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WildinSync - eDNA sampling of marine coastal areas

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WildinSync



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We use this protocol and it's working

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Abstract

This protocol was developed to support long-term global biodiversity monitoring under the WildinSync initiative, which uses environmental DNA (eDNA) to assess anthropogenic impacts and nature-positive actions on ecosystems. Marine water sampling is performed to capture DNA traces left by animals, enabling detection of species presence and distribution across diverse global marine habitats. The method consists of standardized marine water eDNA sampling to obtain DNA traces from aquatic animals. It provides guidance on required materials, field sampling steps, and preparation of samples for later molecular analyses (e.g., DNA extraction and sequencing). The protocol is applicable across a wide range of habitat types and environmental conditions worldwide. It focuses on surface water sampling either from a boat or from the shore. Applicability may be limited in marine environments where water sampling is not feasible or safe (e.g., high-energy coastlines, rough seas, or inaccessible offshore areas).

Materials

Durable equipment

- Naturelink peristaltic pump (2)
- USB-C Wire (2)
- GPS (1)
- Battery Lithium USB type-C 12 V, 22 Ah (2)
- Timer (or a watch) (1)
- Easily cleanable lab tray dedicated only to the eDNA protocol (2)
- Rope Ø3 mm, length 3 m (to attach the peristaltic pump to the boat) (2)
- Lead sinker 1 kg (for dynamic sampling in transects) (1)
- Cisors or knife (1)

Consumable equipment

- Naturelink eDNA filtration Kit (gloves, capsule, tube)(2)
- Buffer kit (gloves, Buffer container)(2)
- Box of nitrile gloves (1)
- Fishing line nylon Ø0.7 mm (for dynamic sampling in transects)(1)
- Duct tape (1)
- Sample ID label stickers (1 set)
- Pencil (2)
- Permanent marker (2)
- Garbage bags (1)

Chemicals

- Ethanol 70% (1 500ml bottle)
- Commercial Bleach (1 500ml bottle)
- Bleach wipes / OH 70% (1 package)

Safety warnings

-  During marine eDNA filtration, personnel must be aware of field hazards such as slips on wet surfaces, strong currents, waves, and boat movement, and must wear non-slip footwear, weather-appropriate clothing, and a personal flotation device when on a vessel. Use personal flotation device (mandatory when working from a boat).

Use nitrile gloves at all times to prevent DNA contamination and avoid touching sterile components or sediment. Handle bleach and preservatives carefully, using gloves to prevent irritation or spills.

Ensure the pump and battery remain dry and well secured. Use proper lifting techniques for heavy equipment and remain alert to entanglement risks from ropes or tubing.



Personnel Required

- 1 A team of two people is more efficient for the good execution of the procedure on a boat, even if the procedure can be done with one person. Sampling from the shore only requires one person.

Safety

- 2 During marine eDNA filtration, personnel must be aware of field hazards such as slips on wet surfaces, strong currents, waves, and boat movement, and must wear non-slip footwear, weather-appropriate clothing, and a personal flotation device when on a vessel. Use personal flotation device (mandatory when working from a boat).

Use nitrile gloves at all times to prevent DNA contamination and avoid touching sterile components or sediment. Handle bleach and preservatives carefully, using gloves to prevent irritation or spills.

Ensure the pump and battery remain dry and well secured. Use proper lifting techniques for heavy equipment and remain alert to entanglement risks from ropes or tubing.

Training Requirements

- 3 Follow the specific protocol for the procedure.

Time Needed to Execute the Procedure

- 4 120 minutes per site.

Preparation

- 5
 - Define a dedicated workspace where you will prepare the filtration equipment away from possible source of DNA contamination. The use of a plastic tray is recommended.
 - Clean the plastic tray with bleach.
 - Make sure no one goes in the water before or during the filtration to avoid contamination.
 - Set up the peristaltic pump on a dedicated place at height to prevent exposure to direct water. While the pump is water-resistant, it is not fully waterproof. Use snap hooks and rope to secure it if you are in a boat.



Naturelink peristaltic pump recommended for the sampling.

- Prepare 1 tubing kit containing tubing and capsule. Prepare on set of labels (three stickers).
- Clean the outer part of the kit packaging with bleach, lay the packages on the plastic tray.
- Use bleach to clean scissors / penknife to open the packaging.
- Open packaging of tubing kit without taking out the content.
- Remove only the gloves. Put the gloves on. Keep the strainer and the screwed coupling end inside the bag.

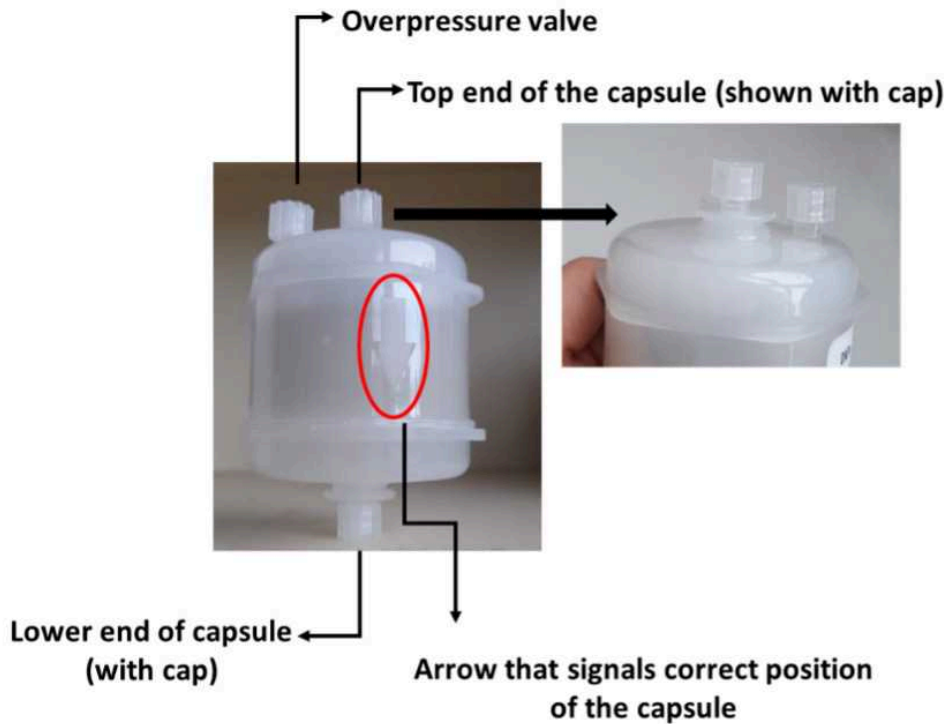


Tubing kit containing the hose with the sterile strainer and the screw coupling as well as the gloves.

Caution: Do not touch the strainer or the screwed coupling end.

Caution: After you put the gloves on, it is imperative not to touch contamination source (skin, clothes, other equipment).

- Take out the plastic bag containing the capsule.



Sampling capsule from top to bottom, with the arrow indicating the flow direction.

Caution: Be careful, together with the kit, you received a set of corresponding labels (stickers) with unique sample ID. Keep them secure.

- Identify the top and lower end of the capsule. Identify the caps of the capsule inside the outer bag and make sure to keep them at the bottom of the bag.

Caution: Be careful not to lose the caps.

- Using Bleach wipes followed by alcohol to clean scissors/penknife and open the outer bag.

- With the easy opener open the inner bag of the capsule (bag with code), and WITHOUT REMOVING THE ENTIRE CAPSULE OF THE CAPSULE peek out the upper part.
- Screw the threaded end of the hose onto the top end of the capsule. SECURE WELL.

Caution: The strainer must not touch ANYTHING before lowering it into the water.

Caution: The lower end of the capsule (drain end) must not touch ANYTHING.

- Unlock and open the peristaltic pump head.
- Position the hose inside the pump, keeping the appropriate distance for filtering on both sides. If you are filtering from the side of the boat, ensure that the hose is long enough to reach the water.

Caution: The arrow on the pump indicates the direction of the water flow, so that the capsule is positioned in the direction of the arrow.

- Lock the head.
- Connect the battery, secure the battery in its dry bag if needed.
- Position the pump in the selected location and the strainer in the water body. Take out the capsule off the bag and keep the capsule drain vertical. Keep the empty filter kit bag as it will receive the capsule when the filtration is done.

Caution: The strainer should be SUSPENDED in the water, it should not touch substrate to avoid sediment removal.

Caution: Capsule must be kept in vertical position (check arrow on capsule, indicating downward position).

Caution: The drain of the capsule must always be kept clear and must not touch anything to avoid DNA contamination.

Caution: Pay attention to the water current. Our setup should align with the direction of the water current to prevent the discharge from the pump from being filtered out.

Note: If you sample from a boat, consider using a clean fishing line. Attach one end to the lead sinker and the other end to the boat. Then, attach the tube along the fishing line using duct tape. This will prevent the strainer from coming out of the water when the boat moves. At this point, the workspace is completely set up.

- Start the filtration by turning on the peristaltic pump. If you are using two pumps on either side of the boat, start the two pumps at the same time.
- Note the start time of the filtration and the coordinates on the field sheet.

Sampling

6 Note: If you carry out the filtration from a boat, initiate the filtration while maintaining a speed of approximately 3 knots to perform a transect parallel to the shore or following the habitat of interest.

- Turn on and set up the pump according to the specifications, recommended 50% speed for 1h time. If you are doing a boat transect, plan the speed of the boat to distribute this filtration time across the entire transect.

Caution: If the water passing through the capsule is turbid the membrane can become clogged. Check regularly that the water flows well through the capsule. If it is not the case, stop the filtration, note the volume that the capsule has filtered and proceed with the next steps.

Caution: During the filtration, the water should completely cover the membrane surface within the capsule. If it is not the case, slow down the water flow on the pump and very carefully unscrew the overpressure valve on the top of the capsule until the water cover the membrane. Be mindful that this operation entails risk, the valve is under pressure and could be ejected.

Note: If you wish to calibrate the pump, we recommend timing how long it takes for the filtered water discharged from the pump to fill a 0.5/1 L container. The optimal operation of the pump targets a flow rate of less than $1 \text{ L} \cdot \text{min}^{-1}$. You can then adjust the strength of the pump by pressing the 2 button and the specific filtration duration using the '3' button. When this allotted time period ends, the pump will automatically switch off.

- Once the system has filtered 30 L of water and/or the filtering time has ended, you need to purge the remaining water from the capsule. This is done with the pump on.
- WITHOUT TOUCHING the strainer remove the strainer from the water and allow only air to pass through the hose, this will serve to empty all the water from the capsule (this may take a few minutes).
- Switch off the pump. Turning over the capsules can help remove the water left.

Caution: The strainer must not touch anything during this step. You can touch the hose. Unscrew the head and remove the hose.

Caution: The unprotected lower end of the capsule must not touch ANYTHING.

- Ensure that the capsule is completely emptied of sea water.

Sample Preservation

7 ▪ CLEAN scissors/penknife with bleach followed by alcohol.



Buffer kit containing the preservation buffer. Several models of kits exist including a squeezing bottle.

- Open the buffer kit bag containing the gloves and the kit.
- Remove the screw cap from the tip of the buffer container.
- Unscrew the end of the hose and capsule, and immediately screw the end of the buffer container onto the capsule.
- Unscrew the pressure valve of the capsule. This will allow air to get out of the capsule as the buffer is injected.

Caution: SLOWLY unscrew the valve, be careful that the valve cap may separate, fall off and become contaminated/lost.

- Always keeping the capsule in an upright position, SLOWLY start pouring the contents of the syringe into the capsule. Regulate the inflow with the valve cap.

Caution: The contents of the syringe should be poured COMPLETELY into the capsule.

- Proceed to secure the top of the capsule with the remaining cap, making sure to still keep the gloves on.



- Once the capsule is closed and the sample is secured, vigorously move the capsule up and down to ensure that the preservative/buffer permeates the entire filter inside for at least 30 s.
- Identify the capsule with its corresponding sticker n°1. Put the capsule in a plastic bag (preferably the numbered bag) and store it inside the box. Store it in a dark place at room temperature, avoiding large temperature variations.
- Identify the box with the corresponding sticker n°2.
- Remember to write in your notebook/field form the metadata associated with the sample in the field sheet.
- Identify the metadata with its corresponding sticker n°3.

Storage

- 8
- Back from the field, store samples in a dark place at room temperature, avoiding large temperature variations. The buffer preserving the DNA is efficient at ambient temperature.

The use of a refrigerator or freezer for storage is strictly prohibited.

Note: It is recommended to send the samples to the molecular laboratory for processing as quickly as possible to the lab that will process them.

Quality Control

- 9
- All samples have a replica sample, which follows the same process either at the same time if two peristaltic pumps are available, or one filtration followed by the other at the same site if only one pump is available.

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