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Plastic ingestion by Atlantic cod (*Gadus morhua*) from the Norwegian coast

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Abstract

This study documents the occurrence of microplastic (b 5 mm), mesoplastics (5–20 mm) and macroplastic (N 20 mm) in Atlantic cod (*Gadus morhua*), a common and economically important species of marine fish in Norway. Fish stomachs (n = 302) were examined from six different locations along the coast of Norway. Three percent of the individual stomachs contained items identified by Fourier transform infrared spectroscopy (FTIR) as synthetic polymers. Bergen City Harbour was a hotspot with 27% of the cod examined found to contain plastic. Polyester was the most frequently detected polymer. All bar one of the stomachs that contained plastic were full of organic stomach content, suggesting a plastic gut clearance rate similar to the ingested food. It is proposed that stomach fullness is an important metric in order to avoid underestimations when assessing the levels of microplastic ingested by fish.

1. Introduction

So common are scenes of beaches covered in litter that most members of the public are aware of litter pollution in the marine environment. In 2010 alone, between 4 and up to 12 million metric tonnes of plastic waste was calculated to have entered the world oceans (Jambeck et al., 2015). The global use and production of plastic has steadily increased since mass production started in the 1940s with 311 million tonnes being produced in 2014 (Plastic Europe, 2015). If plastic production continues to increase at the current rate of 9% per year, as estimated by Hopewell et al., 2009, 400 million tonnes will be produced annually by 2017.

The extent of marine litter found along the Norwegian coast and the coast of Svalbard has been described as unacceptable (Hals et al., 2011). Most of the litter found on beaches, in the water column and on the sea floor is plastic (reviewed in e.g. Derraik, 2002; Cole et al., 2011). This is in part due to the large volume of plastic waste that is generated because of the popularity of cheap single-use plastic items. Around 50% of the plastic produced is designed for single-use (Hopewell et al., 2009) and much of these plastic items are not disposed of in an appropriate manner. Additional factors that intensify the pollution problem are related to the physical properties of plastics, its persistence (Andrady, 1994) and the density of certain polymers (Klyosov, 2007) with around 50% of polymers floating in seawater (Nerland et al., 2014). This allows plastics to be transported by ocean currents to remote areas such as Arctic Svalbard Archipelago, as recent modelled by Sebille et al. (2016). In 2004 Thompson and colleagues demonstrated that in addition to this visible litter pollution, plastic in the form of very small particles also pollute much of the marine environment (Thompson et al., 2004). Since then the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP, 2015) have defined synthetic polymers found in the environment based upon size; microplastics (< 5 mm), mesoplastics (5–25 mm) and macroplastics (> 25 mm). Microplastics can enter the environment in the form of primary microplastic, manufactured as < 5 mm for different purposes (e.g. the cosmetics industry; reviewed by Cole et al., 2011) as well as secondary microplastics formed by weathering of meso- and macroplastics. We know that microplastics enter both pelagic and demersal habitats and can be found inside the organisms living in these habitats (Wright et al., 2013). A great variety of fish species from around the world have been shown to ingest synthetic polymers (Possatto et al., 2011; Boerger et al., 2010; Foekema et al., 2013; Lusher et al., 2013; Rummel et al., 2015). A total of 35% of five mesopelagic and one epipelagic fish species from the North Pacific were found to have ingested plastic with an average of 2.1 pieces of plastic per fish (Boerger et al., 2010). Also three different species of bottom feeding catfish were investigated in a Brazilian estuary whereof 17–33% of the fish had ingested plastic (Possatto et al., 2011). Lusher et al. (2013) found that ten fish species from the English Channel to have ingested microplastics (both pelagic and demersal), with a total of 37% of the fish containing ingested plastic. Foekema et al. (2013) found that only 2.6% of fish from the North Sea contained microplastic, but this was after excluding textile fibres from their data analysis. Of the seven species investigated, five of these contained plastics. Atlantic cod (*Gadus morhua*) was one of the species studied and, dependent on location, 0–15% of the individuals contained plastic (Foekema et al., 2013). Another recent study also found relatively low plastic content in fish (both pelagic and demersal species) from the North Sea and the Baltic Sea; 5.5% of all fish contained plastic (Rummel et al., 2015).

Atlantic cod is one of the most common and economically important marine fish in Norway and in 2015 it was added to the IUCN red list (Cook et al., 2015). It is a widespread bottom dwelling species in the North Atlantic and its habitat ranges from the continental shelf edge of Norway to the innermost parts of fjords all along the Norwegian coastline (Hansen et al., 2016). Stationary costal cod are found along the entire coast of Norway and due to how they opportunistically feed, they are known to ingest a whole range of prey from plankton, shrimps and crayfish to fish including its own species (Hansen et al., 2016). This makes them prone to ingesting anthropogenic matter from both pelagic and benthic habitats. Microplastic is widespread and found in both pelagic and benthic environments (reviewed in e.g. Cole et al., 2011) with Atlantic cod known to ingest microplastic (Foekema et al., 2013). However, no studies have previously looked at Atlantic cod from the Norwegian coast and from stationary cod found in fjords. Therefore, the aim of this study was to establish the level of micro-, meso- and macroplastic ingested by Atlantic cod from the Norwegian coast in order to provide a much-needed insight into the level of microplastics pollution in Norwegian biota.

2. Methods

“The Norwegian Environmental Specimen Bank (ESB)” provided the cod stomachs used in this project. The cod were caught during routine catches on behalf of the ESB at six different locations along the Norwegian coastline: Oslo, Bergen, Sørfjorden, Karihavet, Lofoten and Varangerfjorden (Fig. 1). Fish were collected using fish fyke nets and trawl. The fishing procedure differed from location to location and from year to year due to the use of different fishermen. The overall mesh size (> 70 mm) of the fish nets used was bigger than the plastic pieces found in the stomachs of the fish. It is unlikely that the plastics found in this study were from accumulated plastics that subsequently were consumed by the fish.

2.1. Stomach analysis for plastic

Frozen fish stomachs were thawed at room temperature in individually closed packages. To prevent contamination of the samples, all analysis was performed in a special laboratory for processing organic samples. All of the work surfaces and tools were cleaned thoroughly with ethanol and all equipment was checked under the microscope for particulate contamination before use. No entry or exiting the laboratory was allowed while dissection was being performed. Cotton lab coats were cleaned with a “sticky roller” and easily recognisable blue nitrile gloves were used throughout the dissection of the stomachs. Once thawed, the stomach was taken out and put on a cleaned glass petri dish. The outside of the stomach was inspected for contamination under a stereomicroscope (Nikon SM2 745 T) prior to opening the stomach. The stomach was opened using forceps and scissors and the stomach content registered as empty, half full or full. Based on a visual assessment (Boerger et al., 2010; Lusher et al., 2013), any ingested items that did not resemble natural stomach content were removed using forceps and placed in a petri dish with a new GF-F filter. The petri dishes were sealed and stored in the dark prior to further analysis. To detect possible aerial contamination, a control petri dish containing a wet white GF-F filter was placed next to each stomach exposed to air for the same amount of time as the stomach sample. The control petri dishes were inspected under the stereomicroscope between every specimen and a minimum of six control petri dishes were used per 30 fish. The items collected from the stomachs were photographed and described according to type (fibre, granule, fragment, film), size (length/ width/breadth) and colour. Size was recorded by measuring the longest stretch of the items found. For example a fibre was measured to be 0.02 mm in diameter and 3.2 mm long. In this case, we report the big- gest size of 3.2 mm as the size of the item.

2.2 FT-IR analysis

All the fragments suspected of being plastic (by observation under stereomicroscope) were analysed by ATR (Attenuated total reflection) using a ThermoScientific Nicolet iS50 FT-IR. The infrared absorption spectrum was recorded by exposing the sample to a beam of infrared light (4000–400 cm^1) at the contact surface with a diamond crystal, using 32 scans and resolution 8. The spectrum was automatically com- pared with a series of libraries containing spectra of standard substances in order to confirm the structure of the sample.

3. Results

Nine of the 302 fish stomachs examined contained items identified as synthetic polymers and several of the stomachs that contained plastic contained more than one plastic item (Table 1). The plastic items ranged in size from 3.2 to 41.7 mm with an average length of 14.1 mm. Seventy- five percent of the synthetic polymers were mesoplastics, 18.8% as microplastic and 6.3% macroplastic (Fig. 2) and nine different polymers were identified by FTIR as present in the fish stomachs with polyester (polycyclohexylenedimethylene terephthalate (PCT)) being the most common polymer (Fig. 3). The stomach of a single fish from Bergen contained all size categories (micro, meso- and macroplastic) with fibres, irregular shaped items, including a cylindrical piece and several bundles of fibres (Fig. 4). In Karihavet, the only polypropylene polymer found had fractures most likely from weathering (Fig. 5). Of all the suspected plastic items visually extracted for FTIR scanning, 59.2% were positively identified as plastic. Other non-plastic anthropogenic items were also identified in the stomachs, such as wool and paint (Fig. 6). One hundred of the 302 fish stomachs analysed contained no food and were empty (no organic content).

Table 1. Sample overview. Study locations, individual of fish analysed, and plastic findings. *The amount of plastic items were in some instances difficult to establish due to the clustering of several smaller threadlike objects into larger ones. The amount reported is therefore as we found the items and is not a result of attempts to differentiate between items in the clusters.

Location	Number of fish analysed	Individuals with (FTIR) plastic	Amount of plastic items found
Bergen City Harbour	30	8	14*
Karihavet	12	1	2
Svolvær Lofoten	56	0	0
Varangerfjorden	58	0	0
Indre Sørfjord	50	0	0
Oslofjord	96	0	0
Total	302	9	16

4. Discussion

The present study provides the first published record of plastic polymers in stomachs of Atlantic cod from the coast of Norway (Fig. 1). Of the 302 fish stomachs examined, 3% contained plastic items (Table 1). This is similar to the occurrence rate of 2.6% reported in the 1203 fish stomachs examined by Foekema et al. (2013) in the North Sea and the recent published findings of 5.5% found in different fish species from the North and Baltic Seas by Sebille et al. (2016). It appears that the plastic content of fish from the North Sea is lower than studies from other localities such as the North pacific (Boerger et al., 2010) and The English channel (Lusher et al., 2013). This may reflect a spatial trend with a lower concentrations of microplastics in northern than southern locations as observed by Foekema et al. (2013). One possible explanation could be the presence of higher population densities further south and the proximity of ocean currents that can transport plastic. According to Jambeck et al. (2015), who calculated the plastic waste input from land to ocean and ranked the top 20 countries by mass of mismanaged plastic waste, Europe (if considering collectively the 23 European Union coastal countries) was ranked at number 18. However, looking at the individual countries, where Norway is ranked amongst the best countries with for plastic recycling s, the Jambeck et al. (2015) ranking supports a possible decreasing trend of plastic occurrence going from southern locations around the English Channel and north towards the Norwegian sea.

This might also be the case of the plastic free stomachs from the cod dwelling in the inner parts of Sørfjorden (Fig. 1). This fjord is located at approximately the same longitude as Bergen City Harbour and Karihavet, but the fjord is not directly exposed to open sea and as such may be sheltered from any plastics travelling with ocean currents. The Bergen City Harbour contributed to 89% of the total plastic pieces found and makes it an obvious hot spot. Furthermore, four of the eight stomachs containing plastic obtained from Bergen City Harbour had bundles of fibres (Fig. 4) giving a FTIR-library match of >80% for a type of polyester (polycyclohexylenedimethylene terephthalate (PCT)) with this polyester being the most abundant polymer. To our knowledge, this is the first documented finding of this polymer in marine wildlife. Analogous findings of similar "bundles" of plastic have been found in Norwegian Lobster from the Clyde Sea (Murray and Cowie, 2011). However, these bundles were suspected to be pieces of polypropylene rope. It is worth noting that polyester is a common polymer and is considered to be the fourth-most-produced polymer (Scheirs, 1998). To speculate, it may be that these polyester bundles come from synthetic clothing or from many of the other related polyester products. Due to the same type of polyester being found in four of the Bergen City Harbour stomachs, it suggests that the cod feed from the similar area (or areas) and that the feeding grounds were littered with polyester. Other studies on plastic ingestion in fish have identified nylon as the most common polymer (Dantas et al., 2012; Ramos et al., 2012) but in our study only one of the sixteen plastic items was nylon. Although no plastic (confirmed by FTIR) was found in tfish from Oslofjord, Sørfjord or the two northernmost locations of Lofoten and Varangerfjord, visual assessment led us to extract suspected plastic items pre- FTIR analysis that were not proven to be plastic. This can be a true negative result, but it is important to note that it could be a result of the analytical challenges (organic content on the extracted items (mucus gut) interfering with the FTIR-scanning or a too small sample size to provide accurate FTIR reading) or it could be other anthropogenic items than plastic polymers.

Nine different polymers were identified (Fig. 3) and they are all common polymers used for a range of applications. PE, PP, PVC and PET(polyester) are all within six of the most common produced polymers worldwide (Plastic Europe, 2013). This study shows that the most common polymers also are those found littering the marine environment.

It is worth noting that 88% of the plastic items found in this study were located in full stomachs. Only one empty stomach contained plastic (see upper left image in Fig. 5). Our study reflects the possible capacity the cod has to effectively rid itself of ingested plastics. It is important for the fish to get rid of the plastic for several reasons; plastic ingestion have the potential to give a false satiation and blockage in marine wild- life (Auman et al., 1997; Moore, 2008) and plastic is also known to contain hazardous substances unwanted in marine wildlife (e.g. Hansen et al., 2013) that can negatively affect the fish health such as early warnings signs of endocrine disruption from a laboratory study (Rochman et al., 2014).

When synthetic polymers end up in the digestive tract and stomach of the fish, digestive fluids are in direct contact with the polymer. Such direct contact can enhance the mass transfer of potential contaminants sorbed to the material (Zarfl and Matthies, 2010). Therefore, the longer the plastic item remains in contact with the digestive fluids, the higher the levels of possible contaminants that could be transferred to the animal and subsequently to humans through seafood. The gut retention time (GRT) for North Sea cod have been reported to be 3.7 days (Daan, 1973), meaning that the plastic can possibly retained in the intestinal tracts for around four days. Koelmans and colleagues modelled the leakage from microplastic of two plastic additives, nonylphenol (NP) and bisphenol A (BPA), to the intestinal tracts of Atlantic cod, concluding (based on these two substances) that ingestion of microplastic did not make a considerable contribution to the field exposure of plastic additives to cod (Koelmans et al., 2014). However, the weathering of plastics (Fig. 5) will increases the surface area and can thereby affect polymer biodegradation (Kawai et al., 2004) and in addition to increasing the rate of secondary microplastic particle formation, can also increase the rate of chemical transfer from plastic to organisms (Koelmans et al., 2013). Since plastic debris has been found in fish sold directly for human consumption (Rochman et al., 2015) and from other reports of plastics found in wild fish, like our study, it high-lights the importance to further investigate the potential of plastic related contaminants that can potentially enter seafood.

Based on our study it appears that Atlantic cod from the coast of Norway (except from Bergen harbour fish) tend to contain low levels of micro-, macro and mesoplastic. However, our analyses are conservative and most likely an underestimate due to several factors. One factor is the visual assessment of the fish guts full of organic content. It is challenging to distinguish between the natural debris in the stomach and small pieces of plastic. Another factor is prey engulfed by cod like small- er fish, shrimps etc. that were not individually inspected. Deudero and Alomar (2015) reported that ingested prey identified in the stomach of swordfish collected in the Central Mediterranean Sea had a high percentage of microplastic in the gut. This contributes to the challenges in the precision of the data generated using existing visual sorting methods. The method of visual sorting of stomachs can be a challenging procedure and is shown giving a yield lower than 60% (Avio et al., 2015). As such, it makes the direct comparison of different studies challenging. Further factors are also differences between fish species due to dissimilarities in their ecology such as the feeding location (inner fjord, outer ocean, close proximity to cities and highly population densities) the diet, feeding behaviour and size (Kortsch et al., 2015; Adlerstein and Welleman, 2011). At last, we investigated only the stomach region of the gastro intestinal tract, whereas other studies have reported finding plastic also in other regions of the GI tract (Lusher et al., 2013). Such methodological differences as mentioned above can represent important ecotoxicological issues when assessing the presence and impact of plastic debris on higher trophic level fish and should be addressed.

5. Conclusion

Microplastic, mesoplastic and macroplastic have for the first time been identified in the stomachs of cod from two out of six locations along the coast of Norway. Nine polymers were found; polyester (here polycyclohexylenedimethylene terephthalate (PCT)), polypropylene, polyvinyl chloride, polystyrene, polyetylene, polytetrafluoroethylene (Teflon), nylon 6.6, styrene acrylonitrile resin and poly(n-butyl methacrylate). Bergen City Harbour was identified as a hot spot for plastic ingestion in Atlantic cod was from with polyester being by far the most abundant polymer in the fish gut. Our findings indicate that plastic pieces are more prevalent inside fish with a full stomach content versus those with empty stomachs. This implies that the gut retention time of plastic is similar to food, but this is also important to considerate when comparing scientific results and for standardization of future analyses.

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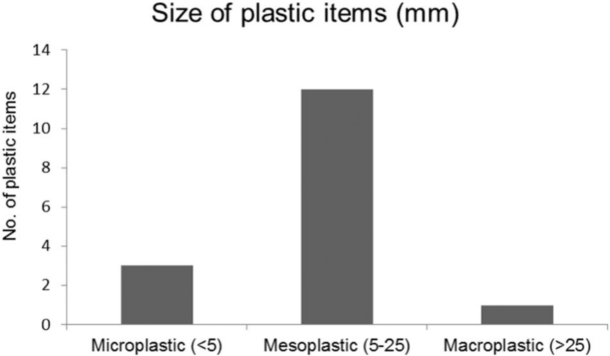
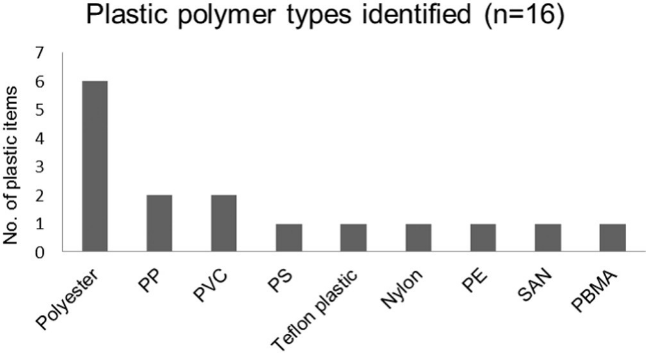
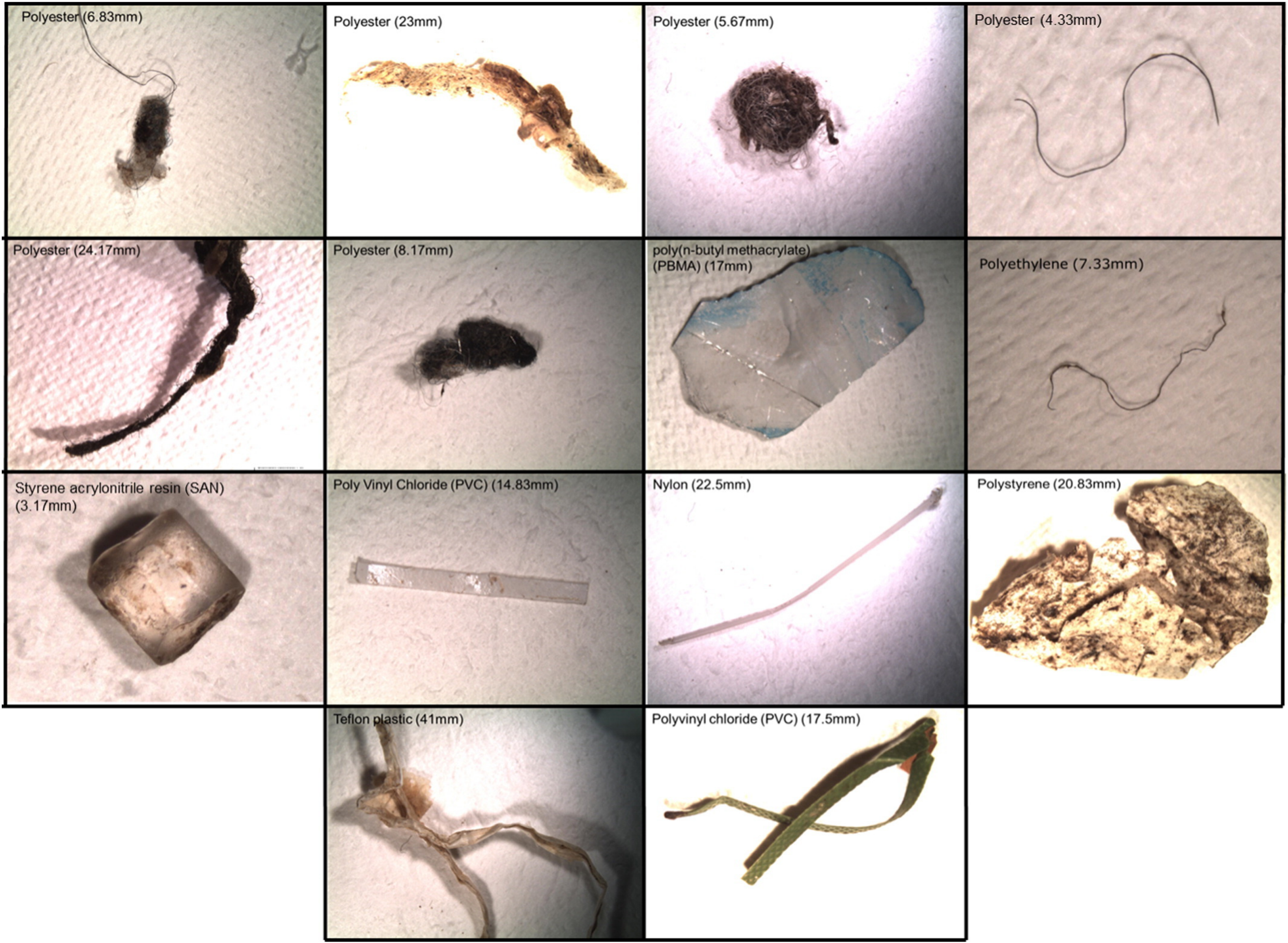
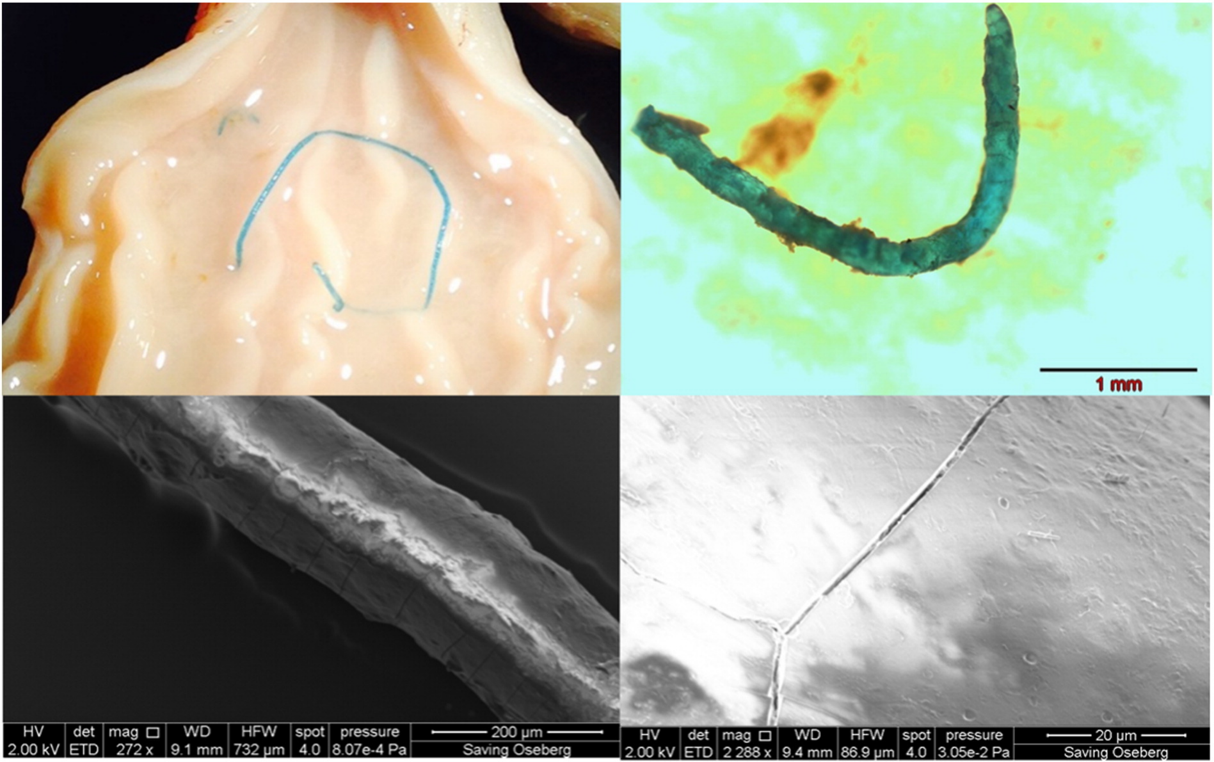
Varangerfjorden
Lofoten
Norway
Europe
Bergen
Karihavet
Sørfjorden
Oslo

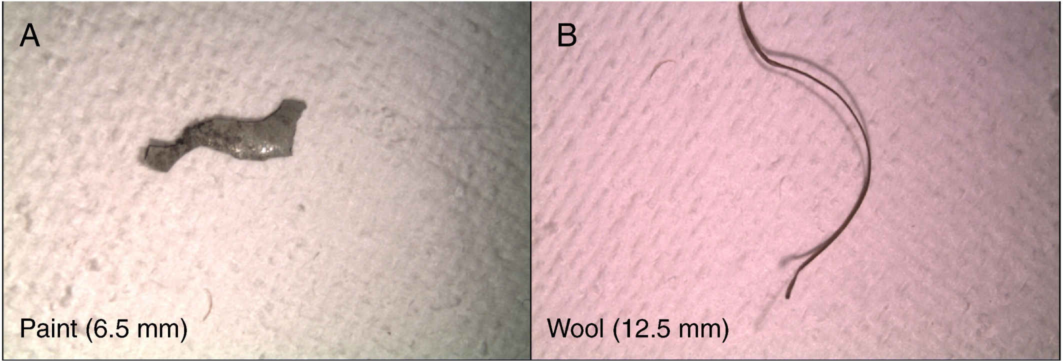


Size of plastic items (mm)
No. of plastic items
0
2
4
6
8
10
12
14
Microplastic (<5)
Mesoplastic (5-25)
Macroplastic (>25)


Plastic polymer types identified (n=16)
No. of plastic items
0
1
2
3
4
5
6
7
Polyester
PP
PVC
PS
Teflon plastic
Nylon
PE
SAN
PBMA


Polyester (6.83mm)
Polyester (23mm)
Polyester (5.67mm)
Polyester (4.33mm)
Polyester (24.17mm)
Polyester (8.17mm)
poly(n-butyl methacrylate) (PBMA) (17mm)
Polyethylene (7.33mm)
Styrene acrylonitrile resin (SAN) (3.17mm)
Poly Vinyl Chloride (PVC) (14.83mm)
Nylon (22.5mm)
Polystyrene (20.83mm)
Teflon plastic (41mm)
Polyvinyl chloride (PVC) (17.5mm)


1 mm
HV
2.00 kV
det
ETD
mag
272 x
WD
9.1 mm
HFW
732 µm
spot
4.0
pressure
8.07e-4 Pa
200 µm
Saving Oseberg
HV
2.00 kV
det
ETD
mag
2 288 x
WD
9.4 mm
HFW
86.9 µm
spot
4.0
pressure
3.05e-2 Pa
20 µm
Saving Oseberg


A
Paint (6.5 mm)
B
Wool (12.5 mm)