Programme: Blue mussels
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Procedure 003:
Collection and sampling of blue mussels

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1. Aim of procedure

This procedure describes all the various steps of collection and sampling of blue mussels (*Mytilus* spp.) for the Environmental Specimen Bank (ESB) for environmental pollutants. This procedure describes every step of the sampling and handling of samples to ensure that the procedure can be used in a clear way. The procedure should cover all aspects of reproducibility, quality and registration of data for the sampling material.

Blue mussels found along the Norwegian coast are three phenotypical very similar species; *M. edulis, M. trossulus* and *M. galloprovincialis* and their hybrids (Ridgway and Naevdal, 2004; Väinölä and Strelkov, 2011; Kijewski *et al.*, 2011, Brooks and Farmen, 2013). These species are assumed to respond slightly different with regards to bioaccumulation and biomarkers (Lobel *et al.* 1990). Hence, sampling for genetic analyses are included in the procedure for the Environmental Specimen Bank, as suggested by Brooks and Farmen (2013).

2. Field sampling

2.1. Trapping methods and handling of the blue mussels

Collection and handling of samples and material should be carried out in order to avoid contamination of potential environmental pollutants and protect the samples from any kind of impact that will affect their usages as research and reference materials.

The timing of the collection should take place in accordance with the programme to avoid any seasonal variations.

For the ordinary sampling programme, at least 150 blue mussels with a length between 3-5 cm should be collected from each area. In addition, ca 20 mussels should be collected and stored for future determination of species by molecular biology methods. The blue mussels are preferably collected from stones or rocks by walking in the tidal zone, by snorkeling or from a boat. Avoid collecting mussels from areas where the substrate could contaminate the samples. If there are no shell in the specified length range, pick mussels in the range of size that is present at the location. Pack the blue mussels in groups in aluminium foil and put them in clean polyethylene (PE) bags. A label with sample identification should be put into the bag and the bag is sealed/closed and frozen as soon as possible after the collection.

2.2. Registration of field data

For each group of blue mussels, a collection sheet with information regarding location, time, method for collection and person responsible for collection should be filled out. Any deviations from procedures should also be written here.

The coordinates for the position of the collection site should be written preferably as UTM/EUREF89 (Universal Transverse Mercator) with the zone 33N (epsg projection 32633), although as an alternative, WGS84 (World Geodetic System 1984) in decimal degrees can be used (Statens kartverk, 2009). The WGS84 system is the reference coordinate system used by GPS.

2.3. Storage before shipping

The samples should be frozen (or kept cool) as soon as possible after the collection, and be kept like that until the samples are being sent to the Environmental Specimen Bank. Location of storage and temperatures should be registered at the collection sheet.

3. Transport of sample

3.1. Packing

The blue mussels should be transported frozen or cooled to the Environmental Specimen Bank in a suitable, isolated parcel to keep the samples cool during transport.

The samples should be wrapped in aluminium foil and put in PE-bags (alternatively; MAGIC VAC® bags) and stored in a cooling bag or i a styrofoam box during transport. The mussels should be packed in groups with 25 individuals/unit. The way of packing should ensure that the samples are transported cold and safe for any kind of damages of the material, and that the mussels are not getting in contact with any areas or compounds that may contaminate the samples. The parcels should be marked clearly with sender's name and address, sent to Environmental Specimen Bank and addressed to a contact person there. The parcel should be marked with a text which states that the parcel contains biological material that needs to be kept cool.

If the blue mussels are collected in coordination with other survey programmes, they can be sent to NIVA/ Environmental Specimen Bank together, as long as the packing and handling of samples are in accordance with this procedure.

3.2. Transport from field

The blue mussels should be transported as quickly as possible to the Environmental Specimen Bank, e.g. as an "express over-night"-parcel. A contact person at ESB should be informed on forehand regarding time of delivery to ensure that the parcel is received in a proper way. If post or shipping companies are used, the parcel must be sent in a traceable manner.

4. Sampling within the lab

4.1. Equipment and cleaning procedures

Before sampling, the staff in the lab should prepare the necessary clean dissection equipment, pre-labelled sampling glasses and a registration sheet. The staff should use clean gloves (nitrile-gloves) and only touch the outside of the blue mussels.

The following equipment is needed for sampling of blue mussels:

- Tweezers
- Knife
- Scalpel and extra scalpel blades
- Scales (precision: 0.01 g)
- Skyvelær
- Clean aluminium foil
- Burned aluminium foil
- Sampling glasses (can withstand freezing down to -25°C)
- Cryo tubes (can withstand freezing down to -25°C)
- Solvents (HPLC grade; acetone, cyclo-hexane)
- Glass for washing of equipment
- Nitrile-gloves

All surfaces that will be in contact with the blue mussels must be covered with clean aluminium foil to facilitate run-off of excess water. The tissue samples should not be in contact with this foil. Any areas that

will get in contact with the tissue samples must be covered with burned aluminium foil. All equipment used for sampling should be of either stainless steel or of glass, quarts or other in-organic ceramic materials. The equipment should be cleaned in accordance with the procedure below.

Cleaning of equipment:
Washing with in-organic (base) soap (Neodisher UW) by the washing machine in the Environmental Specimen Bank (Miele G4230, 75 °C, 2h washing). The equipment is thereafter rinsed three times with distilled water or ion-changed MilliQ-water and then washed with solvents (HPLC grade). First, 5 minutes in acetone and then 5 minutes in cyclo-hexane. This rinsing is done twice. Cleaned equipment can be stored wrapped in clean aluminium foil.

Sample glass cleaning: The sample glasses should be un-used and any organic residues should be burned by heating the glasses for 2 hours at 500 ° C. The glasses are sealed with burned aluminium foil under the lids.

4.2. Dissection of samples

The blue mussels are allowed to defrost. Any organisms growing on the shell of the mussels that will be stored should be removed by a knife or a scalpel. Open the mussels and put them with the open side down on clean aluminium foil to allow excess water to drain out (figure 1a).

Nothing but clean equipment is allowed to be in contact with the content of the mussels. Scrape out the content with a scalpel and collect it in clean, burned glasses (Figure 1b). Scalpel blades and other sampling equipment should be changed between each location sample batch.

The blue mussels are divided in six sample glasses, with ~25 gram in each replicate. Both the weight of the sample material and the weight of the sample glass without the lid and its aluminium foil cover should be registered. With these two weights registered, any loss due to drying during the freezing storage can be calculated.

For future determination of species by molecular biology methods, individual subsamples of ~100 mg of the soft tissue should be taken from ca 20 mussels individuals. Each individual sample should be out in a cryo tube and all cryo tubes are collected and stored in a box marked with location and year.

Shell from ~25 blue mussels are dried in an oven at 60°C for 24 h and are thereafter put in a plastic bag that is labelled and stored in room temperature.

Figure 1a. *Opened blue mussels that are spread out for run-off of excess water before further sampling.*

Figure 1b. *Sample glass with the content of blue mussels before freezing.*

5. Registration of data, marking and freezing samples

Data from the field and sample schemes are transferred to the database. Every sample are given a unique sample number (P_ID), generated from the database of the Environmental Specimen Bank. In the data base, information about where the samples are stored will be included. This information should include which rack and section of it, which shelf and box where the sample is stored. If samples are frozen at -80°C in an ultra freezer, that information should also be noted here.

All glasses and/or boxes should be labelled with (freezing safe labels) unique sampling numbers. After transfer of sample material to the glasses/bags, the glasses/bags should be closed and frozen at -25 °C in the freezer of the Environmental Specimen Bank.

The boxes with cryo tubes for species determination should be stored in an ultra freezer (at -80°C).

6. References

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