

Microplastic in cod stomachs:

Methods for laboratories in rural communities



Contents

Acknowledgements	4
1. Introduction	5
1.1 Anthropogenic development in Arctic regions	5
1.2 Plastic in the High North	5
1.3 Necessity for method standardization	8
1.4 The FINPlast project	9
2. Microplastic analysis methodologies	11
3. Method development	13
3.1 The Icelandic samples	13
3.2 The Faroe Islands samples	16
3.3 Challenges and experiences	19
4. Proposed methodology protocol for small-scale laboratories	21
4.1 Digestion	21
4.2 Filtration	22
4.3 Density separation	22
4.4 Visual and chemical plastic analysis	22
4.5 Quality assurance	23
5. Iceland Case Study	25
6. Faroe Islands Case Study	28
6.1 Data summary	28
6.2 Contamination control and sample condition	29
6.3 Visual identification	29
6.4 Chemical identification	31
7. Discussion	33
7.1 The need for method standardization	33
7.2 Potential obstacles in small-scale laboratories	36
8. Concluding remarks	38
References	39
Appendix A Metadata Faroe Islands case study	46
Appendix B Material List proposed method	48
Appendix C Fact sheet – English	50
Appendix D Fact sheet – Icelandic	51
Appendix E Fact sheet – Faroese	52

Appendix F Fact sheet - Norwegian	53
About this publication	54

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1. Introduction

1.1 Anthropogenic development in Arctic regions

The Arctic and Sub-Arctic waters have historically been considered pristine environments with a relatively low pressure on the environment from a low population density (Kummu & Olli, 2010). However, economic activities in the Arctic have been occurring for centuries, especially since the whaling period in the 17th and 18th century (Kruse, 2016), resulting in changes to its natural environment. Presently, anthropogenic effects in the Arctic are increasing due to not only activities based in the Arctic, but also the global impacts of climate change - largely stemming from activities based outside of the Arctic (Huskey, Mäenpää, & Pelyasov, 2014). These impacts are complex and link together non-Arctic and Arctic issues. As an example, between 2012-2019 there has been a slight overall increase in Arctic fisheries and cruise activity with trends of stretching operational seasons and expanding navigational areas for the fisheries and cruise ships (Stocker, Renner, & Knol-Kauffman, 2020).

Even though from a global perspective the population density decreases in the higher latitudes, the GDP (Gross Domestic Product) increases steadily towards the higher latitudes and only decreases again in the most northern latitudes between 80°N and 90°N (Kummu & Olli, 2010). The moderate increase of the population in the Arctic (Jungsberg et al., 2019) but moreover the anthropogenic development in these northern regions places pressures on the environment (Huntington et al., 2007). The growing development of human activities in the Arctic, plus the growing importance of understanding the interconnectedness of Arctic ecosystems has prompted further research on global to local aspects of anthropogenic impacts. One of the more recently discussed topics is the presence of microplastics in Arctic marine ecosystems (Halsband & Herzke, 2019; Tirelli, Suaria, & Lusher, 2020; Van Sebille et al., 2015).

1.2 Plastic in the High North

The Nordic and Arctic are underrepresented in research regarding plastics in the marine environment (Halsband & Herzke, 2019; Tirelli et al., 2020). Even though relatively little is known about plastics in these remote regions it is evident that microplastics are also present in Arctic marine, terrestrial, and freshwater ecosystems. The origin of the plastics in the Arctic ranges from local to international (Cózar et al., 2017; Falk-Andersson & Strietman, 2019). An exploration into the origin of plastics from selected beaches on Svalbard indicated that the majority of identifiable litter originated from Norwegian and Russian fisheries (Falk-Andersson & Strietman, 2019). Additionally, objects were found with text indicating origins from Denmark, Germany, and England.

On land, plastics enter the natural environment and are then transported towards the oceans. This can be via sewage into nearby water bodies or rivers, from further

inland by runoff into the rivers, or via airborne transport. In the Arctic, sewage is often not treated, leaving a high proportion of smaller non-compostable items being washed out into the nearby waterbodies which eventually lead into the oceans (Gunnarsdóttir et al., 2013; Magnusson et al., 2016; von Friesen et al., 2020). Rivers are seen as the main transporter of plastics from land to sea (Li, Tse, & Fok, 2016). Recent research has shown that larger debris of low-density polymers are transported all the way out to sea, while the smaller and higher density polymers are most likely retained within the river sediments or at the beaches due to the hydrodynamics of rivers (Schwarz et al., 2019). There is little or scarce research on Arctic rivers, but a pilot study has detected microplastic fibres in a Svalbard river (Carlsson and Lusher, in prep) as well as the rivers releasing into the Siberian Arctic (Yakushev et al., in press).



Figure 1: Washed up plastic debris on a beach at Hórnstrandir nature reserve in the northwest of Iceland. Collected pile of marine debris with mainly plastics (picture by Silja Rán Guðmundsdóttir).

Plastics can also be deposited directly into the sea from fishing, aquaculture, shipping and other activities in the offshore and coastal areas (GESAMP, 2020; Lusher & Pettersen, 2021). Once plastics enter the marine environment, they can either float or sink depending on the particle characteristics and environment conditions. Plastics that are denser than the surrounding seawater will sink when they enter the water. However, plastics that float due to their lower density can be subjected to biofouling, thereby increasing their weight and leading to sinking as well (Thevenon, Carroll, & Sousa, 2014). Photo-, mechanical or biological degradation can also influence the buoyancy of plastics (UNEP, 2016). When floating, plastic can be transported by the waves and currents (Van Sebille et al., 2020). Based on modelling data and field observations it is suspected that plastic debris can come to the Arctic

from much farther away via the poleward branch of the Thermohaline Circulation (Cózar et al., 2017). From research it is now known that there is indeed marine litter observed throughout the Barents Sea, of which the majority is plastics (Grøsvik et al., 2018). The majority of the beached materials on Svalbard originated from the fishing industry (Falk-Andersson & Strietman, 2019). This was also observed during the annual OSPAR beach clean-up on Hórnstrandir in Iceland (Figure 1).

The impacts of plastics on marine ecosystems are varied and depend on the size of the plastic encountered (GESAMP, 2020). For example, a large plastic item might have more visible and physical effects on an organism, whereas smaller microplastics may be internalised and have sublethal impacts (e.g. Halsband & Herzke, 2019; Kühn & Van Franeker, 2020; Provencher et al., 2020). One of the more obvious issues is the entanglement of organisms in plastic waste (Figure 2). This might obstruct the organisms in their movement, which can lead to lower fitness, mobility and eventually death (Laist, 1987). Another possibility is that organisms would ingest the particles (e.g. Wesch et al., 2016). There have been several studies investigating the presence of plastics (mostly microplastics) in the digestive tracts of fish (reviewed in Collard et al., 2019; Markic et al., 2019). As research progresses the number of different species identified to show some level of microplastic contamination is growing, for example, during their critical review Markic et al. (2019) found that of the 494 species examined, 65% presented plastic ingestion. Some of the criticisms surrounding field observations is that it is hard to determine consequences of ingestion, on the other hand laboratory studies on the effects of plastic ingestion often use concentrations of exposure materials which are not environmentally relevant. To add more complexity to the situation, plastics in the ocean might act as a passive sampler of (toxic) compounds present in the water and thereby increase the content of a suite of hydrophobic compounds in the plastics. Whether these compounds are delivered to the animal (when plastics are ingestion), or that plastics actually adsorb compounds from the animal will depend on physical-chemical factors (UNEP, 2016). However, in field studies it is argued that the current concentrations of plastics are not high enough to cause severe harmful effects to marine life and that it is more likely that plastics act as a passive sampler than vector for persistent organic pollutants (POP) (Herzke et al., 2016; Koelmans et al., 2014).



Figure 2: North Sea crab entangled in a ghost net (picture by Pernilla Carlsson).

1.3 Necessity for method standardization

So far the presence of plastics has been recorded in every possible marine environment from washing up on coastal areas worldwide (Browne et al., 2011), sinking to the deep sea (Peng et al., 2018; Taylor et al., 2016), floating in tropical and polar waters (Lebreton et al., 2018; Van Sebille et al., 2015), to being locked in and drifting with the sea ice (Obbard et al., 2014; Peeken et al., 2018). The Arctic is no different, marine plastics are widespread and at the same time there are many areas of research on marine plastics that lack basic information (Halsband & Herzke, 2019). One of these research areas is the limited knowledge on plastic ingestion in fish (Bråte et al., 2017; Halsband & Herzke, 2019).

Litter and microplastics have gained increasing interest and awareness during the last years. There is research on the ingestion of plastics by the Northern Fulmar that has shown that the further north one goes the fewer plastics are observed in their stomachs (Trevail et al., 2015). These records spanning multiple years are helpful in plotting trends in plastic occurrence in the marine environment over time. This sort of monitoring is not yet possible for all species due to the lack of a successful standardization of methodologies.

Methods for ingested microplastic analyses are under constant development. However, there are many reports on microplastics with low or lacking quality assurance of the data process, e.g. lack of contamination control (including surrounding air), replicates, data treatment and upscaling of recordings. Some of these problems come from the nature of developing a new method, some from little understanding of chemical analyses and the lack of sufficient amount of (financial) resources. There is a need for standardisation and establishing methods for analyses

that are robust enough to give good data, also in small laboratories with little equipment, within set limits.

The extraction and identification of microplastics and other anthropogenic particles from biota samples can be a complex and challenging scenario (Lusher et al., 2020a,b). In the early days of microplastics research, visual sorting was the primary method for separating microplastics from biota samples (reviewed in Lusher et al., 2020c; Lusher et al., 2017a). In particular, there was a specific focus on stomach contents and intestines of fish (e.g. Lusher et al., 2013; Bråte et al., 2016; Murphy et al., 2017). The methods involved opening and visually sorting observed material with the use of a microscope. This method has also been adopted by the Marine Strategy Framework Directive Technical Subgroup of Marine Litter which recommend that the entire gastrointestinal tract (GI) is assessed under a dissecting microscope (MSFD-TSML, 2013).

Dissection followed by visual sorting is inexpensive and relatively easy for gastrointestinal tract (GI) and whole bodies of some organisms. Visual sorting is also advantageous as there are no chemical hazards and the cost is mostly minimal (apart from work hours). However, there are some limitations with this approach, firstly this method of investigation is relevant for microplastics >500 µm in size as isolation is limited to the visual acuity of the researcher carrying out the task (Isobe et al., 2019; Lusher et al., 2020b). Secondly, in some instances there is a large amount of undigested, or partially digested food items that can hinder visual sorting. Thirdly, visual sorting can lead to false positives or elevated particle counts because (i) samples can be at risk of procedural contamination and (ii) identification is dependent on the personal skills to separate anthropogenic from biological particles. Thus, visual identification (especially of particles < 500 µm) is not as reliable as chemical identification due to human error. Lastly, visual sorting is reliant on confirmation of isolated particles using further analytical techniques.

To assist visual identification, there are several methods available such as digestion of organic matter prior to the analysis of plastic in fish digestive tracks as will be discussed in later sections. The digestion of organic matter facilitates the visual identification of anthropogenic particles. Currently a wide range of chemicals, procedures and technologies are used in microplastic research to assist in the visual identification of anthropogenic particles. The lack of methodology standardization complicates comparison between studies. Contributing to the development of a standardized method for microplastic analysis in fish digestive tracks was the main goal of the FINPlast (Faroes Iceland Norway Plastic ingestion in fish) project.

1.4 The FINPlast project

The study subject for the development of a standardized methodology was Atlantic cod (*Gadus morhua*). Cod was chosen due to its economic importance for many of the Nordic and Arctic countries. Pre-existing data from Norway were compared along with samples from Iceland and the Faroe Islands. In each sample location, the method was adjusted based on lessons learned from the previous sample location, starting with Norway and ending with the Faroe Islands. Microplastic in Atlantic cod in the North Atlantic and European Arctic has been recorded for eastern Canada

where 2% of the examined cod contained plastics in their stomachs (Liboiron et al., 2016) and in Norway where no plastic particles were found in the samples from the Arctic region of the study but up to 27% of the sampled cod in Bergen harbour contained plastics (Bråte et al., 2016). With the variations in methodological approaches and concentrations of plastics observed in cod as elaborated on in the background, the FINPlast project aimed to:

1. Create a list of best practices and protocols for the standardization of the analysis of plastic in fish stomachs, taking into account small laboratory resources in many rural areas -i.e. we worked towards a protocol that can be applied with little resources available.
2. Produce new knowledge on the presence of plastics in fish (cod) stomachs in Iceland, Norway and the Faroes Islands.
3. Transfer knowledge between countries and institutions on both the results of the analysis and the working methods to identify and quantify amounts and types of plastics in fish stomachs.

The focus of this report is to come to a method that can be applicable from high tech laboratories to small rural (science) communities with minimal laboratory resources, which are quite common in Arctic areas. This was done by means of applying a method development strategy.

Based on previous research and our own experiences prior to the current project, we identified a base method that could be adapted to improve aspects such as digestion and filtration efficiency with the goal to have a more successful microplastic identification at the end of the process.

To evaluate whether the methodology fulfilled our goals for this project there have been several criteria formulated to which the method should at least adhere, and some in which it should outperform other methodologies known to be suitable in microplastic analysis in digestive track samples.

The method should:

- be applicable in small/rural communities that have minimal laboratory resources;
- require minimal amount of high technological material to perform the digestive tracks processing;
- be based on a relatively low budget;
- successfully digests biological material to isolate microplastics; and
- be straight forward with the aim to make it easy to communicate and implement.

To achieve this, FINPlast build on existing knowledge which was implemented in different Nordic laboratories during the project.

2. Microplastic analysis methodologies

At the start of the project a choice was made to do a full digestion of the stomach and intestines instead of visual identification of particles and manual separation from other stomach content as was done previously by Bråte et al. (2016) in Norway. The underlying idea was that in this way more and smaller particles would be captured due to a smaller human factor involved as the identification part of the study would be done on a 'cleaner' sample, i.e. with less organic material obscuring the particles. Therefore, this section will only discuss those methods which are used to digest organic materials in microplastics analysis.

In general, there are four ways to treat fish stomachs with the aim of digesting organic material, namely acid, alkaline, oxidizing or enzyme treatments. Acid treatments have been used on a wide range of biological tissues from cellulose, fatty to protein and calcareous based materials. Alkaline treatments on the other hand can degrade cellulose, fatty and protein-based tissues but does not work well on calcareous materials. The oxidizing treatment is also effective on cellulose, fatty and protein-based tissues. Finally, an enzyme treatment breaks down the widest range of 'soft' biological materials.

During an evaluation study of different treatments it was shown that acid was least effective (72–83% efficiency), followed by an optimized alkaline treatment of 10M NaOH at 60°C for 24h with an efficiency of $91.3 \pm 0.4\%$ (Cole, et al., 2014). The significantly most efficient treatment was the optimized Proteinase-K treatment (>97%). However, this is much more expensive than the other treatments as well. Dehaut et al. (2016) looked at six chemical dissolvent treatments, including the NaOH treatment from Cole et al. (2014). Dehaut et al. (2016) showed that five of these resulted in degradation of the microplastic particles and/or insufficient tissue digestion. The protocol in the study with a 10% KOH solution incubated at 60°C for 24 hours resulted in efficient digestion and no significant damage to the tested polymers, although cellulose acetate (found for example in cigarette butts) was affected. KOH is cheaper compared to the enzymatic treatment and has minimum damage on a range of polymers (Dehaut et al., 2016).

Though all treatments will digest organic tissues, they each work differently. Below an overview is given regarding the advantages and limitations of each of these general methodologies (Table 1). One must remember that for each methodology there are several chemicals or enzymes available which can be used with each their own advantages and limitations. The overview here is focussed on the more general advantages and limitations.

Table 1: Overview of the general known advantages and limitations of each of the four types of treatments for degradation of biological material. Based on several comparison studies (Cole et al., 2014; Dehaut et al., 2016; Enders et al., 2017; Karami et al., 2017; Lusher et al., 2020).

Treatment	Advantages	Limitations
Alkaline	Degrades biological material Cheap and easily available Easy to use	Affects cellulose acetate Can bleach (viscose) fibres
Acid	Degrades biological material Degrades calcareous material Cheap and easily available Easy to use	Can severely affect plastics
Oxidization	Degrades biological material Relatively cheap Easy to use	Not as effective as the other treatments
Enzyme	Very effectively degrades biological material	Expensive (Often) complicated, requiring several steps

In the past years there have been multiple promising treatments up for consideration as an international standardized methodology. The KOH treatment has shown potential in this due to the relatively low cost, accessibility, effectiveness and minimal damage to the subject of study – plastic polymers - and is therefore the treatment of choice in this study (Table 1). However, there are still many issues to be considered. For example, the varying digestion efficiency among studies. There have been different attempts at using either a different KOH:sample ratio (de Vries et al., 2020; Dehaut et al., 2016; Foekema et al., 2013) or decreasing the incubation temperature and increasing exposure time (Thiele et al., 2019). There have been developments trying a multi-digestion methodology where the sample is first treated with KOH, then filtered and immediately afterwards treated with nitric acid (HNO₃) which showed promising results (Schirinzi et al., 2020). Lessons were also learned from other methodologies developed for different matrices such as soil by means of a density separation (Hurley et al., 2018; Karlsson et al., 2017; Thompson et al., 2004). A density separation step could further clean-up the sample as is demonstrated later in this study. However, to be taken into consideration is that each new step complicates the process, can bring a higher contamination risk, becomes more time consuming and increases the costs.

3. Method development

3.1 The Icelandic samples

The study described below was recently published (de Vries et al., 2020).

3.1.1 Sample collection

Cod digestive tracts (n=39) were sampled in July 2018 during a sampling campaign by MFRI (Icelandic Marine and Freshwater Research Institute) in cooperation with local fishermen. The cod originated from the Westfjords Fishing Grounds in northwest Iceland. The fish were caught by means of a commercial Danish seine with 135mm mesh size.

Before dissection, the length and weight of the fish were recorded. Thereafter, the digestive tracts were cut out at the throat and the anus, wrapped in aluminium foil, and put inside a plastic bag to be frozen until further processing.

3.1.2 Sample digestion and filtration

The alkaline digestion method with a 10% KOH solution (100 g KOH in 900 ml prefiltered tap water) was chosen considering the available resources at the time. To get effective and efficient digestion the method was adapted from the study of Foekema et al. (2013) to a ratio of 1:6 (sample:KOH v/v). After adding the KOH the samples were placed in an oven for 24 hours at 60°C as suggested by (Dehaut et al., 2016). This was done to speed up the digestion process. As explained later a lower temperature of 40°C is nowadays advised due to damages to some plastic with higher temperatures. Following incubation, 7.7 g of citric acid was added per 100 mL 10% KOH to neutralize the sample with the aim of making the filtration process easier (Thiele et al., 2019). The samples were placed in a dark storage room for approximately 24 hours prior to filtration over 47mm Whatmann glass fibre filters (GF/D: pore size 2.7 µm) (Figure 3).



Figure 3: Filtration unit with an electricity driven vacuum pump (picture by Anne de Vries).

After filtration, the samples were analysed under a stereomicroscope. The particles were classified as either sheets, beads, fibres or fragments and recorded with the colour of the particle. For the visual identification three criteria were used to rule out non-plastic compounds by establishing whether (Norén, 2007):

1. there were no cellular or organic structures visible;
2. if the particle seemed to be a fibre it had to be the same width along its length, and thus not taper towards the ends; and
3. when the particle was difficult to classify as plastic, extra care was taken to examine it under microscope from different angles and lighting as particles might be white or transparent

To minimise identification bias, dual identification was used where one researcher carried out the initial count and was verified by a second person. Following the visual identification of particles, the filters were sent to NIVA (Tromsø, Norway) for chemical identification by using an Agilent Cary 630 FTIR (Fourier Transformation Infrared). The FTIR sends light through an interferometer onto the sample which reflects and refracts the wavelengths. The resulting radiation is collected by a detector which registers the variations in energy versus time of the wavelengths. The results yield a graph which is the signature of the particle. This signature was matched with the MicroLite software libraries database. If the match was >60% and certain identifying peaks corresponded between the library and the particle signature, the type of plastics was considered confirmed. In total 10% of the observed particles were chemically confirmed by FTIR.

3.1.3 Quality control

In microplastic research it is paramount to have a good quality control for potential contamination through the handling of the samples and airborne contamination (Torre et al., 2016; Wesch et al., 2017). This was done by implementing some preventive measures such as cleaning all materials with filtered water between samples and inspecting them under a microscope before use. In addition, clothes worn were made from near 100% non-polymer-based materials such as cotton or wool. Plastic materials for the laboratory were avoided to the largest extent possible. Finally, materials that are in contact with the samples and the air, such as the filtration unit and digestion jars, were covered with aluminium foil to avoid air-borne contamination (Bråte et al., 2016; Dehaut et al., 2016; Hermesen et al., 2017) (Figure 4).



Figure 4: Jars containing dissolved fish guts covered with aluminium foil to prevent air contamination (picture by Anne de Vries).

Besides these preventive measures there were three types of contamination controls in place throughout the processing of the samples from the catch of the fish until chemical identification:

1. **Field air blank** - using double-sided tape placed inside petri dishes or a wetted (coffee) filter which were exposed onboard the ship during the handling of the samples. One blank was used per shift onboard the vessel.
2. **Laboratory air blank** – using a wetted (coffee) filter in a petri dish to observe potential air-borne contamination during handling of the samples in the laboratory. Here one blank was used per ten samples.
3. **Laboratory process blank** - to verify for any contamination during the processing of the samples in the laboratory. This blank was treated in every way the same except for adding organic sample material. For example, if samples required additional stirring during the digestion of the digestive tracks this was done also with the process control. One blank was used per batch.

Each of the field, environment and process controls were linked to an individual sample. Hence, the contamination per sample could be calculated by summing up any observations in the different controls and subtracting that from the observations in the corresponding sample. For example, if four black fibres were found in the sample, and one black fibre was found in the environment control and two black fibres in the process control but none in the field control the end result of

the microplastic analysis of the sample will be one black fibre. Or, if there were four blue fibres in the sample and three black fibres in the controls the result will be four blue fibres.

3.2 The Faroe Islands samples

3.2.1 Sample collection and selection for microplastic study

The cod from the Faroe Islands were caught on Mýlingsgrunnur, located to the northwest of the Faroe Islands, using a 116 feet box trawl with a mesh size of 40 mm in the codend of the trawl. The fish were caught in October during an annual survey by the Faroe Marine Research Institute (FAMRI). Fish were of a consistent size range of 50 cm $\pm$ 10 cm conform the OSPAR guidelines.

During the survey the fish were frozen whole and dissection was done in the laboratory of the Faroese Environment Agency. The individual tissues were afterwards stored in the freezer for future research.

The selection of the stomach samples for this study from the archives was done randomly from three different years.

3.2.3 First sample processing attempt: successes and challenges

Adaptations based on the Iceland study

Based on the experiences in Iceland a list of proposed improvements to the processing of the samples was made with the aim to make the digestion and subsequently the filtration more successful. Other than these the processing of the stomach samples stayed the same as described in 3.1.2 *Sample digestion and filtration*. The following steps were changed and improved from the method applied on the Icelandic samples:

- Only the stomach content was used by rinsing the stomach out with KOH. This was meant to improve the digestion and minimize the formation of a fat layer that occasionally burn in the oven. In turn this would lead to better filtration.
- The sample:KOH ratio was increased to 1:10 to improve the digestion of the samples.
- A lower digestion temperature with a longer incubation period was applied (40°C for 48 hours) to decrease potential damage to polymers as studies have found (Thiele et al., 2019).
- Acetic acid was added to dissolve calcium carbonate (e.g. bone and shell fragments) to improve filtration and visual analysis. This replaced the addition of citric acid to the sample as acetic acid also neutralizes the sample besides degrading calcium carbonate. The method that was applied was developed as part of a Nordic Council of Ministers project (Bråte et al., 2020).
- Metal filters were tried out with a larger pore size of 75 μ m to improve the filtration process. The detection limit of FTIR are expected to be around this size making a finer filter redundant for the purpose of this study. In this study the detection limit was set on 200 μ m.

Quality control

The stomach samples from the Faroe Islands are from archived samples. The fish were caught and frozen to be defrosted for the sampling of different tissues in the laboratory at a later stage. Microplastic research was at that time not the aim for collecting the stomachs which means that there was no control on air contamination during the collection of the stomach samples.

As will become clear in the next paragraphs the processing of the archived samples from the Faroe Islands required some additional adaptations. The first attempt described here was executed under the same quality control measures as adopted in the Iceland study and described in *3.1.3 Quality control* with the exception of the air contamination control during the collection of the stomach samples.

3.2.4 Second sample processing attempt: successes and difficulties

Since the first attempt on processing archived stomachs from the Faroe Islands could not be continued a choice was made to move to a larger equipped laboratory with easier access to the necessary equipment, technologies and expertise to move the project forward.

Adaptations based on the first attempt

When the operations moved to the laboratory of NIVA in Oslo, Norway there were several adaptations made compared to the first attempt based on previous experiences in the FINPlast project and the expertise in Norway on processing stomach samples for microplastic analyses.

- The stomach samples were processed in different parts out of necessity since the samples were already partially digested and not contained within the stomachs. This was probably due to defrosting during transportation. The necessity to process the stomachs in different parts could however have been beneficial for the digestion and filtration part of the process.
- While the samples were incubated, they were in constant motion (120 rpm) with the aim of improving the digestion.
- Prior to filtration over a glass fibre filter (GF/C 1.2 µm) the sample was sieved through a 150 µm metal sieve as the methodology aimed to collect samples > 200 µm due to the detection limit for the FTIR.
- Samples that contained a high load of undigested biological material were treated using density separation prior to filtration.

Quality control

During the second attempt the steps in the laboratory processing which could introduce procedural contamination were identified and monitored using blanks. As was noted earlier there are no environmental controls to account for contamination during the dissection of the fish.

The contamination verification controls were conducted as follows:

- **Laboratory air blanks** – A damp filter paper left exposed to air in a petri dish during the separation of stomach contents from sample bag into respective glass Erlenmeyer flasks. There was an air blank per stomach (n=20).
- **Laboratory process blanks (KOH)** – KOH used for the digestion of samples was added to a clean Erlenmeyer (no sample) to test for contamination during the digestion phase. Three replicates per sample batch were performed. They were treated in the same way as the samples (incubator, sieving, filtering)
- **Laboratory process blanks (density separation)** – The density solutions were added to clean falcon tubes (no sample) and treated the same way as the samples requiring density separation. There were three replicates performed during the density stage.

Sample digestion and filtration

A 10% KOH solution (1.5–1.8 M, pH =14, 20°C) was prepared with pre-filtered reverse osmosis (RO) water. Once the solution was thoroughly mixed it was filtered again through a GF/C filter to remove any impurities introduced at the chemical preparation stage. The solution was maintained at room temperature until required.

The stomach samples were processed in batches (n = 3 batches) between August and September 2020. Stomachs were defrosted at room temperature prior to processing. It was noted that some of the stomachs were in different stages of decomposition (unforeseen delays in transport caused thawing, followed by refreezing until analyses). The level of decomposition was recorded for each stomach, where; (i) stomachs were solid, no decomposition was observed; (ii) stomachs were partially decomposed, with a proportion of the sample in liquid form no longer contained within the stomach; and (iii) sample was decomposed and the sample bag was filled mostly with liquid and no possibility to distinguish between different parts.

Further, it was unclear how clean the sample bags or the outside of the stomachs were before storage, and this process was identified as a possible source of contamination. As mentioned before, no field blanks are available for these archived samples (the main aim was not to look for plastics), it was necessary to try ensuring that the outside of the stomach was treated differently to the inside. Therefore, partially decomposed stomachs should be treated with caution. As a result, some of the samples needed to be treated differently. Intact stomachs were taken from the bag and the outside of the stomach rinsed to remove any external contamination, this was combined with the bag rinse (C). The stomach was then opened and the contents flushed using KOH (A/B). Each sample bag was rinsed on the inside to ensure that all material was assessed (C). When samples were decomposed it often meant that the contents were just poured out only amounting to one sample (C). The volume of sample and KOH used was also recorded (see tables in *Appendix A* for details).

After preparation, additional KOH was added to each glass beaker to increase the volume and aid digestion. Samples were put in an incubator at 40°C, 120 rpm for 48 hours. Originally the time was 24 hours but digestion was incomplete in a number of samples.

Once samples were removed from the incubator, they were filtered through a 150

µm sieve to remove small particulate matter that could clog the filtering apparatus. This method was targeted at particles >200 µm.

Samples were rinsed with filtered RO water in the sieve. The remaining material was resuspended and filtered under vacuum (Büchner) onto 47mm Whatmann GF/C (1.2 µm) filter papers. The sides of the filter funnel were rinsed three times with filtered RO water ensure any particles adhering to the sides are washed onto the filter paper and sealed in a petri dish until dry for visual analysis.

Samples that could not be filtered due to excessive undigested biological material were put to one side for density separation. Samples were subject to two density separation steps, first a freshwater extraction using filtered RO water, and secondly a saltwater extraction (density of 1.4 g/cm³) with NaCl. For each density step the solution was added and the falcon tube containing the sample was vigorously shaken for 30 second. The sample was left to stand for 4 hours before filtering away the overlaying water.

3.3 Challenges and experiences

3.3.1 Access to equipment

Access to enough laboratory equipment (e.g. glassware) and an oven large enough for processing a suitable amount of samples was challenging in Iceland. A cooperation with a local company made it possible to process the samples in an industrial sized oven (Kerecis ehf).

In the Faroe Islands the local University provided access to glassware and two suitable ovens for the processing of the samples. The processing of the Faroe Islands samples was finalized in Norway. The successful processing of the samples in Norway was partly due to access to enough and suitable equipment. For example, the use of a filtration unit with sufficient power to process the digested solution.

3.3.2 Digestion and filtration of the samples

In Iceland there were difficulties experienced with especially the filtration of the samples. In the oven some of the samples formed a thick jelly solution or had a brown (slightly burned) layer floating at the surface of the solution (Figure 5). The thickness of the solution was tried to be resolved by neutralizing the sample with citric acid, which worked to some extent. The brown layer however was difficult to remove or filter. It is thought that this is formed by the chemical reaction between the fatty stomach wall and the KOH.

For the first attempt on the samples in the Faroe Islands a change was made to only use the stomach contents (not the stomach wall), increase the sample:KOH ratio to 1:10 and change the oven temperature and duration to 48 hours at 40°C. These initial changes to the method improved the quality of the solutions prior to the filtration process. There were no more issues of a 'thick' solution or brown layer floating on top. Also, the organic material seemed to be better digested when using the higher sample: KOH ratio. Unfortunately, the metal filters did not work as sufficiently as was hoped. It was difficult to make a good seal for the filtration unit

with the metal filters. This resulted in some leakage and the metal filters were no longer used during the first attempt on the samples in the Faroes Island. Even though the solution was better digested there was still very fine organic material left that clogged the Whatmann GF/C filters (Figure 6).

On the Faroe Islands a choice was made to change to acetic acid to neutralize the samples as this would in addition dissolve any potential calcium carbonate particles such as bone or shell fragments. This additional step, however, doubled the amount of solution that needed to be filtrated, and thus increasing the load for the filters which did not work satisfactory. This step was therefore removed from the protocol as the issues outweighed the potential benefits.

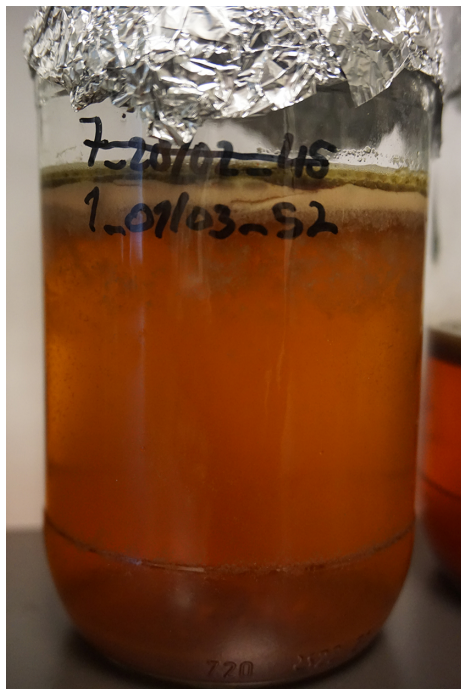


Figure 5: Floating brown layer after oven incubation step (picture by Anne de Vries).



Figure 6: Very fine organic leftover after filtration (picture by Sjúrdur Hammer).

4. Proposed methodology protocol for small-scale laboratories

Based on the experiences during the FINPlast project from the start in 2018 in Iceland to the processing of samples from the Faroe Islands in Norway in 2020 we summarize the proposed method for analyses of microplastics in fish digestive tracts in relatively simple equipped rural science communities. In *Appendix B* a list is included with an overview of the materials required for the execution of this methodology.

4.1 Digestion

Samples should be worked in batches depending on the size of the oven and the amount of shakers that are available. The protocol can be done without a shaker, though this can result in a less effective digestion.

1. Take the digestive tracks out of the freezer and let it thaw at room temperature.
2. Prepare the expected amount of 10% KOH solution (100 g KOH in 900 mL water).
 - a. For the preparation of the KOH solution and rinsing of laboratory equipment and filters prefiltered water is required. This can be made by filtering tap, demineralized or reverse osmosis treated water over glass fibre filters.
3. Place an environmental control (see 4.5 *Quality assurance*) near the workplace, open when the sample is exposed to air.
 - a. This control is used again during the filtration and the plastic analysis under the microscope of this batch.
4. Put some of the prepared KOH solution in a wash bottle.
5. Take a jar and label it with the sample ID.
6. Cut the stomach and intestines from the rest of the digestive tracks and rinse the outside of the stomach and intestines to remove any contamination.
7. Put the intestines in the jar.
8. Cut open the stomach, put content in the jar and rinse the stomach lining thoroughly with the KOH solution from the wash bottle into the jar.
9. Add the KOH solution as needed for a 1:10 sample:KOH ratio and wrap the top of the jar with aluminium foil.
 - a. Repeat steps 4–8 for the entire batch.
10. Prepare a process control (see 4.5 *Quality assurance*) with about 100ml of KOH solution.
11. Place the jars in the oven for 48hrs at 40°C on a shaker at 120 rpm.

4.2 Filtration

1. Remove the samples from the oven.
2. Prepare the filtration unit:
 - a. Make sure it is clean, inside and out, filtrate about a litre of water to clean the system.
 - b. Wrap the top with aluminium foil that can easily be lifted.
3. Prepare the petri dishes by writing the sample ID on the sides.
4. Place the environmental control of the corresponding batch near the workspace, and open when starting the filtration process of the batch.
5. Sieve the sample to remove the finer leftovers in the sample, the mesh size of the sieve depends on the lower detection limit in the study.
6. Rinse the residue from the sieve into a flask and resuspend it with prefiltered water.
7. Filtrate the solution over glassfibre filter(s), leave excess solid leftovers at the bottom.
8. Rinse off the sides of the filter funnel with prefiltered water to catch microplastics that might adhere to the sides.
9. Place the glassfibre filter in the petri dish for storage in a cupboard to dry or if necessary, in the freezer until further processing.

4.3 Density separation

In case there is still leftover that cannot be filtered sufficiently a density separation step can be added. Assuming the sample has a salinity close to freshwater the following steps can be followed for a density separation. Additionally, it is assumed that most of the organic material has been dissolved, sieved or filtrated off during the previous two processing steps. The protocol is based on, and adapted from, the work from Hurley et al. (2018) and is suitable for sediment samples from 1–35 grams.

1. Prepare a seawater density (1.4 g/cm^3) by dissolving as much NaCl as possible in water.
2. Transfer the residue of the sample to a falcon tube.
3. Add water for the first density separation (freshwater), shake rigorously for 30 seconds and leave for 4 hours before filtering off the top on a glass fibre filter.
4. Add the NaCl solution to the falcon tube, shake rigorously for 30 seconds and leave for 4 hours before filtering off the top on a glass fibre filter.

4.4 Visual and chemical plastic analysis

1. Place the environmental control for this batch next to the working space around the stereomicroscope.
2. Work through the sample systematically horizontally and vertically.
 - a. Note down for each particle its category, colour and length.
3. The environment control is analysed last.
4. Store the samples for chemical verification.

Any particles that have been visually identified as potentially plastic, or in case of many observation a randomly selected amount of samples, are afterwards analysed with for example an FTIR or Raman to confirm the chemical composition of the particle.

4.5 Quality assurance

4.5.1 Dual identification

Unless all of the particles are chemically confirmed by for example FTIR, dual identification is required to decrease potential bias in the identification process. First, one person performs the entire visual analysis as explained in 4.4 *Visual and chemical plastic analysis* and then the second person verifies whether the same particles are observed based on category type of the particle and colour though one should not rely too heavily on colour as it is a subjective particle characteristic. In the event when only one person observes a particle it should not be included in the final database.

4.5.2 Recovery rate test

To test for the effectiveness of the method in capturing the microplastics from the samples a recovery test should be done. A 75g digestive tract sample is spiked with a known number of plastic particles and processed as described in the previous paragraphs.

4.5.3 Contamination verification

Digestive tract collection environmental control

During the sampling of the digestive tracts an environmental control is placed near the handling station (field air blank). The environmental control is a petri dish with a wetted filter placed inside it. Depending on the sampling method one can choose to have an environmental control per net raised, or per work shift.

Laboratory environmental control

Each batch of samples should have at least one environmental control (laboratory air blank), meaning at least one per approximately ten samples. The controls are wetted coffee filters in petri dishes. These controls should be used during the processing of the samples in the laboratory, and during the visual analysis every time samples are exposed to air.

Laboratory process control

Potential contamination occurring during the processing of the samples is accounted for by means of a procedural blank. The process control is handled the same as the samples, but without the addition of a digestive tract sample.

4.5.4 Preventive measures

In addition to the various contamination controls, measures should be taken aimed at preventing contamination.

- During the processing of the digestive track samples in the laboratory all materials used should be cleaned in-between samples with tap water and soap and dried with paper towels to limit cross contamination.
- Clothes worn in the laboratory should be non-polymer based.
- Whenever possible non-plastic laboratory materials should be used.
- To prevent air contamination during digestion and filtration, the sample flasks/ jars and filtration beakers should be covered with aluminium foil to reduce air exposure. This should also be done with all jars in the oven if the jar lids have a plastic lining on the inside.

5. Iceland Case Study

Prior to the start of the FINPlast project a study was done on the microplastic ingestion by cod and saithe from the west coast of Iceland (de Vries et al., 2020). The results are shown as an example of implementation of a relatively simple methodology in a rural area with minimal laboratory resources. The methodology applied is the one described in 3.1 *The Icelandic samples*.

In the study by de Vries et al. (2020) the Fulton's Condition Index (CI) was used as an indication of the health of the fish with 1 being a fish in perfect health, <1 indicates a 'slim' fish, and >1 a 'fat' fish. This was calculated by the means of dividing the weight (W) in grams by the length (L) in centimetres to the power 3 and multiplying the outcome by 100 as shown in equation 1 (Nash, Valencia, & Geffen, 2006).

$$1. K = 100 \times \frac{W}{L^3}$$

A total of 39 cod and 46 saithe were analysed for microplastic occurrence in their stomachs and intestines. The saithe were on average smaller (59 cm; 1.95 kg) than the cod (85.3 cm; 6.09 kg), and the cod were older (average: 7–8 years) than the saithe (4–5 years) (Table 2). Finally, the CI of cod was higher (0.96) compared to the saithe (0.86). Of the 39 cod digestive tracks that were analysed, 20.5% contained anthropogenic particles, while of the 46 saithe digestive tracks 17.4% contained anthropogenic particles (Table 2). On average there were 0.23 anthropogenic particles per individual in cod, and 0.28 anthropogenic particles per individual in saithe. Among the detected microplastics one polypropylene (PP) and one polyethylene (PE) particle were confirmed by FTIR in cod, and one particle of PP was confirmed in saithe. The other particles could not be confirmed by FTIR as they were too small for analysis, but they were confirmed through dual ID instead.

Table 2: Overview of the detection of anthropogenic particles in the cod and saithe from the west coast of Iceland (de Vries et al., 2020). The average length, weight, age, and condition index have been included.

	Cod (n=39)		Saithe (n=46)	
	Average	Range	Average	Range
Length (cm)	85.3 (SD=8.7)	69–106	59 (SD=11.7)	40–83
Weight (kg)	6.09 (SD=1.80)	3.16–10.01	1.95 (SD=1.25)	0.54–5.47
Age (based on weight-age relationship data MFRI (2018))	7–8	6–12	4–5	<3–8
CI	0.96 (SD=0.09)	0.68–1.16	0.86 (SD=0.07)	0.71–1.00
Total number of fibres	5		8	
Total number of fragments	4		5	
Total number of sheets	0		0	
Total number of beads	0		0	
Particle occurrence per species (%)	20.5		17.4	
Average number of particles/individual fish	0.23		0.28	

As was indicated earlier the use of a contamination protocol is paramount in microplastics research. This has become evident when comparing the corrected and uncorrected results of the study (Figure 7). If the results would not have been corrected for the recorded (microplastic) contamination the particle occurrence would have undoubtedly been overestimated.

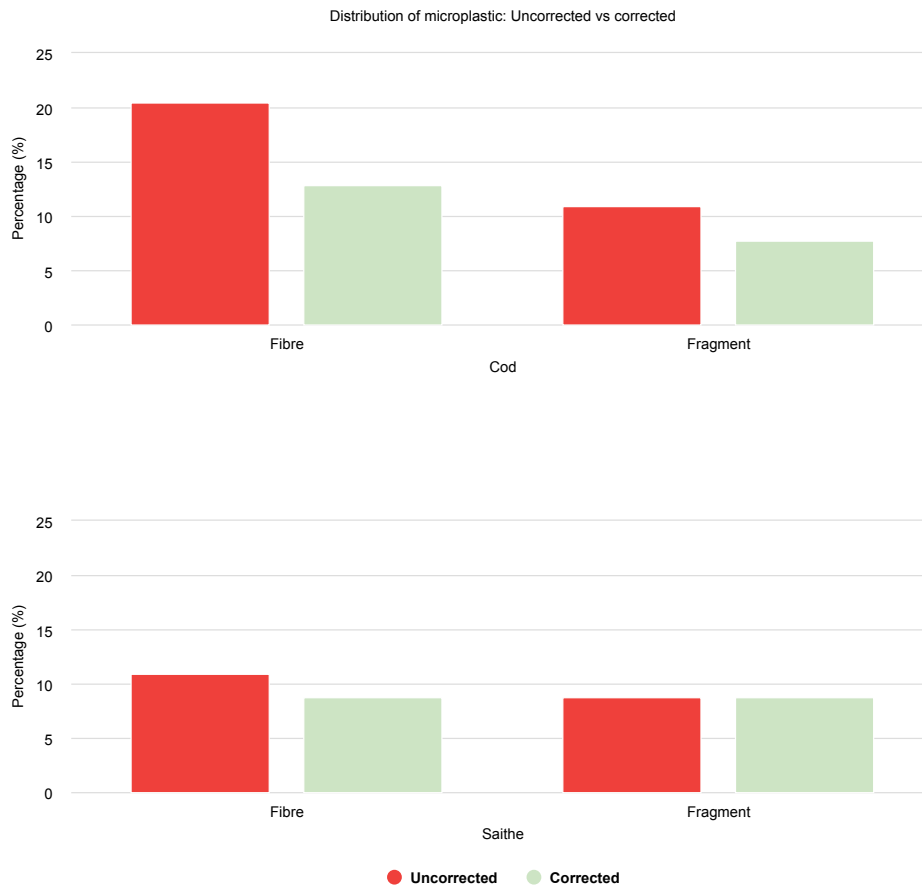


Figure 7: The relative distribution of microplastic in cod and saithe (in percentage) in the Iceland study (de Vries et al., 2020). The uncorrected results and corrected results are shown side-by-side to indicate the importance of using a contamination protocol.

The statistical analysis did not indicate a significant difference in particle occurrence between the two species. Neither did any of the biological parameters (length, weight, CI, digestive track fullness) explain the presence of particle or total number of particles observed. When highlighting solely the fish containing anthropogenic particles a difference was identified between the species. All cod containing anthropogenic particles were longer than the maturity length (i.e. length >71.4 cm (Marteinsdottir & Begg, 2002), while not all saithe with anthropogenic particles were longer than maturity length (i.e. length >58.4 cm (Tu et al., 2018)).

6. Faroe Islands Case Study

6.1 Data summary

From the Faroe Islands a total of 20 cod stomachs were analysed. The cod were on average 49.6 cm in length and weighed 1,44 kg. The cod had an average condition index of 1,13 and were 2 years old. See Table 3 for an overview of general information concerning the cod samples and the detected anthropogenic particles. To be noted is that only the data from (A) fluid stomach content and (B) solid stomach content is included in the overview due to the high likelihood of contamination from in the (C) bag +rinse and the "whole sample" category. The (C) the bag + rinse is specifically meant for removing any potential contamination that occurred during the handling of the samples prior to the laboratory processing. As for the "whole sample", it was not possible to distinguish between content within the bag. This made it impossible to distinguish contamination from the actual particle occurrence in the digestive track samples.

Table 3: Overview of the detection of anthropogenic particles in cod from the Faroe Islands. The average length, weight, age, and condition index of the fish samples have been included. Data from the A and B sample categories have been used for the anthropogenic particle information.

	Cod (n=20)	
	Average	Range
Length (cm)	49.6 (SD = 5.7)	40 – 58
Weight (kg)	1.44 (SD = 0.5)	0.63 – 2.30
Age (based on length-age relationships conform OSPAR guidelines)	2–3	2–4
CI	1.13 (SD = 0.10)	0.95 – 1.32
Sample category	Fluid stomach content (A) (n=15)	Solid stomach content (B) (n=12)
Total number of fibres	46	12
Total number of fragments	2	0
Average particle occurrence (%)	60	33
Particle length (µm)	150 – 5989	552 – 1831
Average number of particles/individual fish	3.3	1.0

6.2 Contamination control and sample condition

Only one of the air blanks (n=20) contained a single anthropogenic fibre, showing the work within the laminary air flow bench did not introduce airborne contamination of major impact at this stage of processing.

Three batches of procedural blanks were processed. Batch 1 (n=3) and Batch 2 (n=3) did not contain any anthropogenic particles. Batch 3 (n=3) contained a single fibre on each filter.

During density separation, none of the freshwater (n=3) nor saltwater blanks (n=3) contained anthropogenic particles.

The condition of the stomach samples varied with 60% of the samples (n=12) being in a solid condition meaning that the stomachs could be taken out of the bag, rinsed from the outside and then cut open to reveal stomach contents. A further 10% of the samples (n=2) were partially decomposed meaning that some of the stomach content was outside of the stomach. The remaining 30% of the samples (n=6) were very decomposed with much of the stomach content outside of the stomach, in 4 instances the stomach lining was no longer distinguishable from the sample, and thus these samples were processed as a "whole" sample.

6.3 Visual identification

See Table 4 for the number of particles per sample category. Of the 20 stomachs, 16 could be opened and the stomach assessed for anthropogenic particles with either fluid or solid stomach content.

(A) Of the stomach flushes containing liquid samples (n=15), 9 contained anthropogenic particle (range 0–10). There were 47 particles identified in total, two of these were fragments, the rest were fibres (96%). Sizes ranged from 150 μm to 5989 μm .

(B) Of the solid contents (n=12), only four of the samples contained anthropogenic particles (range 0–4). A total of 12 particles were identified. They were all fibres ranging in sizes from 552 μm to 1831 μm .

In total 16 of the bags were rinsed. (C) This includes those where there was some stomach content in the bag but the stomach was still distinguishable. A total of 99 particles were identified (range 0–19). The size of the particles ranged from 120 to 3666 μm .

Four of the bags were processed as whole samples. Two of these did not show any particles the remaining had 9 and 11 particles, of which were fibres in the size range from 207 to 7878 μm .

Table 4: Number of particles identified in each sample with processing observations included. The "-" indicates when no sample was available in this category.

Sample name	(A) Fluid stomach contents	(B) Solid stomach contents	(C) Bag Rinse/ external stomach rinse	Whole	Comments
GM0404	5	-	6		Stomach was empty
GM0405	5	0	4		
GM0407	0	3	10		
GM0415	0	0	19		
GM0454	9	-	4		Stomach was empty
GM0455	10	-	13		Bag was leaking, poss. sample loss
GM0622	3	-	4		
GM0625	0	0	9		
GM0458	7	0	0		After D2.
GM0465	0	4	6		
GM1407	-	-	-	9	Extra KOH added - WHOLE
GM1408	-	-	-	0	WHOLE
GM1410	0	0	5		
GM1411	-	3	0		
GM0416	0	0	6		
GM0460	5	2	5		
GM0599	4	0	5		
GM0601	1	0	3		
GM0624	-	-	-	11	Could not distinguish stomach – WHOLE
GM1409	-	-	-	0	Could not distinguish stomach – WHOLE

See Figure 8 for a boxplot indicating the range of particles found per sample and the average number of particles per sample category.

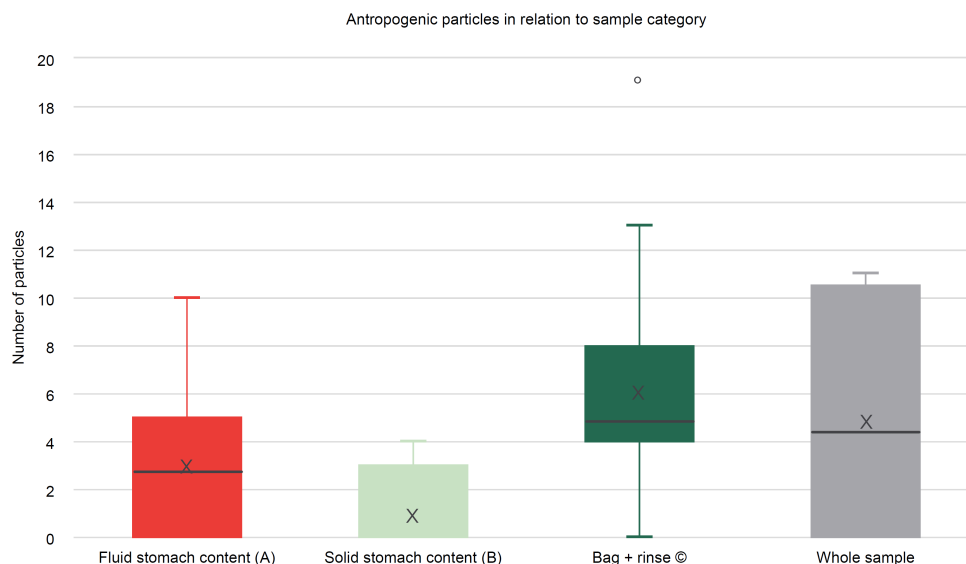


Figure 8: Anthropogenic particles observed (n) per sample category. The coloured box shows where 50% of the data is located, with the whiskers indicating the lowest and highest value observed excluding the outliers. The outliers are indicated by points, and the mean are marked by X.

6.4 Chemical identification

Based on the data available it was necessary to compare the values using an Attenuated Total Reflection (ATR)-FTIR. This allowed for identification of the polymer make-up of the particles. To avoid bias, all particles were assigned a unique number which were subsequently entered into a random number generator by one researcher. A proportion of the particles from each category (A, B, C, Whole) were subsequently run through chemical identification by another researcher.

A total of 37 (20%) particles were assessed. The polymer breakdown is displayed in Figure 9. Almost half of the particles (17) were identified as cellulose (45%), which is often hard to distinguish between natural cellulose and modified cellulose, a further 22% (8 particles) were viscose, a form of chemically modified cellulose, 16% (6 particles) were cotton, and 8% (3 particles) polyester. Finally, 2% (1 particle) was composed of acrylic – the only fully synthetic particles and the remaining 5% (2 particles) were of a cotton-elastane blend.

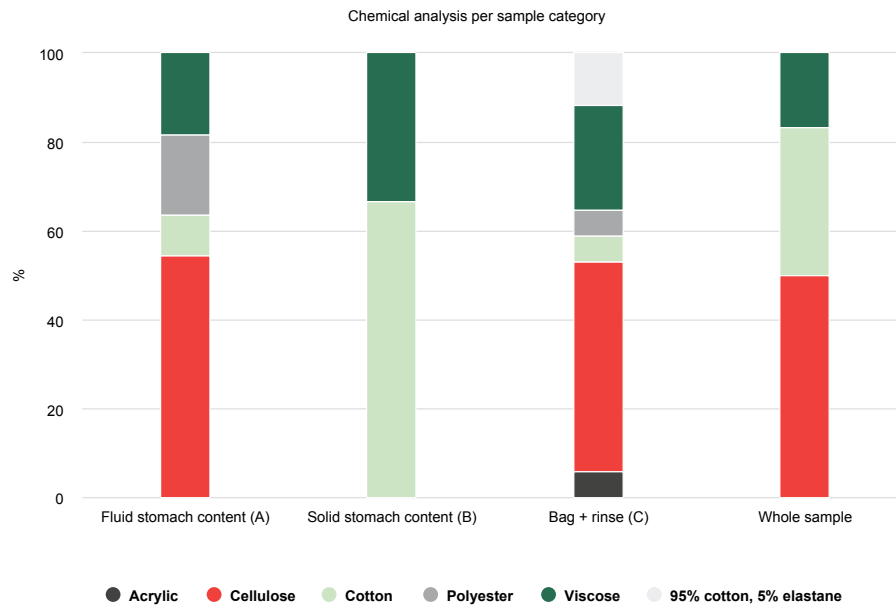


Figure 9: Chemical identification of 20% of the visually identified particles with 5 different polymers identified over the four different sample categories.

7. Discussion

7.1 The need for method standardization

Plastics have been found in the marine environment for decades and have now become so widespread that it is observed in every ecosystem (Browne et al., 2011; Lebreton et al., 2018; Peeken et al., 2018; Peng et al., 2018). For scientists to be able to record data on plastic occurrence in this wide range of habitats and species there have been an equally vast array of methodologies developed. Each matrix requires its own considerations in terms of the methodology that is applied.

Studies concerning plastics in fish go back as far as the 1970s (e.g. Carpenter & Smith, 1972; Markic et al., 2019) and current research is fuelled in many cases by understanding the consequences that plastic could have on wild fish populations in terms of sustainable food production (Gamarro, et al., 2020; Lusher & Welden, 2020). With the need for, and will to, expand our knowledge on plastic ingestion by fish there has been a whole range of approaches developed to research the subject. There are many successful ways to gather data on microplastic ingestion by fish, however, the main problem with this is, that studies have become difficult to compare as there are too many things to consider for comparisons between studies. The first steps towards harmonising methodological approaches has begun by researchers striving to optimising methods (Avio, Gorbi, & Regoli, 2015; Cole et al., 2014; Dehaut et al., 2016; reviewed in Markic et al., 2019), but still a lot of questions remain especially in terms of validating different methods to ensure they can be compared effectively (Cowger et al., 2020; Lusher et al., 2020a,b; Markic et al., 2019).

7.1.1 Standardization in sample collection

Standardization starts with the collection of the samples to be analysed. With the aim is to be able to monitor the status of the environment the question of which species to use as an indicator is important to explore. When considering a species for plastic monitoring in the marine environment, the feeding behaviour, habitat preferences and life stage should be taken into consideration. These components are closely interlinked and variations can create problems in standardizing data. For example, one species can have different habitat preferences and feeding behaviour at different stages of its life. As a fish grows continuously over its lifespan, length can be a substitute for life stage/age. Differences in length and thus life stage/age within one species of different lengths, especially juveniles versus adults, could potentially yield different plastic ingestion rates due to their differences in behaviour. Selecting fish based on their length decreases the influence of different feeding behaviours. Young cod for example prefer the benthic environment and have a diet with more crustaceans compared to older cod, which are more benthic-pelagic dwellers and have a more fish based diet (FishBase, 2018). Similarly, different species have incredibly varied life histories, so the same amount of plastic in two similar-sized species can have a very different applied meaning. Therefore, putting a

list together of suitable indicator species depending on their habitat choice and sampling a specific length will standardize the studied group.

Studies on plastic occurrence in the Northern Fulmar (*Fulmarus glacialis*) (Kühn & Van Franeker, 2012; Trevail et al., 2015) has become the standard for monitoring plastic pollution within OSPAR (Van Franeker, 2019). The Northern Fulmar was considered a suitable monitoring species as it is a surface feeder and therefore can tell something about the developments in the occurrence of floating plastics in the marine environment. To evaluate the occurrence of plastics in coastal waters there has been developments in studies with filter feeders such as muscles, oysters and clams (Bråte et al., 2020; Lusher et al., 2017a). As they are often sessile or have a small distribution range, and additionally are not selective in what they consume, the data is thought to give a good indication of plastic occurrence in coastal waters. However, further offshore it could be more prudent to consider a benthic fish species to research plastic occurrence at the seabed since filter feeders are less common in deeper water or on soft substrates.

Also to be considered is the continuous discussion regarding sample size. There is evidence that with an increased sample size there is a higher detection of plastics (Markic et al., 2019). There has been a significant positive correlation found between sample size up to 10 individuals and plastic detection. The Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic and the MSFD-TSML recommended researchers to use 50 individuals per site and is supported by recent reviews (e.g. GESAMP, 2020; Markic et al., 2019; MSFD-TSML, 2013). However, the number of individuals chosen are often also linked to the complexity of processing samples.

7.1.2 Standardization in processing samples

The main method utilised for (micro)plastic ingestion research in fish was at first visual sorting by dissecting the fish (reviewed in Lusher et al., 2017b; Lusher et al., 2020b). In particular, there was a specific focus on stomach contents and digestive tracts of fish (e.g. Anastasopoulou et al., 2013; Bråte et al., 2016; Lusher et al., 2013; Murphy et al., 2017). In the past years especially, this has become less common (Markic et al., 2019). The desire to also investigate plastic particles <5mm (microplastics) required a different approach. This mainly because visual sorting is limited to the visual acuity of the researcher and thus only to be relevant to be considered for particles >500 µm (Isobe et al., 2019; Lusher et al., 2020b).

Digesting the organic matter and filtering the sample aims to provide the researcher with a 'cleaner' sample to analyse. This has resulted in a higher detection of (micro)plastics in fish (Markic et al., 2019). By observing a 'cleaner' sample it becomes easier to identify anthropogenic particles among the organic and other inorganic matter. Additionally, it allows for analysis of smaller particles. As became clear in section 3 there are many ways of digesting the samples. Some are relatively easy and needs minimal (financial) resources while others are complex and consequently requires more technological expensive materials and expertise.

For the filtration step, it is important to consider which size range to consider for monitor plastic occurrence in fish. Factors such as matrix, mesh size and filtration equipment need to be considered. Based on this a decision needs to be made on the lower size limit in the studies. When mesh size for sieves and/or filters in different

studies varies, it becomes difficult to compare (micro)plastic findings between studies unless the size distributions of the particles are known (de Vries et al., 2020).

When choosing a suitable standardized sample processing method, the main goal should be a method that effectively digests organic biological matter with no or minimal damage to the subject of study – the plastic polymers – and separates remaining biological matter from the microplastics. As secondary goal, a relatively simple but robust method that requires minimal amount of (financial) resources is preferred. In keeping the methodology straightforward, it will increase the potential for smaller (scientific) communities to participate and provide their input into understanding and monitoring microplastics and not to keep the research within elite, financially well supported institutes.

7.1.3 Standardization in data recording

There are many subjects to consider in making microplastic studies comparable and reproducible. It is paramount to provide as much information as possible. Cowger et al. (2020) published a checklist on guidelines curated by a global network of multi-disciplinary researchers actively participating in microplastics research. It included e.g. laboratory policies (clothing, glassware cleaning), calibration of instruments, data on study subject (length, catch location, date, time of collection), particle descriptions (length, shape, colour), controls (positive controls, replicates, contamination protocol), etc.

To the same extent as transparent reporting procedures, researchers must also strive to generate their data in a repeatable and comparable way. Currently, most of the plastic occurrence data in research is based on visual identification of microplastics after either visual sorting or chemical processing of the samples (Lusher et al., 2020a,b). Visual identification alone is highly subjective and relies solely on the visual acuity on an individual. Dual identification decreases the influence of subjective observations. The use of chemical identification provides a much higher level of confirmation regarding whether a particle is plastic and which kind of plastic material it is (Primpke et al., 2020). Unfortunately, it can be difficult to isolate and analyse small or thin particles, such as fibres with e.g. FTIR or Raman spectroscopy.

In addition, one should be cautious and minimise the dependence on and interpretation of colour and state of the particle in the assessment of plastic identification and contamination verification. Colour and state are subjective recordings and even with dual identification can lead to discussion on categorizing particles as plastic or not. Besides, these might be affected by the treatment such that the original colour is bleached, or the state of the particle is severely affected.

There have been developments regarding standardization on particle identification and data recording (Lusher et al., 2020a). The two main parameters to record about a particle are its size and shape. Among the many publications there is a lack of consistency in recording the sizes and shapes of plastics. In general, plastics can be placed into classes based on size from macro- (>5mm), micro- (<5000–0.1µm) to nanoplastics (<0.1µm) (Lusher, et al., 2017). When considering recording the sizes of particles measuring particles along their longest diameter can give a skewed image on how large particles are. For example, if a fibre is measured along its longest dimensions it can lead to categorizing them as large particles while measuring them

over their diameter will place them in a much smaller size class. With this in mind it has been suggested to use Feret's diameter as an appropriate measure (as reviewed in Lusher et al., 2020a). Feret's diameter is based on the slide gauge principle where it is defined as the shortest possible length measurement of an object.

Besides size there are different shapes a plastic can occur as. Numerous terms to describe morphology are used such as sheets, beads, pellets, sphere, granule, fragment, films, fibres, filaments, and foams to name some of the common ones (Lusher et al., 2020a). In an attempt to standardize the recordings of this parameter it is suggested to record the main category as either bead, fibre, or fragment and within these categories further differentiate by using sub-categories.

The final subject consider is how to categorize and control for the occurrence of fibres in (micro)plastic studies. Fibres, whether they are plastic or not, are in general of anthropogenic origin, e.g. (treated) wool and cotton from textiles. Since these non-plastic fibres are not occurring naturally, it is important to report fibres in studies since it provides knowledge regarding their fate, occurrence, and distribution. However, instead of reporting them as plastic particles, the scientific community has begun to use 'other anthropogenic particles' or similar terms with subclasses such as fabrics, processed wood, and metalwork in scientific articles.

7.1.4 Standardization of contamination protocols

Contamination from surrounding air is of high concern in microplastic analysis (Hermesen et al., 2017; Torre et al., 2016; Wesch et al., 2017). The main problem in microplastics research is contamination by fibres from the working environment. As they are small and light, transportation through air occurs easily. In general, there are some common approaches adopted across studies regarding preventive measures such as (i) covering exposed samples with lids or foil, (ii) rinsing materials thoroughly with filtered water in between samples, (iii) wearing cotton during laboratory work, (iv) decreasing air flow or working in a clean-air cabinet and (v) decreasing handling of the samples as much as possible since each time a sample is exposed there is risk of (air) contamination. Besides preventive measures one must be able to verify the contamination that occurred. The best practice is implementation of environmental and procedural blanks in the same way as is the routine in trace chemical analysis.

7.2 Potential obstacles in small-scale laboratories

One of the main issues observed during the Icelandic samples was the formation of a brownish layer floating on top of the solution which occasionally burned in the oven. This has also been observed during a study by Enders et al. (2017). This made the filtration especially difficult. It is thought that this is because of the fat in the cod stomach wall. KOH is known to have difficulties with digesting hard and fatty materials (Enders et al., 2017). Possible solutions are adding citric acid to neutralise the sample before filtration (Thiele et al., 2019), treating the leftover portion of the sample with nitric acid (HNO_3) to digest the remaining organic matter and potential calcareous materials (Lusher et al., 2020b; Schirinzi et al., 2020), or removing the stomach wall and only using the stomach content as was adopted in this study.

In the Faroe Islands samples improvements were seen in the overall digestion of stomach samples by only using the stomach contents and intestines. Besides removing the problem of the fatty stomach wall, it also decreased the digestive load and consequently improved the digestion and therefore the filtration. The improvements in the digestion of the Faroe Islands samples were also due to the increase of the KOH:sample ratio and increase of the incubation period in the oven at a lower temperature.

In using a sieving step prior to filtration of the Faroe Islands samples some of the smaller (organic) matter was washed out. This decreased the load for the filtration unit. The pore size of the sieves is dependent on the lower detection limit in the study which is dependent on the aim of the study itself. By removing the smaller matter from the sample and using glass fibre filters with a larger pore size led to less clogging. Besides the selection of applicable filters for the study it is recommended to use a suitable filtration system with high enough capacity for this type of samples. Additionally, a density separation following the first filtration works well to separate particles from biological material. It allows to capture 'locked' particles in the leftover undigested material.

Especially fibrous particles are incredibly complex to work with when assessing them in environmental samples. As already discussed, they can come from procedural contamination through the settling of airborne fibres or from the clothing of the researcher performing the work.

The methodology used for this project was designed to target particles >200 µm, therefore visual ID could be suitable for fragments supported by ATR-FTIR, unfortunately the ATR-FTIR is not always suitable for fibres due to their thin shape. It is difficult to determine the difference between natural based fibres and plastic fibres by visual analysis alone. Visual analysis can be used to help differentiate, but it is less reliable compared to chemical analysis. From both studies described in this report it became evident that with a complete quality control protocol it is possible to include fibres in the study.

8. Concluding remarks

The aim of the present study was to provide a best practice guide for the standardization of the analysis of plastics in fish stomach. The method described can be applied in small-scale laboratory with minimal infrastructure, is relatively cheap and easy to implement.

The present study has contributed towards making studies comparable and facilitate monitoring of the Atlantic marine environment regarding plastic pollution. To achieve this, we have developed a methodological protocol which can be applied where laboratory resources are limited.

The method protocol is focused on small-scale laboratories and reducing the need for specialized equipment. Hence, the major equipment needed are an oven large enough to process a reasonable amount of samples in one, a sufficient filtration unit, a microscope and access to chemical analyses, e.g. FTIR, Raman or pyrolysis-gas chromatography. The chemical analyses can be performed in collaborating laboratories with such resources available.

The FINplast project has brought in new knowledge on plastic in cod stomachs from Iceland, the Faroe Island and Norway. Since Norway is one of the countries at the forefront of method development and data gathering on microplastics in the environment our focus was mainly on establishing a method in the Faroe Islands for microplastics in fish and share knowledge. A comparison between cod stomachs in these countries showed a distribution of microplastics particles ranging from 0–27% in detection frequency. The large range is mainly due to different site types included (e.g. open ocean versus harbour and coastal areas).

Finally, the FINPlast project aimed to transfer knowledge between countries and institutions. Despite the worldwide difficulties around the COVID-virus we have managed to keep a continuous dialogue among the participating institutions. The combined efforts led to a suitable proposed methodology which can be applied in small-scale laboratories.

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Appendix A Metadata Faroe Islands case study

Table A1: Sample details. State of samples i – solid, ii – partially decomposed, some solid content still present, iii- decomposed and the bag content was pure liquid.

Sample name	Date sampled	State of samples	(A) Fluid stomach contents	(B) Solid stomach contents	(C) Bag Rinse/ external stomach rinse	Air blank	Density separation needed?	Comments
GM0404	Oct. 2008	ii	Yes	No	Yes	Yes		Stomach was empty
GM0405	Oct. 2008	i	Yes	Yes	Yes	Yes	Yes (B)	
GM0407	Oct. 2008	i	Yes	Yes	Yes	Yes	Yes (A,B)	
GM0415	Oct. 2008	i	Yes	Yes	Yes	Yes	Yes (B,C)	
GM0454	Oct. 2010	i	Yes	No	Yes	Yes	Yes (B)	Stomach was empty
GM0455	Oct. 2010	iii	Yes	No	Yes	Yes		Bag was leaking, poss. sample loss
GM0622	Oct. 2016	iii	Yes	No	Yes	Yes		
GM0625	Oct. 2016	i	Yes	Yes	Yes	Yes	Yes (A, B)	
GM0458	Oct. 2010	i	Yes	Yes	Yes	Yes	Yes (A,B,C)	
GM0465	Oct. 2010	i	Yes	Yes	Yes	Yes	Yes (B)	
GM1407	Oct. 2018	iii	No	No	Yes	Yes	Yes (C)	Additional KOH added
GM1408	Oct. 2018	iii	No	No	Yes	Yes	Yes (B)	
GM1410	Oct. 2018	i	Yes	Yes	Yes	Yes	Yes (B)	
GM1411	Oct. 2018	i	No	Yes	Yes	Yes	Yes (A, B)	
GM0416	Oct. 2008	ii	Yes	Yes	Yes	Yes	Yes (A, B)	
GM0460	Oct. 2010	i	Yes	Yes	Yes	Yes	Yes (B)	
GM0599	Oct. 2016	i	Yes	Yes	Yes	Yes	Yes (B)	
GM0601	Oct. 2016	i	Yes	Yes	Yes	Yes	Yes (B)	
GM0624	Oct. 2016	iii	No	No	No	Yes	Yes (C)	Could not distinguish stomach
GM1409	Oct. 2018	iii	No	No	No	Yes	Yes (C)	Could not distinguish stomach

Table A2: Volume of sample and amount of potassium hydroxide (KOH) added (measured in g).

Sample category		A		B		C		Comments
Sample name	Date sampled	Liquid stomach contents	KOH	Solid stomach contents	KOH	Liquid in bag (+ bag rinse)	KOH	
GM0404	Oct. 2008	15.40	95.40	-	-	20.45	64.79	Additional KOH was added to each glass beaker to supplement the digestion- a total of 150–250 ml combined sample went into the incubator. The additional volume was not recorded
GM0625	Oct. 2016	0.99	86.75	5.53	55.0	16.39	48.52	
GM0415	Oct. 2008	-	99.12	5.92	60.0	5.79	49.20	
GM0407	Oct. 2008	-	117.66	4.00	30.0	8.51	45.64	
GM0454	Oct. 2010	-	69.38	-	-	-	46.20	
GM0622	Oct. 2016	-	75.29	-	-	25.58	98.00	
GM0405	Oct. 2008	-	91.07	5.65	56.0	-	70.56	
GM0455	Oct. 2010	-	89	-	-	3.00	50.00	
GM1410	Oct. 2018	-	-	8	90	34.10	150.00	
GM1411	Oct. 2018	7	70	15	90	-	50	
GM0465	Oct. 2010	10.44	100	7	30	-	65	
GM1408	Oct. 2018	-	-	-	-	65	150	
GM1407	Oct. 2018	-	-	-	-	30	200	
GM0458	Oct. 2010	-	71.8	8	80	3	45	
GM0460	Oct. 2010	-	79.68	11.51	10.78	-	60.01	
GM0416	Oct. 2008	-	92.60	7.74	82.48	18.57	68.56	
GM0599	Oct. 2016	-	112.85	3.53	85.73	7.72	74.41	
GM0624	Oct. 2016	-	-	-	-	36.05	87.67	
GM1409	Oct. 2018	-	-	-	-	34.01	100.82	

Appendix B Material List proposed method

In the table below there is a list of the equipment and chemicals needed. The amounts can vary depending on the sample size and processing methodology. This list is based the methodology described in 4. *Proposed methodology*.

Table B1 List of materials for microplastic analysis in fish stomach.

Material
General materials
Latex gloves
Lab coat
Eye protection
Scalpel
Scalpel blades
Tweezers
Weighing scale (g)
Aluminium foil
Stirring rod/spoon
KOH and NaCL solution preparation
Beaker for weighing KOH
Funnel for preventing spilling and splashing
Mixing beaker for KOH solution (2L)
Demineralized/Reverse osmosis treated water (KOH, density separation, rinsing materials)
Falcon tube
KOH[s]
NaCl[s]
Digestion and density separation
Glass beakers or jars for digestion (600 mL)
Wash bottle for rinsing outside and inside of stomachs with KOH (100 ml)
Oven able to hold 20 jars at a time
Shaker

Filtration and (visual and chemical) identification

Petri dishes for storing filters and controls

Sieve (mesh size depending on lower detection limit in study)

Glass fibre filters (pore size depending on study, here assumed 1.2 µm)

Filtration unit

Vacuum pump

Stereomicroscope

FTIR (or other, suitable instruments)

Appendix C Fact sheet – English

PLASTIC IN ATLANTIC COD STOMACHS

Scientists have developed various methods to investigate plastic particles in fish stomachs, but the results from different methods can't always be compared. Some laboratories have highly-skilled researchers and high-end instruments, while other laboratories are small and have fewer resources. To be able to compare results, we developed a method that works for everyone.

Why do we care?



WIDER PUBLIC
Cares about the food we eat and the environment



FISHING INDUSTRY
Cares about the quality of their product



POLICY MAKERS
Need good scientific data to make decisions on food safety and environmental protection



SCIENTISTS
Need reliable standardized method to monitor plastic in fish and the environment

The best method so far



DISSOLVE GUT



FILTER



INSPECT & IDENTIFY

1. Divide up stomach and its content.
2. Use an alkaline solution (potassium hydroxide) to remove biological material and leave only plastics.
3. Place in the oven at 40°C for 48 hours to speed up the process.
4. Sieve the liquid to remove any remaining biological material.
5. Wash the sieve contents onto a very fine filter.
6. Add a high density liquid to the leftovers and let heavier pieces sink.
7. Filter the water over the very fine filter (not the solids at the bottom!).
8. Look under a microscope for plastic particles.
9. Confirm plastic particle findings by analytical machines.

What we know

Between Norway, Iceland, and Faroe Islands, anywhere from 3-27% of cod have plastic in their stomachs.

* We developed the method along the way, so our results are not comparable, but we share them here for general information.

We still have questions

- + Does the amount of microplastics in cod guts correspond with fish size, gut fullness, or body condition? And how would other species differ?
- + Does it matter when and where the fish are caught? How can we better involve the fishing industry in this research?
- + Is it only plastics we should be exploring? How do we account for cotton and other man-made items?

 Nordic Council of Ministers

 Háskólaásetur Vestfirðja
University Centre of the Westfjords

 umhvervisstofan
environment agency

 NIVA
Norwegian Institute for Water Research

AUTHORS: Anne deVries, Pernilla Carlsson, Catherine Chambers, Sjúður Hammer, Rachel Hurley, Amy Lusher, and Jónur Póldóttir.
This project was funded by the Nordic Council of Ministers.

Appendix D Fact sheet – Icelandic

PLAST Í MÖGUM ÞORSKA Í ATLANTSHAFINU

Margar aðferðir hafa verið þróaðar af vísindafólki til að rannsaka plast í mögum fiska en ólíkar aðferðir standast ekki alltaf samanburð. Stórar rannsóknarstofur geta verið útbúnar fullkomnum tækjum og mjög sérhæfðu starfsfólki á meðan smærri rannsóknarstofur hafa oft úr litlu að möða. Til þess að geta borið saman niðurstöður ólíkra aðila höfum við þróað aðferð sem hentar öllum.

Hvers vegna er okkur ekki sama?



ALMENNINGUR
Lætur sig umhverfið varða og einnig matinn sem við borðum



FÓLK Í SJÁVARÚTVEGI
Er umhugað um gæði sinnar vöru



RÁÐAFÓLK
Þarfnast vísindalegra gagna til þess að taka ákvarðanir í tengslum við gæði matvæla og umhverfisvernd



VÍSINDAFÓLK
Þarf áreiðanlegar, staðlaðar aðferðir til að vakta plast í fiski og umhverfinu

Besta aðferðin hingað til



LEYSA UPP INNYFLI



SÍA



SKOÐUN OG GREINING

1. Aðskilið maga og magainnihald.
2. Notið basa (kalíumhýdroxíð-laun) til að leysa upp líkamsvefi og annað lífrænt efni svo aðeins plast verði eftir.
3. Látið sýnin standa í ofni við 40°C í 48 klst. til að hraða ferlinu.
4. Sigtíð vökvann frá sýninu til að fjarlægja upplestar lífrænar leifar.
5. Skolið það sem eftir situr í sigtinu á fingerða síu.
6. Bætið saltvatni saman við afgangsfnið og látið þyngri agnir sökkva.
7. Síð saltvatnið í gegnum fingerðu síuna (ekki fasta efnið á botninum).
8. Rannsakið sýnið undir smásjá til að finna plastagnir.
9. Notið greiningartæki til að staðfesta hvort um plastagnir sé að ræða.

Vitað er að

Við Noreg, Ísland og Færeyjar eru 3-27% þorska sem veiðast með plast í maganum.

* Aðferðirnar voru þróaðar samhliða rannsókninni svo þessar niðurstöður eru ekki fulllega sambærilegar milli landanna þriggja en eru þó settar hér fram til glöggvunar.

Nordic Council of Ministers

Háskóla- og Vestfirðis Háskóla- og Vestfirðis University Centre of the Westfjords

umhvervisstovan environment agency

NIVA Norsk Institutt for vannforskning Norwegian Institute for Water Research

Fleiri spurningar vakna

+Er samband á milli fjölda plastagna í þorskmögum og stærðar fiska, maga- fylli eða ástands? Hvernig kæmu aðrar fiskitegundir út í samanburði við þorsk?

+Skiptir máli hvernig fiskurinn var veiddur eða hvenær? Hvernig má tengja þessar rannsóknir betur við fiskiðnaðinn?

+Ætti að einblína á plast í þessum rannsóknum? Hvað með bómull og önnur manngerð efni?

HÖFUNDAR: Anne deVries, Pernilla Carlsson, Catherine Chambers, Sjúður Hammer, Rachel Hurley, Amy Lusher, og Jónur Póldóttir. Þetta verkefni var styrt af Norrænu ráðherranefndinni.

Appendix E Fact sheet – Faroese

PLAST Í MAGUM FRÁ TOSKI ÚR ATLANTSHAVI

Vísindafólk hava ment ymiskar arbeiðshættir at kanna plastbitlar í fiskamagum, men stundum ber ikki til at samanbera úrslit, tí at mannagongdirnar eru ymiskar. Summar starvsstovur hava væl útbúnað granskarar og framkomna útgerð, ímeðan aðrar starvsstovur eru smáar og hava meira avmarkað tilfeingi. Fyri at kunna samanbera úrslit, hava vit ment eina mannagongd, sum riggar fyri øll.

Hví leggja vit í?



Almenningurin leggur í matin vit eta og í umhvørvið.



Fiskivinnan leggur dent á góðskuna á fiskavørini.



Politikkarar hava tørv á góðum vísindaligum dátum, fyri at taka avgerðir viðvíkjandi matvørutrygd og umhvørvisvernd.



Vísindafólk hava tørv á álitandi, standardiseraðum arbeiðshættum, fyri at halda eftirlit við plasti í fiski og í umhvørvi.

Besta mannagongdin higartil



UPPLOYS MAGAN



SÍLA



KANNA OG EYÐMERK

1. Být upp maga og magainnihald.
2. Brúka eina basiska uppløysing (kaliumhydroxid) til at skilja livfrøðiligt tilfar frá, so einans plast liggur eftir.
3. Set í ovn á 40°C í 48 timar, fyri at fáa prosessina at ganga skjótari.
4. Síla vætuna, fyri at fáa burtur alt livfrøðiligt tilfar.
5. Vaska sílaða innihaldi á einum sera finum filtri.
6. Tilset vætu við stórari evnisnøgd, so sum eina konsentreraða saltvatnsloysing, til tað, sum loypur av, og lat tyngri petti botnfella.
7. Síla vatnið ígjøgnum sera fína filtrið (íkkí botnfallið!).
8. Kanna plastbitlarnar undir mikroskopi.
9. Vátta at talan er um plast við plastgreiningarútgerð.

Hetta vita vit

Ímillum Noreg, Ísland og Føroyar hava ímillum 3-27% av toski plast í maganum.

* Vit mentu mannagongdina so við og við, so okkara úrslit eru ikki samanberilig, men vit vísa á úrslitini her sum kunning.

Vit hava framvegis spurningar

+ **Hvur tað týðning nær og hvar** fiskurin er fingin? Hvussu kann fiskivinnan betri gerast partur av hesi gransking?

+ **Eiga vit einans at kanna plast?** Hvussu við bummull og øðrum mannagjørdum tilfari?

+ **Er samanhangur ímillum mongdina av mikroplasti** í toskamagum og stødd á fiskinum, hvussu fullur magin er og hvussu fiskurin er fyri? Og hvussu er hetta í mun til onnur fiskasløg?

Høvundar
Anne deVries,
Pernilla Carlsson,
Catherine Chambers,
Sjúrður Hammer,
Rachel Hurley,
Amy Lusher, og
Jórun Pólsdóttir.

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NIVA
Norsk institutt for vannforskning
Norwegian Institute for Water Research

Verkætlanin er fíggað
av Norðurlandaráðnum.

Appendix F Fact sheet - Norwegian

PLAST I TORSKEMAGER FRA NORD-ATLANTEN

Forskere har utviklet forskjellige metoder for å undersøke plastpartikler i fiskemager, men man kan ikke alltid sammenligne resultater fra forskjellige metoder. Noen laboratorier har dyktige forskere og avanserte instrumenter, mens andre laboratorier er små og har færre ressurser. For å kunne sammenligne og bruke resultater fra mindre laboratorier har vi videreutviklet en metode som fungerer for de fleste.

Hvorfor bryr vi oss?



FOLK FLEST
Bryr seg om maten vi spiser og miljøet



FISKEINDUSTRI
Bryr seg om kvaliteten på varene

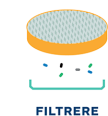


BESLUTNINGSTAKERE
Trenger kunnskap og forskning for å ta beslutninger om mattrygghet og miljø



FORSKERE
Trenger gode, robuste metoder for å overvåke plast i fisk og miljø

Den beste metoden i dag



1. Del opp magen og innholdet.
2. Bruk en alkalisk løsning (kaliumhydroksid) for å fjerne biologisk materiale men platen blir igjen.
3. Sett i ovnen ved 40 °C i 48 timer for kjappere prosess.
4. Sikt væsken for å fjerne eventuelle gjenværende biologisk materiale.
5. Skyll innholdet fra silen til et fint filter.
6. Tilsatt vann med høy densitet til restene for å la tunge, ikke-plast-stoffer synke.
7. Filtrer vannet over filteret (ikke ta med det som sank til bunns!)
8. Se etter plastpartikler i mikroskop.
9. Bruk analytiske instrumenter for å bekrefte type plast.

Hva vi vet

Mellom 3-27% av de norske, islandske og færøyske torskene vi undersøkte hadde plast i magen.

* Metoden ble utviklet mellom analysene av de forskjellige prøvene, så resultatene er ikke direkte sammenlignbare, men gir en idé om hvor mange torsker som har plast i magen.

Vi har fortsatt spørsmål om plast

- + **Er mengden mikroplast** i torskemager relatert til størrelsen på fisken, hvor mye mat den hadde i magen eller i hvor god kondisjon den var? Hvordan ser det ut for andre arter?
- + **Spiller det noen rolle når og hvor** fisk fanges? Hvordan kan fiskere og mindre lokalsamfunn bli mer engasjert?
- + **Er det kun forekomst plast vi skal utforske?** Eller skal vi også rapportere bomull og andre menneskeskapte ikke-plast-materialer?

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Norwegian Institute for Water Research

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