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Distribution of microplastics in marine species of the Wadden Sea along the coastline of Schleswig-Holstein, Germany

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Summary

Within the project "Distribution of microplastics in marine species of the Wadden Sea along the coastline of Schleswig-Holstein, Germany" the central research question investigated was whether, to what extent and under which conditions marine species in the Wadden Sea are affected by microplastics. The aim of this project was to contribute to the assessment of the current status of microlitter ingested by marine species with a focus on microplastics and to provide an initial assessment of possible monitoring strategies for marine species along the North Sea coastline of Schleswig-Holstein.

Within the project, a total number of 12 species were studied at four sites along the Wadden Sea coast of Schleswig-Holstein. The species included demersal and pelagic fish species (Atlantic herring - *Clupea harengus*, viviparous eelpout - *Zoarces viviparus*, European plaice - *Pleuronectes platessa* and common sole - *Solea solea*) as well as benthic species represented by molluscs (soft-shell clam (sand gaper) - *Mya arenaria*), common cockle - *Cerastoderma edule*, blue mussel - *Mytilus edulis*, Pacific oyster - *Magallana gigas*, common periwinkle - *Littorina littorea*), polychaetes (lugworm - *Arenicola marina*) and arthropods (green shore crab - *Carcinus maenas* and brown shrimp - *Crangon crangon*). These were sampled twice, in October 2017 and May/June 2018, with 16 to 66 individuals and examined for microplastics.

After sampling, the benthic individuals were incubated for 24 h in filtered seawater to determine the amount of microplastic particles from faecal residues. After dissection of the tissue, the samples were prepared by destroying the biological organic material with a digestion solution of potassium hydroxide and sodium hypochlorite followed by filtration. Microplastic particles were identified by staining with Nile red and subsequent examination under a fluorescence microscope. A subset of particles identified in this way, were also examined for their polymer type using µRaman spectroscopy.

The results show that all investigated species except common sole are affected by microplastic contamination. The proportion of affected individuals was 38.9% (demersal chordates), 42.3% (pelagic chordates) and 88.2% of all invertebrates. In invertebrates, microplastic particles were most frequently found in individuals of molluscs (96.6% of all individuals), followed by polychaetes (92.7%) and arthropods (64.8%).

With regard to the concentrations of microplastics (in particles per g net weight), the following median values were determined in decreasing order: Common periwinkle (5.50), lugworm (2.27), viviparous eelpout (1.72), common cockle (1.69), blue mussel (1.38), followed by Atlantic herring (0.95), Pacific oyster (0.86), green shore crab (0.45), soft-shell clam (0.40), brown shrimp (0.12) and European plaice (0.01).

The dominant type of microplastics was broken fragments of larger items, which also show a clearly different pattern than fibres in the frequency distribution of the particle sizes. The number of particles recorded increases steadily with decreasing particle size across all particle types. Considering this distribution in relation to fragments separated by species, it becomes evident that in particular Atlantic herring has a different distribution pattern. The median particle size for herring is 287 µm. In comparison to all other species, whose median values lie between 107-168 µm, a significant difference can be observed, which indicates an active uptake of particles of a certain size.

With regard to seasonal differences, the results are difficult to assess, especially due to varying weights of individual animals. Also with regard to the spatial distribution of the four locations List, Pellworm,

Büsum and Friedrichskoog only weak tendencies of increasing concentrations with increasing geographical latitude can be determined. Significantly higher values are observed for the species soft-shell clam, green shore crab and blue mussel at the southernmost site of Friedrichskoog.
The polymer types analysed by µRaman spectroscopy on 113 particles are dominated by polyethylene, ethylene-vinyl acetate copolymer, polyethylene terephthalate and polypropylene, which together comprise over 80% of the polymer spectrum.

Based on the results, several species can be identified as suitable biological indicators for future monitoring programs. From the point of view of statistical representativeness, species with high individual weights such as soft-shell clam and lugworm should be considered in particular. However, other species could also be integrated, provided that a sufficient number of individuals are sampled and analysed as composite samples and the background signals from environmental factors, in particular the mudflat sediment, are recorded.

1. Introduction

The Marine Strategy Framework Directive (MSFD) and the "Commission Decision on Good Environmental Status" in 2017 lay down "criteria and methodological standards on good environmental status of marine waters and specifications and standardized methods for monitoring and assessment" (European Commission, 2017a). For implementation, the member states are requested to develop and regularly review a strategy for its marine waters in the respective European marine regions addressing 11 environmental descriptors. The strategy shall, among others, include the initial assessment of the current environmental status, the establishment of environmental targets and associated indicators to achieve good environmental status (GES) by 2020, and the set-up of monitoring programs for an ongoing assessment and the regular update of targets (European Commission, 2017b).

Descriptor 10 "Marine Litter" sets the target that properties and quantities of marine litter do not cause harm to the coastal and marine environment and lists 4 criteria. Besides macro- and micro-litter on the coastline, in the surface layer of the water column, in seabed sediment (D10C1 and D10C2) and the influence of macro-litter on biota such as entanglement (D10C4), the criteria D10C3 addresses the influence of litter and micro-litter ingested by marine animals.

The current project aims to contribute to the assessment of the current status on microlitter ingested by marine species focusing on microplastics and to provide a first evaluation of potential monitoring strategies of marine species along the North Sea coastline of Schleswig-Holstein.

2. Objectives and research approach

The project "Distribution of microplastics in marine species of the Wadden Sea along the coastline of Schleswig-Holstein, Germany" addresses the central research questions whether, to what extent and under which conditions marine species in the Wadden Sea are affected by the presence of microplastic particles.

In detail, the following research questions are investigated:

(1) Are marine species in the area of the National Park of the Wadden Sea threatened by microplastic contamination and which species are affected the most?

(2) Is there any correlation between microplastic concentrations in marine species and specific parameters and conditions such as season, habitat, trophic level, feeding strategy, body weight and size?

(3) Do microplastic concentrations found in individuals and species show spatial patterns?

(4) Is it possible to evaluate the eligibility of certain species as biological indicators for future monitoring programmes based on the findings?

3. Microplastic contamination in marine species - state of research

3.1 Definitions and general findings

In the last decades the contamination of aquatic ecosystems and coastlines with microplastics has been identified as emerging research focus (Galgani, 2015; Galgani et al., 2013a; Wright et al., 2013; Thompson et al., 2004).
Microplastics are defined as synthetic polymer particles being less than 5 mm in their longitudinal orientation. The further differentiation refers to their origins as primary particles such as pre-production pellets or microbeads used in cosmetics, or secondary particles predominately derived from physical abrasion (e.g. textile fibres, tyre wear) or from degradation of larger meso- or macroplastics (Andrady, 2011; Barnes et al., 2009; Moore et al., 2011).

Marine ecosystems serve as a sink in which plastic litter of any size accumulates, abrades and fragments. Marine sources of plastic litter cover any direct input of plastic at sea (on the German North Sea coast mostly related to fishing and shipping activities), from terrestrial sources such as input via rivers, sewage outlets and litter deposited on the shoreline as well as atmospheric deposition.

Numerous studies undertaken in laboratory and natural environments reveal the ecological consequences of plastic pollution of the marine ecosystem. Whereas harm from larger plastic items is mostly related to entanglement (Gregory, 2009; Thompson et al., 2014), for smaller particles it occurs through intended or unintended ingestion. Once ingested plastic may lead to (physical) obstruction or starvation (Battaglia et al., 2015; Cole et al., 2013; Lusher et al., 2015a, b; Van Franeker et al., 2011; Wedemeyer-Strombel et al., 2015). There are several pathways for microplastic incorporation in aquatic species: incorporation through ingestion, transfer to the digestive tract (Boerger et al., 2010; Lusher et al., 2013) and further translocation (Browne et al., 2008) or uptake via the gills (Watts et al., 2014). Plastic particles can be ingested by benthic and pelagic species directly or via consumption of prey species (Cole et al., 2013; Gutow et al., 2015; de Sá et al., 2015; Rummel et al., 2016; Watts et al., 2015). Additional contamination with plastic particles can be through the adhesion of plastic particles to organism surfaces (Dabrunz et al., 2011).

The potential uptake of ecotoxicological substances, which are an integral part of the plastic itself or which are adsorbed to its surface, is of central interest to many studies on trophical transfer (Camedda et al., 2014; Cole et al., 2011; Farrell and Nelson, 2013; Galgani, 2015; Van Cauwenberghe et al., 2015) or general ecotoxicological consequences (Beyer et al., 2014; Fossi et al., 2014; Galloway, 2015; Koelmans et al., 2016, Rochman, 2015). Most ecotoxicological studies rely on laboratory exposure experiments investigating the consequences of microliter ingestion on reproduction, fertility, energy budget and trophical transfer. In general, these exposure studies are apply high concentrations of plastics in order to achieve a detectable signal or effect and are not carried out under realistic environmental conditions and background values.

3.2 Microplastic abundance in marine species of the southern North Sea

The North Sea is marked by very intense fishing and shipping activities, thus, it is highly susceptible for plastic contamination. Nevertheless, there are only a few studies on microplastic ingestion of invertebrates and fish species under natural environmental conditions in the North Sea region and most of these refer to fish species.

The ingestion of microplastic by demersal and pelagic fish from the North and Baltic Sea was investigated by Rummel et al. (2016). They were able to show that 5.5 % of all investigated individuals were affected by microplastic contamination. The results showed that demersal species were less affected than pelagic species (3.4 vs. 10.7 %) without showing an effect on condition factor K, expressed as the ratio of individual length and weight. In contrast, Lusher et al. (2013), investigating 261 individuals of 10 fish species, found that 11 % of the investigated individuals were contaminated with microplastic particles >500 µm. In his study a significant difference between pelagic and demersal species was not apparent. Another investigation on microplastics (>100 µm) in demersal and pelagic fish species in the Danish regions of the North and the Baltic Seas addressed the species cod (*Gadus morhua*) and herring (*Clupea harengus*) in coastal and off-shore locations (Lenz et al., 2016). In this study microplastic was found in 30 % of all herring individuals in coastal and 16 % of individuals in off-shore locations. Comparing cod and herring in the North Sea, the total number of affected cod was higher with 39 individuals compared to 23 individuals in herring. Though, taking into account the ratio between microplastic particle concentration and the weight of stomach tissue that was analysed, the microplastic concentrations in the herring exceeded those of cod (median values herring about 0.45 vs. 0.05 microplastic particles per g stomach tissue for cod). Comparing the results for herring between the North and Baltic Seas, Lenz et al. (2016) found "notably higher" numbers of individuals with ingested microplastics in the North Sea.
In a project carried out in the North Sea by Foekema et al. (2013) the abundance of microplastic particles (>200 µm) in 1,203 individuals of 7 species was investigated (cod - *Gadus morhua*, whiting - *Merlangius merlangus*, haddock - *Melanogrammus aeglefimus*, herring - *Clupea harengus*, horse mackerel - *Trachurus trachurus*, gray gurnard - *Eutriglia garnardus* and atlantic mackerel - *Scomber scombrus*). Plastic particles were found in 2.6 % of the fish individuals and in 5 of 7 species. Regarding spatial differences, significantly more microplastic was determined in fish from the southern North Sea than from the northern part. Here again there was no apparent relationship between plastic particle contamination and condition factor.

Only few studies on invertebrates in the North Sea have been conducted. Lusher et al. (2017) investigated bivalves and sediments from 13 sampling sites around the coastline of Norway in order to test and develop methodologies and to evaluate potential monitoring recommendations. Microplastics (>150 µm in size) were detected in 76.6 % of all individuals of blue mussels (*Mytilus edulis*) (n=252) showing an average concentration of 1.84 particles per individual (1.85 particles per g wet weight). The dominating type of microplastic was fibre (85 %) mostly consisting of semi-synthetic cellulose. Spatial patterns showed lower contamination close to urban sites compared to rural sites, a result that is affected by other environmental factors and differences in the size of individual mussels.
Leslie et al. (2015) investigated microplastic abundance along the Dutch coastline in the five benthic species Pacific oyster (*Magallana gigas*), common periwinkle (*Littorina littorea*), blue mussel (*Mytilus edulis*), green shore crab (*Carcinus maenas*) and amphipods (*Gammarus sp.*) (3-16 individuals, respectively). Results on filter feeding Pacific oyster and blue mussel were found to be higher (30/87 and 19/105 particles per g dry weight) than in common periwinkle (20 particles per g dry weight) and amphipods (11 particles per g dry weight). No microplastic contamination was detected in green shore crab. Another project along the French-Belgian-Dutch coastline focused on blue mussel (*Mytilus edulis*) and lugworm (*Arenicola marina*) sampled at six locations. Microplastics were present in all individuals investigated visually down to sizes of 35 µm with mean values of 0.2 (blue mussel) and 1.2 particles (lugworm) per g tissue, respectively (van Cauwenberghe et al., 2015).

4. Material and methods

4.1 Investigation site and sampled species

Within the area of the national park "Schleswig-Holstein Wadden Sea" four sampling sites were selected representing the North-South extent of the area.

Area	Investigation site	Mean tidal range (m)	Wadden type
North Frisian Wadden Sea	Lister Tief	1.8	sand mudflat / mixed sediment mudflat
North Frisian Wadden Sea	Pellworm	3.3	sand mudflat / mixed sediment mudflat
Dithmarscher tidal flat	Büsum	3.2	Sand mudflat / mixed sediment mudflat
Dithmarscher tidal flat	Friedrichskoog	3.2	mixed sediment mudflat / intertidal estuarine mudflat

Table 1: Location and basic characteristics of sampling sites

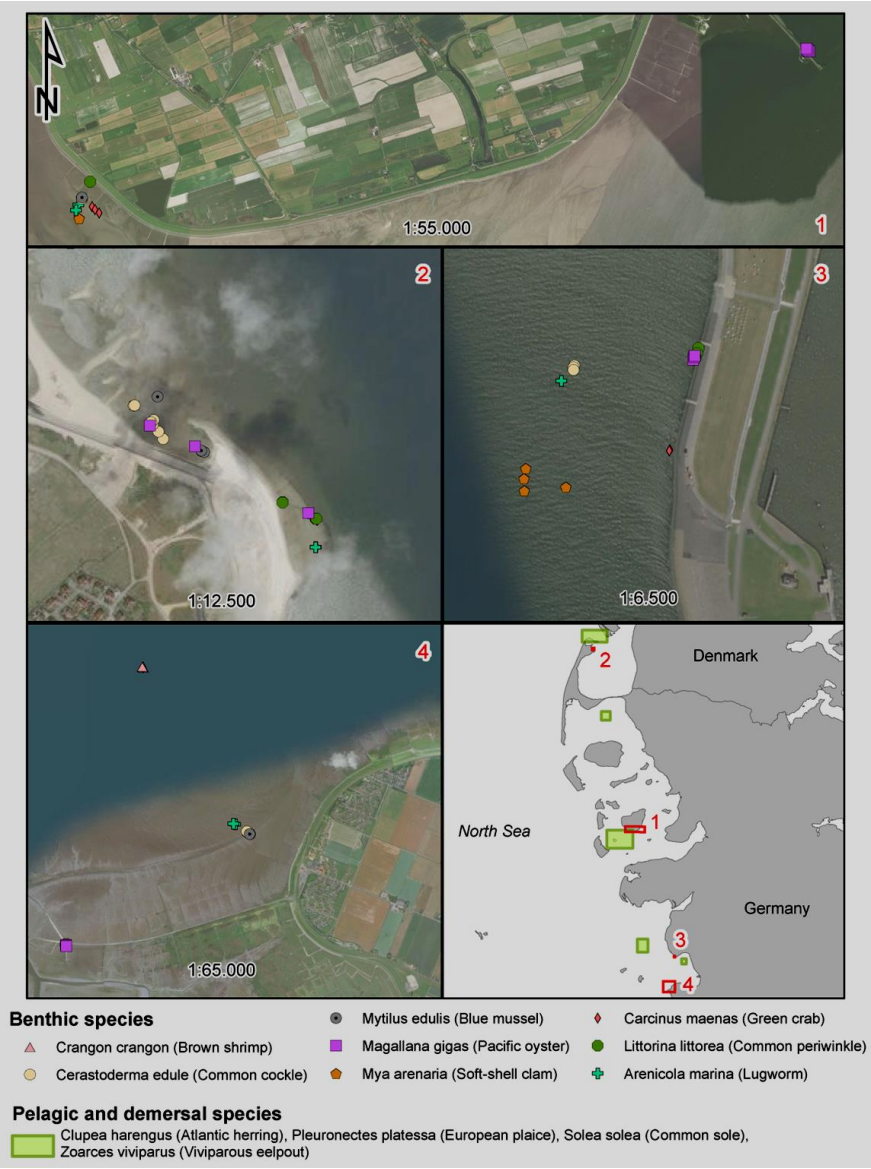

Figure 1: Map of the sampling sites

The investigated species cover different trophic levels and groups of organisms:

Species	Phylum Class Family	Habitat	Feeding strategy / type	Life stage	Behavior	Investigated material
Blue mussel *Mytilus edulis*	Mollusca Bivalves Mytilidae	benthic	filter feeder	adult	sessile	faecal residues, soft tissue whole organism
Pacific oyster *Magallana gigas*	Mollusca Bivalves Ostreidae	benthic	filter feeder	adult	sessile	faecal residues, soft tissue whole organism
Soft-shell clam (sand gaper) *Mya arenaria*	Mollusca Bivalves Myidae	benthic	filter feeder	adult	sessile	faecal residues, soft tissue whole organism
Common cockle *Cerastoderma edule*	Mollusca Bivalves Cardidae	benthic	filter feeder	adult	sessile	faecal residues, soft tissue whole organism
Common periwinkle *Littorina littorea*	Mollusca Gastropodae Littorinidae	benthic	Grazing feeder	adult	motile	faecal residues, soft tissue whole organism
Lugworm *Arenicola marina*	Polychaeta Sedentaria Arenicolidae	benthic	deposit feeder	adult	motile	faecal residues, soft tissue whole organism
Green shore crab *Carcinus maenas*	Arthropoda Malacostraca Carcinidae	benthic	predator carnivorous	adult	motile	faecal residues, soft tissue whole organism
Brown shrimp *Crangon crangon*	Arthropoda Malacostraca Crangonidae	benthic	predator omnivorous	adult	motile migratory	faecal residues, soft tissue whole organism
Viviparous eelpout *Zoarces viviparus*	Chordata Actinopterygii Zoarcidae	demersal	predator carnivorous	adult	motile, migratory	stomach / gut
Common sole *Solea solea*	Chordata Actinopterygii Pleuronectidae	demersal	predator carnivorous	juvenile	motile migratory	stomach / gut
Common sole *Pleuronectes platessa*	Chordata Actinopterygii Pleuronectidae	demersal	predator carnivorous	juvenile	motile, migratory	stomach / gut
Atlantic herring *Clupea harengus*	Chordata Actinopterygii Clupeidae	pelagic	predator omnivorous	juvenile	motile migratory	stomach / gut / liver

Table 2: Investigated species and key characteristics

Location	List			Pellworm			Büsum			Friedrichskoog			TOTAL
Season / Species	10/17	05/18	total	10/17	05/18	total	10/17	05/18	total	10/17	05/18	total	
Blue mussel *Mytilus edulis*	5	9	**14**	5	8	**13**	6	10	**16**	6	9	15	**58**
Pacific oyster *Magellana gigas*	6	5	**11**	6	6	**12**	6	6	**12**	5	6	11	**46**
Soft-shell clam (sand gaper) *Mya arenaria*	1	3	**4**	1	9	**10**	3	5	**8**	0	2	2	**24**
Common cockle *Cerastoderma edule*	5	3	**8**	6	9	**15**	6	8	**14**	3	8	11	**48**
Common periwinkle *Littorina littorea*	7	8	**15**	8	8	**16**	6	4	**10**	8	8	16	**57**
Lugworm *Arenicola marina*	7	6	**13**	4	6	**10**	3	3	**6**	6	6	12	**41**
Green shore crab *Carcinus maenas*	9	5	**14**	6	0	**6**	1	0	**1**	4	0	4	**25**
Brown shrimp *Crangon crangon*	0	7	**7**	18	0	**18**	20	0	**20**	11	10	21	**66**

Table 3: Numbers of sampled individuals of invertebrate species, location and season

Location	Northfrisian Wadden Sea (Lister Tief, Pellworm)			Ditmarscher Tidal Flat (Friedrichskoog / Büsum / Kronenloch)			TOTAL
Season / Species	10/17	05/18	total	10/17	05/18	Total	
Viviparous eelpout *Zoarces viviparus*	0	3	**3**	13	0	**13**	**16**
European plaice *Pleuronectes platessa*	7	6	**13**	52	0	**52**	**65**
common sole *Solea solea*	0	9	**9**	7	0	**7**	**16**
Atlantic herring *Clupea harengus*	18	12	**30**	24	0	**24**	**54**

Table 4: Numbers of sampled individuals of fish species, location and season

4.2 Sampling of fish and invertebrate species

Sampling of invertebrate and fish species took place in September 2017 and May/June 2018. A total number of 365 invertebrate and 151 fish individuals were sampled and subsequently analysed. Due to seasonal programmes of shrimp and monitoring cruises, fish species in the Dithmarscher tidal flat were only investigated in 2017.

The sampling of benthic species was done according to van Cauwenberghe et al. (2015). Benthic species were recovered, thoroughly rinsed with filtered seawater and placed in 250 to 400 ml brown glass jars filled with 150 ml of filtered seawater. Brown shrimp, blue mussels, common periwinkles, lugworms and common cockle were kept in glasses with three or four individuals, pacific oysters and soft-shell clams were kept single, in addition soft-shell clams were sealed with a tight rubber band during incubation in

order to mimic the pressure of the surrounding sediment under natural conditions. All species were incubated in the dark over a 24 h period to allow complete gut clearance.
To minimize any re-uptake of faeces residues, the filtered seawater was replaced every 6 h and collected as a composite sample in 600 ml brown glass jars. The residual suspensions were rinsed over a 63 µm sieve. The residue from the sieving process was transferred, with a little water, into glass vials and stored at 4°C until further analyses. Subsequent to incubation, the individuals were anaesthetised and placed individually in aluminium foil and vacuum packed in bags. The sampled individuals were frozen at -18°C until further treatment.

Fish species were caught during regular shrimp trawls in the North Frisian Wadden Sea and the Dithmarscher Wadden Sea or the during fish monitoring cruises (North Frisian Wadden Sea - Alfred-Wegener-Institut awi List, Dithmarscher tidal flat - Marine Science Service, R. Vorberg). Fish individuals were placed into polyethylene bags, frozen immediately at -8 to -18°C and transported in freezer boxes for further analyses in the laboratory.

4.3 Laboratory analyses

Recording of basic data on individuals and extraction of sample material

Basic data such as individual length, width, net and gross weight were recorded and individuals were photographed.

Soft tissue of benthic species and stomach/gut tissue was dissected, weighed and transferred into glass beakers.

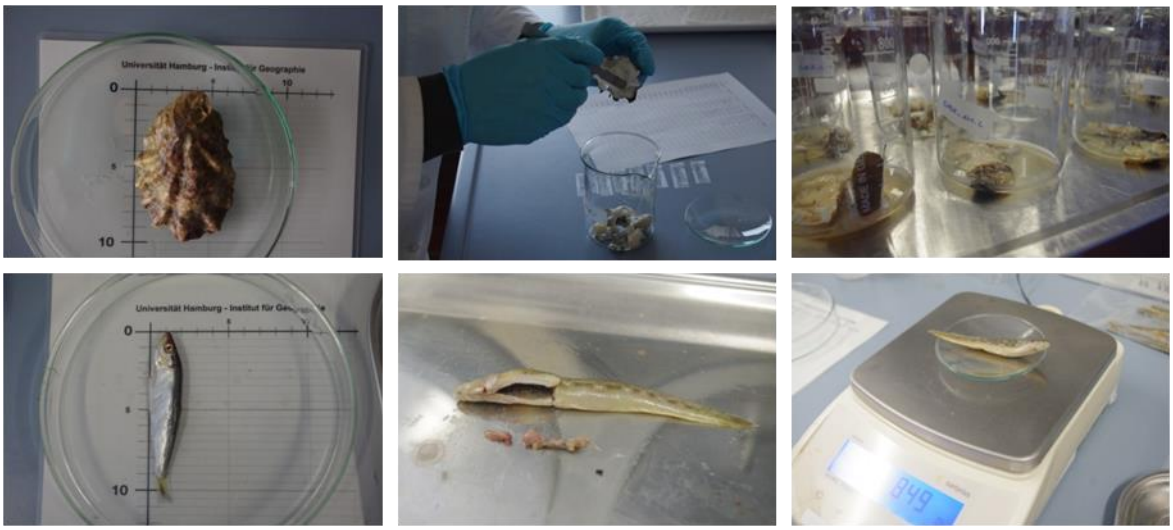

Figure 2: Recording of basic data, dissection of tissue

Digestion protocol - destruction of organic material

The subsequent organic digestion protocol was carried out using a protocol modified from Enders et al. (2016) and Strand & Tairova (2016). For preparing the digestion solution 150 ml potassium hydroxide (KOH, 1120 g/L, 20 M) and 150 ml sodium hypochlorite (6-14 % reactive chlorine) were transferred into a 1000 ml volumetric flask and filled to level with MilliQ-water. For each gram weight of tissue 5 ml of the digestion solution were added to the samples of biota tissue in the glass beakers, which were then covered with watch glasses. The beakers were placed under a fume hood for 18-24 hours at room

temperature. If digestion was incomplete this step was repeated by adding another aliquot of the digestion solution and slowly stirring the suspension with a magnetic stirrer for 10 minutes.

Density separation of faeces residues

After 24 hours of incubation in filtered seawater the faeces residues were treated according to a protocol modified from Claessens et al. (2013). Residues were rinsed with a little water into 15 ml glass vials, sealed and centrifuged for 5 minutes at 2000 g. The supernatant was decanted and transferred to 50 ml brown glass jars. 5 ml of sodium iodide solution (NaI, 3.3 M, density approximately 1.6 g*cm³) was added to the vials, suspensions were manually shaken and centrifuged for 5 minutes at 2000 g. The supernatant was decanted and added to the respective brown glass jars. This treatment step was repeated twice. The resulting decanted suspensions were transferred onto glass fibre filters (613, VWR International, 5-13 µm retention) through vacuum filtration (Sartorius Stedim 16828-CS). Filters were placed in glass petri dishes, covered and dried at room temperature under a fume hood. The identification of polymer particles was carried out by dying the particles using the protocol described below and subsequent µRaman-spectroscopy.

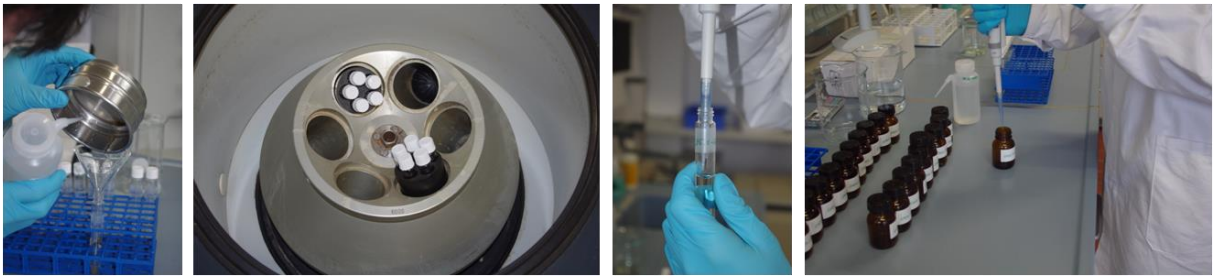

Figure 3: Density separation of faeces residues

Background contamination - quality assurance and procedural blanks

In order to minimize background contamination several provisions were undertaken. Labcoats were worn throughout the analyses, glass and stainless steel material was used as far as possible, all material was thoroughly rinsed with deionized water and/or acetone before use, vessels were covered at all times, all surfaces were cleaned daily, an additional air-cleaning device was employed and air humidity was increased artificially. Moreover, procedural blanks were carried out in parallel to each sample series. In total, a number of 90 blank samples were treated in the same way as faeces residues and samples of individual animals and analysed for background contamination. Since lab conditions varied, the respective median number of polymer particles found in blank samples was subtracted from the respective sample series. Results on procedural blanks are given in chapter 5.1.

4.4 Polymer identification via staining and µRaman-spectroscopy

The polymer identification was done according to a protocol modified from Tamminga et al. (2017) and Maes et al. (2017). Nile red solution was prepared at a concentration of 1 mg/ml chloroform (99 % AnalaR Normapur). A volume of 1.0 ml of Nile red solution was applied in two aliquots of 0.5 ml each directly onto the glass fibre filters. Filters were immediately covered with watch glasses and stored under a fume hood for 12-18 hours for drying. For documentation, filters were photographed prior to staining and after staining in a self-constructed black-photo box under UV light ((Pentax K-30, exposure time 2", ISO 100, resolution 2420 ×2343, Omnilux UV 18W G13, 365 nm). Filters were placed under a fluorescence microscope (AxioLab2.0, Zeiss, modified to light source (LED 1850, AHF) and filter TRITC

HC 543/22 and 593/40 Bright Line, AHF). Filters were scanned in a 4*4 mm grid for visual appearance of potential plastic particles. Particles were photographed (Canon EOX), measured and counted with Adobe PhotoShop (version CC2017) and finally classified to fragment/fibre shapes. Pre-tests and training of processors on a reference sample set covering various polymers and organic material were carried out. Besides emission intensity and colour, other criteria such as homogeneity of staining and lack of cell structure were taken into account for the identification of synthetic polymer particles. For comparable results, the final counting and measurement was done by one single trained processor.

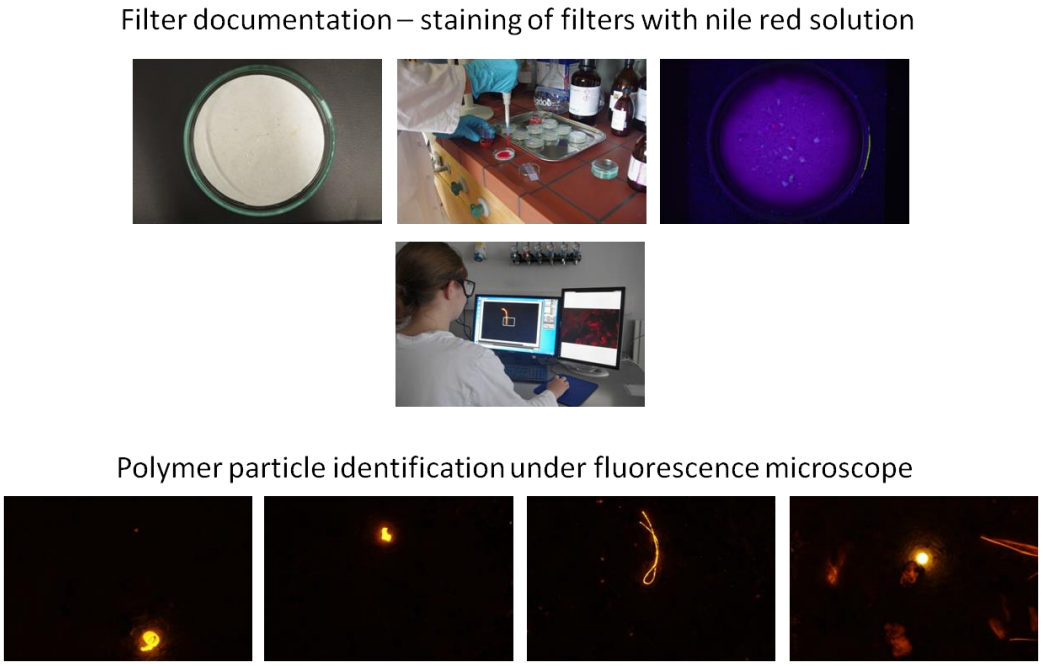

Figure 4: Documentation and staining of filters, polymer identification via fluorescence microscopy

A subset of 2-3 samples per species was transferred to aluminium oxide filters (Whatman Anodisc 0.2 µm, 25 mm diameter) applying vacuum filtration. Filters were dried in covered petri dishes and subsequently screened for polymer particles with µRaman-spectroscopy (DXR2xi, Thermo Fisher Scientific). After recording of polymer spectra of identified particles (n=55), the filters were stained with Nile red solution and investigated under fluorescent microscope as described above. Particles that were additionally identified through staining were then investigated again with micro-Raman-spectroscopy.

Another subset of 60 particles >300 µm were removed from stained filters with tweezers, placed on microscope slides and investigated with µRaman-spectroscopy for polymer identification. Raman spectra and size dimensions of all particles were recorded and evaluated (see Annex A5 and A6 for examples of identified polymer fragments and fibres).

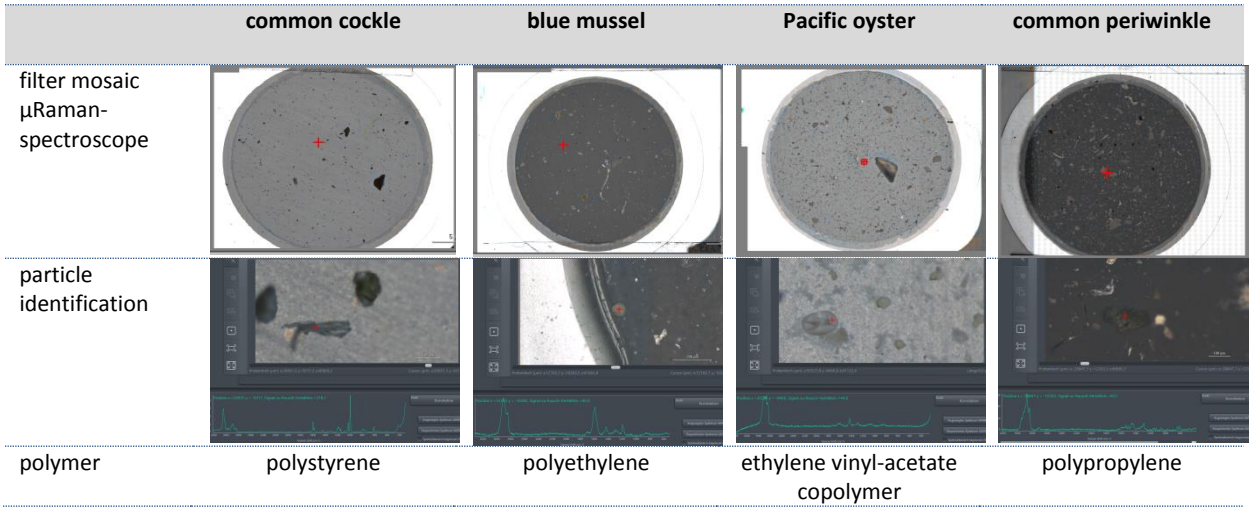

Figure 5: Polymer identification with µRaman-spectroscopy

4.5 Statistical evaluation of data

Statistics on all data were performed with RStudio 1.1.456 (R_Development_Core_Team, 2018). Assumptions of normality of data sets were tested using Lilliefors test, correlation of selected variables were performed using non-parametric SPEARMAN rank correlation test. An analysis of variance (ANOVA) was applied to determine significant differences between results in relation to species, locations, individual size and weight.

Since data are not normally distributed, median values are reported throughout the text. For comparison, mean values and standard deviation are given in the tables on descriptive statistics, as well.

5. Results

In the following, major results on microplastic concentrations found in species are given. Additional descriptive statistics, figures and illustrations are presented within the Annex. An explanation of the statistical variables displayed in boxplot figures can also be found in the Annex (A2).

5.1 Procedural blank analyses

Although diverse prevention measures were implemented, contamination of samples with microplastic could not be prevented completely. Thus, in the current project, a concise analysis with procedural blanks encompassing all steps of sampling and analysis has been undertaken. The results from the analyses of a total number of 90 blanks, which were taken through the same procedure as the samples, from sampling to analysis in the laboratory, were integrated in the final results. Within a conservative approach, mean values + 1x standard deviation were subtracted from the results to take into account background contamination levels. This was done separately for the both fragments and fibres. The analyses on faeces residues were all carried out within a few days, therefore, overall means + 1x standard deviation were considered.

Procedural blanks carried out in parallel to the tissue analyses varied according to the laboratory conditions (mostly due to the level of lab frequentation). The overall statistics are also given in table 5, monthly data on tissue analyses that were considered in the results are shown in figure 6.

	faeces residues (invertebrates)			**tissue (invertebrates)**			**tissue (fish species)**		
	procedural blank sampling and laboratory analyses			**procedural blank laboratory analyses**			**procedural blank laboratory analyses**		
number of blank samples	23			48			19		
shape	fragments	fibres	all particles	fragments	fibres	all particles	fragments	fibres	all particles
minimum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45
maximum	1.79	0.45	2.24	3.13	1.79	3.13	0.89	2.24	2.68
mean	0.39	0.16	0.54	0.53	0.18	0.71	0.45	0.93	1.38
median	0.00	0.00	0.45	0.45	0.00	0.45	0.45	0.45	0.89
standard deviation	0.53	0.22	0.63	0.74	0.39	0.86	0.28	0.86	0.97
blank correction value (mean +1x s.d.)	**0.92**	**0.37**	**1.18**	**1.19**	**0.39**	**1.31**	**0.73**	**1.31**	**1.86**

Table 5: Descriptive statistics on procedural blank results and blank subtraction per individual (number pf particles/blank)

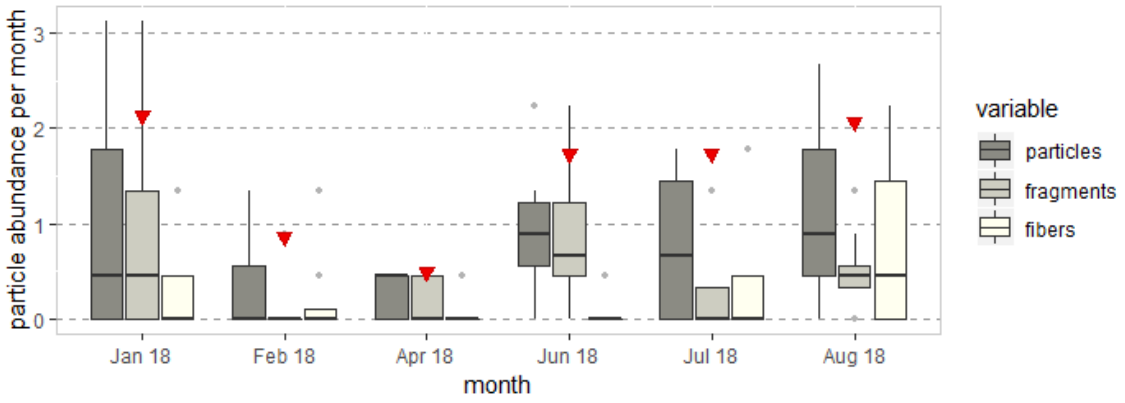

Figure 6: Results on procedural blank samples of tissue analyses according to shapes and value of blank subtraction applied per individual (in red triangles)

5.2 Microplastic abundance and concentrations of fragments and fibres in investigated species

Microplastic particles were abundant in all investigated species except in common sole. For all species together, a proportion of 77.9 % individuals were contaminated with microplastic.
Concerning the fish species the proportion varied from 38.9 % in pelagic fish (Atlantic herring) to 42.3 % in demersal fish (viviparous eelpout, common sole, and European plaice).
Taking into account both residual faeces and microplastic particles in soft tissue 88.2 % of all individuals of the invertebrate benthic species were contaminated. The values for species groups were 96.6 % for molluscs (blue mussel, pacific oyster, soft-shell-clam, common cockle, common periwinkle), 92.7 % for polychaetes (lugworm) and 64.8 % for arthropods (green shore crab, brown shrimp).
Fish species in general were less affected, especially common sole, which showed no microplastic contamination. The invertebrates generally showed higher numbers of microplastic abundance per individual, least influenced were brown shrimps, where only tissue samples and no faeces residues were investigated.

	n (affected / investigated)	percentage (%)
Atlantic herring *Clupea harengus*	33 / 54	61.1
Viviparous eelpout *Zoarces viviparus*	14 / 16	87.5
European plaice *Pleuronectes platessa*	27 / 65	41.5
Common sole *Solea solea*	0 / 16	0.0

Table 6: Total number and percentage of fish species individuals affected by microplastic contamination

	Faeces residue		**Soft tissue**		**Sum**	
	n (affected / investigated)	percentage (%)	n (affected / investigated)	percentage (%)	n (affected / investigated)	percentage (%)
Blue mussel *Mytilus edulis*	40 / 58	69.0	54 / 58	93.1	56 / 58	96.6
Pacific oyster *Magellana gigas*	43 / 46	93.5	42 / 46	91.3	46 / 46	100.0
Soft-shell clam *Mya arenaria*	21 / 24	87.5	24 / 24	100.0	24 / 24	100.0
Common cockle *Cerastoderma edule*	37 / 48	77.1	40 7 48	83.3	47 / 48	97.9
Common periwinkle *Littorina littorea*	33 / 57	57.9	46 / 57	80.7	52 / 57	91.2
Lugworm *Arenicola marina*	37 / 41	90.2	36 / 41	87.8	38 / 41	92.7
Green shore crab *Carcinus maenas*	23 / 25	92.0	25 / 25	100.0	25 / 25	100.0
Brown shrimp *Crangon crangon*	NA		34 / 66	51.5	34 / 66	51.5

Table 7: Total number and percentage of benthic species individuals affected by microplastic contamination

Based on microplastic particle counts per individual, highest numbers were found in lugworm (median 15.7), followed by soft-shell clam (median 13.9) and Pacific oyster (median 13.1). Lowest concentrations per individual were present in fish species (European plaice < Atlantic herring < viviparous eelpout).

With regard to the different types of microplastic, fragments dominated in all species with, however, significant differences between soft-shell clam, blue mussel, Pacific oyster and viviparous eelpout ANOVA, $\alpha<0.05$). In total, only 8 microbeads of spherical shape and sizes between 150 to 300 µm were recorded, with five of them found in Atlantic herring samples.

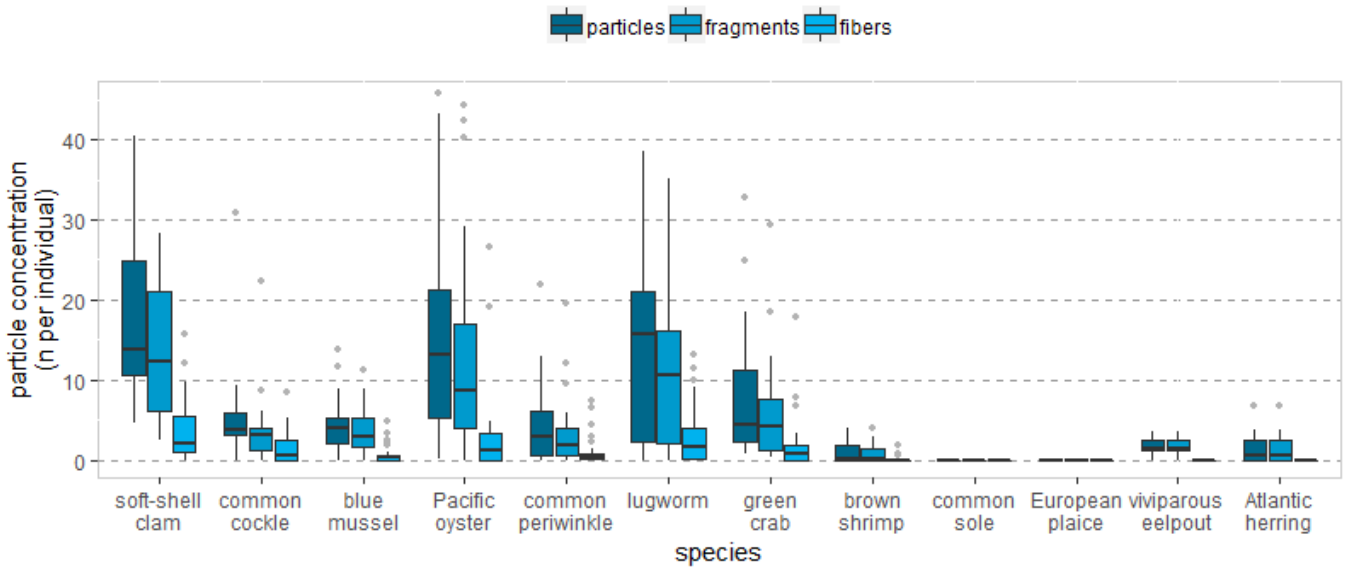

Figure 7: Microplastic concentrations (n per individual) according to shape and species

Datasets varied in total range and statistical dispersion, considerably. Above all, particle concentration for soft-shell clam, Pacific oyster and lugworm showed large variation, which was mostly a result of larger differences in individual weight and thus, weight of investigated soft tissue.
Net weight of individuals was recorded after dissection of soft tissue from shells, carapaces or the dissection of gut and stomach tissue from fish. Recalculating the results according to weight of analysed soft tissue reveals a different pattern, with highest concentrations per g net weight found in common periwinkle (median 15.9 particles per g tissue), followed by Atlantic herring (10.6 particles per g tissue) and lugworm (median 3.64 particles per g tissue) (figure 8a/8b).

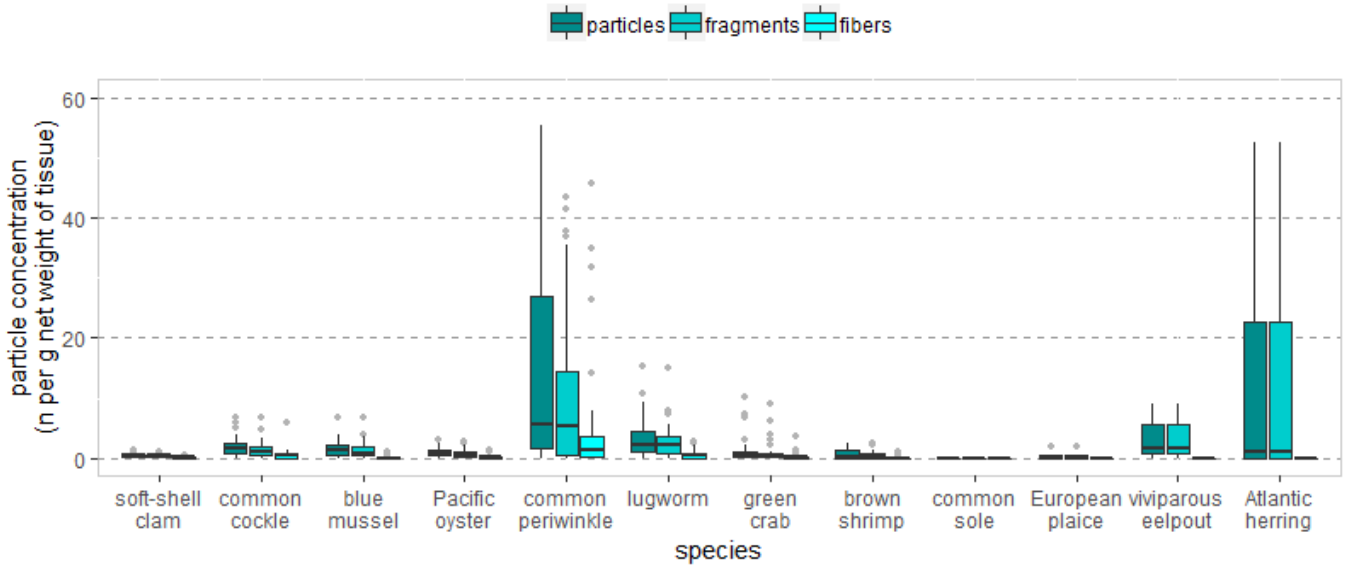

Figure 8a: Microplastic concentrations per g weight of analysed tissue according to shape and species (all species)

For better graphical resolution figure 8b displays the results for concentrations per g tissue without the species common periwinkle and Atlantic herring.

Figure 8b: Microplastic concentrations per g weight of analysed tissue according to type of particle and species (all species except common periwinkle and Atlantic herring)

Summarizing all species investigated, the median concentrations of microplastic contamination per g tissue followed the order common periwinkle > lugworm > viviparous eelpout > common cockle > blue mussel > Atlantic herring > Pacific oyster > green shore crab > soft-shell clam > brown shrimp > European plaice.

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	0.61	**1.38**	1.58	2.07	6.80
brown shrimp	0.00	0.00	**0.12**	0.70	1.31	2.44
common cockle	0.00	0.82	**1.69**	2.04	2.54	6.81
common periwinkle	0.00	1.64	**5.50**	15.90	26.92	67.91
European plaice	0.00	0.00	**0.00**	0.22	0.36	2.00
common sole	n.d	n,d,	**n,d,**	n.d.	n.d.	n.d.
green shore crab	0.05	0.17	**0.45**	1.53	1.17	10.27
Atlantic herring	0.00	0.00	**0.95**	10.64	22.55	52.38
lugworm	0.00	0.97	**2.27**	3.64	4.50	15.40
Pacific oyster	0.01	0.37	**0.86**	0.99	1.33	3.00
soft-shell clam	0.15	0.32	**0.40**	0.51	0.57	1.30
viviparous eelpout	0.00	0.79	**1.72**	3.34	5.65	9.16

Table 8: Descriptive statistics on microplastic particles per g net weight of tissue according to species[1]

Microplastic particles in benthic species result both from faeces residues after 24 hours of incubation and the analysis of dissected soft tissue. Median results from tissue (table 9) analyses were higher compared to faeces residues (table 19) throughout all species except for common cockle, however, the difference is only significant for the soft-shell clam.

species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	0.04	**0.28**	0.60	0.71	2.79
common cockle	0.00	0.01	**0.50**	0.93	1.40	5.86
common periwinkle	0.00	0.00	**0.26**	2.15	1.40	29.71
green shore crab	0.00	0.01	**0.08**	0.62	0.25	5.08
lugworm	0.00	0.06	**0.25**	0.52	1.00	2.08
Pacific oyster	0.00	0.10	**0.18**	0.34	0.37	1.62
soft-shell clam	0.00	0.01	**0.04**	0.07	0.10	0.36

Table 9: Descriptive statistics on microplastic particles per g net weight of tissue in faeces residue of invertebrate species

[1] Overall descriptive statistics is given in Annex A7

species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	0.25	**0.56**	0.98	1.48	4.02
common cockle	0.00	0.06	**0.39**	1.11	1.52	6.70
common periwinkle	0.00	1.45	**4.76**	13.75	15.91	66.70
green crab	0.02	0.12	**0.25**	0.91	0.71	6.30
lugworm	0.00	0.30	**2.27**	3.11	3.63	13.49
Pacific oyster	0.00	0.14	**0.50**	0.65	0.98	2.41
soft-shell clam	0.12	0.24	**0.35**	0.44	0.52	1.04

Table 10: Descriptive statistics on microplastic particles per g net weight of tissue in soft tissue of invertebrate species

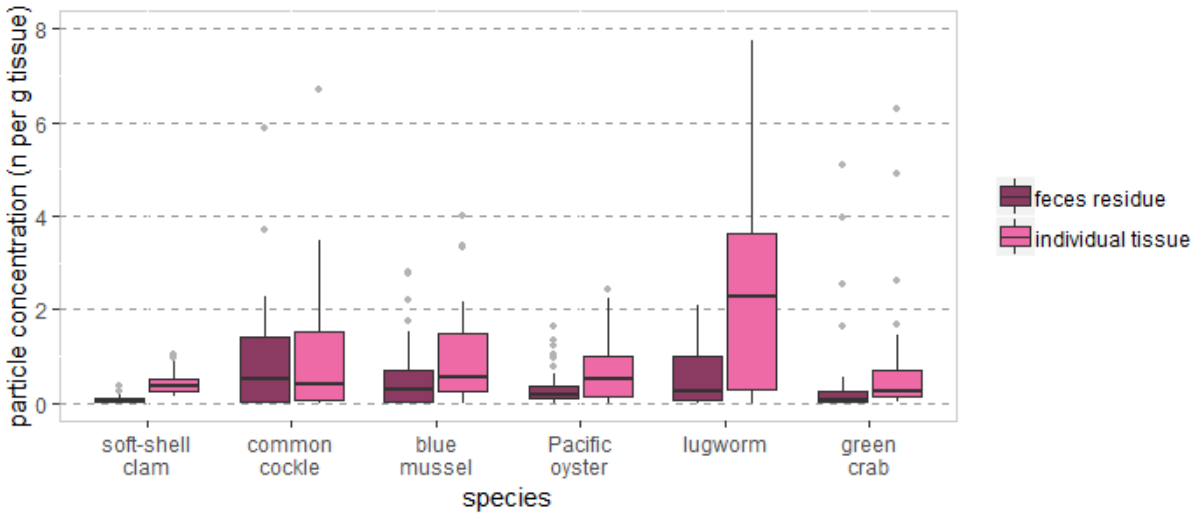

Figure 9: Microplastic particle concentration found in invertebrate species according to faeces residues and tissue analyses (common periwinkle not displayed, see Annex A3)

5.3 Size distribution of microplastic particles according to investigated species

The overall size distribution of polymer particles reveals that 93 % of all particles are smaller than 1 mm and 84 % are smaller than 0.5 mm with constantly rising frequencies with decreasing particle sizes (the lower size limit is set to 63 µm based on the applied method). Fragments and fibres showed a distinct difference in frequency distribution based on the definition of microplastic sizes according to their longitudinal orientation (see figure 10). However, no significant differences between size distribution of all particles (fragments and fibres) were recorded between the species investigated. A significant difference in size distribution of fragments could only be detected between Atlantic herring and all other species (single-way ANOVA, $p<0.001$)). Descriptive statistics on size distribution of fragments according to species are displayed in table 11 showing a median value of 287 µm for Atlantic herring and 107 to 168 for the other species, respectively. Annex A4 illustrates the size distributions of particles for all species.

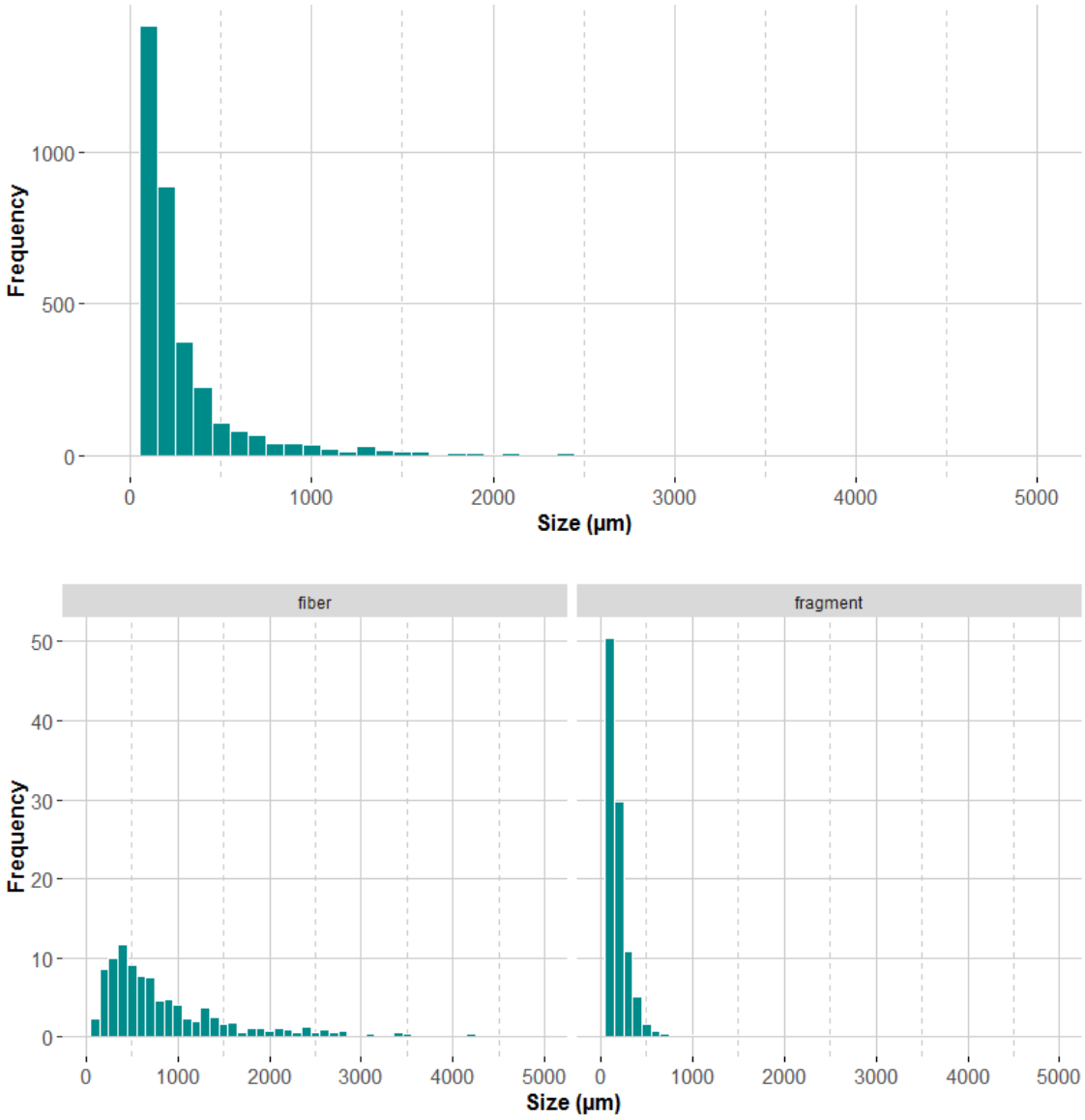

Figure 10: Size distribution of identified microplastic as absolute values (total number of particles counted) and relative frequencies (%) according to shape

Species	shape	min	1st qu.	Median	mean	3rd qu.	max
soft-shell clam	fragments	65	114	**168**	206	240	2244
	fibres	82	366	**628**	872	1089	4432
common cockle	fragments	64	103	**137**	182	195	1765
	fibres	155	400	**693**	943	1276	3547
blue mussel	fragments	67	110	**152**	183	214	1114
	fibres	94	460	**664**	903	1147	2618
Pacific oyster	fragments	65	103	**138**	176	201	2627
	fibres	95	410	**655**	1054	1295	4796
common periwinkle	fragments	65	116	**168**	203	234	1354
	fibres	84	441	**768**	1156	1459	4939
lugworm	fragments	65	104	**147**	187	212	3917
	fibres	68	322	**654**	883	1220	3699
green shore crab	fragments	65	100	**128**	170	216	699
	fibres	126	326	**619**	928	1009	4916
brown shrimp	fragments	65	109	**145**	204	218	1261
	fibres	169	359	**609**	1021	1312	4101
Atlantic herring	fragments	67	195	**287**	328	400	1452
	fibres	163	429	**534**	959	844	2956
European plaice	fragments	74	93	**130**	185	226	589
	fibres	443	507	**707**	1081	1028	3893
common sole	fragments	106	107	**107**	145	165	223
	fibres	136	143	**189**	827	874	2795
viviparous eelpout	fragments	64	101	**133**	162	167	700
	fibres	198	247	**285**	459	734	832

Table 11: Descriptive statistics on size distribution of microplastic fragments and fibres according to species (µm)

5.4 Microplastic concentrations according to phyla and feeding type

Concerning the results according to phyla and feeding type it is important to note that the phylum "polychaetes" is only represented by a single species (lugworm) and the feeding type "grazer" only is represented by the species "common periwinkle".

Focusing on the phyla molluscs, polychaetes, arthropods and chordates, median values vary from n.d. (not detectable) for chordates (range 0.0 to 32.8 particles per individual) to median values of 2.3 (arthropods), 5.3 (molluscs) and 15.7 (polychaetes) particles per individual, respectively. Despite the large variations in median values the data reveal no significant differences between the invertebrate phyla. However, significantly less particles per individual were recorded in invertebrates than in chordates (figure 11, tables see annex A7, table 18a-18c).

No significant differences between phyla occur for particles per net weight of tissue (figure 12). Median values are again below the detection limit (median 0.0 / mean 4.1 particles per g tissue) for the chordate species and range from 0.4 (arthropods) to 1.1 (molluscs) to 2.3 (polychaetes) particles per g tissue in invertebrate species.

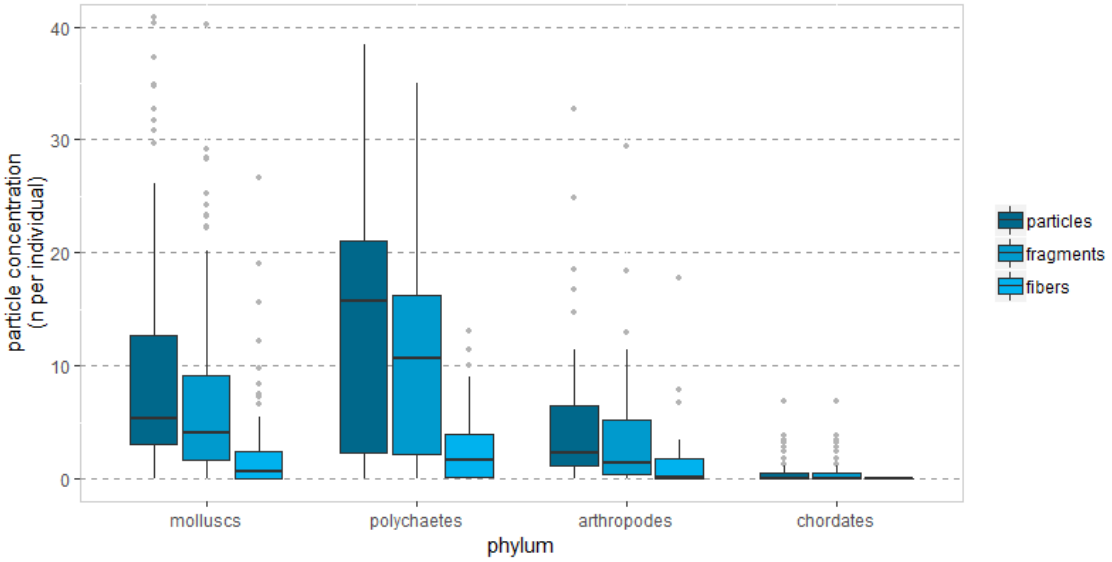

Figure 11: Microplastic particle concentrations per individual according to particle type and phyla

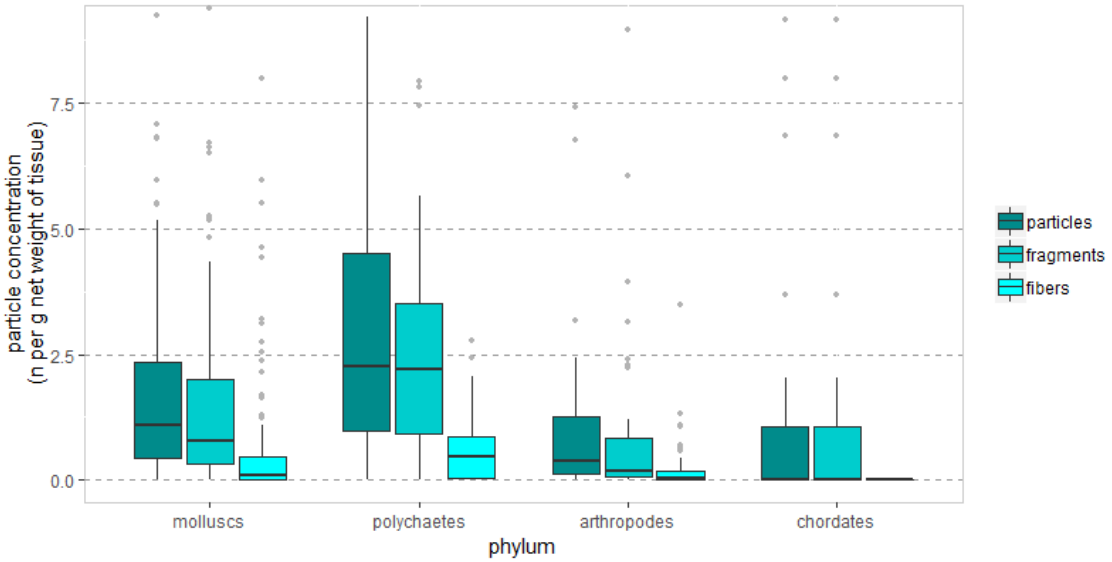

Figure 12: Microplastic particle concentrations per g net weight of tissue according to particle shape and phyla

5.5 Seasonal differences of microplastic concentrations in investigated species

Seasonal differences in microplastic concentrations were detected. Median values varied between 3.27 particles per individual in samples taken in October 2017 and 6.02 particles in samples taken in May/June 2018 (table 12). After standardizing data to g per weight of investigated tissue this median values are higher in autumn 2017, with 1.03 particles per g weight of analysed tissue, than in spring 2018, with 0.72 particles per g weight of analysed tissue (table 13). This finding strongly relates to different body size, and thus, with the amount of tissue analysed in each season. This is mainly the result of the larger weights of the soft-shell clams, green shore crabs, viviparous eelpout and Atlantic herring sampled in spring 2018. This difference was significant at $\alpha<0.05$ for the Atlantic herring (see figure 13a and 13b).

Season	min	1st qu.	median	mean	3rd qu.	Max
2017	0.00	0.65	**3.27**	6.33	8.13	38.45
2018	0.00	1.34	**6.02**	10.54	15.23	57.74

Table 12: Descriptive statistics on microplastic particles per individual (all species) according to season

Season	min	1st qu.	median	mean	3rd qu.	max
2017	0.00	0.22	**1.03**	5.08	3.37	67.91
2018	0.00	0.33	**0.72**	1.23	1.66	8.00

Table 13: Descriptive statistics on number of microplastic particles per g net weight of tissue (all species) according to season

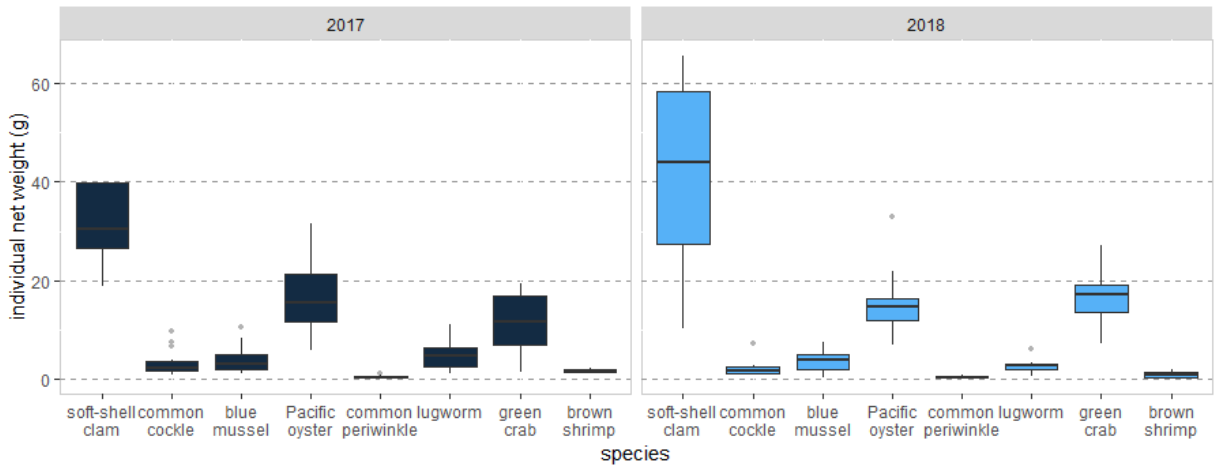

Figure 13a: Net weight of analysed tissue per individual according to invertebrate species and season

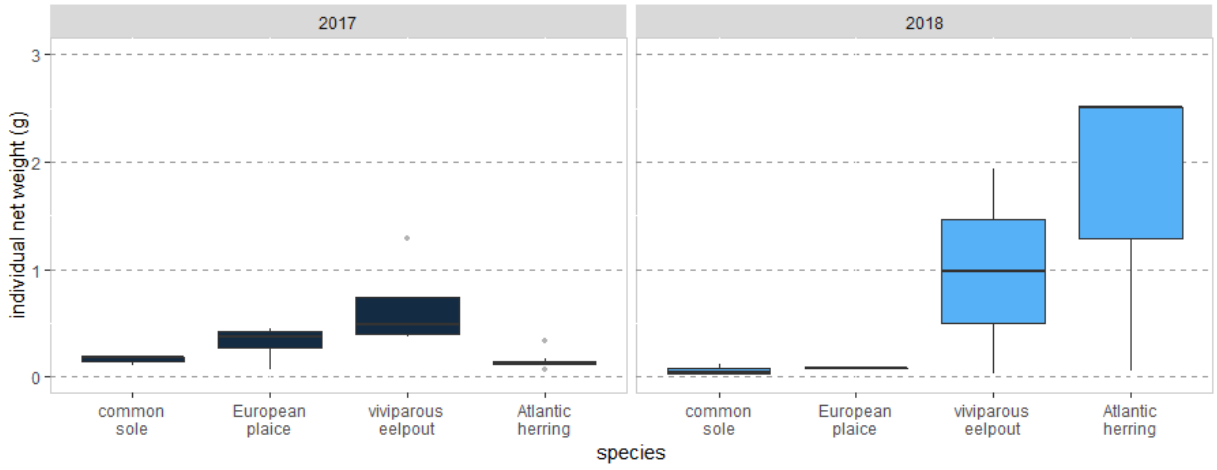

Figure 13b: Net weight of analysed tissue per individual according to chordate species and season

5.6 Spatial gradients of microplastic contamination of investigated species

Differences in the results for the different sampling sites could be detected. This was especially the case for the most southern site, Friedrichskoog. These results are significant for the soft-shell-clam, green shore crab and blue mussel (α=0.05), in which the highest concentrations per weight of investigated tissue were observed. Except for the common periwinkle, a tendency for rising concentrations with increasing geographical latitude was detected, although this was only significant for Friedrichskoog (figure 14).

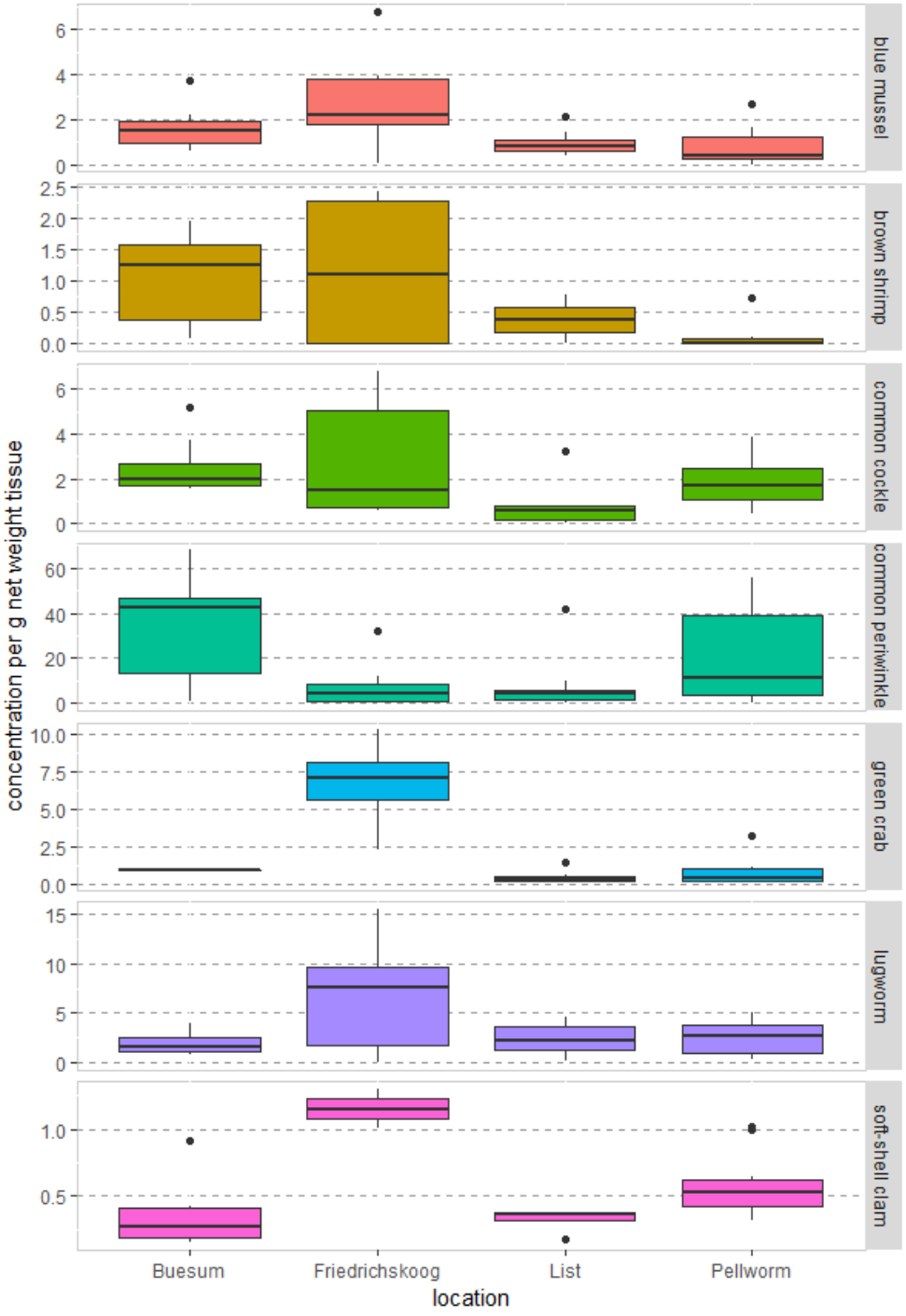

Figure 14: Microplastic concentrations in investigated invertebrates (n per g tissue) according to location

5.7 Polymer classification of microplastics

The polymer types were identified using µRaman spectroscopy. A total of 113 plastic fragments and fibres were collected manually from the filters using a fluorescence microscope. They were scanned on aluminiumoxide filters. A comparison between the Nile Red/fluorescence microscope and µRaman-spectroscopy methods showed that 93% of the plastic particles could be identified correctly using the Nile Red/fluorescence microscope method. The dominant polymer type found in the samples was polyethylene (38.8%) followed by ethylene-vinyl-acetate-copolymer (19.3%), polyethylene-terephthalate (14.0%) and polypropylene (10.5%) (Fig. 15a).

Figure 15b displays the polymer distribution according to species, which shows that polymer types are almost equally distributed over all species. Because of the small number of particles analysed a statistical analysis was not carried out and results have to be regarded as first screening values, only.

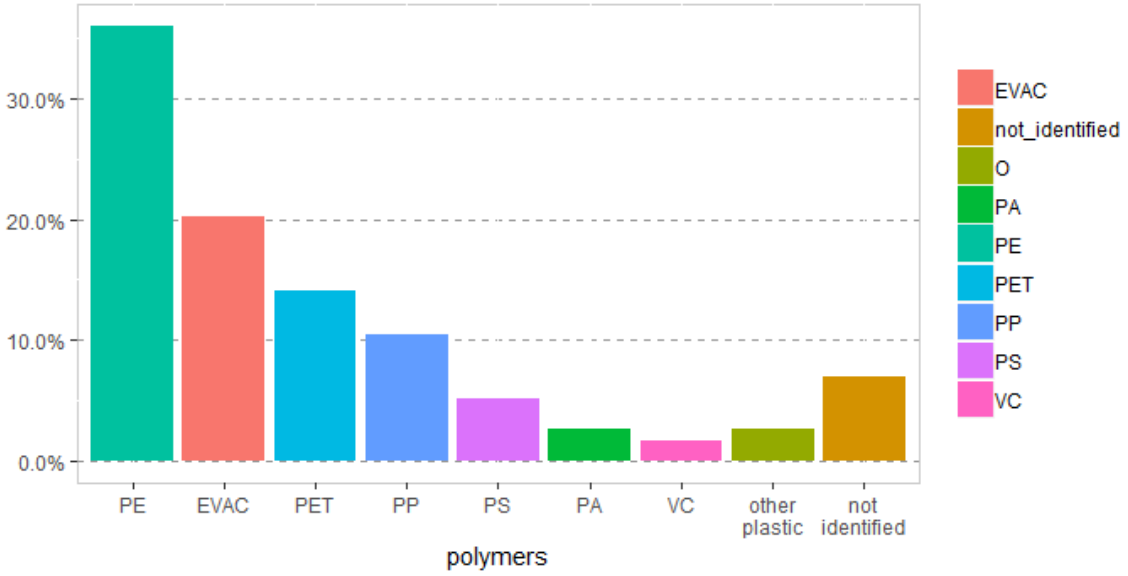

Figure 15a: Distribution on polymer types based on identification with µRaman-spectroscopy – all samples

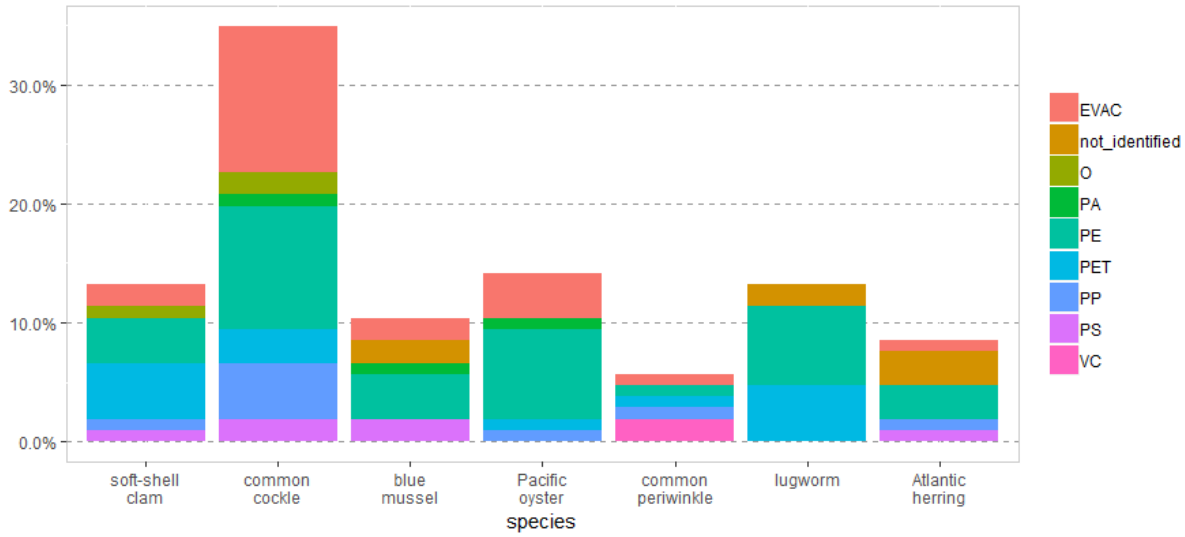

Figure 15b: Distribution on polymer types based on identification with µRaman-spectroscopy according to species

6. Discussion

A direct comparison with findings from other investigations is hampered because only a few comparable studies exist. This is especially because the methods and detection limits vary between studies. Most other surveys focus on particle sizes from >150 to >500 µm. As shown, 84 % of all particles detected within this study are <500 µm. In addition and in contrast to other studies, the present project addresses juvenile instead of adult fish.
The use of the combination of tagging and fluorescence microscopy complemented by Raman-microscopy to identify the plastic particles proved to be highly efficient and appropriate. This was especially the case in comparison to visual identification with light microscopy only. The correlation between particles identified under fluorescence light after staining with Nile red and identification of polymer type using Raman-microscopy for a total number of 113 particles (by manual selection from filters and complete scans of filters with subsequent staining) reached a value of 93.4 %. Most of the misidentified particles were fibres of cellulose origin, which display similar emissions to synthetic polymers under fluorescent light and spectra under the µRaman-spectroscope.

Research on fish species conducted in the North Sea indicated percentages of contaminated individuals per species of 2.6 % (Foekema et al., 2013), 5.5 % (Rummel et al., 2016), 11 % (Lusher et al., 2013) and 30 % (Lenz et al., 2016). Compared to the percentage of affected chordates found within this investigation (38.9-42.3 %) these values are low, which is most probably due to the different lower size limits of the plastic particles applied in this study. In accordance to the outcomes of Rummel et al. (2016) and Lenz et al. (2016) but in contrast to Lusher et al. (2013) demersal species were more affected than pelagic species. However, the results of the present study regarding Atlantic herring were influenced by large differences in the weights of the individual fish.

Contrary to findings of Lusher et al. (2017) and Lenz et al. (2016) in this study fragments were found to be more frequent than fibres in all investigated species. These results were significant for soft-shell clam, blue mussel, Pacific oyster and viviparous eelpout. Comparing herring and cod, Lenz et al. also found that the overall ratio between fibres and fragments was markedly higher in herring. The authors hypothesize that this difference reflects the predominately predatory feeding strategy of Atlantic herring, which, however, also filter feeds via the gills. Lenz et al. conclude that the higher proportion of fibres found in herring is because the fibres are held back by the gills. This effect could not be shown in the present study.

Benthic species are highly prone to ingest microplastic and have a high tendency of bioaccumulation (Rijsgard and Banta, 1998). Lusher et al. (2017) found that 76.6 % of blue mussel individuals were affected along the coastline of Norway, with mean concentrations of 1.85 particles per g wet weight of tissue. These values match very well the contamination of blue mussels recorded in this study for the Schleswig-Holstein coastline (mean value 1.58 particles per g tissue and 96.6 % affected individuals). As mentioned above, the predominant shape found in the Norwegian investigation was fibre. Despite differences in the lower size limit used (150 µm versus 63 µm), this difference is also likely to be a result of polymer identification. The Nile Red staining method helps considerably to reduce the misinterpretation of cellulose and cotton fibres. Lusher et al. identified most fibres as semi-synthetic cellulose. This chemical composition was also detected for particles investigated in this study, however, since Raman spectra of pure cellulose and cotton fibres can easily be mistaken for microplastics, such particles were not considered. The similar values and proportion of microplastic contamination in blue mussel recorded by Lusher and in this study might simply be due to the fact that a lower size limit used

in this study, leads to a higher number of particles counted, and the exclusion of cellulose fibres to a lower number.

Leslie et al. (2015) presented results in benthic species along the Dutch coastline as particles per g dry weight. Therefore, there data cannot be compared directly with this study. A conversion of their dry weight values to wet weight leads to estimated mean values of 6.6 particles per g wet weight (common periwinkle), and 2.4 and 12.2 (Pacific oyster, two sampling seasons), 3.6 and 13.7 (blue mussel, two sampling seasons), 1.1 (amphipod) and n.d. (green shore crab) in their study. Though only estimations, these values are in good accordance with the present study for common periwinkle (5.5 particles per g tissue), however, considerably higher for the other species (median values as particles per g wet weight: blue mussel – 1.4, Pacific oyster – 0.9, green shore crab – 0.45).

The results of van Cauwenberghe et al. (2015), along the French-Belgian-Dutch coastlines, revealed values for the total of faeces residues and tissue analyses for the blue mussel of 0.3 and for the lugworm of 1.5 particles per g tissue. These values are considerably lower than the values recorded in this study, with 1.4 particles per g tissue (blue mussel) and 2.3 particles per g tissue in lugworm. Also here, the different lower size limits of plastic particles applied in the two investigations, differing sampling locations and dissimilar particle identification methodology have to be considered when comparing these data. As found by van Cauwenberghe et al. the concentrations in tissue were higher than those in faeces residues (except for common cockle in the present study). This tendency, although not significant, points to several aspects that need to be addressed in the future. The target of 24 hours of incubation in filtered seawater was to achieve complete gut clearance. When postulating that ingested microplastics are egested completely after passage through the gut and thus that they are not accumulated over time within the gut, the outcome should have been the other way around. This indicates that gut clearance was not completed after incubation, the volume of filtered seawater was not sufficient or the frequency of exchange of the filtered seawater was not set at an appropriate level. In both the latter cases, individuals could re-ingest particles that they had egested previously. Here, further tests are required.

In particular, the findings for Atlantic herring point to relationships between various parameters. With the exception of the Atlantic herring, the size distribution revealed increasing frequencies of fibres and fragments with decreasing particle size. Foekema et al. (2013) and Boerger et al. (2010) suggested that fish mainly ingest plastic fragments, which have a similar colour and shape as their food. This was clearly so for Atlantic herring, but could not be shown for the demersal species viviparous eelpout, European plaice and common sole. The findings for herring indicate that particles of a specific size and shape are actively selected. Based on visual inspection, rounded oval shaped particles, both of organic and polymer origin, dominated in the sample residuals investigated under the fluorescence microscope. A striking though not significant observation is the presence of 5 spherical microbeads detected in Atlantic herring compared to 3 found in all other species, which also indicates that an intentional uptake is taking place. This should be further investigated.

The results for microplastic concentration based on the number of particles per individual and concentrations per net weight of soft tissue clearly demonstrate the necessity of investigating sufficient numbers of individuals and samples. Data show that the larger the net weight of individuals the less the results vary and the smaller the statistical variability. Whereas the number of particles per individual e.g. for soft-shell clam and lugworm, with large net weights, varied considerably. The use of numbers of particles per net weight instead of numbers per individual reduced the variability in the data set to the

level where mean and median values were almost equal, hence transforming the data set close to normal distribution for these two species. This especially qualifies these species, which are representatives of filter and deposit feeders, as suitable bioindicators for microplastic pollution.

To a lesser extent this effect can be shown for all other species, with the exception of the common periwinkle and the Atlantic herring. The reasons for this are that the results for Atlantic herring differed markedly between the two investigation areas of the Northfrisian Wadden Sea and the Dithmarscher tidal flat. This has to be considered as a cross-correlating effect of the seasonal sampling. Atlantic herring, where it has been shown that they intentionally uptake particles with a certain size and shape, showed a seasonal pattern with larger concentrations in samples recovered in May 2018 than in October 2017. Since fish species were only sampled in the Northfrisian Wadden Sea in the spring season this directly influences the results with regard to the spatial distribution of plastic particles.
With the common periwinkle the large statistical variability of the data set is related to the low amounts of sampled material per individual (ranging from 0.11 to 1.07 g wet weight of tissue). When calculating the amount in gram per tissue, the results and any random noise e.g. via background contamination have to be multiplied by values which lie magnitudes higher than for larger species. Thus, the results for the common periwinkle have to be regarded critically. Further investigations with larger sample size using a number of individuals as a composite sample would need to be carried out to enhance statistical representativeness.

The hypothesis that pelagic organisms and small crustaceans are more likely to encounter less dense, floating microplastic particles (Desforges et al., 2015, Long et al., 2015) whereas benthic organisms are more likely to be exposed to and tend to ingest denser polymer particles (Mathalon and Hill, 2014, Browne et al., 2008, Thompson et al., 2004) could not be demonstrated in this survey. Even though the polymer composition was identified via µRaman-spectroscopy for a total number of 113 particles this number (accounting for 4 % of all detected particles) is too low to draw any conclusions, especially at a species level. Even though it is postulated that in general, polystyrene is more common than polyethylene in the marine environment (Browne et al., 2007; de Sá et al., 2018) the proportion of PS found in this study do not reveal this, neither in the stained samples, which are susceptible to polystyrene melting through the applied chloroform, nor in the none destructive method of µRaman-spectroscopy. In accordance with Rummel et al. (2015), the dominating polymer type in this investigation was polyethylene. Another high proportion is represented by ethylene-vinyl-acetate-copolymer, which shows a very similar spectrum to polyethylene, and therefore cannot being clearly distinguished in all cases.

Microplastic uptake is dependent on species size and weight and accordingly, their ingestion or sequestration/filtration capacity. Size of individuals is, above all, a factor of life stage and basic surrounding conditions such as temperature and salinity. Concerning fish species, their distribution in the marine environment is influenced through the factors temperature, water depth, atmospheric and sea currents and the temporal and spatial occurrence of prey (Lenz et al., 2016). As for benthic species the duration of filtration or ingestion time per tidal event plays a major role in intake of microplastics. In the Schleswig-Holstein Wadden Sea area several gradients of environmental factors are present e.g. radiation which influences primary production of algae and water temperature, as well as salinity and morphological criteria. For example, mean water temperature increases from North to South whereas the morphological activity of sediment and salinity decline. An additional parameter affecting the food uptake is surface chlorophyll concentration, which generally increases from North to South, but is highly

variable between sub-basins (Büsum/Friedrichskoog: 5.6-7.7, Pellworm 2.2-2.6, List 2.6-3.5 mg Chlorophyll/m³ (according to model layers from 2000-2004, HZG, 29.08.2018)

All the above-mentioned factors vary in relation to seasonal and diurnal/tidal changes. Morphologic hydrodynamics as well as morphologic differences, resulting in decreasing mean tidal range, also vary from North to South between micro- to mesotidal about 1.5 to 3.7 m. Therefore, the spatial extension of eulitoral areas and potential habitats varies (Behrends et al., 2004).
Thus, the slight tendency of increasing microplastic abundance in species with increasing latitude found in this study might be a result of varying environmental factors, though further analyses is needed to improve statistical significance.

In summary and in reference to the aims and results of this study the following conclusions can be drawn:

(1) With the exception of the common sole all marine species investigated within the project in the area of the National Park of the Wadden Sea are contaminated with microplastic. Contamination, based on median number of particles per unit weight of investigated tissue, ranks highest for the common periwinkle followed by the Atlantic herring > lugworm > viviparous eelpout > common cockle > blue mussel > green shore crab > Pacific oyster > brown shrimp > soft-shell clam > European plaice > common sole

(2) A significant correlation between microplastic concentrations in marine species and weight of tissue could be shown, revealing the urgent need to standardize reporting values in the future. Concerning trophic level and habitat, the benthic species had significantly higher amounts of plastic particles than demersal and pelagic species. Based on phyla, median contamination per g tissue was highest in polychaetes followed by molluscs > arthropods > chordates. Grazing feeders showed higher median microplastic concentrations than deposit feeders, filter feeders and predators.

(3) No distinct spatial pattern in the contamination levels could be determined, though, the most southern sampling site Friedrichskoog revealed significantly higher microplastic contamination in soft-shell clams and green shore crabs.

(4) The eligibility of selected species as biological indicators of microplastic pollution for future monitoring programs could be shown on the basis of statistical representativeness and reproducibility. The species potentially most eligible, based on statistical criteria, is the soft-shell clam, however, other species qualify as well, if sufficient numbers of individuals are analysed and the representativeness of habitat and feeding strategies as well as background signals from environmental conditions of water and sediment are considered.

7. Evaluation of indicator qualification of investigated species for monitoring purposes

The evaluation of the qualification of different species as indicators of microplastics in the marine environment has to take a number of factors into consideration e.g. feasibility of sampling, seasonal and spatial representativeness, morphological and habitat induced limitations, detection limits and site specific factors such as representativeness of ecosystem compartments / zones, trophic levels and feeding strategies of the species.

Feasibility of sampling - seasonal and spatial representativeness

The feasibility of sampling is strongly related to a number of factors, but above all, to seasonal and spatial occurrence of potential indicator species. Most of the investigated benthic species are ubiquitous and common within the Schleswig-Holstein Wadden Sea area, with slight restrictions concerning brown shrimp and green shore crab, which showed varying season-dependent abundances. Concerning fish species, high seasonal and yearly variations in abundance were reported from the people that supplied the animals for this study.

Statistical representativeness of species – sampling numbers and replicates

As demonstrated, the standardization of results to the amount of microplastics per given unit of wet weight of tissue is of utmost importance. If this factor is taken into consideration, all species investigated would be eligible as biological indicators, with the exception of the common periwinkle and Atlantic herring. However, also these two species satisfy a number of criteria necessary for monitoring purposes and, with sufficient sample size and homogenous size classes, could achieve statistical representativeness.

Habitat induced limitations

In order to achieve representative and comparable results throughout a monitoring area the basic environmental conditions for each sampling site should be more or less equal. This is especially the case for the duration of filter or deposit feeding of benthic species. Feeding times are strongly related to tides and the position of the sampling site in the investigation area. For example Pacific oysters and blue mussels are sessile and often cling to natural or anthropogenic hard substrates, such as dikes and harbour walls, at different levels below MtHw. They are thus exposed to different depths of sea water for varying periods of time as tides rise and fall.

The fish species investigated are widely distributed within the North Sea, which makes them eligible for monitoring purposes. Though, abundances differ according to season and other environmental criteria such as currents, and the abundance of prey, etc. Higher numbers of individuals would need to be sampled in order to produce statistically firm results.

Morphological induced limitations

Especially when not only the soft tissue of benthic organisms but also their catabolic products are taken into consideration, as it was done in this study based on faeces residues, the morphological criteria of the different species play a major role. Whereas some species are characterized by the smooth and even surfaces of their shells or skin other species, like pacific oysters, green shore crab and blue mussels, have rough or at least uneven surfaces, where particles can easily adhere and are difficult to remove prior to incubation and analyses. If the particles attached to the surface of the animals investigated

cannot be excluded from the samples they will lead to an overestimation of the number of microplastic particles really ingested and this will be higher for animals with rough or uneven surfaces.

Detection limits

The lower limits of detection of microplastic particles are determined by contamination levels of samples during sampling and analysis in the laboratory, as measured by procedural blanks. Though taking maximum care to avoid contamination from background levels of microplastics in the laboratory or during sampling, procedural blank results show that fragments as well as fibres contaminate the samples. Since the possibility of contamination of all environmental samples with microplastic from background levels is present, the detection limit of microplastic particles must be based on a sound analysis of procedural blank samples. It is advised that at least 5-10 % of the samples should be blanks in order to take this into consideration. On an environmental analysis basis, the detection limit often is set at the mean value of blank sample analyses plus up to three times the standard deviation. If this approach is taken, microplastic concentrations of environmental samples are very low or lie beyond detection limits. This means that in addition to the effort taken to avoid or reduce background contamination the results from the environmental samples should be analysed with caution. With regard to biota samples for monitoring purpose this means that only species with a clear concentration signal should be taken into account or large sample numbers of selected species and/or composite samples of several individuals need to be processed.

8. Monitoring prerequisites and recommendation

Environmental monitoring is defined as "survey to be repeated over time, enabling trends in some variable of interest to be determined" (Newton, 2008: 3). In order to achieve this objective, some basic criteria are defined: objectivity, representativeness in space and time, validity (of the methods applied and the results obtained), reliability / reproducibility (i.e. comparability of the methods used for sampling and analysis).

On behalf of the European Commission, the Technical Subgroup on Marine Litter has defined requirements for such monitoring (Galgani et al. 2013b). These include:

(1) The monitoring shall enable a continuous assessment of the environmental status.

(2) Monitoring programs shall be coordinated, compatible, coherent, consistent and comparable.

(3) Monitoring should build on and integrate existing monitoring programs.

(4) Data and information resulting from the monitoring programs should be made available for interoperable use.

(5) Monitoring programs must be adapted and respond appropriately to changes in the marine environment.

(6) Monitoring should be linked to requirements of assessment criteria (flexible monitoring approach).

(7) Monitoring programs should take into account differences in scientific understanding in relation to other descriptors.

In order to fulfil these requirements, the following proposals and recommendations for monitoring microplastics in biota on the Schleswig-Holstein North Sea coast are made on the basis of the experience and results of the current project:

Ad (1): Establishment of a long-term and regular sampling program with defined species, sites, sampling frequencies and the inclusion of further environmental factors such as water and sediment samples with long-term coordination and financing.

Ad (2): Assignment of selected institutions for sampling and analysis of microplastic concentrations using the same sampling and laboratory protocols including central coordination and documentation of results. Setting of sampling and analysis strategies.

Ad (3): Consideration of the use of parallel samples from other monitoring programs, e.g. on environmental contamination and/or fish monitoring programs.

Ad (4): Integration of the data into a coordinated (open source) environmental database.

Ad (5): Definition of a statistically reliable time frame for data evaluation (e.g. 5, 10 or 15 years) to allow changes and adjustments of target values.

Ad (6): Consideration of specific assessment needs related to microplastic concentrations for specific monitoring objectives such as assessment of damage at different trophic levels or in the food chain.

Ad (7): Consideration of the different physical and chemical properties of microplastic particles. While other descriptors such as chlorophyll-α are easily measurable although they are heterogeneously distributed, microplastic particles occupy an intermediate position between chemical and physical parameters.

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List of abbreviations

EC	European Commission
EVAC	ethylene vinyl-acetate copolymer
GES	Good Environmental Status
KOH	Potassium hydroxide
M	Mol
MSFD	Marine Strategy Framework Directive
NA	“not available” / “not acquired”
NaClO	Sodium hypochlorite
NaI	Sodium iodide
n.d.	not detectable
PA	polyamide
PE	polyethylene
PET	polyethylene terepthalate
PP	polypropylene
PS	polystyrene
PVC	polyvinylchloride

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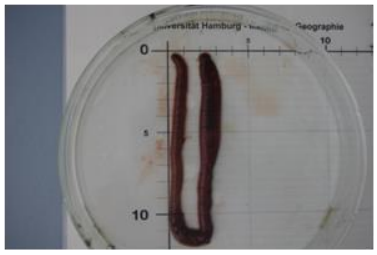
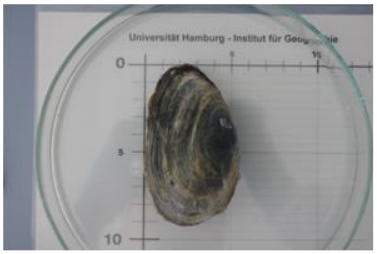
Annex

A1: Documentation of species

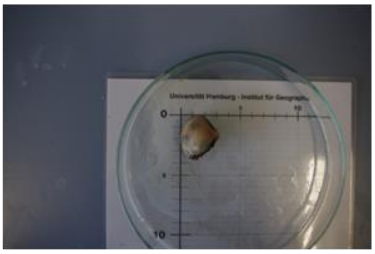
Lugworm
Arenicola marina



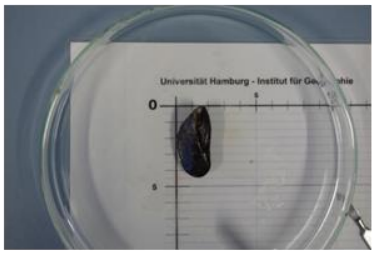
Soft-shell clam
Mya arenaria



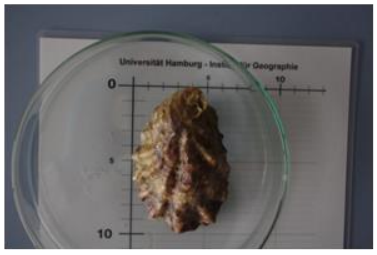
Common cockle
Cerastoderma edule



Blue mussel
Mytilus edulis



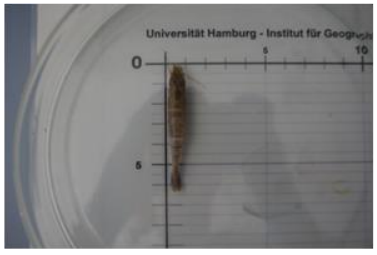
Pacific oyster
Magallana gigas



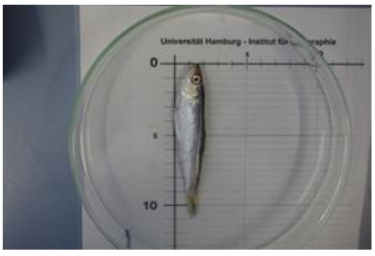
Common periwinkle
Littorina littorea

Green crab
Carcinus maenas

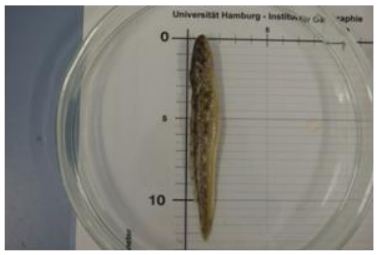
Brown shrimp
Crangon crangon



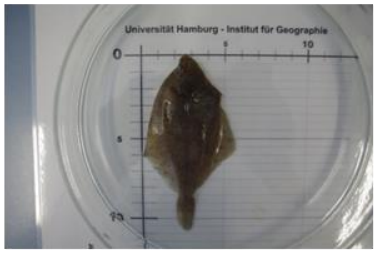
Atlantic herring
Clupea harengus



Viviparous eelpout
Zoarces viviparus

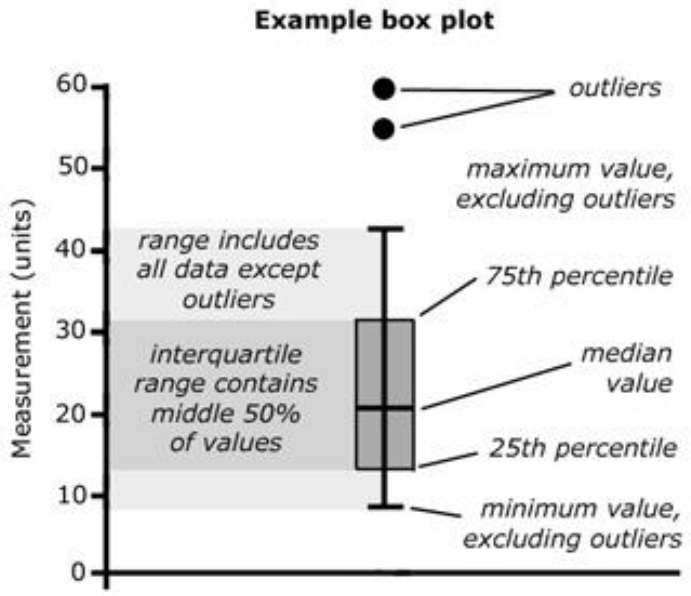


European plaice
Pleuronectes platessa



Common sole
Solea solea

A2: Explanation on figures displaying boxplots



Source: WordPress, 13.09.2018

A3: Microplastic concentrations in invertebrate species according to sample type

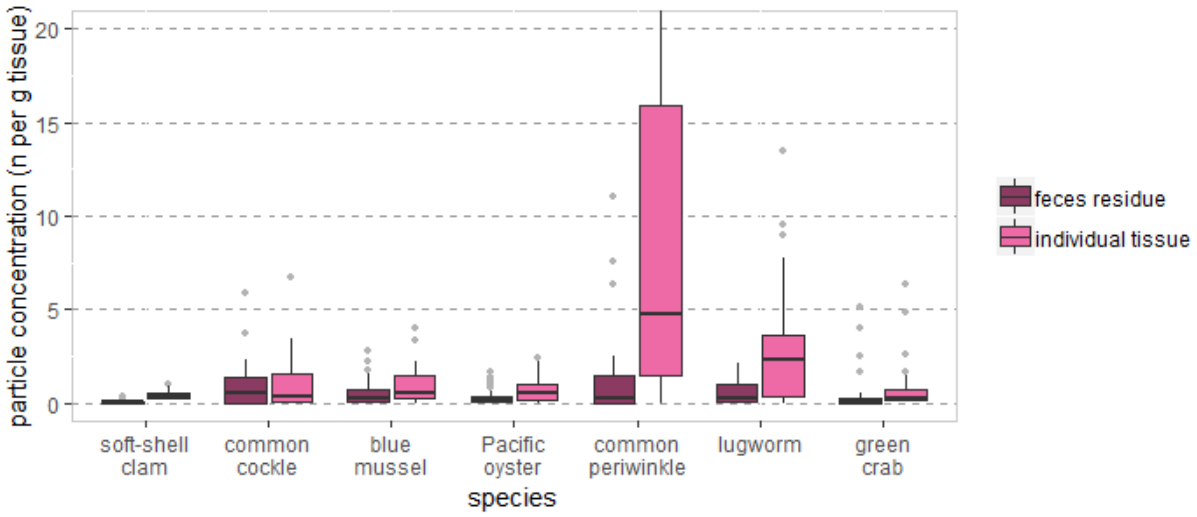
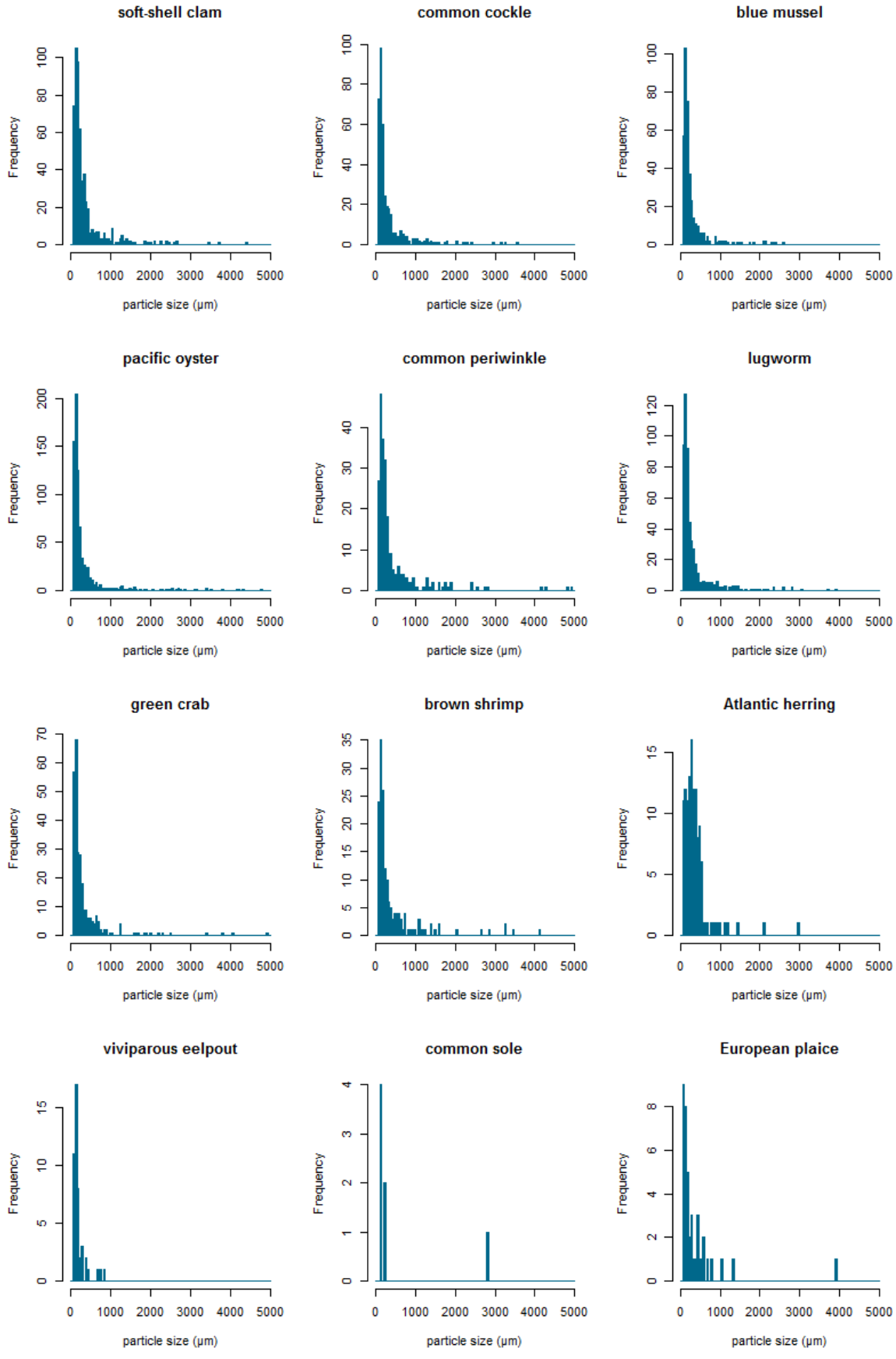
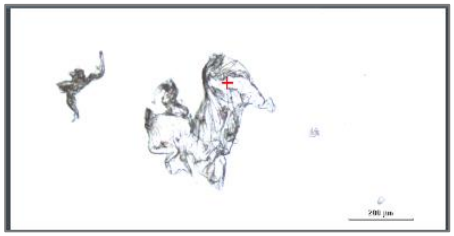
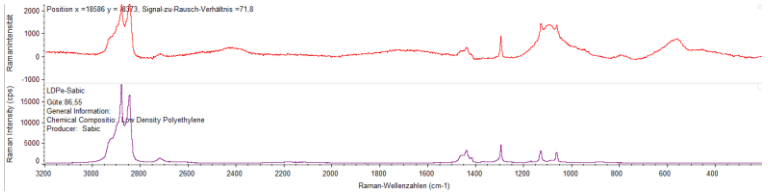
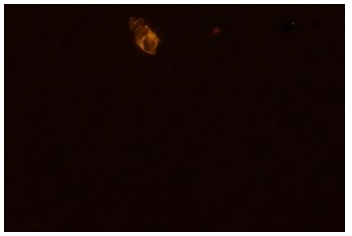
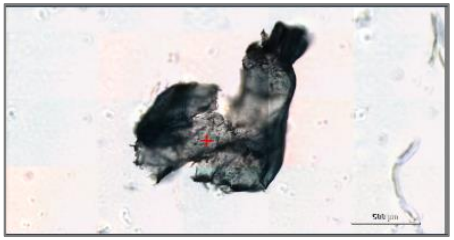
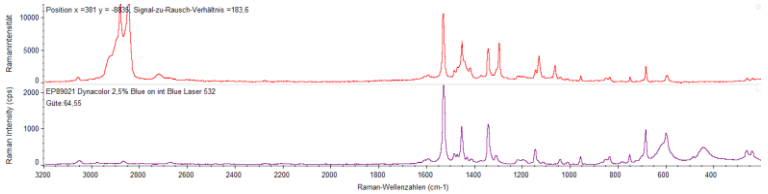
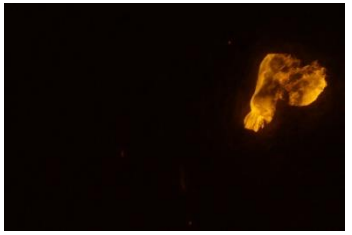
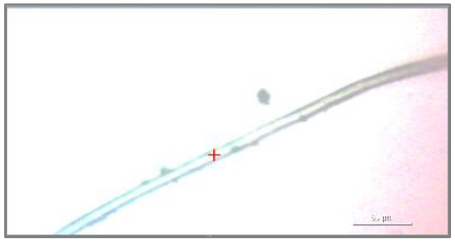
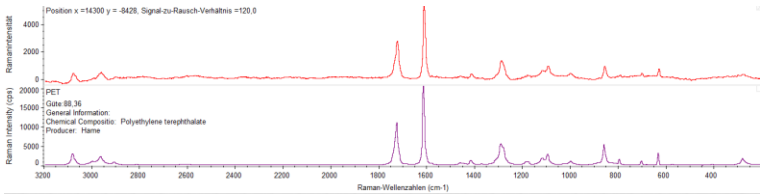
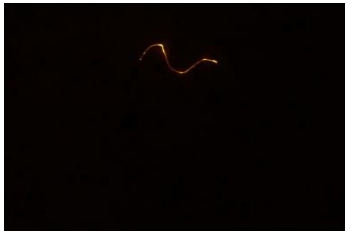
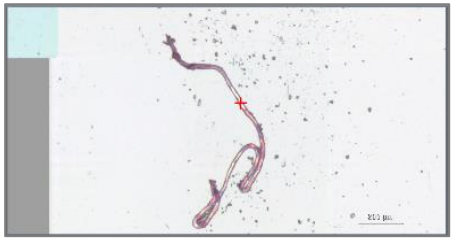
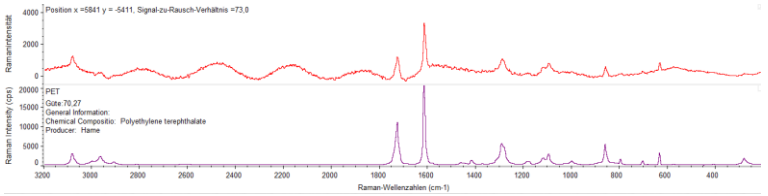
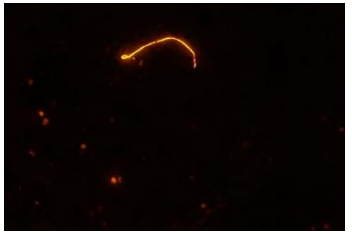


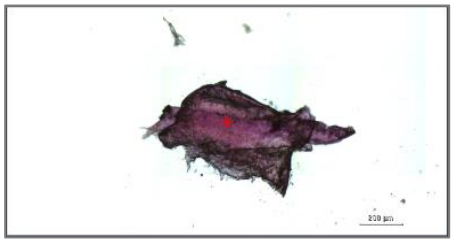
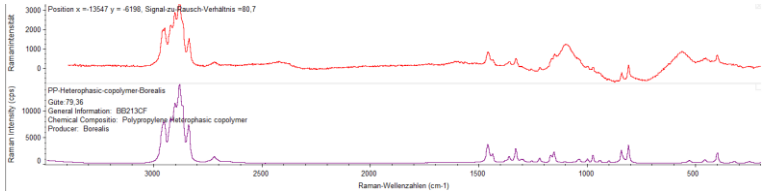
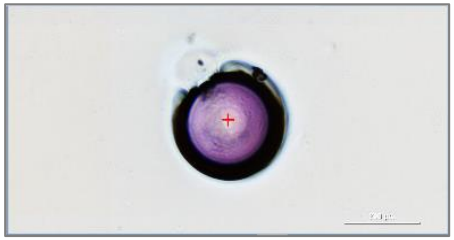
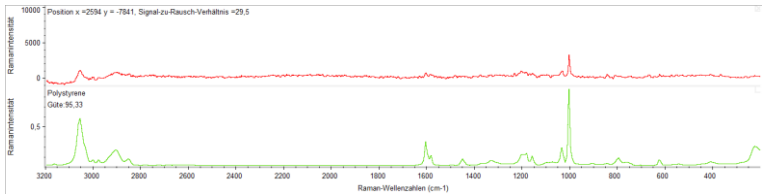
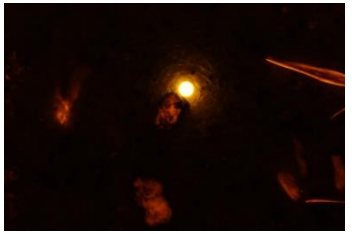
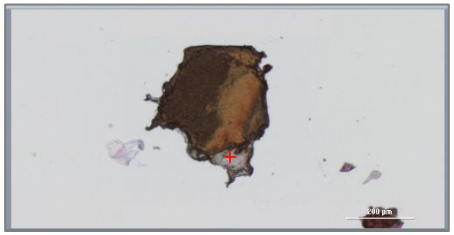
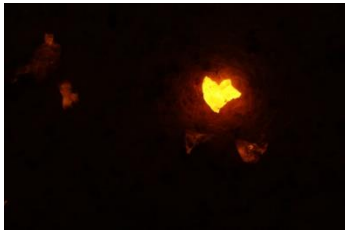
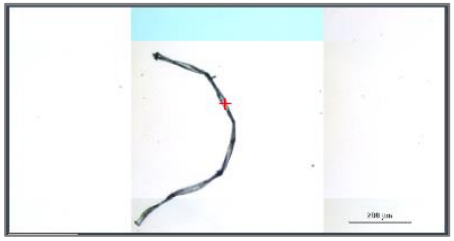
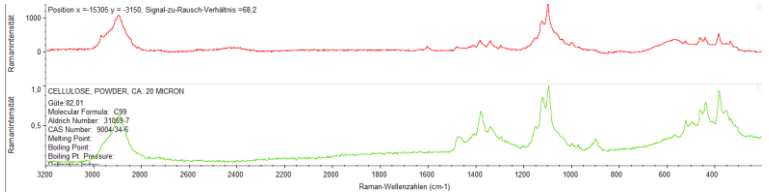
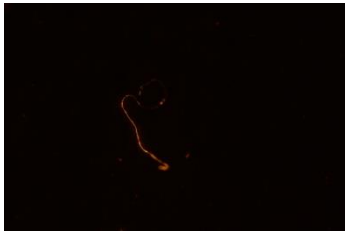
Figure 16: Microplastic concentrations found in invertebrate species according to faeces residues and tissue analyses (all invertebrate species)

A4: Size distribution of microplastic particles (fragments + fibres) according to species



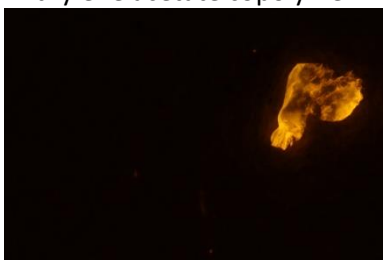
A5: Examples of identified polymer fragments and fibres comparing tagging / fluorescence microscopy and Raman-microscopy

polymer dimensions (mm) (species)	Screenshot particle µRaman	Screenshot Spectra µRaman	Image (fluorescence microscope - Nile red)
PE / polyethylene 420 x 349 (lugworm - 39.1-2)			
PE / polyethylene with blue pigment 1400 X 1100 (common cockle - 60.1-3)			
PET / polyethylene terephthalate 2400 X 12 (lugworm - 79.3)			
PET / polyethylene terephthalate 1836 x 20 (soft-shell clam - 121.1)			

polymer dimensions (mm) (species)	Screenshot particle µRaman	Screenshot Spectra µRaman	Image (fluorescence microscope - Nile red)
PP / polypropylene 990 X 535 (blank – LB7)			
PS / polystyrene 268 X 246 (Atlantic herring - 204.1-3)			
Polypropylene 438 x 430 (Atlantic herring – 204.1-3)			
Cellulose 975 x 19 (blue mussel – 25.1-3)			

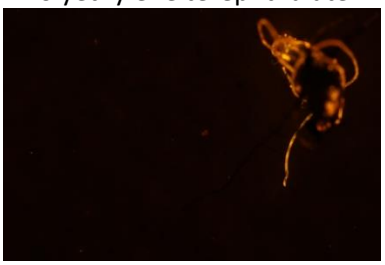
A6: Examples of microplastic and organic particles identified with Nile red tagging and fluorescent microscopy

Ethylene acetate copolymer

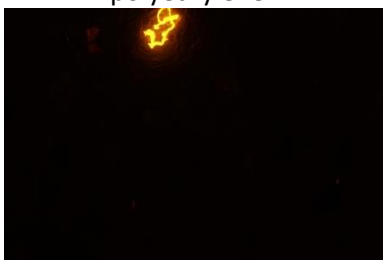


polyetheylene

Polyethylene terephthalate



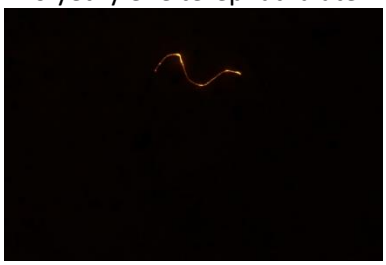
polyethylene



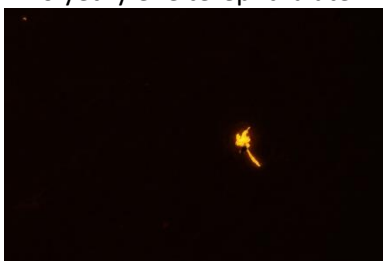
polyethylene

polyethylene

Polyethylene terephathalate

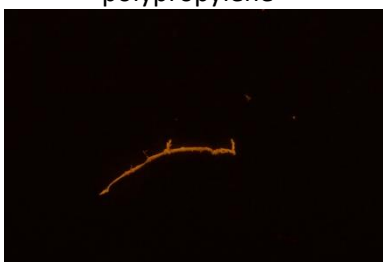


Polyethylene terephthalate

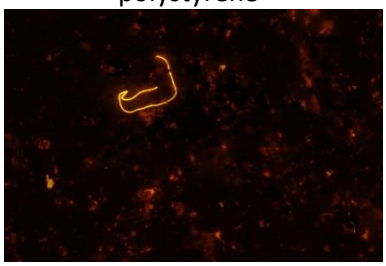


Polypropylene

polypropylene

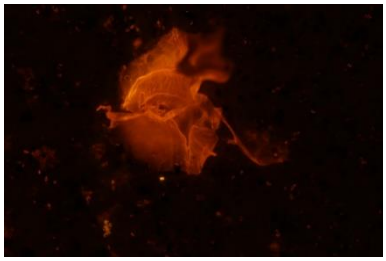


polystyrene

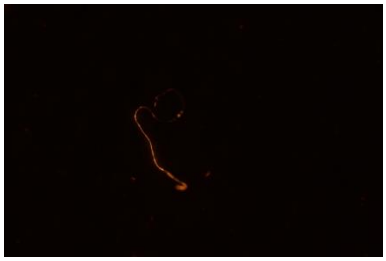


polyethylene

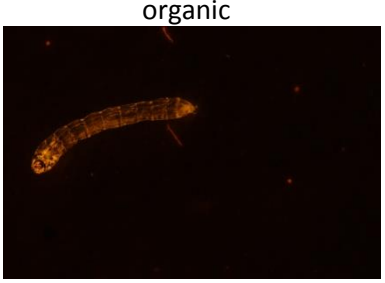
chitin



cellulose



organic



A7: Descriptive statistics

Species	min	1st qu.	Median	mean	3rd qu.	max
blue mussel	1.28	3.43	**4.05**	**4.01**	4.65	5.60
brown shrimp	4.00	5.71	**6.02**	**5.95**	6.28	7.00
common cockle	1.75	2.13	**2.39**	**2.57**	2.77	4.30
common periwinkle	1.00	1.30	**1.50**	**1.63**	1.83	3.60
European plaice	6.43	9.70	**10.63**	**10.07**	10.93	12.60
common sole	5.60	6.32	**8.87**	**8.37**	10.37	10.60
green crab	2.90	4.20	**5.10**	**4.77**	5.40	5.90
Atlantic herring	7.93	8.30	**8.47**	**9.77**	9.67	19.13
lugworm	6.27	13.25	**19.80**	**18.88**	23.50	30.47
Pacific oyster	4.90	6.93	**7.75**	**7.77**	8.67	10.50
soft-shell clam	5.50	7.98	**9.35**	**8.88**	10.03	10.50
viviparous eelpout	9.00	11.71	**12.28**	**14.48**	17.58	22.50

Table 14a: Descriptive statistics on individual length (mm) according to species

Species	min	1st qu.	Median	mean	3rd qu.	max
blue mussel	1.03	1.80	**2.15**	**2.10**	2.40	2.60
brown shrimp	0.72	0.86	**0.92**	**0.91**	0.96	1.07
common cockle	1.60	1.94	**2.34**	**2.36**	2.78	3.30
common periwinkle	0.90	1.10	**1.28**	**1.29**	1.43	1.90
European plaice	0.35	3.90	**4.90**	**4.74**	5.27	10.27
common sole	1.67	2.65	**3.20**	**3.16**	4.01	4.15
green crab	3.20	5.10	**6.00**	**5.75**	6.50	6.80
Atlantic herring	1.53	1.60	**1.67**	**1.81**	1.77	3.03
lugworm	0.70	0.95	**1.20**	**1.12**	1.30	1.60
Pacific oyster	3.40	4.60	**5.50**	**5.36**	6.00	7.50
soft-shell clam	3.20	4.90	**5.65**	**5.35**	5.93	6.60
viviparous eelpout	1.20	1.56	**1.79**	**2.03**	2.49	3.20

Table 14b: Descriptive statistics on individual width (mm) according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.24	1.95	**3.51**	**3.63**	4.90	10.48
brown shrimp	NA	NA	**NA**	**NA**	NA	NA
common cockle	0.82	1.55	**2.11**	**2.98**	2.82	9.78
common periwinkle	0.11	0.27	**0.35**	**0.43**	0.50	1.07
European plaice	0.07	0.26	**0.35**	**0.78**	0.43	9.99
common sole	0.03	0.06	**0.11**	**0.11**	0.17	0.18
green crab	1.44	7.27	**13.47**	**12.47**	17.18	27.11
Atlantic herring	0.06	0.12	**0.13**	**0.41**	0.17	2.51
lugworm	0.52	2.27	**3.88**	**4.27**	5.85	11.11
Pacific oyster	5.92	11.71	**15.17**	**16.02**	18.69	32.92
soft-shell clam	10.26	26.48	**41.03**	**40.39**	56.68	65.52
viviparous eelpout	0.03	0.39	**0.49**	**0.77**	1.11	1.94

Table 15a: Descriptive statistics on individual net weight (g) of investigated tissue according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.92	5.41	**8.38**	**9.24**	13.40	20.38
brown shrimp	0.18	1.21	**1.43**	**1.44**	1.87	2.28
common cockle	1.87	3.39	**5.05**	**6.69**	7.29	22.31
common periwinkle	0.55	0.99	**1.53**	**1.91**	2.25	4.59
European plaice	0.35	2.81	**11.71**	**9.36**	13.20	22.54
common sole	1.57	2.86	**6.66**	**6.38**	10.04	10.66
green crab	8.23	25.43	**42.97**	**39.42**	52.48	62.35
Atlantic herring	2.52	2.68	**2.98**	**6.22**	4.49	27.69
lugworm	NA	NA	**NA**	**NA**	NA	NA
Pacific oyster	15.07	44.38	**55.67**	**60.95**	70.21	141.42
soft-shell clam	14.61	41.89	**69.43**	**64.15**	88.34	104.24
viviparous eelpout	2.91	6.64	**7.64**	**21.19**	26.79	69.45

Table 15b: Descriptive statistics on individual gross weight (g) according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	2.14	**3.91**	4.26	5.28	13.76
brown shrimp	0.00	0.00	**0.14**	1.07	1.85	4.08
common cockle	0.00	3.05	**3.88**	4.93	6.02	30.78
common periwinkle	0.00	0.63	**2.88**	4.28	6.10	21.87
European plaice	0.00	0.00	**0.00**	0.06	0.14	0.14
common sole	0.00	0.00	**0.00**	0.00	0.00	0.00
green crab	0.89	2.31	**4.42**	8.11	11.34	32.78
Atlantic herring	0.00	0.00	**0.48**	1.40	2.48	6.81
lugworm	0.00	2.30	**15.70**	14.14	21.12	38.45
Pacific oyster	0.08	5.24	**13.12**	15.26	21.24	45.74
soft-shell clam	4.74	10.51	**13.90**	18.98	24.90	57.74
viviparous eelpout	0.00	1.14	**1.48**	1.73	2.56	3.48

Table 16a: Descriptive statistics on microplastic particles per individual according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	0.00	**0.30**	0.64	0.55	4.87
brown shrimp	0.00	0.00	**0.00**	0.23	0.02	1.85
common cockle	0.00	0.00	**0.63**	1.45	2.43	8.43
common periwinkle	0.00	0.10	**0.37**	1.20	0.89	7.30
European plaice	0.00	0.00	**0.00**	0.00	0.00	0.00
common sole	0.00	0.00	**0.00**	0.00	0.00	0.00
green crab	0.00	0.00	**0.89**	2.14	1.89	17.77
Atlantic herring	0.00	0.00	**0.00**	0.00	0.00	0.00
lugworm	0.00	0.09	**1.73**	3.07	4.00	13.06
Pacific oyster	0.00	0.00	**1.25**	2.97	3.31	26.63
soft-shell clam	0.00	1.01	**2.08**	3.90	5.42	15.64
viviparous eelpout	0.00	0.00	**0.00**	0.00	0.00	0.00

Table 16b: Descriptive statistics on microplastic fragments per individual according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	1.57	**2.83**	3.62	5.18	11.32
brown shrimp	0.00	0.00	**0.14**	0.84	1.46	4.08
common cockle	0.00	1.15	**3.05**	3.48	4.05	22.35
common periwinkle	0.00	0.49	**1.90**	3.07	3.90	19.57
European plaice	0.00	0.00	**0.00**	0.06	0.14	0.14
common sole	0.00	0.00	**0.00**	0.00	0.00	0.00
green crab	0.39	1.23	**4.23**	5.97	7.65	29.39
Atlantic herring	0.00	0.00	**0.48**	1.40	2.48	6.81
lugworm	0.00	2.16	**10.69**	11.07	16.19	35.02
Pacific oyster	0.00	3.91	**8.66**	12.29	17.08	44.23
soft-shell clam	2.57	6.23	**12.23**	15.08	20.98	56.23
viviparous eelpout	0.00	1.14	**1.48**	1.73	2.56	3.48

Table 16c: Descriptive statistics on microplastic fibres per individual according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	0.61	1.38	**1.58**	2.07	6.80
brown shrimp	0.00	0.00	0.12	**0.70**	1.31	2.44
common cockle	0.00	0.82	1.69	**2.04**	2.54	6.81
common periwinkle	0.00	1.64	5.50	**15.90**	26.92	67.91
European plaice	0.00	0.00	0.00	**0.22**	0.36	2.00
common sole	0.00	0.00	0.00	**0.00**	0.00	0.00
green crab	0.05	0.17	0.45	**1.53**	1.17	10.27
Atlantic herring	0.00	0.00	0.95	**10.64**	22.55	52.38
lugworm	0.00	0.97	2.27	**3.64**	4.50	15.40
Pacific oyster	0.01	0.37	0.86	**0.99**	1.33	3.00
soft-shell clam	0.15	0.32	0.40	**0.51**	0.57	1.30
viviparous eelpout	0.00	0.79	1.72	**3.34**	5.65	9.16

Table 17a: Descriptive statistics on microplastic particles per g net weight of tissue according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	0.43	0.91	**1.40**	1.81	6.62
brown shrimp	0.00	0.00	0.12	**0.55**	0.79	2.41
common cockle	0.00	0.47	1.13	**1.49**	1.99	6.72
common periwinkle	0.00	0.53	5.19	**10.29**	14.55	43.39
European plaice	0.00	0.00	0.00	**0.22**	0.36	2.00
common sole	0.00	0.00	0.00	**0.00**	0.00	0.00
green crab	0.02	0.12	0.24	**1.19**	0.88	8.95
Atlantic herring	0.00	0.00	0.95	**10.64**	22.55	52.38
lugworm	0.00	0.91	2.19	**2.99**	3.52	15.00
Pacific oyster	0.00	0.26	0.60	**0.82**	1.10	2.90
soft-shell clam	0.06	0.26	0.36	**0.41**	0.46	1.19
viviparous eelpout	0.00	0.79	1.72	**3.34**	5.65	9.16

Table 17b: Descriptive statistics on microplastic fragments per g net weight of tissue according to species

Species	min	1st qu.	median	mean	3rd qu.	max
blue mussel	0.00	0.00	0.07	**0.19**	0.17	1.08
brown shrimp	0.00	0.00	0.00	**0.15**	0.02	1.09
common cockle	0.00	0.00	0.39	**0.56**	0.69	5.97
common periwinkle	0.00	0.10	1.32	**5.61**	3.51	45.63
European plaice	0.00	0.00	0.00	**0.00**	0.00	0.00
common sole	0.00	0.00	0.00	**0.00**	0.00	0.00
green crab	0.00	0.00	0.09	**0.34**	0.23	3.48
Atlantic herring	0.00	0.00	0.00	**0.00**	0.00	0.00
lugworm	0.00	0.03	0.45	**0.64**	0.87	2.77
Pacific oyster	0.00	0.00	0.09	**0.17**	0.23	1.24
soft-shell clam	0.00	0.02	0.09	**0.10**	0.13	0.45
viviparous eelpout	0.00	0.00	0.00	**0.00**	0.00	0.00

Table 17c: Descriptive statistics on microplastic fibres per g net weight of tissue according to species

Phylum	min	1st qu.	median	mean	3rd qu.	max
arthropodes	0.00	1.18	**2.31**	5.16	6.45	32.78
chordates	0.00	0.00	**0.00**	0.71	0.48	6.81
molluscs	0.00	3.04	**5.29**	9.43	12.68	57.74
polychaetes	0.00	2.30	**15.70**	14.14	21.12	38.45

Table 18a: Descriptive statistics on microplastic particles per individual according to phylum

Phylum	min	1st qu.	median	mean	3rd qu.	Max
arthropodes	0.00	0.39	**1.48**	3.82	5.23	29.39
chordates	0.00	0.00	**0.00**	0.71	0.48	6.81
molluscs	0.00	1.65	**4.06**	7.44	9.19	56.23
polychaetes	0.00	2.16	**10.69**	11.07	16.19	35.02

Table 18b: Descriptive statistics on microplastic fragments per individual according to phylum

Phylum	min	1st qu.	median	mean	3rd qu.	Max
arthropodes	0.00	0.00	**0.13**	1.34	1.77	17.77
chordates	0.00	0.00	**0.00**	0.00	0.00	0.00
molluscs	0.00	0.00	**0.61**	1.99	2.43	26.63
polychaetes	0.00	0.09	**1.73**	3.07	4.00	13.06

Table 18c: Descriptive statistics on microplastic fibres per individual according to phylum

Phylum	min	1st qu.	median	mean	3rd qu.	Max
arthropodes	0.00	0.11	**0.37**	1.18	1.27	10.27
chordates	0.00	0.00	**0.00**	4.11	1.05	52.38
molluscs	0.00	0.44	**1.08**	4.38	2.34	67.91
polychaetes	0.00	0.97	**2.27**	3.64	4.50	15.40

Table 19a: Descriptive statistics on microplastic particles per g net weight of tissue according to phylum

Phylum	min	1st qu.	median	mean	3rd qu.	Max
arthropodes	0.00	0.07	**0.17**	0.92	0.83	8.95
chordates	0.00	0.00	**0.00**	4.11	1.05	52.38
molluscs	0.00	0.31	**0.78**	3.00	2.00	43.39
polychaetes	0.00	0.91	**2.19**	2.99	3.52	15.00

Table 19b: Descriptive statistics on microplastic fragments per g net weight of tissue according to phylum

Phylum	min	1st qu.	median	mean	3rd qu.	max
arthropodes	0.00	0.00	**0.03**	0.26	0.19	3.48
chordates	0.00	0.00	**0.00**	0.00	0.00	0.00
molluscs	0.00	0.00	**0.10**	1.38	0.47	45.63
polychaetes	0.00	0.03	**0.45**	0.64	0.87	2.77

Table 19c: Descriptive statistics on microplastic fibres per g net weight of tissue according to phylum

sub-phylum	min	1st qu.	median	mean	3rd qu.	max
chordates	0.00	0.00	**0.00**	0.71	0.48	6.81
invertebrates	0.00	2.00	**5.00**	9.20	13.13	57.74

Table 20a: Descriptive statistics on microplastic particles per individual comparing chordates and invertebrates

sub-phylum	min	1st qu.	median	mean	3rd qu.	max
chordates	0.00	0.00	**0.00**	0.71	0.48	6.81
invertebrates	0.00	1.38	**3.94**	7.20	9.35	56.23

Table 20b: Descriptive statistics on microplastic fragments per individual comparing chordates and invertebrates

sub-phylum	min	1st qu.	median	mean	3rd qu.	max
chordates	0.00	0.00	**0.00**	0.00	0.00	0.00
invertebrates	0.00	0.00	**0.61**	2.00	2.43	26.63

Table 20c: Descriptive statistics on microplastic fibres per individual comparing chordates and invertebrates

sub-phylum	min	1st qu.	median	mean	3rd qu.	max
chordates	0.00	0.00	**0.00**	4.11	1.05	52.38
invertebrates	0.00	0.37	**1.07**	3.73	2.39	67.91

Table 21a: Descriptive statistics on microplastic particles per g net weight of tissue comparing chordates and invertebrates

sub-phylum	min	1st qu.	median	mean	3rd qu.	max
chordates	0.00	0.00	**0.00**	4.11	1.05	52.38
invertebrates	0.00	0.26	**0.76**	2.63	2.16	43.39

Table 21b: Descriptive statistics on microplastic fragments per g net weight of tissue comparing chordates and invertebrates

sub-phylum	min	1st qu.	median	mean	3rd qu.	max
chordates	0.00	0.00	**0.00**	0.00	0.00	0.00
invertebrates	0.00	0.00	**0.10**	1.10	0.47	45.63

Table 21c: Descriptive statistics on microplastic fibres per g net weight of tissue comparing chordates and invertebrates

feeding type	min	1st qu.	median	mean	3rd qu.	max
deposit feeder	0.00	2.30	**15.70**	14.14	21.12	38.45
filter feeder	0.00	3.54	**6.11**	10.82	14.04	57.74
grazer	0.00	0.63	**2.88**	4.28	6.10	21.87
predator	0.00	0.00	**0.48**	2.77	2.85	32.78

Table 22a: Descriptive statistics on microplastic particles per individual according to feeding type

feeding type	min	1st qu.	median	mean	3rd qu.	max
deposit feeder	0.00	2.16	**10.69**	11.07	16.19	35.02
filter feeder	0.00	2.32	**5.06**	8.62	11.23	56.23
grazer	0.00	0.49	**1.90**	3.07	3.90	19.57
predator	0.00	0.00	**0.39**	2.15	2.48	29.39

Table 22b: Descriptive statistics on microplastic fragments per individual according to feeding type

feeding type	min	1st qu.	median	mean	3rd qu.	max
deposit feeder	0.00	0.09	**1.73**	3.07	4.00	13.06
filter feeder	0.00	0.00	**0.63**	2.20	2.63	26.63
grazer	0.00	0.10	**0.37**	1.20	0.89	7.30
predator	0.00	0.00	**0.00**	0.62	0.00	17.77

Table 22c: Descriptive statistics on microplastic fibres per individual according to feeding type

feeding type	min	1st qu.	median	mean	3rd qu.	max
deposit feeder	0.00	0.97	**2.27**	3.64	4.50	15.40
filter feeder	0.00	0.41	**0.95**	1.29	1.75	6.81
grazer	0.00	1.64	**5.50**	15.90	26.92	67.91
predator	0.00	0.00	**0.24**	2.76	1.20	52.38

Table 23a: Descriptive statistics on microplastic particles per g net weight tissue according to feeding type

feeding type	min	1st qu.	median	mean	3rd qu.	max
deposit feeder	0.00	0.91	**2.19**	2.99	3.52	15.00
filter feeder	0.00	0.31	**0.61**	1.04	1.48	6.72
grazer	0.00	0.53	**5.19**	10.29	14.55	43.39
predator	0.00	0.00	**0.15**	2.64	0.88	52.38

Table 23b: Descriptive statistics on microplastic fragments per g net weight tissue according to feeding type

feeding type	min	1st qu.	median	mean	3rd qu.	Max
deposit feeder	0.00	0.03	**0.45**	0.64	0.87	2.77
filter feeder	0.00	0.00	**0.08**	0.25	0.25	5.97
grazer	0.00	0.10	**1.32**	5.61	3.51	45.63
predator	0.00	0.00	**0.00**	0.12	0.00	3.48

Table 23c: Descriptive statistics on microplastic fibres per g net weight tissue according to feeding type

location	min	1st qu.	median	mean	3rd qu.	Max
Buesum	0.00	0.14	**3.06**	4.78	6.23	31.76
Friedrichskoog	0.00	1.69	**5.02**	9.00	15.38	43.13
List	0.00	0.92	**3.48**	7.13	11.01	30.78
Pellworm	0.00	1.71	**5.62**	11.15	13.72	57.74

Table 24a: Descriptive statistics on microplastic particles per individual according to location

location	min	1st qu.	median	mean	3rd qu.	Max
Buesum	0.00	0.14	**1.95**	3.92	4.65	28.39
Friedrichskoog	0.00	1.65	**3.87**	7.73	12.09	42.39
List	0.00	0.50	**2.73**	5.27	7.35	29.23
Pellworm	0.00	1.32	**3.57**	8.43	9.99	56.23

Table 24b: Descriptive statistics on microplastic fragments per individual according to location

location	min	1st qu.	median	mean	3rd qu.	max
Buesum	0.00	0.00	**0.19**	0.86	0.93	9.77
Friedrichskoog	0.00	0.00	**0.13**	1.27	1.63	7.87
List	0.00	0.00	**0.07**	1.86	1.77	19.06
Pellworm	0.00	0.00	**0.89**	2.71	3.31	26.63

Table 24c: Descriptive statistics on microplastic fibres per individual according to location

Species	min	1st qu.	median	mean	3rd qu.	max
Buesum	0.00	0.19	**0.91**	4.83	1.98	67.91
Friedrichskoog	0.00	0.74	**1.89**	3.88	5.86	32.19
List	0.00	0.18	**0.57**	2.57	1.78	42.06
Pellworm	0.00	0.32	**0.73**	3.79	2.16	55.44

Table 25a: Descriptive statistics on microplastic particles per g net weight of tissue according to location

Location	min	1st qu.	median	mean	3rd qu.	max
Buesum	0.00	0.10	**0.56**	3.77	1.84	52.38
Friedrichskoog	0.00	0.61	**1.53**	3.16	4.86	18.00
List	0.00	0.11	**0.44**	2.30	1.62	40.14
Pellworm	0.00	0.11	**0.47**	2.21	1.65	43.39

Table 25b: Descriptive statistics on microplastic fragments per g net weight of tissue according to location

Location	min	1st qu.	median	mean	3rd qu.	Max
Buesum	0.00	0.00	**0.02**	1.06	0.22	35.00
Friedrichskoog	0.00	0.00	**0.08**	0.72	0.38	14.19
List	0.00	0.00	**0.01**	0.28	0.23	4.42
Pellworm	0.00	0.00	**0.11**	1.57	0.67	45.63

Table 25c: Descriptive statistics on microplastic fibres per g net weight of tissue according to location

Habitat	min	1st qu.	median	mean	3rd qu.	max
benthic	0.00	2.00	**5.00**	9.20	13.13	57.74
demersal	0.00	0.00	**0.00**	0.35	0.14	3.48
pelagic	0.00	0.00	**0.48**	1.40	2.48	6.81

Table 26a: Descriptive statistics on microplastic particles per individual according to habitat

Habitat	min	1st qu.	median	mean	3rd qu.	max
benthic	0.00	1.38	**3.94**	7.20	9.35	56.23
demersal	0.00	0.00	**0.00**	0.35	0.14	3.48
pelagic	0.00	0.00	**0.48**	1.40	2.48	6.81

Table 26b: Descriptive statistics on microplastic fragments per individual according to habitat

Habitat	min	1st qu.	median	mean	3rd qu.	max
benthic	0.00	0.00	**0.61**	2.00	2.43	26.63
demersal	0.00	0.00	**0.00**	0.00	0.00	0.00
pelagic	0.00	0.00	**0.00**	0.00	0.00	0.00

Table 26c: Descriptive statistics on microplastic fibres per individual according to habitat

Habitat	min	1st qu.	median	mean	3rd qu.	max
benthic	0.00	0.37	**1.07**	3.73	2.39	67.91
demersal	0.00	0.00	**0.00**	0.75	0.40	9.16
pelagic	0.00	0.00	**0.95**	10.64	22.55	52.38

Table 27a: Descriptive statistics on microplastic particles per g net weight of tissue according to habitat

Habitat	min	1st qu.	median	mean	3rd qu.	max
benthic	0.00	0.26	**0.76**	2.63	2.16	43.39
demersal	0.00	0.00	**0.00**	0.75	0.40	9.16
pelagic	0.00	0.00	**0.95**	10.64	22.55	52.38

Table 27b: Descriptive statistics on microplastic fragments per g net weight of tissue according to habitat

Habitat	min	1st qu.	median	mean	3rd qu.	max
benthic	0.00	0.00	**0.10**	1.10	0.47	45.63
demersal	0.00	0.00	**0.00**	0.00	0.00	0.00
pelagic	0.00	0.00	**0.00**	0.00	0.00	0.00

Table 27c: Descriptive statistics on microplastic fibres per g net weight of tissue according to habitat

season	min	1st qu.	median	mean	3rd qu.	max
autumn 2017	0.00	0.65	**3.27**	6.33	8.13	38.45
spring 2018	0.00	1.34	**6.02**	10.54	15.23	57.74

Table 28a: Descriptive statistics on microplastic particles per individual according to season

Season	min	1st qu.	median	mean	3rd qu.	max
autumn 2017	0.00	0.35	**2.42**	4.43	5.58	35.02
spring 2018	0.00	1.23	**5.23**	9.38	14.23	56.23

Table 28b: Descriptive statistics on microplastic fragments per individual according to season

Season	min	1st qu.	median	mean	3rd qu.	max
autumn 2017	0.00	0.00	**0.30**	1.90	2.30	26.63
spring 2018	0.00	0.00	**0.08**	1.16	1.37	15.64

Table 28c: Descriptive statistics on microplastic fibres per individual according to season

Season	min	1st qu.	median	mean	3rd qu.	max
autumn 2017	0.00	0.22	**1.03**	5.08	3.37	67.91
spring 2018	0.00	0.33	**0.72**	1.23	1.66	8.00

Table 29a: Descriptive statistics on microplastic particles per g net weight of tissue according to season

Season	min	1st qu.	median	mean	3rd qu.	max
autumn 2017	0.00	0.11	**0.62**	3.82	2.42	52.38
spring 2018	0.00	0.24	**0.55**	1.03	1.41	8.00

Table 29b: Descriptive statistics on microplastic fragments per g net weight of tissue according to season

Season	min	1st qu.	median	mean	3rd qu.	max
autumn 2017	0.00	0.00	**0.08**	1.27	0.62	45.63
spring 2018	0.00	0.00	**0.02**	0.20	0.10	5.97

Table 29c: Descriptive statistics on microplastic fibres per g net weight of tissue according to season