











MONICEPH: cephalopod and cetacean feces collection Season 2023



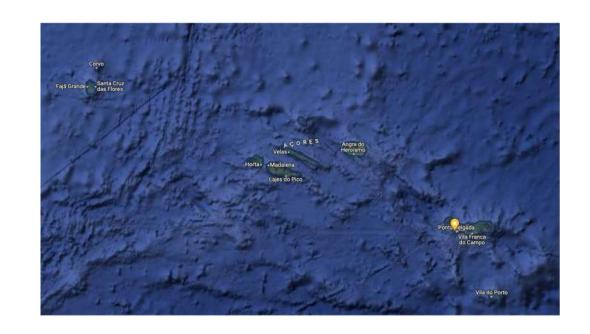
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Wayne Osborne

MONICEPH and the study of trophic ecology of cetaceans in the Azores

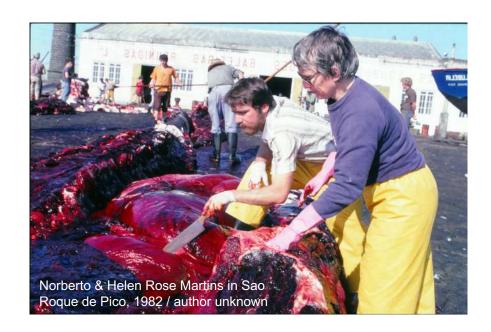
Introduction

How can you help?: Protocol



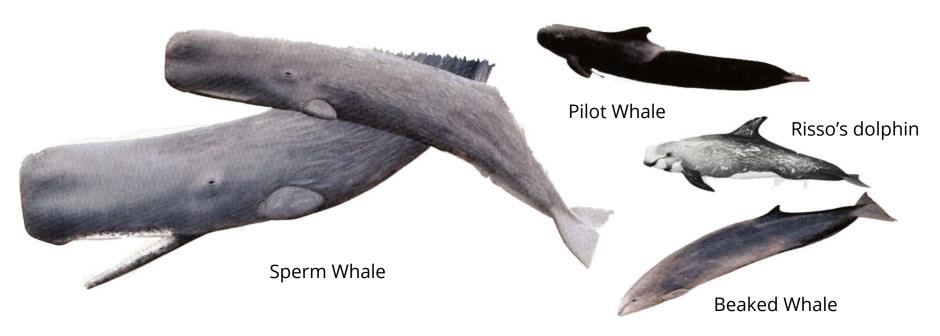
Historically, sperm whales diet study was based on **stomach content analysis**

- Clarke (1993), The Diet of Sperm Whales off the Azores
- During his work, Clarke reported 36 species of cephalopods, including species not previously recorded in the North Atlantic





The study focus on the diet and eating behavior of teuthophagous cetaceans in the Azores



MONICEPH: MONItoring CEPHalopod

In collaboration with whales watching companies, MONICEPH is monitoring cephalopods pieces found and cetaceans feces during touristic activities in the Azores





How did it start?

On several occasions, whale watching companies found cephalopods pieces at the surface of the water during touristic activities



Haliphron atlanticus found by Terra Azul in 2019



Haliphron atlanticus found by Futurismo

MONICEPH is a collaborative network

- Started to collect bio-samples in 2020 in São Miguel and expanded to the central group in 2021
- Created to collect samples of floating pieces of cephalopods found at sea (2020), and continued with sperm whales' feces collection (2022)





















The methodology based on participatory science was published in Arquipelago:

Full Article

SHORT COMUNICATION

Whale-watching contributions for the study of cetaceancephalopod interactions

STÉPHANIE R.A. SUCIU, JASMINE ZEREBA, LORENZO FIORI AND JOSÉ M.N. **AZEVEDO**



Suciu, S.R.A., J. Zereba, L. Fiori and J.M.N. Azevedo 2021. Whale-watching contributions for the study of cetacean-cephalopod interactions, Arguipelago, Life and Marine Sciences 38: 1 - 6.

Cephalopods are the primary source of food for several species of odontocetes. The unstable nature of this trophic resource is likely to affect the ecology of their cetacean predators. This can be reflected in whale conservation status but also in the tourist activities which focus on cetacean observation. However, the study of cetacean-cephalopod interactions is limited by the complicated and heavy logistics of dedicated scientific campaigns. Fortunately, this limitation can be overcome by coupling modern molecular tools with indirect sampling methods. In this note we present the first results of a project to involve whale watching companies, which represent an intense observation effort worldwide, in the collection of biological material and information for studies of cetaceancephalopod interactions and cephalopod distribution. In early 2020 we contacted all whale watching companies on São Miguel Island, Azores. All of them welcomed the invitation and received training and a sampling kit. Nine cephalopod tissue samples were collected, most of them in close association with sperm whales. All samples were determined by DNA barcoding (confirmed in a few cases by morphological observation) to belong to the gelatinous giant octopod Haliphron atlanticus (Octopoda, Alloposidae). We believe that, although the Azores may have particularly favourable conditions for participatory science, similar programs can be replicated elsewhere.

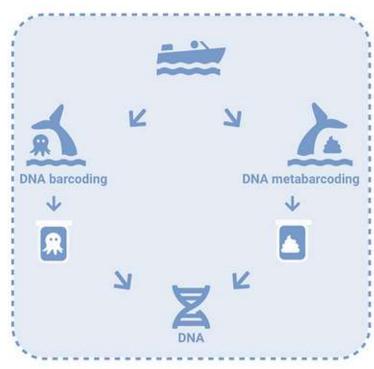
Key words: cephalopods, DNA barcoding, teuthophagous cetaceans, whale watching, participatory science



MONICEPH is currently collecting:

1. Cephalopod remains





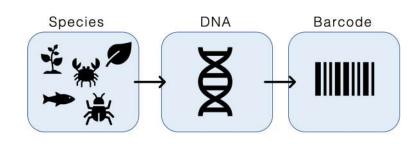
2. Sperm whale feces





Barcoding on cephalopods remains and metabarcoding on whales' feces allow to identify cephalopods species present in the diet of the whales





DNA analysis allow to study deep-ocean ecology and cephalopod/cetacean interaction

MONICEPH and the study of trophic ecology of cetaceans in the Azores

- Introduction
- How can you help? : Protocol

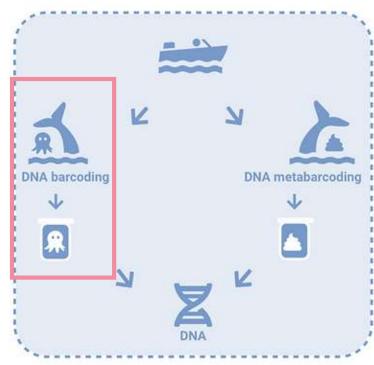




Collect and secure samples of cephalopods from the Azores

1. Cephalopod remains









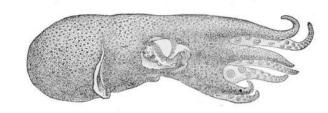
Protocol for cephalopod remains

1/ Take pictures

- Take several pictures, from several angles.
- Focus on distinctive features that could help identify the animal, but also to understand which part of the animals have been found.
- Try to include an element in the picture that can be used to estimate the size of the animal.



Taking pictures of cephalopod can allow to identify species, and to understand which part has been eaten



2/ Collect and store cephalopod remains of the material in the sampling kit

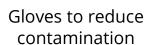




Ziplock for the beak (if present)



Disinfected pocket knife







Ethanol 96%



2/ Collect and store cephalopod remains of the material in the sampling kit

- Once you caught the cephalopod remains in a bucket, make the sampling on land (or in the boat)
 - Use **gloves** if needed to avoid intersample contamination
 - With the pocket knife cut a small piece (approx 1-2 cm3) from the animal remains
- Place the **sample in a numbered pot**, and fill it with <u>96% ethanol</u>
- Close the pot well!
- If present, **extract the beak with its muscle** and keep it in a <u>zip</u> <u>lock bag in the freezer</u>
- After each collection, wash the tool used with detergent
- Change ethanol of the sample after 24h.

3/ Fill in the Data Form



Be as precise as possible What did you see just before, just after finding the cephalopods:

- Species of cetaceans in the area
- Gender of the individuals
- Age category of the individual (adult, juvenile, calf)
- Number of individuals for each gender and age category
- Behavior



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Date

Time

GPS coordinates

Name of collector

Company

Observations



4/ Contact the MONICEPH team

Place the cephalopods sample(s) in a refrigerator

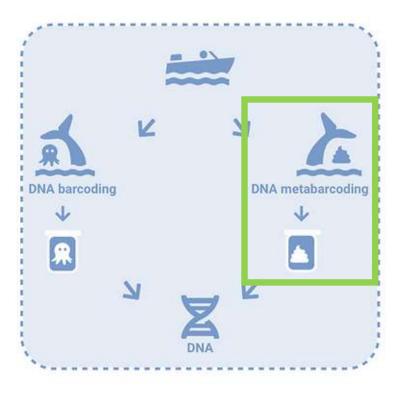
Contact the project team for help and pick-up

- <u>Stéphanie Suciu</u>: +351 914 892 298

Send pictures to

- ✓ Whatsapp +351 914 892 298
- ✓ <u>stephanie.ra.suciu@uac.pt</u>

Collect and secure samples of cetacean feces



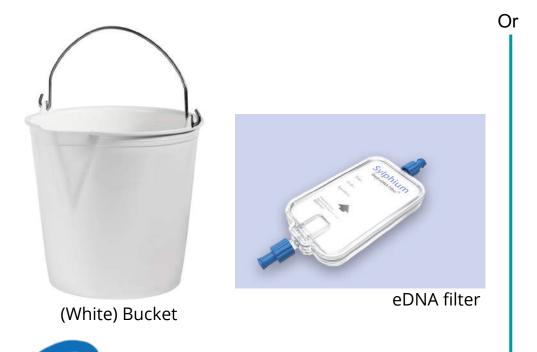
2. Sperm whale feces



How to collect whales' feces?



Protocol for whales' feces collection: sampling kit











Pot

Protocol for sperm whales' feces collection

1/ Take pictures

- Fluke for photo ID





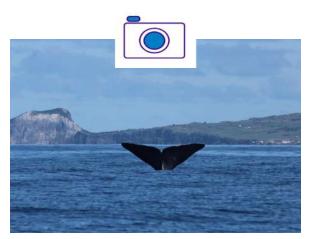
Sperm whale are defecating at the surface before a feeding dive



Fluke picture allows to identify individuals







2/ Approach the "fluke print" and use a bucket to collect feces







Bucket containing feces diluted in sea water

3/ Filter whales' feces

use a eDNA filter collection kit



eDNA filter kit (syringe, eDNA dual capsule, preservation buffer)



Connect the valve connector to the syringe

Watch video for detailed protocol: https://www.youtube.com/watch?v=EaQfMxnw2IQ&t=4s

3/ Filter and store whales' feces

use a eDNA filter collection kit (1/3)



Immerge the lower part of the capsule and use the syringe to pump the water containing the feces in the capsule



Pump the water out. The feces sediments are retainined in the capsule



Repeat (about 16 times): Pump until you see the capsule contains enough feces suspension

3/ Filter and store whales' feces

use a eDNA filter collection kit (2/3)



Drying the capsule:
When finished, remove all water from the eDNA dual filter capsule
Be aware: not removing all water, will result in degradation of eDNA.



Close the bottom of the capsule with the blue cap

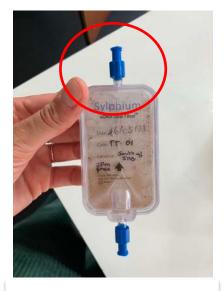




Add the preservative buffer:
Turn the capsule up side down and connect the 5ml syringe containing the buffer.
Then, add the buffer.

3/ Filter and store whales' feces

use a eDNA filter collection kit (3/3)



Close the other part of the capsule, with the second blue cap.



Place all the material in the storage bag. Keep at room temperature (or in the fridge)

It is ready to be sent to the lab!

(don't forget the data form – see step 4)

Watch the video: https://www.youtube.com/watch?v=EaQfMxnw2IQ&t=4s

3/ Collect and store whales' feces, the "creative way"

- Be creative and use other methods of collection: bucket, net, pot,...
- Storage: in alcohol or in a freezer.

In alcohol 96%





The <u>"solid parts"</u> (chunks of feces / cephalopod beak) can be kept in alcohol 96%

Frozen (-20°C)



If a sample is <u>highly</u> <u>concentrated</u>, it can be kept frozen

4/ Fill in the Data Form



Be as precise as possible

- Species of cetaceans
- Gender of the individuals
- Age category of the individual (adult, juvenile)
- Behavior
- Number of boats



Number of sample pot

Date

Time

GPS coordinates

Name of collector

Company

Observations



5/ Contact the MONICEPH team

Contact the project team for help and pick-up

- <u>Stéphanie Suciu</u>: +351 914 892 298

Send pictures to

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- ✓ <u>stephanie.ra.suciu@uac.pt</u>

MEMO

CEPH



- Take pictures at sea and on land
- Put CEPH sample in pot with <u>96% alcohol</u> and keep at RT
- If present, put BEAK (including muscle) in bag at -20°C
- Fill in DATA FORM!



FECES



- Take PHOTO ID picture
- Collect feces in a BUCKET
- FILTER with <u>eDNA capsule</u> and keep at RT
- Fill in DATA FORM!





SKIN

- Put SKIN sample in pot with 96% alcohol and keep at RT
- Fill in DATA FORM!

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- Apoio ao desenvolvimento de projetos exploratórios de investigação : M1.1.C/PROJ.
 EXPLORATÓRIOS/013/2022/

Hosting entities







PhD advisors

Supervisor: José Azevedo

- Co-supervisor: Jean-Luc Jung





Current collaborators

























AQUA AÇORES









More info:



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Thank you!

Any questions, any doubts: CONTACT Stephanie

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3/ Concentrate whales' feces

You can also use a coffee filter (time consuming)

