

# Guidelines for the conduct of benthic studies at aggregate dredging sites

This report has been produced by the  
Centre for Environment, Fisheries and Aquaculture Science  
on behalf of the  
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# Preface

These guidelines for the conduct of benthic surveys at commercial aggregate extraction sites have been produced in response to the rapid increase in survey work for Environmental Statements to accompany dredging applications, and to impending legislation which will bring extraction activity under statutory control (see Introduction). The guidelines are designed to promote a comprehensive and consistent approach to the assessment of the seabed environment (i.e. sediments and the associated benthic fauna) as part of the planning process and, on granting of a permission to dredge, in response to any monitoring requirements. They have been written by scientists at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) on behalf of the UK Department for Transport, Local Government and the Regions, who will shortly assume the role of the regulator. Since the inception of the requirement for such benthic surveys, CEFAS, as an Executive Agency of the Department for Environment, Food and Rural Affairs (DEFRA), has led on the provision of scientific advice regarding their conduct, as well as carrying out related R&D programmes of a strategic nature in UK waters. The production of these guidelines was overseen by a Steering Group, membership of which is given at Annex I.

The increased demand for evaluations of environmental status at and around aggregate extraction sites, whether for Environmental Statements prepared by the industry or in connection with R&D and monitoring programmes, spans a period of less than ten years. Historically, the scientific study of coarser substrata has presented a significant challenge, largely on account of the difficulties in obtaining reliable quantitative samples. As a consequence, information on the nature and distribution of benthic assemblages, and on their wider role in the marine ecosystem, is considerably more limited than in areas of soft sediments.

Developments in sampling practices, such as the use of acoustic techniques for accurate discrimination of substratum type, thereby allowing inferences to be made concerning biological status, are proceeding rapidly. At the same time, there is increasing emphasis in national and international fora on the development of more holistic (ecosystem-level) approaches to marine environmental management, including evaluations of the scope for 'cumulative' or 'in-combination' effects. Given this, a question may reasonably be asked as to the correct timing for the production of study guidelines. In terms of the operational need for greater consistency in sampling and analytical approaches the answer is, unquestionably, now. However, a document of this nature cannot anticipate with certainty the consequences of all ongoing R&D effort, or of future developments in environmental policy, in specifying present requirements for the conduct of routine benthic surveys. The account therefore serves a dual purpose, namely the provision of guidance on established approaches accompanied, where appropriate, by evaluations of the 'state of the art' of parallel developments in UK methodologies which may influence the direction of future studies. It is recommended that the guidance is updated at appropriate intervals to incorporate significant improvements to current practices arising from such developments.

Finally, this document is targeted at experienced marine scientists (especially benthic ecologists, sedimentologists and geophysicists) working on behalf of the industry or the regulator in the conduct of R&D or, more usually, on the implementation of environmental assessment and monitoring programmes. **However, it is not intended as a substitute for appropriate consultation at critical stages in the environmental assessment process.** This is especially true at the initial design stage, when the guidelines contained herein are adapted to meet the circumstances prevailing at individual sites.



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# CHAPTER 1

## Introduction

### 1.1. Background and scope of guidelines

The control of marine aggregate dredging in the U.K. under the Government View (GV) Procedure dates back to 1968. Under this non-statutory system, the Crown Estate, as owners of most of the seabed, would only issue a dredging licence if the Government was satisfied that predicted impacts on the environment were viewed to be acceptable. The level of information required to assess these impacts has progressively increased as more has become known about the marine environment. The GV procedure was revised in 1989 and requires that an Environmental Impact Assessment (EIA) is undertaken by the dredging applicant as part of the application process for a dredging licence/permit. In view of the move towards statutory control of aggregate dredging activity through the impending introduction of the Environmental Impact Assessment and Habitats (Extraction of Minerals by Marine Dredging) Regulations, there is an increasing need to harmonise approaches to benthic surveys associated with the activity of marine aggregate extraction. Under these regulations, monitoring and other requirements will be specified in conditions attached to Dredging Permissions.

Aggregate extraction can have a number of environmental effects on the seabed including the removal of sediment and the resident fauna, changes to the nature and stability of sediments accompanying the exposure of underlying strata, increased turbidity and redistribution of fine particulates particularly from screening. The activity is of concern not only from the standpoint of effects on the benthic fauna during and after the event of aggregate extraction, but also in terms of its effects on the wider resource including dependent fish/shellfish populations and associated fisheries and other legitimate interests such as conservation and recreation. These concerns are addressed in Environmental Statements (ESs). Methodology for appraisals of the distribution of commercial fish stocks and fishing activity is beyond the scope of these guidelines. Rather, the focus of this report is on the conduct of surveys of the seabed and the associated benthic fauna, the results of which are submitted in ESs in support of dredging applications. The dredging industry or their consultants have carried out many of these surveys and, to date, a notable feature has been the wide variation in their scope and the analytical methodology employed. This is, in part, an inevitable consequence of differences in prevailing environmental conditions present across the areas where extraction permissions are concentrated. Despite this, there is clearly scope for greater harmonisation of approaches, leading to improvements in the quality of samples collected during seabed surveys, and in the resulting data.

The purpose of this report is to provide detailed guidance on the conduct and reporting of benthic surveys to facilitate consistency of approaches among consultants employed by the

industry to conduct ESs and when carrying out monitoring surveys. In addition, this report has been produced to foster compatibility between ongoing regulatory monitoring activity and related R&D.

The report begins with an account of the rationale for benthic surveys at aggregate extraction sites (Chapter 1.2), presents a strategy for their planning and design (Chapter 2) and then documents current and developing methodologies for their conduct and should be of use to both the regulatory authorities and the industry. This is followed by a general review of the range of equipment available for sampling the marine benthic fauna from coarse substrata (Chapter 3), and then a discussion of the approaches for processing faunal samples both in the field and the laboratory (Chapter 4). Recognising the role of remote acoustic techniques in complementing conventional approaches, Chapter 5 describes a number of devices for use in characterising attributes of the physical habitat. Coverage of this topic was considered to be important, as the production of high-resolution biotope maps of the seabed, using data derived from a combination of conventional sampling devices, acoustic and visual techniques, has potential in assisting with future site-specific environmental assessments of aggregate extraction sites (Brown *et al.*, 2001).

It has long been recognised that abiotic factors such as the sediment grain size and tidal current strength are responsible for determining broadscale benthic community patterns (Cabiocch, 1968; Warwick and Uncles, 1980; Rees *et al.*, 1999). Therefore, interpreting trends in the status of benthic assemblages in areas which have been subjected to dredging should include consideration of variations in sediment particle size and the hydrodynamic regime. Thus, Chapters 6 and 7 briefly describe a range of techniques for characterising the wave and current climate and for the collection and particle size analysis of sediments. The report then details a framework for analysing benthic community data and for linking the output to environmental variables (Chapter 8). Throughout the report, good practice in terms of Quality Assurance (QA) procedures is presented within each of the sections describing methodological approaches. This is supplemented by generic guidance on QA matters in Chapter 9. Recommendations are also made on the format for presenting findings from environmental surveys (Chapter 10).

Finally, in addition to providing detail on established methodological approaches with the aim of fostering continuity and harmonisation among the various establishments carrying out such work, the report also identifies “state-of-the-art” approaches (Chapter 11). These are likely to evolve further in line with the outcome of ongoing R&D, e.g. strategies for the evaluation of cumulative effects.

## 1.2. Rationale for benthic surveys at aggregate extraction sites

As the extraction of marine aggregate has its primary impact at the seabed, assessment of the effects of this activity has conventionally targeted bottom substrata and the associated benthic fauna. Benthic communities are a logical target for investigations of the effects of aggregate extraction since:

1. They may be valued in terms of their links with other resources, as well as containing representatives which are themselves commercially harvested (e.g. crabs, shrimps,

flatfish). They may also have intrinsic value in terms of their rarity and hence conservation status (which may also apply to individual species). Because of the open nature of the marine environment, evaluations of benthic biodiversity, productivity and trophic interactions may all bear upon wider ecosystem integrity.

2. They are constant features of the seabed, and vary predictably in association with the physical habitat and in response to man-made changes. Furthermore, unlike shifting populations of planktonic organisms or many pelagic fish species, adults of most benthic invertebrate species are either sessile or mobile within narrow spatial ranges. Thus they are good indicators of locally induced environmental changes.

Attendant sampling of sediments is also essential for assessing the physical properties of the seabed environment and for interpreting any biological changes (Kenny and Rees, 1994, 1996; Kenny *et al.*, 1998; Newell *et al.*, 1998; Desprez, 2000; Brown *et al.*, 2000). Remote methods for surveying such as sidescan sonar and photography can also be employed in order to provide an indication of the spatial distribution of sediments in the wider area encompassing the dredged site and to estimate the likely spatial extent of dredging disturbance (Kenny and Rees, 1994, 1996; Kenny *et al.*, 1998).

In recent years, greater consideration has been given to identifying mitigation measures to reduce the impact of aggregate extraction which are translated into appropriate permit conditions. To ensure that such permit conditions are effective in minimising environmental disturbance and that predictions regarding the extent and significance of effects are sound, a monitoring programme is usually initiated. Monitoring is required to document both pre- and post- extraction conditions at dredging sites and to determine whether unacceptable impacts are occurring, or if conditions that could lead to an unacceptable impact are developing, within and in the vicinity of new and existing extraction sites. The outcome of monitoring programmes can therefore usefully contribute to judgements on the acceptability or otherwise of continued dredging within an extraction site. Monitoring will also be appropriate to determine whether permit conditions are being properly implemented, and to improve the basis on which future dredging applications are assessed by improving knowledge of field effects.

## CHAPTER 2

# Planning and design of benthic surveys at aggregate extraction sites

### 2.1. Introduction

This section provides a strategy for assessing the environmental status of an area of seabed, which may be targeted for its commercially exploitable reserves of sand and gravel, and then setting up a monitoring programme to evaluate the effects of dredging, in the event that extraction is permitted.

The strategy consists of a series of logical steps which are comparable to those employed in national and international guidelines for the evaluation of the effects of marine waste disposal activities (e.g. Rees *et al.*, 1990, 1991; Anon., 1996, 1997). Similar principles apply to studies of the effects of aggregate extraction in that all are manifestations of man-made perturbations (see also Davies *et al.*, 2001 for draft guidelines in relation to the monitoring of marine nature conservation sites). However, there are some important differences in practical approaches, which are accounted for here. In keeping with these earlier guidelines, it is not possible to provide a definitive design blueprint applicable to all areas. Thus the design of surveys, along with sampling effort, must be tailored to local circumstances. These may vary according to the nature and perceived sensitivity of the environment, the amount and area to be dredged, and the need to address other activities nearby, including the possibility that cumulative consequences may arise.

Examples of approaches to the design of sampling programmes at aggregate extraction sites are given at 2.3 below. Useful general sources of information concerning the evolution of sampling designs in benthic studies include Elliott (1971), Cohen (1977), Green (1979), Holme and McIntyre (1984), Andrew and Mapstone (1987), Skalski and Robson (1992) and Underwood (1997).

### 2.2. Objectives of benthic surveys at aggregate extraction sites

The outcomes of benthic surveys provide essential information on environmental status at the pre-application stage, and on the consequences of dredging activity in cases where permits are issued. The objectives of surveys are:

- To provide a spatially extensive description of the seabed environment within and around the proposed extraction area including the identification of important/sensitive habitats or species.
- To assess the progress of any changes over time (in nature, intensity and spatial extent) which may be attributable to the effects of aggregate extraction.
- To determine whether the permit conditions are appropriate and that they are having their desired effect of minimising the effects of aggregate extraction.
- To determine whether permit conditions have been properly implemented and adhered to.
- To determine whether unacceptable impacts are occurring, or if conditions that could lead to unacceptable impacts are developing, within and in the vicinity of new and existing extraction sites.
- To establish the nature and rate of recolonization by benthic invertebrates following cessation of dredging.

## 2.3. Stages in the planning, design and conduct of benthic surveys

### 2.3.1. Desk study

This is an essential pre-cursor to all field sampling effort. The outcome should allow a preliminary evaluation of the likely environmental consequences of extraction activity and hence provide a rationale for appropriate sampling design and sampling frequency, as well as an indication of the suitability of various sampling devices to meet survey needs.

Information on the study area may be obtained from the published literature, geological maps and Admiralty charts. Industry surveys at the prospecting stage (especially the output from acoustic surveys and the sampling of sediments using vibrocores or hydraulic grabs) may provide valuable information on local conditions, although wider access may be limited by commercial considerations. An evaluation of the possible physical consequences for the shoreline environment arising from aggregate dredging, especially the risks of coastal erosion, is now required for all dredging applications. The mathematical models employed in this evaluation may also aid in the design of sampling programmes, in the event that a permit is issued, for example in respect of predicted water movements or particulate transport.

Contacts with governmental and research agencies may reveal ongoing research and monitoring initiatives in the area of interest, including the existence of GIS and archived oceanographic data, as well as providing information on nearby discharges, disposal sites, species or habitats of conservation value, and so on. Access to unpublished literature, including earlier Environmental Statements in the vicinity, and consultations with individuals with local sampling experience may provide useful background information, which will reduce uncertainties at the planning stage, and hence increase the cost-effectiveness of sampling programmes.

A component of the desk study will be an evaluation of the scope for cumulative impacts at the seabed arising from aggregate extraction, which may influence survey effort. Cumulative impacts have been defined as effects on the environment, either from the summation of individually minor but collectively significant impacts, or as a result of the interaction of impacts from one or more source (DETR, 2001). The scope for such effects may be enhanced by a wide range of man-made activities, which may raise issues for resolution such as the extent to which a company responsible for a new application should, in planning an environmental survey, consider the influence of all other existing activities in the vicinity. This evaluation will generally be carried out as part of a more holistic appraisal of the scope for cumulative effects on the marine environment (Baskerville, 1986) for which detailed guidelines on aggregate extraction activity are awaited (see Chapter 11.1). In the meantime, an assessment should be made on the basis of locally available information on the nature, extent and disposition of documented impacts at the sea bed arising from man-made activities, as a result of which survey design and effort should be adjusted accordingly. The outcome of research by CEFAS into the cumulative impacts of aggregate extraction (see Chapter 11) will, in due course, help in the planning of sampling programmes.

A summary of the key information requirements at this stage of the process is given in Table 1. **The outcome, namely a plan of survey intentions, should be accompanied by a concise rationale, for appropriate consultation.** In certain cases, it may be necessary to conduct a pilot survey (see Chapter 2.3.3 below) before evolving and then submitting a plan.

## 2.3.2. Survey planning

Information gained during the desk study will inform decisions regarding the range of sampling equipment needed which will, in turn, determine the size and capability of the survey vessel required for field sampling. Critical issues regarding the suitability and seaworthiness of chartered vessels, along with safe working practices for scientists at sea, must be considered at this stage by competent and experienced individuals. Where possible, the Maritime and Coastguard Agency workboat code of practice should be followed (DETR, 1998). As a general rule, the larger the size of vessel needed, the more notice will be required of the intended period of charter. Larger vessels are also more expensive, but there are circumstances where the extra cost can be offset against the facility to work in a wider weather window, thereby achieving survey aims in a shorter time, or increasing the likelihood of success where only a narrow time-frame is available to guarantee year-on-year comparability in an ongoing monitoring programme.

Approximately four weeks before any survey work is to be carried out, it is strongly recommended that the Clerks of the appropriate Sea Fisheries Committees (SFCs) and the relevant DEFRA District Inspectors are provided with a survey plan. The plan should include details of the timing of the survey, the name and contact number of the survey vessel, station positions and the type of gear to be used. These measures will help to avoid conflict with local fishing activities (e.g. fixed fishing gear) during the period of the survey. Relevant port authorities should also be notified if all or part of the survey falls within their jurisdiction. Contact must also be made with aggregate extraction companies who may be actively dredging at extraction sites within the survey area.

If epibenthic trawling is a survey requirement, the District Inspectors will also be able to provide advice on local regulations regarding trawl mesh sizes, since the finer meshes employed in sampling may break minimum legal requirements (see Chapter 3.3.3). The use of such gear will require a dispensation which should be applied for 4 weeks prior to the

**Table 1 Summary of strategy for the planning, design and conduct of sampling programmes at marine aggregate extraction sites**

<b>Desk study</b>	<b>Survey planning</b>	<b>'Pilot' survey</b>	<b>'Baseline' survey</b>	<b>'Ongoing' survey</b>
1. Seek information on: <ul style="list-style-type: none"> <li>• wave climate</li> <li>• tidal/residual currents</li> <li>• substratum type</li> <li>• benthic communities</li> <li>• valued resources (e.g. fish/shellfish)</li> <li>• man-made activities/impacts</li> </ul>	1. Determine: <ul style="list-style-type: none"> <li>• survey timing</li> <li>• suitability/availability of charter vessel</li> <li>• availability of sampling gear</li> </ul> 2. Attend to issues of safety at sea and other relevant matters	1. Determine: <ul style="list-style-type: none"> <li>• local hydrography</li> <li>• suitable sampling gear</li> <li>• substratum type (qualitative)</li> <li>• benthic fauna (qualitative)</li> <li>• boundaries of survey area</li> </ul> 2. Evaluate findings	1. Carry out: <ul style="list-style-type: none"> <li>• quantitative spatial survey</li> <li>• initial sampling at representative stations</li> <li>• analysis/AQC of samples</li> </ul> 2. Analyse data/report and act on findings           3. Refine hypotheses for dredging-induced changes           4. Repeat at intervals	1. Carry out: <ul style="list-style-type: none"> <li>• sampling at representative stations over time</li> <li>• analysis/AQC of samples</li> <li>• hypothesis-testing for dredging-induced changes</li> </ul> 2. Report and act on findings           3. Review sampling design/frequency
2. Determine: <ul style="list-style-type: none"> <li>• survey needs/sampling gear</li> <li>• QA strategy</li> <li>• hypotheses for dredging-induced changes</li> </ul>				
3. Submit plan, with accompanying rationale, for approval				

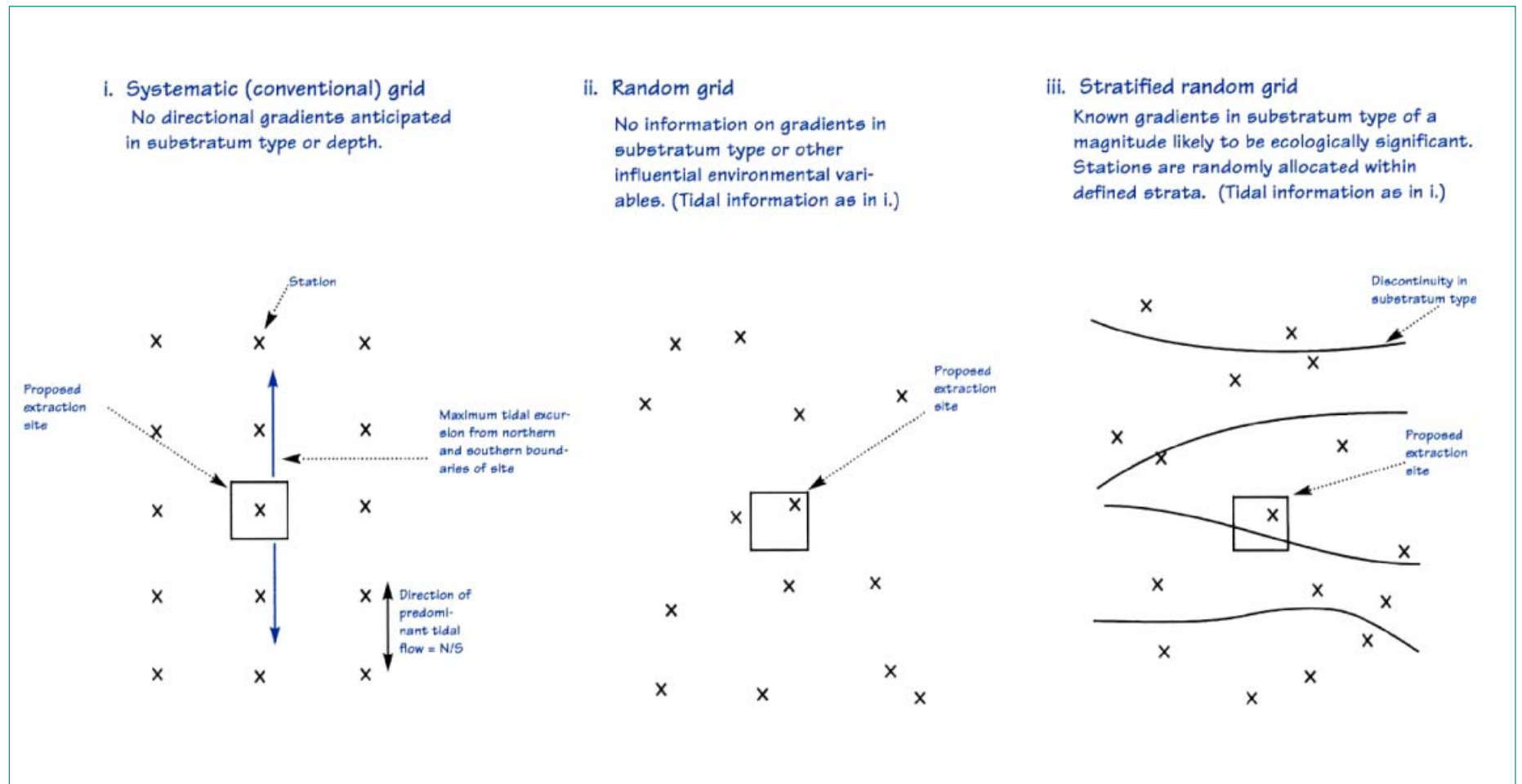


Figure 1 Hypothetical examples of approaches to sampling design in a Pilot Survey. (The numbers and locations of stations are purely indicative)

survey from the DEFRA Sea Fisheries Conservation Division (Branch B, Room 425, Nobel House, 17 Smith Square, London, SW1P 3JR). The Sea Fisheries Conservation Division will require information such as the nature and timing of the work being carried out, the name of the vessel to be used for the survey and the rationale behind the work, before a dispensation will be granted.

A summary of the key stages involved in survey planning is given in Table 1.

### 2.3.3. 'Pilot' survey

The necessity for such a survey will depend upon the availability of existing information for the area of interest. A well-studied location may provide all the information necessary for selecting suitable sampling tools, and designing a 'baseline' survey (see Chapter 2.3.4). In its absence, preliminary sampling over a wide area encompassing the proposed extraction site may be required, using a range of mechanical sampling devices, along with acoustic and visual methods for ground discrimination. Any deficiencies in local knowledge of water movements and their influence on particulate transport may be made good by the deployment of current and turbidity meters.

The adopted sampling design may be random, systematic, stratified or even selective (e.g. for confirmation of the presence of features), depending upon the extent of prior knowledge of the area. Hypothetical examples of alternative designs are given in Figure 1. The options are similar to those available for subsequent 'baseline' surveys, further details of which are given in 2.3.4 below. Thus, in terms of *design*, the two may differ only in respect of the number of stations visited, if the 'pilot' survey is successful in confirming prior inferences concerning variability.

For bottom sediments and the accompanying fauna, on-board qualitative or semi-quantitative assessments of collected samples will usually suffice at this stage. The purpose will be to determine the most effective sampling tools to meet the aims of future monitoring, to establish the distribution of habitat types which may influence subsequent sampling design, and to provide a preliminary characterisation of the benthic fauna, which may influence decisions on the size and number of samples to be taken. For example, larger numbers of samples are likely to be required in order to reduce the variance of counts of organisms that are present in uniformly low densities, or are patchily distributed. In many areas around the UK coastline, sufficient information may already exist on a larger scale, and pilot sampling may only be necessary to confirm that local conditions conform with the wider pattern. Such an investigation may be conducted immediately prior to a 'baseline' survey, in order to 'fine tune' the sampling design or sampling practices, but need not involve a separate sampling trip. A summary of targets for determination during a 'pilot' survey, and its relationship to the overall strategy is given in Table 1.

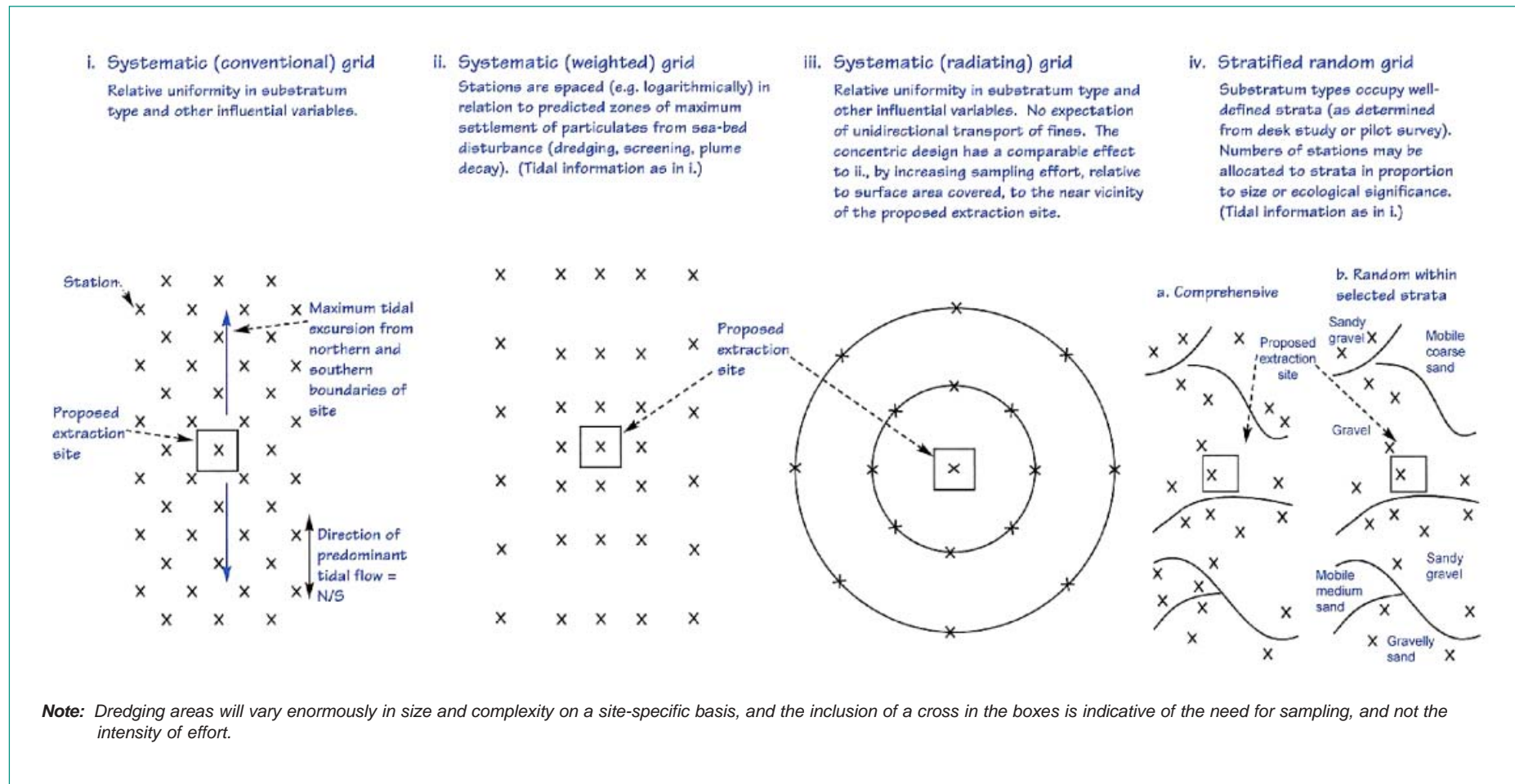


Figure 2 Hypothetical examples of approaches to sampling design in a Baseline Survey. (The numbers and locations of stations are purely indicative)

### 2.3.4. 'Baseline' survey

The purpose of this survey is to provide a quantitative description of the benthic fauna and associated sediments over an area encompassing predicted effects of dredging activity, before the event of permit issue. In an area of relative uniformity, this will typically take the form of a systematic grid of stations extending at least one tidal excursion beyond the limit of proposed dredging. In practice, this is the most commonly employed sampling design and provides a convenient basis for determining the distribution of benthic assemblages, and for exploring relationships with environmental variables. The design may be modified to enhance coverage near to anticipated future dredging activity and along predicted dispersal pathways for finer material released during dredging. More complex and spatially extensive sampling designs may be necessary to account for other man-made activities or features of conservation interest in the vicinity. A stratified random sampling design may be more appropriate where prior information (e.g. from desk study or 'pilot' survey) reveals well-defined spatial partitioning of habitat types. Hypothetical examples of approaches to 'baseline' survey design are given in Figure 2. **It must be strongly emphasised that sampling intensity within prospective dredging areas will be in proportion to their size and complexity, and therefore will commonly involve multiple stations.** Ideally, the same sampling device will be employed at all stations but alternative methods may be necessary in some circumstances, e.g. in the presence of significant rock outcropping supporting a valued epifauna.

As the emphasis in such a survey is on the elucidation of spatial pattern, a strategy involving the collection of single samples from several stations is favoured over repetitive sampling at fewer stations. The latter approach is more appropriate for 'ongoing' monitoring surveys at representative stations (see Chapter 2.3.5), but selective sampling at this stage in anticipation of the future need is likely to be cost-effective. A summary of the procedures involved in the conduct and reporting of a 'baseline' survey is given in Table 1.

### 2.3.5. 'Ongoing' survey

The main emphasis in this activity is on the monitoring of temporal trends before, during and after dredging activity. However, a spatial component is also essential to establish whether any trend at a location within the sphere of dredging influence is distinct from that occurring at a comparable but distant 'reference' location, i.e. whether any trend is attributable to natural or man-made influences. Ideally, a limited number of sampling stations occupying identical habitats within and beyond the predicted influence of dredging activity should be identified for this purpose. The approach is comparable to the 'Control/Treatment Pairing' principle of Skalski and McKenzie (1982) and developments (by Underwood, 1992) of the 'Before/After and Control/Impact' (BACI) design of Stewart-Oaten *et al.* (1986). (However, the term 'reference' is preferred to 'control' since, *sensu stricto*, examples of the latter do not exist in natural communities). Examples of the application of this approach to the monitoring of waste disposal activities are given in Rees and Pearson (1992) and MAFF (1993).

Properly designed, an 'ongoing' survey will allow a statistical evaluation of outcomes in relation to earlier predictions for dredging-induced changes. Stations may be located along a transect where effects are predicted to occur principally along a well-defined gradient away from a dredging area, or at representative locations within physically comparable zones. The number of stations will vary with the complexity of the physical habitat, the dispersive properties of the environment

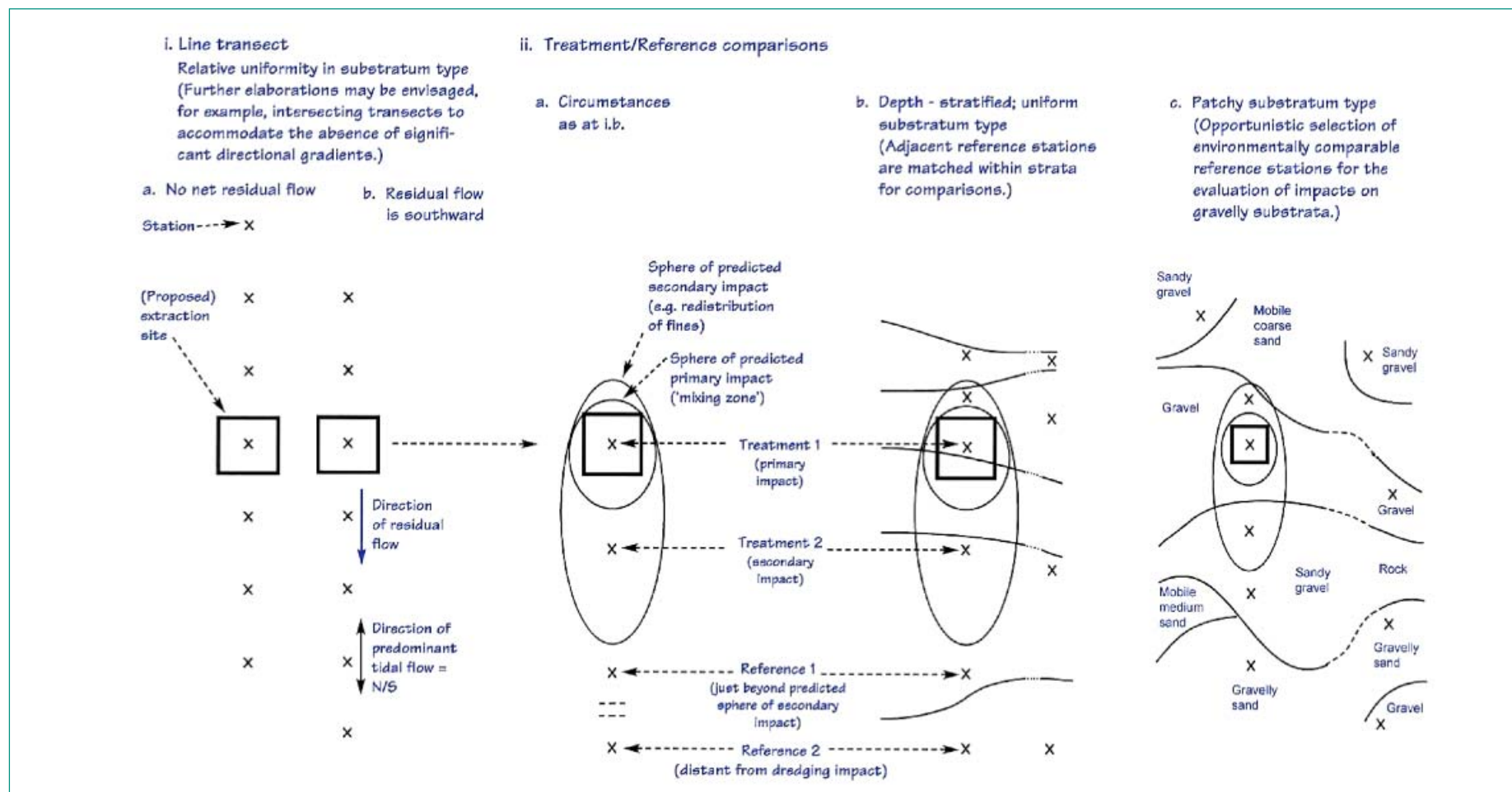


Figure 3 Hypothetical examples of approaches to sampling design in an Ongoing Survey. Note: these examples assume that dredging effort is uniform throughout the licensed site. In practice, many sites are more complex and subject to variable rates of dredging, so that zones of primary impact may be confined to locations within the licensed boundaries

within which dredging is to occur, the pattern and intensity of dredging, and the proximity of other man-made influences. As a minimum, an ongoing sampling design will consist of one 'treatment' station located within the predicted sphere of dredging influence, but peripheral to the centre of intensive dredging activity (see below), accompanied by two 'reference' stations, one just beyond the predicted sphere of influence, and one at some distance away.

The rationale for locating a 'treatment' station peripheral to the centre of dredging activity is that (by analogy with the 'mixing zone' concept applied to waste discharges: Water Authorities Association, 1988) any standards governing permissible biological changes in the surrounding environment would not be expected to be met at the point of immediate impact. Nevertheless, sampling at one or more stations within this area may often be necessary, since 1) in cases where dredging proceeds in sequence across zones within a licensed area, or following cessation of dredging, there will be a management interest in the recovery process, and 2) experience suggests that, depending on the spatial distribution and intensity of dredging, licensed zones are rarely lifeless, and there is a wider scientific and management interest in the responses of animal populations to ongoing physical perturbations, especially if there are specific sensitive features within the permit area which are being protected by dredging exclusions (e.g. *Sabellaria* reef). Hypothetical examples illustrative of this approach to the monitoring of aggregate extraction sites are given in Figure 3.

The number of samples to be collected at each station will reflect a balance between the statistical requirements of data analysis, the nature of the fauna and any resource constraints. Commonly, a minimum of 5 replicates will be collected either from a fixed point or randomly within a well-defined habitat type, and a minimum of three subsequently analysed.

The frequency of sampling will depend (*inter alia*) upon the perceived sensitivity of the environment within which dredging is taking place, and the amounts of material to be removed. In general, the frequency is likely to be higher in the period just prior to, and soon after, the onset of dredging, and then lower following demonstration that the environmental consequences conform with expectation (i.e. are acceptable), and are stable over time.

Sampling will be carried out at the same time of the year, preferably in the period February – May (i.e. before the main recruitment period for pelagic larvae), but only rarely will there be a need for seasonal sampling.

The choice of sampling locations should be informed by the outcome of the 'baseline' survey, and indeed sampling to generate the first (pre-dredging) data points in an 'ongoing' monitoring series may be feasible during this survey. As part of an overall quality assurance strategy, it will be important to check on the continued validity of stations selected as representative of impacted and reference conditions. This may be achieved by periodically repeating the 'baseline' survey, at intervals appropriate to local circumstances, but typically once every 3 – 5 years.

In 'ongoing' monitoring programmes, allowance must therefore be made for the possibility of modifications to sampling design or survey frequency in response to unanticipated man-made or natural influences. In some circumstances, design modifications may be justified in response to changes in dredging patterns within extraction sites. With the advent of Electronic Monitoring Systems which accurately record vessel movements during dredging, sampling may be precisely targeted at locations of varying dredging intensity (Boyd and Rees, in press and Chapter 2.4 below). Such an approach may also be useful in evaluations of the recovery of dredged areas after cessation of the activity.

A summary of the requirements of an 'ongoing' survey is given in Table 1.

## 2.4. The use of EMS information for designing surveys

Since 1993, every vessel dredging on a Crown Estate licence in the UK has been fitted with an Electronic Monitoring System (EMS). It consists of a PC electronically linked to a navigation system and one or more dredging status indicators. This automatically records the date, time and position of all dredging activity every 30 seconds to disk. Many of the dredgers operating in UK waters are fitted with Differential GPS navigation systems, which allow the EMS to operate with an accuracy of  $\pm 10$  m. This information can be collated and displayed as intensity plots showing the location of active dredging for any period of time. The information can also be interrogated to locate areas of the seabed within extraction sites, which have been subjected to different levels of dredging intensity. This information can be used in the design of seabed surveys and for interpreting the results (see, for example, Boyd and Rees, in press).

# CHAPTER 3

## The conduct of benthic surveys at aggregate extraction sites

### 3.1. Introduction

The type of gear selected for sampling seabed substrata and the benthic macrofauna at aggregate dredging sites is primarily determined by the hardness/compactness of the substrata. Whilst a wide variety of sampling methods are available (see Holme and McIntyre, 1984), only a small proportion of these have the ability to effectively collect samples from areas of relatively coarse sediments which are characteristic of dredging sites. Recommendations for equipment, which are capable of collecting samples of the benthic macrofauna and/or sediments from such areas, are provided below. Future innovations may improve sampling efficiency in such deposits and it should therefore be noted that certain techniques which are presently favoured may be superseded as new equipment is developed, tested and applied.

The majority of grab sampling devices are unsuitable for the collection of coarse sediments in environmental monitoring programmes. Typically, the downwardly-directed jaws are vulnerable to incomplete closure due to the presence of stones. For this reason, only a small number of grabs are presently appropriate for use at aggregate extraction sites (see Table 2). Whilst grabs allow quantitative evaluation of the macrobenthic infauna and a proportion of the epifauna, their size and mode of action means that they do not effectively sample the larger, rarer epifaunal species, or those capable of rapid avoidance reactions. Towed gear, such as trawls and dredges, are more appropriate for sampling these species, although usually at the expense of accurate quantification due to their inherent inefficiency (see below). For this reason, the overall aims of the survey should be taken into consideration when selecting the most appropriate sampling equipment and, in certain situations, it may be necessary to use more than one technique in order to sample the full range of benthic organisms present in an area. Finally, the important issues of position-fixing and vessel heading associated with the field sampling process are covered in Chapter 3.4.

## 3.2. Quantitative methods for sampling the benthic macrofauna and sediments

### 3.2.1. The Hamon Grab

The Hamon grab is the recommended tool for sampling the benthic macro-infauna from coarse substrata (Oele, 1978). This grab, originally designed by the Netherlands Institute for Applied Geosciences, consists of a rectangular frame forming a stable support for a sampling bucket attached to a pivoted arm (see Figure 4). On reaching the seabed, tension in the wire is released which activates the grab. Tension in the wire during inhauling then moves the pivoted arm through a rotation of 90°, driving the sample bucket through the sediment. At the end of its movement, the bucket locates onto an inclined rubber-covered steel plate, sealing it completely (Figure 4). This results in the sediment rolling towards the bottom of the sample bucket, thereby reducing the risk of gravel becoming trapped between the leading edge of the bucket and the sample retaining plate, and thus preventing part of the sample being washed out. Weights are attached to the grab to minimise the lateral movement of the supporting frame during sample collection. Weighting of the grab should be adjusted to obtain optimum sampling efficiency. A grab stand should support the grab before and after sampling (Figure 5). The stand should allow enough space for a container to be placed under the grab to receive the sampled material following its release from the bucket.

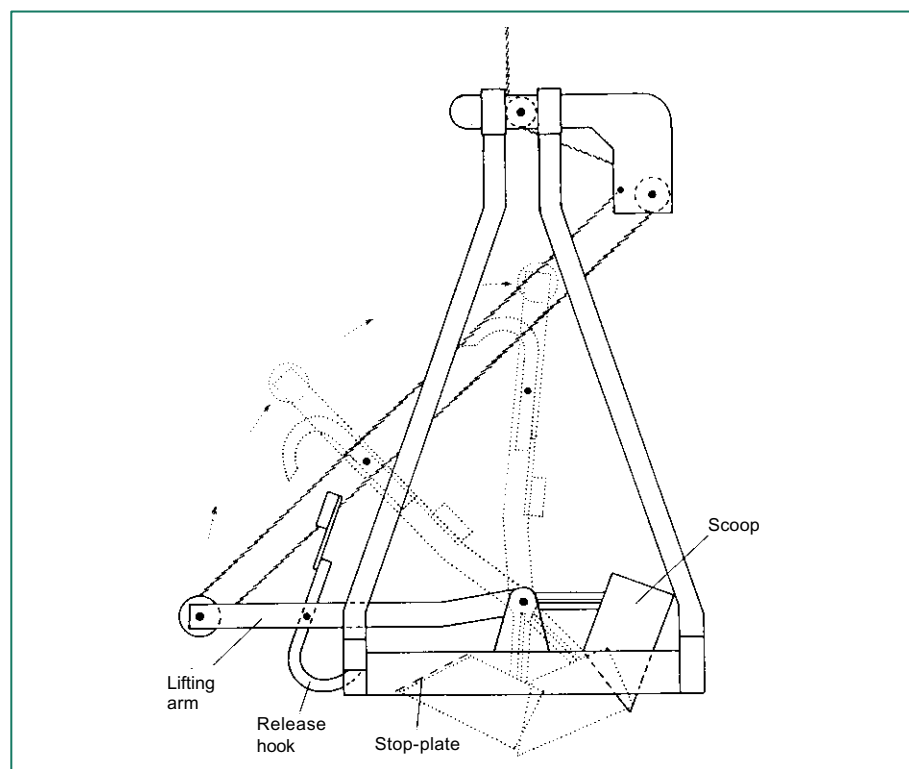


Figure 4 Hamon grab, showing mode of action. The lifting arm rotates through 90° to drive the sampling bucket (scoop) through sediment, closing against the stop plate. Plate taken from Eleftheriou and Holme (1984)



**Figure 5 A 0.1 m<sup>2</sup> Hamon grab supported on an open frame to facilitate retrieval of the sample into a moveable container following controlled release from the bucket. Note the nearside rack supporting lead weights to increase sampler efficiency (a comparable rack on the other side of the sampler is hidden from view in this photograph)**

The Hamon grab is robust, simple to operate and has been shown to be particularly effective on coarse sediments. It has been employed as a quantitative benthic sampler in several studies designed to assess the impacts of marine aggregate extraction on the macrofauna (van Moorsel and Waardenburg, 1991; Kenny and Rees, 1994, 1996; Kenny *et al.*, 1998; Seiderer and Newell, 1999). The original design was for a grab which samples an area of about 0.25 m<sup>2</sup>. Since then, CEFAS has introduced a smaller device, sampling an area of 0.1 m<sup>2</sup>. The height clearance (approximately 3 m) required for the larger device has caused operational difficulties when deployed from small research vessels (<25 m). However, when used from larger ships, it can be safely deployed and retrieved in most sea states (up to Beaufort Scale Force 5 to 6). Nevertheless, the smaller version (Figures 5-6) has a greater utility due to its ease of handling, which potentially widens the weather window for sampling and allows it to be used on smaller vessels. Furthermore, 0.1 m<sup>2</sup> is the conventional surface sample unit employed in most benthic surveys of continental shelf sediments, and conformity with this size therefore allows direct comparison of results with those from a wide array of other sources using a range of other sampling devices. This grab also takes quantitative samples of a more manageable volume than the large Hamon grab: up to a volume of 15 litres compared with up to 35 litres from the larger grab. Thus, the smaller grab is the preferred sampler for collecting samples of the macrobenthic infauna in a cost-effective manner. There may be locations with a very sparse fauna where the collection of a larger surface area is justified, but this can be achieved by increasing the numbers of replicates using the smaller-sized sampler. Enhanced replication also has the potential advantage of increasing the statistical power of the resulting data.

A drawback of the Hamon grab is that the sediment sample is 'mixed' during the process of collection and retrieval, thereby precluding the examination or sub-sampling of an undisturbed sediment surface.



**Figure 6 A 0.1 m<sup>2</sup> Hamon Grab being retrieved. Note the use of a winch-controlled lateral supporting rope for increased stability (and therefore safety) during deployment and recovery. It is unhooked before descent of the sampler**

### 3.2.2. The modified Day grab

The Day grab evolved from the spring-loaded Smith-McIntyre grab (see Holme and McIntyre, 1984), and represents an attempt to simplify this earlier type of sampling device, without loss of operational efficiency. It incorporates a frame to keep the grab level on the seabed and two trigger plates to activate the release, but there are no springs to force the hinged buckets into the bottom. The modifications over the original Day grab design (see Eagle *et al.*, 1978) consist of 'stub axles', with closing flaps that hinge from the exterior of the buckets, rather than centrally (Figure 7). This device samples an area of 0.1 m<sup>2</sup>, to a maximum depth of 14 cm. The jaws are supported within an open framework, which will cause minimal down-wash as it lands on the seabed (Figure 8). Lead weights are usually added to obtain optimum penetration of the sediment. The grab should not be allowed to bite too deeply into the sediment, as this results in the sediment surface making contact with the closing flaps of the sample bucket, which can ultimately lead to loss of material on retrieval and disturbance of the surficial layers. The jaws of the grab and the flaps on top should seal well to ensure no loss of material when the grab is retrieved.

This grab was designed for sampling soft sediments i.e. ranging from sands to muds. It does not function well on coarse sediments due to the tendency of larger particles to prevent closure of the buckets, causing loss of sample and is therefore not well suited for use at aggregate dredging sites. However, where there is a high percentage of soft sediment (sands or muddy sands) associated with a gravelly component, this grab could be used, albeit with the likelihood of a relatively high failure rate.

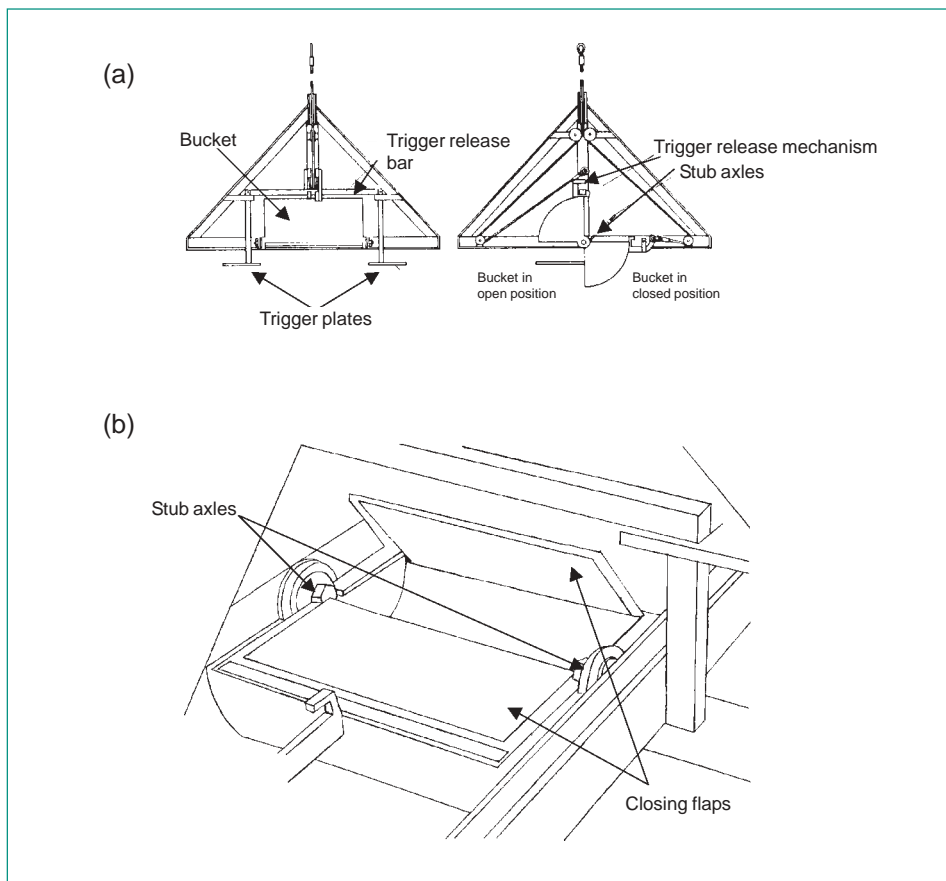


Figure 7 **Modified Day grab (source Eagle *et al.*, 1978). a) Side views of the grab showing the opened and closed bucket positions; b) Top view of the sampling buckets. In the original Day grab design a solid axle ran across the top opening between the two bucket pivots to which the closing flaps were hinged. In the modified design the solid axle has been replaced by stub axles and the closing flaps are hinged from the outer edges of the buckets to allow better access to the sample**



Figure 8 **A 0.1 m<sup>2</sup> Day grab during deployment. Note that the sample buckets are in the open position prior to sampling. Also note the addition of triangular lead weights to improve sampling efficiency**

### 3.2.3. The Shipek grab

The Shipek grab employs a semicircular bucket activated by powerful springs (see Holme and McIntyre, 1984). It has proved very effective in sampling coarse substrata and is widely used in marine geophysical and geochemical surveys (Figure 9). The spring loaded bucket rotates through 180° on closure, ensuring that no wash-out of sediment occurs during recovery through the water column. The strong spring mechanism also allows samples to be collected from relatively hard and consolidated sediments, albeit with an increased failure rate due to larger particles preventing proper closure. Unfortunately, due to its small size (sampling an area of approximately 0.04 m<sup>2</sup>), this device is unsuitable in routine macrofauna investigations, but may be useful in 'pilot' surveys aimed at preliminary characterisation of variability in habitat type and the associated fauna.

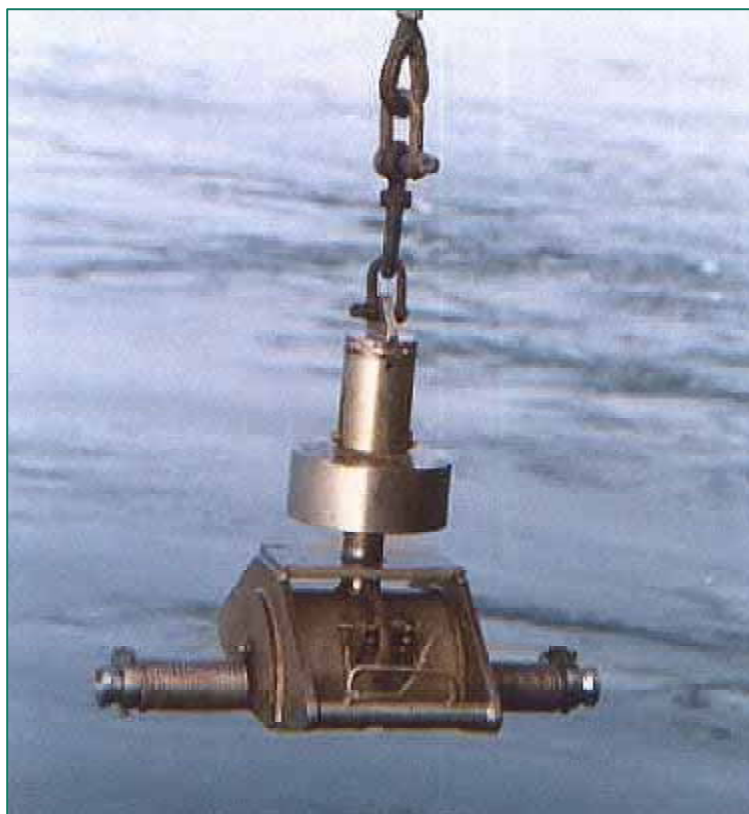


Figure 9 **A Shipek grab prior to deployment. Note the powerful spring on the side-arms, and the top-mounted weight under warp tension which, on release following contact with the seabed, induces firing of the closing mechanism and, at the same time, increases collection efficiency by downward pressure**

### 3.2.4. The van Veen grab

The van Veen grab (van Veen, 1933), in common with many other grabs, relies on the closure of two opposing jaws for the collection of a sediment sample. The difference between this and the Petersen grab (see Holme and McIntyre, 1984), is that the van Veen grab has long arms attached to each bucket, thus giving better leverage during closure. This mode of action is not ideally suited for the collection of coarse sediments as large particles of

gravel tend to become caught between the jaws, resulting in loss of the sample upon retrieval of the grab. Thus, whilst this type of grab has been used widely in benthic macrofauna studies, it is not recommended for use on coarser substrata. On softer substrata, (i.e. with a reduced gravel component) its performance characteristics are likely to match those of the Day grab and, in some instances, may be preferable on account of the greater leverage provided by the side arms. In such localities, the success rate, and therefore the cost-effectiveness of the device relative to, e.g. a Hamon grab, will be a matter for judgement by experienced survey scientists.

### 3.2.5. Other grabs

Canadian scientists have devised a hydraulically operated benthic grab, which incorporates a top-mounted camera for precision sampling (Gordon *et al.*, 1997; Rowell *et al.*, 1997). This has been found to work efficiently on gravel deposits in the Grand Banks area, but presently is employed as a research tool, and is an expensive option. Future developments may widen the scope for its application in routine monitoring. Similar considerations apply to industrial-scale samplers such as the hydraulic clam shell grab, and the scope for their adaptation to scientific-scale sampling merits further exploration.

### 3.2.6. Deployment and recovery of grabs

Despite substantial differences in the design and operation of grab samplers, there are a number of important general issues relating to their deployment and recovery (see for example, Rumohr, 1999). During retrieval of the gear from the seabed, the first 5 metres of warp should be hauled slowly so as to maximise sampling efficiency. The grab can then be hauled to the surface at a faster rate. When the grab reaches the sea surface, it should be swung onboard as soon as possible, as the device presents a danger on a rolling vessel. Once the grab is recovered, it should be lowered on to a supporting frame, designed to allow efficient placement and removal of a sample container underneath the sample bucket. In rough seas, the bow of the vessel should, where possible, face into the direction of oncoming swell, thus minimising the roll of the vessel, and hence reducing the potential for loss of control of the grab during deployment and recovery.

### 3.2.7. Corers

A large number of corers have been designed for the collection of sediments and the associated macrobenthic fauna (see Holme and McIntyre, 1984). On coarse or well-consolidated sediments many of these devices will have a low sampling efficiency, as coarse sediment particles will prevent penetration of the sampling device and will hinder the proper sealing of the core barrel. Therefore such devices are not appropriate for routine surveys of the macroinfauna from marine aggregate extraction sites. However, devices such as vibrocorers (James and Limpenny, *unpublished*) will be appropriate for collecting samples from coarse substrata in order to evaluate vertical structure and integrity. Vibrocorers are widely used by the industry in prospecting surveys and therefore may provide information relevant to 'pilot' surveys. Again, adaptation of industrial scale vibrocorers for use in environmental sampling programmes merits further attention.

<b>Sampling device</b>	<b>Surface area sampled</b>	<b>Approximate weight without sample</b>	<b>Suitable for sampling coarse substrata</b>	<b>Quantitative/ semi-quantitative</b>	<b>Easily and safely deployed from small (&lt;25 m) vessels</b>	<b>Advantages</b>	<b>Disadvantages</b>
Small Hamon grab	0.1 m <sup>2</sup>	300kg + weights up to 300 kg.	Yes	Quantitative	Yes	Easy to handle. Surface area sampled conforms with the conventional sampling unit for continental shelf sediments.	More replicates may be required in patchy environments, compared with its larger counterpart.
Large Hamon grab	0.25 m <sup>2</sup>	350kg + weights up to 150 kg.	Yes	Quantitative	No	Large sample may be more representative of coarser or more sparsely populated sediments.	Large size makes it more difficult to handle than the smaller version. Large sample volumes (35 litres max) can be relatively time consuming to process. Surface area sampled not directly comparable to other sampling devices.
Day grab	0.1 m <sup>2</sup>	80 kg + weights up to 80 kg.	No	Quantitative	Yes	Easily deployed. Standard sampler for most U.K. infaunal soft sediment surveys.	Not effective in coarse substrata.
Small van Veen grab	0.1 m <sup>2</sup>	80 kg	No	Quantitative	Yes	Easily deployed. Widely used for infaunal surveys, especially in continental Europe.	Not effective in coarse substrata.
Large van Veen grab	0.2 m <sup>2</sup>	100 kg	Will meet with variable success depending on coarseness of substrata	Quantitative	Yes	Easily deployed. Widely used for infaunal surveys, especially in continental Europe.	Unreliable in very coarse substrata, but may be more effective in some coarse sediments than 0.1m <sup>2</sup> version.
Shipek grab	0.04 m <sup>2</sup>	80 kg	Yes	Unsuitable for infaunal assessments	Yes	Can be used effectively for physical characterisation of substrata.	Sample too small and variable for quantitative faunal assessment.
Newhaven Scallop dredge	Variable	Single dredge 90 kg. Three dredges on beam 400 kg	Yes	Semi-quantitative	Single dredge configuration easily deployed. Two or three dredge option more cumbersome.	Extremely robust design is suitable for use over coarse unconsolidated substrata. Under favourable conditions can sample effectively for the duration of a fixed distance tow.	Sampling efficiency variable under poor weather conditions. Heavy. Selective towards epifauna.
Rallier du Baty dredge	Variable	80 kg	Yes	Semi-quantitative	Yes	Robust design will work in most unconsolidated substrata. Circular mouth increases sampling efficiency. Easy to deploy. Can be fitted with mesh liner for retaining smaller organisms.	Uncertain mode of sampling, especially over coarse or rocky terrain.
Modified Anchor dredge	Variable	65 kg	Yes	Semi-quantitative	Yes	Inexpensive and easy to handle. Will operate either side up. Easy to deploy.	Uncertain mode of sampling, especially from large vessels. Can be damaged on rocky ground.
Rock dredge	Variable	140 kg	Yes	Semi-quantitative	Yes	Can be used over very coarse ground, including bedrock. Useful for 'blind' sampling during pilot surveys. Can be fitted with mesh liner for retaining smaller organisms.	Heavy when full. Uncertain mode of sampling.
Heavy duty 2m Beam trawl	Variable	60 kg	Yes	Semi-quantitative	Yes	Can yield relatively consistent samples over coarse unconsolidated substrata, under calm sea conditions.	May be damaged if towed over very coarse or rocky terrain. May lose bottom contact during unfavourable sea or tidal states, or as a result of vessel speed and size.

### 3.3 Qualitative and semi-quantitative methods for sampling the benthic macrofauna

Much of the following account is taken from Rees and Service (1993) and Rees *et al.* (1990) and is mainly concerned with epifauna sampling. However, certain devices referred to (notably the Anchor dredge) will also be appropriate for the sampling of infaunal populations.

#### 3.3.1. Background

The epibenthos comprises animals and plants living on - as distinct from within - the seabed. They may be sedentary, e.g. hydroids and bryozoans, or motile e.g. decapods, starfish and flatfish. Animals in the former category are typically filter-feeders, whilst the latter are typically carnivores or omnivorous scavengers. Some groups spend their entire adult life intimately associated with the seabed, e.g. hydroids, most crabs and flatfish, while others may only be transiently associated, e.g. shrimps and many ground fish species. Subtidally, the most well-developed epibenthic assemblages normally occur on mixed substrata with a significant coarse component, where the range of micro-habitats can allow colonisation by a wide array of species.

There are several attributes of the epibenthos of coarse substrata which can make this group an important target in environmental assessment. For example:

- i. on predominantly rocky areas or tide-swept grounds, they may be the only significant component of the benthos. Such areas may support an exceptionally high diversity and biomass of species, e.g. associated with subtidal mussel beds;
- ii. sedentary epibenthic species provide a direct route for carbon from the water-column to the seabed via filter-feeding;
- iii. many species are preyed upon by fish;
- iv. complementary surveys of the epifauna provide additional information, beyond that obtained from infaunal investigations, about the status of an area, e.g. in terms of the range and relative abundance of species present, or their mode of feeding.

#### 3.3.2. Sampling approaches

Because of the much wider size range of organisms encountered compared with the infauna, as well as factors such as the motility and comparative rarity of some of the component species, small (0.1 m<sup>2</sup>) grab samplers are generally unsuitable for quantitative assessment of the epifauna. Moreover, on mixed substrata or hard ground, grab sampling devices may operate at low sampling efficiency or not at all. A wide range of dredges and trawls have been devised for remote epibenthic sampling, with varying efficiency of organism retention (see e.g. Eleftheriou and Holme, 1984 and below). In addition, a number of devices, more usually associated with epifaunal sampling, can collect large volumes of sediment. In this

mode of operation, such devices can be useful tools for semi-quantitative or qualitative sampling of the infaunal fraction of the benthos during 'pilot' surveys.

Given suitable tidal and weather conditions and adequate water clarity, diving probably provides the best means for quantitative assessment of the epifauna through a combination of direct observation and sampling, but these conditions are not typical for much of the U.K coastline. For these and other logistical reasons diving can be an expensive - and potentially hazardous - option for the conduct of regular offshore monitoring programmes, although guidelines for survey and sampling of inshore (mainly rocky) habitats by this means are well-defined in the UK, largely through the efforts of conservation interests (e.g. Hiscock, 1990; Davies *et al.*, 2001).

Alternative methods for *in situ* assessment include remotely deployed underwater video and still photography. These, along with a range of other imaging methods, have been reviewed by Rumohr (1999); further detail is provided below. Again, water clarity is an important limiting factor but, in general, this option is likely to be cheaper and less weather-dependant than diving surveys. Moreover, they may be operated in areas deeper than those normally accessible to divers.

A combination of *in situ* observation by photography and efficient remote sampling of sediments offers the most promising tool for routine assessment of epifaunal communities at aggregate extraction sites. This may be achieved by attaching a video camera and light in order to collect an image of the seabed adjacent to or in front of the sampling device.

### 3.3.3. Trawls

Small-sized Beam and Agassiz trawls are commonly used for remotely sampling the epifauna in a 'semi-quantitative' or qualitative manner (see Holme and McIntyre, 1984). These trawls are designed to sample at and just above the surface of the seabed and, because of the relatively large area that can be covered in one deployment, they are appropriate for collecting the larger, rarer or more motile species. A 2-m beam trawl is generally to be recommended for sampling the epifauna at marine aggregate extraction sites. The small size of this type of gear makes it easy to deploy and usually results in the collection of a manageable sample size. On coarser substrata, such as those likely to be encountered during surveys of aggregate extraction sites, the use of a heavy duty 2-m beam trawl is advised (Figure 10). This consists of a metal beam, a chain mat designed to prevent the collection of larger boulders, and chafers to limit net damage (see Jennings *et al.*, 1999 for design information). Standard 2-m Lowestoft beam trawls with wooden beams and tickler ground chains (Riley *et al.*, 1986) have also proved useful for epifaunal sampling on finer substrata (Rees *et al.*, 1999) (see Figure 11). The net consists of a belly (98 rows m<sup>-2</sup>) and codend (157 rows m<sup>-2</sup>), with a 3mm mesh codend liner to capture smaller organisms.

On each deployment, 2 m beam trawls should be towed over a distance which will produce a sufficiently large sample to adequately characterise the epibenthic community, but not so large that the sample is unmanageable. The appropriate towing distance will vary according to ground type and the density of the epibenthic fauna. For this reason, it is prudent to assess the towing distance to be used for the survey by carrying out a trial tow or tows before commencing the survey proper. As a general guide, towing distances of between 200 m and 800 m produce manageable sample sizes, i.e. when the objective is a full census of all animals retained, whilst covering sufficient ground to adequately characterise the communities. Start and end positions should be recorded for each tow, even in cases where towing over a fixed time interval is the primary goal. This allows calculation of the appropriate distance



Figure 10 **A heavy-duty 2-m beam trawl with chain mat being recovered over the stern of the research vessel. Note the metal beam between the two trawl shoes and the chain mat attached to the underside of the beam**



Figure 11 **A standard Lowestoft 2-m wooden beam trawl (Riley *et al.*, 1986) at the water surface prior to deployment. Note the wooden 2 m beam and the pair of towing bridles attached to the trawl shoes**

covered (see also below). The start position should be recorded at the point at which the trawl winch is stopped, and the end position should be recorded at the point of commencement of hauling.

Beam trawls can be towed on a pair of bridles attached to a single tow-rope or line. As a guide, the length of the warp should be approximately three times the maximum expected water depth. Clearly, the speed at which the beam trawl is towed will depend on local circumstances and the type of vessel employed; however, a maximum speed of 1.5 knots over the ground is recommended. The beam trawl is likely to be damaged if it meets an obstruction on the seabed whilst being towed, which can limit the use of the gear to relatively uniform areas. Trawls should be inspected before and after tows and any repairs should be carried out immediately, with any damage being noted in the field log. A second tow may be necessary, and its contents assessed, before a station is finally abandoned. Clearly, serious damage to the net or frame will be taken to indicate that the station is unsuitable for beam trawl deployment. If this is the case, careful consideration should then be given to nearby locations where trawling may be possible.

Evidence that the device has maintained good bottom contact during towing should be sought from an examination of the warp under tension, and of the beam trawl shoes on retrieval. Inspection of the underside of trawl shoes will give an indication of seabed contact and whether the gear has been sampling in the correct orientation. Clearly this cannot provide confirmation on the extent of bottom contact. An odometer wheel attached to one of the shoes can also provide useful information on seabed contact, although its function may be impaired on mixed substrata or in the presence of significant quantities of hydroid colonies, which can become entangled in the mechanism. (Research is in progress at CEFAS to produce a more reliable electronic device for determining the duration of bottom contact). A weight placed in the cod-end of the trawl can also be used to prevent the net fouling the beam during deployment.

The efficiency of the sampling gear will often be dependent on the different tidal and wind conditions that prevail at the time of sampling and, for offshore surveys, it is rarely practicable to co-ordinate effort in such a way as to ensure close comparability on all sampling occasions. Thus sample size and quality may vary, irrespective of whether tows are conducted over fixed times or fixed distances. Therefore it is essential that information on tidal state and weather conditions are recorded, as they may contribute to observed differences between stations and/or sampling times. It is to be expected that the efficiency of capture of epibenthic organisms by the trawl will vary with substratum type and weather conditions, and will always fall well short of 100%.

A degree of expert judgement regarding sampling efficiency will be a routine requirement during trawl survey, and samples will accordingly be accepted or rejected on this basis. This also emphasises the need to recognise that the data generated are, at best, 'semi-quantitative' in nature. Further work is required in order to improve the quality and comparability of epifaunal data generated from trawl surveys.

### 3.3.4. Dredges

In general, the use of towed dredges for evaluation of epifaunal community structure should be avoided when other sampling tools (e.g. beam trawls) can be effectively employed. However, where the hard or uneven nature of the substrata precludes the use of a trawl it is often possible to obtain adequate samples using dredges, a variety of which are available (Holme and McIntyre, 1984).

## NEWHAVEN SCALLOP DREDGE

The Newhaven Scallop dredge (Franklin *et al.*, 1980) (Figure 12) is a commercially-used towed device that may be operated over very coarse terrain but would be likely to suffer damage if towed over bedrock or through large boulders. The dredge itself consists of a triangular steel frame supporting, on its underside, a spring-loaded plate to which a tooth bar, designed to dig into the sediment, is bolted. When the dredge encounters rock or large stones, the springs allow the tooth-bar to swing back thus avoiding snagging and reducing the quantity of stones caught. The tooth bar is normally 0.8 m wide and bears about 10 teeth up to 7 cm long. The mouth of the dredge is approximately 800 mm wide and 110 mm high during deployment. Also attached to each frame is a bag whose lower surface is made up of heavy-duty metal links (outside diameter ~55 mm, inside diameter ~42 mm) with an upper surface of heavy gauge nylon mesh. The maximum diameter of particle likely to be retained within the dredge is approximately 20 mm. A number of these dredges may be attached to a robust metal beam which is fitted with large rubber rollers at each end.

The dredges are deployed over the stern or side of a vessel and towed for a pre-determined time. Care must be taken to ensure that the dredge is deployed the right way up. The sampling efficiency of the dredge for each tow can be assessed on deck, normally by the quantity of material collected. Variables such as the duration of the tow or the length of warp paid out can be adjusted each time in an attempt to increase the quantity of material collected. In general, the same considerations employed during the beam trawl survey (Chapter 3.3.3) regarding towing duration should be applied. Samples collected using the Scallop dredge should only be treated as at best semi-quantitative in nature. The use of this device is recommended for the collection of qualitative samples as a last resort in areas of coarse, unconsolidated sediments which are too rough or uneven to permit the deployment



Figure 12 **A Newhaven Scallop dredge. Note the robust metal beam with rubber rollers on each end. Three dredges are attached to the beam, and the upper nylon mesh side of the collection bags are visible**

of less robust gear (e.g. small trawls). They may also be used to sample for 'keystone' species, such as horse mussels, from an area of interest. The Scallop dredge may be used to test the suitability of the ground prior to the deployment of less robust gear (e.g. beam trawl). This may be particularly useful if the ground is thought to be very coarse or uneven. There are other types of commercial Scallop dredges (e.g. French Scallop dredge) which may also be used in environmental sampling, but which differ in that they are heavier in design and lack the spring-mounted teeth (see Franklin *et al.*, 1980).

### **RALLIER-DU-BATY DREDGE**

The Raillier-du-Baty dredge (Figure 13, see also p.498 in Cabioch, 1968) is designed to work in a range of substrata from sands to cobbles, and has a long and successful history of use in the English Channel and Celtic Sea (e.g. Cabioch, 1968). It consists of a robust metal ring (inside diameter 550 mm large version, 390 mm small version) attached to a central towing arm. An open ended bag of the desired mesh size (e.g. 500  $\mu\text{m}$  or 1 mm) is attached to the ring, and the trailing end of the bag is tied to prevent loss of material during collection of the sample. This inner bag is protected by an outer, coarser bag which is, in turn, enclosed by a heavy duty apron of fishing net, in order to reduce chafing. The warp is attached to a fixing point on the metal ring, and a weak link is placed between this point and the central arm. This optimises the digging capability of the edge of the ring and reduces the chances of the edge being lifted away from the seabed.



**Figure 13 A Raillier-du-Baty dredge. The dredge consists of a robust circular metal mouth to which a collection bag is attached. The dredge is towed from the bridle attached to the outer rim of the dredge. A weak link between the towing bridle and the central towing arm of the dredge is designed to break if the dredge meets an obstruction on the seabed**

The dredge is deployed over the stern or side of a vessel, and the warp is paid out to a length of approximately three to five times the water depth. Contact with the seabed can be judged by the vibration of the warp as the device is towed. The dredge should be towed at not more than 1.5 knots for a pre-determined time which should not normally exceed 5 minutes. On completion of the tow, the dredge is recovered and the mesh bag untied. For convenience, the dredge can be suspended and the sample released onto the deck. Samples collected by this method should be treated as semi-quantitative or qualitative.

The circular nature of the mouth of the dredge allows it to roll as it is towed across the seabed, which has the advantage that the device can continue to sample over uneven terrain. The device is suitable for collecting both infaunal and epifaunal organisms. The disadvantage of this gear is that it can collect very large volumes of sediment (e.g. occasionally > 100 litres) which may be very time consuming to process. It can also be difficult to judge whether the dredge fills up immediately upon reaching the seabed, or whether it fills gradually as it is towed along the seabed. This uncertainty can complicate interpretations of the resulting macrofaunal data. This device is not for routine use other than in cases where recommended by CEFAS, and where other sampling tools prove ineffective.

## ANCHOR DREDGE

The Anchor dredge (Forster, 1953) is designed to be operated from a small vessel in sandy sediments, although it can produce acceptable samples when used on coarser substrata (see Eleftheriou and Holme, 1984). It consists of a rectangular metal frame, forming the mouth



Figure 14 **A CEFAS modified Anchor dredge. Note the solid rectangular metal collection box (as opposed to a net bag as in the original design), the open side of which forms the mouth of the dredge. The warp is attached to a hinged wishbone arm which enables the dredge to collect a sample irrespective of which side it lands on the seabed**

of the dredge, which is towed by hinged wishbone arms. In the original version a net collection bag is attached to the rear of the device to retain the sample (Holme and McIntyre, 1984). This design has been modified by CEFAS to make the device more robust (Figure 14). In the modified version, the net collection bag is replaced by a sealed metal plate: the dredge therefore consists of a metal box, the open anterior end of which is 450 mm wide by 225 mm deep.

The Anchor dredge is deployed over the side or stern of a vessel and after sufficient warp is paid out (three to five times water depth) the warp is secured. As the name suggests, the dredge is intended to collect a discrete sample from a single point as it digs into the sediment under the weight of the drifting vessel. On larger vessels, it may be employed deliberately or by default as a towed device but, as with the Rallier-du-Baty dredge, uncertainty in its mode of action at the seabed may complicate interpretations of the resulting data. Again, the data generated are, at best, semi-quantitative in nature. Advantages of this dredge are that it can fall either side up and will still collect a sample, its small size makes it relatively easy to handle and deploy, and it is relatively inexpensive.

## ROCK DREDGE

The Rock dredge (Nalwalk *et al.*, 1962) (Figure 15) is an extremely robust device that was originally designed for the collection of rock samples from deep-water locations. It is comprised of a heavy gauge rectangular metal rim to which a heavy-duty mesh made of interlaced metal rings is attached. The dimensions of the mouth of the dredge are 595 mm wide by 400 mm high and the diameter of the rings is 55 mm outside diameter and 42 mm inside diameter. The largest particle which can pass through the mesh is approximately 20 mm. It can be used successfully over most substrata including gravels and cobbles, and will even collect surface scrapings of bedrock. It is possible to fit a fine mesh bag inside the outer metal mesh enabling the dredge to collect finer material. The mesh size used will depend on the requirement of the survey. The dredge is deployed in a similar fashion to that described above for other dredges. On return to the deck the dredge is lifted by its trailing end and the sample is tipped onto the deck. As with other dredges, the data generated should be treated as, at best, semi-quantitative.



Figure 15 **A Rock dredge. Note the heavy-duty rectangular metal rim and the collection bag consisting of interlaced metal rings**

The robust nature of this type of gear permits deployment in areas where little is known about the nature of the substratum, and it may therefore be useful during 'pilot' surveys, at locations where difficult sampling conditions are a possibility. Advantages of this dredge are that it can fall either side up and will still collect a sample, its small size makes it relatively easy to handle and deploy and it is relatively inexpensive.

### 3.3.5. Underwater video and stills techniques for surveying hard ground

The following account is an extended version of that provided by Rees and Service (1993). Underwater video and stills photography are valuable, non-destructive methods for the assessment of all types of seabed habitat. They can be particularly useful over hard and consolidated ground where the sampling efficiency of other physical sampling methods is low. Remote-control underwater photography has been in use for a number of years to obtain static images of the seabed, and high quality images can be obtained which enable the identification of much of the macro-invertebrate fauna present. These images cover a small area of seabed and, while useful in pilot surveys, do not give information on the overall distribution of faunal communities.

To allow wider coverage of the seabed, photographic and video cameras have been mounted on a variety of platforms (Figures 16-17). Cameras have also been attached to a variety of grabs to provide real time images of the nature of the substratum sampled. However, in most instances platforms will fall into one of the following categories:

- devices which are capable of moving or being directed under their own power such as Remotely Operated Vehicles (ROVs).
- samplers which are lowered to a point above the seabed (e.g. remotely operated hoisted platforms), or are towed along the seabed, such as photographic sledges.

The most commonly used method for photographing coarse aggregate environments is the camera sledge (Figure 16), which is robust and simple to operate. It is usually towed over the seabed at slack water and typically includes a vertically mounted stills camera and a forward-, or sideways-pointed television camera linked by way of an electrical 'umbilical' cable to a recording unit on the survey vessel. This allows still photographs to be taken at selected locations of interest, or at regular fixed distances. By using a fixed frame, the area in view at any one time can be calculated and this, coupled with knowledge of the distance covered in any one haul, allows transect-type studies to be conducted.

Cameras can also be mounted on ROVs, which are self-propelled vehicles controlled by commands from the surface which are relayed down an umbilical cable which also carries the video and other telemetry signals. The apparent advantage held by ROVs over towed vehicles is their manoeuvrability, which offers the freedom to move in three dimensions. This should allow objects to be viewed from a variety of angles and the vehicle can be stopped or moved back onto an object for further study. However, small ROVs are restricted by their limited capability to operate in currents in excess of 1.5 knots. The area covered by ROVs is generally restricted by the length of umbilical and the water depth.

A third alternative to the systems listed above is the Remotely Operated Towed Vehicle (ROTV), whose depth and altitude are controlled by rotors. Such devices allow relatively fast towing speeds and the possibility of midwater observations. However, the cost of the elaborate control systems required for these devices will tend to limit their use by smaller organisations.

Camera systems, both video and stills, may also be attached to other sampling platforms such as grabs, corers and trawls. This enables the collection of images which not only relate directly to the substratum being sampled, but in many cases also allows some assessment of the sampling efficiency of the device to be made.

The following guidelines for the deployment of underwater camera systems are recommended:

- i. underwater photographic systems should normally comprise at least one video camera and a stills camera;
- ii. where towed sledges are used, the field of view of each camera should be known from previous calibration;
- iii. the distance travelled by the sledge should be known, either using the ship's electronic navigator or a meter wheel attached to the sledge;
- iv. towing should be at constant speed;
- v. still photographs should be taken at fixed intervals either on a distance or on a time basis. These can be backed up by opportunistic shots taken of 'interesting subject matter', e.g. dredge tracks as identified on the video monitor.
- vi. where ROVs are used, the distance travelled, heading, height above seabed and field of view should be calculated.

The quality of photographs largely depends on water clarity and this can vary considerably, even at the same location, depending on the state of the tide and season of sampling. The chances of encountering good visibility can be increased by deploying the equipment at slack water periods. Use of towed devices is also dependent on the tidal/current speeds, requiring towing speeds of less than 1 knot to obtain clear images. Therefore, the slow towing speeds necessary to obtain high quality images when using towed sledges means that, at most UK extraction sites, transects will be run in the form of controlled drifts along the direction of the prevailing tidal current. The data from such surveys can be treated at a number of levels, which will be partially determined by the quality of the images obtained (see Figure 18). Still photographs taken at regular intervals along the transect can be treated as point quadrats, the fauna identified to the appropriate taxonomic level and quantified. Data obtained by 'freezing' the video image at regular intervals can be treated in a similar manner. It should also be noted that advances in digital video and stills technology are improving image quality, and such systems may become the preferred choice as the price of these continues to fall. Digital video has the advantage that near-photographic quality images can be obtained by 'freezing' the video image. It may therefore become unnecessary to have both video and stills equipment mounted on underwater survey platforms.

Two of the most common platforms used for the collection of photographic data in the UK are shown in Figures 16 - 17. It should be recognised, however, that other systems exist, which are currently not in use in the UK and that may be useful for the collection of video and stills images. In particular, a number of pieces of equipment recently developed in Canada are worthy of further investigation for application in the UK. The BRUTIV (Bottom Referenced Underwater Towed Instrument Vehicle) is a video sledge that is towed a few metres above the seabed and collects good images of conspicuous epifauna and their



Figure 16 **A CEFAS camera sledge. Note the downward pointing 35 mm stills camera at the front of the sledge and the forward looking video camera towards the rear of the sledge. The large buoy on the back of the sledge floats at the surface during deployment, and provides a visual indication of the position of the sledge. It also acts as a means of recovery for the main towing cable parts**



Figure 17 **A drop-camera frame. The video and stills cameras, lights and flash unit are housed within the protective metal frame, orientated to collect images of the seabed directly below the frame**

associated substrata (Gordon *et al.*, 1997; Rowell *et al.*, 1997; Gordon *et al.*, 2000). This device may be less effective in UK waters where water turbidity would preclude the collection of images at distances of several metres from the seabed. An elaborate videograb has also been developed which allows scientists to evaluate, and if necessary reject, a sample whilst the grab remains at the seabed. The grab has a video camera directed at the seabed through the jaws of the device. When a suitable substratum is located, the grab is dropped and the jaws are closed hydraulically. This system has worked well on many substratum types and has the advantage that poor samples can be rejected, and further sampling attempts can be made whilst the grab is still at the seabed. This may be particularly advantageous where the collection of good quality samples from coarse substrata is problematic. Another device which utilises photographic techniques is the Aquareve III Epibenthic sledge (Gordon *et al.*, 1997; Rowell *et al.*, 1997). This is primarily a device for the collection of surface substrata, and macrofaunal species living at and just below the sediment surface. It has a backward pointing video camera which monitors the performance during sample collection and provides information on the undisturbed nature of the substrata collected.

These devices are expensive in comparison to the video and stills equipment currently in use in the UK for environmental surveys, and furthermore are not easily available. Nevertheless, they merit further consideration as an alternative to conventional devices.



Figure 18 **An example of a digital still image of a 'gravelly' substratum taken using a Benthos™ DSC4000 digital stills camera mounted on a drop camera frame (Figure 17)**

### 3.4. Positioning

It is essential that a geographical reference position can be assigned to any sample or datapoint that is generated from a survey. The most frequently used positioning system both in UK waters and worldwide is the Global Positioning System (GPS) which can provide the latitude and longitude of a point to within a few metres. Differential GPS (DGPS) improves on the accuracy of GPS by using precisely-surveyed reference ground stations to generate a position which is accurate to within a metre (Ashjaee, 1986). The absolute accuracy of GPS depends on numerous factors, but by using DGPS the antenna of a vessel may be confidently and precisely located anywhere in the world.

On larger vessels, the position from which gear is deployed may be tens of metres away from the antenna location, thus generating an inaccurate sampling position, and this potential source of error should be corrected for (e.g. with the use of survey software to allow manual input of an offset) in surveys requiring a high degree of accuracy.

If very precise sample or equipment positioning is required this can be achieved using acoustic positioning systems. Acoustic positioning is used for the location of underwater objects and is available in two different forms. Long baseline systems (LBL) involve a network of accurately positioned seabed beacons, which are used to calculate a triangulated position from a transponder fitted to the sampling equipment. In contrast, short baseline systems (SBL) rely on vessel-mounted sensors, which can detect the incoming direction of an acoustic signal from a remote beacon or transponder fixed to the item being tracked. Short baseline systems are especially aimed at tracking towed sensors such as a sidescan sonar fish, where cable length measurements are not sufficiently accurate for the aims of the survey.

Concerns about positional errors must be weighed against the aims of the survey. In most cases, horizontal accuracies to within a few metres are sufficient for routine assessments at aggregate extraction sites.

Information relating to the horizontal datum, projection and grid used for the survey must be documented and the same versions of these variables should be applied when post-processing the positional data.

### 3.4.1. Heading

Vessel heading is measured by magnetic or gyro compass systems. Magnetic systems are lower cost but less accurate. In contrast, gyro compass systems are expensive and mechanically complex, but solid-state sensors are now available using laser interferometry to give accurate heading and attitude information. GPS based systems are also available which can provide more accurate measurements than gyro-compasses. However this level of accuracy is rarely required in the execution of routine surveys.

# CHAPTER 4

## Approaches to processing benthic samples

### 4.1. Introduction

For convenience, the process of extracting macrobenthic organisms from sediments or (in the case of trawls and dredges) other residual material is usually separated into two stages. Initially, samples are processed over sieves of appropriate mesh sizes on board the survey vessel in order to reduce the bulk of the material transported back to the laboratory. Having reduced samples to a manageable size in this way, the retained material is preserved and the final sorting of the fauna from the residue can then proceed in the laboratory at a later stage. In cases where, for logistical reasons (e.g. due to restricted deck space or limited numbers of personnel), it is impractical to carry out sample processing onboard the survey vessel, entire samples may be preserved in the field (see Chapter 4.4.3), and then dealt with on return to the laboratory.

This chapter describes the treatment of benthos samples obtained using grabs, trawls and dredges.

### 4.2. Approaches to processing quantitative samples collected by grabs

#### 4.2.1. Estimation of sample volume

On retrieval of the grab, an estimate of the sample volume should be made, along with a description of the sediment type. This information is required, since it provides an indication of the performance of the grab and should be noted in the survey log (see Chapter 4.4.6). With, for example, a Day grab, where a relatively undisturbed sample can be accessed *in situ* via opening flaps, an estimate of the volume can be made by measuring the depth of sediment at its deepest point (usually at the point of closure of the grab buckets) and then applying a standard conversion factor. Alternatively, with a Hamon grab sample, an estimate may be made by measuring the depth of the sediment following release of the material into an underlying sample container.

In some cases, it may be necessary to reject grab samples and the following criteria should be used:

1) Sample inspection

If the jaws of the grab are not fully closed (e.g. due to the presence of stones) and there is associated evidence of the winnowing of surface material, then the sample should be rejected.

2) Acceptable sample volume

For the Hamon grab, the aim should be to collect a minimum sample volume of 5 litres, and samples smaller than this would normally be rejected. However, in very coarse substrata, the failure rate may be very high, and expert judgement should be exercised regarding the collection of the occasional sample of less than 5 litres. The reasoning behind this judgement should be documented in the survey log and in any subsequent reports, and the sample(s) flagged on account of their failure to meet the above quality criterion.

Pooling of collected material, i.e., the practice of amalgamating two or more samples individually rejected due to insufficient volume to provide a composite sample of acceptable size, **should not** be carried out. This procedure is invalid, as faunal occurrences are expressed in terms of unit area, not volume. Volume is used as a practical measure of sampling efficiency (see above). The relationship between volume and faunal content is more complex and more unpredictable than that of surface area: **the two measures should not be confused.**

## 4.3. Separation of benthic infauna from the sediment

Sediment samples should be slowly released into appropriately sized sample containers, ensuring there is no spillage of material. Once an acceptable sample has been obtained a sub-sample for sediment particle size analysis is taken (see Chapter 7.2.1).

The contents of the sample container should then be transferred to a purpose-built sieving table where it should be washed with seawater (under gentle hose pressure) over a removable 5mm square mesh screen. A range of equipment for washing and sieving sediment samples is available and these have been reviewed by Eleftheriou and Holme (1984) and Proudfoot *et al.* (*in prep.*). They vary from purpose-built sieving tables (e.g. Figure 19) to automated methods such as the 'Wilson autosiever' (Proudfoot *et al.*, *in prep.*). However, the utility of automated methods for extracting fauna from mixed sediments has not been fully assessed and consideration should be given to any recommendations arising from an ongoing 'best practice' review (Proudfoot *et al.*, *in prep.*).

For convenience, the description below relates to the treatment of samples using a sieving table, as this is the device most frequently employed for processing benthic samples in the U.K. This device consists of an open wooden box, the base of which slopes downwards towards an outlet pipe. The interior of the box is coated with epoxy resin to present a smooth surface for ease of washing, and to act as a wood preservative, thereby prolonging the life of the device. Small blocks mounted on the interior of the box provide support for a removable square stainless steel frame with 10 mm or 5 mm square mesh aperture. The



**Figure 19 Processing of a macrofaunal sample using a purpose built sieving table on a survey vessel. The sample is being washed over a 5 mm square mesh aperture sieve supported by a removable square stainless steel frame. Note also the 1mm mesh sieve held within a sieve holder beneath the outlet pipe of the table**

entire device is supported on legs that can be adjusted to allow the table to be positioned at a suitable height (normally waist height) for ease of use.

Collected sediment in the sample container should be slowly transferred to the 5 mm mesh sieve supported within the sieving table, using gentle hose pressure. In this way, much of the lighter fraction (including the more delicate organisms) is separated from the residual coarser material at an early stage. Once the sample has been emptied onto the sieving table, the larger animals and all encrusting fauna present on shell and gravel which are retained on the 5 mm mesh can be removed and transferred to sealable plastic bottles or buckets (of appropriate size). It is recommended that this material is preserved separately in order to prevent damage through abrasion to the smaller and more delicate organisms collected on a finer mesh sieve at the outlet pipe. Any remaining material on the 5 mm mesh sieve screen may then be discarded. The finer sediment fraction is washed over a 1 mm or (exceptionally) a 0.5 mm mesh sieve, the choice depending on the objectives of the investigation (see below).

This sieve is supported beneath the outlet pipe of the sieving table. Inevitably, some sediment will also be retained and animals must be separated at a later stage in the laboratory. Periodically, the fine-mesh sieve may become blocked with sediment, thus reducing the effective mesh size and the efficiency of the extraction process. In such cases, care should be taken to ensure that the sieve does not overflow. Accumulations of sediment on the mesh can usually be removed by gentle 'puddling' (see Figure 20), involving vertical motions of the sieve in a seawater-filled container. Horizontal movements of the sieve should be avoided as this can result in damage to the fauna through abrasive action. The remaining material should then be carefully transferred to an appropriate sample container (see Chapter 4.4.2). If spillage/loss of material occurs at any stage during processing, a repeat sample should be taken.



Figure 20 **‘Puddling’ - the removal of accumulations of fine sediment through gentle vertical motion of the sieve. Note that the seawater-filled container is placed at waist height for ease of processing**

For most routine surveys at gravel extraction areas, it is recommended that a stainless steel 1mm mesh sieve is used, conforming to British Standard 410. The effects of different sieve apertures on the results of macrofaunal surveys, based largely on experiences of working in soft sediments, have been examined by a number of authors including Eleftheriou and Holme (1984), Rees (1984) and Kingston and Riddle (1989). Sieves should be discarded at the first sign of damage to the mesh.

## 4.4. Approaches to processing epifaunal samples from trawls and dredges

On retrieval of the trawl, the catch should be concentrated in the cod-end of the net. The contents of the cod-end should then be released into a suitable sample container, and an estimate made of the total volume of the catch. This should be recorded in a logbook, along with a summary of the contents, noting especially the presence of stones, rock etc. The presence of any infaunal organisms arising from the fouling of soft sediment should also be noted, together with the occurrences of pelagic species. However, these additional faunal records should be excluded from the final compilation of the data for most monitoring and baseline surveys. It is essential that all the fauna is retrieved from the full length of the net and included in the analysis of material.

The structure and dimensions of a 2-m beam trawl (the most commonly used device for epifaunal sampling at extraction areas) is given in Chapter 3.3.3. It is recommended that all samples be processed over a frame-supported 5mm mesh. Any material passing through the sieve should be discarded.

Ideally, the contents of the trawl should be processed on board the ship. This entails counting and, where possible, identifying all solitary species. Counts of very abundant solitary species may be derived from sub-sampling, as appropriate. This may be achieved through sub-dividing the catch after it has been evenly distributed over the 5 mm mesh sieve, or by transferring the sample contents to a container and then removing an appropriate volume of material for subsequent processing.

Colonial species (notably hydroids and bryozoans) are generally recorded on a presence/absence basis or on a scale of relative abundance such as the SACFOR scale (**S**uper abundant, **A**bundant, **C**ommon, **F**requent, **O**ccasional, **R**are) employed by the UK Marine Nature Conservation Review (<http://www.jncc.gov.uk/mit/sacfor.htm>). This scale has been in use since 1990 as a system for recording the cover/density of marine benthic flora and fauna.

At least one individual of each species should be retained for inclusion in a reference collection. Specimens which cannot be reliably identified whilst on the ship should be preserved for later laboratory attention (see Chapter 4.5.2).

Epifaunal samples obtained with other gears, e.g. dredges, should be processed following the same procedures as above.

Because of the relatively high sampling error associated with epifaunal sampling, the data are generally considered unsuitable for the detection of subtle numerical trends.

#### 4.4.1. Washing equipment

Both the grab and sieving table (see Chapter 4.3) should be washed between sampling occasions to avoid any risk of cross contamination. Finer-mesh sieves should also be cleaned (e.g. with a scrubbing brush) to prevent excessive clogging with sand particles.

Furthermore, to avoid potential cross-contamination of epifaunal samples, trawls should be washed out, after every deployment, by towing the trawl at the sea surface for approximately 5 minutes with the cod-end of the net open.

#### 4.4.2. Transfer of processed material to sample containers

During this stage in the process there is the potential for loss of sample material and therefore appropriate means to avoid this should be instituted. The material should be backwashed into the container using a funnel or other 'foolproof' device. The entire process should be carried out within a large receptacle, in such a way that any accidental spillage can be retrieved. Any enmeshed fauna should be carefully removed with forceps and transferred to the sample container.

### 4.4.3. Sample preservation

Biological material will require fixing with a solution of formaldehyde. Fixation hardens the tissues and limits the scope for fragmentation of the specimens, as well as preventing decomposition. Improperly fixed specimens may cause problems during laboratory identification. There should be a final concentration of 4-5% formaldehyde in the sample for effective fixation. If necessary, prior to fixation, any excess supernatant in the sample container can be poured off through a sieve mesh of the same size as that used in initial processing, and any animals retained by the sieve should be returned to the sample. Samples should be stored in the fixative for a minimum of three days before any further processing (Gray *et al.*, 1992; Eleftheriou and Holme, 1984). For very large samples, containing large quantities of gravel, care should be taken to ensure that there is sufficient fixative and that it is adequately mixed through the sample.

Since formaldehyde tends to become acidic during storage, a buffering agent, such as sodium acetate trihydrate (25 g l<sup>-1</sup> for 30% formaldehyde), should be added as this will help to prevent the dissolution of any calcareous material, including mollusc shells, which may make subsequent identification of specimens difficult. **Formaldehyde is a toxin, a carcinogen and an irritant and therefore extreme caution should be exercised whilst preparing dilutions of this substance**, particularly when transferring neat chemical from one container to another. Eye protection, disposable gloves and waterproof clothing must be worn and the procedure must be carried out in a well-ventilated area. Alcohol (70% ethanol/IMS) is often used for later preservation of samples, but it should not be used during initial field preservation, as it is an inadequate fixative and can cause the production of a precipitate when mixed with seawater. However, specimens can be transferred to alcohol (70% ethanol/IMS), after fixation, if long-term preservation of samples is anticipated. Further information on the use of fixatives, preservatives and buffering agents is provided by Lincoln and Sheals (1979).



Figure 21 **An example of a visual record of the contents of a 0.1 m<sup>2</sup> Hamon grab. Such photographs can be reviewed at a later stage during data interpretation.**

### 4.4.4. Sample staining

Rose Bengal, a vital stain, may be added to the fixation fluid, to enhance the colour contrast between inconspicuous specimens and the sediment, thereby potentially increasing subsequent sorting efficiency. **Rose Bengal is an extremely hazardous carcinogen** and, in its powder form, should only be handled under fume extraction. It should therefore be added to the concentrated formaldehyde solution in the laboratory under carefully controlled conditions or made up as an aqueous solution for use in the field. The final concentration of Rose Bengal should be around 0.01% in 10% formaldehyde.

### 4.4.5. Sample labelling

Each sample must be suitably labelled as follows: date, research cruise number or code, the type of sample, station code and sample location. Labels should be applied to both the outside and inside of any sample container. The internal label should be waterproof and chemically resistant and annotated with a soft-carbon pencil which will not fade in formaldehyde. This label should accompany the sample through each stage of processing.

### 4.4.6. Sample logging

All surveys should be logged in a pre-designed field log or electronic datasheet. Each log sheet should contain prompts for all the information required. It may also be useful to offer a list of options within the survey log for recording certain variables (e.g. sediment type), to improve objectivity. A book format has the advantage that it keeps all the information together and is perhaps less likely to be damaged in the field situation.

Information that should be recorded during the survey will include the state and direction of tide, wind direction and strength and any swell. Photographs of collected samples should also be routinely taken, as they provide a visual record (see Figure 21). The names of all personnel involved in sample collection/processing should also be logged. In addition, for each sample collected a record of the following information should be made:

- date of sampling
- sampling position (this is usually recorded at the instant that the sampling device makes contact with the seabed)
- survey datum
- type and size of sampler employed
- any modifications to the sampling device (including the addition of weights)
- type of sample retrieved (e.g. macrobenthos sample)
- sieve mesh size employed (e.g. 1 mm)
- depth or volume of sediment sample obtained
- water depth at each sampling position
- time in GMT that the device landed on the seabed
- whether the sample was retained or rejected (and the criteria used to reject the sample)
- volume of any material removed for additional analyses (e.g. particle size analysis)
- a brief description of the sediment
- presence of any artefacts
- size of sample container(s) used to store preserved samples
- any deviation from standard operating procedures

## 4.5. Laboratory processing of grab samples

### 4.5.1. Elutriation and sorting

The formaldehyde fixative must first be removed from a sample prior to processing. Formaldehyde is toxic and carcinogenic and this initial phase must be carried out under fume extraction whilst wearing disposable gloves and a laboratory coat. The 5 mm sample fraction (see Chapter 4.3) is first washed with freshwater over a 1 mm sieve, to remove excess formaldehyde solution. The sample material is then backwashed into a sample container for examination. Attaching a flexible tube to the freshwater supply is highly recommended, as it greatly increases the control over the direction and strength of the water flow.

The finer sample fraction (usually  $>1$  to  $<5$  mm: see Chapter 4.3) is initially washed over a 1mm sieve and then backwashed into a 10 litre sample container. This container is then filled with fresh water and the sample is gently agitated in order to separate the smaller and lighter animals from the sediment. Once the animals are in suspension, the supernatant is decanted over a sieve. This procedure should be repeated until no further specimens are recovered. This stage in the process allows the smaller and more delicate animals to be elutriated from the bulk of the sediment and, by doing so, improves the speed and efficiency of the sorting process. The decanted fine fraction is placed in labelled petri-dishes for identification and enumeration under a binocular microscope with a light source.

A few remaining animals, such as heavy-shelled bivalves (e.g. *Nucula* spp.), will not be recovered in the decanting process. These are dealt with by washing aliquots of sediment into the sieve using a very low water pressure. The contents of the sieve are then backwashed onto a shallow sorting dish, preferably divided into grid sections. Sufficient water should be added to the dish so that the entire sample is immersed. This dish is then examined under an illuminated magnifier of at least  $\times 1.5$  magnification. The amount of material on the sorting dish should not obscure grid markings. Animals can be removed from the sediment using watchmaker's forceps or, if exceptionally small and delicate, using a pipette, and transferred to a suitable container, one for each species or faunal group. Ideally, an independent analyst should check each aliquot of sediment to ensure that all animals have been enumerated and extracted.

After the entire sample has been processed the sieve residue is returned to the original container, formaldehyde or alcohol applied, and the material stored until satisfactory completion of AQC procedures.

### 4.5.2. Identification and enumeration

All specimens of solitary taxa should be enumerated and identified down to the lowest possible taxonomic level, usually species, using standard taxonomic keys. It is essential that competent personnel are employed, in order to ensure accurate and consistent identification of specimens. The skills of personnel involved in species identification should be regularly assessed and updated through attendance at training workshops and participation in exercises designed to test proficiency.

Distinction should be made between adult and juvenile macrobenthic specimens. Only individual specimens with an anterior end are counted. Common species that are readily identifiable can be counted using digital counters. Colonial species (e.g. hydroids and bryozoans) are usually recorded on a presence/absence basis or using the MNCR SACFOR scale (see Chapter 4.4).

Nomenclature should conform with that of Howson and Picton (1997). All taxonomic references employed for identification should be documented. In cases where specimens cannot be assigned to the level of species due to damage, the lowest definitive taxonomic level should be recorded. An indication of the level of uncertainty associated with identifications should be denoted by a question mark before the second epithet for a species binomen (e.g. *Sabellaria ?spinulosa*), and before the generic name at the genus level (e.g. *?Sabellaria*). Following standard convention, if there is only a single species within a particular sample, then this is acknowledged by 'sp.' following the genus (e.g. *Sabellaria* sp.). However, if it is established that there are more than one species present within a sample (or dataset), then this is indicated by 'spp.' (e.g. *Sabellaria* spp.). Occasionally, due to taxonomic disputes, collective groups may have to be assigned (e.g. *Eteone flava/longa*). Identified specimens of each species should be transferred to numbered vials (one per species) containing preservative.

### 4.5.3. Biomass determination

If biomass estimates are required, they may be determined as wet weight and then converted to Ash Free Dry Weight (AFDW) using standard conversion factors (e.g. Rumohr *et al.*, 1987; Ricciardi and Bourget, 1998). Ideally, samples should be left in the fixative solution for a minimum of three months prior to weighing to allow for weight loss stabilisation (Brey, 1986). The wet weight is measured after the specimen has been blotted dry by moving it around on dry absorbent paper until no wet mark is left. Once blotted dry, the specimen should be transferred to a weighing balance as soon as possible, and the blotted wet weight recorded once equilibrium has been attained, or after a fixed time interval. Fauna should be weighed to the nearest 0.1mg.

Taxa containing fluid (e.g. heart urchins) should be punctured and drained before blotting. Tube-dwelling taxa should be weighed after removal from their tubes. Where possible, faunal fragments (lacking an anterior end) should be assigned to the appropriate species and included as part of the biomass estimate for the species; otherwise they should be weighed separately and then allocated across appropriate taxonomic groups. Ideally, estimates of biomass should be provided for each identified species and these, together with the estimated total biomass for each sample, should be reported.

### 4.5.4. Sample re-analysis

To ensure that laboratory processing is carried out to an acceptable standard, a random selection of 10% of the samples processed should be re-analysed. AQC criteria, typically those employed by the UK NMBAQC (see Chapter 9 for more details) are used to assess whether any error is acceptable for the purposes of the investigation. If the error is unacceptable then a repeat analysis of the entire batch of samples may be necessary. The outcome of AQC activity should be included in any reporting of the data.

### 4.5.5. Preservation and storage

After completion of identification, enumeration and estimation of biomass, specimens from each sample should be transferred to a single container, and a preservative solution of 70% ethanol/IMS applied. Sample containers should be labelled and kept in storage until all quality assurance issues have been resolved to the satisfaction of the responsible authority.

### 4.5.6. Reference collections

A separate reference collection should be catalogued and maintained in a curatorial manner for all benthic surveys. This involves the preservation of at least one individual of each species found in the survey in a separate container. Specimens should be preserved using an alcohol-based preservative (70% ethanol/IMS) and labelled with at least the following information: species name, station number, station replicate number, date of sampling and name of identifier. This collection can be used to validate identifications between samples and surveys.

### 4.5.7. Sample tracking

Collected samples constitute a valuable resource, both financially and in terms of the data they provide. Sample tracking i.e., information concerning the location and status of samples at all stages following collection, is an essential part of any Quality Assurance programme.

# CHAPTER 5

## Remote acoustic methods for examining the seabed

### 5.1. General Introduction

Remote acoustic techniques have been employed for many years to complement the physical sampling methods traditionally used to carry out benthic surveys. The most useful and, consequently, the most commonly applied technique is sidescan sonar. However, more recently, other techniques such as acoustic ground discrimination systems (AGDS), sub-bottom profiling, swathe bathymetry, and more conventional line bathymetry (some of which are commonly used by the industry in resource-driven surveys) have become more readily available to the environmental surveyor, especially in an R&D context. The application of these tools may significantly enhance the capability to accurately interpret biological data, as well as having 'stand-alone' value in impact assessments, although many have not yet reached the stage at which routine deployment can be recommended (e.g. on grounds of cost or ease of interpretation).

The following Chapter describes a variety of acoustic methods for use in support of environmental surveys of the seabed. Further details on operating procedures are provided in General Instructions for Hydrographic Surveyors (GIHS) and other standards issued by the International Hydrographic Organisation ([www.iho.shom.fr](http://www.iho.shom.fr) IHO Publication S-44: Standards for Hydrographic Surveys) and various national organisations (listed at [www.marinenav.net/hydro\\_nav\\_ofc/](http://www.marinenav.net/hydro_nav_ofc/)). Hydrographic survey standards exist for single, multibeam and sidescan sonar operations, but there is no current unified international reference. However a new International Hydrographic Survey Manual is being co-ordinated by the IHO with contributions from its various member states expected in 2002.

### 5.2. Bathymetric surveys

#### 5.2.1. Introduction

The generation of bathymetric data may serve a number of purposes when it is integrated into an environmental survey. Bathymetry is an easily viewed backdrop over which other data might be draped, particularly when a range of datasets are presented in a GIS format. Information from a line bathymetry survey is able to quickly and cheaply provide an interpolated map of the general topography of the seabed. Gross features such as channels and banks can be accurately mapped, and associated changes in the horizontal distribution of habitats might be more easily interpreted and explained when provided with a bathymetric map. Swathe bathymetry is able to generate a 100% coverage map of the seabed enabling far

better feature definition and object resolution than line bathymetry is able to provide. Swathe bathymetry is able to discern comparatively small seabed features such as rippled sand and suction trailer dredge tracks, and therefore provides the surveyor with a more detailed view of those features which might affect the distribution of habitats. Swathe systems also offer backscatter information, albeit of a relatively reduced quality, at little extra cost, and some systems also claim to provide concurrent swathe acoustic ground discrimination. A disadvantage of swathe bathymetry is that it is still a relatively expensive tool, although costs are gradually reducing.

Bathymetric survey data may be used to map both the large- and fine- scale topography of the seabed and also to monitor changes in depth over time. Bathymetric data are usually gathered under contracted procedures aimed at specific survey needs (e.g. pipeline, aggregate extraction sites, cable route or hydrographic surveys).

Bathymetric data are most frequently collected using a single beam echo-sounder with a transducer attached either to the hull of a vessel, or to a pole mounted over the side or bow of the vessel. The sounder is operated in tandem with a motion reference unit which monitors change in the attitude and height of the transducer due to heave, pitch and roll of the vessel. Standard single beam echo-sounders collect data from a narrow zone directly beneath the vessel track and generally the data are presented either in line form, with gaps between each data point, or as an interpolated plot of these data.

Multibeam (or swathe) sonar is a relatively new remote survey method. The hire and purchase costs of these systems are decreasing and their reliability and performance is improving. There are two main types of multibeam system: true multibeam (or focussed multibeam) and interferometric (or bathymetric sidescan sonar) systems. True multibeam consists of a transmitter and receiver capable of projecting and detecting multiple beams of sound energy which ensonify the seabed in a fan-shaped swathe. Multiple soundings are thus taken at right angles to the vessel track, as opposed to a single sounding underneath the vessel with a conventional single beam echosounder. This gives a far greater density of soundings enabling quicker coverage of the survey site. An interferometric sonar is a variant of sidescan sonar technology where electronic techniques are applied to a multiple set of sidescan sonar-like transducers arranged to give phase information in the vertical plane. This phase information is used to determine the angle of reception of reflected sound from the seabed and, given the time of flight of the return pulse, a range/angle measurement can be made of the seabed. The main difference is that soundings for a multibeam system are denser directly under the vessel, and sparser at full swathe range. The inverse is true for interferometric systems. Both systems are prone to greater errors on the outer limits of the swathe. Both are also dependent on a very high quality motion reference unit (MRU) to determine the position and attitude. This apparatus significantly increases the cost of the system but is essential for accurate and precise depth measurement. The application of swathe bathymetry techniques demands a lengthy calibration procedure to define any systematic errors in the installation (e.g. heading, latency and roll).

## 5.2.2. Survey design

Bathymetric surveys should be planned with the following considerations in mind:

- The navigational limitations of the survey vessel in relation to the objective of the survey. For example, shallow water or sandbanks may prevent access to some areas.

- The limits of the survey should be pre-defined and should always encompass, and ideally overlap, the area of interest. The degree of overlap will depend on the aims of the survey.
- The spatial separation of the survey lines and the density of the soundings need to be defined. This will again depend on the specific aims of the survey, and should take into consideration the size and shape of the features likely to be encountered, the complexity of the bathymetric variability of the seabed and the intended scale of the final survey chart.
- The horizontal datum, projection and grid to be used.

### 5.2.3. Survey execution

Bathymetric surveys using either single or multibeam systems should only be undertaken by an experienced hydrographic surveyor. The method for installing the survey equipment will depend, amongst other things, on the objectives of the survey, vessel size and configuration and specification of the equipment to be used. A recognised set of guidelines for the conduct of bathymetric surveys such as the International Hydrographic Organisation Standards S-44 for Hydrographic Surveys, should be followed.

### 5.2.4. Post-processing and reporting of data

Modern swathe bathymetry systems collect data with a width up to about seven times the water depth. The data are collected digitally and the high-resolution multibeam swathe bathymetry information can be used to produce ‘synoptic’ maps of the seabed. Until recently swathe bathymetry systems have been a prohibitively expensive option for the collection of routine data. However, these systems are becoming steadily cheaper, encouraging greater future utilisation by the dredging industry, and others involved in the assessment and monitoring of impacts from aggregate extraction. Both can provide backscatter information similar to sidescan sonar but, unlike sidescan sonar, the hull-mounted sensors are not optimised to the best grazing angles for the provision of textural information.

The processing and reporting of the acquired data is a task that should only be undertaken by an experienced hydrographic surveyor who is fully familiar with the technical specifications of the survey. As with all stages of the bathymetric survey process an accepted Standard Operating Procedure should be followed when carrying out the data post-processing.

The reporting of bathymetric data can take a number of forms. Single beam data is usually presented as a large format paper record with a sub-set of the total dataset plotted along the ships' track. The full XYZ dataset can be processed using bespoke software to produce an interpolated map of the bathymetric setting. The software may also be used to overlay other relevant datasets such as sampling positions (Figure 22). Of particular benefit is the use of bathymetric data as a backdrop for other datasets (e.g. sidescan sonar backscatter or Acoustic Ground Discrimination Systems (AGDS): Figure 23 and Figure 25). This can often be achieved using GIS packages.

Swathe data are most frequently presented as sun-illuminated plots or colour-shaded relief maps.

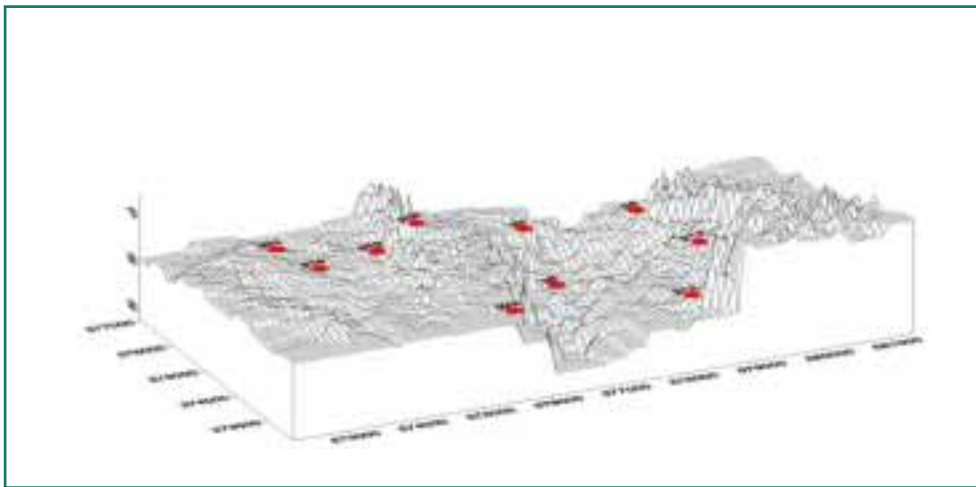


Figure 22 **Interpolated wireframe plot of bathymetric data gathered using single beam echo-sounder. Sampling positions are overlaid as red circles**

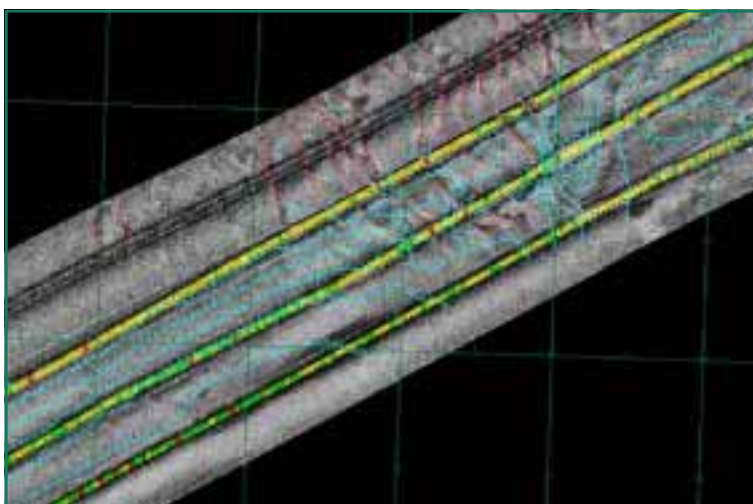


Figure 23 **Single beam bathymetric data and AGDS data overlain on backscatter data from a sidescan sonar survey. The green and yellow central tracks represent different substratum types distinguished by AGDS. The blue lines are interpolated bathymetric contours. The red lines track the crests of large sand waves**

## 5.3. Sub-bottom profiling

### 5.3.1. Introduction

Seismic or sub-bottom profiling techniques obtain data in the vertical plane defining the layers of sediment or bedrock strata below the seabed. The equipment applies high-energy acoustic pulses to the seabed, and sensitive arrays detect the reflected energy. The scale and resolution of this technique varies enormously, from deep seismic (where powerful air or water guns project high-energy, low-frequency sound through kilometres of substratum, to be picked up by thousands of metres of hydrophone arrays, towed or streamed behind the vessel), to centimetric resolution of sub-bottom echosounders operating in the decimetre layers of fine silts and muds. An example of the output from a high resolution chirps sub-bottom system is shown in Figure 24. (Chirps systems generate an acoustic pulse containing a narrow band of wavelengths, rather than a single wavelength pulse).

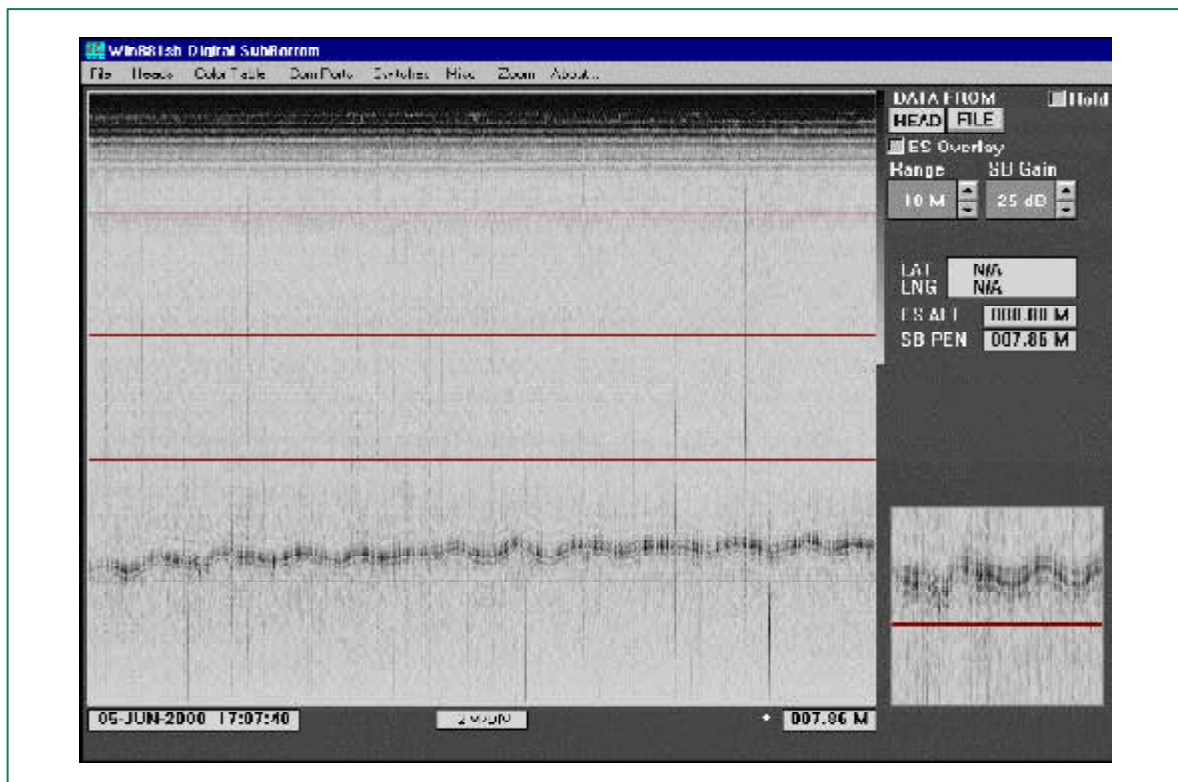


Figure 24 **Example of the output from a digital chirps sub-bottom profiling system when used in coarse substrata. The seabed is the dark line towards the bottom of the image. The indented box to the right of the main image is a 'zoomed in' section of the seabed. The vertical scale is 10 m overall, and the horizontal scale is approximately 90 m. There is no apparent structure below the highly reflective seabed surface**

Sub-bottom profiling techniques are used principally by the aggregate industry to assess the nature, quantity and distribution of a buried aggregate resource. None of the currently available sub-bottom profiling systems are able to effectively resolve distinct layers within the top 0.5 m – 1.0 m below the surface of coarse, and consequently highly reflective, seabeds characteristic of aggregate extraction sites. As this is the part of the sediment that is of most interest to the benthic ecologist, acoustic sub-bottom techniques rarely provide data

that are of significant value in assisting the interpretation of benthic data collected as part of environmental surveys at aggregate extraction sites. However, access to industry generated resource data may provide useful background information on the bulk disposition of sediments, buried geological deposits, and the nature and scale of seabed features such as sandwaves. This information may serve to assist decisions relating to survey design.

A review of sub-bottom profiling techniques, and their effectiveness in environmental assessments at aggregate extraction sites, is given by James and Limpenny (*unpublished*). It is beyond the scope of these guidelines to produce detailed procedures for the execution of sub-bottom profiling surveys.

## 5.4. Acoustic Ground Discrimination Systems (AGDS)

Acoustic ground discrimination systems are designed to detect the acoustic reflectance properties of seabed substrata. The acoustic differences reflected by the seabed are linked to differences in the physical, or occasionally biological nature (e.g. mussel beds) of these substrata. AGDS are one of a range of tools currently being utilised in the field of habitat mapping. For the most frequently applied systems, a vessel mounted single beam echosounder is used to generate a single frequency acoustic pulse which travels through the water column, is reflected off the seabed either once or twice, and is received back on board the vessel by the transducer. The signal may then be displayed in a conventional depth format by the echosounder, and is also received and processed by the AGDS.

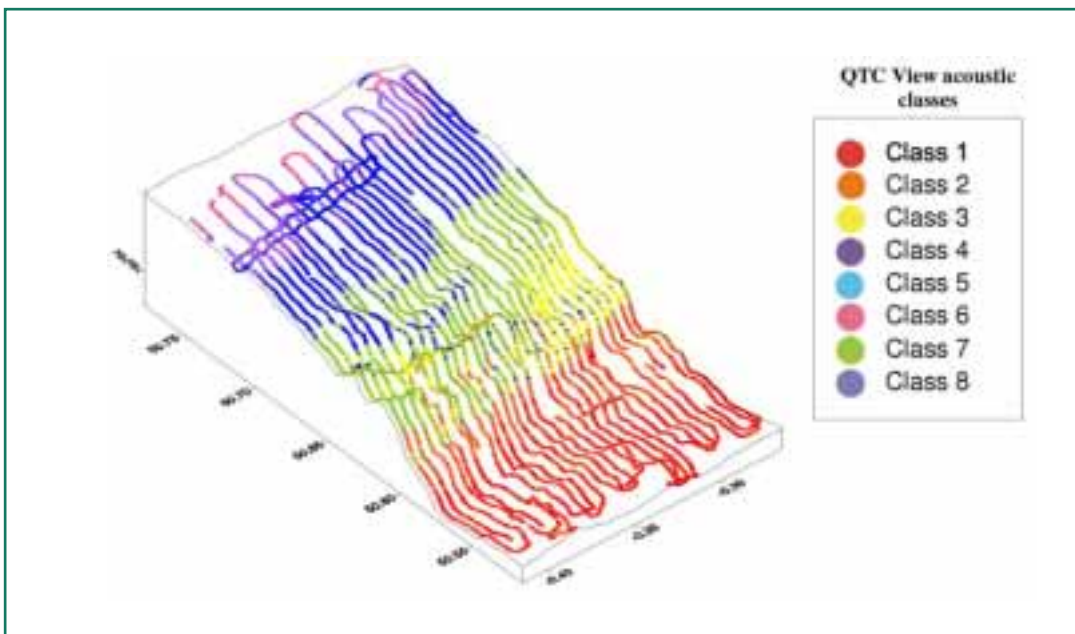


Figure 25 **Plot of QTC™ View data from Shoreham following post-processing using the Quester Tangent IMPACT™ software. 8 acoustically distinct classes are illustrated and have been overlaid on the bathymetry interpolated from the QTC™ data (Brown *et al.*, 2000). Although these classes represent acoustic differences, ground truthing enables some physical distinctions to be made, e.g. Classes 1 and 2 represent clean offshore gravels with sand veneers, and Class 3 represents clean mobile sand**

Presently there are very few systems in commercial supply. The first extensively used system was RoxAnn<sup>™</sup>, and this system remains in common usage world-wide. RoxAnn<sup>™</sup> uses analogue features associated with the first and second echo returns to allocate values for reflective properties of the seabed. The first return is the direct bounce off the seabed to the transducer. The second return is caused by the transmitted echo bouncing off the seabed a first time, off the sea surface back to the seabed, from where it is reflected again to be finally received by the transducer. The first return is sensitive to bed roughness (E1), whilst the second return is sensitive to bed hardness (E2). Because of the multipath nature of the second return, this parameter is also affected by its interaction with the sea surface. By plotting the relationship between E1 and E2 on an XY scale, and associating that relationship with a substratum type (defined using ground truthing methods), a spatial distribution map of substrata can be produced.

More recent advances in AGDS technology have resulted in the development of a system known as QTC<sup>™</sup>-View. This product converts the analogue echo return into a digital format which is then analysed by a large number of algorithms to provide a detailed interrogation of the features associated with the first return only, to produce its interpretation of ground type. Bespoke software may be used to identify natural clusters within the dataset, which can then be groundtruthed and assigned a substratum type. This system has advantages in that it provides a more reliable pathway for the acoustic pulse, with no sea surface interference. The wider utility of AGDS, and guidelines for the application of these systems in environmental assessment programmes, is the subject of much ongoing research (Hamilton *et al.*, 1999; Brown *et al.*, 2000; Foster-Smith *et al.*, 2001; Brown *et al.*, 2001; Brown *et al.*, 2002). However, at present, it is recommended that AGDS are not used in isolation, but rather that they be used in combination with other more conventional methods such as sidescan sonar, underwater TV and physical sampling methods when producing high-resolution biotope maps of an area (Brown *et al.*, 2001).

## 5.5. Sidescan sonar

### 5.5.1. Introduction

A sidescan sonar survey provides information on the texture of the substrata within the survey area, and from this it is possible to predict the particulate nature of the sediments and assign sediment descriptions to regions of the seabed (e.g. gravel, mud). Sidescan sonar also enables sediment transport features such as sand waves and ripples, lineated gravel features and scour marks to be identified. The nature of the substrata and their associated features are key elements in determining the distribution of faunal communities. Sediment transport features identified using this method suggest bed sediment transport pathways and allied hydrodynamic effects that will also affect the structure of benthic communities. This information may be used to direct subsequent monitoring surveys.

Geological features such as outcrops of bedrock, and aggregate deposits associated with submerged river valleys may also be mapped using this technique, especially when used in conjunction with other acoustic techniques such as bathymetry and AGDS. Examples of sidescan sonar output are shown in Figures 26-27.

Sidescan sonar surveys can be used to map changes in the physical composition and morphology of the seabed. The technique can also be used to assess the rate at which

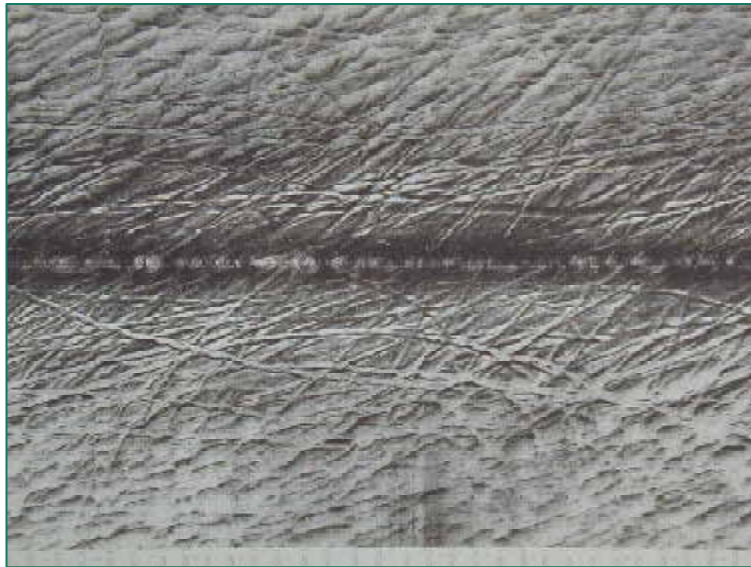


Figure 26 **Sidescan sonar image showing the scars on the seabed left as a result of trailer suction dredging activities. The image shows a sidescan sonar swathe approximately 300 m across, with an along-track distance of approximately 400 m**

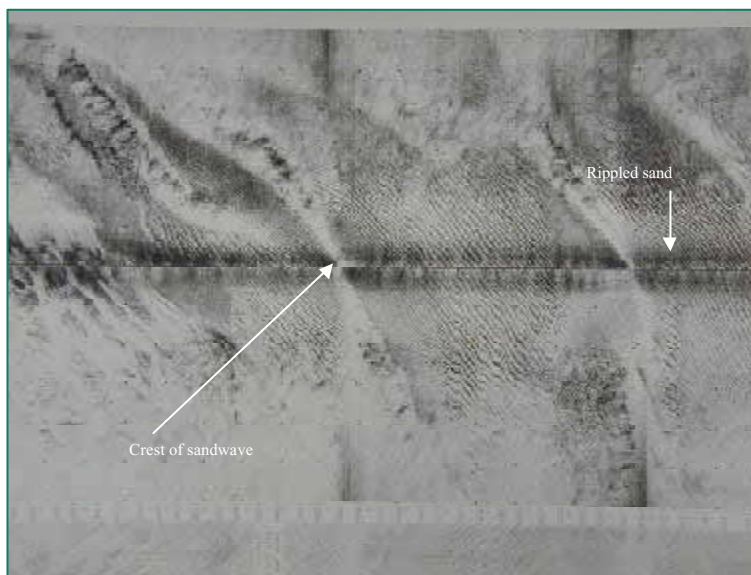


Figure 27 **Sidescan sonar image showing the crests of two large sandwaves, with rippled sand covering their flanks. The image shows an area of seabed approximately 300 m by 400 m**

dredge tracks become infilled with sediment. Sidescan sonar data are produced using towed or hull mounted transducers which ensonify a swathe of the seabed to either side of the transducers. The reflected portion of the acoustic signal is received by the transducers, amplified and converted into a paper or on-screen image showing levels of strength of return across the ensonified swathe of seabed indicating hardness of the substrata and orientation of seabed features. The use of sidescan sonar technology as part of a suite of sampling techniques is commonplace when carrying out benthic surveys, as its application fulfils a number of objectives.

## 5.5.2. Equipment

Several sidescan sonar systems are available to achieve a number of different aims. Coastal sidescan sonar systems are generally designed to work in water depths of up to approximately 300 m. They are designed to be routinely operated at frequencies of between 100 – 500 kHz. Systems operating at the lower frequencies will give a wider swathe with lower feature resolution. In contrast, systems operating at the higher frequencies will produce a narrower swathe but with far greater feature resolution. Systems are available that can operate at more than one frequency with the advantage that relatively broadscale surveys can be carried out using the lower frequency, followed by more detailed investigations using the higher frequency setting. Other systems are able to survey using a high and a low frequency signal simultaneously and combine the received signals into a single output. These systems produce the wide swathe of the low frequency systems, and utilise the improved image quality of the higher frequency signal at the shorter ranges. Some newer systems utilise chirp technology to produce a pulse of sound within a frequency bandwidth. These systems send a more powerful signal out to a greater range than single or dual frequency products and hence generate an improved image at greater distances from the transducer. Most modern sonar systems generate a digital signal at the towfish which is sent to an acquisition system onboard the vessel. Some systems generate an analogue signal at the towfish which is sent up the tow cable and either digitised and stored electronically for later processing on board the vessel, or plotted as an analogue signal on a paper record. Whilst the analogue systems produce a satisfactory image, the digital image is of superior quality. Factors such as the nature and aim of a survey, cost limitations, and equipment currently held by and familiar to the surveyor, will determine the most appropriate sonar system employed to complete any particular sidescan sonar survey. Useful reviews of sidescan sonar techniques are given in Fish and Carr (1990) and Blondel and Murton (1997).



**Figure 28 Datasonics™ SIS 1500 Chirp sidescan sonar fish. This device acts as a stable platform for the transducers, and is towed behind the vessel during the survey**

### 5.5.3. Survey design and execution

The factors mentioned above, which define the choice of survey equipment, are similarly important when designing a sidescan sonar survey. The model survey design might consist of 125% coverage of the survey area using a variety of survey line orientations, utilising both high and low frequency systems and towing the transducer at a range of depths above the seabed. Ideally, the survey should be carried out in favourable weather conditions with no cross-fish tidal effect and the vessel used should produce little or no turbulent wake.

Similarly, the survey vessel should not be subjected to forced changes of course due to other shipping, or due to fixed fishing gear, which may present a risk to the safety of the towed transducer. The positional co-ordinates of the towfish should be logged alongside the data to within 1m accuracy. However, it is generally accepted that a combination of cost, and the range of variables encountered, rarely allow the execution of the model survey and, in practice, the final survey will be limited to some degree.

The high cost of survey vessels, particularly all-weather vessels, is the main factor that limits the amount of data that can be collected. Therefore, it is frequently the case that a survey design must be limited to some degree at the outset, and it is the task of the scientist to judge how best to minimise the effects of this limitation.

### 5.5.4. Coverage

Increased coverage is achieved either by increasing the swathe width, increasing the vessel speed or by increasing the number of survey lines. Increased swathe width will generally result in reduced resolution as the ground distance displayed per pixel increases with increased swathe range. This need not be a problem if the finer detail of the substratum is of lesser importance than the broader scale information that is generated.

Vessel speed over the ground may be increased up to a point, but will eventually have a detrimental effect on the quality of the sidescan sonar record. It is the speed of the vessel over the ground (SOG), not the speed of the vessel through the water (TTW) which should be used to select the appropriate survey speed for the vessel. A vessel travelling at 3 knots TTW directly into a 3 knot tide will have an SOG of 0 knot and consequently would be surveying the same seabed location. In this situation the TTW would need to be around 8 knots to achieve an SOG of 5 knots.

The optimum speed for a survey is the one that will give the best along-track time/distance ratio without reducing the quality of the output. This optimum speed will vary from survey to survey and will depend on factors such as the depth of the transducers below the ships wake, the prevailing sea state, the in-water stability and the frequency of the transducers, the width of the ensonified swathe and the ability of the on-board PC to acquire the data at a sufficiently fast rate. It is largely a subjective decision for the surveyor to evaluate when the acoustic signal is beginning to be adversely affected due to vessel speed. A frequent sign that excessive speeds are being reached is a blurring of the signal at the extreme margins of the record. A safety margin of around 0.5 knot below the optimum speed should be maintained to allow for short-term fluctuations in vessel speed during the survey. As a rough guide vessel SOG should not exceed 7 knots, and sidescan sonar surveys should normally be undertaken at an SOG of around 5 knots. However these recommended speeds might be increased or decreased subject to survey conditions and the sidescan sonar system used.

The maximum coverage a survey might expect to achieve would be 100% with some degree of overlap of each adjacent line. This approach would ensure that all parts of the seabed within the survey area were ensonified. This will of course increase the distance covered by the survey vessel and hence the time required to complete the survey. For this reason, it is often the case that less than 100% coverage is achieved, particularly when carrying out surveys over a large area. In these circumstances it is necessary to design the survey in such a way that maximum information is gained from the survey time available. In this instance, the most effective method of increasing survey coverage is to increase the density of the survey lines.

### 5.5.5. Survey structure

As mentioned above, whilst it may be desirable to achieve 100% or even 125% coverage of the survey area, operational limitations, or limitations of cost often preclude this objective. In these circumstances it is necessary to pre-select the density of coverage. This may involve a systematic reduction over the entire area or a tiered approach with some areas receiving more attention than others. It is the specific requirements of the survey that will dictate the most effective approach. For example, a survey of a large area of seabed, the nature of which is largely unknown, might take the form of a regular grid which will cover the whole area at a reduced density. This will allow the scientist to identify broadscale differences in substratum type and provide information that might guide subsequent investigations. In some cases the scientific objectives may require a detailed look at a relatively small area of seabed, such as an aggregate extraction site, but there may also be a requirement to describe the nature of the substrata surrounding it. These aims might suggest a two-tiered approach, with at least 100% coverage over the extraction site and less dense coverage over the surrounding area. Each situation will require an approach that best suits the objectives.

The orientation of the lines is important when designing a survey. Features on the seabed that show some relief such as rocky outcrops, and sediment transport features such as sand waves and ripples will manifest themselves differently on the sidescan sonar record depending on their orientation on the sea-bed in relation to the sonar transducers and the acoustic signal produced. A sand ripple whose long axis runs parallel to the vessel track will present a relatively steep face to the acoustic signal which will readily be reproduced on the sidescan sonar record. However, if the same ripple runs at right angles to the vessel track the acoustic signal will run along the ripple, not meeting any steep faces, and consequently the feature may not appear at all on the sonar record (see Figure 29). It is possible to miss, or mis-interpret substratum types or bed features because of this. Therefore, it is extremely important to include lines of differing orientation within the survey grid. The orientation of bed-sediment transport features can often be predicted after consideration of local tidal axes or the direction of the dominant wave climate. The surveyor might choose to orientate the primary grid lines in such a way that these features are best represented on the sidescan sonar record. A reduced number of survey lines placed at right angles (or some other orientation) to the primary grid would serve to identify other features that might not be effectively represented whilst surveying using the primary grid.

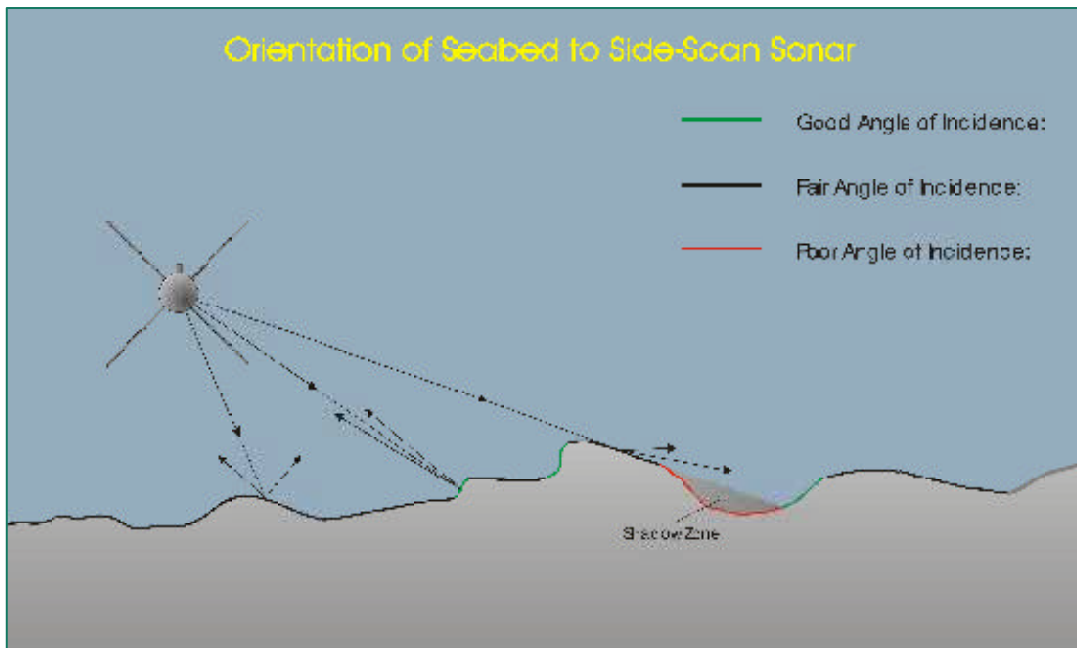


Figure 29 **Diagram illustrating the angles of orientation of sidescan sonar beams in relation to the seabed**

### 5.5.6. Operational considerations

The most appropriate choice of sidescan sonar system for a specific survey has already been discussed in Chapter 5.5.2. It should be emphasised that the most efficient balance between quality, coverage and cost should be struck when selecting the data gathering equipment.

Weather conditions and sea state at the time of the survey are important factors that will affect the quality of the sidescan sonar image. A rough sea surface will interfere with the sonar signal, as will the consequent aeration of the surface water layer. This will manifest itself as a non-systematic greying of the water column on the sidescan sonar image, and a reduction in contrast of the seabed image. Ideally, the transducer should be lowered below the influence of this “surface noise”, but this may not be possible when surveying in shallow water depths. The sea state will also affect the movement of the vessel, and therefore the stability of the transducer. If the vessel is subject to rapid motion due to a rough sea state, the sonar image can have a smeared or blurred appearance. If the transducer cannot be lowered below the influence of detrimental surface noise then the survey should either be postponed or terminated, and ideally recommenced when weather conditions have improved. If the ship's motion due to the poor sea state is having an adverse effect on the sonar image, an attempt to run each survey line with a following sea should be made.

Whilst this approach will significantly increase the survey time, it will often allow the collection of acceptable data during poor weather conditions. The size of the vessel will of course affect the motion of the vessel under poor sea conditions, and a larger vessel will generally provide a more stable platform than a smaller one. Therefore it may be more cost effective in some circumstances to pay a higher cost for a larger vessel on the basis that the weather window for good quality data will be wider than if a smaller, cheaper vessel were used.

The accurate geographic position of the transducer throughout the survey is an important factor for the collection of good quality data. Under normal circumstances the data gathering system will receive a GPS or DGPS input string providing positional information for each data point. The location of the transducer would be interpolated by the data gathering software using a calculation based on the length of tow cable and the depth of the transducer below the surface. This system frequently relies on the operator manually entering the cable-out information to the on-line survey PC as the transducer is raised and lowered through the water column during the survey. This method does not take into account horizontal deviations of the towed transducer from the central line of the vessel track which are most likely to occur during changes in the vessel course. This may lead to inaccuracies of up to several tens of metres depending on the length of tow cable out and the speed, degree and sharpness of the course change. However, this is the most frequently applied method of producing positional information for routine environmental surveys at aggregate extraction sites.

The utilisation of an Ultra Short Baseline (USB) positional system is an effective but expensive method of ensuring very accurate positional information relating to the towed transducer. Again, it is the specific requirements of the survey which will dictate the level of positional accuracy that must be achieved.

Sidescan sonar surveys should be carried out by a personnel who have an understanding of both the technical nature of the surveying equipment and the onboard data gathering system, and also appreciate the scientific requirements of the exercise. An individual who has knowledge of the technical side of the operation will ensure that the data being gathered is of optimal quality, and will also be able to identify and repair or replace faulty equipment. A thorough understanding of the scientific objectives of the survey will allow online interpretation of the image, and will consequently allow informed modifications of the survey design when appropriate. Davies *et al.* (2001) provide a useful guide to the execution of sidescan sonar surveys.

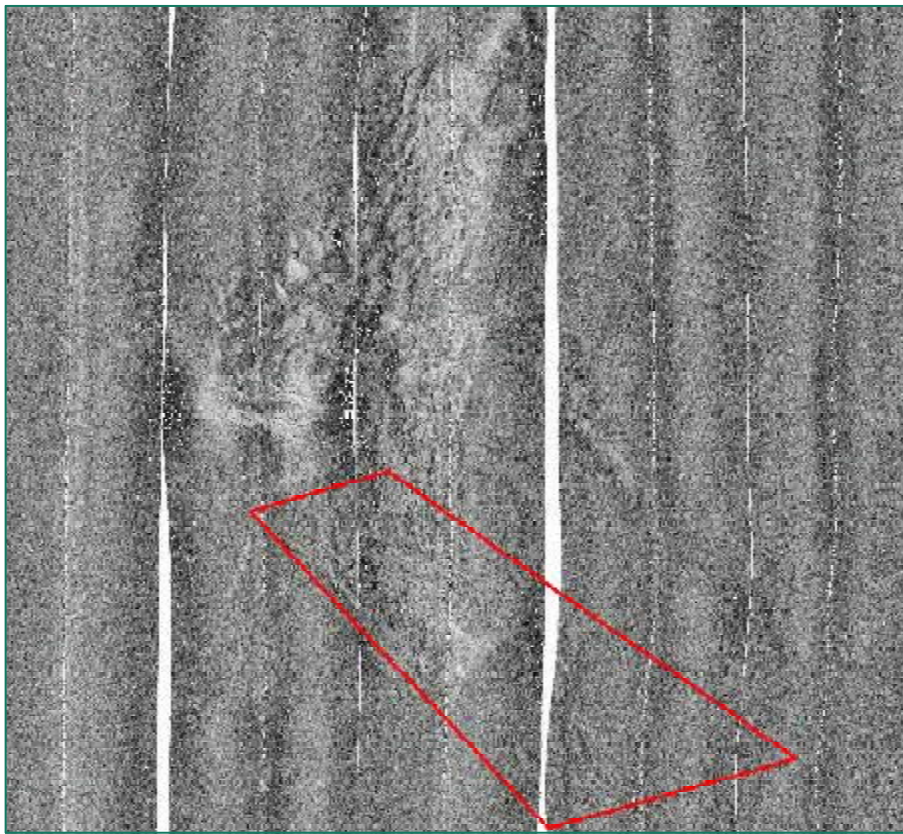
### 5.5.7. Presentation of sidescan sonar data

Data generated during a sidescan sonar survey may be presented in a number of ways depending on the nature of the data and the requirements of the end user. Data collected in analogue form is recorded directly on to a paper or higher quality plasticised film roll, using a custom-built printer. This paper record should be annotated automatically with time and position to aid subsequent interpretation. It can also be annotated by hand to highlight points of interest, to note survey system adjustments or to provide an *aide memoir* for the operator. When the survey is complete, the paper record can be cut into individual lines and laid out in rough georeferenced position to each other to provide an overall view of the survey, thus facilitating the interpretation of the data.

More frequently, data are gathered and stored digitally, and this digital information may be presented using a variety of methods. The favoured method of post-processing is to georeference the data using bespoke software packages and produce a mosaiced image of the backscatter information. This technique effectively presents the data as a map with all survey lines georeferenced to each other (Figure 30). This image would normally be

viewable on a PC screen. However, the scale at which sidescan sonar surveys are routinely carried out are not easily represented on a relatively small PC screen. If the entire survey is viewed at once, little detail can be observed. If it is necessary to view small-scale features, only a small portion of the survey can be viewed on the screen and the overall survey may not be viewed in context. It is therefore useful to produce a paper version of the mosaic at a scale that allows much of the small-scale detail to be viewed in relation to the survey area as a whole. This can be achieved with the use of large format printers and continuous paper rolls. A disadvantage of the computerised presentation is that an expensive viewing software package must be owned by each person wishing to view the survey in that format. A distinct advantage of the computerised version is that the software generally allows the surveyor to overlay other relevant datasets such as bathymetry, sampling positions, outlines of extraction sites etc onto the backscatter data (Figure 30). These multi-layer plots can then be printed out as large format paper records as described above, and aid significantly in the interpretation of the data.

The digital dataset may be used to provide a layer towards any Geographical Information System (GIS) that might be prepared as part of the overall survey work.



**Figure 30** Example of a section of a mosaiced sidescan sonar survey. Bespoke software enable, in this case, five sidescan sonar lines to be geographically referenced, and aligned with each other to provide a sidescan sonar coverage map over which other datasets may be viewed. Seabed features which extend over more than one survey line can be more readily viewed in mosaic form than is possible when they are viewed as individual lines. The red box indicates the limits of an aggregate extraction site

## 5.6. Archiving acoustic data

The archiving of data is becoming increasingly complex as the size of acoustic datasets increase. Most new data are electronically stored in digital format. This means that, with cost per unit decreasing, it is possible and economic to duplicate and back-up data as they are collected. The long-term storage aspects must also be considered, for example, with magnetic media the deterioration with age is a recognised process. The newer optical media are not as well researched with no track history available. If possible, data should be duplicated on more than one type of medium.

Various compression techniques exist to reduce dataset sizes. This is acceptable for electronic transfer of data and for duplicate data but, if at all possible should not be employed on original archival material. Many smaller files are also more resilient to data loss than a few large files; this should be factored into survey operations.

It is not possible to be prescriptive for archiving methods. However, a robust process should be employed which is easily understood, and will stand up to external scrutiny. It is also essential that all relevant details on the methods and circumstances of data collection are archived; without this information the data integrity can easily be challenged.

# CHAPTER 6

## Oceanographic surveys

The significance of oceanographic factors, particularly the hydrodynamics of an area, for controlling the distribution of benthic species has been recognised for many years. For example, Cabioch (1968) noted the important influence of tidal current action on the structure of benthic communities, mediated through its effect on substratum characteristics, from large-scale surveys of gravelly substrata in the English Channel. Warwick and Uncles (1980) also correlated the variability in the fauna of the Bristol Channel to bed stresses arising from tidal currents. Their findings were comparable with those of Rees *et al.* (1999) who examined the benthic biodiversity around the U.K. coast and demonstrated a link between the degree of physical disturbance of sediments and broad trends in the numbers and densities of taxa. On the basis of such evidence, it has been suggested that it is the hydrodynamic regime (mainly the tidal currents) that largely determines the characteristics of superficial sediments of an area and which is ultimately responsible for determining broad scale community patterns. It is therefore apparent that any changes in the status of benthic assemblages in areas which have been subjected to commercial aggregate extraction will need to be referenced against variations in sediment particle size distributions (see Chapter 7) and the hydrodynamic regime. The local hydrodynamics will also affect the dispersal of sediment plumes arising from marine aggregate extraction. It is essential that such information is accounted for in the design of 'baseline' benthic surveys in order to address any secondary consequences of dredging, especially the release and then redeposition of fines beyond the boundaries of the extraction permit. Much of this information (e.g. on tidal currents and wave climate) already exists and therefore new surveys to characterise the hydrodynamic regime may not always be required (see Chapter 2).

The following chapter briefly describes the range of oceanographic techniques which can be employed to help ascertain the hydrodynamic regime and, in particular, to determine the wave climate and the strengths and direction of tidal currents for a locality. However, care must be taken when extrapolating data obtained during short periods of observation, especially in areas known to experience wide variations in oceanographic conditions. For a comprehensive review of oceanographic techniques which may be employed during surveys of marine aggregate extraction sites, reference should be made to general texts such as UNESCO (1988, 1993), Emery and Thompson (1997) and ICES (2000).

### 6.1. Currents and tidal elevation

For any survey site, it is important to understand the tidal conditions present in terms of mean, maximum and minimum tidal currents, directions and residual current rate and direction. Either a string of single point current meters, a current profiler, or an Acoustic Doppler Current Profiler (ADCP) can be used to observe the current dynamics. An ADCP

emits an acoustic signal from a series of transducers and measures the Doppler Shift in a series of depth strata (bins) thus giving a profile of currents vertically through the water column. Backscatter information from the bins may also give additional information on the suspended sediment profile. ADCPs can either be mounted on the seabed and be directed vertically upwards or mounted on a vessel and be directed downwards. The time-series should ideally be long enough to observe spring to neap tidal variations i.e. to resolve the  $M_2$  tide (Principal Lunar tide - 28 days) and the data should be collected at a sufficiently high capture rate to correctly monitor the daily tidal regime. Measurements of current velocities close to the seabed can be used to calculate the shear stress exerted on the seabed and, from this, the range of sizes of sediment particles which can be set in motion by the prevailing tidal currents can be deduced.

Several current meter locations may be required in areas where the current varies significantly in either speed or direction. Typically this could be due to bathymetric changes or the convergence/divergence of two or more tidal streams.

Data from current meters should be calibrated in accordance with standard oceanographic procedures (ICES, 2000) and analysed to produce time-series of currents, histograms of current speed and direction, residual tidal drift and so on. Tidal elevations measured using a tidal gauge mounted on the seabed give site-specific information on the daily tidal curve. Abnormal events such as tidal surges will also be evident.

## 6.2. Suspended sediment and turbidity

The suspended load climate, measured in terms of concentration and mean particle size, is an important influence on the benthic community. Measurement of the suspended sediment concentration should be collected over a sufficiently long period to observe any tidal resuspension due to spring tides, and should also be at sufficiently high resolution to observe short-term events such as waves and anthropogenic disturbance events e.g. dredging activity, fishing activity or cable laying, which might occur during a deployment. This can be used to determine the driving mechanism of suspended sediment transport. The suspended load can be monitored by two types of sensors: optical backscatter sensors for the fine particles in suspension and acoustic backscatter sensors for the coarser sands in suspension. These instruments should be deployed as close as possible to the seabed and configured in such a way that they encounter minimum impedance of current flow generated by the structure upon which they are mounted, as shown in the CEFAS Minipod seabed lander system (Figure 31) or the CEFAS loggerpot (Figure 32). Water samples can also be taken using a variety of modern oceanographic samplers (e.g. Rosette, Niskins and displacement water bottles) at various depths, times and positions. The analysis of the sediment contained within such samples can provide a view of the suspended sediment climate.



Figure 31 **CEFAS Minipod.** The Minipod consists of a hard-disc based burst logger, capable of recording the current velocities (from a Nortek Vector® Acoustic Doppler Velocimeter), pressure (using a DigiQuartz® sensor), suspended sediment sensors (acoustic for sands and optical for finer material) and seabed position. Passive and timed sediment traps (Booner tubes) are also evident on two legs

## 6.3. Waves

Exposure to waves, especially winter waves, is an important factor in controlling the stability of the seabed and hence the benthic environment. An assessment of the exposure to waves, from all directions and all seasons, can be used as a disturbance indicator. Wave statistics such as significant wave height ( $H_{sig}$ ), wave period ( $T$ ), significant wave height for a return period of 50 years ( $H_{50}$ ), combined with water depth, can give estimates of the wave orbital velocity at the seabed which can be contoured to show regional variations.

Waves can be recorded by two main groups of instruments, namely surface-mounted buoys and seabed mounted pressure recorders. The former use accelerometers to record wave dynamics, whereas the latter use pressure sensors to record pressure variations. The latter should not be used in deep water (>30 m water depth) as the short waves are attenuated with depth. Surface buoys normally give direction information but pressure recorders need an auxiliary velocity recorder to infer directional information.



Figure 32 **CEFAS loggerpot which contains an ESM2 burst logger recording suspended sediment concentration, tidal elevation, wave statistics, conductivity and temperature. Also mounted in the Loggerpot is a Falmouth Scientific Instrument 2D Acoustic Current Meter (ACM)**

## 6.4. Sediment dynamics

Sidescan sonar surveys can be used to give an indication of the sediment transport pathways (see Chapter 5.5). Sediment transport features such as sand waves, sand ribbons and scour marks can be interpreted to predict transport vectors. Care must be taken in interpreting these surveys as the image represents the most recent sediment transport event and not necessarily long-term pathways.

Sediment traps can also give useful information on the timing, rates, and direction of horizontal sediment transport around an aggregate extraction site. This may be important in regions where active sediment transport is taking place, e.g. at the edges of sandbanks, or during storms. Sediment traps measure rates of horizontal sediment transport and usually take three forms: passive, active or directional. Passive traps are simple and widely used, whereas active traps include some sort of mechanism to infer the timing or sequence of sediment transport events. Direction traps infer the directional source of the material. These data can be used to calibrate the suspended sediment sensors and, when combined with the current information, provide an indication of the sediment transport direction. Bedload transport can also be estimated using traditional bedload transport formulae (Soulsby, 1997).

## 6.5. Horizontal and vertical structure (temperature or salinity)

Horizontal or vertical gradients of temperature or salinity (due to freshwater inputs) may exist over a survey area and can affect distributions of species. These can be assessed by either vertical CTD (Conductivity, Temperature and Depth) and Rosette casts (Figure 33) or by undulating CTD systems.

The Rosette can carry up to 24 Niskin bottles for collecting water samples for various determinands. The CTD system sends conductivity, temperature and pressure (depth) data via a cable in real-time to a surface control system. Water samples can then be taken either at the seabed, at the sea surface, in thermoclines or at other locations of scientific interest. Auxiliary sensors include transmissometers, suspended sediment sensors and fluorometers.



Figure 33 **CEFAS Rosette and CTD system being deployed from *RV Corystes***

# CHAPTER 7

## The collection and analysis of sediment samples for particle size analysis (PSA)

### 7.1. Introduction

The following account describes recommended methods for generating particle size data which may then be associated with the analysis of benthos samples, but they do not exclude other suitable methods. The Standard Operating Procedure (SOP) described in Annex III is included as an example of one recommended analytical approach for the analysis of aggregate samples. Other standard procedures such as those contained within BS1377 may also be adapted for this purpose (British Standards Institution, 1990). An SOP for the analysis of the fine fraction has not been included, as the analytical methodologies used for this fraction vary widely.

### 7.2. Field methods

#### 7.2.1. Subsampling sediment for particle size analysis from a macrofaunal sample

Sampling of sediments for later particle size analysis (PSA) is an essential accompaniment to macrofaunal surveys. Small-scale heterogeneity at the seabed dictates that a PSA sediment sub-sample should be collected from the same sample as that collected for the benthic fauna. PSA samples must also be collected from each replicate biological sample. This allows the macrofaunal data to be accurately referenced against variations in particle size characteristics.

The currently recommended quantitative sampling device for the collection of benthic samples at aggregate extraction sites is the Hamon grab. A description of the use and operation of this grab is given in Chapter 3.2.1. The Hamon grab collects a sediment sample in such a way that an undisturbed sediment surface is rarely available. Samples that do retain some degree of integrity on retrieval can be viewed and sampled before the grab is emptied, by means of an access door fitted to the uppermost surface of the grab bucket. However, it is most often the case that the sample will be mixed to some degree prior to subsampling.

After the contents of the grab have been emptied into a sample container, it is important that the subsample which is removed for PSA is as representative of the whole sample as possible. For coarser substrata, this demands that proportionally larger quantities are required than would be the case for sand or muds. However, a balance must be struck between the quantity of sample that is removed for particle size analysis, and the amount of sample that remains for the assessment of macrofaunal species composition. Typically when using a 0.1 m<sup>2</sup> Hamon grab, a sub-sample of approximately 500 ml is removed using a plastic scoop. After the sample has been emptied from the Hamon grab into a sample bin, a plastic scoop should be used to remove a number of randomly distributed aliquots to generate the 500 ml PSA sample. This procedure should allow for sorting of the sediment within the sample container. The PSA sample should be stored frozen in a sealed container, preferably in the dark, prior to later laboratory analysis. If cobbles (>63 mm) are present in the sample, they should not be included as part of the PSA subsample. Cobbles should be measured across their smallest axes so that they can be included in later data analyses. Methods for the field analysis of the particle size distribution of the coarsest material do exist (R. Nunny, *pers. comm.*) but have not been widely assessed.

Whilst the relatively small sample size specified above would generally not satisfy geological criteria for accurate characterisation of particle size distributions (e.g. in connection with industry-scale resource surveys), it represents a practical compromise which best serves the primary survey objective of evaluating macrofaunal community structure in relation to the physical habitat.

## 7.3. Laboratory analysis of sediment samples

The results generated as a result of the PSA of sediment samples provide important insights into the interpretation of benthic faunal, acoustic and hydrodynamic data. Sediment samples collected from aggregate extraction sites frequently possess a wide particle size distribution. A typical sample collected from an aggregate extraction site might comprise an admixture of cobbles, gravel, sand and silt/clay. The following text describes how the particle size distribution of a typical aggregate sample should be determined. An example of a more detailed Standard Operating Procedure is provided in Annex III.

### 7.3.1. Splitting the sample into a coarse and fine fraction

It is not possible to accurately analyse the coarser end of the particle size range of a typical aggregate sample using the same methods as those employed for the analysis of the finer end. Therefore the whole sample should initially be wet sieved on an automated sieve shaker (Figure 34) using a 500  $\mu$ m sieve if optical techniques are to be used for the analysis of the finer fraction, or a 63  $\mu$ m sieve if settling techniques are to be used. Sediment that is finer than the mesh size of the sieve being used will be washed through the sieve into a collecting pan. Material coarser than the aperture size will remain on the



**Figure 34 The automated wet sieve shaker is used to split a sediment sample into a coarse fraction and fine fraction. The coarse fraction remains on the sieve, and the fine fraction passes through the sieve to be retained in a collecting pan. The two fractions may then be treated separately for further particle size analysis**

sieve. Before the sieving process begins care should be taken to ensure that lumps of consolidated fine material are disaggregated and pass through the sieve. In addition, care should be taken to ensure that all material finer than the sieve aperture size has passed through the mesh during at this stage.

The coarser fraction should be dried to constant weight in an oven at a temperature of not more than 80°C. The subsequent treatment of the fine fraction will depend on the intended method of particle size analysis to be used.

### 7.3.2. Analysis of the coarser fraction using a dry sieving process

The oven-dried coarser fraction is left to cool to room temperature. It should then be sieved on a double gyratory jolting sieve shaker (e.g. Pascall Inclyno™) using a stack of sieves nested at 0.5φ intervals. The coarsest sieve, placed at the top of the stack, would typically have a mesh size of 63 mm and the finest sieve at the base of the stack would have a mesh size of either 500 μm or 63 μm depending on the method of subsequent analysis of the finer fraction. A collecting pan at the bottom of the stack retains the fraction passing through the finest sieve. The sample should be tipped onto the coarsest sieve, and the sieve stack should be shaken for a standard time (at least 10 minutes). At the end of this time, the components of the sample will have been shaken as far down the sieve stack as their diameter will allow. The weight of the sediment in each sieve should be recorded, as well as relevant observations on the nature of the material present (e.g. shell material or organic debris). The weight of the sediment in the collecting pan should also be recorded.

### 7.3.3. Analysis of the finer fraction

The fine fraction of the sediment should be either freeze-dried, air dried or oven dried at a low temperature ( $<30^{\circ}\text{C}$ ) to reduce the likelihood of concretion of the silt/clay fraction during the drying process. The analysis of the finer fraction may be carried out in a number of ways. Commonly used methods include settling techniques such as pipette and Sedigraph® analysis and optical methods such as laser sizing (Figure 35). It is important that comprehensive SOPs, produced by laboratories experienced in the use of these techniques for the analysis of marine sediments, are followed.



**Figure 35 Coulter™ LS 130 laser sizer. This equipment uses laser diffraction technology to measure particle diameters. It is most frequently used to measure the finer component ( $<500\ \mu\text{m}/<63\ \mu\text{m}$ ) of a sediment sample**

### 7.3.4. Synthesis, expression and reporting of results

Particle size can either be quoted in metric (millimetres, microns) or logarithmic (Phi) units (Krumbein, 1934). Sediment descriptions as defined by their size class should be based on the Wentworth classification system (Wentworth, 1922). Sediment mixtures should be described using the classification system developed by Folk (1974). Statistics relating to particle size distributions should be calculated and described using the formulae given in Dyer (1986).

The data generated from the analysis of both the coarse and the fine fractions should be combined to produce a complete particle size distribution for each sample, which can then be plotted. When the full distribution has been constructed the sample should be assigned a description based on the Folk classification system.

Statistics which should typically be calculated for a distribution include the following:

1. Percentage gravel, percentage sand, percentage silt/clay
2. Mean particle size
3. Sorting coefficient
4. Skewness
5. Modal size
6. Kurtosis

The grain size composition of a sample may also be presented according to a standard classification system such as that produced by Folk (1970). It is extremely useful to provide graphical representations of particle size distributions in the form of size frequency histograms (Figure 36), or cumulative frequency curves to accompany these statistics.

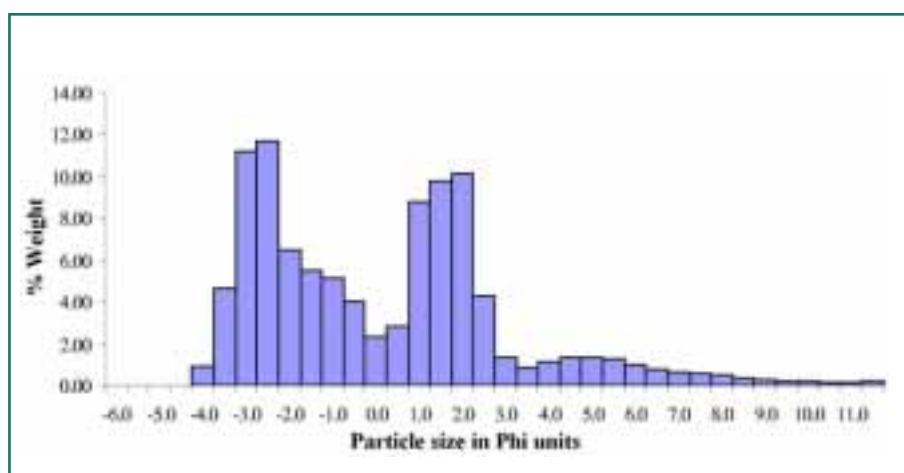


Figure 36 **Histogram showing particle size distribution of a typical mixed sediment sample. The Phi scale along the x axis ranges from coarse material at the left to fine material at the right**

### 7.3.5. Quality Control Procedures

The application of appropriate QC procedures when conducting particle size analysis is essential. The use of certified reference material to check the performance of laboratory equipment is recommended. Additionally, the use of an internally produced standard sediment is another valuable method of checking equipment on a more frequent basis.

Subscription to accredited QC schemes, such as the NMBAQC Scheme (see Chapter 9.1) and the Laser Diffraction Proficiency Testing Scheme (LDPTS) introduced by Beckman Coulter UK Ltd., is recommended. BS1377 also lists recommendations for laboratory apparatus specifications and calibrations which are valuable for checking analytical performance.

# CHAPTER 8

## Methods for data analysis of benthic samples

### 8.1. Objectives of data analysis

The objectives of the analysis of data arising from the monitoring of marine aggregate extraction sites include:

1. the identification of spatial patterns in the macrofaunal assemblage(s) under investigation and the relationship of these to environmental information including the spatial extent of any dredging related disturbance (baseline/exploratory data);
2. the detection and quantification of effects and temporal trends which are attributable to aggregate extraction, and the identification of other forcing factors (ongoing monitoring data);
3. the monitoring of the recolonization of aggregate extraction sites following the cessation of dredging until a 'stable' state is demonstrated (post-dredging data).

A typical dataset arising from a survey of a marine aggregate extraction site in U.K. waters usually contains well over 100 species. There are numerous techniques that can be employed to simplify and elucidate structure in the data. The following section outlines a basic suite of statistical approaches for analysis of biological data typically obtained during the monitoring of marine aggregate extraction sites. Each of these techniques can be used to partially fulfil the objectives of data analysis. However, it is recommended that parallel application of a range of techniques will help both to differentiate patterns and confirm real trends in the data. For a comprehensive review of statistical methods, the reader is referred to general texts such as Green (1979), Sokal and Rohlf (1987), Clarke and Warwick (1994) and Underwood (1997). For convenience, emphasis in the following account is placed on statistical techniques which are included in the PRIMER (Plymouth Routines In Multivariate Ecological Research) software package developed at the Plymouth Marine Laboratory (Clarke and Warwick, 1994; Clarke and Gorley, 2001). This is because the package is widely employed and has gained general acceptance as a tool for analysing benthic datasets. However, it is also recognised that there are many other software packages and statistical techniques which are equally suited to the task of handling benthic community datasets such as CANOCO (Jongman *et al.*, 1987). It should be noted that both novel statistical approaches for the analysis of biological data and new statistical software packages are continually emerging.

In addition to biological data, analysis will generally include consideration of physical data such as sediment particle size distributions and other environmental variables. Many of the approaches described below can be applied equally to other forms of data. However, non-parametric methods may be more appropriate than parametric measures when analysing count data.

## 8.2. Initial data processing

Prior to data analysis, there are several stages of initial data processing that must be conducted. These stages are summarised by Clarke and Green (1988) and are briefly considered below (see Figure 37). Firstly, the data must be collated and classified using a coding system based on hierarchical taxonomic levels such as that described by Howson and Picton (1997). A species-sample matrix is then created of taxon abundance per replicate sample. This should document both quantitatively and qualitatively measured taxa. In addition, a matrix detailing wet-weight biomass of individual taxa by sample can be prepared and then converted to ash-free dry weight (AFDW) using standard conversion factors (Rumohr *et al.*, 1987; Ricciardi and Bourget, 1998). Finally, a matrix of the corresponding physical data should be prepared.

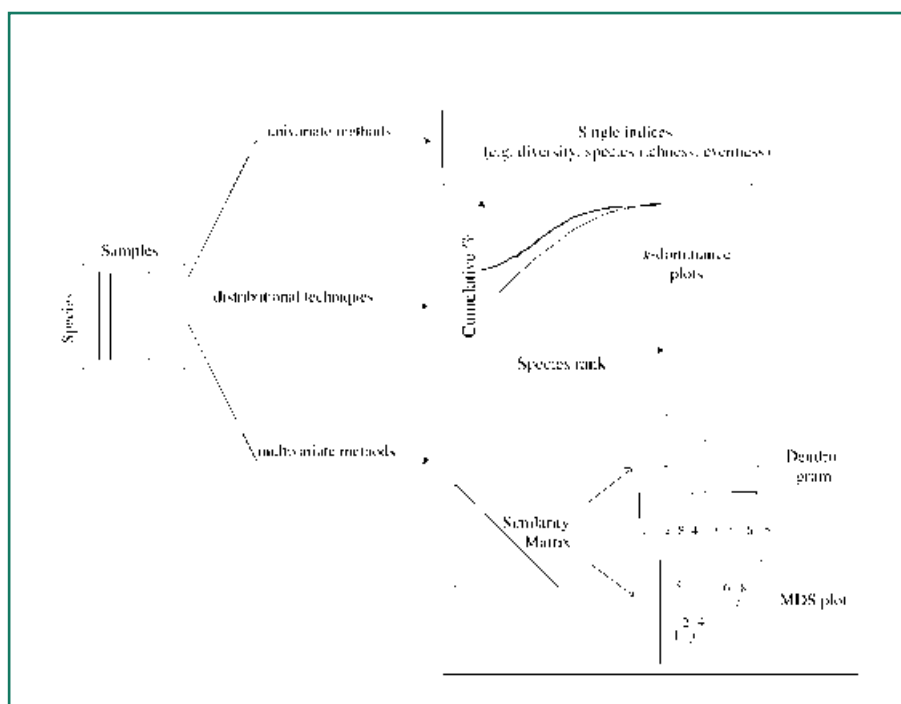


Figure 37 **Statistical methods used to analyse macrobenthic assemblage structure (after Schratzberger, 1998)**

Once data are collated in a suitable matrix, it may be appropriate, in some cases, to remove rarer species from the data analysis. However, if such a procedure is followed then the criteria adopted and reasoning behind species removal should be transparent. Colonial species, which are not amenable to counting, are normally removed from datasets prior to analysis of quantitative data. Again, although this is common practice, the action should be clearly documented in any reports.

Statistical methods used for describing assemblage structure can be grouped into three categories:

- (1) Univariate methods
- (2) Distributional techniques
- (3) Multivariate methods.

For each of these classes, appropriate statistical tests have been developed to determine the significance of differences between replicated samples.

## 8.3. Univariate methods

Diversity measures take into account two factors. These are species richness (number of species) and species evenness (how equally abundant the species are). Distribution-free indices are commonly used since they make no assumption about the underlying species abundance distribution. There are two categories of distribution-free indices (Magurran, 1988):

- (1) Information theory indices (e.g. Shannon-Wiener Index  $H'$ ).
- (2) Dominance indices (e.g. evenness).

More information about the structure of assemblages and their change due to aggregate extraction can be obtained by the use of a variety of different univariate indices including total number of individuals, total number of species, diversity (Shannon-Wiener Index  $H'$ ), dominance (Simpson Index  $C$ ), species richness (Margalef's  $d$ ) and evenness (Pielou's  $J'$ ). In general, such measures tend to be highly correlated and therefore there is limited value in calculating a large number of indices, as many will show similar trends in the data. Those indices that are less dependent on sample size (see Table 3) may be more appropriate for data arising from coarse substrata.

### 8.3.1. Characteristics of univariate measures

Magurran (1988) provides a summary of the performance and characteristics of a range of univariate indices to show their relative merits and shortcomings (Table 3). The column headed "richness or evenness/dominance" shows whether an index is biased towards either species richness or evenness.

There are indices which reflect the species richness element of diversity and measures which express the degree of evenness in the data. The number of species detected in a sample usually changes much more in relation to sample size or sampling intensity than does the distribution of relative abundances (Huston, 1996).

Therefore, indices in the first category are generally better at discriminating between samples but are more affected by sample size than the evenness of diversity measures.

**Table 3 Summary of the performances and characteristics of diversity statistics (modified from Magurran, 1988)**

	<b>Discriminant ability</b>	<b>Sensitivity to sample size</b>	<b>Richness or evenness/dominance</b>	<b>Calculation</b>
Diversity ( $H'$ )	moderate	moderate	richness	intermediate
Dominance ( $C$ )	moderate	low	dominance	intermediate
Species richness ( $d$ )	good	high	richness	simple
Evenness ( $J'$ )	poor	moderate	evenness	simple

### 8.3.2. Biodiversity indices

The latest version of PRIMER (version 5) which has recently become available (Clarke and Gorley, 2001) allows the calculation of new biodiversity indices including taxonomic distinctness indices. These indices capture the structure not only of the distribution of abundances amongst species but also the taxonomic relatedness of the species in each sample. In practice, these taxonomic distinctness indices have the important attribute that they are not, generally, dependent on the degree of sampling effort involved in the data collection, implying that results can be compared across studies with differing and uncontrolled degrees of sampling effort (Clarke and Warwick, 1999). Although these indices are now beginning to be used more widely in the marine field, they are still in need of methodological refinement and wider testing (Clarke and Warwick, 1999).

### 8.3.3. Analysis of variance (ANOVA)

When the species abundance information in a sample is reduced to a single univariate index, the existence of replicate samples from each treatment allows formal statistical treatment by one-way analysis of variance (ANOVA) (Clarke and Warwick, 1994). This analysis relies on the following assumptions:

- (1) that the data follow a normal distribution,
- (2) that the variance of the sample is independent of the mean, and
- (3) that the components of the variance are additive.

In general, the variance and mean tend to increase together and therefore the second condition is never fulfilled. Transformations are an essential procedure before the application of most methods associated with the normal distribution (Elliott, 1971).

In cases of significance, multiple comparisons tests can be performed to identify assemblages that are significantly different at  $p < 0.05$ .

## 8.4. Distributional techniques

Diversity profiles can be visualised by plotting  $k$ -dominance curves (Lambshead *et al.*, 1983) (Figure 38). Species are ranked in decreasing order of dominance along the x-axis and the percentage cumulative abundance ( $k$ -dominance) is then plotted against the species rank  $k$  (Platt *et al.*, 1984). The purpose of such curves is to extract information on the dominance pattern within a sample, without reducing the information to a single summary statistic, such as a diversity index.

Diversity can only be assessed unambiguously when the  $k$ -dominance curves from the assemblages to be compared do not overlap. In this situation the lowest curve will represent the most diverse assemblage. If the curves overlap it is impossible to discriminate between the assemblages according to diversity as different diversity indices may rank them in opposite ways. Diversity indices focus on one aspect of species abundance relationships and emphasise either species richness or dominance. Plots which overlap therefore illustrate the shift of dominance relative to that of species richness (Magurran, 1988).

Sets of macrofauna species counts and biomass can be summarised in abundance and biomass  $k$ -dominance curves applying the ABC procedure (Warwick, 1986) (see also Figure 38). This method is based on the assumption that, in the event of environmental disturbance, the distribution of numbers of individuals among species in macrobenthic assemblages behaves differently from the distribution of biomass. Under stable undisturbed conditions, the biomass will become increasingly dominated by one or a few large species, each represented by rather few individuals which are in equilibrium with the available resources. However, the numerical dominants, are smaller species which are out of equilibrium with resources and thus an undisturbed state is indicated if the biomass  $k$ -dominance curve falls above the abundance curve throughout its length. As disturbance becomes more severe, macrobenthic communities become increasingly dominated numerically by one or a few very small species, and few larger species are present although these will contribute proportionally more to the community biomass in relation to their abundance than will the small numerical dominants. A strongly disturbed state is therefore indicated if the abundance  $k$ -dominance curve falls above the biomass curve throughout its length.

## 8.5. Shortcomings of univariate methods and distributional techniques

Univariate methods and distributional techniques allow a visual interpretation of any trends (e.g. increasing or decreasing diversity at different sampling locations) and their statistical significance. However, both procedures share the property that comparisons between samples are not based on the identity of species. Two samples can have exactly the same diversity or distributional structure without possessing a single species in common (Clarke and Warwick, 1994). In order to better address the complexity of ecological systems, with their numerous species and discordant time-courses of change in populations, species-dependent multivariate analysis of community structure is required (Underwood, 1996).

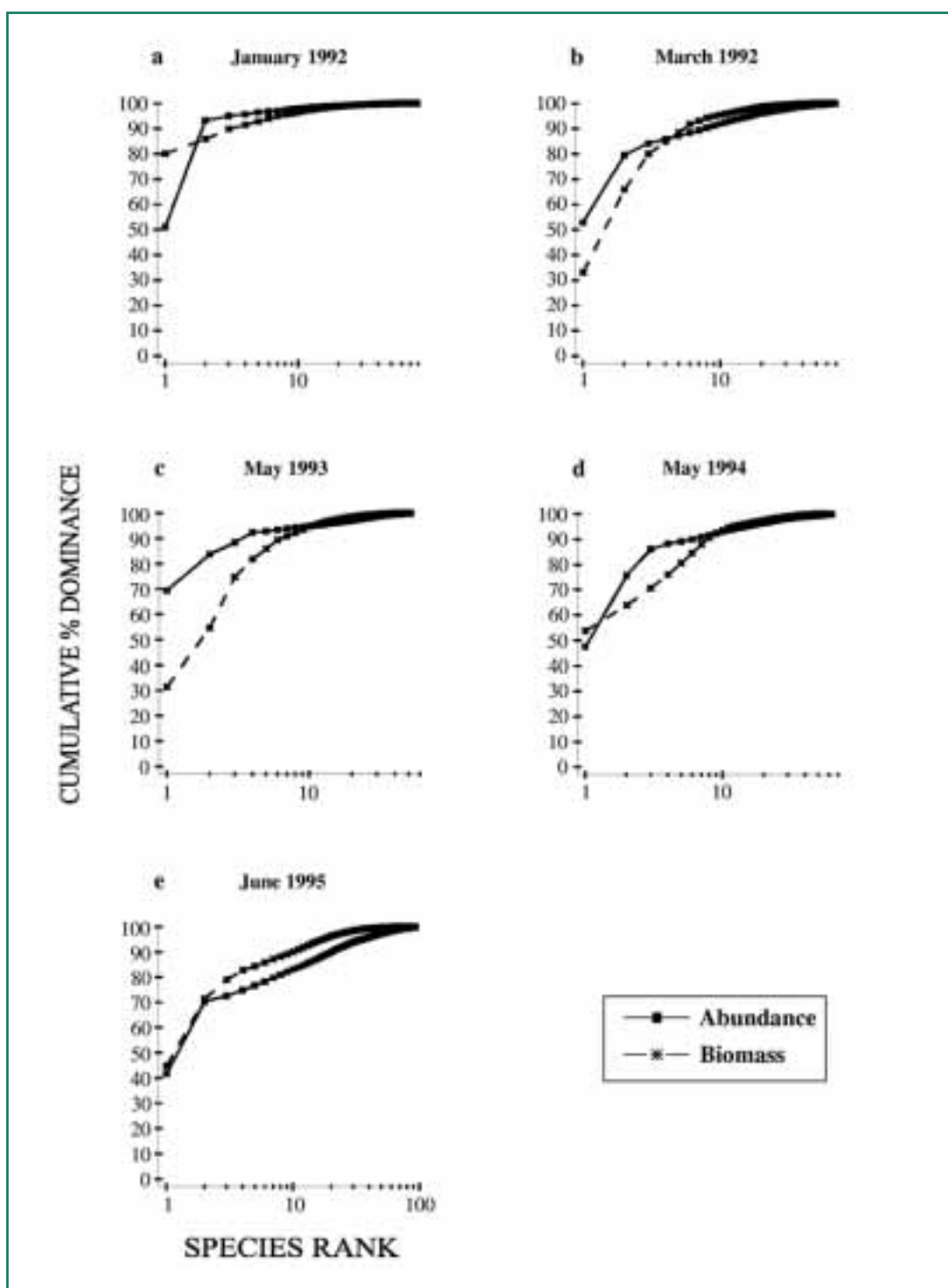


Figure 38 **Abundance and Biomass Comparison  $k$ -dominance curves (ABC plots) for macrofauna at an experimentally dredged site. Dredging was carried out in April 1992 (after Kenny *et al.*, 1998)**

## 8.6. Multivariate methods

Multivariate analyses are conducted to determine whether biological assemblages respond to different types of disturbance by small, but consistent changes in the relative abundances of species. These changes might not be detected by comparisons of univariate indices.

Field *et al.* (1982) described the steps involved in the multivariate analysis of marine biological survey data:

(1) Data Transformation

Several options are available on analytical outcomes. Untransformed data may have the undesirable property of accentuating the influence of very abundant species. In such cases, increasingly powerful transformations will have the effect of increasing the influence of rarer species at the expense of the commoner ones. For example, log-transformation has the powerful effect of ‘scaling down’ very abundant species and thus increasing equitability of the dataset. The square-root transformation has a similar effect in reducing the weighting of abundant species but has the advantage that, when similarity is assessed by the Bray-Curtis measure, the similarity coefficient is invariant to a scale change (i.e. it doesn’t matter whether scores are expressed per cm<sup>2</sup> or m<sup>2</sup>).

(2) Similarity Measurement

The overall similarity between every pair of samples is expressed, taking all the species into consideration. The Bray-Curtis measure gives more weight to abundant species than to rare ones.

(3) Classification

Hierarchical sorting strategies are used to produce a dendrogram from the similarity matrix. The most commonly used method in marine benthic studies is group average sorting (Lance and Williams, 1967), which joins two groups of samples together at the average level of similarity between all members of one group and all members of the other.

(4) Ordination

An ordination of the  $n$  samples is produced in a specified number of dimensions. In the multi-dimensional scaling (MDS) ordination, the identity of each species is retained and used integrally with, for example, abundance data to compare assemblages (Austen and Warwick, 1989). The purpose of the MDS is to construct a configuration (“map”) of samples, which attempts to satisfy all the conditions imposed by the underlying similarity matrix. The distances between pairs of samples in the resulting plot reflect their relative dissimilarity in species composition (see Figure 39 for an example of a MDS ordination using data from an experimentally dredged site).

Initially, the samples are placed in two-dimensional space at entirely arbitrary locations, and then their relative positions are gradually refined by an iterative analytical process. The intention is to move samples into positions in which the rank order of their distances from each other becomes ever closer to the rank order in the original similarity matrix. The extent to which the two disagree is reflected in the stress value (Clarke and Warwick, 1994). This coefficient indicates the degree to which the two-dimensional plot provides an acceptable summary of the multi-dimensional sample relationships. Stress values of  $< 0.05$  indicate an excellent representation with no prospect of misinterpretation, whereas MDS plots with stress values  $> 0.3$  should be treated with caution as the points are close to being arbitrarily placed in the two-dimensional ordination space (Clarke and Warwick, 1994).

Non-parametric multi-dimensional scaling (MDS) ordination, employing the Bray-Curtis similarity measure (Bray and Curtis, 1957), is a commonly used analytical technique which is available on the PRIMER software package (Clarke and Warwick, 1994; Clarke and

Gorley, 2001). MDS ordinations can be carried out on data which have been transformed in a variety of ways.

Analysis of similarity (ANOSIM) (Clarke, 1993) can be conducted to test for statistically significant differences in macrofaunal assemblage structure between samples or stations.

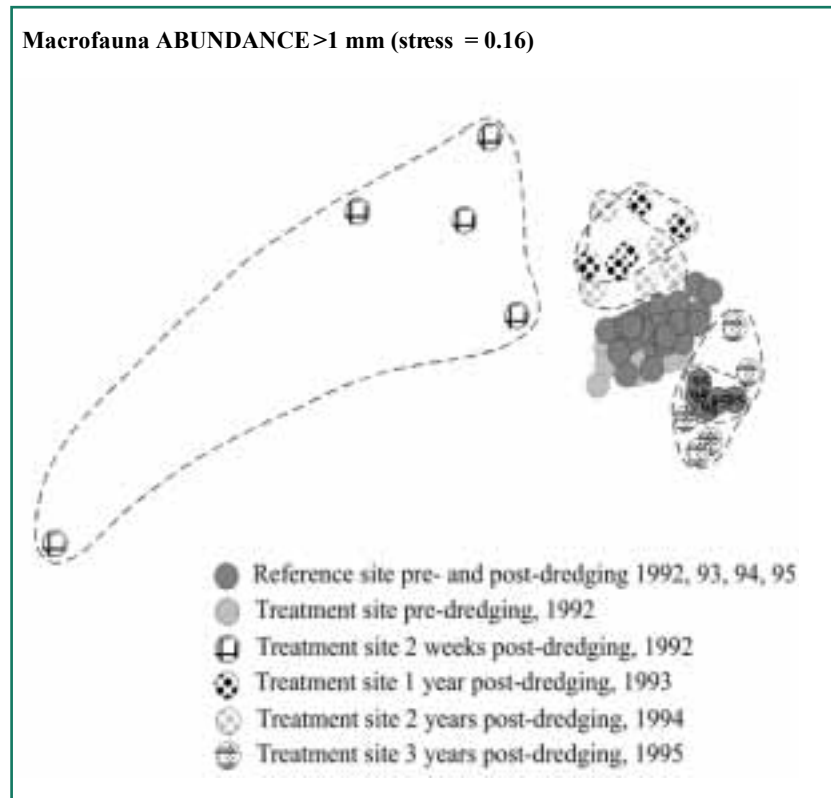


Figure 39 **An example of a non-metric multidimensional scaling ordination (after Kenny *et al.*, 1998)**

## 8.6.2. Species analyses

It is important to establish which species contribute to observed differences in the data. This can be achieved by ranking species in terms of abundance or by examining the degree to which species contribute to measures of similarity/dissimilarity between individual samples or sample groups (SIMPER) (Clarke and Warwick, 1994). Dominant species can be identified from the original raw data but, often, those species are not the ones that discriminate between an impacted and a reference site. The strength of the SIMPER analysis lies in identifying the discriminant species. In the case of large and complex data sets, this would be impossible without computerised programmes such as SIMPER.

## 8.7. Linking biological data with environmental information

The techniques described above are not ends in themselves; they only identify patterns in the faunal data and do not attempt to establish the causes of the faunal distributions. This can, however, be partially achieved by further statistical analyses, if the physical characteristics of the sediment have been determined or other properties of the habitat have been established. Analysis of variance can be used to determine which environmental variables are significantly different between the groups of stations, and correlation analyses can show which variables are correlated with features of the faunal data. Therefore, insights into the causative factors may be gained through computing correlations between environmental variables and faunal attributes such as the densities of selected species, diversity indices, or numbers and densities of all species at each station. More sophisticated techniques employing multivariate approaches to data analysis can also be used to link biological patterns to environmental variables (see e.g. Clarke and Warwick, 1994). One useful visual approach is to superimpose environmental data upon the output from station ordination or classification of biological data (see Figure 40).

## 8.8. Interpretation of the data

For the final stage in the interpretation of the results, a knowledge of the biology of the various species (e.g. feeding habits, environmental preferences, functional significance) is required to assess whether variables which are empirically related to the faunal distributions might be causative factors. Thus, it is possible to assess which environmental factors, either natural or resulting from marine aggregate extraction or other anthropogenic perturbations, are affecting the benthic environment and to what degree. This information may then be employed in a predictive manner to assess the likely consequences of any alterations in the intensity of aggregate extraction in a given area.

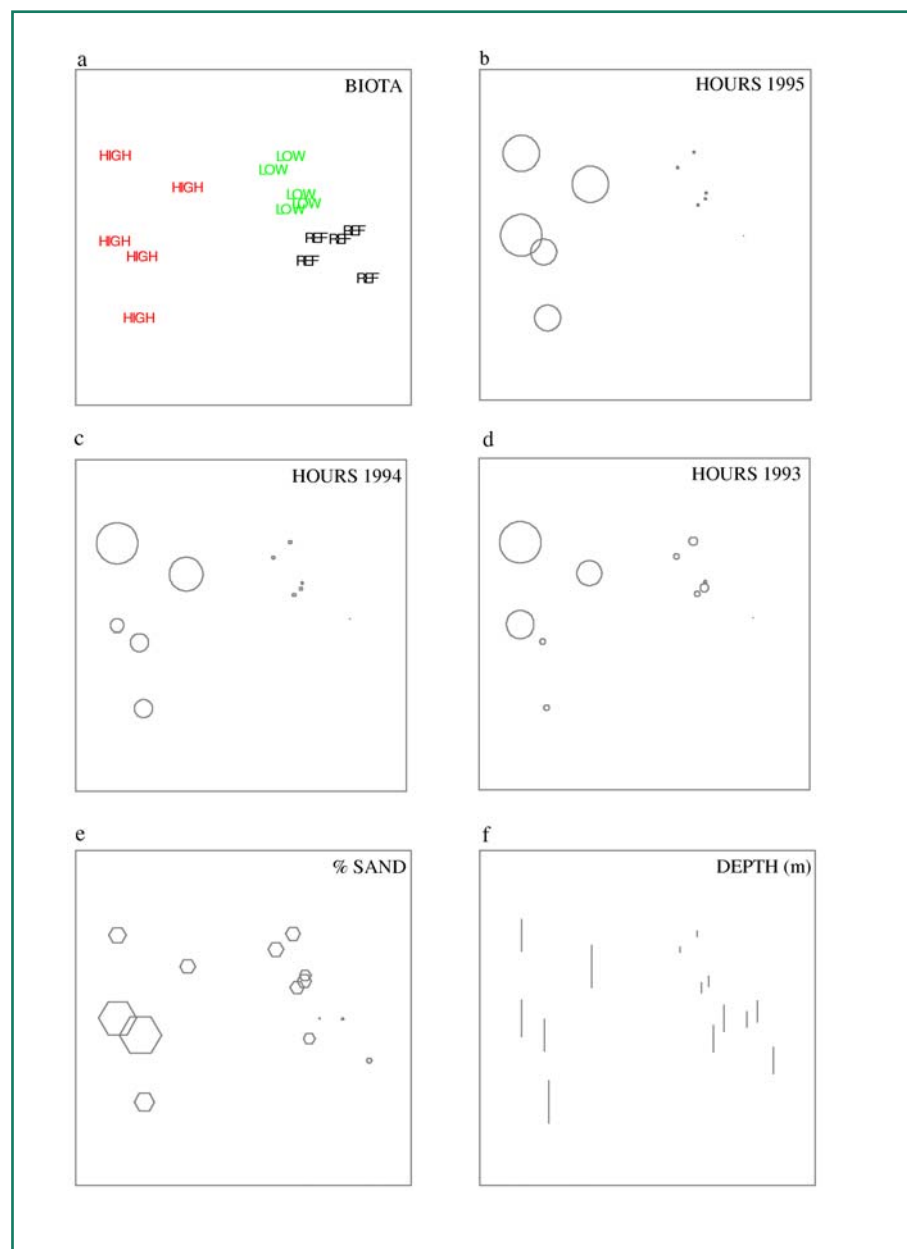


Figure 40 **a) MDS of Bray-Curtis similarities from double square-root transformed species abundance data at three stations of different dredging intensity; b-f) the data are shown with superimposed symbols on the original faunal groupings in linear dimensions proportional to the selected environmental variables b-d) hours of recorded dredging derived from EMS for each year; e) % sand and f) depth of water (Stress = 0.09) (Boyd *et al.*, 2002)**

# CHAPTER 9

## Quality assurance

### 9.1. Definitions and scope

Quality Assurance (QA) is the total management scheme required to ensure the consistent delivery of quality controlled information fit for a defined purpose. The scheme must take into account as many steps of the analytical chain as possible in order to determine the contribution of each step to the total variation. Analytical Quality Control (AQC) encompasses procedures which maintain the measurements within an acceptable level of accuracy and precision.

A QA strategy should be evolved at the outset of an investigation, and should encompass the objectives and design of programmes, as well as practical matters relating to their execution. Thus the adoption of consistent and reliable practices in accordance with documented procedures, both at the field sampling and laboratory analytical stages, will provide confidence in the validity of the output but cannot make up for a sampling design which no longer serves its intended purpose. QA must therefore include regular re-evaluation of reported outcomes in relation to the original objectives of a study.

For benthic ecological studies, all-encompassing QA/AQC systems are still evolving, but the trend is towards increased involvement by individuals and institutes, especially those engaged in collaborative work requiring the synthesis of data from several sources. In the UK, an example of this trend is the recent establishment of a National Marine Biological AQC scheme. Although designed principally to service the needs of the National Marine Monitoring Programme, participation (at cost) is available to a variety of other interested parties. Further details are available at [www.NMBAQCS.org](http://www.NMBAQCS.org). Another example, involving certification of individual competence in species identification, is the IdQ scheme operated by the Natural History Museum, details of which may be obtained from [www.nhm.ac.uk/science/consulting/text/te.html](http://www.nhm.ac.uk/science/consulting/text/te.html).

Draft guidelines for the setting up of quality systems are given in Anon (2002), with the emphasis on marine biological studies. The degree of sophistication will clearly depend upon laboratory size, and it would be inappropriate to attempt to cover the needs of all recipients in the present document. However, one of the most important practical tools in a QA system is the Standard Operating Procedure, details of which are given below.

### 9.2. Standard Operating Procedures

Standard Operating Procedures (SOPs) are an integral part of any Quality Assurance programme and help to ensure that data collected by a laboratory are scientifically valid, comparable and adequate to meet the study objectives. A SOP is defined as 'a written

procedure which describes how to perform certain routine laboratory tests or activities normally not specified in detail in study plans or test guidelines' (Good Laboratory Practice Regulations, 1997). An absolute requirement that all laboratories carry out tasks in exactly the same way would be unrealistic, as procedures are often legitimately tailored to local circumstances (e.g. vessel size). However, where approaches differ between laboratories, it is essential to establish that these do not have adverse implications for the comparability of data. Specific examples relating to studies at aggregate extraction sites are given at Annex II and III. The following general guidance on the structure and content of an SOP is taken from Anon (2002).

A well-written SOP will help inexperienced members of staff in a laboratory to quickly develop expertise in a sampling or analytical area which is consistent with past practice at that laboratory, while being compatible with established approaches elsewhere. For those seeking laboratory accreditation, the production of SOPs will be essential as part of a wider QA package but, even for those who are not, they provide an important means to foster good practice internally. However, SOPs are clearly not, in themselves, guarantors of data quality.

SOPs should describe all steps performed in biological measurement. They should be established to cover the following areas of activity:

- station selection and location, navigational accuracy;
- handling, maintenance and calibration of field and laboratory equipment;
- handling and use of chemicals (i.e., fixatives, preservatives, reagents) used in marine environmental surveys;
- collection of biological material;
- storage of biological material including labelling and the checking of preservation status;
- distribution of biological material to external contractors/taxonomic specialists;
- analytical methods for biological material;
- identification of biological material including taxonomic expertise of the personnel;
- recording of biological and environmental data; data management;
- analysis of biological and environmental data;
- QA of report writing and documentation including signed protocols in all steps of analysis.

In considering "best practice", it is recommended that SOPs should:

- be structured logically by heading and sub-heading to cover the full sequence of activities in field sampling and laboratory analysis;
- carry an issue number, date and name(s) of the individual(s) responsible for its drafting and updating. This anticipates a likely requirement for changes to SOPs in response to new equipment, guidelines and so on;
- document in-house AQC procedures;
- account for the specific practices of the individual laboratory. At the same time, SOPs must of course reflect agreed guidelines applicable at national or international level, for example, relating to nomenclature and coding systems employed in documenting the outcome of the analysis of field-collected specimens;

- contain a full listing of taxonomic keys used for laboratory identification, and other useful reference works relating to procedures;
- be filed as paper copies in an accessible place, as well as being available on a computer network;
- be freely available to all interested parties (especially funding agencies);
- contain explicit instructions for the tracking of samples from the point of collection to the point of archiving of analysed material.

SOPs may usefully contain:

- diagrams depicting gear, especially where local modifications to equipment are made;
- a summary flow-chart as an accompaniment to a lengthy SOP, as an *aide memoire* for field and laboratory bench operators;
- details of local suppliers, manufacturers, etc., where relevant.

SOPs should not:

- contain vague generalisations;
- contain excessive detail: a sensible balance needs to be achieved which takes into account the basic level of training and common sense that a new operator will possess;
- cover too many activities: for example, it is logical to have separate SOPs for field and laboratory procedures. Different types of field activity such as intertidal core sampling and shipboard sampling are also sensibly treated separately.

The preparation of SOPs to cover field and laboratory analytical activities is one of the most important practical steps that a laboratory/institute can take in seeking to improve the quality and consistency of its scientific products and is, therefore, to be strongly recommended. This having been done, interlaboratory comparisons of SOPs may then provide a useful tool in identifying any remaining inconsistencies, and hence in promoting harmonisation of methodology at a national and international level (see, for example, Cooper and Rees, *in press*). Such periodic comparisons of SOPs are also to be strongly recommended.

# CHAPTER 10

## Format for reporting findings from environmental surveys

The appropriate authority should be consulted at an early stage regarding the requirements for reporting findings from benthic surveys. It is recommended that paper copies should be the default report form unless recipients specify otherwise. For example, it may be acceptable to supply electronic copies of reports and these should be in a format readily accessible (e.g. pdf format) to the Regulator, nominated agencies, consultees and industry consortia. For electronic reports, it is recommended that these are posted on industry or Government websites.

Guidelines for the structure of a report on the outcome of a benthic survey are as follows:

- Title page including authorship and date
- Executive Summary
- Introduction
- Materials and Methods
- Results
- Discussion
- References
- Appendices

All reports detailing the findings of marine aggregate extraction monitoring should include relevant raw data and basic statistics as appendices, including details of sub-sampling procedures adopted. Each report should include an appendix containing taxonomic and faunal notes. It may also be useful to include an appendix containing relevant photographic images of samples and equipment.

The methods adopted throughout the environmental appraisal should be clearly described and any modifications to standard procedures should be highlighted. Limitations to the chosen methodology should also be reported. A figure showing the location of sampling stations in relation to the boundaries of the proposed or existing extraction permit should be included in the report. It is also useful to incorporate a figure of block analysis of EMS data for those sites exposed to ongoing extraction effort. The faunal patterns can then be compared with the level of dredging effort within an area. Simple graphical presentations should also be used where possible to summarise major trends in the data. A statement detailing the results of QA exercises should be included in the report as well as references to SOPs and guidelines followed.

All reports should stand alone without the need for reference to previous reports, and all reports should be freely available.

## 10.1. The use of Geographical Information Systems (GIS)

The use of Geographical Information Systems (GIS) for presenting and reporting data is a relatively recent development, but the use of such systems is likely to become more widespread and therefore their future utility is discussed below.

A GIS is a computer-based system designed to input, store, manipulate, analyse and output spatially referenced data. Its application in an environmental context serves a number of purposes. A database provides the basis for any GIS and offers an efficient and manageable format for the storage of all types of information generated as part of an environmental survey. Once stored in the database the information can be easily extracted, and discrete but related datasets can be georeferenced and visualised together. A simple visualisation may take the form of a two dimensional map of the distribution of a number of variables. For example, plots of faunal distributions could be superimposed over values of recorded dredging intensity allowing inferences to be drawn concerning the relationships between the two spatially referenced datasets. Furthermore, the addition of a bathymetric dataset allows insights into the relationships between a number of variables viewed in three dimensions. Datasets which provide 100% coverage maps such as those produced during sidescan sonar surveys can also be draped over, for example, bathymetric plots within a GIS. A further advantage of a GIS is that it allows the inclusion of photographic images or video clips within the GIS, which can be reviewed during subsequent data interpretation. GIS also provides an opportunity to display and compare time-series data. For example, changes in particle size distributions due to changes in the intensity of dredging activity over time could be observed and quantified using GIS. In addition, some systems enable the scientist to interrogate and analyse the information stored within the database.

A number of GIS e.g. Mapinfo™ and ARC View™ are readily available for use by the environmental scientist. GIS lend themselves to wider dissemination in CD format. However, an alternative to the inclusion of bespoke viewing packages is to distribute the GIS maps in pdf format.

# CHAPTER 11

## Future developments

The approaches described above are those which can be routinely applied when conducting baseline surveys of a proposed application area or in assessing the effects of aggregate extraction. However, work continues on the development of new or improved monitoring methods for application in the field, in the laboratory and in the work-up of the resulting data. Significant developments from ongoing areas of research will be incorporated into proposed future editions of these guidelines and/or reported in the wider published literature. A summary of areas of continuing CEFAS research that are likely to have future utility in the assessment of the effects of marine aggregate extraction are briefly described below. Ongoing developments that are being pursued by other organisations are discussed under appropriate earlier sections.

### 11.1. Assessment of cumulative environmental impacts

Cumulative impacts have been defined as effects on the environment, either from the summation of individually minor but collectively significant impacts, or as a result of the interaction of impacts from one or more source (DETR, 2001). This definition includes both additive and interactive effects and is not limited to consideration of a single type of human activity such as aggregate extraction.

Surveys designed to assess the potential for cumulative environmental impacts arising from a prospective aggregate extraction site should consider its likely effect in combination with the sum of individual impacts already established for other sites, both in space and time (including changes projected into the foreseeable future).

Thus the main features which distinguish cumulative environmental impact assessment from conventional seabed surveys can be summarised as follows:

- Emphasis is placed on interactions between impacts arising from aggregate extraction and/or impacts of other perturbations. For example, it considers the additive impacts of multiple small-scale actions which might otherwise have been dismissed or judged to be insignificant for a single extraction application or other activity.
- It aims to evaluate the combined impacts of extraction activity on larger-scale ecological processes including effects on valued resources.

Methods for the assessment of the potential for cumulative impacts arising as a result of aggregate extraction are still evolving and will be presented in due course, following completion of CEFAS research.

## 11.2. Habitat mapping techniques

The facility to map the distribution of physical habitats and their associated biological assemblages is essential to evaluations of the acceptability of proposed or ongoing dredging activity. For example, the production of a habitat map prior to the commencement of dredging may allow subsequent physical changes to the seabed, and the associated benthic communities, to be assessed during follow-up monitoring surveys. This may be achieved using conventional grab sampling techniques, but cost considerations invariably limit the degree of spatial resolution in areas of habitat complexity. Presently, a number of alternative mapping techniques are under investigation by CEFAS and other research institutes.

Acoustic methods, such as sidescan sonar, and the acoustic ground discrimination systems QTC™ View and RoxAnn™, used in conjunction with traditional benthic macrofauna sampling techniques, such as those covered under Chapter 3, are under evaluation to assess their utility for high resolution mapping of benthic assemblages.

Preliminary findings (e.g. Brown *et al.*, 2000; Brown *et al.*, 2001; Brown *et al.*, 2002) suggest that acoustic methods can be used to divide an area into discrete seabed types, which can then be used to derive an optimal sampling design for determination of the spatial distribution of associated benthic communities. Results to date indicate that, in areas of high substratum homogeneity, a close correlation between discrete assemblage types and acoustically distinct regions can be established, which may thus have predictive value. However, the relationship between acoustically detectable habitat regions and discrete benthic assemblages is less obvious in areas of complex, heterogeneous sediments. Whilst the outcome of this work is promising, further research into appropriate techniques and methodology is required before the approach can be routinely adopted.

## 11.3. Assessment of meiofauna

As outlined in Chapter 1.2, the assessment of the effects of aggregate extraction has conventionally consisted of an analysis of large visible organisms, i.e. the macroinfauna and epifauna (> 1mm), that can readily be counted and identified. Due to their small size, the meiofauna, an assemblage of marine benthic metazoa with dimensions between 500 and 63  $\mu\text{m}$ , has been largely neglected in applied sampling programmes (but see e.g. Somerfield *et al.*, 1995; Boyd *et al.*, 2000; Schratzberger *et al.*, 2000a). This size spectrum separates a discrete group of organisms whose morphology, physiology and life history characteristics have evolved to exploit the interstitial matrix of marine sediments.

There are a number of devices suitable for sampling the macrofauna from gravelly sediments (see Chapter 3.2 and 3.3) whereas time- and cost-effective meiofauna sampling is currently restricted to areas of finer sediments. Compared with studies of the macrofauna, the time and effort required for processing of meiofauna samples prior to species identification is generally higher due to the extended effort involved in the extraction of fauna in the laboratory.

The labour- and time-intensive task of meiofauna sample collection and processing must be weighed against the high intrinsic information value of each sample. Thus, as a discrete benthic component the meiofauna have an important role in ecosystem function and as a result of their high abundance, ubiquitous distribution, rapid generation times and fast metabolic rates, the status of meiofauna assemblages may therefore reflect the overall health of the marine benthos (Kennedy and Jacoby, 1999).

Although published information on meiobenthic communities inhabiting marine gravel is currently limited, studies of meiofaunal taxonomy and ecology have increased considerably in the last 20 years. Meiobenthic assemblages have also increasingly been used to assess the effects of perturbations in the marine environment and, in the last 25 years, more than 200 meiofauna papers have been published with a pollution theme (see review by Coull and Chandler, 1992).

Due to their small size, meiofauna assemblages are ideal for follow-up laboratory work, and experiments to simulate the effects of aggregate extraction, using samples of the indigenous fauna as test material may be envisaged. Information from such experimental studies may offer important insights into the processes operating in perturbed assemblages and may provide a cost-effective way to develop and improve future field sampling designs (Katz and Elias 1996; Schratzberger *et al.*, 2000b and c).

# CHAPTER 12

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# ANNEX I

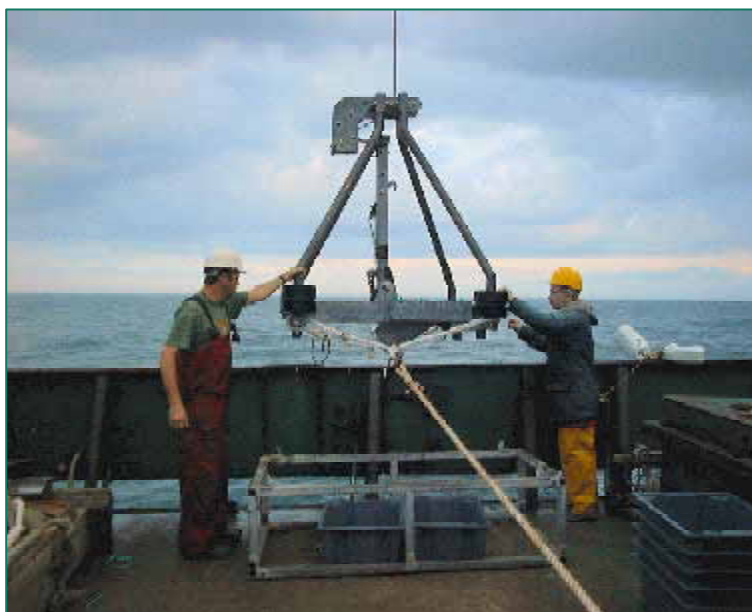
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Dr Tom Simpson (Chairman)	Department for Transport, Local Government and the Regions
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Mr Alan Clayton	(as above)
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Dr Tony Murray	The Crown Estate
Dr Paul Gilliland	English Nature
Mr Mark Russell	British Marine Aggregate Producers Association

# ANNEX II

## An example of an SOP for the collection and analysis of macrofaunal samples using a Hamon grab

	Procedure No. FET 002
	Page of
BENTHOS QUALITY MANUAL	Issue no. 2
TITLE OF PROCEDURE	Issue Date: September 2001
<b>Grab Sampling for Marine Sub-tidal Gravelly Sediments</b>	Issued by:
	Authorised by:



### 1. INTRODUCTION

Sediments, and particularly the associated benthic fauna, can act as a useful indicator of environmental disturbance and as a result samples are routinely collected for analysis of a wide variety of biological and physical determinands. For gravelly sediments in the vicinity of marine aggregate extraction sites, sampling is aimed principally at assessing the biological and physical impacts of such activities.

Many samplers (e.g. Day grab and Box corer) are unsuitable for use in gravelly sediments as coarse particles of sediment prevent the effective operation of the devices resulting in a loss of sampled material. However, the Hamon grab (Oele, 1978), has proved to be an effective sampler of coarse sediments.

This grab consists of a rectangular frame forming a stable support for a sampling bucket attached to a pivoted arm. On reaching the seabed, tension in the wire is released which activates the grab. Tension in the wire during in-hauling then moves the pivoted arm through a rotation of 90°, driving the sample bucket through the sediment. At the end of its movement, the bucket locates onto an inclined rubber-covered steel plate, sealing it completely.

This procedure deals specifically with the collection of samples, from areas of coarse sediment, for the analysis of the benthic macrofauna and particle size distribution.

## **2. SAMPLING VESSELS**

CEFAS Research Vessels conform to the International Maritime Organisation's '*International management code for the safe operation of ships and prevention of pollution*'. These vessels do not require checks for suitability.

If using a charter vessel, the CEFAS document '*Standing Instructions for the use of Vessels other than Research Vessels in the Directorate's Field Programmes, January 1993*' [currently being updated] should be consulted. For the purposes of grab sampling, the vessel should have a winch with a  $\geq 1$  tonne capacity, fitted with sufficient wire to extend beyond the sampling depth. The wire should lead from the winch to either a derrick, gantry or 'A' frame which allows the grab to be deployed safely clear of the vessel. The boat should have sufficient deck area to carry out the processing of samples. The vessel should also be fitted with a DGPS satellite positioning system and a deck-wash hose.

## **3. PERSONNEL**

In addition to the skipper and crew, personnel must comprise a minimum of two scientists, at least one of whom is experienced in benthic sampling, according to the procedure described below. One person should also be experienced at operating the winch (normally the skipper or member of the crew of the vessel).

## **4. SAFETY**

Hazards are presented by the improper use of reagents used in the procedure. Survey staff should be familiar with the use of hazardous substances and should be provided with the relevant safety documentation in the form of COSHH and risk assessment forms. Copies should also be provided to the captain or nominated safety officer of the survey vessel.

The working environment on board the sampling vessel also presents a number of hazards. Personnel must have the appropriate training and safety equipment and be aware of the risks associated with working onboard ships at sea.

## 5. EQUIPMENT

### 1) 0.1 m<sup>2</sup> Hamon grab (see Figure 1).

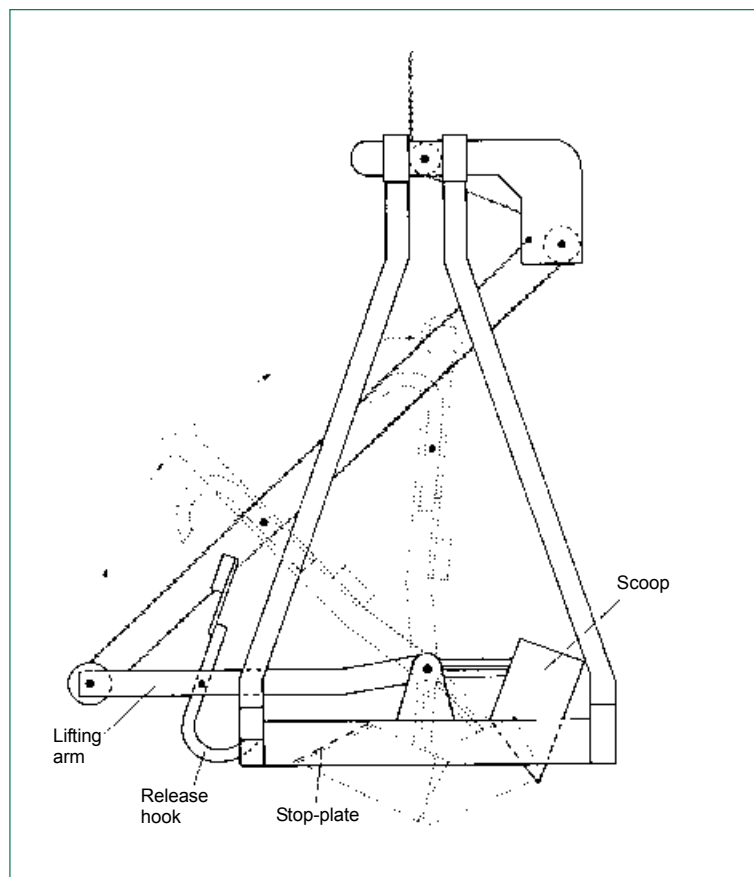
The grab consists of a rectangular frame forming a stable support for an articulated sampling bucket. On reaching the seabed, tension in the wire is released allowing uncoupling of the release hook. This allows the lifting arm to rotate through 90° driving the bucket laterally through the sediment. At the end of its movement, the bucket locates on a rubber-covered steel plate, sealing the bucket mouth completely, and preventing any wash-out of sample material. The device samples an area of 0.1 m<sup>2</sup> and penetrates up to 15 cm into the seabed.

Lead weights can be attached to the grab, allowing greater penetration of the sediment, and should be adjusted according to the prevailing substratum type.

A larger version of the same device, sampling an area of 0.25 m<sup>2</sup>, is available for use in certain circumstances, but the smaller (0.1 m<sup>2</sup>) version has now been adopted for general use because of its versatility and ease of handling.

### 2) Grab stand (see Figure 2).

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



**Figure 1 Hamon grab, showing mode of action. The lifting arm rotates through 90° to drive the scoop through the sediment, closing against the stop plate. (Reproduced from Eleftheriou and Holme, 1984)**

### 3) Large 50-70 litre sample containers

Suitable watertight boxes, small enough to be placed under the grab stand but with sufficient capacity to receive the collected sediment and supernatant water without spillage should be used. These containers typically have a capacity of 50-70 l and may be calibrated for determining sediment volume. There should be sufficient containers to allow processing to be carried out at a later stage, if replicate samples are being taken.

### 4) Sieve table

This device consists of an open-ended box whose interior sides slope towards an outlet pipe (see Figure 3). The interior of the box is coated with epoxy resin that facilitates easy washing and which also prolongs the life of the device. Small blocks mounted on the interior of the box provide support for a removable, square stainless steel frame with a 10 mm or 5 mm square mesh aperture. The entire device is supported on legs that can be adjusted to allow the table to be positioned at a suitable height (normally waist height) for ease of use.

### 5) Sieve holder

This device consists of an aluminium frame designed to support a circular sieve of 1mm or 0.5 mm square-mesh aperture (30 cm diameter 'Endecotts' Laboratory Test Sieves certified to BS410; (0.5 mm, 1.0 mm and 2.0 mm stainless steel meshes). The sieve holder is supported on the top of an open plastic box, which allows the sieve to be positioned underneath the outlet pipe of the sieve table (see Figure 3). The choice of sieve mesh size will depend on the objectives of the investigation. Sieves should be discarded at the first sign of damage to the mesh.

### 6) Plastic funnel and stand

A large, wide-bore funnel, the spout of which will fit into the necks of the sample containers, should be used. The stand holds both the funnel and smaller sample containers, minimising the risk of loss of material (see Figure 4). Where larger (e.g. 10 l) buckets are to be used, the funnel may be placed directly inside, for transfer of the sample contents.

### 7) Sample containers

Sample containers should be spill proof, air tight and strong enough to withstand rough handling during transport and storage. The size of the container will be determined by the size of the sample. Choose from 125 ml, 250 ml, 500 ml, 1000 ml bottles and 2.5 l, 5 l, and 10 l buckets.

### 8) 500 cm<sup>3</sup> plastic scoop

This is used for the collection of aliquots of sediment for subsequent particle size analysis.

### 9) Waterproof pen and labels

Labels made from water resistant paper are used inside the sample container. Water resistant ('Nalgene® Polypaper') sticky labels should be used for external labels. All labels should be annotated with a permanent marker.

## 10) Log book

A standard field log should be used. Pencil should be used to record information in the log. The log should be annotated with prompts for all the information required. The following information should be routinely recorded (highlighted information will be recorded on the bridge by the crew on CEFAS research vessels): cruise details, station number and code, **coordinates of sampling position**, survey positional datum, equipment used and any modifications to the equipment (including addition of weights), type of sample taken, mesh size used for sieving, depth or volume of sediment sample obtained, brief description of the sediment including any artefacts, **water depth**, time of sample collection (GMT), size of container(s) used to store preserved samples, any deviation from standard operating procedure, personnel involved, **tide direction and strength**, **wind direction and strength** and **sea state**. On charter vessels it will be necessary to record all of the above information.

## 11) Surveying software (SEXTANT™)

This is a software package that allows station positions to be accurately recorded at the precise moment of sampling. Positional information is taken from a DGPS receiver interfaced to the computer. The system is particularly useful on large research vessels as it allows any offset between the position of the DGPS receiver and the position of the grab to be taken into account. The software also allows the vessel to be positioned within a set distance of a pre-determined sampling location. On smaller vessels where this system is not available the position of the vessel as indicated by the DGPS should be recorded manually as the sampling position.

## 12) 500 ml standard laboratory 'wash bottle'

## 13) Calibrated measuring bucket (minimum 10 litre capacity)

## 14) 0.75 l plastic boxes for PSA samples

## 15) Chemical aspirator for the storage of 10% formaldehyde

## 16) Water hose / deck wash (ideally with variable pressure)

## 6. REAGENTS

*Preservative* – 10% formaldehyde solution

Composition: formaldehyde 30%, pH 7.0 (buffered with sodium acetate trihydrate 25g/litre)

– seawater

At CEFAS, buffered 30% formaldehyde solution is stored in 10 litre drums at the Lowestoft Laboratory. A working solution of 10% formaldehyde is prepared by diluting approximately 3-fold with clean seawater.

**Formaldehyde is a toxin, a carcinogen and an irritant and should only be handled whilst wearing eye protection, disposable gloves and waterproof clothing. All containers must be clearly labelled. A funnel must be used when transferring the neat chemical from container to container. All samples fixed with formaldehyde must be thoroughly washed under fume extraction before they are handled in the laboratory.**

## 6.1. Preparing dilutions of formaldehyde

At sea, prepare dilutions of formaldehyde on deck, whilst wearing safety glasses, gloves and waterproof clothing. Details on procedures for dilution, storage and transport of the chemical are contained in the relevant Control of Substances Hazardous to Health (COSHH) Risk Assessment (Sea\_COSHH\_01: Storage of 30% formaldehyde solution, dilution of 30% formaldehyde to 10% and use of 10% formaldehyde for preservation of benthos samples). The aspirator used for the storage of 10% formaldehyde solution should be labelled with the following information: 10% formaldehyde solution, toxic, carcinogen as well as the carrying of Harmful and Flammable adhesive tape labels. The container should also be securely lashed to the deck of the vessel. When working on small vessels it may be advisable to preserve samples on return to the laboratory. The length of time between the collection of samples and returning to the laboratory will determine whether this is feasible.

*Sample stain - Rose Bengal*

Rose Bengal, a vital stain, may be added to the fixation fluid to enhance the colour contrast between specimens and the sediment, thereby potentially increasing subsequent sorting efficiency.

**Rose Bengal is an extremely hazardous carcinogen and, in its powder form, should only be handled under fume extraction.** It should therefore be added to the concentrated formaldehyde solution in the laboratory or made up as aqueous solution for use in the field. The final concentration of Rose Bengal should be around  $0.1 \text{ g l}^{-1}$ .

## 6.2. Preparation of concentrated Rose Bengal solution

The concentrated solution should be stored in labelled “safe break” Winchester bottles inside plastic carrying containers. Both the Winchester and the plastic container should be suitably labelled. The 1% Rose Bengal solution should be added to the 10% formaldehyde solution in a ratio of  $1 \text{ cm}^3$  to  $1000 \text{ cm}^3$  to give the required concentration of Rose Bengal (approximately 0.01%).

## 6.3. Use of Rose Bengal in the field

Measure out the required volume of concentrated Rose Bengal solution (0.01%) using a measuring cylinder. Add this solution to the aspirator containing the 10% buffered formaldehyde. Ensure the solutions are well mixed. This procedure should be carried out in a well ventilated area whilst wearing safety glasses, disposable gloves and waterproof clothing.

# 7. PROCEDURE

## 7.1. Pre-survey checks

At the laboratory, all items required for field survey work, including disposables, should be checked against the equipment list and inspected for damage (e.g. damaged sieve meshes). Replace or repair damaged items as necessary. Once on board the survey vessel ensure all equipment is present and safely stowed.

## 8. PREPARATION OF EQUIPMENT

Position the grab and stand beneath the derrick or gantry and attach the wire of the Hamon grab to the winch wire from the survey vessel using a shackle and swivel. Check that the weights are securely fastened.

Set the Hamon grab by pulling the lifting arm down from the vertical position allowing the release hook to engage (see Figure 1).

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

Place a clean, large plastic box under the grab stand hopper.

## 9. DEPLOYMENT AND RECOVERY

When the boat is stationary and the skipper has given permission, the grab is deployed, typically at a rate of approximately  $1 \text{ ms}^{-1}$ . As the grab approaches the seabed the wire should be released more slowly to avoid the creation of a 'bow wave' which could wash away surface material. Once the Hamon grab has reached the seabed, slackening of the winch wire provides a signal to stop the winch. The grab should then be raised, initially very slowly to maximise sampling efficiency. When the grab reaches the surface it should be stabilised and then swung on-board, as soon as possible, as the device presents a danger on a rolling vessel. The grab is then lowered gently onto the supporting frame. Enough winch cable should be released to enable the lifting arm (and grab contents) to be released.

In rough seas, the vessel should be orientated 'head to wind' thus minimising roll and reducing the risk of loss of control of the grab during deployment and recovery. An inhauler should be used to facilitate safe retrieval. This device consists of a rope attached to a winch



**Figure 2 Hamon grab primed and ready for deployment. Note the winch controlled lateral supporting rope for increased stability and therefore safety during deployment and recovery. It is unhooked before the descent of the sampler**

which is then attached to the grab by means of a hook (see Figure 2 for set-up). As the grab is lifted above the rail of the vessel, the inhauler is used to pull the grab on board.

## 10. COLLECTION OF SAMPLES

Should the bucket of the grab fail to engage fully with the stop plate (e.g. as a result of a stone obstructing closure), resulting in the loss of sample material, the contents should be discarded and the grab re-deployed.

Slowly release the sediment into the sample container by pulling down the lifting arm to the horizontal position. The container should be moved in synchrony with the grab bucket. Any material remaining in the grab should be carefully washed into the container.

The volume of the sample should be measured by transferring it into a calibrated bucket. This action should be carried out over the sieving table so that any water within the sample is not lost. Samples with a volume of less than 5 litres of sediment are discarded and a repeat sample taken. At least three attempts should be made at each sampling station before abandonment of sampling at the station position. At the discretion of the Scientist-in-Charge a smaller sample may be accepted if there is some merit in obtaining indicative (e.g. qualitative) information from a location. Alternatively, further attempts can be made at increasing distance (typically 50-100 m intervals) from the original site. Again this will be at the discretion of the Scientist-in-Charge.

Once an acceptable sample has been obtained, the volume of sediment and the nature of the material should be recorded in the log book (e.g. 'gravel', 'sandy gravel'). In describing the nature of sediments, the component making up the smallest fraction of the sample should be described first. For example, sediment composed of mainly fine sand with a little gravel would be described as 'slightly gravelly fine sand'.

### 10.1. Particle size analysis

Using the plastic scoop, transfer a representative sub-sample of 500 ml of sediment to a sealable plastic bag which should then be placed inside a 0.75 l plastic box. Add labels (see Section 13) and freeze the samples.

## 11. SIEVING THE SAMPLE USING A PURPOSE-BUILT SIEVING TABLE

After measuring the volume of the sample the sediment should be washed, using gentle hose pressure, whilst still in the calibrated sample container. This should be conducted over the sieving table and the appropriate meshes and sieves should be in place. This will allow many of the lighter organisms to be released from the sediment with the minimum amount of damage to specimens. Allow the supernatant water, containing any fine sediments and benthic organisms, to overflow from the sample container and pass through the 5 mm sieve. The remaining sediment should then be gently washed over the 5 mm removable square mesh screen. Larger individual animals retained on the 5 mm mesh and all encrusting fauna present on shell and gravel are removed and transferred to plastic bottles or buckets (depending on the size of the sample). Any sediment remaining on the 5 mm screen (with no attached concealed fauna) may be discarded. The nature of the coarse material,

including the presence of any artefacts, should be recorded in the log. If any material is lost a repeat sample should be taken.

The material passing through the 5 mm mesh is sieved over a stainless steel sieve with either 1 mm or 0.5 mm precision steel mesh screens, the choice depending on the objectives of the investigation. This sieve is held within a sieve holder beneath the outlet pipe of the sieving table. Temporary blockage of fine meshes can occur and care should be taken to ensure that there is no loss of animals as a result of overflow. Periodically, the sieve should be removed from beneath the outlet pipe, and replaced by another. Accumulations of fine sediment on the mesh screen can usually be removed by gentle ‘puddling’ in a large plastic container filled with seawater (using a vertical motion as horizontal motion can cause animals to be damaged through abrasion).

## 12. SAMPLE PRESERVATION

On completion of the sieving process, retained animals and residual sediment on the mesh screens are transferred to plastic bottles via a large funnel in a frame support (Figure 3). The stainless steel sieve should be supported at about 45°, and rinsed using a hose under gentle water pressure from top to bottom. This whole process should be carried out within a large plastic container so that any accidental spillages can be contained and rinsed back onto the sieve. If the water pressure from the hose is too high and cannot be adjusted, a 500 ml wash bottle should be used. Any material trapped within the mesh of the sieve should be carefully removed using forceps. A scoop should not be used to remove material from the sieve as this may cause damage to specimens.

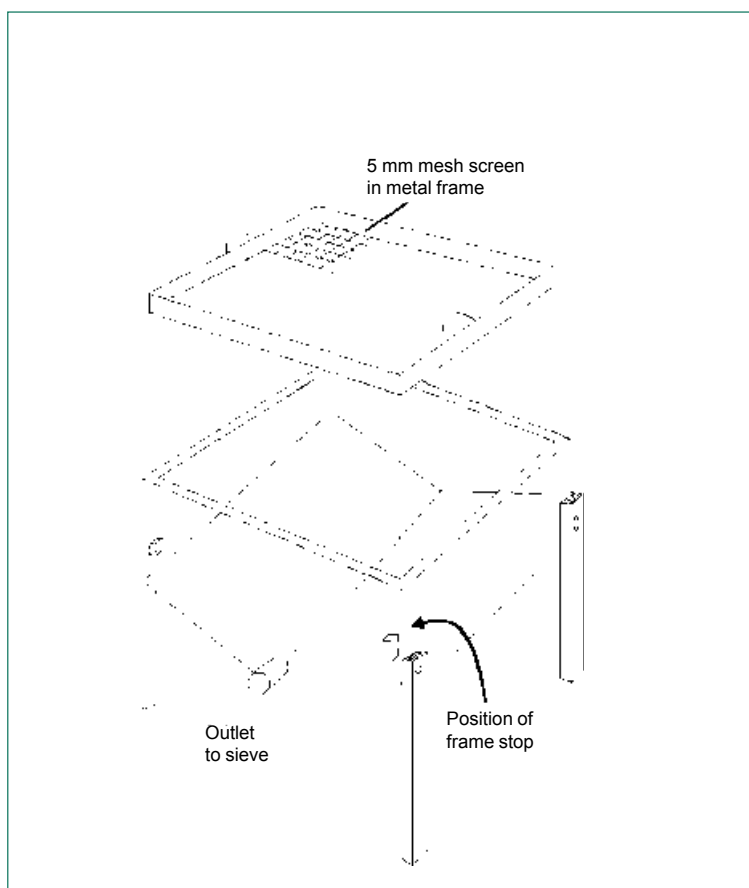
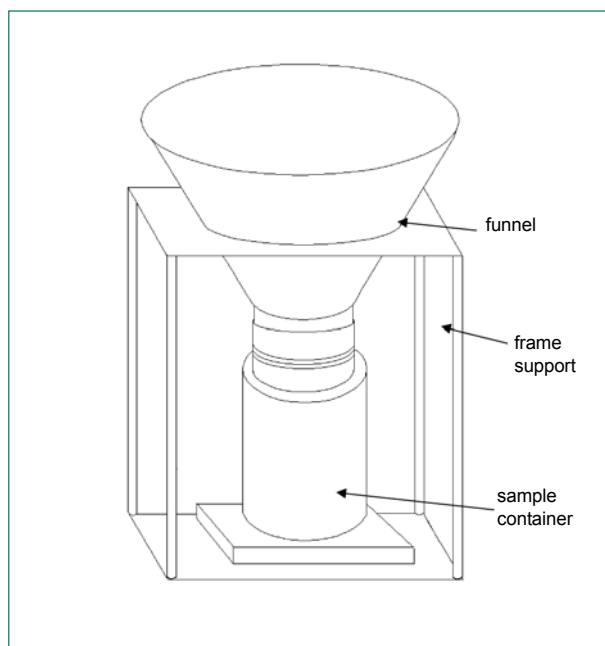


Figure 3 Sieving table used for the processing of grab samples



**Figure 4 Funnel in frame support**

The 10% formaldehyde preservative solution, with or without added Rose Bengal (see Reagents), should be added to fresh samples with the aim of achieving a final concentration of 5% of formaldehyde in the sample; i.e. add approximately the same volume of the 10% formaldehyde solution as the volume of fresh sample (including any liquid).

### **13. SAMPLE LABELLING**

An adhesive label should be attached to the outside surfaces of the sample container and an internal waterproof label inserted (so that any damage to the external label does not prevent the later identification of the sample). Polythene bags for sediment sub-samples should be labelled directly onto the panel of the bag, prior to use. The plastic boxes used for subsequent storage of PSA samples should also be directly labelled. All labels should contain the following information:

- Research cruise number or code (e.g. prefix - vessel name: Cir – Cirolana, Cor – Corystes followed by cruise number/year)
- Date
- Station number and code (stations are numbered sequentially from the start of a cruise).
- the type of sample (e.g. macrofauna, PSA etc)
- Survey area

### **14. SAMPLE STORAGE AND TRACKING PROCEDURE**

Details of the samples taken are recorded in the cruise log book. This acts as the sample record. On completion of the cruise this should be signed and dated by the Scientist-in-Charge. On return to the laboratory, samples and log book data should be dealt with in accordance with the storage and sample tracking procedure (FET 004).

## **15. QUALITY CONTROL**

Check operation of position-fixing equipment, winch and deck-wash prior to departure.

Check the condition of the sampling equipment (particularly sieves and large volume sample containers) and replace as necessary.

Comply with the criteria for sample rejection.

## **16. ANALYTICAL PROCEDURES**

For analysis of macrobenthic samples see Procedure FET 003.

For analysis of sediment particle size, refer to the appropriate SOPs.


## **17. REFERENCES**

Eleftheriou, A. and Holme, N.A., 1984. Macrofauna techniques. In: Holme, N.A. and McIntyre, A.D. (eds). *Methods for the study of marine benthos*. Oxford: Blackwell, pp 140-216.

Oele, E., 1978. Sand and gravel from shallow seas. *Geologie en Mijnbouw*, 57: 45-54.

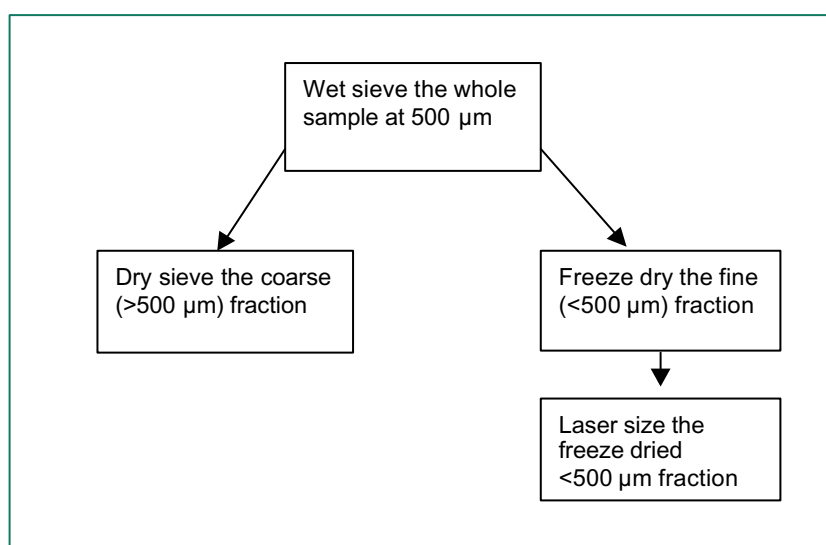
# ANNEX III

## An example of an SOP for the laboratory particle size analysis (PSA) of samples collected from coarse substrata

 <b>CEFAS</b> <i>The Centre for Environment, Fisheries &amp; Aquaculture Science</i>	Procedure No.
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	Issue no. 2
	Issue Date: September 2001
BENTHOS QUALITY MANUAL	Issued by:
TITLE OF PROCEDURE	Authorised by:
<b>An example of an SOP for the laboratory particle size analysis of coarse substrata</b>	

### INTRODUCTION

The SOP outlined here is one example of many possible SOPs available for the PSA of coarse substrata.



Flow chart showing the breakdown of the main processes involved in completing this type of PSA

## 1. WET SIEVING AT 500 $\mu\text{m}$

Check that all sieving equipment is clean, in working order, and that sufficient stocks of consumables (bags, labels, foil trays etc.) are available. If necessary, remove a sub-sample from the whole sample prior to analysis. Prepare the sieving apparatus by placing a clean bucket in the sink with the closed off drain tap lying in it, and mounting a clean 500  $\mu\text{m}$  sieve with receiving pan onto the sieve shaker.

Place the sample onto the 500  $\mu\text{m}$  sieve and screw down the lid.

Pour sufficient tap water through the hole in the shaker lid to cover the sample and then insert the rubber bung into the hole to prevent leakage during operation.

Adjust the amplitude of vibration to a level high enough for water to splash onto the lid of the sieve shaker. Set the timer dial to ten minutes and begin the sieving process.

Label a clean plastic bucket lid (for the resultant  $<500 \mu\text{m}$  fraction) and a foil tray ( $>500 \mu\text{m}$  coarse fraction). After ten minutes of sieving, open the drain tap and allow the fine material ( $<500 \mu\text{m}$ ) to run into the plastic bucket. Close the drain tap and add more tap water to the sample (following instructions above). Sieve for a further five minutes. Drain off the water again, and repeat this step until the water in the top of the sieve, and the water being drained, is clear. Remove the lid of the sieve shaker, and, using a washbottle, wash any sediment from the lid of the sieve shaker onto the sieve. Remove the sieve, and use a washbottle to wash any sediment from the retaining pan into the bucket. Cover the bucket and leave to settle.

Using a clean plastic scoop, remove the bulk of the material retained on the sieve into the foil tray, and wash the remainder out with tap water from a washbottle. Place the foil tray into an oven at  $80^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for at least twelve hours. Clean the sieving apparatus in preparation for the next sample by washing water through the retaining pan and tubing. Wherever possible, avoid interruptions whilst wet sieving a sample. Where interruptions are unavoidable, finish the processing of the current sample before leaving the laboratory for more than one hour.

## 2. DRY SIEVE ANALYSIS OF THE $>500 \mu\text{m}$ COARSE FRACTION

Remove the coarse fraction from the laboratory oven and allow to cool to room temperature.

Sediment samples are dry sieved using stainless steel laboratory test sieves, with mesh sizes at  $\frac{1}{2}$  phi intervals. The coarsest sieve used should be larger than the largest particle in the sample. The finest sieve has a mesh size of 500  $\mu\text{m}$  (+ 4 phi), followed by a collecting pan. The dry sieve shaker can accommodate a stack of up to 12 standard height sieves, plus the collecting pan and lid. Given the wide range of particle sizes encountered when analysing aggregate samples it is frequently necessary to use two stacks of sieves, the coarse stack ranging from 2.8 mm upwards, and the fine stack from 2.8 mm to 500  $\mu\text{m}$ . If both stacks are to be used for one sample, the samples should be sieved through the coarse stack first. The sample retained in the collecting pan from this first process should then be sieved through the fine stack.

Prepare the sieves by ensuring firstly that they are clean. If particles are visible either on, or trapped within the mesh of the sieves, brush them using a nylon sieve brush (a hard bristle for the coarse sieves and a soft bristle for the finer sieves i.e. 90  $\mu\text{m}$  and less). Stack sieves in phi class order (coarsest at the top), including a lid and collecting pan for every stack used. Pour the sample from the foil tray into the top of the selected stack of sieves. Ensure that no sample remains in the foil tray by gently tapping and brushing it onto the sieve. Place the lid onto the stack. Place the sieve stack into the sieve shaker, and clamp the retaining plate down. Switch the shaker on by turning the timer to ten minutes, then close the door to the sound proofed cabinet. After ten minutes the shaker will switch itself off, and the sieve stack can be removed.

Place a clean foil tray onto a top-pan balance and tare it. Remove the lid of the stack, then remove the coarsest sieve. Carefully pour the contents of the sieve into the foil tray, brushing any residual sediment into the tray using a clean sieve brush. Record the weight to 0.01 g on a data sheet. Weigh each successive sieve in the same way. If the coarse stack of sieves is being used, the contents of the collecting pan should be poured into the top of the fine stack of sieves and sieved as above. If the fine stack is being used, the contents of the collecting pan should be weighed and recorded. Place the sieved sample into a labelled sealable plastic bag. Ensure that the sieves are clean, then re-stack them and load another sample.

### **3. FREEZE DRYING OF THE <500 $\mu\text{m}$ FINE FRACTION IN PREPARATION FOR LASER SIZING**

Remove the majority of the supernatant from the plastic bucket using a tap fitted filter pump valve system. Take care not to disturb or remove any sediment during this process. Pour the remaining contents of the bucket into a labelled plastic petri-dish (or dishes if necessary), washing any remaining sediment from the bucket using a washbottle containing tap water. Place the lid on the dish and place into a freezer at  $-10^{\circ}\text{C}$  until the sample has frozen solid.

When the samples are frozen, switch on the freeze dryer. Close the chamber door and allow the temperature to drop to between  $-40^{\circ}$  and  $-60^{\circ}\text{C}$ . Take the samples from the freezer, remove the lids and place them under the base of each petri dish. Place the samples onto the shelves of the freeze dryer as quickly as possible to prevent them from thawing. Cover the samples with the plastic bell-housing, close the drain tap and switch on the vacuum pump. Apply pressure to the chamber door to ensure that a vacuum is created. The samples may take upto 5 days to dry. Observation of the underside of the petri-dishes will usually reveal whether all of the ice has been removed. A dark patch is evidence that some ice remains, and that freeze drying is not yet complete.

When the samples are dry, switch off the vacuum pump, and gradually open the drain tap to release pressure. Once the pressure has equalised, the drain tap may be fully opened, and the bell housing removed. Remove the samples from the freeze dryer and replace the lids. Allow the freeze dryer to defrost. Transfer the sample from the petri-dish to a tared, labelled, sealable plastic bag. Weigh the sediment and the bag to 0.01 g on a top pan-balance. Record this weight on the bag.

#### 4. LASER SIZER ANALYSIS OF THE <500 $\mu\text{m}$ FINE FRACTION USING THE COULTER™ LS130 LASER SIZER

The Coulter™ LS 130 Laser sizer is capable of analysing sediments with a particle size of up to 900  $\mu\text{m}$ , although it is more routinely used for the analysis of particles up to 500  $\mu\text{m}$  in diameter. Analysis is carried out by a series of measurements of the angle of diffraction of a laser across sediment particles. Actual analysis of one sample takes approximately 90 seconds, although the full cycle of rinse and calibration takes around 6 minutes.

It is important that the laser sizer is NOT switched off (except for emergencies) as warm-up takes up to 4 hours. The laser sizer is operated via a PC running Coulter™ LS software. The laser sizer computer should be kept on. If it is switched off, turn on the PC and double click on the LS icon to open the software.

It is important that the laser sizer is rinsed at the start of each day in order that any material which may have settled is removed. This procedure should be carried out as follows. From the menu bar, select Control, then Rinse. The water in the sample vessel should begin to flush up and down. If the vessel empties completely use blue roll to dry the metal sensors in the sample vessel.

After approximately 2 minutes, click Cancel in the fluid module box. The sample vessel should automatically fill. Check that the water in the vessel is clean. If particles are visible, repeat the rinse, as above.

The appropriate quality control should be run prior to any samples being analysed. The Coulter™ control samples should be run on the first day of the week that the laser sizer is used. On each day that the laser sizer is used a test sand sample should be run. The results should be checked to ensure the samples are within acceptable limits.

The procedure for running a sample is as follows.

From the menu, select Run, then Cycle. Click on the New Sample button. Accept the defaults for rinse, calibration etc. Click on Start. The machine will run a series of measurements for offsets, alignment and background. Once Obscuration:PIDS is reached, observe the percentages gradually decreasing as the machine rinses. The two numbers should read around zero percent before the sample can be added. If this looks unlikely, it may be that a full manual rinse needs to be carried out.

Weigh out ~1.5 g of the sample into a 50 ml glass beaker. Add 40 ml of 0.1% sodium hexametaphosphate. Place the beaker in an ultrasonic bath and sonicate for 10 minutes. Add a magnetic stirring rod and stir the sample at a speed that ensures all the sample is mobilised (i.e. none of it is settling in the bottom of the beaker).

When Obscuration and PIDS are at around zero percent, add sample using a pipette as described. While the sample is mobilised take a subsample using a pipette. This should be done with care to ensure a representative sample is taken. Place the end of the pipette at the bottom of the beaker and raise it diagonally through the liquid at a steady rate to remove a subsample. Drop the subsample into the laser sizer sample vessel. Ensure that the pipette is shaken in a downward motion to get a fair representation of each subsample into the sample vessel. Repeat pipetting until the laser sizer indicates that sufficient sample has been added, and that the obscuration level is at around 45%. Click on Done. A sample details dialogue box will appear.

Complete the sample details as appropriate. In the comment box, insert details such as the fraction size and also the sediment weight.

Click OK. A run information box appears. Check that the default run length is 90 seconds, and that the optical model is Fraunhofer and that PIDS is included. Select OK. The sample will now run for 90 seconds. Following this, the screen displays a histogram of particle size distribution, which prints automatically. Close the graph by double clicking in the top left hand corner.

At the end of the day, it is important that the laser sizer is cleaned by running a manual rinse (see above). Because the laser sizer is not switched off, it is essential that the pump is not left running. On the PC, select Control from the menu, then Pump Off. Switch off the pump at the plug.