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Title: The Floating Lab: Standard Operational Procedure for Collecting and Filtering Seawater Samples from Operating Ferries for Environmental DNA Analysis

Authors and Affiliations:
Alessia Rota[1,2,3], Elena Valsecchi[1,2]

[1]Department of Earth and Environmental Sciences, University of Milano-Bicocca
[2]Marine Research and Higher Education Center (MaRHE Center)
[3]National Biodiversity Future Centre (NBFC)

Corresponding Authors:
Elena Valsecchi elena.valsecchi@unimib.it

Email Addresses for All Authors:
Alessia Rota a.rota58@campus.unimib.it
Elena Valsecchi elena.valsecchi@unimib.it

Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **MM/DD/YYYY**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 12
Number of Shots: 27

Introduction

REQUIRED: What is the scope of your research? What questions are you trying to answer?

1.1. **Elena Valsecchi:** The aim of our research is to survey the biological communities of the high seas using commercial vessels as platforms for the collection of environmental DNA samples. In our specific case the focus is on cetaceans, trying to identify offshore areas ecologically strategic for their protection.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1*

What are the current experimental challenges?

1.2. **Elena Valsecchi:** While the acquisition of eDNA samples is extremely easy and the analytical technology is constantly progressing, the real challenge is how to reach the environments to be monitored, especially when these are difficult to access, e.g. offshore or deep waters.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What research gap are you addressing with your protocol?

1.3. **Alessia Rota:** Protocols that allow for easy and reproducible large-volume sample processing in the field are missing. As eDNA degrades in a timespan of hours, it is pivotal to filter and store seawater eDNA samples after collection, to ensure maximal retrieval and efficiency.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Elena Valsecchi:** This protocol relies fully on operating ferry-boats for data collection. Ferry-based monitoring offers 1) repeatability as routes are constant and run year-round, allowing seasonal monitoring, 2) access to off-shore waters, 3) emission-free sample collection, 4) sampling possible also during night-time hours, 5) sampling costs dramatically reduced.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.2*

How will your findings advance research in your field?

1.5. **Alessia Rota:** This study is relevant as we target marine organisms that live in open sea, namely marine mammals, as the information obtained from their eDNA can shed light on their distribution, habitat preference and, ultimately, conservation status.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.6.2*

Protocol

NOTE: Different takes of the same shots are labeled as a, b, c

2. Collecting Seawater from a Marine Ferry

Demonstrators: Elena Valsecchi

2.1. To begin, note down the current or average cruising speed of the ferry on the route to estimate the extension of the sampling stretch **[1-TXT]**.

2.1.1. Talent recording coordinates and speed with GPS. **TXT: Discuss the Fixed Sampling Station (FSS) with the crew**

2.2. After sanitizing the filtering surface, slightly open the sample collection tap for the duration of the cruise **[1-TXT]**. Use the continuous flow of seawater to rinse the collection pipe with local water, disposing of the excess water in the bilge **[2]**.

2.2.1. Talent opening the sample collection tap. TXT: **TXT: Keep the BiBs (Bag-in-Box) ready**

2.2.2. Shot of collection pipe being rinsed.

2.3. To prepare the filtering station, connect the vacuum pump to the suction nozzle of the vacuum flask **[1]** and place the stopper with the support on the neck of the flask to accommodate the filtration cylinder **[2]**. Use a permanent marker to label the BiB *(B-I-B)* with the required details **[3] [4]**.

2.3.1. Close-up shot of connecting the vacuum pump to the suction nozzle of the vacuum flask.

2.3.2. Close-up shot of placing the stopper with the support on the neck of the flask.

2.3.3. Talent labeling the BiB with a permanent marker. *Video Editor: Use a split screen to show both 2.6.3 and 2.5.4 shots simultaneously*

2.3.4. TEXT ON PLAIN BACKGROUND:

Details:

- The unique alphanumeric identification code of the sample on both sides of the BiB
- Date of sampling
- Exact time of starting the sample collection in BiB

2.4. Remove the sealing film from the BiB opening **[1]** and fill it with approximately 13 Liters of seawater **[2]**.

2.4.1. Talent removing the sealing film from BiB opening.

2.4.2. Talent filling the BiB with water.

2.5. When the BiB is nearly full, remove the sealing film from the lid, leaving the film around the tap intact **[1]**. After sealing the BiB tightly, note the exact time when sample collection is completed and record the total duration of the sample collection **[2]**.
  2.5.1. Talent removing the sealing film from the lid, leaving the film intact around the tap.
  2.5.2. Talent noting the end time and duration of the sampling process.

2.6. Write the completion time on the BiB using a permanent marker and transfer the information onto the sample collection and filtration form **[1-TXT]**. Place the filled BiB in the storage room or filtration area **[2].**
  2.6.1. Close-up shot of labeling the BiB with the completion time. **TXT: Enter the geographic coordinates at the beginning and end of sampling**
  2.6.2. ~~Close-up shot of recording the data on the form~~. **NOTE**: Not filmed, VO adjusted
  2.6.3. Talent placing the BiB in the designated area for storage or filtration. **TXT: Calculate the length of the sampled sea segment**

3. Filtering Seawater for Environmental DNA Sampling

Demonstrators: Alessia Rota and Elena Valsecchi

3.1. Prepare all necessary materials and assemble the filtering system in the designated space for filtration **[1]**. Use a new cylinder for each station and a new pair of single-use tweezers or sterilized tweezers **[2-TXT]**. To minimize the contamination risk, place an insulating sleeve, such as a plastic bag, between the tap and the filtration cylinder to isolate the water flow **[3]**.
  3.1.1. Close-up of the filtration station with required equipment.
  3.1.2. Close-up shot of setting up the filtration cylinder with sterilized tweezers. **TXT: Complete the sample collection-filtration data log**
  3.1.3. Talent applying an insulating sleeve to prevent contamination.

3.2. Activate the vacuum pump and start the timer to record the filtration time **[1]**. Pass up to 4 liters of water per filter, ensuring the cylinder remains full throughout the process to avoid air entry **[2]**.
  3.2.1. Talent activating the vacuum pump and starting the timer immediately.
  3.2.2. Shot of water getting filetered through the filter unit.

3.3. When the filtered water reaches the 4-liter mark, turn off the vacuum pump and stop the timer immediately **[1]**. Record the filtration time in the data log sheet **[2]**.
  3.3.1. Talent stopping the pump and the timer.
  3.3.2. Talent recording the time in the data log sheet.

3.4. Then, use tweezers to carefully recover the first filter or filter A from the cylinder, avoiding any damage **[1]**. Fold it in half with the side retaining biological material folded inward and wrap it in aluminum foil **[2]**. Label the foil with the sample number and filter replicate ID before storing it in the freezer **[3]**.

3.4.1. Talent extracting filter A with tweezers.

3.4.2. Close-up Shot of folding the filter properly and wrapping it with foil.

3.4.3. Talent labeling the wrapped filter.

3.5. Mount a new filter inside the filtration cylinder for the next round of filtration and empty the flask **[1-TXT]**.

3.5.1. Talent replacing the filter and emptying the flask. **TXT: Blank-bag control: Drinking water from a sealed bottle**

3.6. After filtration, store all filters between minus 4 degrees Celsius and minus 20 degrees Celsius until further laboratory processing **[1]**. Just before disembarkation, retrieve all samples and place them in low-temperature transportable coolers for transport **[2-TXT]**.

3.6.1. Close-up of placing filters in refrigerators.

3.6.2. Close-up of placing the samples in transportable coolers. **TXT: Analyse the marine environmental DNA in the collected samples**

Results

4. Results

4.1. Marine environmental DNA collection and analysis using metabarcoding identified multiple vertebrate species, including teleost fishes, elasmobranchs, and cetaceans, along the sampled route **[1]**, enabling the reconstruction of vertebrate community composition and trophic structure **[2]**.

4.1.1. LAB MEDIA: Figure 13. *Video editor: Highlight the chart/box on the left*.

4.1.2. LAB MEDIA: Figure 13. *Video editor: Mark the chart/boxes on the right*.

1. Metabarcoding

Pronunciation link:
https://www.howtopronounce.com/metabarcoding
IPA: /ˌmɛtəˈbɑːrˌkoʊdɪŋ/
Phonetic Spelling: meh-tuh-bar-koh-ding

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2. Elasmobranch

Pronunciation link:
https://www.howtopronounce.com/elasmobranch
IPA: /ɪˈlæzməˌbræŋk/
Phonetic Spelling: ih-laz-muh-brank

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3. Cetacean

Pronunciation link:
https://www.howtopronounce.com/cetacean
IPA: /sɪˈteɪʃən/
Phonetic Spelling: sih-tay-shun

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4. Teleost

Pronunciation link:
https://www.howtopronounce.com/teleost
IPA: /ˈtiːliˌɒst/
Phonetic Spelling: tee-lee-awst

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5. Vacuum flask

Pronunciation link:
https://www.howtopronounce.com/vacuum-flask
IPA: /ˈvækjuːm flæsk/
Phonetic Spelling: vak-yoom flask

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6. Bag-in-Box (BiB)

Pronunciation link:
No confirmed link found
IPA: /ˈbæg ɪn bɑks/
Phonetic Spelling: bag-in-box

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7. Trophic

Pronunciation link:
https://www.howtopronounce.com/trophic
IPA: /ˈtroʊfɪk/
Phonetic Spelling: troh-fik
