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Title: Environmental DNA Sampling from Whale-Watching Vessels for Cetacean Monitoring

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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. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one.**

☒ Interviewees self-record interview statements. JoVE can provide support for this option.

3. Proposed interview filming date: Please indicate the proposed date that your group will self-film interviews: **08/04/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 25

Number of Shots: 48

Introduction

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

We have chosen 5 best answers

INTRODUCTION:

Author's NOTE: I made a single video for 1.1, 1.2 and 1.3 - A001_04081827_C107_intro

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Belén García Ovide:** This protocol provides a standardized approach to whale eDNA sampling during whale watching cruises.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the research gap or limitation in existing methods and how this protocol addresses them.**~~

- 1.2. **Belén García Ovide:** Many different eDNA sampling methods are available. This hinders comparative data analysis. Our protocol has been optimized across environmental conditions and safeguards against contamination.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the research contexts or systems in which this protocol can be applied.**~~

- 1.3. **Belén García Ovide:** With the support of an eDNA-ready laboratory, this protocol can be carried out by non-experts and citizen scientist during whale-watching activities around the globe.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~**What does this protocol allow researchers to study or measure?**~~

- 1.4. **Belén García Ovide:** This protocol enables the non-invasive detection and monitoring of cetaceans and co-occurring taxa via eDNA contained in flukeprints collected from whale-watching vessels.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **FILE: A001_04081836_C112_conclusion_1**

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Belén García Ovide:** This protocol can be used for future large-scale eDNA sampling campaigns to investigate marine biodiversity or whale population genetics.
- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **A001_04081836_C112(1)_conclusion_2**

Protocol

2. Equipment Preparation and Sterilization

Demonstrators: Belén García Ovide and Anna Gaia Iacono

- 2.1. To begin, prepare field documentation materials [1], including the field data sheets and the laminated step-by-step field protocol for cetacean environmental DNA sampling [2].
 - 2.1.1. WIDE: Talent standing in the lab and looking at the field document.
 - 2.1.2. LAB MEDIA: 1.1. Preparing docs. 00:00-00:04
- 2.2. Put spare gloves, spare filters with one filter per sample replicate, a waterproof marker, and a dedicated waste container in the box [1].
 - 2.2.1. LAB MEDIA: 1.2 Preparing box.MOV *Video editor: Please show the initial 5 seconds and the last 5 seconds*
- 2.3. Prepare one or more rigid buckets suitable for the vessel type and onboard configuration [1], a secondary environmental DNA container [2] and a portable peristaltic or vacuum water pump [3].
 - 2.3.1. LAB MEDIA: 1.3 Preparing buckets.MOV
 - 2.3.2. LAB MEDIA: 1.3.1 Preparing additional eDNA container.MOV 00:10–00:20
 - 2.3.3. LAB MEDIA: 1.4 preparing pump.
- 2.4. Wear gloves during all equipment handling and change gloves between tasks [1].
 - 2.4.1. LAB MEDIA: 1.5 Putting gloves_caution.MOV
- 2.5. To sterilize the equipment, apply a diluted household bleach solution by splashing evenly across all surfaces of the buckets, lids, containers, and pump tubing [1].
 - 2.5.1. LAB MEDIA: 2.4 rinsing bucket, 2.3 Cleaning container; 2.3 Cleaning tubes.MOV
Use first few seconds in all these footage.

2.6. Rinse thoroughly with tap water three times [1].

2.6.1. LAB MEDIA: 2.4 Rinsing container

3. Flukeprint Water Collection

3.1. Bring all the materials onboard before passengers arrive [1] and store them in a designated clean area [2].

3.1.1. LAB MEDIA: 3.1 Bringing materials onboard.

3.1.2. LAB MEDIA: 3.1 SamplingMaterials on zodiac

3.2. Coordinate sampling timing and positioning of the vessel with the vessel crew [1]. Observe surfacing patterns on the water to anticipate whale dives [2].

3.2.1. LAB MEDIA: 3.2 GX010109.MOV;00:15–00:25

3.2.2. LAB MEDIA: 3.3 GX010158.MOV

3.3. Collect 20 liters of seawater from the flukeprint as soon as possible, ensuring no more than a 10 minute delay after the whale dives [1]. Generate two 10 liter sample replicates [2]. Obtain samples from various regions using customized logistical conditions appropriate to each location [3].

3.3.1. LAB MEDIA: 3.4 GX010159.MOV

3.3.2. LAB MEDIA: 3.4 GX010166.MOV 00:00-00:10

3.3.3. LAB MEDIA: 3.4. water collection from zodiak azores.MOV and 3.4. Water collection from motorsailor Italy.MOV. 00:00-00:20 *Video editor: Play the 2 videos side by side using split screen*

3.4. Now, transport the collected water to a designated filtration station on board, avoiding spillage [1].

3.4.1. LAB MEDIA: 3.5 GX010167.MOV

3.5. Record species identity, geographic positioning system coordinates, time, and sample replicate identification in the data sheet [1]. Change gloves immediately after reporting [2].

3.5.1. LAB MEDIA: 3.6 GX010124.MOV

3.5.2. LAB MEDIA: 3.7 GX010168.MOV

4. Environmental DNA Filtration Onboard

4.1. Open the filter bag and handle the filter only by the outlet [1]. Insert the inlet into the snorkel [2].

4.1.1. LAB MEDIA: 4.1 GX010129.MOV 00:00-00:06

4.1.2. LAB MEDIA: 4.1 GX010129.MOV 00:312-00:20

4.2. Then, connect the pump tubing to the outlet [1].

4.2.1. LAB MEDIA: 4.2 GX010133.MOV 00:10-00:25

4.3. Submerge the snorkel in the water sample without submerging the filter housing [1] and set the pump to a fast, steady flow rate [2].

4.3.1. LAB MEDIA: 4.2 GX010133.MOV 00:25-00:30

4.3.2. LAB MEDIA: 4.3;4.4 GX010138.MOV 00:30-00:40

4.4. Filter 10 liters continuously while monitoring flow stability [1]. Next, invert the filter and run the pump for 30 to 60 seconds to remove residual water [2]. Customise the filtering pump setup according to the field conditions [3].

4.4.1. LAB MEDIA: 4.5 GX010141.MOV

4.4.2. LAB MEDIA: 4.6 GX010145.MOV 00:20-00:30

4.4.3. LAB MEDIA: 4.5 Filtering set-up Azores.MOV and 4.5 Filtering set up in Italy,(2).MOV *Video editor: Play the 2 videos side by side using split screen* **Authors: I have added this as per your suggestion**

4.5. Switch off the pump and disconnect the tubing [1]. Place the filter in its original bag [2] and label it immediately [3]. Change gloves and prepare to perform filtration for the second replicate [2].

4.5.1. LAB MEDIA: 4.7 GX010149.MOV 00:00-00:08

4.5.2. LAB MEDIA: 4.7 GX010149.MOV 00:20–00:30

4.5.3. LAB MEDIA: 4.8 GX010171.MOV

4.5.4. LAB MEDIA: 4.10 GX010168.MOV

5. Filter Processing and Lysis

5.1. Filter one field negative control after every five flukeprint samples. Use target DNA-free water immediately after returning to shore and before equipment cleaning [1]. Store self-preserving environmental DNA filters in a safe, dry container in a shaded, ambient-temperature room or in a refrigerator if ambient temperature exceeds 20 degrees Celsius, for no longer than 3 months before shipping [2].

5.1.1. LAB MEDIA: 5.1;5.2 GX010190.MOV 00:44–00:55

5.1.2. LAB MEDIA: 6.1 GX010178.MOV

5.2. Perform all laboratory procedures in dedicated ultraviolet-sterilized rooms with adequate ventilation [1] and clean the workbenches after donning DNA-free gloves and protective clothing [2].

5.2.1. LAB MEDIA: 7. IMG_0939.MOV

5.2.2. LAB MEDIA: 7. IMG_0946.MOV

5.3. Using DNA-free forceps, fold the filter membrane into triangles [1] and transfer it to a 2-milliliter reaction tube with the tip oriented downward [2]. Add 380 microliters of TES buffer and 20 microliters of Proteinase K to each reaction tube to obtain 400 microliters of lysis buffer [3].

5.3.1. LAB MEDIA: 7.1.3 IMG_1079.MOV 00:05-00:10 and 00:25-00:30

5.3.2. LAB MEDIA: 7.1.3 IMG_1083.MOV

5.3.3. LAB MEDIA: 7.1.4 IMG_1090.MOV 00:15-00:25

5.4. Vortex briefly and incubate at 56 degrees Celsius for at least 3 hours with agitation [1]. After incubation, centrifuge the tubes at 18626 *g* for 10 minutes at room temperature [2].

5.4.1. LAB MEDIA: 7.1.5 IMG_1092.MOV 00:016-00:25

5.4.2. LAB MEDIA: 7.1.6 IMG_1097.

6. DNA Extraction and Quantitative PCR

6.1. Pipette extraction reagents into wash plates and elution plates [1].

6.1.1. LAB MEDIA: 7.2.2 IMG_0959.MOV 00:20–00:30

6.2. Transfer 300 microliters of lysate into each well in the bind plate [1]. Vortex and add 30 microliters of magnetic particles per well in the first Bind plate [2].

6.2.1. LAB MEDIA: 7.2.4 IMG_0989.MOV 00:05–00:10

6.2.2. LAB MEDIA: 7.2.4 IMG_0990.MOV

6.3. Run the preliminary DNA uptake program for two bind plates and the subsequent DNA extraction program on the robot. [1].

6.3.1. LAB MEDIA: 7.2.6 IMG_0998.MOV 00:2–00:16

6.4. Next, prepare the master mix for PCR [1]. In a dedicated space for preparing PCRs, add the extracted DNA standards to each well containing master mix [2] and then add 3 microliters of DNA extract to each subsequent well [3].

6.4.1. LAB MEDIA: 7.3.4 IMG_1039.MOV 00:28–00:30 **Authors: This step is moved here**

6.4.2. LAB MEDIA: 7.3.5 IMG_1053.MOV 00:10–00:24

6.4.3. LAB MEDIA: 7.3.5 IMG_1051 00:03–00:15

6.5. After sealing the prepared PCR plate, centrifuge it at 3500 *g* for 10 seconds [1] and load the plate into the real-time quantitative PCR thermocycler [2]. Finally, run the assay under optimized cycling conditions [3].

6.5.1. LAB MEDIA: 7.3.7 IMG_1058.MOV 00:00–00:10

6.5.2. LAB MEDIA: 7.3.7 IMG_1060.MOV 00:05–00:15

6.5.3. LAB MEDIA: 7.3.8 IMG_1061.MOV 00:08–00:17

Results

7. Results

7.1. 247 Environmental DNA or eDNA samples were collected in Skjálfandi Bay, Iceland and in the Azores involving the collection of up to 10 liters of seawater with two self-preserving eDNA filters per flukeprint [1].

7.1.1. LAB MEDIA: Figure 9. *Video editor: Show the map and highlight the black dots marking sampling locations in Iceland and the Azores*

7.2. Among the 44 eDNA filters analyzed for humpback whale eDNA, 20 yielded amplification signals [1], whereas 24 showed no amplification [2].

7.2.1. LAB MEDIA: Table 4. *Video editor: Highlight the rows with yellow-orange background*

7.2.2. LAB MEDIA: Table 4. *Video editor: Highlight the rows with white background*

7.3. Extraction blanks, field controls, and quantitative PCR controls showed no detectable amplification, indicating absence of contamination [1].

7.3.1. LAB MEDIA: Table 4. *Video editor: Highlight the rows towards the end with grey background*

7.4. Among positive reactions, 9 eDNA filters yielded at least one cycle threshold value below the limit of detection and were classified as reliable detections [1].

7.4.1. LAB MEDIA: Figure 10. *Video editor: Highlight the dark blue bars*

7.5. The remaining 11 eDNA filters produced cycle threshold values above the limit of detection, indicating plausible detections [1].

7.5.1. LAB MEDIA: Figure 10. *Video editor: Highlight the light blue bars*

7.6. The mean cycle threshold value was marginally lower in the Azores eDNA filters at about 34.7 [1] compared to the Iceland filters with a value of approximately 36.9 [2].

7.6.1. LAB MEDIA: Figure 11. *Video editor: Highlight the Azores box plot*

7.6.2. LAB MEDIA: Figure 11. *Video editor: Highlight the Iceland box plot*

7.7. The difference in cycle threshold values between Azores and Iceland eDNA filters was not statistically significant [1].

7.7.1. LAB MEDIA: Figure 11.

1. Cetacean
Pronunciation link: <https://www.merriam-webster.com/dictionary/cetacean>
IPA: /sɪˈteɪʃən/
Phonetic Spelling: sih·tay·shuhn
2. Peristaltic
Pronunciation link: <https://www.merriam-webster.com/dictionary/peristaltic>
IPA: /ˌpɛrəˈstɒltɪk/, /ˌpɛrəˈstæltɪk/
Phonetic Spelling: pehr·uh·stawl·tik, pehr·uh·stal·tik
3. Ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
IPA: /ˌʌltrəˈvaɪələt/
Phonetic Spelling: uhl·truh·vye·uh·luht
4. Forceps
Pronunciation link: <https://www.merriam-webster.com/dictionary/forceps>
IPA: /ˈfɔrsɛps/, /ˈfɔrsəps/
Phonetic Spelling: for·seps, for·suhps
5. Membrane
Pronunciation link: <https://www.merriam-webster.com/dictionary/membrane>
IPA: /ˈmɛmbreɪn/
Phonetic Spelling: mem·brayn
6. Proteinase
Pronunciation link: <https://www.merriam-webster.com/dictionary/proteinase>
IPA: /ˈproutəˌneɪs/, /ˈproutɪn/, /ˈproʊtiənˌeɪz/
Phonetic Spelling: prout·uh·nays, prout·teen, prout·tee·uhn·ayz
7. Lysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysis>
IPA: /ˈlaɪsəs/
Phonetic Spelling: lye·suhs
8. Lysate
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysate>
IPA: /ˈlaɪˌseɪt/
Phonetic Spelling: lye·sayt
9. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /'sentrəˌfjudʒ/

Phonetic Spelling: sen·truh·fyooj

10. Quantitative

Pronunciation link: <https://www.merriam-webster.com/dictionary/quantitative>

IPA: /'kwʌn(t)əˌteɪtɪv/

Phonetic Spelling: kwahn·tuh·tay·tiv

11. Amplification

Pronunciation link: <https://www.merriam-webster.com/dictionary/amplification>

IPA: /ˌæmpləfəˈkeɪʃən/

Phonetic Spelling: am·pluh·fuh·kay·shuhn

12. Threshold

Pronunciation link: <https://www.merriam-webster.com/dictionary/threshold>

IPA: /'θreʃhoʊld/, /'θreʃəld/

Phonetic Spelling: thresh·hohld, thresh·uhld