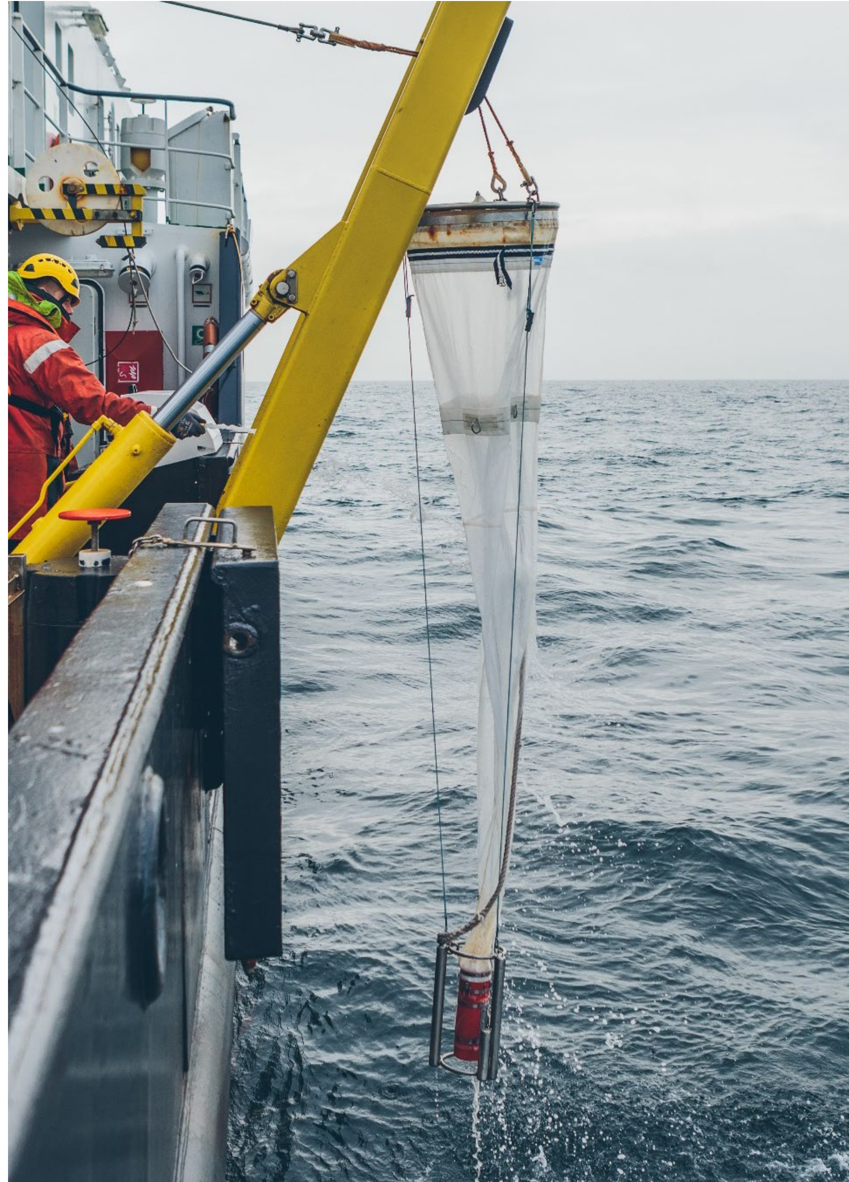
Guidelines for DNA-based sampling of Non-Indigenous-Species



Genetic tools for Ecosystem health Assessment in the North Sea region

Guidelines for DNA-based sampling of Non-Indigenous Species

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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 Non-Indigenous Species (NIS) pilot (VLIZ, SeAnalytics, Senckenberg, Aarhus University) in several sampling campaigns.

2. Study design

2.1 Sample types

NIS are a heterogenous group of organisms that may comprise species with very different life histories, sizes, reproductive cycles, behaviours, and microhabitat preferences. In order to obtain a representative overview of the NIS present in the study area, the design should consider the different microhabitats where the NIS may occur as well as the seasons, in which the NIS are abundant. A broad range of sampling types is needed to cover benthic, epibenthic, planktonic, or nektonic NIS.

2.2 Sampling season and deployment periods

Plankton and water eDNA samples should be taken during the spring bloom as well as at summer maximum. The settlement plates should be deployed at the first plankton sampling event and retrieved at summer maximum to allow for the fouling community to develop into a species rich state. Grab and scrape samples should be collected at summer maximum.

2.3 Sampling sites and replicates

For an adequate representation of the study area, at least three microhabitats should be chosen. In a harbour this might be a site with different connectivity to the open sea or nearby different vessel types. At each sampling site, at least three replicates should be taken for plankton, grab, scrape or eDNA samples. These replicates should be take at a distance of at least 15 m from each other.

2.4 Prevention of contamination

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).

Clean the workspace and equipment that comes in contact with the samples with 10 % bleach. Rinse with purified water and let air dry. Make sure to wear gloves and protective clothing while handling bleach. Clean the sampling material with 10 % bleach between biological replicates (i.e. different sampling sites). It is not necessary to clean between field replicates (i.e. different hauls at the same site). Do not clean nets with bleach as it damages the fabric. Instead, rinse it thoroughly with water from the sampling site before each use.

3. Water filtering for eDNA

3.1 Sampling

Water samples should be taken at approximately 30 cm depth using 2 L sterile bottles or with a submersible filtration device. Sample volume may range from 0.5 to 2 L depending on how fast the filter clogs. If water filtration is not done in the field, the samples should be stored in a cooled container and filtered in the laboratory immediately after return. Filtration may be conducted with a vacuum or a hand pump over a filter of 0.45 to 1.2 µm pore size.

3.2 Sample fixation and storage

Filters may be stored at -20 °C without preservatives until further processing. For long term storage, the use of a -80 °C ultrafreezer is necessary. If filters cannot be frozen immediately after storage, they should be fixed in a preservation buffer (e.g. Longmire's solution or ATL buffer from Quiagen) and stored in a cool and dark place before they can be transferred to a freezer.

4. Plankton sampling

4.1 Sampling

Mesh sizes of the plankton nets used should be 20 µm, 100 µm and 300 µm. For each of the mesh sizes hauls should stop 1 m before the bottom and not exceed a hauling rate of about

0.25 – 0.3 m/s for the smallest mesh size and 1 m/s for the larger mesh sizes.

4.2 Sample fixation and storage

Samples should be fixed in ethanol at a final concentration of 70 % or higher and stored in a cool and dark place until further processing.

5. Sediment samples

5.1 Sampling

Van Veen grab samples should have a sediment penetration of approximately 10 cm. These samples may be sieved before fixation with a mesh size of 0.5 – 1 mm.

5.2 Sample fixation and storage

Samples should be fixed in ethanol at a final concentration of 70 % or higher and stored in a cool and dark place until further processing.

6. Scrape samples

6.1 Sampling

Scraping should be done in the sub littoral zone on a pile or projecting steel facing of a wharf, berth, pier or dolphin. A representative sampling surface of 0.1 m^2 should be selected and scaped with a handheld scraping tool directly into a net with a mesh size of 0.5 mm or less.

6.2 Sample fixation and storage

Samples should be fixed in ethanol at a final concentration of 70 % or higher and stored in a cool and dark place until further processing.

7. Deployment and retrieval of settlement plates

7.1 Deployment

Settlement plates should be made of sanded grey PVC plates (15 * 15 cm) and suspended in the water column by means of a rope that passed through a hole in the middle of each plate. The rope should be tied to a pier or other fixed structure and on the other end to a heavy weight that keeps the plate in position (e.g. a brick). Two settlement plates should be deployed per location with the upper plate suspended at 1 m and the lower plate at 7 m depth.

Alternatively to settlement plates, Autonomous Reef Monitoring Structures (Obst *et al.* 2020) can be deployed according to the protocols from the ARMS MBON (https://github.com/arms-mbon/).

7.2 Retrieval, sample fixation and storage

Upon retrieval, the biofouling communities on the settlement plates should be scraped off with a steel blade scraping device. The scaped material should then be sieved through a mesh of 0.5 mm or less and then be fixed in ethanol at a final concentration of 70 % or higher and stored in a cool and dark place until further processing. If sample fixation is not possible in the field, samples should be transferred into the laboratory alive. Oxygenation of the transport containers with air pumps may be required in this case.

8. References

Obst, M., Exter, K., Allcock, A.L. et al. (2020) A Marine Biodiversity Observation Network for Genetic Monitoring of Hard-Bottom Communities (ARMS-MBON). Frontiers in Marine Science https://doi.org/10.3389/fmars.2020.572680

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