FISH GASTROINTESTINAL TRACT SAMPLING PROTOCOL FOR MICROPLASTICS EXTRACTION AND QUANTIFICATION

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1. Introduction

To monitor and survey time and spatial trends in marine microplastic contamination, fish gastrointestinal tracts (GIT) are a suitable biomonitoring matrix: various fish species are accessible to catch, and species may be selected to either cover a wide geographic area or a specific functional niche (e.g. due to their feeding behaviour) in the ecosystem. This sampling protocol describes a procedure for sampling fish GIT at fishing vessels and subsequent sample storage, with the aim to process samples for microplastics extraction and identification in a laboratory. As microplastics are ubiquitous in the surrounding environment, including fishing vessels, specific measures should be considered to minimise microplastic background contamination and to ensure high quality data.

2. Background measures

- Before starting the dissection of each individual fish, all dissection material should be thoroughly cleaned with tap or seawater to remove microplastics.
- Avoid wearing fleece or easily shedding clothing.

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- Note the colour of all plastic and rubber materials (clothing, conveyer line) in the surroundings of the sampling area. To do so, please use the fish gastrointestinal tract (GIT) sampling sheet, attached to this protocol.
- Minimise the dissection time.
- Cover the sample in the storage container as soon as possible.
- Only use glass, metal or aluminium containers for sample storage (Fig. 1).
- In each sampling day, a blank sample (fish fillet) should be taken (see below) as procedure blank.

3. Blank samples

- A blank sample (fish fillet) should be collected daily, during a sampling campaign.
- To do so, dissect and collect 5 to 20g of fillet from one (1) fish, following the same measures to mitigate sample contamination with airborne particles.
- Place the fillet into a sampling container and store in the freezer (-20 °C) or on ice, as for GIT samples.
- Note the species and the fish blank number on the fish GIT sampling sheet. The number of the blank can be noted by the sampling year, month and day, followed by “blank”. E.g. 20200815-blank.

4. Fish gastrointestinal tract (GIT) sampling

- For each sampling station, note down the sampling location and the sampling day on the fish GIT sampling sheet.
- Before initiating the sampling of the GIT (stomach and intestines), prepare the sample container provided by the analytical laboratory, inspecting for any fibres or particle contamination, and which should be made of a glass, metal or aluminium (Fig. 1). Keep the container closed as long as possible.
- Check the mouth of the fish before dissection: do not dissect a fish with plastic or other materials in the mouth.
- Note the fish species, measure the length of the fish (head to tail, cm) and record the total weight of the fish (g) on the fish GIT sampling sheet.
- Dissect the fish (Fig. 2). Carefully take out the GIT (stomach and intestines) without sampling other organs (Fig. 3). Only sample GIT with a normal, non-everted stomach. Do not sample empty stomachs.
- Store the GIT in the container and close the container as soon as possible.
- Label the container with a unique sampling number, e.g. sampling year, month and day, followed by a sample indication (20200815_1). This unique sampling number should also be indicated on the fish GIT sampling sheet.
- Store the sampled GIT in the freezer (-20 °C) or in ice.
- At the end of the campaign, samples should be transported to or picked up by the analytical laboratory as soon as possible.

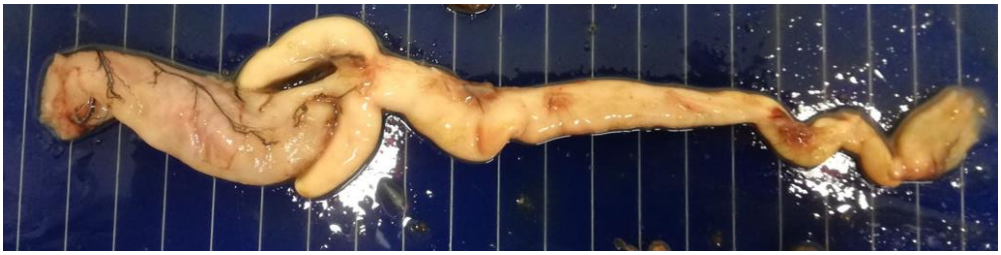
Figure 1. Metal sampling container to store and transport GIT and filet samples

(A)

(B)

Figure 2. Dissection of (A) common dab (Limanda limanda) and (B) whiting (Merlangius merlangus)

(A)



(B)

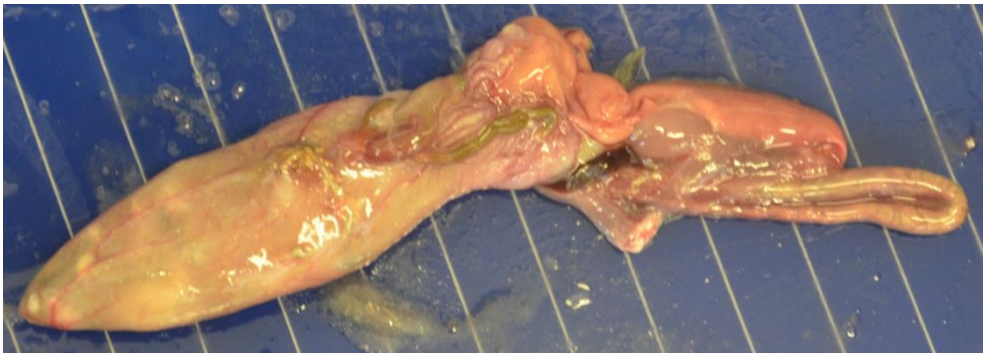


Figure 3. Gastro-intestinal tract of (A) brill (Scophthalmus rhombus) and (B) whiting (Merlangius merlangus)

5. Funding

This sampling protocol was developed within the MICROFISH project, funded by CEFIC.

FISH GIT SAMPLING SHEET FOR MICROPLASTIC EXTRACTION AND QUANTIFICATION

One sheet for each sampling location

General information

Sampling day	
Sampling location (start longitude)	
Sampling location (start latitude)	
Sampling location (end longitude)	
Sampling location (end latitude)	

Background information

Description of plastic/rubber material near sampling area (clothes/conveyor belt/…)	Colour

Sample information

Sample blank number	
Sample blank species	

Sample number	**Species**	**Fish length (cm)**	**Fish weight (g)**