Field Protocol

eDNA sampling via water bottles and Metaprobes for researchers and partner tour operators

Overview

Use a blank sampling data sheet for each sampling event. Once back on campus, log the data from the data sheets into the main data sheet on OneDrive.

Sampling events are identified by an event ID. The event ID has three parts: the date, a code identifying whether it was a citizen science (CS) or researcher/tour operator (R) collected sample, and a letter, e.g. the second sampling event on the 3rd June 2020 collected by a tour operator/researcher = 03062020-R-B.

Samples from each sampling event are labelled with the sampling event ID and two more pieces of information: the type of sample (metaprobe (MP), water bottle treatment (WBT), water bottle control (WBC)), and a number denoting whether it was the first, second, third, etc. sample of that type taken that day. For example, a metaprobe taken in the second sampling event on the 3rd June 2020 would be labelled as 03062020-R-B-MP2, and each tube (n=3) containing one piece of cotton from that metaprobe (MP2) would have this sample ID written on the label.
Labelling for water bottle samples works similarly but differently: the water bottles are labelled separately, with each bottle having a unique identifier; Sterivex filters, once water has been filtered through, should be labelled with the sampling ID. For each sample and sampling event, record on the data sheet which bottles are used for the treatment (seawater sample), and the control (distilled drinking water), noting their unique IDs in the designated column.

Before leaving the harbour, make sure all Greiner tubes and all bottles are appropriately labelled. And then when sampling, ensure to use the equipment that corresponds to the sampling event, type and order.

Before leaving:

1. Prepare equipment as per the Field Packing List.
2. Pre-fill in the event IDs, and sample IDs on the data sheet.
3. Label bottles AND/OR Greiner tubes according to the sample IDs

Water bottle sampling

4. At the sampling event location, record the GPS coordinates (Lat/Long), note who is sampling, the vessel/tour operator.

5. Wearing disposible medical gloves, open the cool box and remove the corresponding sample bottles (two 1L treatment bottles, WBT), and shut the cool box. Take one bottle, collect sample avoiding surface debris, plants, and sediment. Close bottle tightly. Repeat with the second treatment bottle. Record on the data sheet the unique identifiers of the bottles that were used as WBTs.
6. Spray the WBTs with 10% bleach solution, and place them back into the bool box.
7. Remove gloves, and put into trash bag outside of the cool box.
8. Wearing a new pair of disposible gloves, open the packaged 1L of distilled drinking water, and pour contents into the third sampling bottle (WBC). Record on the data sheet the unique identifer of the bottle that was used as WBC.
   If trips have 2+ sampling events, repeat as required for the sampling events, ensuring that the bottles with corresponding event and sample IDs are removed and used as and when appropriate

Metaprobe sampling

4. At the sampling event location, record the GPS coordinates (Lat/Long), note who is sampling, the vessel/tour operator.
5. Wearing disposible medical gloves, open the cool box and remove the corresponding Metaprobe (MP), and shut the cool box. Ensure the fishing line is attached to the D-ring and that the D-ring is securely attached to the shut MP and in the locked position. Finally, check that a small weight is tied to the D-ring.
6. Deploy the MP by gently tossing the MP overboard into the sea, avoiding surface debris and plants where possible. Record the TIME IN on the data sheet (24hr clock).
7. Remove your gloves and dispose of them in the small trash bag. Spray the top of the cool box with 10% bleach solution, this will be your processing area.
8. Put on a new pair of disposible gloves, remove the 50mL Greiner tubes with corresponding sample IDs, and place on top of the cool box. Haul in the MP using the hand reel or rope. Place the MP on top of the cool box, and cut the zip ties holding the MP together. Record the TIME OUT (24 hr clock)
9. Remove the three rolls of gauze, carefully cut the zip ties holding them together.
10. Unravel the gauze, and retrieve the small piece of cotton from inside. Place the cotton in the 50mL Greiner tubes, into the ethanol solution. Twist the tubes firmly to close, place into the sample bag with the corresponding sample ID, and replace this bag into the cool box.
11. Remove gloves, and put into trash bag outside of the cool box.
    *If trips have 2+ sampling events, repeat as required for the sampling events, ensuring that the metaprobes, 50mL tubes, and sample bags, with corresponding event and sample IDs are removed and used as and when appropriate