Census of Antarctic Marine Life (CAML)

Benthic Protocols

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Aim

A uniform benthic sampling protocol is regarded to be necessary in order to consolidate work on a global scale, for which data must be comparable. A standard protocol is therefore needed for sampling each size class, and for both morphological and molecular taxonomy, in order to enable datasets to be globally comparable. Progress is currently being made for many groups of organisms towards achieving a consistent taxonomy, a standard sampling methodology and a common suite of environmental measurements.

The benthic realm is the largest marine habitat in terms of geographic coverage. It comprises shallow shelf areas down to abyssal plains and hadal troughs. Its sediment, organic carbon availability and faunistic composition differs tremendously with geographic area, depth and age of the environment.

The technologies for studying benthic habitats over a wide range of scales are advancing rapidly. Neverthtless, for this purpose, only traditional faunal sampling and processing in the study of shallow to deep-sea benthic communities will be considered here. Traditionally, the choice of a method ultimately relies on the aims of the study, the biological properties of the community of interest as well as pragmatic considerations. Sampling methods and sample processing vary according to the size class of the benthos that is targeted. Three size classes are commonly studied, the mega-, macro- and meiofauna, based on both biological and pragmatic considerations. The megafauna include all epibenthic vertebrates and invertebrates visible to the naked eye. The macrofauna is defined as the invertebrates retained on a sieve of defined mesh size (usually 0.25 or 0.3 mm) with the exceptions of meiofaunal taxa like Nematoda, Copepoda, Ostracoda and Foraminifera. The meiofauna is defined by those invertebrates passing through a 1 or 2 mm mesh sieve (facultative) and retained in a 40 or 42 µm mesh sieve.

For molecular work, it is critical to process material quickly before DNA degradation takes place, e.g. in cold 95% ethanol or acetone. However, some taxa require specialist fixatives for both morphology and/or molecular work (e.g. foraminifera). In some cases, ethanol-fixed samples are rendered useless for morphological studies. Formalin-fixed material is considered to be a superior preservative for morphological studies. It is therefore dependent on the questions that are being asked as to whether or not specimens will be retained for molecular or morphological studies, or both.

Digital images of fresh pre-preserved material can be important for identification purposes, and must be taken prior to preservation, after which specimens tend to lose their colour.

Community structure in the sedimentary environment

Megafauna

Trawls are used to sample the megafauna and video/photography used to image these large organisms without direct recovery of specimens. Different trawls are available (i.e. otter trawls, beam trawls, bottom trawls like the Agassiz trawl). The trawls are towed at a constant speed of about 1 knot. The speed and duration of trawling is used to estimate the area sampled. In order to enhance the accuracy of the estimate a pinger can be use to track the trawl.

On board, the fauna collected by the trawl is identified to the lowest possible taxonomic level, preferably species level, and fixed in 4% borax-buffered formaldehyde. It is advised to weigh the organisms individually prior to fixation. For molecular studies, tissue has to be fixed in precooled ethanol or be frozen.

Megafauna can also be quantified from photographs or videotapes. These may be taken by a variety of camera devices towed from the ship (e.g., camera sleds, drop cameras) or attached to deep submersible or autonomous vehicles (e.g., on AUV, ROV or submersibles). Lasers can be used to provide a scale in these images. However, identification of species from photographic or video images usually require collection of reference specimens (e.g., by trawl or manipulator) unless the area is well known.

Macrofauna

A variety of gear has been used to sample the macrofauna. Besides epibenthic sledges, (e.g. Brenke 2005), the two main devices deployed from the surface are the USNEL box corers and the multicorers. The box corers typically sample an area of 0.25 m². An improved box corer, the vegematic box corer has also been widely used. The box core is then divided in 25 10 x 10 cm subcores. Usually, only the 9 central subcores are used for biological purposes. The area sampled is thus 0.09 m².

Box core
Once on deck the corer should be checked to decide if the core is acceptable. Sample acceptance should be based on the following criteria:

- cores > 10 cm in length
- core surfaces essentially level (excepting relief deemed to be natural)
- sediment surface covering the full cross-sectional area of the box (excepting *limited*, (5 cm or less), lateral compression)
- essentially clear supernatant water - *limited* resuspension, particularly following a recoveries where the box core impacts the ship's hull is deemed acceptable.

If the core is judged to be acceptable the overlying water is carefully drained onto sieve and the surface of the core is examined and a record made (including either a drawing or photograph) of any surface features and/or fauna of note.

- Picking of megafauna, large macrofauna, nodules, stones.
- Slicing of 0-1cm, 1-5cm, 5-10cm layers.
- Sliced layers are placed into buckets of pre-chilled seawater.
- Buckets are placed in cold room, if available.
- Elutriation of sample and sieving in ideally <4 ºC water
- Careful sieving procedures to minimise damage to fauna e.g. in pooled, chilled seawater

The multicorer (termed megacorer in the United Kingdom) has 8 to 12 tubes of approximately 10 cm diameter. The area sampled by a single drop ranges between 0.06 and 0.09 m². Multicorers are hydraulically damped, which reduces the bow wave effect and preserves the water-sediment interface virtually undisturbed.
Comparisons of the box corer and Megacorer are important because they may allow us to use historical data from the box cores – it is necessary to determine a conversion a factor I order to compare the two gears.

Megacore/Multicorer (~10cm internal diameter cores)

- On recovery of the corer, the function of each coring unit should be checked and recorded.
- Core lengths should be measured and recorded and any surface and profile features noted.
- Sample acceptance is based on the following criteria: cores > 10 cm in length; core surfaces essentially level; the sediment-water interface intact. The latter criterion was partly relaxed where localised disturbance had been caused by the dislodgement of gravel during core penetration.
- Acceptable cores should be removed from the corer and transferred to the ship's laboratories for subsequent processing.
- In all cases, processing begins with the careful removal of the supernatant water to bucket/sieve using gentle overflow, pump siphon and/or syringe as appropriate to the sediment type.

- For macrobenthos samples, cores should be further processed as follows. Cores are extruded (by plunger from below) and sectioned into appropriate horizons, e.g. 0-5 cm and 5-10 cm. Corresponding horizons from successive cores are pooled.
- Samples are then carefully sieved. The resultant residues are then fixed and preserved in 10 % borax buffered formalin.

The epibenthic sledge which is equipped with an epi- and a suprabenthic sampler has the advantage to cover a larger geographic area and to preserve even fragile macrofauna in a good way to allow systematic an phylogenetic investigations.
The samples are usually fixed in 4% to 8% borax-buffered formaldehyde prior or after to sieving and later transferred in 70% ethanol.. The samples can be sieved over a stack of sieves with different mesh size or over a single sieve. The stack of sieves allows a wider range of comparisons with data previously gathered in the deep-sea but it also increases the damage to organisms. The sieve mesh sizes most commonly used in the study of deep-sea macrobenthic communities are 500 µm, 300 µm and 250 µm. A sieve size larger than 300 µm should not be used in the deep-sea. A 500 µm mesh sieve has been successfully used to highlight large scales patterns in shallow and bathyal communities. Neverthtless, about 15% of the species may be missed on such sieve size (Gage et al., 2002).

The organisms sorted from sediments are identified to taxonomic groups and counted under a stereomicroscope.

Meiofauna
Meiofaunal samples were in the past typically collected by sub-sampling a box-core with tube cores of various sizes. Now multicorers are widely used. The samples are sieved over a 42 (or 64) µm mesh sieve. The residues of the finer sieve are fixed in 4% borax-buffered formaldehyde. The metazoan meiofaunal taxa can be sorted under a microscope either manually or by using the centrifuge density gradient method (Ludox or another gradient-inducing chemical). Benthic foraminifera are typically processed in the same way as metazoan meiofauna, but with sediment volume quantified. Rose Bengal may be used
to distinguish live from dead foraminifera, though the reliability of this technique is debated.
In some cases foraminifera are sorted alive.
Processing on board:
- On recovery of the corer, the function of each coring unit should be checked and recorded.
- Core lengths should be measured and recorded and any surface and profile features noted.

- The overlying water is collected separately including the phytodetritus layer if present. It is sieved over a 32µm sieve which is defined as the lower size limit of the meiofauna.
- The cores are processed immediately after retrieval on board. They are kept cool and if possible processed in a cold room at in situ temperature.
- Virtual undisturbed cores are sliced in vertical layers.
- The sediment is sliced in layers of 0.5 cm for the upper cm, followed by layers of 1 cm up to 5 cm. Also the 5 to 10 cm layer of sediment is collected as bulk or sliced in 1 cm layers depending on the study. For biodiversity studies we recommend to analyse the upper 5 cm of the sediment. For ecosystem functioning studies it is often relevant to analyse the sliced core up to 10 cm depth.
- The sediment is fixed in borax buffered formaldehyde to an end concentration of 4 %. Filtered seawater is used for dilution of the formaldehyde.

Replication:
Replicates are retrieved from different deployments. Cores from the same deployment are considered as pseudoreplicates.

The number of replicates required depends on the question. In many cases a high number (> 5) is requested, but limitation in ship-time, and time of processing will generally allow less. A minimum of 3 replicates is recommended.

Extraction from the sediment:
Centrifugation (3 times at 4000 pm) with Ludox or Levasil (40%) with kaolin added is recommended for extracting metazoan meiofauna from fine sediments.

For protozoans (foraminiferans) hand sorting is recommended.

Samples are stained with rose Bengal

Molecular analysis

- Foraminiferans freeze, dry
- Nematode fix in acetone
- Others fix in ethanol

Microfauna
Protists are recognized as an abundant component of the deep-sea sedimentary fauna but they are rarely quantified and there are few standard protocols.

Environmental Parameters
Food inputs are a major structuring agent of deep-sea benthic communities. The study of

carbon fluxes in the benthic system gives a better understanding of the function and largescale
patterns of benthic communities. Ideally, such a study requires data on primary production, particle flux from the euphotic zone to the seafloor, benthic oxygen consumption,
and the biomass of the different size classes of the benthos. The profiles of organic carbon and chemical species involved in early diagenetic processes are also useful.
The main chemical components important for benthic studies are oxygen, sulfide, and at seeps, methane.
In addition to food inputs, large-scale patterns of benthic communities can depend on water masses (characterized by their temperature and salinity), currents, grain-size and bottom
water oxygen content. At smaller scales, the topography of the sea floor is an important variable. Seafloor imagery can be obtained with multibeam echosounders and/or side-scan sonars. These are ideal to guide the exploration and sampling strategies.

Which environmental parameters do we need to measure?

Priority measurements
Grain size diversity/granulometry – general descriptions more useful/easier
Sediment organic carbon (POC) – organic matter quality
Oxygen
Temperature
Salinity
Pigments
Core photos – stratified layer, sediment water interface

Secondary measurements
Sediment accumulation rates
x-radiography
Pb-210 mixing depth
Pore water oxygen
Sulphide

Tertiary measurements
POC flux
Flow regime - ADCP
Nephelometry