop plate properly, resulting in the loss of material, then the contents should be discarded and the grab re-deployed.

Slowly release the sediment into the large plastic box, by pulling the lever arm to horizontal.

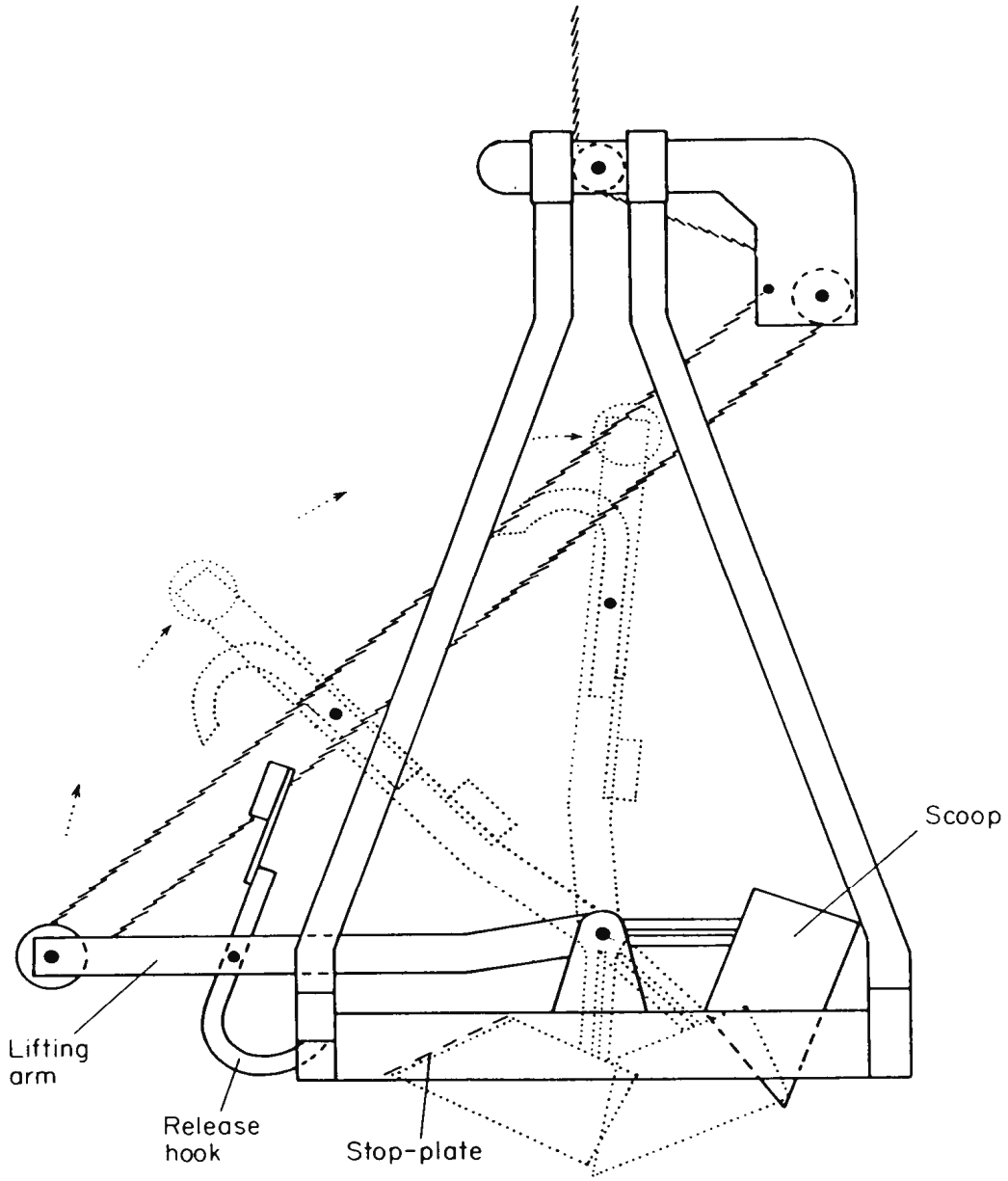

Figure 1: Diagram of the Hamon grab, showing mode of action. The lifting arm rotates through 90° to drive scoop through sediment, closing against the stop plate. Plate taken from Eleftheriou & Holme

0.01m² HAND CORER

1. GENERAL DESCRIPTION

The hand corer is a simple hand held device used for sampling intertidal soft sediments primarily for macrofaunal studies. The corer is made up of a length of 11.3cm internal diameter stainless steel tubing. There are two handles at top of the corer, and a marker on its external surface 15cm from its cutting edge. A rubber bung of appropriate size to fit into one end of the tube is provided. A plunger of the same dimensions as the internal diameter of the corer is also provided.

2. OPERATING PROCEDURE

2.1 Holding the corer by its handles, the sampler should be placed vertically on the sediment surface and pushed straight down until buried up to the 15 cm mark.

2.2 To remove the corer it should be rotated and rocked to overcome the cohesive nature of the surrounding sediment, and then pulled out. In particularly cohesive sediment it may be necessary to free the corer at its base by hand.

2.3 The corer should be removed swiftly after being inserted so that deeper burrowing animals do not escape.

2.4 Where sediments are particularly soft, the bung should be inserted into the top of the corer so that the sample is retained by suction as the sampler is pulled from the sediment.

2.5 Samples can be removed from the corer using the plunger from the underside and pushing the sample out of the top of the corer. However, extremely soft cores will fall out as soon as the bung is released.

COLLECTION, PROCESSING AND PRESERVATION OF MACROINVERTEBRATE SAMPLES FROM ESTUARINE AND COASTAL INTERTIDAL SEDIMENTS FOR COMMUNITY ASSESSMENT.

1. INTRODUCTION

1.1 Purpose

Environmental monitoring programmes are undertaken with the aim of assessing natural conditions and to identify the causes of any changes greater than that related to natural variability, and to identify the causes of change. This information is used in the setting of standards and the effective management of environmental systems. Marine and estuarine waters are managed with the aim to protect the ecosystem as a whole, rather than just the water itself as a potential resource. Such an approach aims to estimate the health of the system while considering both the environment and man's use of it.

Due to the transient nature of water in coastal and estuarine systems, periodic analysis of saline waters provides little useful information applicable to the management of the ecosystem. Obtaining meaningful data from the analysis of seawater is made difficult by the number of solutes occurring naturally, and by the fact that pollutants may be present at concentrations below the limit of detection. Furthermore, simple dissolved concentrations fail to provide comprehensive information concerning the amounts of biologically available material in the system, and give no indication of possible biological impacts. As sediments are the ultimate sink for most materials discharged into marine and estuarine environments, analysis of sediment bound pollutant levels will give a time integrated indication of contamination patterns in the system.

As the majority of marine macrofauna live within the sediment, feeding upon other sediment dwelling organisms, or consuming the sediment itself, they represent a suitable group to target for biomonitoring studies. Since most macrofaunal species are relatively long-lived (>1 year) and sedentary, they also provide a measure of contaminant effects integrated over time. Therefore, biological information, combined with data from sediment and chemical analyses, will provide important indications as to the quality of marine and estuarine environments.

1.2 Principle of Method

Many natural factors will affect the distribution and structure of macrobenthic communities, such as substrate, salinity, currents. Similarly, anthropogenic inputs into marine and estuarine systems can influence benthic communities. Pearson and Rosenberg (1978) describe the community changes observed along an organic pollution gradient. As the level of organic contamination increases diversity tends to fall, and the resulting lack of competition can lead to a proliferation of pollution tolerant species. Chemical pollution is unlikely to favour any particular species, and therefore species number, abundance and biomass will continually decrease toward a chemical discharge (Pearson & Rosenberg, 1978), although patterns will vary depending on the contents of individual effluents (Gray, 1981). In all cases of gross pollution all species are affected, and in extreme cases may be excluded completely. More subtle levels of contamination

may elicit community responses difficult to differentiate from the effects of the natural variability of the system.

The principle underlying the method requires the collection of samples representative of the benthic communities to be studied. Samples should also be taken from reference sites for comparison. Quantitative techniques should be employed to enable direct comparisons between sites to be made. Similarly, methods should be consistent so that any differences observed can be attributed to environmental factors rather than to variation in sampling procedures.

1.3 Sampling Considerations

Although quantitative intertidal benthic samples could be obtained from a boat at high tide they have traditionally been collected by workers accessing sites on foot at low tide. Samples are taken using corers of a suitable size and weight to enable them to be carried over considerable distances. Large box corers of standard area and depth have been used, which can produce considerable amounts of material for processing. Small diameter corers are also used, which, although necessitating the collection of more replicates, will produces less material than the box corer.

Whatever type of sampler is employed, the depth to which samples are obtained is an important factor. Samples taken to a depth of 15cm should sample the majority of fauna. Barnett (1984) noted that taking samples any deeper than 15cm contributed little to the fauna compared with the time taken to sort the extra material, and Smith (1982) found that almost 100% of intertidal fauna in the Humber was present in the surface 10cm of sediment. However, it may be necessary to sample to 40cm in some sediments to obtain all deep dwelling fauna (Wolff, 1987). The appropriate sampling depth for any intertidal work should be determined in a pilot study, or by reference to the literature and/or previous work in the area to be investigated.

The chosen sampling device must be able to sample efficiently in different sediments, and be easily transported by hand over large distances, as must the samples collected. Furthermore, it must be considered whether the surface area sampled and the number of replicates taken adequately address the objectives of the survey. Historically, the Environment Agency (and its predecessors) has predominantly used small hand corers, with large box cores being employed on some occasions (see NRA, 1993).

2. SAMPLING DEVICES

2.1 0.01m² Hand Corer

For operating instruction refer to *0.01m² Hand Corer* operating instruction.

2.2 0.1m² Intertidal Box Corer

For operating instruction refer to *0.1m² Intertidal Box Corer* operating instruction.

4. SAMPLING PROCEDURE

4.1 The location of each site can be determined by reference to fixed landmarks. As anchored structures move with the wind and currents they should not be used as reference points. The compilation of a site directory is recommended for each survey including grid references, latitude/longitude, landmarks (including site markers such as stakes and paint) and photographs. These details should enable other workers to relocate the site on subsequent sampling occasions.

4.2 Different shore heights can be determined at known states of the tide with reference to tide timetables and a chronometer. These sites can be visited successively down the shore on a retreating tide, with low shore sites being sampled one hour before the tide turns. It should be ensured that samples are taken at a consistent tidal height (± 0.2m).

4.3 The type and number of cores required are dependent on the objectives of the survey, habitat and sediment type, and should be determined prior to commencing field work. However, for temporal trends, a minimum of five cores should be collected.

4.4 Generally, samples should be collected using a 0.01m² corer. However, supplementary samples may be required at sites where sediments are predominantly coarse. Methods for the determination of sediment type and the required supplementary sampling techniques are described in Appendix 1

4.5 The sampling device should be inserted vertically into the sediment to provide a representative sample.

4.6 Samples should be transferred to clean water-tight containers. Light and easy to carry containers such as plastic buckets or polythene bags should be used.

4.7 Containers should be labelled with site, replicate number, and date. A suitable waterproof label should also be added to the sample. This label should remain permanently with the sample as a unique identifier that can be used to track the sample for subsequent audit purposes.

4.8 A visual inspection of the sample should be made and observations on colour, smell, texture, depth of Redox Potential Discontinuity (RPD) layer (that part of the sediment where redox potential changes from positive to negative i.e. E_h = 0, which is often characterised by a change in sediment colour to black) and presence of surface features (accretions, algae, fauna, etc.) recorded on the Intertidal Macrofaunal Sampling Record Sheet (Appendix 2) along with any other relevant information. It is recommended that a photograph of the sediment be taken for future reference.

5. TRANSPORT

5.1 Sampling equipment must be light and easy to carry.

5.2 Due to the physically demanding nature of intertidal sampling, the weight which any worker should be expected to carry must be kept to a minimum.

5.2 Where access to sites involves traversing tidal flats equipment and samples should be transported on a handcart or sledge.

5.3 If very large distances are involved, the use of a hovercraft is recommended.

6. PROCESSING AND PRESERVATION

6.1 Samples should be returned to the laboratory for processing. It is recommended that sieving is not done in the field due to the lack of control over conditions, and the associated problem of sample contamination.

6.2 It is recommended that samples are processed within 24 hours of collection, although they may be stored for up to 48 hours if refrigerated. Samples should not be frozen.

6.3 Samples are processed by washing through a sieve with tap water. Sieve mesh size should be decided prior to commencing sampling and will be determined by the objectives of the work and the characteristics of the environment to be sampled. Normally 0.5mm mesh is used for intertidal work, although 1.0mm mesh may be employed for processing coastal samples.

6.4 Sandy sediments can be washed into a sieve over a bucket of water. The mesh of the sieve should kept below the water as the sediment is gently washed from above. By agitating the sample in the sieve the heavier sediment particles will pass through the mesh while the lighter fauna are suspended in the water and retained in the sieve. Care must be taken to prevent clogging of the sieve.

6.5 Muddy samples can be broken down in a bucket with a jet of water and run into a sieve. However, to avoid undue damage to specimens, it is advisable to gently elutriate off the lighter fraction of the sample, which should be transferred to a suitable container. The remainder of the sample can then be washed more vigorously, preferably by “puddling” in a bucket or sink filled with water. Both fractions of the sample can then be recombined.

6.6 Samples should not be washed with a direct jet of water against the mesh. If necessary a jet of water may be applied to the sieve only when the mesh and sample are covered by water, this allows sediment to be washed while the fauna should be suspended in the water and will not impinge on the sieve.

6.7 During sieving large animals should be picked out of the sample using plastic forceps to avoid undue damage. Any stones and large shells should also be removed (and discarded if appropriate) to minimise any grinding effects on the fauna and sieve.

6.8 When possible, all retained material should be washed into an appropriately labelled water-tight container with a gentle stream of water directed from behind the mesh ("backwashing"). This can be facilitated with the use of a funnel. Any fauna enmeshed on the sieve should be removed with forceps.

6.9 It must be ensured that the label added to the sample in the field should remain with the processed sample.

6.10 If the sample is transferred with an appreciable amount of water, this should be decanted off through a sieve. Any material caught on the sieve is then transferred back to the sample with a minimum of water using a wash bottle.

6.11 All processing equipment must be thoroughly washed between samples to avoid cross-contamination. Special attention should be given to sieves which should be scrubbed thoroughly between replicates to avoid gradual clogging of the mesh.

6.12 Samples should be fixed in 5% formaldehyde solution buffered with disodium tetraborate (borax). For muds, a 3:1 ratio of formaldehyde solution volume/sample volume should be used; for sandy samples the ratio should be 2:1. Particularly organic muds will require a 10% formaldehyde solution to ensure that samples are adequately preserved.

6.13 Stain may be added to the sample at this point to facilitate laboratory analysis, although this is left to the discretion of the biologist concerned.

7. QUALITY ASSURANCE

7.1 All personnel participating in sampling should have received training/instruction from a suitably experienced person in how collect and process samples correctly.

7.2 The depth to which samples are taken should be kept consistent. This can be achieved with reference to a suitable marker on the outside of the sampling device.

7.3 When transferring samples from the sampler to receiving containers it must be ensured that no material is lost. If loss occurs then the sample should be rejected.

7.4 The sampling device must be thoroughly washed between sites to avoid cross contamination of samples.

7.5 Waterproof labels clearly stating site name, replicate number, and date should be added to samples immediately after decanting from the sample device. This label should remain permanently with the sample as a unique identifier that can be used to track the sample for subsequent audit purposes. It is recommended that labels should be made of Dymotape, or be written in pencil on waterproof paper.

7.6 Care must be taken to avoid sample loss during sieving. Samples should be replaced if material is lost.

7.7 To avoid damage to specimens during sieving, water pressure should not be too high, and samples should not be shaken vigorously in the sieve. The adoption and consistent use of appropriate sieving techniques can be addressed by correct training being received by all staff prior to commencing processing.

7.8 During the sieving process as soon as any large specimens are observed they should be picked out with plastic forceps and placed in the appropriate sample container.

7.9 All sieves should conform to BS 410 and be replaced at the first signs of damage to the mesh.

7.10 Care must be exercised when transferring washed samples from sieve to container. Any samples where material is lost should be replaced.

7.11 All sample containers should be labelled with waterproof ink stating all relevant information such as site name, replicate number, and date. Both containers and lids should be labelled, but not lids alone as these can become confused between containers or be easily lost.

7.12 When samples are processed using tap water, material should be fixed as soon as possible to maintain specimen quality which can be impaired by prolonged exposure to freshwater.

7.13 Details of all samples collected should be recorded on Intertidal Macrofaunal Sampling Record Sheet (Appendix 2). All records should be made in ink, and any changes initialled and dated.

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APPENDIX 1.

Supplementary Sampling

Principle

In areas where coarse sediments are predominant, 0.01m² cores are likely to underestimate the populations of rare fauna. To compensate for this, an increased number of replicates should be collected. The method of collection of these supplementary samples is dependent on both sediment type and the habitat to be investigated.

Supplementary sampling will not be necessary in areas of fine material as the "Core" method should adequately assess the communities present.

Sediment Classification

In order to determine the general sediment characteristics of a site, any or all of the following measures may be employed:

(i) If a site has been visited in the past, the records made on previous sampling occasions should be referred to. However, when considering this information it must be appreciated that any activity on the shore, especially construction, is likely to affect the sediment characteristics of an area.

(ii) Visual inspection from a distance. This will only give broad scale indications of sediment characteristics, and, where spatial variation is evident, should be used in conjunction with other assessment techniques.

(iii) Visual and physical examination achieved by visiting the survey area and taking sediment samples for *in situ* assessment.

(iv) A pilot study of particle size to assess median grain size carried out by a simple sieving exercise. As a general rule coarse sediments are those which are retained by a 500μm mesh sieve

Habitat Classification

Although the delineation between estuarine and coastal areas is somewhat subjective, for the present purpose the distinction can be based on either salinity and/or geography.

Sampling Methods

(i) Estuaries: Estuarine sands are likely to be fine or prone to siltation, therefore, collection of additional 0.01m² cores may be appropriate. The number of supplementary samples required will be dependent on the objectives of the survey. All additional cores should be processed as in section 6.

(ii) Coasts: The sediments of coastal beaches tend to be characterised by coarse material for which the 0.1m² box core is the most suitable device for collecting supplementary samples. The number of supplementary samples taken in addition to the "Core" method required will be dependent on the objectives of the survey. Box cores should be sieved to 1mm in the field if possible, and then returned to the laboratory for subsequent processing as in section 6.

APPENDIX 2

Intertidal Macrofaunal Sampling Record Sheet.

N.B. Complete in ink and initial and date any corrections.

Survey:		Date:	
Site:		Time:	
Position Fix/NGR		Sampling Device	
Shore Height:		Sampler:	

I. "Core" Method

Sampling Device (e.g. 0.01m² corer): _____

Sample No.	Container Code	Sediment Type*/ Observations+	Sieving Observations	Sieved By

Subsamples: Metals PSA
Organic Carbon Organic Chemicals Coal
Bacteriological

PTO…

II. Supplementary Samples

Sampling Device (e.g. 0.25m² Box corer/0.01m² corer): _____

Sample No.	Container Code	Sediment Type*/ Observations+	Sieving Observations	Sieved By

*Sediment types: Mud (M)

Sediment Texture: Fluid (l)
Sandy mud (SM)

Soft (s)
Muddy sand (MS)

Firm (f)
Sand (S)
Gravelly sand (GS)
Sandy gravel (SG)
Gravel (G)

e.g. Soft Muddy Sand = sSM

+Observations should include: Colour
Smell
Stability (stable-mobile)
Sorting (well-poor)
Surface relief (even-uneven)
Depth of RPD layer
Accretions (e.g. coal, illmenite, etc.)
Surface flora/fauna
Presence of burrows, casts and tubes

STANDARD OPERATING PROCEDURE FOR THE BOWERS AND CONNELLY MULTIPLE CORER

Field sampling

Deployment of Bowers and Connelly Multiple-corer

Plate 1 shows a complete view of the Bowers and Connelly Multiple Corer. The following is an operating procedure adopted by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS):

Put the required amount of weight on their mounts. The four cut-out weights must be placed on first (two on each side). Tighten down the wing-nuts.

Remove core retainers by lifting the bolt and pulling out using the fine string.

Swing the bottom catchers out to the side and insert the core tubes (55mm internal diameter) fitted with stainless steel tips. Core tubes need to be pushed up firmly through the 0-rings. If it proves difficult to insert the core tubes a smear of petroleum jelly on the O-rings may help.

Replace core retainers and press in bolt.

Push in the L - shaped pieces on both core units on the same block and rotate the adjustment bars out to the side.

Push in the plunger to raise top-caps and engage the top-cap bar in to the notch.

Pull out the bottom closing plate until it latches, rotate the plate until it is below the core tube and slide it up around the tube.

Continue to lift the closing plate up the tube until it latches around the tube.

Slide out the L - shaped pieces as far as they will go towards the ends of the top cap arms.

Repeat procedure for the other two units.

To ensure ease of handling the corer is deployed from and returned to the Day grab table.

When the weight of the corer is taken on the warp remove the pin from beside the latch, slide the latch bar to the right and insert the pin from below so that it holds the latch bar open.

Lower the corer at approximately 1metre per second.

Leave the corer on the sea bed for at least 15 seconds. If sampling conditions allow (e.g. wind speed and tides), the corer should be left on the sea-bed for longer but no greater than one minute. The warp needs to be slack whilst the corer is on the bottom

and therefore the winch operator will need to compensate for any movement of the corer away from the ship, by paying out extra warp.

The length of the core obtained depends on the sediment compaction, the amount of weight loaded, the length of time the corer is left on the bottom and how far the frame sinks into the sediment. The relative values of these parameters must be determined empirically.

Raise the corer slowly off the seabed. Recovery can then take place at any reasonable speed (up to 2m per second).

Safety

Avoid firing the closing plates in air to prevent injuries caused by fingers becoming trapped. The edges of the linear springs that operate the bottom closing plates are sharp and it is advisable to wear gloves when handling the corer.

Recovery

When the corer comes within reach take the pin, which is now dangling, and put it downwards through the plate at the top of the frame. This will prevent the latch from being disengaged and locks up the corer head.

Before redeployment of the corer, it is a good idea to hose all the moving parts of the corer to prevent jamming by sediments.

Meiofaunal sample collection

Multiple samples collected from one deployment of the corer are pseudo-replicates and cannot be considered as replicates. However, cores from the same deployment can be used for different purposes i.e., P.S.A, chemical analysis, bacterial analysis.

There are generally three types of samples collected for meiofaunal analysis. These are:

a) intact sediment cores with a diameter of 55mm. retained for whole community analyses;

b) sliced core samples for analysis of vertical community composition and;

c)sub-samples taken from both cores and Day grabs using a syringe (26mm diameter).

(1) To remove intact cores for meiofaunal analysis the procedure below is followed:

Push back the L-shaped pieces towards the rear of the top-cap arm.

Position a 2.5l bucket under the end of the core tube.

Pull out the bottom closer until it latches.

Crack open the seal on the top-cap and allow sediment core to slip in to the collecting bucket.

(2) To remove the core tubes for slicing cores or removal of sub-samples the procedure below is followed:

Push back the L-shaped pieces towards the rear of the top-cap arm.

Lower and rotate the bottom closer clear of the end of the tube and insert a rubber bung.

Remove the core retainer.

Crack open the seal on the top cap and twist the tube clear of its 0-ring seal.

The core tube can now be removed for slicing or sub-sampling.

Sample description

The sediment type, length of cores retained and whether the cores were retained intact, sub-sampled or sliced needs to be recorded in the log-book. It is also necessary to record the diameter of the core tubes and whether the M8 bolt was removed during sample collection.

Storage and Maintenance of Multicorer.

On return to the laboratory the corer should be thoroughly washed with fresh water and left to dry. Any core tubes must be removed and the bottom closing plates closed. The drop bars should be lifted through the blocks and the white protective sheaths pushed around the bars. Finally the corer should stored with the head in the lowered position and covered by the blue canvas frame.

Plate 1: The Bowers and Connelly Multi-corer (CEFAS, 1997)

STANDARD OPERATING INSTRUCTION FOR THE 0.05m² VAN VEEN GRAB

The following is an operating instruction for the 0.05m² van Veen grab adopted by the Environment Agency of England and Wales:

1. GENERAL DESCRIPTION

The van Veen grab (Plate 1) is a remote sampler primarily used to obtain sediment samples from coastal and estuarine soft sediments, for both biological and physico-chemical studies. Due to its relatively small size and weight, the van Veen grab may be deployed from a large survey vessel and small boats equipped with a winch. However, in either case a minimum of two workers are required to operate the grab, one to man the grab, and one to operate the winch when deployed from a survey vessel, and two to deploy and retrieve the sampler when used from a smaller vessel.

The grab consists of two hinged self supporting jaw buckets with long arms attached to each bucket through which the warp passes. The arms help to prevent the grab being jerked off the seafloor should the survey vessel roll as the grab is closing. The whole grab is fabricated from stainless steel. The shape of the jaw buckets is a quarter circle, so that when they close they form a large bucket semicircular in cross-section.

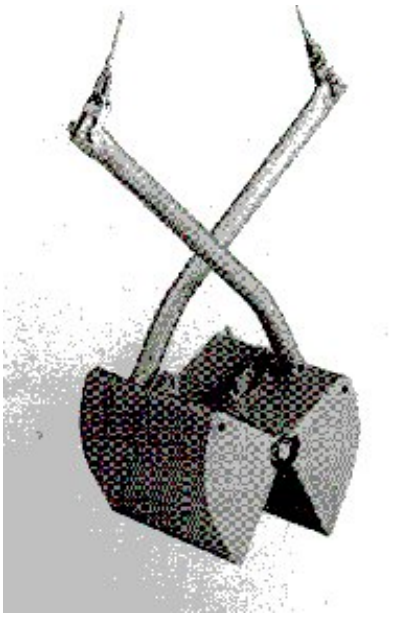

Plate 1: van Veen Grab

The grab is lowered in a cocked (open) position with the jaws being held open by a locking bar that is released as the tension on the bridle is relaxed when the grab settles on the seafloor. When cocked, the surface area of the jaw buckets is 0.05m^2. The weight of the grab can be altered with lead weights attached to top of the buckets so that efficient penetration can be achieved on a variety of sediments. The warp is attached to the arms on each jaw bucket, so that the closure of the grab is completed by mechanical leverage when hauling commences. The upper sides of each jaw bucket are fitted with lids that are closed throughout deployment. On retrieval these lids can be opened to

allow access to the sample enabling sub-samples to be taken of surface, and for observations of the sediment/water interface to be made.

2. OPERATING PROCEDURE

2.1 When being deployed from a survey vessel two workers are required to operate the grab, one operating the winch, and one controlling the grab.

2.2 When being deployed from a small boat two workers are required to deploy and retrieve the grab. To avoid loss of the grab the end of the warp must be securely attached to the boat.

2.3 Before deployment, the grab must be cocked (see Plate 1). Before cocking, the lids on the buckets are fastened shut. The arms are pushed apart to open the jaws, and the locking bar lifted into place. The bar will only be automatically held in place when strain is taken up when lifting the grab, before which it must be held by hand.

2.4 When deployed from a survey vessel the grab is lifted above the level of the transom by the winch and then directed outboard by the hydraulic gantry, while the one worker manually controls any swinging. Once clear of the stern, lowering can commence. On retrieval, the grab will be lifted above the level of the transom, and brought inboard and lowered onto the deck or into a receiving container, all the while being guided manually.

2.5 When deployed from a small boat two workers must deploy and haul the grab. The retrieved grab should be placed in suitable receiving container

2.6 While the grab is being deployed drifting of the survey vessel must be minimised as the grab is easily toppled when on the seafloor.

2.7 Once the grab has been retrieved the lids can be opened and the sample examined and sub-samples collected.

2.8 Once all inspection and sub-sampling is completed the grab can be emptied by fully opening the buckets.

3. SAFETY

3.1 Suitable personal protection equipment should be worn:

(i) Twin chambered lifejackets must be worn by all staff working in RIBs or on the afterdeck of vessel, alternatively a safety harness may be worn.

(ii) Hardhats must be worn when working with grab onboard survey vessel.

(iii) Steel toe-cap boots to be worn by all workers involved in operating the grab.

3.2 All personnel operating sampling equipment should have received training/instruction from a suitably experienced person in how to operate the gear correctly.

3.3 While the winch is in operation all workers on the afterdeck are responsible for being aware of where the cables are. Stepping over moving cables should be avoided wherever possible.

3.4 All workers on the afterdeck are responsible for being aware of the hydraulic gantry, and must keep away from moving parts when in use.

3.5 Only suitably qualified individuals should operate the winch. All other workers must be aware of which drum is being used, and keep clear of the winch and cables when in operation.

3.6 When the grab is being deployed and retrieved the line of sight of the winch operator must not be obstructed.

3.7 Hands and fingers should never be placed within the open jaws of the grab, or interfere with the warp. When man handling the grab, especially when cocked, workers should guide the grab with the flat of their hands placed on the outside of the arms. However, when the grab is cocked, no downward force should be applied to the arms as this could release the locking bar resulting in closure of the jaws.

3.8 The use of benthic sampling gear in rough weather is extremely dangerous. The decision of whether sampling proceeds is down to the Master of the vessel, as the safety of all on board is his responsibility.

3.9 Only suitably fit and able-bodied individuals should handle heavy sampling gear or lift or move samples. All workers must be suitably trained in lifting techniques and should refer to Health and Safety Manual, Section ?

3.10 Prior to undertaking work on any Agency vessel all workers should refer to the National Boatwork Code of Practice (Health and Safety Manual, Section 3.6.1), and should be aware of all safety procedures and equipment applicable to the vessel used.

APPENDIX 6

Notes made by Participants during the Biomass Exercise

Lab 21 (ISC IRTU)

Blot until no moisture left on tissue paper. Blot once again and wait ca. 30 seconds - weigh.

Bivalves opened, *Echinocardium* etc. punctured & drained.

Lab 23 (EA North East)

data, but no comments.

Lab 5 (EA South West – Blandford)

Gently blot on paper until no more liquid appears. Leave on balance for 30 seconds and take reading.

A. (*Macoma*) Would usually open valves using a scalpel to facilitate draining. Roll bivalve along gape to help draining.

B. (*Nephtys*) Larger, more robust worms, used blotting 'sandwich' i.e. gently blot top & bottom at same time.

C. (*Lanice*) Small worms just blot by lifting specimens up & down on paper using forceps

D. (*Bathyporeia*)- As C.

E. (Oligochaete) - As C.

Lab 18 (SEAS)

Specimen A - 4 bivalves, would normally pierce twice allowing to drain (like blowing an egg).

Alcohol with a touch of glycerol to store in future after biomass.
Dry tweezers between samples.

M.D. notes. Very light blotting used. Samples usually placed into vial with water on balance so that there is less evaporation & the reading is more stable. Used filter paper on the balance and replaced with a new one for each specimen.

EA South West - Exeter

M.D. notes. Very light blotting

Lab 03 EA Welsh

M.D. notes. Light blotting. Weighed after 30 secs. Used plastic boat for weighing.

Lab 04 (MBCC Mentec)

Plastic boats used. Lightly dabbed/dried in tissue.

Lab 19 (SEPA West)

Boat placed on balance and zeroed. Fauna placed on 'blue roll' and gently pressed and rolled.

Fauna transferred to boat and placed on balance. Wait for 30 seconds and record weight.

Entec

Re-zero tray in machine
Remove animal - worm blot on paper until no more meths is let out.
Weigh
Wait until close to stabilisation
Record!

Problem with biomass is time = money and actual numbers of samples to get through.

APPENDIX 7

North East AES Biomass Protocol

Principle

Animals separated and identified in the course of benthic analysis are weighed in order to derive an estimate of their dry weight by a non-destructive method. In order to achieve this, the animals are blotted dry before weighing. A factor is to be applied to the results by Entec to estimate the dry, ash free weight.

Note that juveniles are to be enumerated and stored separately and are to be excluded from this analysis.

Application

This procedure assumes that all animals in the test sample have been identified, enumerated and stored in separate, suitably labelled specimen tubes in 70% IMS. It will normally be appropriate to separate animals to family level. For this project, animals should be separated into the following groups:

1 Nephtyidae	7 Polychaeta other	13 Mactracea
2 Cirratulidae	8 Amphipoda	14 Veneracea
3 Spionidae	9 Other crustacea	15 Other mollusca
4 Magelonidae	10 Echinodermata	16 Miscellanea
5 Terebellidae	11 Nuculacea	17 Juveniles - Not To Be Weighed
6 Pectinariidae	12 Tellinacea	

Only if another, distinct taxon is found to be dominant in a set of samples should it be separated for weighing. The same taxonomic groupings are retained when samples are supplied to the external auditor.

Reagents

70% Industrial Methylated Spirit (IMS)

Equipment

Balance, weighing to 0.0001g

Weighing boats both small and large **or** small GFC filter papers.

Tissue paper

Microscope

Fine forceps, both rigid and flexible

Procedure

1. Record the sample number names of each taxonomic group on a suitable recording form.

2. Remove all specimens of a taxon from its specimen tube onto a piece of dry tissue paper.

A microscope should be employed to check you've got all small specimens out of a tube for weighing. This may be facilitated by emptying the animals into a small petri dish or even a tea-strainer. The check that all specimens are accounted for should be made against the identification/enumeration results.

3. Move the specimens around the absorbent paper until no wet patch is left behind, this process must be carried out without delay. **Specimens must then be transferred without delay to the balance as soon as this blotting is complete.** It will be necessary to actively blot larger specimens, in order to dry off excess preservative medium in this way.

   Do not, for this project, pierce or crack shells of animals but aim simply to dry off excess moisture from the surface of animals.

4. When the specimens are blot-dry, transfer them immediately to a pre-weighed (and weight recorded) or tared weighing boat and place on the balance pan, closing the balance windows, or whatever, to stop air currents, excess evaporation etc.

5. Record the new weight after allowing 30 seconds to elapse since transferring to the weighing boat. This result must be recorded carefully against the taxon name on the result sheet. Derive the blotted wet-weight of the animals by subtraction if appropriate. Record the weight of animals down to 0.0001g. Where the weight of the animals in a taxon is less than this then record the weight as 0.0001g.

6. Count the specimens back into their labelled tubes, making sure none are lost or left behind and top up with preservative.