A protocol to address the study of microplastic intake in stranded cetaceans

Jun 28, 2021

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dx.doi.org/10.17504/protocols.io.bcfxitpn

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Citation: Tania Montoto-Martinez, Raquel Puig-Lozano, Nuno Marques, Antonio FernÃÂ¡ndez, JesÃÂºs De La Fuente, Maria Dolores Gelado Caballero (06/28/2021). A protocol to address the study of microplastic intake in stranded cetaceans. https://dx.doi.org/10.17504/protocols.io.bcfxitpn

ABSTRACT

Marine debris can impact biodiversity in a number of ways, and its effects may vary depending on the type and size of the debris and the organisms that encounter it [1]. Since the first evidence of a marine mammal's interaction with plastic intake, there have been a number of studies on this subject, together with alarming images of stomachs full of marine debris and a growing concern about it.

However, very little is known about the presence of microplastics in higher trophic level species such as cetaceans [2]. Up to more recently, they were primarily focused on the study of particles larger than 2.5 cm, and therefore failing to assess the microlitter presence, which remains a challenging task due to large gut content volumes and the difficulties of sampling following careful airborne contamination prevention protocols.

Working with stranded cetaceans (n=12), which represent a significant opportunity to study the interaction of marine fauna with plastic debris, we have validated a protocol for microplastic ingestion studies that serves to obtain samples from different multidisciplinary teams (i.e. veterinary and marine sciences schools), without interfering in the work of any of the parties.

The successful table set up used for the extraction of microplastic particles from the gastrointestinal contents was proofed advantageous and applicable by any research group that already counts with the necessary facilities to perform cetaceans autopsy analysis, fulfilling the harmonisation needs as explicated by Panti et al. [3].

This approach is fully compatible with necropsy protocol in cetaceans [4], and at the same time complies with the recommendations for reporting ingested plastics in marine megafauna [5]. The proposed workflow allows the collection of valuable data for different interdisciplinary research teams, aiming to harmonize data, facilitate large-scale comparisons of plastic ingestion and also give scientific basis to future conservation policies.

References:

1. Secretariat of the Convention on Biological Diversity and the Scientific and Technical Advisory Panel - GEF. Impacts of marine debris on biodiversity: current status
and potential solutions. Montreal; 2012. Available: http://www.deslibris.ca/ID/242832

2. Moore RC, Loseto L, Noel M, Etemadifar A, Brewster JD, MacPhee S, et al. Microplastics in beluga whales (Delphinapterus leucas) from the Eastern Beaufort Sea. Mar Pollut Bull. 2020;150: 110723. doi:10.1016/j.marpolbul.2019.110723

3. Panti C, Baini M, Lusher A, Hernandez-Milan G, Bravo Rebolledo EL, Unger B, et al. Marine litter: One of the major threats for marine mammals. Outcomes from the European Cetacean Society workshop. Environ Pollut. 2019;247: 72–79. doi:10.1016/j.envpol.2019.01.029

4. Kuiken T, García-Hartmann M, editors. Cetacean pathology: dissection techniques and tissue sampling. 1993. Available:
https://www.researchgate.net/publication/285819905_Cetacean_Dissection_techniques_and_tissue_sampling

5. Provencher JF, Bond AL, Avery-Gomm S, Borrelle SB, Rebolledo ELB, Hammer S, et al. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Anal Methods. 2017;9: 1454–1469. doi:10.1039/C6AY02419J

DOI

dx.doi.org/10.17504/protocols.io.bcfxitpn

PROTOCOL CITATION

Tania Montoto-Martinez, Raquel Puig-Lozano, Nuno Marques, Antonio Fernández, Jesús De La Fuente, Maria Dolores Gelado Caballero 2021. A protocol to address the study of microplastic intake in stranded cetaceans. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bcfxitpn

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Manuscript currently under revision.

KEYWORDS

microplastics, protocol, stranded cetaceans, necropsy, megafauna

IMAGE ATTRIBUTION

Acknowledgments: This work has been supported by a postgraduate research scholarship from the University of Las Palmas de Gran Canaria and carried out with funding from the European Regional Development Fund through the Madeira-Açores-Canarias Territorial Cooperation Operational Program (POMAC) 2014-2020 through the MARCET Project (MAC/1.1b/149).

CREATED

Feb 12, 2020

LAST MODIFIED

Jun 28, 2021

PROTOCOL INTEGER ID

32983

GUIDELINES

The airborne contamination is proved to be reduced as the preventive methodology is improved, as it is recommended on the study of Norén and Naustvol (2010). That's why, we recommend to take into consideration the following common measures to prevent contamination of the samples during lab processing:

MATERIALS TEXT

MATERIALS

MilliQ water **Contributed by users**

Distilled Water **Contributed by users**

KOH Potassium hydroxide **Fisher Scientific**

Glass microfibre filter (0.7 µm; GF/F ø=47 mm) **Contributed by users**

SAFETY WARNINGS

You will be working with Potassium Hydroxide, which is very corrosive and can cause severe skin burns and eye damage. Please, ensure you are using the appropriate lab protective equipment, including: googles, lab coat, vent hood and proper gloves.

BEFORE STARTING

(i) Wear cotton clothes while manipulating the samples,
(ii) Clean all containers using distilled water prior to its reuse,
(iii) Perform blank controls filtering MilliQ water,
(iv) Place a clean petri dish with a filter paper close to the manipulation area to register possible airborne contamination.
(v) Cover all samples with aluminium foil after each single step and during the procedure.

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Extraction of the digestive tract during the necropsy 2m

1 2m
After opening the thoracic cavity, locate the final part of the oesophagus (between both lungs) before passing through the diaphragm by the oesophageal hiatus. Separate a little bit of the connective tissue that holds it in place and with some string (or ties) make a tight and secure knot around this part of the oesophagus to seal it.

Repeat the procedure about five centimetres cranially and cut between the two knotted areas.

2 2m
Subsequently, after block extraction of the thoracic organs, locate the proximal area of the oesophagus (after the laryngeal appendix) and seal as explained in the previous step.

Cut the sealed area skull and dissect it by carefully separating this first part of the digestive system.

3 2m
Remove the oesophagus, rinse externally and set aside.

4 2m
After opening the abdominal cavity, seal the final part of the intestine in the same way as explained for the oesophagus, by making two ligatures with a little string (or ties) around this intestinal portion.

5 6m
Extract the gastrointestinal package in block and separate it from the rest of the structures (pancreas, spleen, mesenteric lymph node...).

Separate the stomach together with the duodenal ampulla from the rest of the intestine. To do this, we will seal the proximal part of the duodenum with two ligatures, leaving a space of about 5 cm between them by making a cut in the central area of this space.

Dissect carefully, separating the intestine from the mesentery.

Rinse the stomach and duodenal ampulla externally on one side and the intestinal package on the other.

Sample collection for anatomopathological, microbiological, parasitological, dietary and microplastic studies

6 ⚠

Throughout the procedure, and especially now that each of the gastrointestinal compartments is going to be opened, precautions to prevent contamination shall be taken.

Recommendations can be found at the "Guidelines & Warnings" section of this protocol.

7 ⚠

A custom-made adaptation to the necropsy table was made to perform the following steps meeting the requirements for microplastic research. The drainage of the necropsy table was connected to a set of three stacked metal sieves (1000, 500 and 200 µm) where the washed stomach contents were retained after thorough GIT rinses.

8 12m
Empty the digestive content of each GIT section (oesophagus, stomach, duodenal ampulla, and intestine) into separate trays. Perform this step one by one so the contents exposure time is reduced. In between the different GIT sections inspections, clean the table thoroughly and place a new air control filter next to the operation area.

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The steps described here correspond to the steps to be followed for each of the successive sections (esophagus, duodenal ampulla, stomach and intestines). The times indicated are the approximate sum of the performance of each action for the four compartments, even if they are done separately.

9 Rinse the inside of each section with filtrated water, taking care to leave all digestive traces on the trays. 12m

10 Carry out the macroscopic examination of the different GIT sections and take samples for histopathology and, if necessary, for microbiology. 12m

11 Wash the inside of the GIT sections with water, rubbing the mucous membranes thoroughly until all the digestive remains have been left on the trays. 12m

12 Now, the digestive content will be retined in each of the sieves described above.

Sample treatment for the study of macrowaste, diet and parasites 12m

13 Carry out a thorough observation and manual extraction of organic remains retained for observation and identification of diet. 12m

The remains extracted will be introduced in an airtight container and stored in freezing at **-20 °C** for their subsequent laboratory study.

14 Carry out a thorough observation and manual extraction of parasites for observation and identification. 12m

The greatest number of parasites present will be collected in order to take a census of the parasite load, introducing them into different containers for each type of parasite and parasitized organ (oesophagus, stomach and duodenal ampulla, and intestine). They will be set in 70º alcohol, including the name of the organ where they were observed on the label.

70 º Alcohol **Contributed by users**

Likewise, in all cases a parasitic load index (1 - 5) will be given in the organs where parasites appear:

- Mild: 1-10 parasites
- Moderate: 20-50 parasites
- Intermediate: 50-100 parasites
- High: 100-500 parasites
- Massive: more than 500 parasites

15 Carry out an exhaustive observation and manual extraction of the non-organic waste deposited on the 5mm sieve for the study of macro-waste. 12m

The extracted residues will be introduced in containers differentiated for each organ (oesophagus, stomach and duodenal ampulla, and intestine) and will be stored in freezing at **-20 °C** for their subsequent laboratory study.

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Sample treatment for the study of microplastics 4m

16 Check that the sieve has no traces of diet indicators (small otoliths, crystals, etc.) that may have been left behind. 4m

17 The filtered remains will be introduced into different containers for each organ (oesophagus, stomach and duodenal ampulla, and intestine) and will be stored in freezing at **-20 °C** for their subsequent laboratory study. 4m

Laboratory study of microplasticsics 2w 6d 12h 20m

18 Following *Foekema et al. (2013)* protocol to remove organic material, prepare the digestion of the stomach content of each of the GIT compartments, pouring it into a glass beaker, with triple the volume of KOH [M]**10 % volume** . 2w 6d

19 Once the material is degraded, follow 2h 30m

Seawater sample preparation for microplastic determination
by Tania Montoto-Martinez,
University of Las Palmas de Gran Canaria

PREVIEW RUN

filtering the remains using a **vacuum pump**.

We used Whatman® glass microfiber filters (Grade GF/F, 47 mm).

19.1 Clean the glass beaker where your seawater sample is transfered: rinse it thoroughly three times with MilliQ water **Contributed by users** 2m

Note down the volume.

19.2 Prepare the **vacuum filtration** system with a glass microfibre filter (**0.7 µm**; GF/F ø=47 mm) and filter the full volume. 15m

19.3 **Rinse** the vacuum filtration system three times while it continues filtrating to the microfibre filter, **so no particles are left behind.** 5m

19.4 Place the filter on a petri dish and let it **dry overnight** in the oven at **60 °C** . 1d

19.5 Observe the filter under a **microscope**.
Take pictures and measures of the identified particles following Lusher et al (2014) guidelines. 10m

20 Dry the filters overnight at **60 °C** . 12h

21 Observe each filter under a microscope taking careful airbone contamination controls. 4d

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