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Burnham Laboratory (Benthic Ecology)

Standard Operating Procedure

(SOP number 1381)

(Issue number 1.0)

SAMPLING AND ANALYSIS OF THE MACROBENTHIC INFAUNA FROM SOFT SEDIMENTS

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Issue and Validation

Production summary

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History of Procedure

Issue	Date Issued	Changes
1	October 2005	The SOP was updated to include information on the use of Digilog, Tower, sample tracking and transport of formaldehyde.
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SAMPLING AND ANALYSIS OF THE MACROBENTHIC INFAUNA FROM SOFT SEDIMENTS: Issue 1.0

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1 Introduction

Properties of marine sediments, and particularly the benthos, i.e. fauna, can act as useful indicators of environmental disturbance. Sediment samples are routinely taken for a number of purposes including analysis of: the benthic macrofauna, particle size, trace organic and inorganic concentrations, redox, and % organic carbon & nitrogen.

Samples from soft sediments are taken using a 0.1m^2 Day grab. This device is lowered to the seabed and on making contact a trigger mechanism fires, releasing the jaws of the grab. On raising, the grab jaws close and a sample is retained. The sample is then processed on board the vessel and returned to the laboratory for analysis.

This procedure deals specifically with the collection of samples, from areas of soft sediment, for the analysis of macrofauna, meiofauna and particle size. There will be areas where this procedure overlaps with the procedures for the collection of other types of sample. The relevant procedures must be consulted if other samples are to be collected.

2 Scope

This SOP describes the procedure for the use of a 0.1m^2 Day grab and the subsequent processing of benthic samples collected with this piece of field equipment. Additionally, it highlights the safe transport and use of formaldehyde for preserving the benthic samples (see COSHH BOC-EQ-Coshh-SAS-Sea-01 and COSHH BOC-EQ-SAS-Formaldehyde which are available on the intranet under the Health and Safety pages)

3 Training (Identify any specific training linked to the SOP)

This procedure may only be carried out by staff who have received training in this SOP and in the use of equipment. Training records must be maintained and archived accordingly.

4 Safety Precautions

Before performing this procedure staff should have read and understood the following COSHH & risk assessments.

4.1 COSHH

BOC-EQ-Coshh-SAS-Sea-01 Storage of 30% formaldehyde solution, dilution of 30% formaldehyde to 10% and use of 10% formaldehyde for preservation of benthos samples
BOC-EQ-Coshh-SAS-Sea-05 Collection of sediment samples at sea from sewage sludge, dredged material and industrial waste disposal sites

BOC-EQ-SAS-Formaldehyde Movement to and storage of samples preserved in 10% formaldehyde solution at Burnham offsite storage facility

4.2 Risk Assessments

G03 Participation in research cruises on CEFAS owned and managed ships. The collection of samples and data, all subsequent processing whilst on-board, including the use of the ships sea-rider

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G04 Scientific work on chartered vessels not owned and / or managed by CEFAS (but NOT including work on commercial, un-chartered vessels or travel on passenger vessels.

G05 Work on beaches or in coastal waters, estuaries, rivers and lakes, whether operating from dinghies, small chartered vessels or from shore

G06 Working on un-chartered, commercial vessels, including observations of discards, sampling fish or shellfish or deployment of scientific gears

5 References/Associated documents

Please refer to SOPs for the use of the Cruise Planner, Tower navigation system, bar-coding system and Digilog, all of which will be used to record information relating to samples collected.

6 Equipment /Apparatus

1) Modified Day grab (see Figure 1)

The modifications consist of 'stub axles', with closing flaps that hinge from the exterior of the buckets, rather than centrally (see Figure 2). The buckets are stainless steel to reduce the risk of contamination in the collection of sub-samples for later analysis of trace contaminants. This device samples an area of 0.1m^2. The jaws are supported within an open galvanised steel framework, which will cause minimal down wash as it lands on the seabed. Triangular lead weights are fitted to, and within, the four corners of the framework. These afford the grab greater weight with which to penetrate the sediment. Weighting of the grab should be adjusted in order to obtain optimum penetration of the sediment. The grab should not be overfilled as this could lead to loss of material on retrieval. The jaws of the grab and the flaps on top should seal well to ensure no loss of material when the grab is retrieved.

2) Purpose built grab table

This structure is made from wood and supports the grab before and after sampling. It consists of a hopper that directs the material released from the grab into a large plastic box, which is placed underneath.

3) Large plastic boxes

Suitable watertight boxes, small enough to be placed under the grab stand hopper but with sufficient capacity to contain the collected sediment and washings before sieving, should be used. There should be enough of these boxes to allow processing at a later stage whilst replicate samples are being taken.

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4) Sieve table

This device is shown in Figure 3 and consists of an open-ended box whose interior sides slope towards an outlet pipe at one end of the bottom of the apparatus. The table is made of galvanised stainless steel that facilitates easy washing and which also prolongs the life of the device. Small blocks mounted on the interior of the box allow a 5mm sieve, held within a metal frame, to be placed inside. The positioning of the blocks allows a 15cm overlap between the top of the sieve frame and the top of the box. The entire device is raised off the floor by legs to allow the table to be utilised at a suitable height (normally waist height).

5) 30cm diameter 'Endecotts' Laboratory Test Sieves certified to BS410 (0.5mm, 1.0mm and 2.0mm stainless steel meshes). Choice of sieve will depend on the objectives of the investigation. Sieves should be discarded at the first sign of damage to the mesh.

6) Sieve frame

The sieve frame consists of a 5mm wire mesh, supported within a stainless steel frame.

7) Plastic funnel and Stand

A large plastic funnel with a wide bore, the spout of which will fit into the necks of the sample containers, should be used. The stand holds both the funnel and sample container, minimising the risk of loss of material. This device is shown in Figure 4.

8) Sample containers

Sample containers should be spill proof, air tight and strong enough to withstand filling with sediment and liquid. The size of the container will be determined by the size of the sample. Choose from 125ml, 250ml, 500ml, 1000ml bottles and 2.5L, 5L, and 10L buckets.

Samples for particle size analysis require resealable plastic bags with a white panel for labelling. Sample bags are then placed in a 0.75l or where replicates samples are taken, a 1.2l plastic box (see sedimentology SOP for detailed information).

9) 3cm diameter corer

This consists of a 3cm diameter graduated perspex corer capable of being deployed to a depth of up to 10cm. This is used for collection of meiofauna and particle size analysis sub-samples.

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10) Depth measurer

This device consists of a metal rod with a moveable ringed collar. The rod, which has a diameter of around 6mm, is placed into the sediment and the collar slides down to rest on the surface of the sediment (see Figure 5). The rod is graduated in such a way that the depth of the sample can be read from the top of the collar. In the absence of this device, a clear 30cm length plastic ruler can be used as a replacement.

11) Waterproof pen and labels

Labels for internal and external marking of samples are pre-printed from the Cruise Planner database (see separate SOP) with cruise code, station code and sample area. Following successful collection of the sample, station number and sample type are added to the label with a permanent marker.

12) Logsheets

Digilog grab logsheets should be used to record sampling information (see Digilog SOP for detailed information). They will prompt the user for the following information:
Cruise code
Area
Time and date of sampling
Water depth
Station code
Station number (sequential from start of cruise)
Replicate
Sediment type
Sample type
Sieve mesh size
Container size
Storage

13) 500ml standard laboratory ‘Wash bottle’

14) Sea water hose / deck wash (ideally adjustable to provide appropriate pressure)

15) DigiLog has been designed to perform the role of a digital logbook. This is an Access database used for keeping sample metadata (For specific details see DigiLog SOP). Data are entered into the database from field data recording sheets as specified in 12) above. Specific recording sheets are available for the different types of gear used during surveys (i.e. grab, trawls, video, sidescan, multibeam, etc.). Additionally, the positional information and environmental data (e.g. sea conditions, winds, etc.) are also recorded on the bridge on separate hand-written recording sheets.

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16) Tower is a navigational software package (live electronic chart) used for plotting intended sampling positions (i.e. grabs, trawls, video, etc.). This software is often used by the bridge officer/vessel skipper to guide the vessel to those positions and also allows the logging of the exact sampling positions. Once the grab hits the seabed the TOWER operator records the actual position of the sample electronically using a keystroke. This operator also enters the sample metadata onto a DigiLog field records sheet.

7 Ingredients/Reagents/Media

7.1 Preservative – 10% formaldehyde solution

Composition: -Formaldehyde* 30%, pH 7.0 (buffered with sodium acetate trihydrate 25g/litre)
- Seawater

Buffered 30% formaldehyde solution is obtained in 10 litre drums from the Lowestoft Laboratory. A working solution of 10% formaldehyde solution is prepared by diluting approximately 3-fold with clean seawater.

7.1.2 Preparing dilutions of Formaldehyde

Prepare dilutions of formaldehyde in a well-ventilated area outside, 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 (BOC-EQ-Coshh-SAS-Sea-01 Storage of 30% formaldehyde solution, dilution of 30% formaldehyde to 10% and use of 10% formaldehyde for preservation of benthos samples).
Label the aspirator with the following information: 10% Formaldehyde solution, Toxic, Carcinogen. Add Harmful and Flammable tape labels.

*Formalin 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.

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7.2 Sample stain - Rose Bengal**

Use of Rose Bengal facilitates sorting of the sample by staining proteinaceous matter bright pink. Rose Bengal, if required, is added to the buffered formaldehyde solution (approx. 10%) at a concentration of approximately 0.01%.

**Rose Bengal is an extremely hazardous carcinogen. The neat chemical should only be handled under fume extraction. Disposable gloves should be worn whenever handling this substance. A concentrated solution of the substance should be prepared for preparation of working solutions in the field where no fume extraction is available.

7.2.1 Preparation of concentrated Rose Bengal solution

Wearing disposal gloves, in the laboratory fume-cupboard make up a Rose Bengal paste using a small amount of tap water. This solution should be stored in a clearly marked watertight container.

7.2.2 Use of Rose Bengal in the field

Add a very small quantity of the paste to the aspirator containing the 10% buffered formaldehyde until the required concentration is obtained (seek advice from the Scientist in Charge regarding the required quantity). Safety glasses, disposable gloves and waterproof clothing should be worn whilst carrying out this procedure.

8 Procedure

8.1 Sampling vessels

CEFAS Research Vessel Endeavour conforms 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, vessels which conform to this code 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 ³1 tonnes capacity, fitted with sufficient wire to extend 1.5 times beyond the maximum sampling depth. The winch operator should have a clear view of the grab during deployment and recovery. The deployment and recovery process should be described in detail to the winch operator and a system of signals should be agreed. The Scientist In Charge (SIC) will have overall responsibility for the safe deployment of the grab and will halt sampling if he deems it unsafe (i.e. under worsening weather conditions). When the SIC is not on watch, he/she will nominate a suitably experienced scientist as a deputy. 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.

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8.2 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).

8.3 Safety

Hazards are presented by the improper use of reagents used in the processing of benthic samples. 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. (see section 4)

8.4 Pre-survey checks

At the laboratory check sampling equipment, including disposables, against the equipment list and inspect for damage (e.g. worn sieve mesh). Replace or repair damaged equipment as necessary. Once on board the survey vessel ensure all equipment is present and safely stowed.

8.5 Preparation of equipment

8.5.1 Position the grab and stand beneath the derrick or gantry and attach the wire of the Day grab to the boat's winch using a shackle and swivel. Check that the weights are securely fastened by means of split pins or wing nuts (depending on grab) and that the jaws of the grab are sealing properly.

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8.5.2 Set the Day grab by raising the retaining bar, opening the jaws to their maximum extent and then lowering the retaining bar so that it catches on the teeth of the trigger mechanism, holding the jaws open.

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

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

8.6 Deployment and Recovery

8.6.1 When the boat is stationary and the skipper has given permission, the grab is deployed, typically at a rate of approximately 1 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 Day grab has reached the seabed, slackening of the winch wire provides a signal to stop the winch (at this point the sample position is recorded by the TOWER operator). Pause for five seconds to allow the jaws of the grab to bite into the sediment under the weight of the grab then raise, slowly at first, to allow complete closure of the jaws and maximize sampling efficiency. When the grab reaches the surface it should be stabilized 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 jaws of the grab to be opened.

In rough seas the bows of the vessel should be facing into the sea thus minimising the roll of the vessel and hence swing of the grab during deployment and recovery. If the grab is deployed from the stern of the boat ensure that it is deployed away from the wash of the propeller to avoid raising of the flaps and loss of sediment due to turbulent flow.

8.7 Collection of Samples

8.7.1 Open the flaps of the grab to allow inspection of the sample. Should the jaws of the grab have failed to close properly with the resulting loss of material the contents should be discarded and the grab re-deployed. If an undisturbed sample is present then measure the depth in the centre of the sample using the purpose-built depth measurer (see Figure 3). If the sediment depth is less than 5cm then the contents should be discarded and the grab re-deployed. At least three attempts should be

made at each sampling station before it is abandoned. At the discretion of the Scientist in Charge (SIC) 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, record the depth of sediment and the nature of the material on Digilog grab logsheets (e.g. 'sand','muddy sand'). When describing sediment the least abundant component should be noted first followed by the next most abundant and so on. An indication of the amount of that first component (e.g. slightly muddy sand) should also be recorded. The depth can be converted into volume using the graph shown in Figure 6. Collect the following samples as required:

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8.7.2 Particle Size Analysis (see specific SOP for greater detail)

Allow surface water to drain. Remove a sub-sample of sediment from the most undisturbed part of the sample using the 3cm diameter corer, deployed to a depth of at least 5cm. Transfer the sub-sample to a sealable plastic bag, which should be placed inside a pre-labelled plastic box (see section 8.10). Samples may either be frozen, or stored in a dark environment to prevent algal growth.

8.7.3 Meiofauna Sample (if required)

Remove a sub-sample of sediment from the most undisturbed part of the sample using the 3cm diameter corer, deployed to a depth of 5cm. Any excess sediment should be extruded from the corer by gently depressing the plunger, and then returned to the bulk sample. Transfer the sub-sample to a 250ml plastic pot. Add preservative and labels according to the instructions in sections 8.9 and 8.10. Wash the syringe thoroughly before taking the next sample.

If collecting samples for purposes other than for macrofaunal or meiofaunal analysis you should refer to the relevant procedures (see section 8.14).

8.7.4 Macrofauna sample

Release the remaining contents of the grab into the plastic bin, placed beneath the chute of the grab table. Any material remaining in the grab or on the sides of the hopper should be carefully washed into the plastic box.

8.8 Sample sieving

8.8.1 The contents of the sample container should be transferred to the sieving table (see Figure 5) where it should be washed with seawater (under gentle hose pressure) over a 5mm mesh sieve. Allow the supernatant water, containing sediment and benthic organisms, to overflow from the box and pass through the 5mm sieve. Sediment

broken up directly on the sieve could damage fragile invertebrates. Where hose pressure cannot be controlled remotely, a hand should be used to produce a less forceful spray as necessary. More consolidated sediment may require prolonged sieving; gentle breakage into smaller lumps by hand may help; smaller quantities may simply be washed through and preserved. This shouldn't cause undue damage to the benthic organisms, as the more delicate individuals will have been washed off with the softer sediment.

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8.8.2 The finer sediment fraction is sieved over the 30cm diameter sieves, the size of mesh depending on the objectives of the investigation. This sieve is held under the outlet shown in Figure 5 at the same time as the above process is taking place. Temporary blockage of meshes can occur when sieving certain types of sediment, especially cohesive muds. Care should be taken in these cases to ensure that no loss of animals occurs as a result of overflow. If excess sediment is retained on the sieve, it 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 bin filled with seawater (using a gentle up and down motion, not side to side, which can cause animals to be damaged). If spillage of sample material occurs at any stage during the processing a repeat sample should be taken.

8.9 Sample preservation

8.9.1 On completion of the sieving process, retained animals and residual sediment on the mesh screens are transferred to plastic bottles or buckets via a large funnel in a frame support (Figure 6). 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.

8.9.2 Transfer any animals retained on the 5mm sieve-table mesh by hand, or forceps for the more delicate individuals, to the appropriate sample container. Remove and dispose of any cobbles or pebbles without encrusting fauna. Separate containers can be used for the fauna retained by the different sieves (e.g. 1-5mm and >5mm fractions). The advantage of this approach is that smaller fauna are less likely to be damaged by some of the more abrasive material which can be retained on a 5mm sieve.

8.9.3 The 10% formaldehyde preservative solution with/without added Rose Bengal (see section 7) should be added to samples with the aim of achieving a final concentration of at least 5% of formaldehyde in the sample. To achieve this add approximately the same volume of the 10% formaldehyde solution as the volume of the sample (including any liquid).

8.9.4 Both the grab and sieving hopper should be given a final wash down between stations with the appropriate mesh size of sieve in place, to ensure collection of the entire sample contents and to avoid cross contamination. It is good practice to scrub sieves between samples as clogging of the sieve effectively reduces the size of the mesh. This will have implications for the size of the fauna retained, as well as the time taken for sieving. This problem is countered to a certain extent in the laboratory if the sample is again washed over a sieve of the same mesh size. However, fixed specimens may be more brittle than their live counterparts and so sieve meshes in the field should be maintained in an 'unclogged' state at all times.

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8.10 Sample labelling

Each sample must be suitably labelled. The use of the Cruise Planner database (see separate SOP) enables pre-printed labels to be used. Labels should be applied both to the outside and inside of any samples so that any damage to the external label does not prevent the identification of the sample. Polythene bags for particle size samples should be labelled directly onto the panel of the bag. The plastic boxes used for PSA samples should also be directly labelled. All labels contain the following pre-printed information:

- Research cruise number or code (e.g. prefix - vessel name: End – Endeavour, Cor – Corystes followed by cruise number/year)
- Station code (allocated at the survey design stage)
- Type of sample (macrofauna/meiofauna/PSA)
- Survey area

On collection and recording of the sample, the station number will need to be written onto the pre-printed label with a permanent marker (stations are numbered sequentially from the start of a cruise).

8.11 Transport of samples from ship to laboratory

Formaldehyde solution should always be carried in containers that are approved for this purpose. The container should be of a material that is impervious to formaldehyde solution or vapour. All containers must be checked prior to transport. If there is any apparent leakage of liquid or vapour from the container, or there appears to be potential for leakage then the container is not suitable for the carriage of formaldehyde solution. Ideally concentrated formaldehyde should be carried as 10l aliquots with no more

than 25l being carried in a single container. At sea, 30% formaldehyde solution is stored on deck in a chemical storage container. Benthic samples containing 10% formaldehyde solution are stored on deck in large labelled crates.

Formaldehyde solution must not be transported to or from a vessel in a situation where fumes generated from a spillage can come into contact with the driver or passengers. All quantities of 30% and 10% formaldehyde solution must be carried in an approved chemical container and securely stored in a vehicle that separates the occupants of the vehicle from the formaldehyde (e.g. box van, flat-bed lorry or van). Containers of formaldehyde solution should be clearly labelled with details describing the nature of the contents. An approved formaldehyde spill kit and chemical notification sheet must always be carried when transporting all concentrations of formaldehyde solution in case of accidental spillage. Spillages of formaldehyde in an enclosed vehicle should, where possible, be irrigated with water. If this is not possible the formaldehyde must be allowed to evaporate and all fumes should be dispersed before the vehicle is used again.

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Refer to the following COSHH forms for full advice on the appropriate procedures:

- **BOC-EQ-Coshh-SAS-Sea-01** Storage of 30% formaldehyde solution, dilution of 30% formaldehyde to 10% and use of 10% formaldehyde for preservation of benthos samples
- **BOC-EQ-SAS-Formaldehyde** Movement to and storage of samples preserved in 10% formaldehyde solution at Burnham offsite storage facility

8.12 Sample storage and archiving of information

Details of the samples taken are recorded in the cruise DIGILOG database. This acts as the sample record for retrieving information on samples, surveys, etc. On completion of the cruise the resultant DIGILOG file is quality assured and a master copy is saved in the cefas Benthos drive for further consultation. On return to the laboratory, samples and DIGILOG database should be dealt with in accordance with the storage and sample tracking procedure (CEFAS – Field Evaluation Team 004).

8.13 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 plastic boxes) and replace/repair as necessary.

Comply with the criteria for sample rejection.

8.14 Analytical Procedures

For analysis of macrobenthic samples see Procedure

For analysis of particle size see Procedure

For analysis of sediment trace metals see Procedure

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For analysis of sediment redox potential samples see Procedure

For analysis of organic carbon and nitrogen in the fine (<63/90µm) fraction see Procedure

For analysis of sediment trace organics samples see Procedure

9 Review

This procedure will be reviewed as a minimum on the time scales given in the review / amendment programme. A record of the review will be made on a separate Review / Amendment Sheet which will be added to the Master Copy file of this SOP. Any amendments arising from such review or from operating requirements will result in the issue of the entire amended procedure as a new Issue.

10 Records

This procedure, its review sheets and its subsequent revisions constitute records in themselves and each master copy will be retained in a file as arranged by the Quality Manager. Records will be retained for a minimum of five years unless otherwise specified.

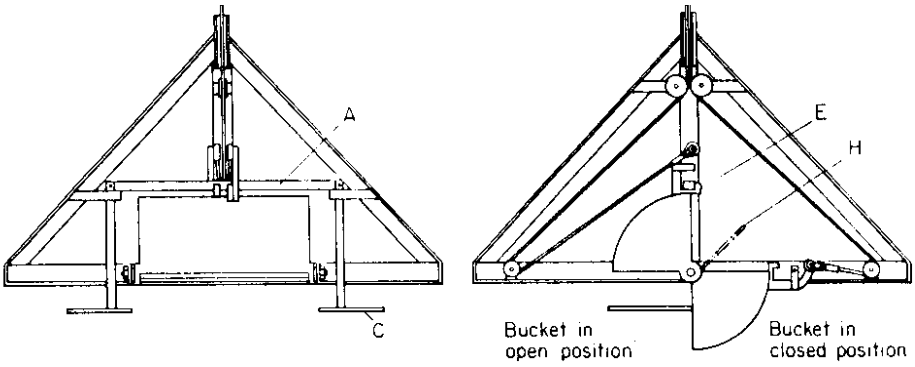

Figure 1. Modified Day grab design

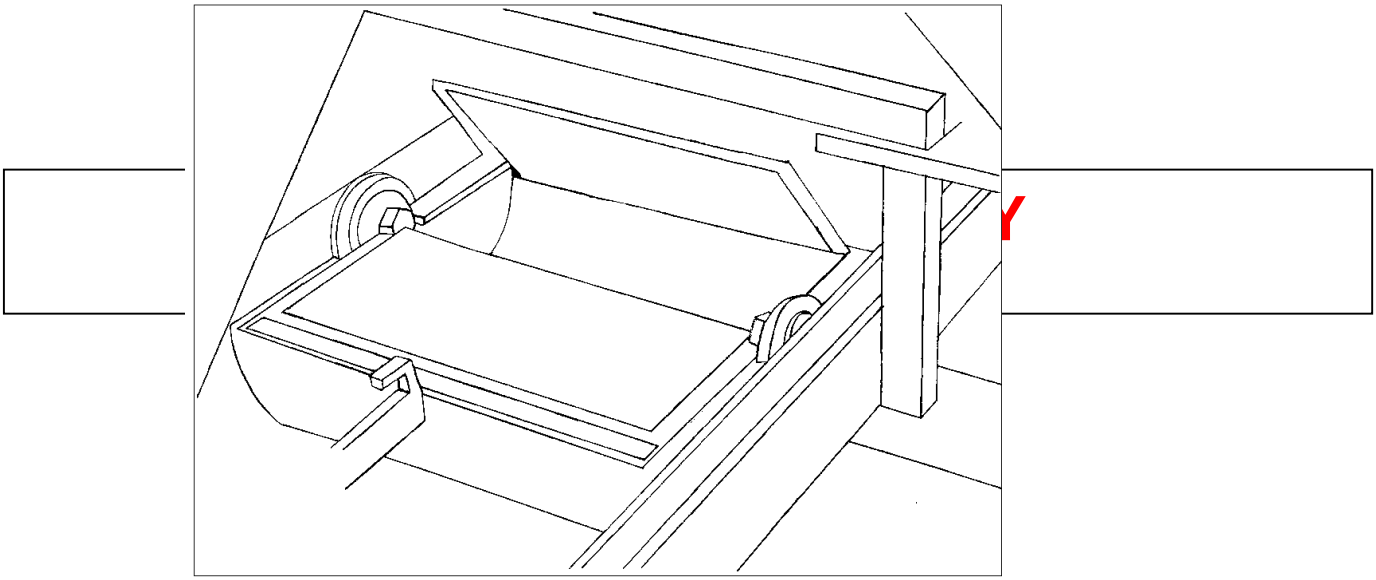

Figure 2. Close-up view of Day grab modifications

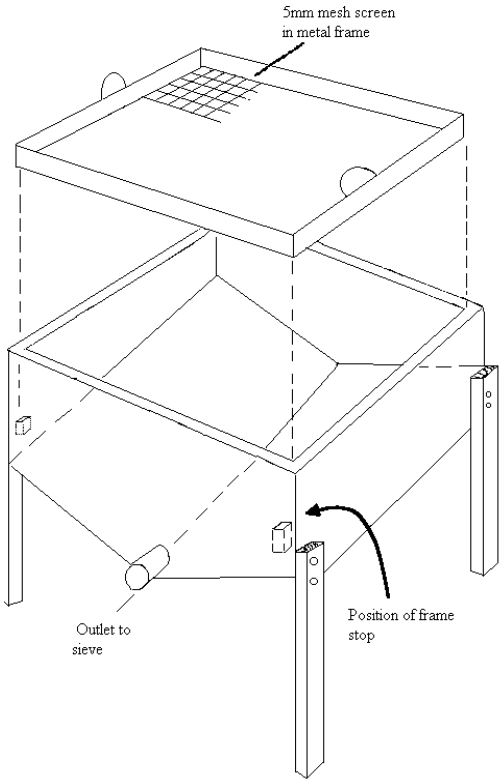

Figure 3. Sieving table used for the processing of Day grab samples

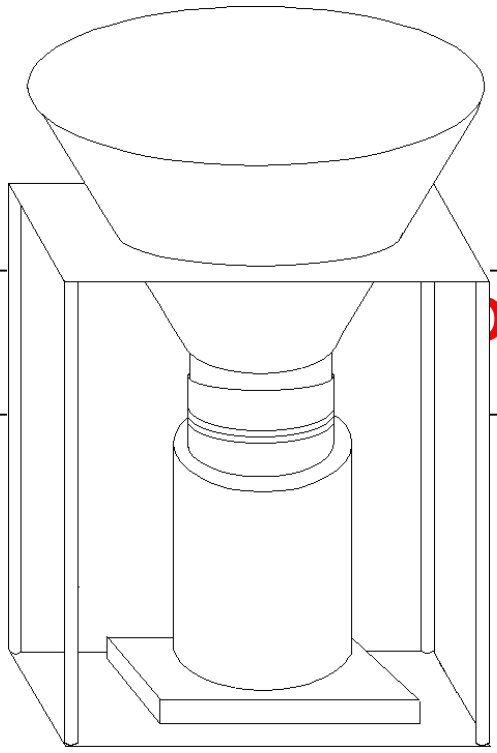

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Figure 4. Funnel in frame support

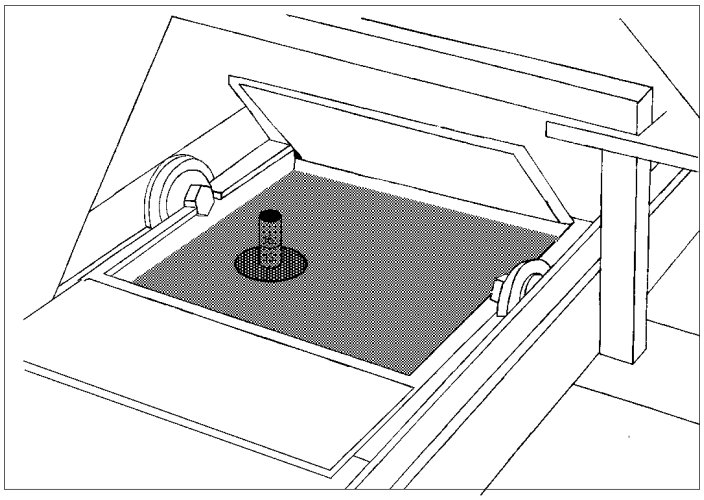

Figure 5. Day Grab Sample with Measuring Probe in position, to estimate depth

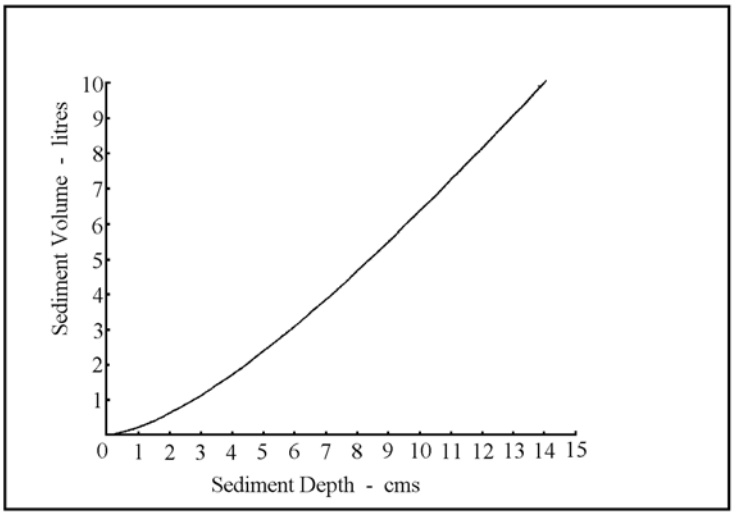

Figure 6. The graphical relationship used to convert sediment depth to volume.