Protocol for DNA-based sampling of macrobenthos in soft sediments

Interreg North Sea Region GEANS
European Regional Development Fund

EUROPEAN UNION

Genetic tools for Ecosystem health Assessment in the North Sea region

Protocol for DNA-based sampling of macrobenthos in soft sediments

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Project:
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https://northsearegion.eu/geans/output-library/

Overview

1. Introduction

The Interreg North Sea Region funded project GEANS (Genetic tools for Ecosystem health Assessment in the North Sea region) strives to harmonize and implement DNA-based tools in routine monitoring programs in support of policy and decision making concerning ecosystem health. To ensure the applicability and implementation of the developed genetic tools in ecosystem health assessment, pilots are being conducted in the partner countries in close cooperation with relevant stakeholder groups. For all pilots, GEANS applies traditional morphological monitoring in tandem with DNA metabarcoding and focuses on macrobenthos from the North Sea. Read more about our project at https://northsearegion.eu/geans/ .

The following protocol is based on experience by several partners of the GEANS soft sediment pilot (ILVO, Naturalis, Senckenberg, Aarhus University) in several sampling campaigns. The samples obtained using this protocol were successfully analyzed in the lab for different studies optimizing the DNA metabarcoding lab protocol (Derycke et al. 2021; Van den Bulcke et al. 2021; Van den Bulcke et al. 2023) and for the GEANS pilot project comparing bulk DNA with morphological identifications (https://www.geans.eu/outreach/fact-sheets).

2. List of materials

- Surgical gloves
- Spoon
- Absolute ethanol solution (>96%)
- Bleach (10%)
- 2 spray bottles (one for absolute ethanol and one for 10% bleach)
- Buckets (thoroughly cleaned with 10% bleach before use)
- Clean containers for storage: the volume of the containers depends on the amount of specimens and shells that need to be collected after sieving
- Sieve (mesh size depending on your study design)
- Freezer (-20°C)
- Sampling instrument (e.g. Van Veen grab, NIOZ boxcore,…)

3. Protocol

When working with DNA, it is important to **avoid contamination**. Therefore, used **material should be thoroughly cleaned between samples** (to avoid contamination between samples) and **gloves** should be used in every step of this protocol (to avoid contamination of the researcher processing the sample). DNA from the previous sample can be removed from used material by cleaning the material with a tissue sprayed with 10% bleach.

a) Take sediment sample

Clean the collection tray with local seawater to make sure no sediment is left of the previous sample. Take a sediment sample with a sediment grab (e.g. Van Veen, boxcore, …)*. Open the grab on the collection tray and carefully gather all the sediment wearing gloves in a clean collection recipient (e.g. a bucket). Rinse the collection tray with seawater to make sure all the sediment is collected in the recipient.

**In case of an Van Veen grab, subsamples for sediment analysis or eDNA can be taken with a plexiglass core or syringe through the smaller lid at the top of the grab*.

b) Sieve the sample

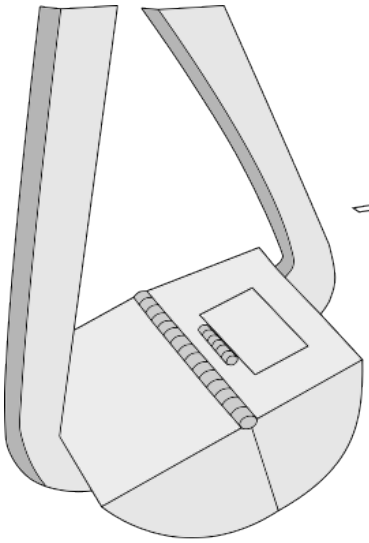
Transfer the content of the collection recipient to the sieve using local seawater. Flush the sediment through the sieve with local seawater. After sieving, gather the remaining specimens and residue (typical shells) on the side of the sieve and use a clean spoon to carefully transfer the sample in a clean container. ***Make sure not to transfer any seawater in the container***. This would dilute the ethanol concentration and hampers proper fixation of the specimens. Use a spray bottle filled with pure ethanol (>96%) to collect the last part of specimens from the sieve. Thoroughly clean the used spoon and bucket with seawater.

c) Fix and store the sample

Fix the sample with pure ethanol (>96%) to avoid DNA degradation. Make sure the specimens and shells are about ¼ of the total sample, so that ¾ of the sample is the ethanol fixative. The final concentration of ethanol in the container should be above 70%. Label the container with the location name and other relevant information. Use stickers and a pencil as ethanol easily removes writing of permanent markers. We suggest to label each container twice: next to writing the location with a marker at the lid of the container, also use a printed label to stick on the side of the container (or using a pencil).

Store the samples cold, ideally in a -20°C freezer or if not available in a refrigerator or cool box. If possible, measure the ethanol concentration of the samples after 24h with an alcohol meter, and replace the ethanol when the concentration is below 70%. Use cooling elements and cool boxes to transport the samples to the lab. Back in the lab, place the samples in the -20°C freezer until further processing.

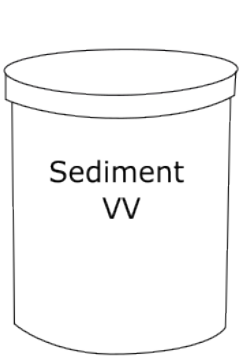
Visual representation

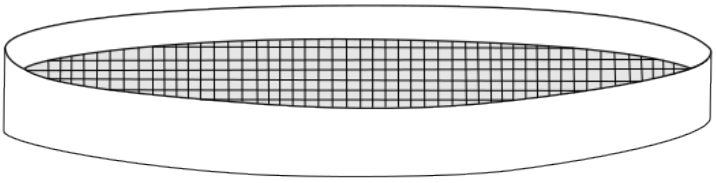


Grab

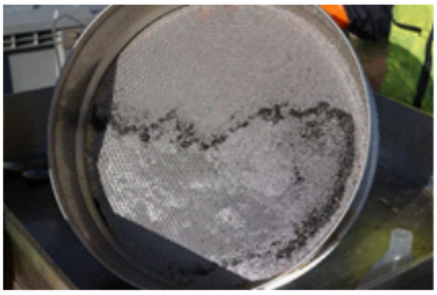
Collection tray

Collection recipient

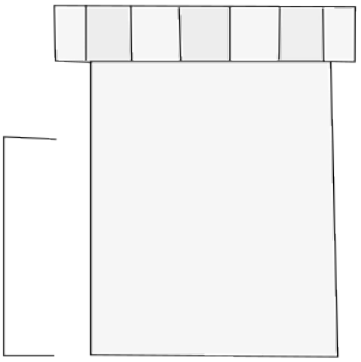




Sieve



3/4
ETHANOL



Container

Freezer

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