JNCC Report
No. 643

Recommended operating guidelines for USNEL box corer

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November 2019

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ISSN 0963 - 8091

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This report should be cited as:

Wilson, I., O'Connor, J. & Gibb, I. (2019). Recommended operating guidelines for USNEL box corer. *JNCC Report No.643,* JNCC, Peterborough, ISSN 0963-8091.

EQA:

This report is compliant with the JNCC Evidence Quality Assurance Policy http://jncc.Defra.gov.uk/default.aspx?page=6675.

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1 Introduction and scope

A box corer is a benthic sampling device designed to take a vertically undisturbed ‘core’ sample from the top of the seafloor, typically to a maximum depth of 50cm (Hopkins 2007). The Joint Nature Conservation Committee (JNCC), Benthic Solutions Ltd. (BSL) and Marine Scotland Science (MSS) have developed the following guidance to inform box coring operations undertaken with a United States Naval Electronic Laboratory (USNEL) Mk2 style 0.25m^2 surface area box corer (Figure 1).

Figure 1. 0.25m^2 sampling surface area USNEL Box Corer (Image © Ellen Last (JNCC), 2016, box corer owned by Benthic Solutions Ltd.)

2 Pre-deployment checks

Prior to the start of a new session of work (e.g. at the beginning of each research cruise), a toolbox talk should be held with all personnel involved in the deployment. This will cover all aspects of the deployment including: what is to be achieved, an overview of all safety aspects involved in the deployment and the roles of all personnel within the process.

The condition of the equipment should be checked and confirmed on deck. This will include mechanical condition as well as cleanliness.

Areas to pay particular attention to include:

- the box should be seated correctly in the lip of the main body prior to fitting the front panel;
- the gimbals should be inspected visually and manually;
- the overall status of bolts, ensuring tightness;
- the condition of wire rope strops;
- the condition of the grease-free winch wire/dyneema and splice;
- ensure the trigger release pin on the stem of the corer is not bent and sits in the sprung trigger hole correctly;
- the correct setup and function of all mechanisms.

An Ultra Short Base Line (USBL) beacon should be attached vertically and in a position which does not impede proper function of the box corer or would result in the loss of the beacon. It should be turned on immediately before deployment.

Where possible, ensure that the site has been surveyed with a camera before the box corer is deployed to reduce the potential for damaging any protected or sensitive habitats or the box corer itself. Where potentially damaging sub-surface substrates are suspected (e.g. buried rocks or boulders), consideration should be given to surveying the site with a Sub-Bottom Profiler or similar equipment before the box corer deployed.

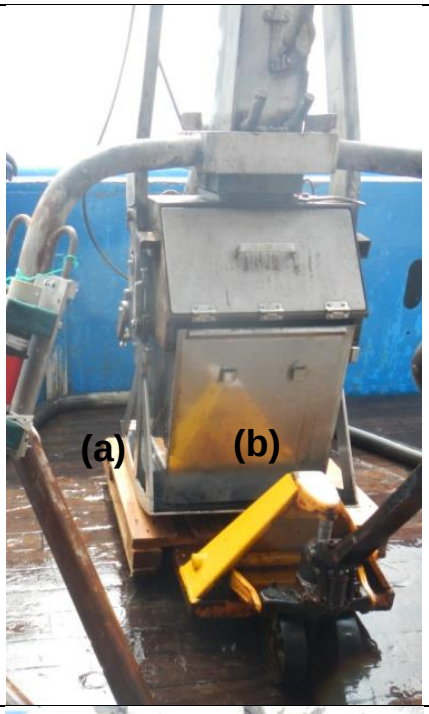
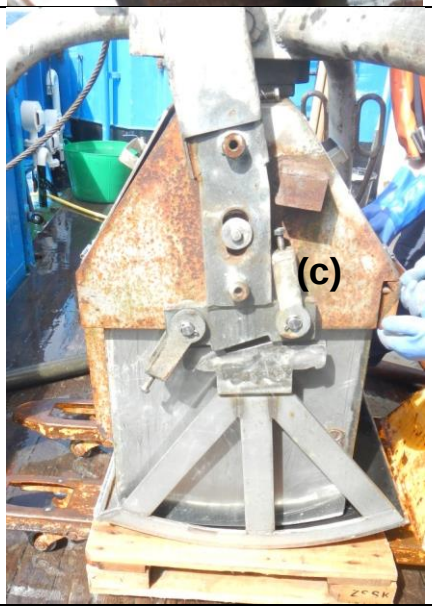
Appropriate personal protective equipment (PPE) should be worn at all times (i.e. hard hat, safety boots, coveralls, gloves and life jacket), with additional PPE including a harness and fall arrestor to be worn by personnel working inside areas not protected by a guard rail, or as required by individual vessel safety requirements. If tag lines are to be used, their condition should be checked.

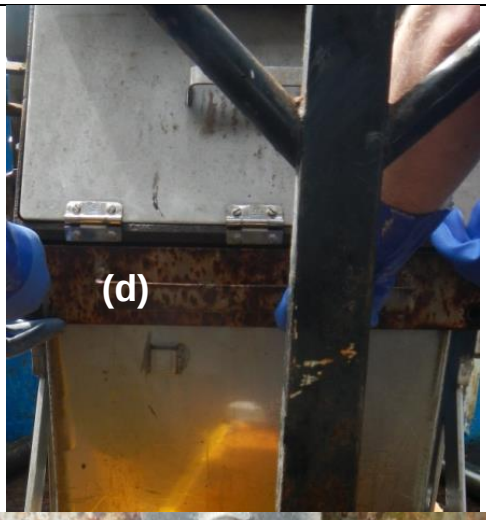
3 Box corer assembly

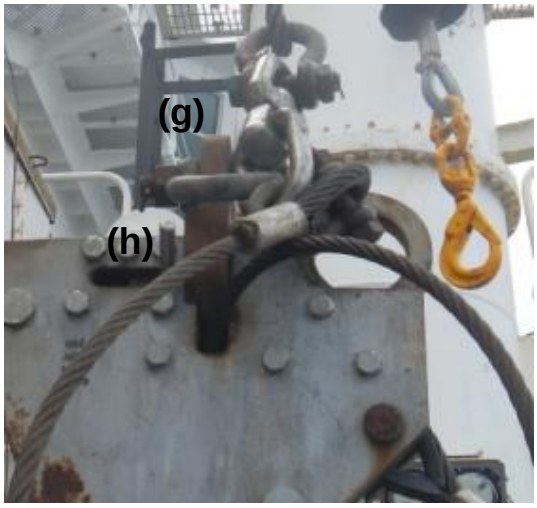
Please note the safety pins should remain in place throughout box corer assembly and removed only during the deployment operation.

Assembly should be carried out as is described in Table 1.

Table 1. Box corer assembly protocol.

1	The pallet truck should be used to position the spade **(a)** and box **(b)** (which should be secured with chocks/wedges once in position) under the main box corer structure.	
2	The pallet truck should be jacked up to allow the box to fit into the front and back brackets of the box corer, and the spade retention arms **(c)** should be fitted.	

3	The front plate **(d)** and the spade retention bolts **(e)** should then be attached and bolted. Ensure that the shaped triangular edges of the box are located inside the 'V' guides inside the main corer housing.	 
4	The pallet truck can be dropped and the box corer arm **(f)** should then be pulled down to the horizontal position.	

5	Using a harness and fall arrestor where required, the lifting plate **(g)** should be inserted into the top of the box corer, ensuring sufficient cable length is available on the trigger release pin side and that the lifting plate is in the correct orientation. Release the sprung trigger release pin on the stem of the unit before pushing the trigger arm **(h)** along the slot allowing the retention pin and assembly to slide up into the bolt. This may require some assistance from below by someone pulling on a rope looped over the lever arm.	
6	The inspection door **(i)** should be put in the open position by pulling on the small stainless cable and twisting the shutter arm across until the cable falls into the slot.	

4 Deployment and recovery

The lead scientist will direct the vessel to a predefined location, as informed by the Survey Plan. When the bridge officer and lead scientist are happy with the position of the vessel, taking into account prevailing weather (i.e. sea state and wind) and wave and tidal current directions, permission to deploy can be given. Note that use of vessels equipped with Dynamic Positioning (DP) is highly recommended, to enhance the capacity of the vessel to hold position while deploying and recovering the box corer. The bridge officer must be informed when the box corer is deployed, when it reaches the seabed, and when it is safely back aboard the vessel.

During deployment/recovery of the box corer, the following shall be carried out or considered as appropriate:

- Ensure the corer arming mechanism has been reset. Once armed, the safety pin should be inserted to prevent it triggering prior to deployment. Once the weight is taken on the cable, while lifting the corer over the side, this pin should be removed. Care must be taken when arming, both during deployment of the corer from the landing table and when removing the pin over the vessel side.
- There should be at least two people, one either side, steadying the corer during the deployment and recovery process.
- When recovering the corer to the landing table, care must be taken to avoid the equipment striking the ship's hull, both to minimise disturbance of the sediment and prevent damage to either box corer or hull.
- Upon completion of all work at a particular site, the corer and deck area must be thoroughly washed down and cleared so that there is no danger of slipping or tripping when it comes to the next deployment. Corer cleanliness is important both to avoid jamming and also for avoidance of sample contamination.
- Potentially mobile equipment (e.g. pallet truck) must be tied securely.

4.1. Deployment of box corer

Prior to any coring operations, the lead scientist will ensure:

- The box corer is clean and free from sample contamination.
- The corer is reloaded/rearmed and ready for deployment.
- Weather conditions are suitable to safely lower the corer to the seabed.

During deployment:

- The box corer should be positioned on deck, as close to the deployment area as possible, and directly beneath the block to avoid the load from swinging under tension.
- If a camera is attached to the box corer, a clapper board should be recorded detailing:
    - Date
    - Station name
    - Attempt
- The box corer should be armed ready for deployment with safety pins still in place.
- Ensure that the inspection doors are in the open position and the trigger plate is in the correct orientation.
- If used, tag lines should have one end secured to a pad eye on deck while the other is passed through the box corer frame (avoiding potential snags) allowing for line recovery immediately after deployment. Tag lines should not be tied directly onto the box corer.

- All personnel should position themselves suitably on deck. Ideally this will be:
    - To the non-seaward side of sampling device;
    - Not blocking winch operator's line of sight;
    - Not beneath cables under tension;
    - Not in potential pinch point;
    - Not on coiled cable on deck.
- Permission must be granted from the Bridge before deployment of equipment.
- Slack should be taken up by the winch operator: enough to lift the central gimbal mechanism but without lifting the box corer off the deck. This will allow the safety pins to be removed (Figure 2).
- Once the safety pins are clear and tag lines are taut, the box corer should be slowly raised and held. Simultaneously, the A-frame or crane should be deployed outwards and the winch controlled to lift the device over the edge of the vessel. The box corer should be kept as close to the deck as possible during the deployment.
- The tag lines on each side must be kept taut to control the movement of the box corer.
- The system is now armed and at its most dangerous, so personnel must stand clear. If a problem occurs the reverse of the above process will be undertaken and the box corer will be placed back on deck. Only when the mechanism has been made safe (and confirmed by the lead scientist), can the problem be resolved.

DO NOT PLACE FINGERS BENEATH THE BOX OR NEAR THE LIFTING STROPS.

- When over the side and clear of the vessel (Figure 3), the box corer should be lowered to the water at a reasonable speed to avoid the chance of pendulum action. The box corer should be slowed before entering the water to avoid premature triggering.
- On reaching the sea surface any tag lines should be retrieved.
- The box corer should be lowered to approximately 10m below the sea surface and it should be confirmed that the USBL beacon is being tracked. Note that some USBL systems will only see the USBL from a depth of 30m or more due to the transducer angle.
- The box corer should then be gently lowered to the seabed at a nominal speed of ~50m/min. The unit should be slowed to around 20m/min immediately prior to touchdown on the seabed.
- Successful seafloor penetration can be visually confirmed by movement of the block and slack appearing in the deployment wire. To assist this process, the cable can be marked off at suitable depth increments (including 10m from the seabed) during the initial deployment or a test deployment.
- The lead scientist shall then confirm a fix with the Surveyor and, following a 1-minute settling period (note a longer settling period may be required on hard substrate), recovery can commence while all relevant station metadata (position, time, depth etc.) is logged on the log-sheet (see Appendix 1).

Figure 2. Removing the safety pins.

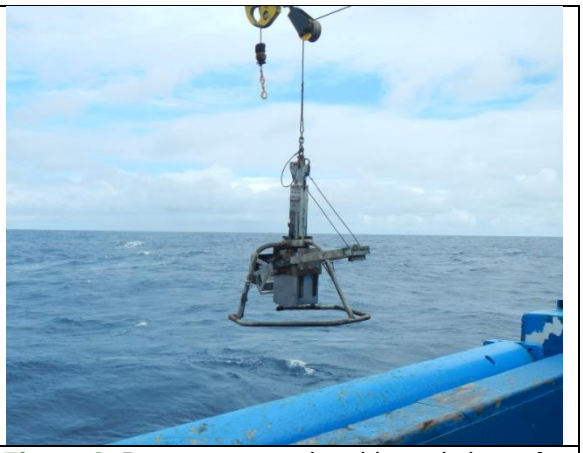


Figure 3. Box corer over the side and clear of the vessel.

4.2. Recovery procedure

- The winch operator should slowly recover the lifting wire and box corer; ensuring smooth and steady bucket closure. Once the box corer is clear of the seabed it can be recovered at full winch speed to the surface.
- The box corer can be recovered through the water column at the maximum winch speed, however the rate of recovery should be reduced to ~0.5m/s (30m/min) prior to the equipment breaking the surface to ensure the integrity of the sample.
- Once at the surface, tag lines can be attached to the box corer using grappling hooks (if required). Attaching tag lines before recovering the box corer should help control any pendulum action. Should vessel movement cause increasing pendulum action of the corer, returning the device to the water and holding just below the surface will stop this motion and allow the winch operator to gauge when it is safe to return the corer to the deck.
- The box corer should be simultaneously hauled in and gently brought inboard; if required, control can be maintained using the tag lines.
- Once the box corer is completely above the deck it should be lowered onto a pallet truck positioned below the spade and box and secured with chocks. Note that the corer should be landed in an appropriate orientation (i.e. with the side from which the box can be removed facing a sufficiently large area of open deck).
- The pallet truck should then be jacked up to allow the safety pins to be inserted.
- The box corer should be secured before processing begins.

5 Sample acceptance and pre-processing

- The inspection doors should be opened as soon as is practical following recovery of the box corer, and the contents inspected for sample quality and acceptability by the lead scientist.
- The spade retention bolts and front plate should be removed, freeing the sample from the main box corer structure.
- The sample is considered to be acceptable if the following conditions are met:
    - Sample was taken inside the acceptable target range;
    - Box corer was retrieved upright and had not been fouled in any way (e.g. no disruption of the sample has occurred through striking the side of the vessel);
    - Inspection/access doors had closed properly enclosing the sample;
    - Box corer spade and box are sealed together (i.e. no sediment washout has occurred);
    - Water above sample is undisturbed;
    - No contamination is visible in the sample by other sampling equipment;
    - Sample depth is greater than 15-20cm (i.e. greater than 30% of the total box capacity);
        - Note sample depth can be measured using a stainless-steel divider with depth markings (e.g. with a horizontal line each 5cm) inserted into the sample box. Alternatively, a ruler or measuring tape can be used.
    - No hagfish (*Myxine glutinosa*) and/or mucus coagulants are present, as processing samples contaminated by hagfish or other mucus coagulants will clog sample sieves.
- Once deemed acceptable by the senior scientist, the overlying supernatant water should be siphoned off the sample (i.e. by submerging one end of a hose in the supernatant water and gently sucking in on the other end until the water is about halfway through the tube, when the tube can be put into the sieve, which should be secured at a lower height than the top of the sample) and passed through a sieve (N.B. sieve size to match smallest fraction to be retained e.g. 0.25mm).
- Depth of sample should be recorded on the log sheet.
- A **LANDSCAPE** digital photograph (Figure 4) of the entire sample should be taken in situ with the clapper board containing the following information:
    - Station name (i.e. 1016S_GSH_A01_S1)
    - Date
- At this point, any delicate surface fauna (e.g. xenophyophores, brittlestars and any tubes) should be removed and placed in a separate sample vessel prior to the sediment being processed.

Figure 4: Landscape digital photograph of entire box core sample with clapper board.

6 Sample processing

One person will be nominated as Sample Manager, to take charge of the sample processing for all samples collected at a particular station. It is the sole responsibility of this person to ensure that the samples are processed according to this guidance and that all relevant details are entered in the log sheet (Appendix A) and on the labels (Appendix B) for each sample from that station. The Sample Manager will sign off the sample processing undertaken by entering their initials in the QA section of the log sheet labelled 'Completed by:' The Sample Manager will be accountable for rectifying any errors that subsequently come to light.

The principal aim of sample on-board processing is to reduce the volume of the substrate in the sample whilst retaining the biological material in good condition through careful sieving and appropriate fixation. Back in the shore laboratory, the samples will be sorted by hand to pick out all the biological material. The time and cost of this final processing is significantly reduced by removing the finer particulate matter (silt, mud and sand) during the on-board sample processing.

6.1. Particle Size Analysis (PSA) sub-sampling

- Labels should be prepared for PSA sub-sample stating:
  - Station name (e.g. 1016S_GSH_A01_S1_PSA)
  - Date
- A clear acrylic sub-sampler should be used to extract a vertical core from the centre of the box core sample to the same depth as the faunal sample, to provide a PSA sub-sample which can be related to the faunal samples (typically to 15cm).
  - Prior to sub-sampling, the sampler should be thoroughly rinsed and cleaned.
- A **LANDSCAPE** photograph (Figure 5) of the vertical core and clapper board should be taken.

- PSA sub-samples are to be placed in a labelled container and a completed waterproof label placed in and on the outside of the container.
- A **LANDSCAPE** photograph (Figure 6) of the vertical core and label in the container should be taken.
- Samples should be immediately transferred to an on-board freezer for storage at -20°C.

Figure 5. Landscape photograph of PSA core and clapper board.

Figure 6. Landscape photograph of PSA core and label in container.

6.2. Faunal sample processing

- Depth of sample to be processed should marked using a pre-cleaned stainless-steel divider (typically inserted sufficiently deep to mark a depth of 15cm) (Figure 7 and 8).
    - Sieving of the upper 15cm should be sufficient to capture macrofauna quantitatively. However, the lower part of the sediment column should be spot-checked for large animals, such as nemertines, bivalves, echinoderms and echiurids that may live very deep in the sediment.

Figure 7. Stainless steel divider and depth marker.

Figure 8. Stainless steel divider inserted into box.

- If dividing the sample into replicates, **LANDSCAPE** digital photographs of the replicate samples with the clapper board should be taken (check the photographs to ensure they are in focus, evenly exposed and any text is clearly visible).
- A preliminary sediment description should be recorded on the log sheet including the following:
    - Sample and site ID
    - Date and time of sample
    - Approximate sediment classification (sand, silt and gravel content)
    - Sediment colour
    - Evidence and description of layering
    - Approximate sediment volume (before sieving)
    - Odour (presence of anoxic matter)
    - Reference to deck or other photographs taken
    - Surface description (burrows, tubes, casts, bioturbation, uneven etc.)
    - Conspicuous fauna
    - Vertical structure (obvious horizons, depth of loose surface layer etc.)
    - Presence of reef and venting gas indicators (*Sabellaria spinulosa* tubes, *Lophelia pertusa*, mussels, *Beggiatoa*, MDAC, serpulids)
    - Presence of anthropogenics such as oil contamination or cuttings.
    - Other factors/comments relating to quality of sample including reasons for failed samples, weather conditions, *etc*.
- All failed samples should be photographed and recorded, with reasons for failure recorded where known.
- Care should be taken throughout the entire sample processing to avoid loss of sample material and cross contamination between samples.
- Once a sample has been deemed acceptable, photographed and described following the above criteria, each faunal subsample should be gently emptied to a depth of 15cm into sample trays.
- A small, waterproof, internal label should be completed and placed in each deck tray to remain with the sample throughout the rest of the processing procedure (this is particularly important when processing two replicate samples simultaneously).
- Megafauna, large macrofauna, nodules and stones are to be picked using tweezers.
- The sample should be transferred from the sample tray to the Wilson AutoSiever[1] (WAS) and/or stacked sieves for sieving– if each sub-sample is large, this should be a gradual process to avoid overburdening the sieves.
    - Large volume macrofaunal samples collected should be sieved using a WAS and/or stacked sieves on a sieving tables in ideally <4ºC filtered seawater through stacked sieves of mesh size 0.25mm and 0.5mm (Figure 9), with both fractions retained separately in suitable containers (note the smallest mesh sized sieves used should be placed in the WAS, and used as the bottom sieves when using stacked sieves).

[1] http://benthicsolutions.com/brochures/BSL_Brochure_Wilson_AutoSiever.pdf.

Figure 9. Wilson AutoSiever (top of image) and stacked sieves of mesh sizes 0.5mm (top sieve in stack) and 0.25mm (bottom sieve in stack) on sieving tables.

- Elutriation may be used instead of, or as well as, the method outlined above.
    - Elutriation is a gentle method used for processing benthic samples, initially described by Sanders *et al*. (1965), which uses a large bucket/dustbin with two hose nozzles made near the top (Clark *et al.* 2016; Eleftheriou & McIntyre 2008).
    - A hose is connected to the slightly higher inflow nozzle allowing a continuous input of filtered seawater to fill the bucket and suspend the sample material. Water and suspended sediment and fauna flow through the outflow nozzle onto a large-diameter sieve below, which retains the fauna, but allows through all sediment particles smaller than the sieve aperture (Figure 10).
    - A secondary hose can be used in the bucket to gently break down large sediment aggregates whilst under water.
    - The sample in the dustbin should not be physically stirred, as this may damage delicate animals. The dustbin and sieve are usually used in cradles that can be tilted to optimise flow, with a height adjustment to prevent splashing of the overflow on the sieve.
    - It is very important to prevent the sieve blocking so that it fills with water and overflows. Constant close scrutiny of the process by the operator is required.
    - Stirring the sample in the dustbin will result in a richer mixture suddenly entering the sieve, which may quickly block it.
    - Material residue on the sieve should be periodically removed and stored in a suitable sample container; periodic removal of washed material from the sieve to the sample jar helps prevent blockages from developing, as does agitating the sieve within the cradle; a gentle rotating movement of the sieve by hand backwards and forwards can be useful, and with stubborn sediment, directing a flow of water upwards through the sieve from below can rapidly unblock it before it starts overflowing.

- Care needs to be taken when using this method if the sediment contains glass sponge spicules; these can block the sieve very rapidly.
- Once elutriation is complete the entire residue can be preserved in the sample container and mixed gently to ensure that all material comes into contact with the fixative.

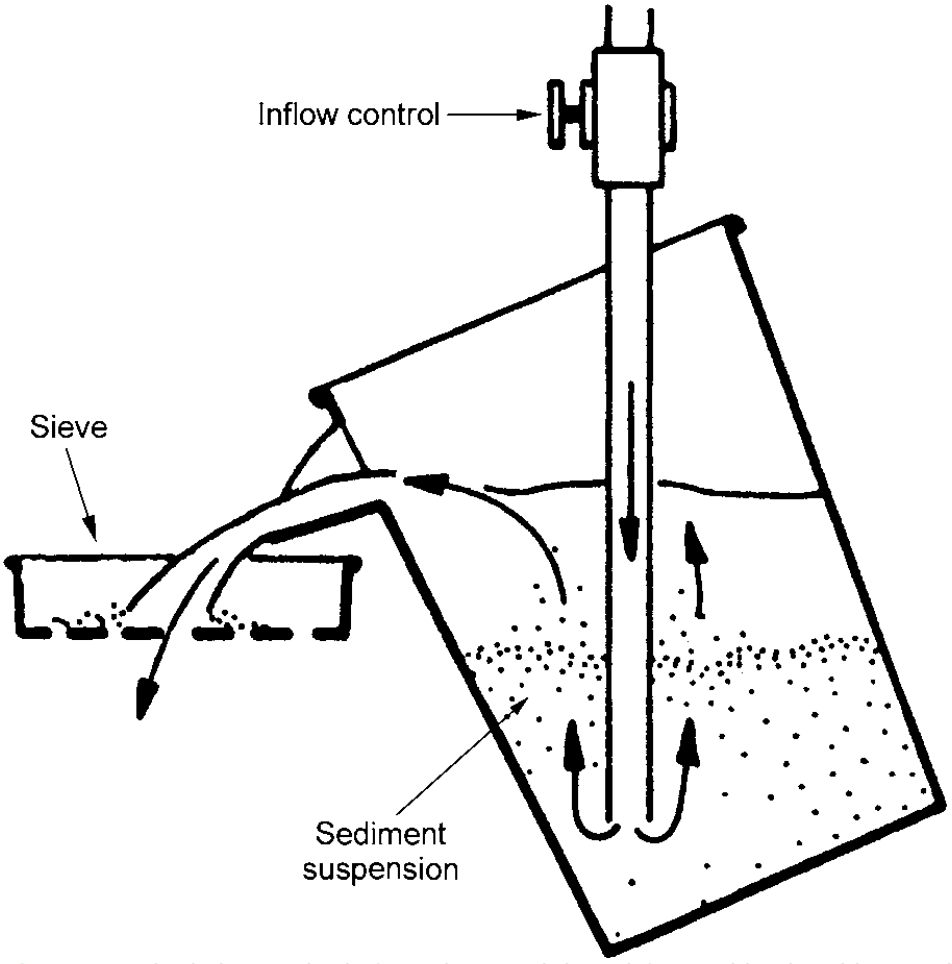


Figure 10. Elutriation method of Sanders *et al.* (1965) for washing benthic samples from soft sediments in the deep sea (from Eleftheriou & McIntyre 2008)

- Once sieved, the remaining sample material should be carefully transferred to a labelled sample container together with the internal label. Any fauna retained from siphoning of the supernatant should be gently sieved through both the 0.5mm and 0.25mm sieves.
  - Note that all sieves should be thoroughly checked for remaining fauna once emptied (e.g. by holding sieve up to light); any remaining fauna observed should be hand-picked by tweezer and added to the relevant sample container.
- Where a sample is split between multiple containers (e.g. large samples, many stones, different size classes) these should be labelled appropriately (e.g. 1 of 2 or 2 of 2). Do not overfill sample containers (samples should be filled not more than 60-70% of the container volume to ensure proper fixation of samples).
- **LANDSCAPE** digital photographs should be taken of all resultant sample containers with the sample label clearly visible (Figure 10 and 11).

Figure 10. Landscape photograph of 0.5mm infaunal fraction sample and label in container.

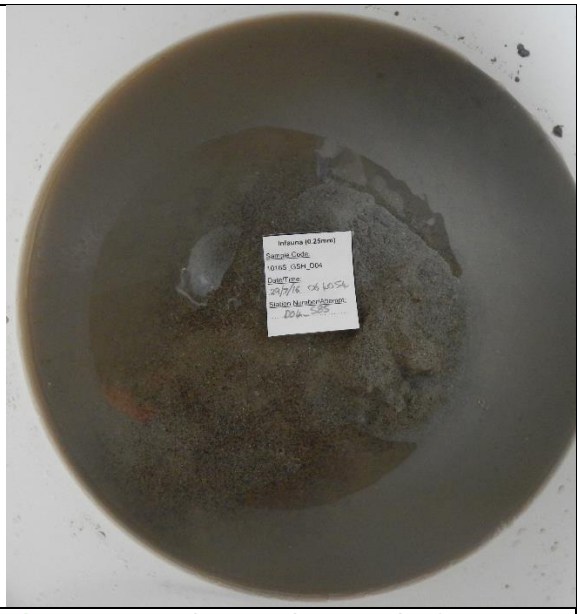


Figure 11. Landscape photograph of 0.25mm infaunal fraction sample and label in container.

- Complete the metadata log sheet entering the remaining sample information.
- All faunal samples are labelled with:
  - Date
  - Site name
  - Sample name
  - Sample equipment type (e.g. BC for box corer)
  - Depth range (e.g. 0-10cm, 0-15cm)
  - Sieve size class (i.e. 0.25mm or 0.5mm)

6.3. Faunal sample preservation

- Please note that use of chemicals at sea should be assessed and documented as recommended by the Control of Substances Hazardous to Health (COSSH) Regulations 2002, and that appropriate Personal Protective Equipment (PPE) should always be worn.
- Samples should be fixed in 4% buffered (borax) formalin saline solution.
- A fixative to seawater ratio of approximately 1 to 4 should be used to ensure the sample is sufficiently preserved.
- The lid should be secured and the sample gently rotated to ensure adequate mixing of the fixative through the whole sample.
- Faunal samples will be stored upright at ambient temperature. Samples should not be stored within the vessel's casing as any spillage may comprise the ventilation systems and closed spaces of the ship.
- All equipment should then be washed and the box corer reassembled before further deployments are carried out.

7 Causes of deployment failure

- If the box corer has triggered but completely failed to collect any sediment, this indicates that the box corer was dragged horizontally over the ground and did not land upright on its frame (there may be some sediment on the frame), pre-tripping of the spade, or hard (impenetrable) substrate. If the lower edges of the box are bent a rock may have been hit.
    - Reason: ship speed too fast (could be mitigated against by use of Dynamic Positioning vessel), substrate impenetrable.
- If the box corer has triggered but failed to collect a valid sample with some sediment remnants (i.e. spade and the box have closed) this indicates pre-tripping of the instrument; in this case the closed legs have penetrated into the bottom and the lower lids grabbed some sediment while the corer was pulled out.
    - Reason: vibrations, wave movements.
- Failure to trigger, with the box remaining open, is often caused when the hook of the release mechanism does not slide out. The release-hook should be well greased so that it can slide out easily, and the siding should be controlled when the release mechanism is prepared before lowering.
    - Reason: the hook on top did not slide out.
    - This may also occur if the seabed is very soft and the stem did not fully penetrate through the gimbal.
- If on recovery to sea surface, the corer is wrapped in steel deployment/recovery wire (may even be inverted) extreme care should be exercised when raising the corer from the sea surface to the deck. Only when the sampler is resting on the deck and when all tension is released from the wire should the corer be approached, and attempts made to unravel.
    - Reason: too much cable paid out from winches after contact with seabed.
- No water sample retained above the box and possible sediment spilling out of the upper doors.
    - This is usually from over penetration. Limiter blocks should be lowered on the stem of the corer reducing penetration through the gimbal.

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Appendix A: Box corer log sheet

Cruise code: 1016S Area name: GSH Stn. code: _____

Stn. number: _____ Attempt: _____ Replicate: _____ Nav log file: _____

Date sampled: _____ Time: _____ Fix: _____

Latitude: _____ Longitude: _____ Cable out: _____

Sample accepted: _____ Sample depth: _____ Sample volume (l): _____

Substrate type: _____

Sediment colour: _____ Layering: _____ Odour: _____

Surface description: _____ Anthropogenic: _____

Conspicuous fauna: _____

Depth range processed (cm): _____ PSA sample label: _____

Sample photo folder: _____ Sieve Mesh: _____

0.25mm infauna sample pot volume: _____ 0.25mm infauna sample label: _____

0.5mm infauna sample pot volume: _____ 0.5mm infauna sample label: _____

Notes:

Completed by..... Checked by..... Entered by.....

Appendix B: Sample labels

PSA	Infauna (0.5mm)	Infauna (0.25mm)
Sample code: 1016S_GSH_A01 Date/time: …… Station number/attempt: ……	Sample code: 1016S_GSH_A01 Date/time: …… Station number/attempt: ……	Sample code: 1016S_GSH_A01 Date/time: …… Station number/attempt: ……