A draft manual for the sampling and analysis of macrobenthic samples from the intertidal-subtidal sandy substrates during the MED-CORE Project

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Equipment:

The equipment described below is for one transect, with five Sampling Points:

1. Pegs (1)
2. Tapelines (1)
3. Masks (2)
4. Snorkels (2)
5. Weight belts (2)
6. Full scuba equipment (2)
7. Cores (two types) (25 for macrofaunal sampling, 10 for sediment, POC and Chl-*a* samples)
8. Core-carriers (2)
9. Rubber mallets (1)
10. Rubber bungs (15)
11. 500μm sieves (5)
12. Sample backets (30)
13. Magnesium chloride (200 ml)
14. Formaldehyde (1l)
15. Rose Bengal (100 ml)
16. Vials (200of 5 ml (ependorfs), 100 of 15 ml)
17. Parafilm (1)
18. Eh meter (REDOX potential), with zobel solution (1)
19. Camping freezers (1)
20. Refractometer (2)
21. Distilled water (1l)
22. Narrow metal pipe (2)
23. Syringe (10, of 25 ml)

Sampling Procedure

The water line must be observed by the point at which the sand is submerged during half of the time (time defined as the period between two successive up streaming water flows). The water line represents Sampling Point (SP) 2 and it is marked with a

peg. In total, 5 SPs have to be determined. Five (random) replicate samples must be collected in each SP.
Location of SPs 1 and 3 are determined, as follows: SP 1 at 1m distance above water line and SP 3 at 1m distance below water line. SP 4 should be located at 1m deep (below water line). The precise depth of the SP 5 varies, from 3m to 5m (again, below water line). At SP 4, a mask, a snorkel, and a weight belt are required for the cores to be taken. SP 5 requires full scuba equipment and the necessary safety procedures to be followed.
Macrofaunal samples must be collected by means of marked plastic cylindrical Corers (9.4 cm diameter, >25 cm length), marked at 5cm intervals, which are pushed into the sand at each SP until submerged to just above the 25cm mark. In the most of the cases a rubber mallet must be used in order to hammer the corer in the sediment. Special attention should be paid to the sound produced as the mallet hits the corer to avoid damage from pebbles or rocks. A rubber bung is then placed in the top. The corer is then lifted out of the sand, the airtight seal of the bung keeping the sand in place. A rubber bung is then placed in the bottom. The top bung is removed to allow the bottom bung to be pushed in fully then replaced. Three replicate samples should be taken at each SP (each replicate is consisted of two cores placed adjacently), approximately 1m apart. The Corers can be carried to the shore in an adapted core-carrier.
On a sheltered part of the beach the bottom 15cm of each core is placed into a 500μm sieve, the top 10cm is placed in another. The cores should be sieved in the sea to remove fine sediment. Care should be taken to encourage water to enter gently from below the sieve, thereby minimizing any damage to the organisms in the sediment.
The remaining sediment was rinsed with filtered seawater from the sieve into a pre-labeled sample backet. Sufficient magnesium chloride (75g/l concentration) is then added to just cover the sample. This chemical is a muscle relaxant that aids specimen preservation by preventing the animals from clinging to sand particles or from curling up excessively. After at least 15 mins ~5% formaldehyde (made from a 10% stock solution with a 1:9 ratio with filtered sea water) is added to preserve the specimens (approx. 20ml), and a couple of drops of Rose Bengal is also added to dye any organic matter making it easier to identify in the lab.
In the laboratory, sorting of the collected macrofaunal organisms must be carried out under a stereomicroscope, and the sorted groups should be kept in vials, containing 70% ethyl alcohol, and labeled with all details from SP, sample replicate etc. Vials should be tightly covered by parafilm to avoid evaporation of alcohol.

Two additional cores (4.5cm diameter, >30cm height) must be collected at each SP for the measurement of environmental variables. These cores should be marked at 5 cm intervals for a more analytic view of the sediment. From the first corer for every 5 cm of sediment the Eh has to be measured and then the samples must be secured in pre-labeled bags for sediment analysis in the lab. The content of the second corer is also divided at 5 cm intervals. Every part should be separated additionally in two parts, one for the POC and one for the Chl-*a* analysis. Whenever the Laboratory facilities are far from the sampling locations, samples for POC and Chl-*a* analysis should be kept frozen.
Salinity can be measured using a refractometer. Distilled water can be dropped onto the lens of the instrument in order to calibrate it. A small water sample (approx 10 ml) can then be extracted from different depths (0-10cm, 10-25cm) at each station using a narrow metal pipe and syringe. A few drops from each sample were then placed on

the lens of the refractometer and the salinity reading is taken ($^{0}/_{00}$). The lens is rinsed with distilled water between each sample to prevent contamination.