Analysis of microplastic in the stomachs of herring and cod from the North Sea and Baltic Sea

Robin Lenz, Kristina Enders, Sabrina Beer, Thomas Kirk Sørensen, Colin A. Stedmon

Photos: Line Reeh, Robin Lenz, Kristina Enders

DTU Aqua
National Institute of Aquatic Resources

Summary

There is a pressing need to assess the distribution of microplastic in aquatic environments and the extent to which they are ingested by fish. This report presents the results of a study contracted by the Danish Ministry of Environment and Food's Nature Agency in 2015 to analyse the microplastic stomach content of demersal and pelagic fish from the North Sea and Baltic Sea. The focus is on particles > 100 µm in size and on comparing distributions in coastal and offshore waters using the sampling already planned as part of DTU Aqua fish monitoring activities. Overall 23% of the analysed fish contained one or more particles or fibres of synthetic polymeric material. Although cod contained plastic more often and also in higher numbers than herring, the latter species showed higher numbers of microplastic items per gram of stomach analysed. A small subsample of the retrieved microplastics was analysed under a Raman microspectrometer revealing common commodity plastic polymer types such as PE, PP and PS, but no quantitative analysis of these was possible within the project's scope. The comparison between offshore and coastal regions is hindered by the fact that many of the coastal stations were close to the outer boundary of the defined coastal zone (12 nautical miles from shore) due to sampling from commercial fisheries and large research vessels.
We recommend the development of an indicator for microplastic in fish based on the present study, where stable coastal populations in close proximity to urban areas could be compared against a reference group unaffected by direct land-based microplastic input.

Introduction

The European Union Marine Strategy Framework Directive lists eleven qualitative descriptors for determining good environmental status (Anon 2008). One of these (Descriptor 10) is focused on marine litter and states that, in order to achieve Good Environmental Status regarding marine litter, member states must ensure that: "Properties and quantities of marine litter do not cause harm to the coastal and marine environment". In this light member states now have to begin to assess the distribution and impact of solid debris such as plastic. In 2010 the European Commission further specified four indicators for descriptor 10, where identifying "trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis)" is one (Galgani and Hanke 2013).

Good environmental status for marine litter is in the Danish marine strategy (NST 2012) described as: 1) litter and its degradation products do not cause harm to marine ecosystems and species and do not support spreading of non-indigenous and invasive species; and 2) litter and its degradation products do not have a significant negative socio-economic impact on marine professions and professions associated with marine areas including tourism. Furthermore three environmental targets have been set, which describe intermediate goals towards reaching good environmental status. Due to lack of knowledge, an operational target has not yet been formulated for microparticles. The Danish marine strategy states that, in order to develop quantitative targets, scientific data is needed to establish reference levels and to specify actions to achieve significant

reductions in litter, including microplastic particles. To inform the development of indicators to support target-setting, Denmark's MSFD monitoring strategy (NST 2014) includes monitoring of microplastic in fish. The current study is part of this monitoring programme and is prescribed in (NST 2014) as follows: Macro- and microliter in fish stomachs as prescribed in EU Guideline "Guidance on Monitoring of Marine Litter in European Seas" (TSG-ML 2014). It is suggested that 2 fish species are included with different feeding strategies, so litter in both water column and seafloor is represented. The chemical composition of microlitter (< 5mm) is determined spectroscopically to gather information on possible sources of microlitter. It is suggested to monitor litter in fish stomachs at least once in the programme period.

Awareness of plastic waste in the sea has been rapidly increasing in recent years, in particular the widespread occurrence of microplastic (Ivar do Sul and Costa 2014). Although macroplastic litter from human activity in the form of bags, containers, ropes etc. were known to persist and be transported over large distances, the realisation of a globally widespread distribution of microplastic particles has obtained much media attention. Microplastic consists of particles less than 5 mm in size, that in part originate from the disintegration of macroplastic in the environment (Barnes et al. 2009) but are also specifically manufactured and eventually released via, for example, waste water effluent (Gregory 1996; Fendall and Sewell 2009).

Concerns on the impact of plastic litter ingestion on marine organisms can be grouped into two categories: physical congestion and damage of digestive tracts and the role of microplastic as adsorbent surfaces for pollutants. The size range of microplastic particles overlaps with that of many planktonic organisms and as a result they are commonly ingested by detritivores and planktivores (Wright, Thompson, and Galloway 2013). Microplastic can be either directly ingested by fish or indirectly through feeding on zooplankton which have ingested microplastic (Cole et al. 2013; Setälä, Fleming-Lehtinen, and Lehtiniemi 2014). Additionally the surface of plastic particles efficiently scavenges hydrophobic persistent pollutants resulting in surface concentrations orders of magnitude above ambient levels (Lee, Shim, and Kwon 2014). Once ingested acidic gut conditions in the gut facilitate the release and potential uptake of pollutants by organisms (Bakir, Rowland, and Thompson 2014).

Scope of this study

The focus of this study is on two specific fish species: cod and herring, which both have a widespread distribution in Danish waters. The goal was to analyse the stomach contents of 100 fish from each species caught in coastal and offshore waters of the North Sea and the Baltic Sea. It was expected that there might be a clear differences between the exposure of coastal and offshore fish to plastic with the latter category being less exposed. Coastal fish on the contrary would be expected to be more exposed. The focus of this survey is on particles > 100 µm in size and using the sampling already planned as part of DTU Aqua fish monitoring activities.

Atlantic cod (Gadus morhua)

The cod is an important commercial species that is caught by commercial and recreational fishermen throughout all Danish seas (Figure 1 and 2). Cod can tolerate low salinities and can be found far into the Baltic Sea.

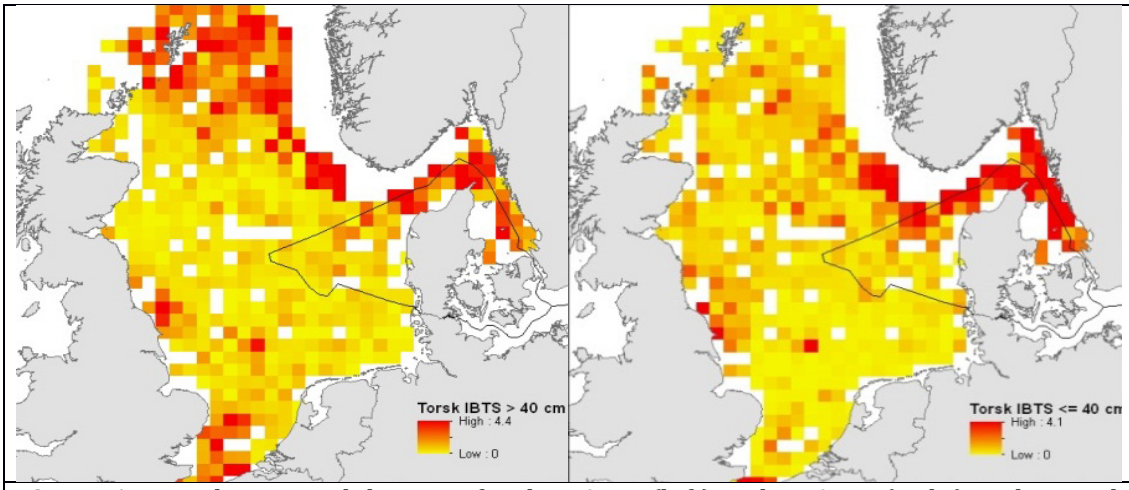


Figure 1: Distribution and density of cod > 40 cm (left) and < 40 cm (right) in the North Sea, Skagerrak and Kattegat. Data is from 2012 IBTS monitoring cruises. The black line marks Danish Exclusive Economic Zone.

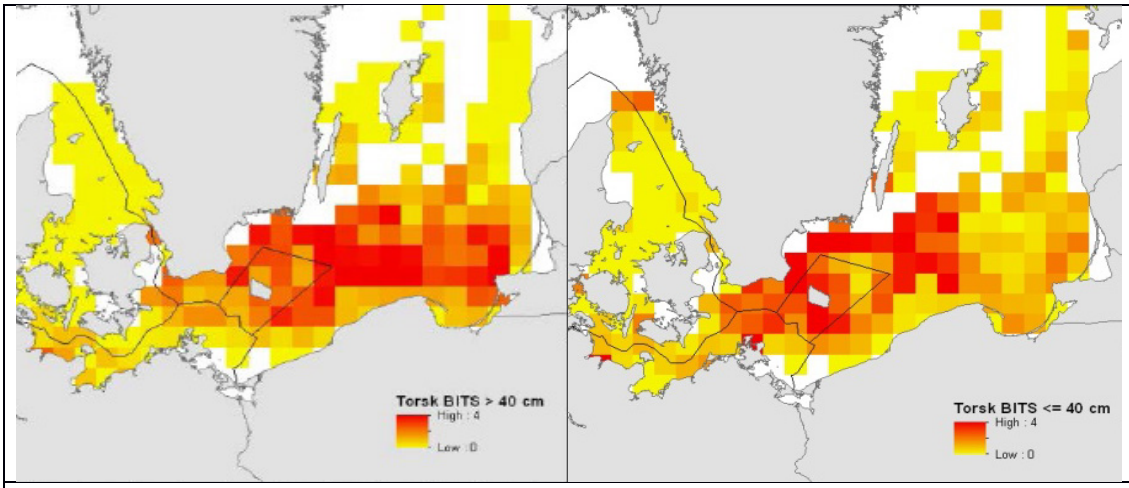
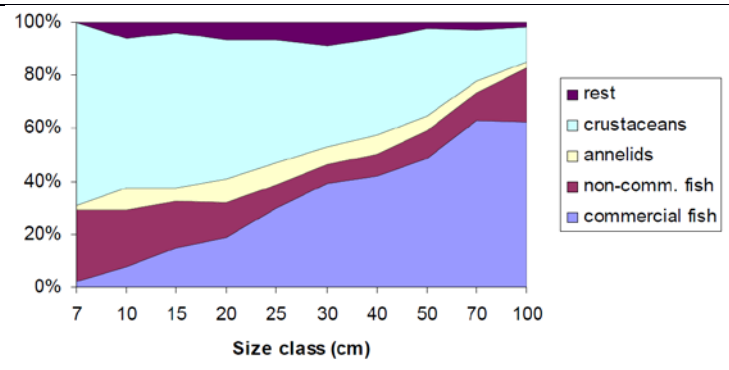


Figure 2: Distribution and density of cod > 40 cm (left) and < 40 cm (right) in the Baltic Sea. Data is from 2012 BITS monitoring cruises. The black line marks Danish Exclusive Economic Zone.

Figure 3: Average stomach contents as percentage weight by size class. Source: Daan, N. (ed). 1989. Data base report of the stomach sampling project 1981. Cooperative Research Report 164. 144 pp



The species can grow to a maximum size of 150 cm or 40 kg, although most are much smaller. Cod can be found at depths ranging from very shallow coastal areas and down to 600 m. Cod are considered benthopelagic demersal fish, i.e. living and feeding near the bottom as well as in midwaters. They feed on both benthic as well as pelagic organisms (Fig. 3). The distribution of adult cod varies greatly, mainly depending on the age of the individual, seasonal changes in temperature and the distribution of prey species. At the age of 2-3 years they become sexually mature adults. At this stage they usually remain near the sea floor, inhabiting many different habitat types. Young cod spend most of spring and autumn in relatively shallow water, but move to deeper waters during warm summer months and cold winter months. As cod grow older, they generally begin to inhabit deeper waters. Tagging experiments indicate that cod are usually quite stationary during feeding periods, i.e. moving less than a few nautical miles per day. During larger, one-directional migrations there are indications that cod move at a maximum distance of up to 15 nautical miles per day (personal communication S. Neuenfeldt, DTU Aqua, 2016).

The diet of juvenile cod is dominated by crustaceans e.g. shrimp, crabs. Larger cod feed mainly on fish such as sandeel, flatfish, clupeids such as herring and even juvenile cod. However, cod feed from both the sea floor and the water column throughout their adult lives. As cod grow older, the size of preferred prey increases (Figure 3). There are many anecdotal examples of fishermen finding large marine litter items when gutting cod. Cod stomach retention time with a meal consisting of sprat in the Baltic ranges between 48 to 72 hours, depending on meal size (Andersen & Beyer 2005a; Andersen & Beyer 2005b).

Herring (Clupea harengus)

Herring is a commercially important clupeid species that is in fact made up of many different races, which are segregated by morphology, differences in spawning seasons, growth among other factors. They can grow to a maximum size of 40 cm at an age of 20-25 years. Herring are schooling fish that are completely pelagic, i.e. inhabiting and feeding only in the water column. However, herring are demersal spawners, i.e. attaching their eggs to gravelly substrates on the sea floor and in some cases vegetation.

Herring feed in the water column predominantly on zooplankton, which the herring schools follow during diurnal vertical migrations. As a result, herring can usually be found higher in the water column during the night and in deeper waters during the day. Herring are able to use their gills to filter-feed. Herring can also visually detect prey, such as an individual copepod or a mysid shrimp, and attack these targets actively.

Stomach retention time for herring is markedly shorter than for cod. Almost independently of the model used to estimate evacuation rates, stomachs can be considered emptied after 24 hours (Darbyson et al. 2003; Bernreuther et al. 2008). No numbers exist for plastic retention times in the fish stomachs. However, it is likely that plastic particles are evacuated from the stomach together with other undigested remains.

The distribution of herring is affected by temperature, depth, frontal systems and mixing of the water column, as well as the abundance and distribution of prey species. Herring are present in all of the seas surrounding Denmark (Figure 4) and different stocks are distributed throughout the entire Baltic Sea (Figure 5).

There are limited data describing the displacement rates of clupeids such as herring and sprat. However, it has been observed in the INSPIRE project (www.bonus-inspire.org) that Baltic Sea fishermen follow moving herring and sprat schools for up to 15 nautical miles per day, which can be seen as an upper limit. However, there are indications that herring are rather stationary during feeding periods, i.e. with movement limited to approx. 2 nautical miles per day (personal communication, S. Neuenfeldt, DTU Aqua, 2016)

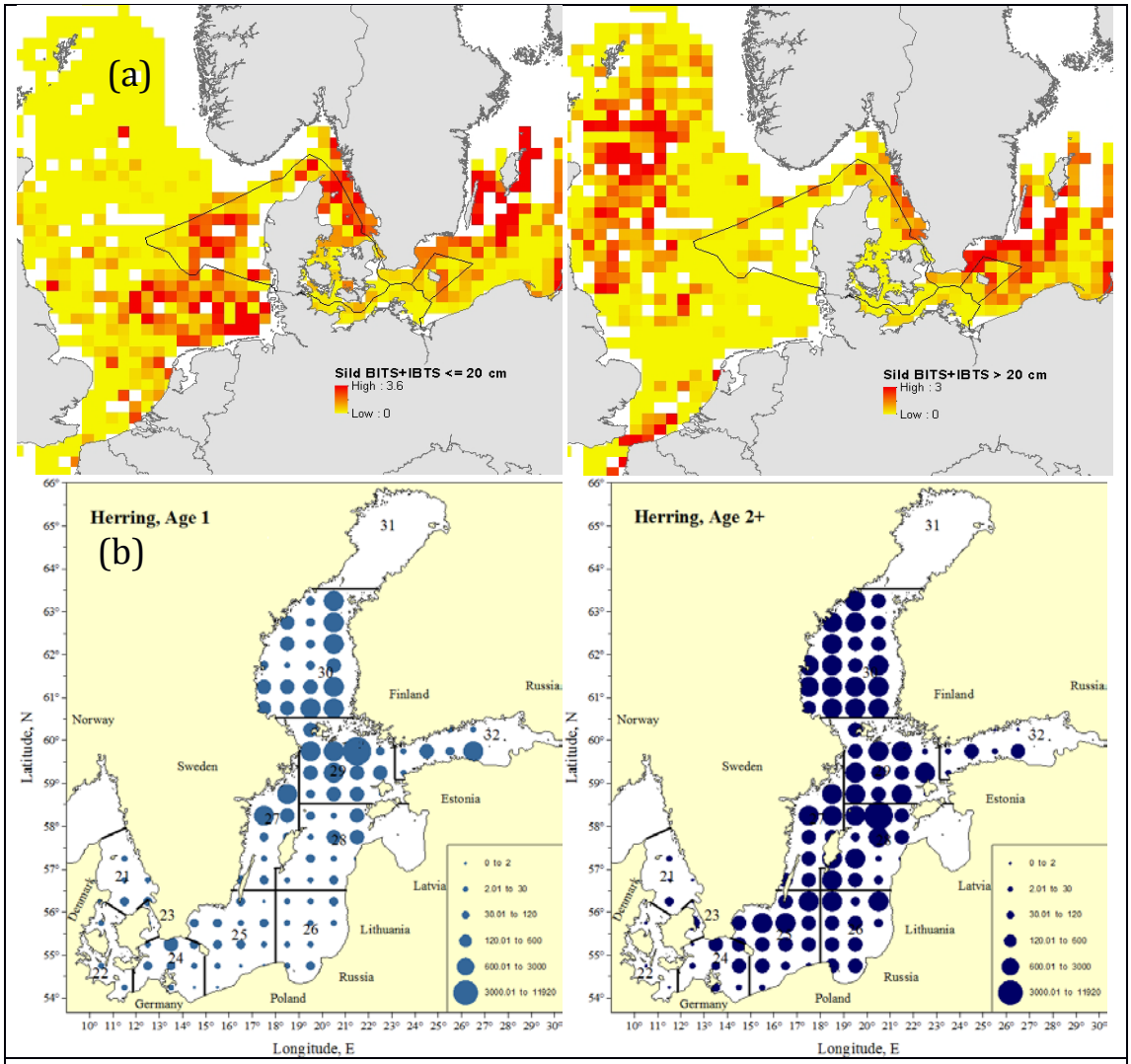


Figure 4 (a): Distribution and density of herring < 20 cm (left) and > 20 cm in the Danish seas. 2012 data from IBTS & BITS monitoring surveys. **(b):** Spatial distribution of herring in the Baltic Sea in Quarter 4 2012 (BIAS survey). Three different stocks are represented: Western Baltic (SDs 22-24), Central Baltic (SDs 25-29, 32) and Bothnian Sea (SD 30). (Casini and Neuenfeldt et al., 2013)

Methods

Sample collection

The original sampling plan agreed upon including a collection of cod and herring from planned DTU Aqua cruises: the International Bottom Trawl Survey (IBTS) in the North Sea, July and August; and the Bio-C3 research cruise in the Baltic Sea, September. The North Sea sampling in particular was plagued by poor catches and inappropriate predetermined trawl locations. The IBTS program that Denmark is also assigned to sample near the south-eastern North Sea coastline is far from Danish waters. Therefore additional samples were arranged through the Swedish IBTS monitoring cruise (Skagerrak, August) and two additional smaller surveys; HG20 (Skagerrak, October) and TNG/SUR (Kattegat, November). Catches of North Sea herring were supplemented by catches from commercial vessels (H218-H10, S349, RI366) and catches from another cruise (SOLEA). An overview of acquired and analysed fish is given in Table 1.

Table 1: Summary of the number of fish stomachs analysed by the project split between regions. Coastal is defined as being within 12 nm off the coast. Numbers in brackets indicate number of fish obtained.

		Cod		
Region	***Cruise / Vessel***	***Coastal***	***Offshore***	***Total***
North Sea incl. Kattegat/Skagerak	IBTS-DK, IBTS-SE, TNG/SUR, HG20	28 (28)	72 (143)	100 (171)
Baltic	BIO-C3	51 (53)	50 (97)	101 (150)
Total		79 (81)	122 (240)	201 (321)
		Herring		
Region	***Cruise***	***Coastal***	***Offshore***	***Total***
North Sea incl. Kattegat/Skagerak	IBTS-DK, IBTS-SE, Solea, H218-H10, S349, RI366	50 (63)	50 (111)	100 (174)
Baltic	BIO-C3	55 (78)	50 (95)	105 (173)
Total		105 (141)	100 (206)	205 (347)

Figures 5 - 8 show location of Cod and Herring caught in the North Sea and Baltic, respectively, that were later analysed for microplastic. The coloured dots indicate stations with number of fish analysed (if no number exists: station was not chosen for analysis). Light blue areas represent 12 nautical miles zones, the orange area the Danish exclusive economic zone (EEZ) and the orange grid ICES statistical rectangles with names. Maps were produced with QGIS (http://qgis.org).

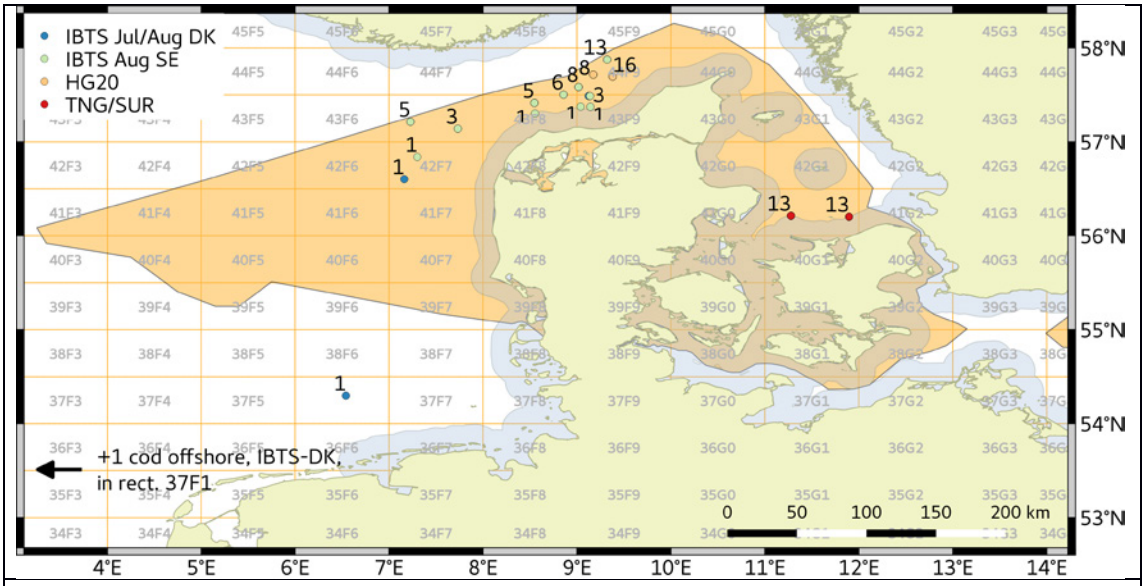


Figure 5: Location of cod catches in the North Sea.

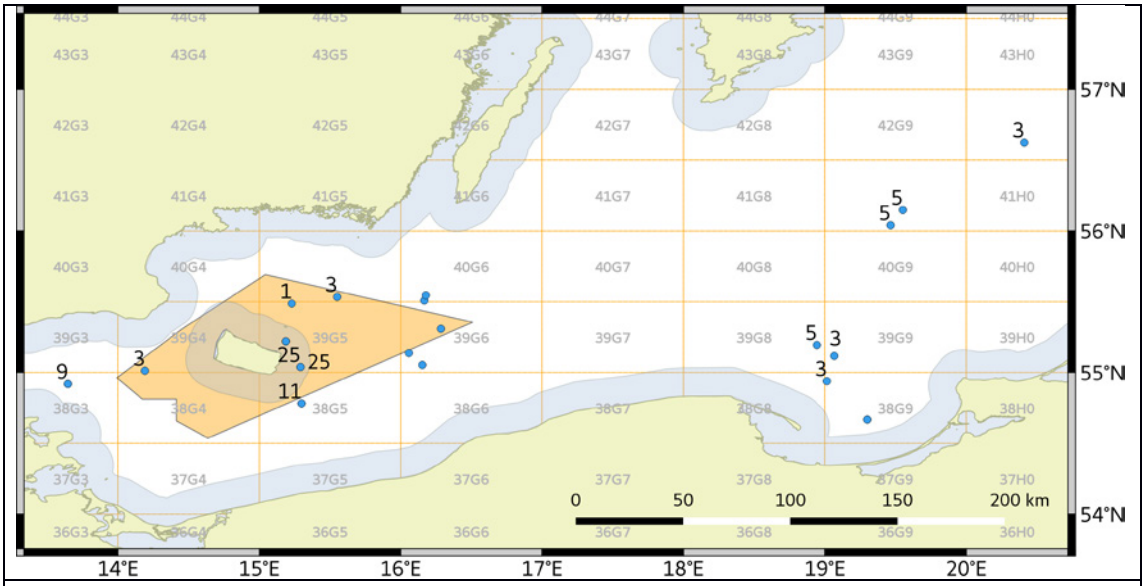


Figure 6: Location of cod catches during the Baltic Sea sampling.

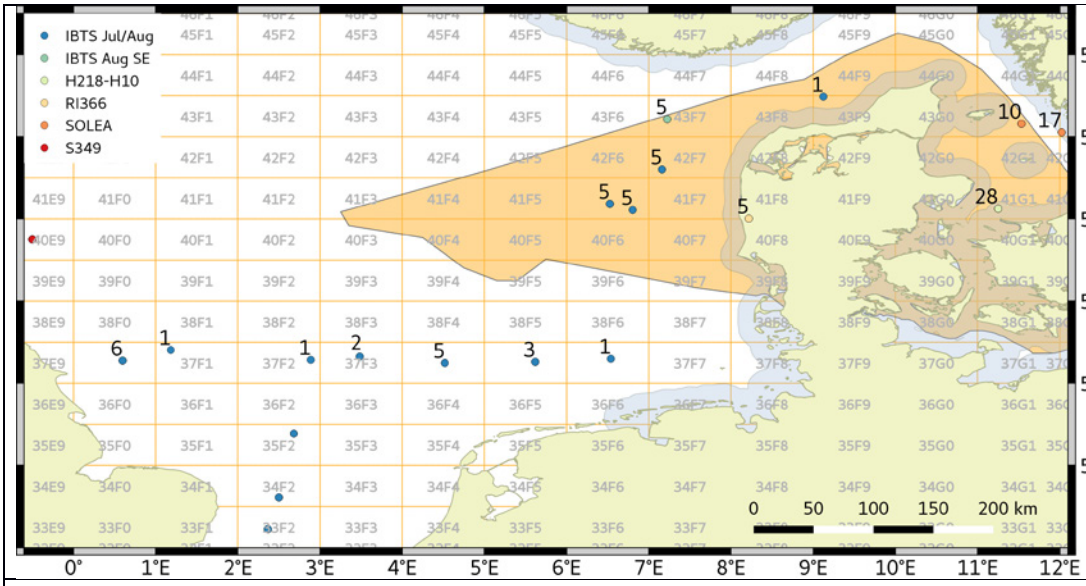


Figure 7: Location of herring catches in the North Sea.

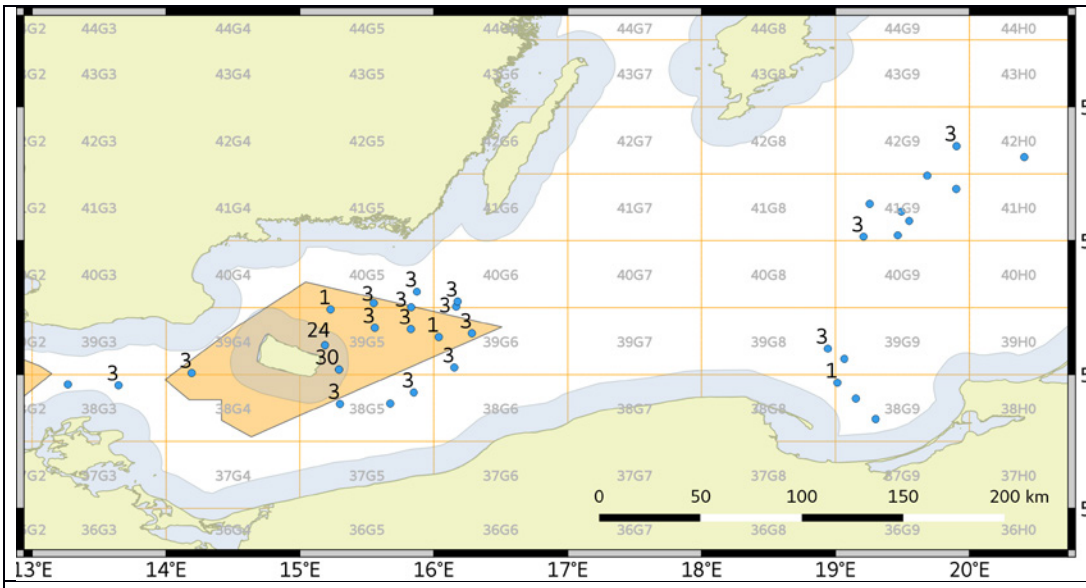


Figure 8: Location of herring catch in the Baltic Sea

Sample processing

For all cruises except the BIO-C3 and IBTS cruise, the fish were frozen immediately after catching and the stomachs were extracted later on return to the laboratory on land. During the BIO-C3 cruise the extraction was partly carried out in the laboratories on board the ship. Cod stomachs were extracted and transferred to zipper bags under clean conditions ensuring minimal exposure time and potential contamination.

Sample digestion

Initially the project followed the recently published guidelines for isolating particle in fish stomachs provided by ICES (ICES 2015) as recommended by the Nature Agency in the contract. However the digestion mixture recommended

was found to be much too harsh and readily dissolved a wide range of polymer types. A study documenting this was therefore carried out and is provided in the appendix. An alternative approach based on what was used in the finally developed digestion method was a result of test series to optimise tissue digestion and removal of fat and oil residues from the samples, as well as the protection of contained microplastics of all major commodity plastics. The protocol was inspired by experiences from an earlier Nature Agency project (Sørensen et al. 2013) and similar recent work (Agersnap 2013; Strand et al., unpublished).The digestion of the stomach tissue was carried out in acid washed glass jars. A digestion solution of 150 ml KOH (1120 g/L) and 150 ml NaClO (14% active chlorine) to 700 ml water was prepared and filtered through a 30 µm filter.

For each stomach sample 5 ml stock solution per 1 g stomach wet weight was dispensed and the lid closed loosely (i.e. not gas tight). The jars were subsequently treated with 10 minutes ultrasound bath and 1 hour on a shaker table. If the stomach tissue was still visible the period on the shaker was extended. Microplastic particles were isolated from the digestion fluid by vacuum filtering through a metallic sieve stack consisting of a mesh of 1 mm and 300 µm and a 100 µm polyamid filter (plankton net) and rinsed with MilliQ water.

Microplastic particles retained on each of the mesh size were classified with respect to shape, colour and size using light microscopy. All microplastic samples were tested using a melting device to confirm their plastic origin. Additionally a sub-fraction from each category was characterised using Raman spectroscopy to allow polymer identification (Lenz et al. 2015).

Results and Discussion

Distribution of plastic between areas and species

Of the 72 offshore North Sea cod analysed 49% were found to have microplastic in their stomachs. Whereas from the 28 coastal North Sea cod 14% contained microplastic. The respective numbers for the Baltic cod were 26% and 16% (Table 2). There was an overall tendency for higher likelihood of stomach plastic content for the offshore cod.

A similar pattern was apparent for the Baltic herring. For the 55 herring sampled from coastal Baltic waters, plastic was found in only 4 fish (7%) (Table 3). For offshore herring from the Baltic twice as many had plastic (16%). For herring from the North Sea the number of fish with microplastic in their stomach content was notably higher, 30 and 16% for coastal and offshore respectively.

The findings presented do not support the hypothesis that fish from coastal stations are more exposed to land-based sources of microplastic and would therefore be contaminated to a higher degree. The opposite is found in the present data for 3 out of 4 groups (Figure 9). There was no significant correlation

between distance from shore and microplastic content in fish from all stations analysed.

North Sea herring was the only group to have more coastal fish containing microplastic. It must be noted that stations marked as coastal and offshore were in fact often in close proximity to one another – a factor which makes robust comparisons between coastal and offshore difficult.

Table 2. Summary of the results for the analyses on cod stomachs

Cod North Sea COASTAL

	fish	percent
Analysed total	28	100.0%
No plast	24	85.7%
All plast	4	14.3%
All plast > 1	1	3.6%
Particle	2	7.1%
Particle >1	0	0.0%
Fibre	2	7.1%
Fibre >1	0	0.0%

Particle length min	500 µm
Particle length max	1000 µm
Particle length mean	750 µm
Fibre length min	300 µm
Fibre length max	10000 µm
Fibre length mean	3767 µm

Cod North Sea OFF-SHORE

	fish	percent
Analysed total	72	100.0%
No plast	37	51.4%
All plast	35	48.6%
All plast > 1	19	26.4%
Particle	12	16.7%
Particle >1	2	2.8%
Fibre	29	40.3%
Fibre >1	2	2.8%

Particle length min	100 µm
Particle length max	1160 µm
Particle length mean	415 µm
Fibre length min	150 µm
Fibre length max	30000 µm
Fibre length mean	3962 µm

Cod Baltic COASTAL

	fish	percent
Analysed total	51	100.0%
No plast	43	84.3%
All plast	8	15.7%
All plast > 1	1	2.0%
Particle	2	3.9%
Particle >1	0	0.0%
Fibre	6	11.8%
Fibre >1	0	0.0%

Particle length min	1400 µm
Particle length max	5600 µm
Particle length mean	3500 µm
Fibre length min	200 µm
Fibre length max	5800 µm
Fibre length mean	2239 µm

Cod Baltic OFF-SHORE

	fish	percent
Analysed total	50	100.0%
No plast	37	74.0%
All plast	13	26.0%
All plast > 1	4	8.0%
Particle	4	8.0%
Particle >1	1	2.0%
Fibre	10	20.0%
Fibre >1	1	2.0%

Particle length min	180 µm
Particle length max	420 µm
Particle length mean	300 µm
Fibre length min	200 µm
Fibre length max	10000 µm
Fibre length mean	1430 µm

Overall microplastic containing fish among cod amounts to 39% in the North Sea and 21% in the Baltic, for herring to 23% and 11%, respectively. There was a general tendency for higher numbers of plastic in larger stomachs (i.e. the large cod stomachs) which seems reasonable simply because more material is available for analysis. However, evaluating the microplastic load relative to the weight of the fish stomach, herring shows a roughly four times higher abundance (Figure 10). Comparing fish body length to microplastic load showed no clear correlation, despite the same slight tendency that highest number of plastic

items were generally observed in fish in the upper half of the group's size spectrum (Figure 11).
In the Baltic Sea, fish were analysed from the Bornholm Basin and the Eastern Gotland Basin / Bay of Gdansk. Figure 12 is providing an overview of the percentages of fish that were found containing microplastic in their stomach in the two respective regions. For cod and herring from the Bornholm basin, relatively more fish were contaminated with plastic. However, it must be noted that the study was intended to focus on Danish waters as much as possible, resulting in small sample sizes from the Gotland basin (n= 24 for cod and n= 10 for herring).

Table 3. Summary of the results for the analyses on herring stomachs

Herring North Sea COASTAL

	fish	percent
Analysed total	50	100.0%
No plast	35	70.0%
All plast	15	30.0%
All plast > 1	3	6.0%
Particle	3	6.0%
Particle >1	0	0.0%
Fibre	14	28.0%
Fibre >1	0	0.0%

Particle length min	160 µm
Particle length max	2500 µm
Particle length mean	1007 µm
Fibre length min	350 µm
Fibre length max	57000 µm
Fibre length mean	5461 µm

Herring North Sea OFF-SHORE

	fish	percent
Analysed total	50	100.0%
No plast	42	84.0%
All plast	8	16.0%
All plast > 1	0	0.0%
Particle	1	2.0%
Particle >1	0	0.0%
Fibre	7	14.0%
Fibre >1	0	0.0%

Particle length min	150 µm
Particle length max	150 µm
Particle length mean	150 µm
Fibre length min	400 µm
Fibre length max	8300 µm
Fibre length mean	1993 µm

Herring Baltic COASTAL

	fish	percent
Analysed total	55	100.0%
No plast	51	92.7%
All plast	4	7.3%
All plast > 1	0	0.0%
Particle	0	0.0%
Particle >1	0	0.0%
Fibre	4	7.3%
Fibre >1	0	0.0%

Particle length min	0 µm
Particle length max	0 µm
Particle length mean	0 µm
Fibre length min	450 µm
Fibre length max	4600 µm
Fibre length mean	1913 µm

Herring Baltic OFF-SHORE

	fish	percent
Analysed total	50	100.0%
No plast	42	84.0%
All plast	8	16.0%
All plast > 1	2	4.0%
Particle	1	2.0%
Particle >1	0	0.0%
Fibre	7	14.0%
Fibre >1	0	0.0%

Particle length min	2600 µm
Particle length max	2600 µm
Particle length mean	2600 µm
Fibre length min	200 µm
Fibre length max	27500 µm
Fibre length mean	5428 µm

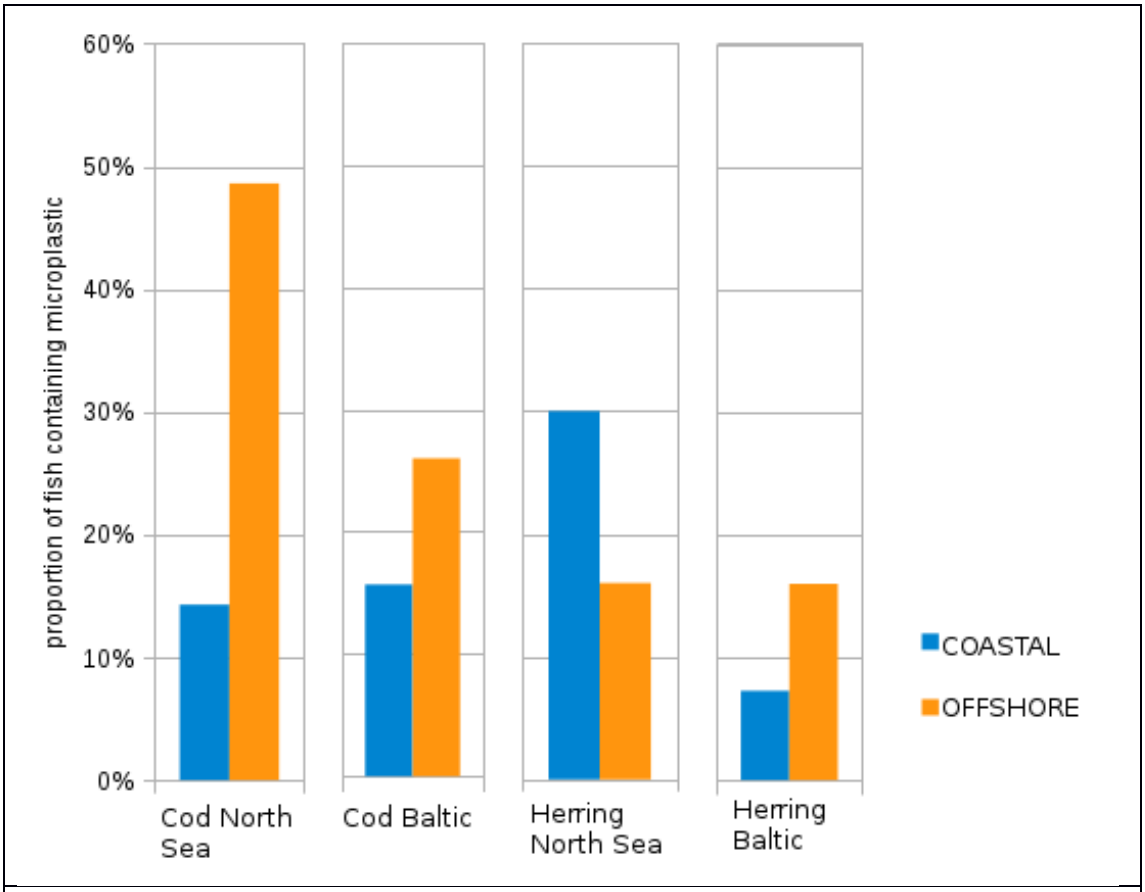


Figure 9. : Summarising bar diagram illustrating microplastic occurrences in Cod and Herring form the North Sea and Baltic, respectively. Sample sizes can be taken from Table 2 and 3 (analysed total)

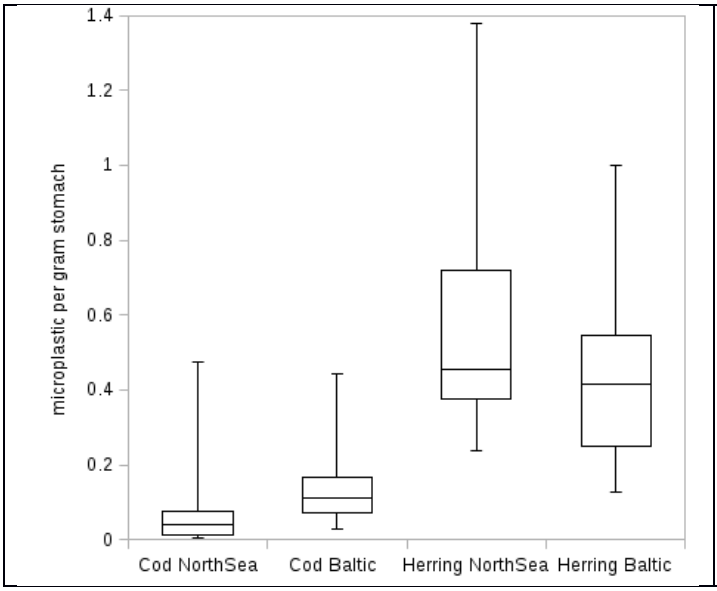


Figure 10: Box plots showing the relative microplastic load per gram fish stomach. Depicted are the median values with 25 and 75% percentiles. Error bars indicate maximum and minimum of the data set.

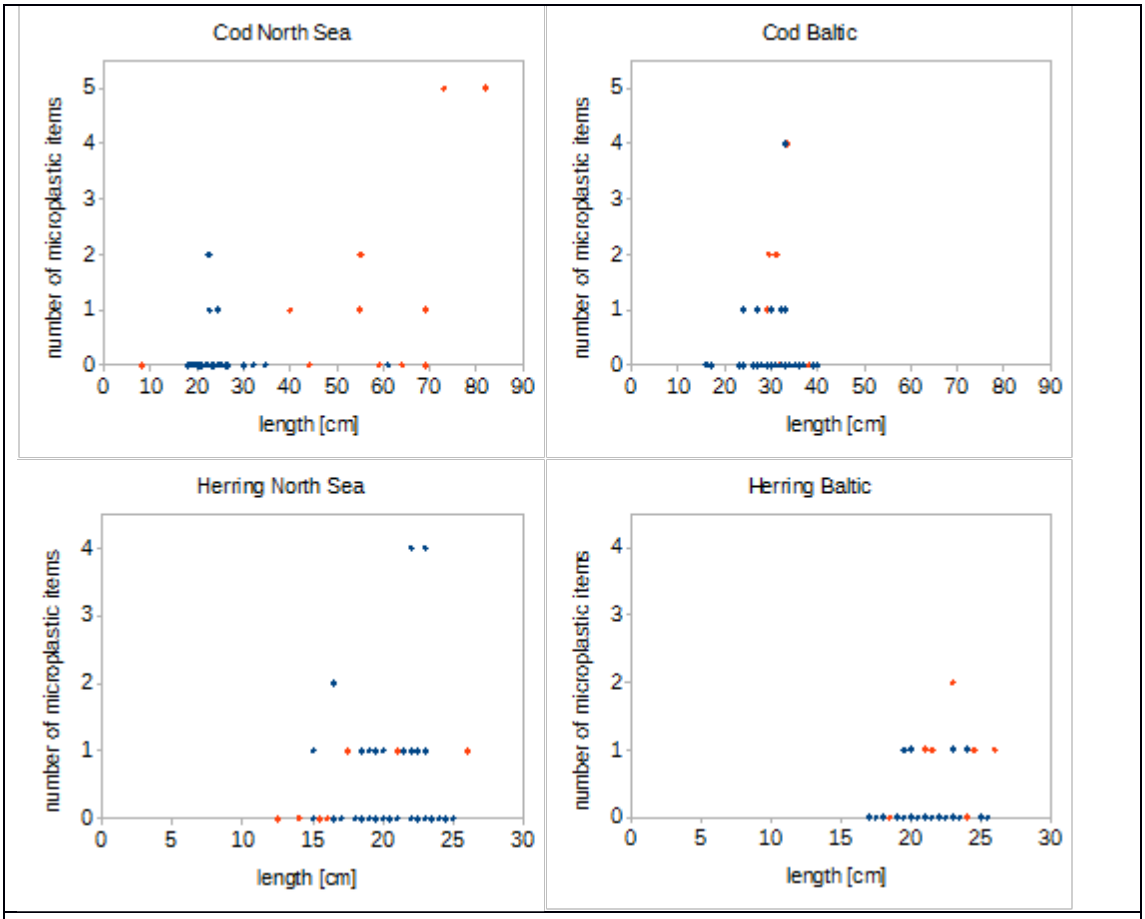


Figure 11: Relation between size of fish (total length) and microplastic particles and fibres. Blue for coastal, red for offshore stations

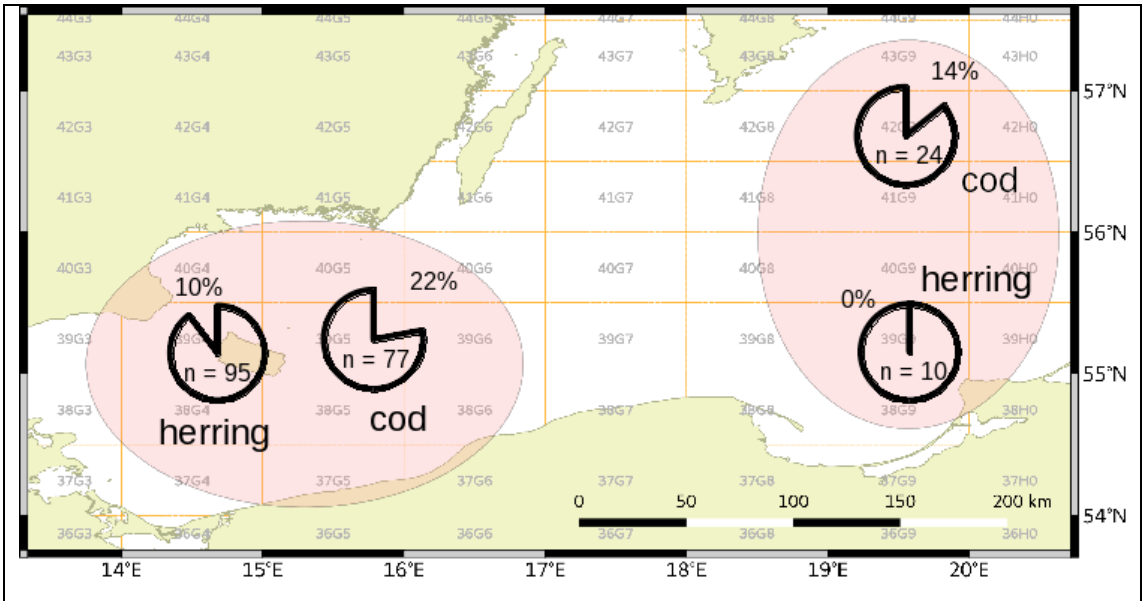


Figure 12: Comparison of percent fish containing one or more pieces of microplastic from Bornholm area and south-eastern Baltic. Cut-off part of the pie charts represents plastic-containing fish.

Plastic categories

Some examples of the types of plastic isolated from fish stomachs are shown in Figure 13. From the 95 pieces of plastic discovered the vast majority (83%) have been fibres ranging in length from 0.15 to 57 mm. Particle sizes have ranged from 0.1 to 5.6mm. The largest piece was found in a cod from the Skagerrak (Figure 13, right).

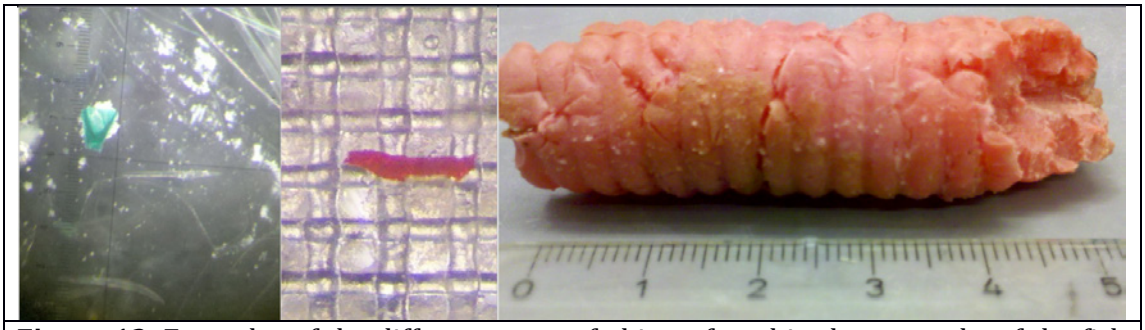


Figure 13: Examples of the different types of objects found in the stomachs of the fish examined. To the left a typical fragment/particle, in the middle a fibre and on the right an extreme example of an approximately 5 cm long piece of rubber or silicon originating from a sport fishing bait found in the stomach of a cod

The results of the colour analysis are shown in Figure 14a showing a similar colour distribution across herring and cod whether them being from the Baltic or the North Sea. Microplastic colour differences are observed between coastal and offshore fish (Figure 14b). Both herring and cod from rather coastal zones appear to contain microplastic of less colour variability than its offshore equivalent.

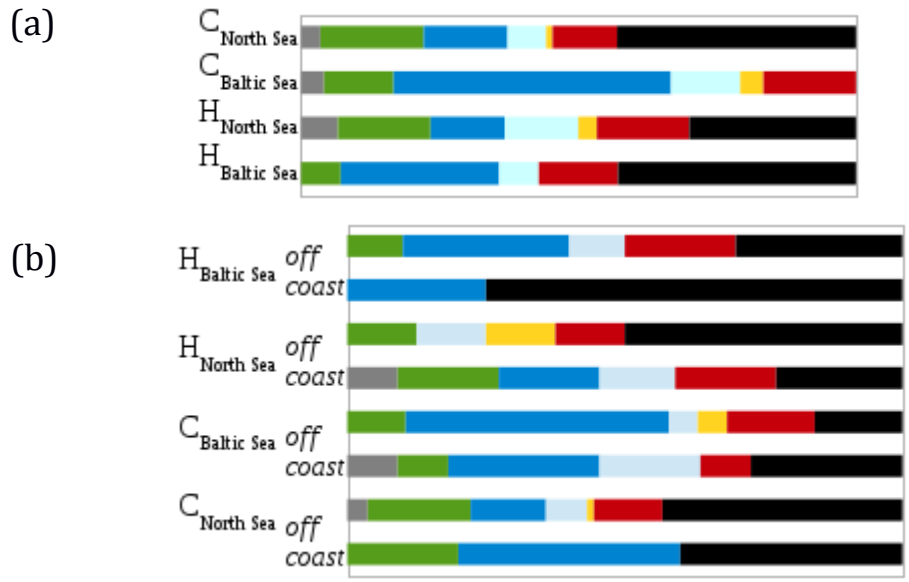


Figure 14: Microplastic colour variability. Whole bars represent 100% of collected micrplastic in each group. Colours were recorded as grey, green, blue, transparent/whitish, yellow, red/pink, black. **(a)** Colour distribution in cod C and herring H from North Sea and Baltic. **(b)** Colour distribution split up for off-shore and coastal samples.

Raman Analysis

The polymer type of a small subsample was analysed in a Raman micro-spectrometer. Exemplary spectra are shown below (Figure 15). Some common polymer references could be matched to a high degree, however in other cases no analysable spectra could be obtained from particles or fibres that otherwise exhibited clearly plastic-like features (morphology, melting behaviour).

A given polymer type found in an ingested microplastic particle cannot be used to trace litter sources. This is due to the fact that the majority of plastic types is applied across all continents and sectors such as the building industry, consumer products, packaging, etc. Shapes such as fibrous microplastics, usually made of polyester, polyamide or of acrylic nature, originate mostly from textiles. However there are no numbers on how much is lost during production compared to consumer waste (from washing machines).

The labour intensive nature of measuring individual particles limits the feasibility of quantitative spectroscopic analysis. Raman signal confounding factors such as fluorescence overlay from strongly Raman-active additive compounds e.g. colourants, degradation state or surface coatings of microplastics are problems that require further development and adaptation of Raman techniques to be more efficiently harnessed for marine microplastic detection and characterisation. The methods and labour time available for this study did not allow for a quantitative microplastic identification based on spectrometric analysis. The presented results are only covering a few selected particles that were in a good measurable condition. The results demonstrate the current limitations but at the same time the fundamental possibilities of Raman microspectroscopy assisted microplastic analysis. Currently, we are intensifying our research in automated spectroscopic microplastic identification where Raman and other techniques will be developed further for rapid microplastic sample analysis, which is urgently needed in all studies investigating microplastic pollution from environmental samples.

FTIR microscopy, an alternatively used spectral identification technique, would require the same if not higher degree of sample purification. It will not be confounded by fluorescent additives but is inferior in identification of dark, light absorbing materials. Both techniques together can give complementary results and are commonly used in combination in analytical chemistry. However, regardless of which spectroscopic technique is applied, there is a fundamental need to develop or adapt automatised procedures for microplastic sample analysis before larger studies on chemical composition of marine microplastic litter becomes cost-efficient.

The proportion of fibre to particles was slightly higher in coastal regions compared to offshore. This appears reasonable as microplastic fibres usually arise from fabric fragments which pass washing machines and waste water treatment plants (Browne et al. 2011). The mentioned difference between the ratios (fibre:particle) 3.7 and 2.9. It has to be noted that most stations that are within the 12 nautical mile zone and declared coastal are still relatively far out at sea which was due to the rather rigid planning of the monitoring cruises.

The overall ratio of fibres to particles was markedly higher in herring compared to cod, 6.4 and 2.4, respectively. This could reflect their feeding strategy where when filter feeding fibres are held back by the gills. A previous study conducted by DTU Aqua in 2013 which analysed 90 whiting and herring from the Belt Sea for microplastic (>0.5 mm) found 31% and 27% in the digestive systems. Also here fibres were the prevailing type of plastic ranging from 0.5 to 4 mm (Sørensen et al. 2013).

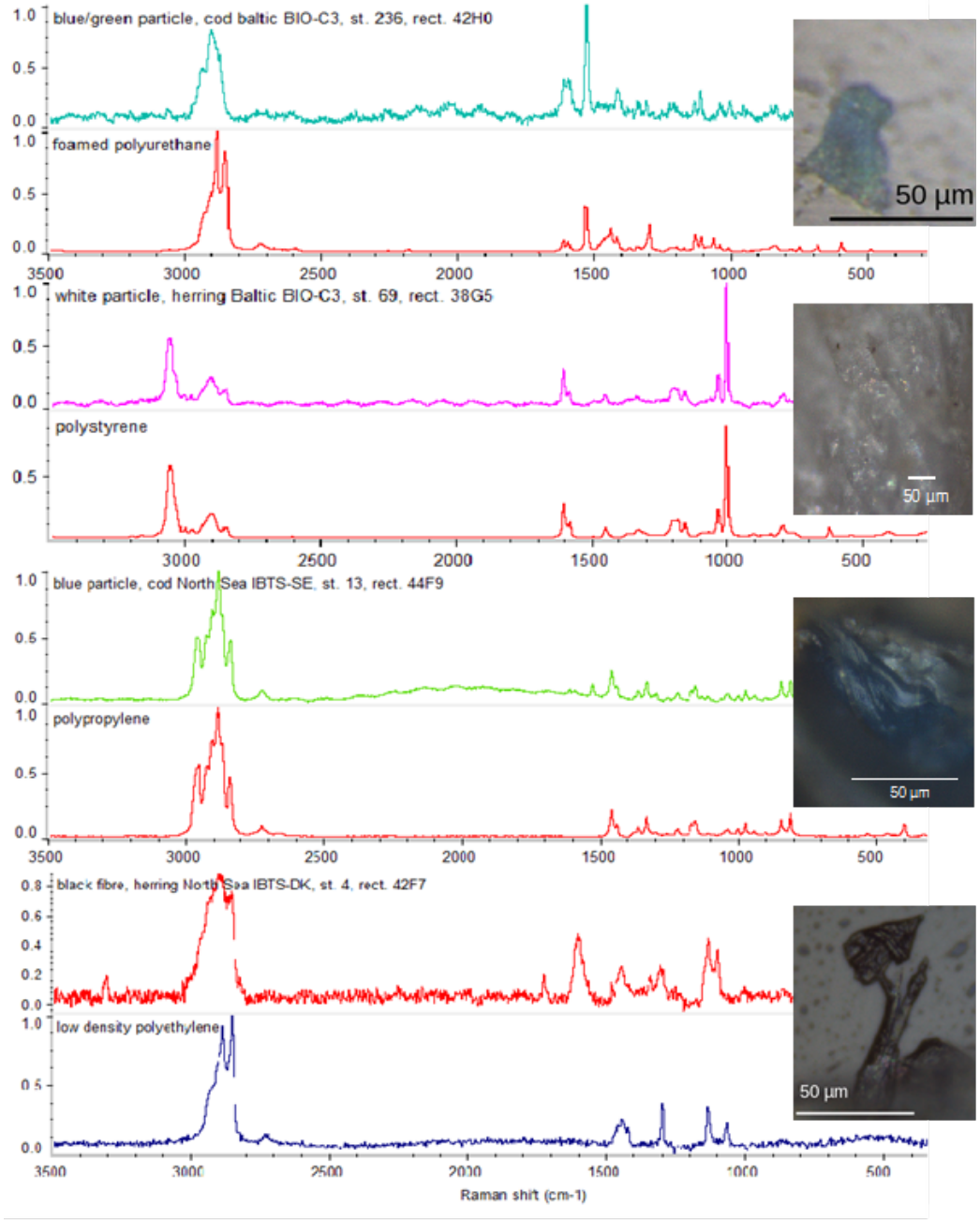


Figure 15: Raman spectra of four plastic items found in fish stomachs (microscopic photographs on the right, scale bars in micrometre). The spectra are each shown with a reference spectrum obtained from commercially available consumer products of known polymer type. From top to bottom: polyurethane, polystyrene, polypropylene and polyethylene.

A similar pattern can be seen in this study where cod reached an overall microplastic occurrence of 30% and herring 17%. Thus it is likely that microplastic can be found in the stomachs of up to about a quarter of fish in Danish waters. The implications of this are currently unknown and the focus of much research and discussion.

With respect to monitoring of microplastic pollution it would be relevant to include a study on fish from very coastal habitats. This could possibly be done near primary microplastic input areas such as large cities and industrial sites or secondary microplastic accumulation zones mainly influenced by present hydrodynamics and density of the specific plastic. Accumulation zones for the North Sea and Baltic region are not fully documented yet and the mapping of macro and microplastic there should be subject to coming research projects in order to identify hotspot areas.

Development of an indicator

The widespread distribution and differing feeding strategies of both species make them ideal for development into indicator species for microplastic ingestion by fish. Additionally the fact that they are both part of routine monitoring surveys and intensively studied provides added economical and scientifically benefit. Comparing the two species, herring is by far the easiest to process due to the smaller stomach size and shorter base-digestion time in the laboratory.

The results also showed that herring has a higher load of microplastic per stomach weight compared to cod (Figure 10). This could be due to the feeding strategy of herring which may bias microplastic uptake. In areas with high prey abundance herring often switches to filter-feeding mode which enhances chances for accidental uptake as shown for mussels and zooplankton. It is also possible that herring mistake microplastic for prey. However, the size of particles found was usually in the sub-millimetre range, far below the usual feeding size of herring. This suggests that plastic entered the fishes accidentally through filtering or along with ingested prey.

When developing an indicator it is very important to clearly specify the goal. In Danish waters to date herring could be used to give indication for trends in microplastic load in pelagic fish species. It would however be beneficial to have a reference beyond local anthropogenic (e.g. oceanic) influence to compare to and distinguish between local influence and general widespread pollution. In addition, before this can reach management levels there needs to be more knowledge of the seasonal variation within microplastic concentrations. For one reason, the fish migrates to coastal zones during spawning seasons which could change the uptake tremendously. Secondly, the pelagic microplastic load in the Baltic Sea is likely to vary with freshwater input and mixing which changes markedly seasonally. Based on the described migration capabilities and stomach retention times it can be expected that microplastics from herring stomachs originate from sites within a maximum of 15 nautical miles from the fish sampling location. For cod the range can be expected to be similar. It should be

noted that no direct data on stomach retention times of microplastics have been measured for the two species. Although plastic is expected to generally pass the intestines with any other non-digestible matter it is possible that special shape and morphological properties of microplastics can lead to longer retention. Fibres or highly edged particles might get stuck in grooves and narrow sections inside the intestines, and worst case entangle permanently.

A current study at DTU Aqua is analysing herring and sprat for microplastic and simultaneously taken water samples tracing back from 2015 to the late 1980's, including a comparison between seasons. While there has been research efforts on determining the plastic ingestion by planktivorous fish in several waters worldwide, to our knowledge there are no investigations focusing on the abundance, distribution and composition of plastic litter over a longer period of time and whether the stomach content reflects the plastic concentrations in the water. This could give valuable insights into correlations between seasons, changes in microplastic density and sprat as a possible indicator species and thus a step forward towards developing an indicator species.

Conclusions

The results of this report present the microplastic (>100 µm) load of cod and herring from the North and Baltic Sea which on average amounts to 23% of all fish. Contrary to expectation we have not seen differences between offshore and coastal caught fish. For future investigations one could consider the analyses of a very coastal population either herring or short spined sea scorpion (dk.: ulk, e.g. not more than 1 nautical mile from shore). Part of this could be to compare between heavily contaminated areas such as harbours, big cities, industries, and sparsely populated regions. Cod as well as herring are appropriate indicator species for microplastic pollution in demersal/benthopelagic (i.e. living near the seafloor as well as in the water column) and pelagic fish species, respectively. However due to stomach size, cod stomach tissue digestion is a more laborious procedure. For future work inclusion of a reference population from an oceanic site such as the open North Atlantic, would facilitate the distinguishing between local and more diffuse global pollution.

Acknowledgements

This study was funded by the Ministry of Environment and Food of Denmark's Nature Agency (Naturstyrelsen). The BIO-C3 cruise on RV DANA in September 2015 was funded by the Danish Centre for Marine Research (grant 2015-04). The authors would like to thank Bastian Huwer for the logistic support and organisation of the cruise as well as Zac Calef for collecting fish for the analysis. Further, the authors would like to thank Frank Ivan Hansen, Kai Wieland, Susanne Hansen, Barbara Bland, Peter Vingaard Larsen, Dennis Ulrik Andersen, Thomas Møller, Jan Pedersen, Helle Rasmussen, Aage Thaarup and Stina Bjørk Stenersen Hansen for organising and collecting fish from supplementary cruises.

References

Agersnap, Sune. 2013. “Mikroplastik I Havmilløet.”

Andersen, N. G., & Beyer, J. E. (2005a). Gastric evacuation of mixed stomach contents in predatory gadoids: an expanded application of the square root model to estimate food rations. Journal of Fish Biology, 67(5), 1413-1433.

Andersen, N. G., & Beyer, J. E. (2005b). Mechanistic modelling of gastric evacuation in predatory gadoids applying the square root model to describe surface‐dependent evacuation. Journal of fish biology, 67(5), 1392-1412.

Anon. 2008. “Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 Establishing a Framework for Community Action in the Field of Marine Environmental Policy (Marine Strategy Framework Directive).” *Offical Journal of the European Union* 164: 19–40.

Bakir, Adil, Steven J Rowland, and Richard C Thompson. 2014. “Enhanced Desorption of Persistent Organic Pollutants from Microplastics under Simulated Physiological Conditions.” *Environmental Pollution (Barking, Essex : 1987)* 185 (March). Elsevier Ltd: 16–23. doi:10.1016/j.envpol.2013.10.007.

Barnes, David K a, Francois Galgani, Richard C Thompson, and Morton Barlaz. 2009. “Accumulation and Fragmentation of Plastic Debris in Global Environments.” *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 364 (1526): 1985–98. doi:10.1098/rstb.2008.0205.

Bernreuther, M., Herrmann, J. P., & Temming, A. (2008). Laboratory experiments on the gastric evacuation of juvenile herring (Clupea harengus L.). *Journal of Experimental Marine Biology and Ecology*, 363(1), 1-11.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T. and Thompson, R. (2011). Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environ. Sci. Technol.*, 2011, 45 (21), pp 9175–9179

Casini and Neuenfeldt et al., Study Group on Spatial Analyses for the Baltic Sea (SGSPATIAL), ICES report, 2013.

Cole, Matthew, Pennie Lindeque, Elaine Fileman, Claudia Halsband, Rhys Goodhead, Julian Moger, and Tamara S Galloway. 2013. “Microplastic Ingestion by Zooplankton.” *Environmental Science & Technology* 47 (12): 6646–55. doi:10.1021/es400663f.

Daan, N. (ed). 1989. Data base report of the stomach sampling project 1981. Cooperative Research Report 164. 144 pp

Darbyson, E., Swain, D. P., Chabot, D., & Castonguay, M. (2003). Diel variation in feeding rate and prey composition of herring and mackerel in the southern

Gulf of St Lawrence. *Journal of Fish Biology*, 63(5), 1235-1257.

Fendall, Lisa S, and Mary a Sewell. 2009. "Contributing to Marine Pollution by Washing Your Face: Microplastics in Facial Cleansers." *Marine Pollution Bulletin* 58 (8). Elsevier Ltd: 1225–28. doi:10.1016/j.marpolbul.2009.04.025.

Galgani, F, and G Hanke. 2013. "Marine Litter within the European Marine Strategy Framework Directive." *ICES Journal of Marine Science* 70: 1055–64. doi:10.1093 / icesjms / fst122.

Gregory, Murray R. 1996. "Plastic 'scrubbers' in Hand Cleansers: A Further (and Minor) Source for Marine Pollution Identified." *Marine Pollution Bulletin* 32 (12): 867–71. doi:10.1016/S0025-326X(96)00047-1.

Ivar do Sul, Juliana a, and Monica F Costa. 2014. "The Present and Future of Microplastic Pollution in the Marine Environment." *Environmental Pollution (Barking, Essex : 1987)* 185 (February). Elsevier Ltd: 352–64. doi:10.1016/j.envpol.2013.10.036.

Lee, Hwang, Won Joon Shim, and Jung-Hwan Kwon. 2014. "Sorption Capacity of Plastic Debris for Hydrophobic Organic Chemicals." *The Science of the Total Environment* 470-471 (February). Elsevier B.V.: 1545–52. doi:10.1016/j.scitotenv.2013.08.023.

Lenz, Robin, Kristina Enders, Colin A Stedmon, David M A Mackenzie, and Torkel Gissel. 2015. "A Critical Assessment of Visual Identification of Marine Microplastic Using Raman Spectroscopy for Analysis Improvement." *Marine Pollution Bulletin*. Elsevier Ltd. doi:10.1016/j.marpolbul.2015.09.026.

NST. 2012. Danmarks Havstrategi - Basisanalyse. Danmarks Havstrategi - Miljømålsrapport. 100 pp.

NST. 2014. *Danmarks Havstrategi Overvågningsprogram*.

Setälä, Outi, Vivi Fleming-Lehtinen, and Maiju Lehtiniemi. 2014. "Ingestion and Transfer of Microplastics in the Planktonic Food Web." *Environmental Pollution (Barking, Essex : 1987)* 185 (February). Elsevier Ltd: 77–83. doi:10.1016/j.envpol.2013.10.013.

Sørensen, Thomas Kirk, Colin Stedmon, Kristina Enders, and Ole Henriksen. 2013. "Analyse Af Marint Affald I Sild Og Hvilling Fra Det Nordlige Storebælt," no. December.

Strand, J., P. Lassen, Y. Shashoua, and J.H. Andersen. "Occurrence of Microplastic Particles and Bio-Geochemical Relationships in Sediments from Danish Waters (in Prep.)."

TSG-ML. 2014. "Executive Summary 2013 Directive Executive Summary of the Guidance on Monitoring of Marine Litter in European Seas," no. January 2016.

Wright, Stephanie L, Richard C Thompson, and Tamara S Galloway. 2013. “The Physical Impacts of Microplastics on Marine Organisms: A Review.” *Environmental Pollution (Barking, Essex : 1987)* 178 (July): 483–92. doi:10.1016/j.envpol.2013.02.031.

Appendix 1

Assessment of existing digestion approaches for isolation of marine microplastic from biota

Introduction

Plastic is accumulating in the oceans where it, exposed to various environmental stressor (UV, waves, sand, salt), breaks down to ever smaller fragments such as microplastic. Every size of plastic is of potential harm to a corresponding feeding size spectrum of a particular group of marine species. The quantification of microplastic in stomachs of various biota is central to assessing the impact and extant of plastic pollution in the environment to organisms and to determining its pathways through food webs and sinks. The development of techniques to isolate and characterise microplastic is, however, still under in progress and methodologies are across different studies are barely standardised. The monitoring of plastics in fish has been integrated into the European Union Marine Strategy Framework Directive (MSFD) and Oslo-Paris Commission (OSPAR) guidelines.

Before microplastic distribution and impacts can be systematically assessed standard sampling and laboratory techniques are required. A major challenge is the removal of the organic matter that is often associated with microplastic particles. This can be both detrital, for environmental samples as the hydrophobic nature of plastics will cause aggregation, but also organic matter in the form of tissue in the case of samples from biota. Before microplastic can be characterised either visually or spectroscopically the organic matrix must be removed.

Various approaches have been made using enzymes, a range of different bases (KOH, alkaline cleaning agents) and acids (HNO_3 $HClO_4$), oxidizer (H_2O_2).

A preliminary protocol for the digestion of organic matter in conjunction with microplastic isolation and sample preparation has been provided issued by the International Council for the Exploration of the Seas (ICES). The procedure recommends the usage of a mixture of HNO_3:$HClO_4$ (4:1) as digestive agents [1].

During preparations for national monitoring activities for microplastics in fish stomachs we found indications that the recommended acid combinations had severe effects on a range of common polymers. Here we report the results of a set of 20 different common polymers that as a result of the tissue digestion treatment during the recommended exposure time some polymers either completely dissolved, others partly disintegrated, or changed colour (surface damages) or were resistant.

Various studies have already applied the recommended acid mixture [2]–[4] or nitric acid alone [5] and should therefore be handled with caution. A validation of

different applied chemicals for method improvement is highly needed and was accomplished in this study by exposing the same test polymers to KOH [6] in combination with NaClO (14% free Chlorine) [7] as well as VIP1 (with 30% NaClO) [8], [9].

Methods

Plastic resistance to test chemicals

Polymer samples were chosen from a selection of consumer plastic items of which the material or recycling label was visible. Several pieces of about 0.5 cm size were cut off each item and transferred into a separate 8 ml laboratory glass vial with a black butyl/PTFE screw top lid. Treatment solutions (5 ml) were added and vials were kept upright during the testing period. Four different digestion solutions were compared (Table 1). The effects of the different treatments were documented photographically before the addition of chemicals and after 30 min, 1 h and 5 h. Following this treatment period at room temperature all samples were exposed to 80°C for 20 min and observed changes thereafter documented in the same manner. In the case of the acid mix treatment an extra image series was recorded after 10 h to better document also weak changes. Observed effects were categorised by severity into four levels (Table 2). Further notes were taken to better describe particular effects observed.

Table 1: Description of the four digestions solutions tested.

Acid mix	4:1 (v:v) HNO_3 69% (AnalR, VWR International S.A.S.) + $HCLO_4$ 70% (Rectapur, VWR International S.A.S.), procedure: Addition of 5 ml/g and digestion for 5 hours and subsequent heating for 10 min in 80°C.
KOH	Solution of KOH pellets (Emsure, Merck) in microfiltrated H_2O 1120 g/L, procedure: see above.
NaClO	NaClO solution, 14% active chlor (VWR International S.A.S.), procedure: see above.
Industrial CIP agent	VIP 1 (Novadan Aps), ready solution containing ~3% Potassium hydroxide, ~1% Potassium tripolyphosphate, ~1% Potassium silicate and ~7% Sodium hypochlorite, procedure: see above.

Table 2: Definition of the four different impact levels observed.

Level of impact	Description
L1	Beginning visual recognisable changes (colour, surface morphology)
L2	Morphological changes, beginning dissolution
L3	Strong morphological disintegration, change of bulk structure
L4	Complete dissolution / disintegration

Raman micro-spectrometry

In order to better evaluate weak changes on the outer matrix of the polymer a range of polymers was measured with Raman micro-spectrometry after the digestive treatments. The spectra were compared to a spectra library of the same polymers in untreated condition. Table 4 shows the respective polymer / digestant combinations tested.

Testing tissue digestion effectiveness

A comparison study was conducted in order to test for digestion effectiveness among the KOH (I), NaClO (II), KOH and NaClO in combination (III) and VIP1 (IV). VIP1 is a ready-made solution which contains both potassium hydroxide (KOH, 3%) and sodium hypochlorite (NaClO, 7%) as the main ingredients which is why these chemicals were tested for their digestive power first separately and later in combination. The test stomach tissues weighed between 8 and 22gram. Per gram of tissue, 5ml test solution was added to the sample. All treatments were first subjected to a 15 minutes ultrasonic bath followed by two hours of thorough shaking. The most effective digestion solution was then further diluted to find an optimum i.e. most economical concentration.

Results

Acid mix treatment

Exemplary observations from the acid mix exposure are shown in Figure 1 – 4 and summarised in Table 3. The strongest effects were observed for Polyamid (PA), Polyurethan (PU) and black tire rubber elastomer, all of which were completely dissolved by the acidic treatment. In case of PA6 the complete dissolution (L4) was observed within seconds to minutes after submersion in the acid mix. Other structurally affected polymers were Acrylonitrile butadiene styrene (ABS), Poly(methyl methacrylate) (PMMA) and Polyvinyl chloride (PVC). The latter one being affected in moderation, mainly colour leaching and softening (L2 – L3). Polymer samples of Polycarbonate (PC), expanded and solid Polystyrene (PS) and Polyethylene terephthalate (PET) were structurally little affected. Only staining or colour loss could be observed visually (L1). No effects were observed for Polypropylene (PP), high density and low density Polyethylene (HDPE, LDPE), Ethylene-vinyl acetate (EVA) and Polytetrafluoroethylene (PTFE). The heating to 80°C after the digestion period was fund to have an exacerbating effect on the polymer destruction in all cases where an effect has been observed beforehand.

Raman micro-spectrometry that has been performed on polymers which did not show severe visible changes after the acid mixture treatment revealed that apart from ABS all remaining polymers gave recognisable spectra although some showed signs of degradation or peak shifts (Table 4).

Table 3: Tested polymer types in the acid test treatment with observed impact levels.

	0.5 h	1 h	5 h	10 h	80°C	no change
PP						x
LDPE						x
HDPE						x
PS					L1	
EPS					L1	
ABS		L1		L2	L3	
PU	L2	L3	L4			
PA	L4					
PA	L2		L3			
EVA						x
PET			L1			
PC	L1					
Nitrile	L1		L3	L4		
PVC 1			L1		L2	
PVC 2			L1		L2	
PVC 3						x
PMMA		L2	L3			
PTFE						x
RB	L3	L4				
RB	L2	L3			L4	
TR	L1	L2	L3		L4	

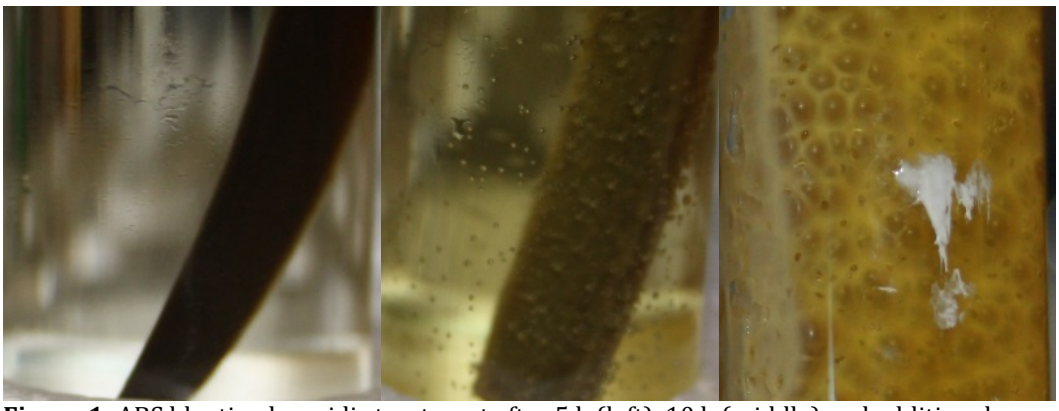


Figure 1: ABS bloating by acidic treatment after 5 h (left), 10 h (middle) and additional heating to 80°C (right).

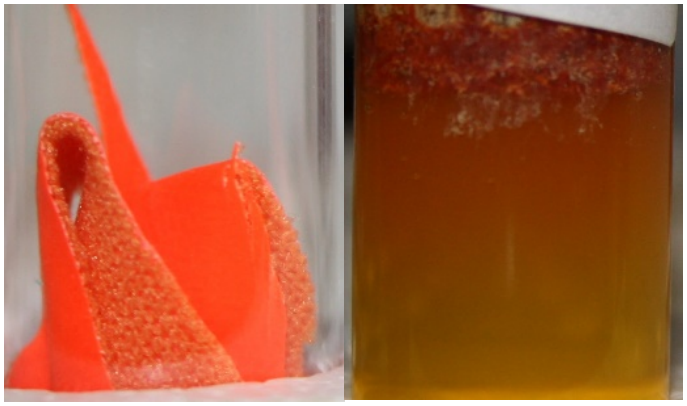


Figure 2: PU before (left) and after 5 h of acidic treatment.

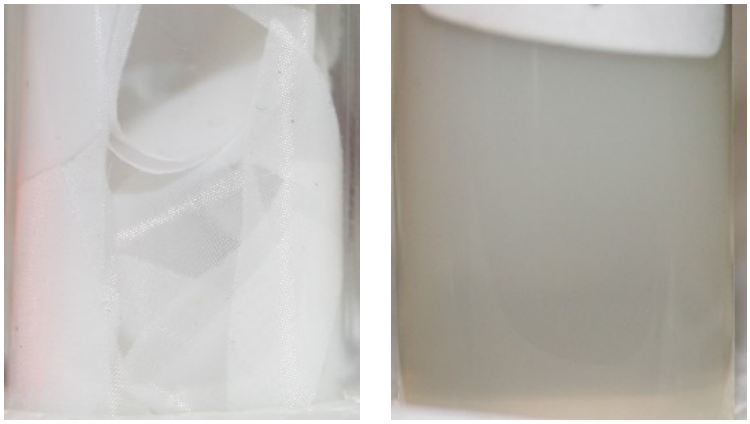


Figure 3: PA-6 before (left) and after first contact with acidic treatment.

Figure 4: No observable changes after acidic treatment of polyolefins (PP left, HDPE right).

Alkaline Treatments

All tested polymers did not show any impact according the defined levels 1 to 4 during the treatments using KOH, NaClO, VIP1 alone or in the described combinations. The characteristics of acquired Raman spectra were not or little changed after the polymer samples were treated with the 30% dilution of the 1:1 ratio mixed KOH:NaClO solution. For the pure and saturated KOH solution strong spectral deviations and lower quality spectra were obtained, however, all

polymer types could be recognised by means of comparing against the library of spectra from the untreated polymers.

Table 4 Evaluation of spectra changes after chemical treatments via Raman.

Legend: Recognisable, widely identical; Recognisable, with noted peak changes or flourescense; Hardly recognisable; Not recognisable; n/a: not measured.

	Acid Mixture	**KOH**	**30 % KOH : NaClO**
PP	Recognisable, widely identical	Recognisable, widely identical	Recognisable, widely identical
LDPE	Recognisable, alterations from the reference are likely to not be from the acid but due to variances among LDPE.	Recognisable	Recognisable, widely identical
HDPE	Recognisable, widely identical	Recognisable, widely identical	Recognisable, widely identical
PS	Recognisable, slightly noisy, florescence	Recognisable, widely identical	Recognisable, widely identical
EPS	n/a	n/a	Recognisable, widely identical
ABS	Not recognisable, change in peak positions and florescence. 1605 peak remains, 1353 peak occurred	Recognisable, slightly noisy	Recognisable
PA	n/a	Recognisable, missing peak at 147	Recognisable, peak at 147 weakened
PET	Recognisable, widely identical	Recognisable, peak at 3080 and 861 enhanced, new peaks at 1418 and 1132	Recognisable, widely identical
PC	Recognisable, widely identical	Recognisable, new peak at 1065	Recognisable, widely identical
PVC	Recognisable, Fluorescence, weaker main peaks at 704 and 637 indicating degradation processes	Recognisable, weaker main peaks at 704 and 637 indicating degradation processes	Recognisable, widely identical
PMMA	Recognisable, new peak at 1054 and 1308	Recognisable, new peak at 1070	Recognisable, widely identical
PTFE	n/a	n/a	Recognisable, widely identical

Testing tissue digestion effectiveness

Table 5 shows that among all chemicals tested KOH in combination with NaClO had the most satisfying effect i. e. it dissolved the sample tissue completely. KOH or NaClO alone are not nearly as effective. VIP1 was closest to the result of KOH: NaClO, however some slimy sediment remained. KOH:NaClO was then tested in three different (IIIa, IIIb, IIIc) proportions of which IIIc (1:1) was found to be optimal. Higher proportions of NaClO causes foam formation, too little reduces the digestion effectiveness (Table 6).Eventually a dilution of the KOH: NaClO (1:1) mixture to 30% was tested due to economical reasons and found to still enable a full digestion. While preparing this solution water should be added first before the two reagents are added to avoid precipitation.

Table 5 Descriptive test results for digestion effectiveness of KOH (I), NaClO (II), KOH: NaClO (III) and VIP1 (IV). a and b indicate when different concentrations were applied. The most effective treatment is marked in bold.

	Treatment	**Post treatment**
I	100% KOH	layer of black/brown slime afloat, no big pieces
IIa	30% NaClO	milky, stomach still floating as one piece
IIb	100% NaClO	only half of mixture added because of very strong foam formation, partly loss of sample.
III	**1:1 (KOH:NaClO)**	**sample tissue completely dissolved**
IVa	30% VIP1	sample mostly dissolved, slimy sediment remaining
IVb	100% VIP1	sample dissolved better, less slimy sediment remaining

Table 6 Testing different proportions of the KOH:NaClO mixture. The most effective one is marked in bold.

	Treatment	Post treatment
IIIa	2:1 (KOH:NaCLO)	many small pieces (food remaining e.g. copepods)
IIIb	1:2 (KOH:NaCLO)	Foam formation (minimized by cooling glass bottle in cold water), few small pieces (food remaining)
IIIc	**1:1 (KOH:NaClO)**	**Foam formation (slightly less than above). Sample tissue completely dissolved**

Conclusion and Recommendations

As we tested and evaluated the effect of the acid mixture on macroscopic plastic items one can assume that when exposing microplastic to the chemicals the destructive effect is even more severe due to its larger surface area and rather fragile nature. This is especially relevant to studies investigating microplastic below 300 µm. Study results based on the use of the ICES protocoll recommending the mixture of HNO3 and HCLO4 should therefore interpreted

with precaution.

Another study (Strand, in prep.) successfully used VIP1 for sediment and tissue digestion purposes. The VIP1 used in this study was already several month old and has been opened before which makes it likely that active chlorine has deteriorated to some extent. This would mean that the effectiveness was limited and perhaps better results can be obtained when using a fresh VIP1 solution. However, its access is more difficult for a wider scientific community (produced in DK) and it contains ingredients of which an auxiliary effect is unknown to us. Because of that and due to the convincing digestion effectiveness the usage of a 30% KOH:NaClO mixture (i.e. for 1 litre: 150 ml saturated KOH solution 1120 g/l + 150 ml NaClO solution with 14% active chlorine + 700 ml microfiltrated water) was found most appropriate and we suggest it therefore as a fast, inexpensive and effective digestion method. We recommend it being further compared under aspects of economic and expedient test execution against existing protocols of enzymatic digestion. Having evidenced the complete dissolution in reasonable work time while not impairing the integrity of all important plastic polymer groups, we argue for the described method being considered in international guidelines when targeting standard protocols for worldwide usage.

References

[1] ICES, "ICES Special Request Advice Northeast Atlantic and Arctic Ocean. OSPAR request on development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys.," IICES Advice 2015, B. 1, no. June, pp. 1–6, 2015.

[2] Z. V. Mechtild, "Impact of microplastic uptake : contamination in sprat and microplastic-mediated uptake of PAHs by European shore crab," 2015.

[3] V. Griet, V. C. Lisbeth, J. C. R, M. Antonio, G. Kit, F. Gabriella, K. M. J. J, D. Jorge, B. Karen, R. Johan, and D. Lisa, "A critical view on microplastic quantification in aquatic organisms," no. 2014, 2015.

[4] B. De Witte, L. Devriese, K. Bekaert, S. Hoffman, G. Vandermeersch, K. Cooreman, and J. Robbens, "Quality assessment of the blue mussel (Mytilus edulis): Comparison between commercial and wild types," *Mar. Pollut. Bull.*, vol. 85, no. 1, pp. 146–155, 2014.

[5] L. Van Cauwenberghe and C. R. Janssen, "Microplastics in bivalves cultured for human consumption.," *Environ. Pollut.*, vol. 193, pp. 65–70, 2014.

[6] E. M. Foekema, C. De Gruijter, M. T. Mergia, J. A. van Franeker, A. J. Murk, and A. a Koelmans, "Plastic in north sea fish.," *Environ. Sci. Technol.*, vol. 47, no. 15, pp. 8818–24, Aug. 2013.

[7] T. K. Sørensen, C. Stedmon, K. Enders, and O. Henriksen, "Analyse af marint affald i sild og hvilling fra det nordlige Storebælt," 2013.

[8] S. Agersnap, "Mikroplastik i havmilløet," 2013.

[9] J. Strand, P. Lassen, Y. Shashoua, and J. H. Andersen, "Occurrence of microplastic particles and bio-geochemical relationships in sediments from Danish waters (in prep.)."