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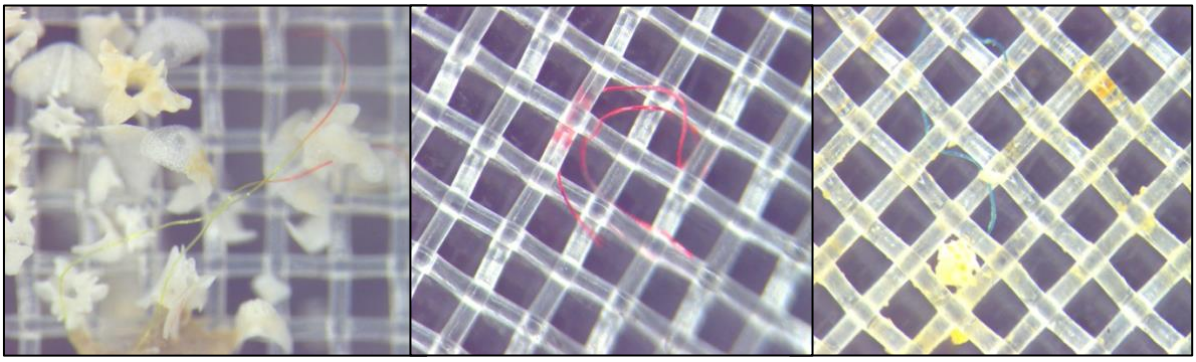
Plastic ingestion and diet composition in two common fish species from the Swedish Skagerrak



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Plastic ingestion and diet composition in two common fish species from the Swedish Skagerrak

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Abstract

Plastic is a serious threat to the marine environment. However, the knowledge on how and to what extent it affects marine species is still limited. Currently, more and more studies focus on plastic ingestion and accumulation in marine biota and sediments. Research is gathering data in order to work towards understanding the underlying processes of plastic in the marine community. This study presents the results from the gut content of two common fish species collected in the coastal and offshore Skagerrak. After identifying the diet composition, each sample was digested with an enzymatic method. The sample leftovers were visually inspected for ingested plastic polymers. Ingested plastic particles were found in 10.8% of the whiting (*Merlangius merlangus*) and 17.6% of the common dab samples (*Limanda limanda*). Plastic ingestion rates did not differ between the coastal and the offshore region. The 60 recovered particles consisted almost exclusively of fibres. The sizes ranged between 240µm and 25mm, while the dab ingested significantly wider size range of plastic particles. The colour spectrum was dominated by translucent plastics. Next to plastic, my study recovered even higher numbers of anthropogenic non-plastic polymers (26.8%), respectively natural and synthetic fibres. The colour spectrum was more diverse, with black particles being most abundant.

In the whiting, the diet composition showed significant dissimilarities between the samples from the coast and offshore. Whitings from the coast predominantly ingested fish and shrimps. While conspecifics from the offshore region contained only 1.8% fish, the rest of the diet was mainly composed of polychaetes, nematodes, shrimps and other crustaceans. The common dab from the offshore regions mainly consumed echinoderms and polychaetes, while bivalves, echinoderms and algae were most abundant in the diet of coastal individuals. The varying diet compositions were likely caused by seasonal and regional differences.

Plastic ingestion is supposed to be linked to the feeding behaviour of the fish. Anthropogenic particles were expected to be accidentally ingested by the common dab due to its feeding strategy, which is focused on ground-living organisms. In the whiting, marine debris was suggested to be ingested secondarily through the prey organisms as well as by accident. However, the drivers of plastic ingestion require further research and discussion. In order to understand the interaction between the diet and plastic ingestion, future research is advised to focus on the role of plastic in food web dynamics.

Keywords: microplastic, plastic ingestion, whiting, dab, diet composition, enzymatic tissue digestion

Popular science summary

Plastic products can most likely be found everywhere in the ocean by now. It enters and distributes in the oceans in various shapes and sizes, for instance large fishing gear, plastic bottles or microplastics. How and to what extent these plastic products affect marine animals is still mostly unknown. Currently, popular scientific questions are: Why does a marine animal take up plastic? How much plastic can be found in the body of marine animals? This knowledge helps to understand the influence of plastic on the marine ecosystem. In this study, the gut content of two common fish species from the coastal and offshore Skagerrak was identified and checked for plastic particles. Plastic particles were found in 10.8% of the whiting (*Merlangius merlangus*) and 17.6% of the common dab samples (*Limanda limanda*). The amount of plastic did not differ between fish from the coastal and the offshore region. In total, I found 60 plastic particles, mainly fibres. The plastic found in the dab samples varied more in length than in the whitings. Even though the plastics had different colours, most of the particles were translucent. Next to plastic, I found even more anthropogenic but non-plastic particles (26.8%) such as cotton and rayon fibres. Here, the particles showed more diverse colours, but black particles were most abundant.

In the whiting, the gut content differed between individuals from the coast and offshore. Whitings from the coast predominantly preyed on fish and shrimps, while whitings from the offshore region mainly ingested bristle worms, roundworms, shrimps and other crustaceans. The common dab from the offshore regions mainly consumed echinoderms and polychaetes, while bivalves, echinoderms and algae were most abundant in the diet of coastal individuals. These differences in the gut content were likely caused by seasonal and regional differences.

The uptake of plastic is supposed to be connected to the feeding behaviour of the fish. The common dab was expected to accidentally feed on plastic that settles down in between the preferred ground-living prey. In the whiting, plastic was suggested to be taken up secondarily through the prey organisms as well as by accident. However, the interaction between the fish diet and plastic uptake requires further research and discussion.

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1 Introduction

We are living in the plastic age. All over the world, plastic products have become an essential part of the everyday life. After plastic was introduced to the global market in the 1950s until now, the manufacturing rates increased exponentially. By now, plastic polymers have spread everywhere, to every continent, country, city, village, household and unfortunately every natural habitat (Thompson et al., 2009). The favourable plastic properties to the modern world pose the most threat to the environment: durability, lightweight, heterogeneity and cheap manufacturing. Thus, plastic is not only persistent over time, but occurs in vast amounts providing every possible shape and colour. The variety in appearances results from the numerous sources from which plastic enters the environmental system. The major sources are represented by the textile, cosmetic and automotive industry, fisheries and sewage (Boucher & Friot, 2017; Gallo et al., 2018; Napper & Thompson, 2016). Boucher and Friot (2017) identified the seven most influential sources of primary microplastics in the oceans: tyres, synthetic textiles, marine coatings, road markings, personal care products, plastic pellets (spills during manufacturing and transportation) and city dust. Primary microplastics are considered small plastic particles that are directly released into the environment. An additional dominant source are secondary microplastics, that originate from bigger plastic items through the process of fragmentation (Sundt et al., 2014). Plastic is not resistant against degradation. It degrades into smaller fragments due to natural forces such as UV light, wind and current (Song et al., 2017). The resulting secondary micro- or nanoplastics can enter even deeper into the system.

By now, plastic polymers have probably reached almost every single corner of our planet and start to accumulate. The biggest sink for plastic accumulation is the ocean, including the coastlines (Lots et al., 2017; Stolte et al., 2015), the open water (Dixon & Dixon, 1983) and the deep sea (Bergmann & Klages, 2012; Van Cauwenberghe et al., 2013). Even though plastic pollution is documented to be of serious concern in terrestrial and freshwater environments (Horton et al., 2017;

Imhof et al., 2013), most of the research has been focused on marine habitats. The major fraction of contaminants runs off from the land into the oceans (Siegfried et al., 2017) and accumulates in the sediment and the open ocean (Eriksen et al., 2014; Munari et al., 2017). Thus, the marine environment requires a lot of attention to monitor and understand the ongoing processes. A study by Siegfried et al. (2017) estimated the microplastic fluxes from land to sea based on point-sources and selected sources in European river systems. According to the modelled processes, 42% of the microplastic run-off originated from tyre and road wear, 29% was abraded from textiles during laundry, 19% resulted from household dust and 10% was released through personal care products. These numbers are highly dependent on the sewage treatment technologies in the single countries. Innovative wastewater technology demonstrates a crucial step towards reducing pollution of freshwater systems, which indirectly affect the oceans.

Next to the indirect pollution of plastic run-off from land to sea, direct pollution represents a major contamination source for the world ocean. It is defined as the release of plastic debris directly into the ocean. The fishing industry represents one of the main sources and proved to be a serious threat to marine organisms (Jones, 1995). Most of the fishing-related plastic material that enters the marine system are big items such as fishing lines and nets. In the literature, these items often occur in connection with entanglements, injuries or even deaths of marine species such as birds (Bond et al., 2012), sea turtles (Bugoni et al., 2001), cetaceans and seals (A. L. Lusher et al., 2018; Unger et al., 2017). Despite that big plastic debris can cause serious harm, small plastic polymers are most likely more dangerous to marine species.

In recent years, numerous studies focused on the impact of microplastic on the marine environment. The size range for the category ‘microplastics’ is still under debate in the current literature (Hartmann et al., 2019). In consensus with previous publications, the following study addresses all plastic particles < 5 mm as microplastic (Löder et al., 2017; A. Lusher et al., 2017; Rummel et al., 2016). Due to their size, microplastics are able to affect not only big mammals but much smaller species likewise, such as zooplankton (Cole et al., 2014; Desforges et al., 2015), annelids (Wright et al., 2013), echinoderms (Graham & Thompson, 2009), cnidarians (N. Hall et al., 2015), bivalves (Van Cauwenberghe & Janssen, 2014), crustaceans (Devriese et al., 2015; Murray & Cowie, 2011) and fish (A. Lusher et al., 2013; Rummel et al., 2016). Thus, numerous studies found traces of plastic polymers in various marine taxa. What drives animals to ingest plastic? Even though several studies already addressed this question, the reasons for plastic ingestion are still unclear. However, finding an answer to the ‘why’ always requires an explanation for the ‘how’. Hence, how do marine species ingest plastic?

The majority of studies suspects plastic particles to be ingested through the diet. Suspension feeders were reported to take up plastic polymers together with the ingested substrate (Graham & Thompson, 2009; Wright et al., 2013). Sediment samples from the sea floor were tested and found to contain microplastic all over the world (Claessens et al., 2011; Munari et al., 2017; Peng et al., 2017; Stolte et al., 2015). Plastic debris that enters the marine system eventually settles down and accumulates on the sea floor. However, some plastic types possess the ability of buoyancy, which allows the particles to float at the water surface or in the water column. Depending on the size, floating plastic polymers might be ingested by filter feeding as well as predatory species. Different filter feeders were reported to contain plastic that was filtered from the water column, in both benthic (N. Hall et al., 2015; Van Cauwenberghe & Janssen, 2014) and pelagic habitats (Desforges et al., 2015; Devriese et al., 2015). Predatory species on the other hand, can take up plastic in two different ways: through active or passive ingestion. By ingesting plastic actively, marine organisms confuse plastic particles with their actual prey or ingest it accidently (Boerger et al., 2010). Passive ingestion results from a trophic transfer. Thus, the predator preys on a species from a lower trophic level, that previously ingested plastic polymers. This process causes at least part of the plastic ingestion in top predator species such as seals (Eriksson & Burton, 2003; Nelms et al., 2018). What drives smaller predatory species such as fish to ingest plastic polymers?

Presumably, plastic polymers are ingested while feeding. Accordingly, plastic ingestion is assumed to be connected to the diet or the feeding behaviour of fish (Morgana et al., 2018), Thus, information on the feeding behaviour and the diet composition are important for studies on plastic pollution in biota. Which factors are most influential to plastic ingestion in fish, whether it is dependent on the habitat or geographical region and if the species ecology plays an essential role is yet to be investigated. In order to understand the underlying processes and potential impact, the role of plastic in the food web dynamics should be analysed more closely. As mentioned, plastic was found in the systems of species from different trophic level. Therefore, it can enter and be transferred through the system in several different ways (Diepens & Koelmans, 2018). If plastics are ingested by organisms instead of their actual prey, it may affect the trophic energy exchange and shift the food web dynamics. From a nutritional point of view, plastic might influence the consumers choice of prey due to its energy requirements (Machovsky-Capuska et al., 2019). Further research is needed in order to understand the pathways in food web dynamics and to include plastic litter into the ongoing interactions. At the start, investigating the feeding behaviour and diet composition of the participating organisms is the first step towards understanding this complex system. Thus, performing a diet analysis as part of an investigation for ingested plastics in organisms would be essential.

The role of plastic in the marine ecosystem leads to an important question: Is ingested plastic detrimental to the health of organisms? Indeed, plastic can harm marine organisms. Apart from entanglements in plastic nets and strings (Unger et al., 2017), smaller plastics were found to affect the health condition of marine animals. Microplastics posed a toxic effect on the liver of Zebrafish (Lu et al., 2016) and was found to affect the endocrine system of the Japanese medaka (*Oryzias latipes*) (Rochman et al., 2014). In addition, the lugworm (*Arenicola marina*) proved to be 30% more susceptible to oxidative stress after plastic ingestion (Browne et al., 2013) and the cell tissue of blue mussel (*Mytilus edulis*) was significantly affected by microplastics taken up into the cells (von Moos et al., 2012). According to these and other studies, plastic can pose a serious threat to the health condition of marine organisms from different taxa and habitats. Nevertheless, why should we care about this?

We should care because according to Miranda and de Carvalho-Souza (2016) we are eating plastic-ingesting fish. Plastic was not only recorded in wild marine organisms as cited above, but as well in fish and mussels that are cultured or caught for human consumption (Miranda & de Carvalho-Souza, 2016; Van Cauwenberghe & Janssen, 2014). Since we as humans are on top of the food chain and bioaccumulation was reported to occur in the marine food web, plastics are likely to eventually enter our body system as well. If and how plastic ingestion affects our health and body functions is unknown. Since plastic can be detrimental to the health of various marine organisms, it is likely to influence the human system as well. Due to the potential threat to the health of humans and marine animals, as well as conservation reasons, investigating the impact of plastic ingestion is a important research topic.

As previously mentioned, conducting a diet analysis on the study species might help to understand the underlying process of plastic ingestion. Furthermore, gathering data of the diet and the amount of ingested plastic potentially reveals yet unknown interaction between plastic ingestion and the feeding strategy of the consumer. For this reason, the following study not only focused on plastic ingestion but addressed the diet composition as well. The study was conducted on the whiting (*Merlangius merlangus*) and the common dab (*Limanda limanda*) that both frequently occur in the Swedish Skagerrak. The fish samples were caught in two different regions, the coastal and offshore Skagerrak. Thus, I explored the data of each species for geographical differences in the body condition, diet composition and plastic ingestion. More in detail, I compared the body length and the condition of the individuals from the coast and offshore. Regarding the diet analysis, I identified the different prey groups occurring in the diet of both species and subsequently compared the diet composition between the two regions. I counted, measured and identified the colours of the recovered plastic particles as well as other

anthropogenic particles, and compared these measures between the two regions. In addition, I analysed the data for inter-specific patterns.

Apart from gaining further knowledge on plastic ingestion and the diet composition in biota, I took special care to establish a well-working laboratory protocol that provides a solid diet analysis, low contamination risk and harmless digestion method.
Plastic research is highly susceptible to contamination during the laboratory procedure. Contamination might occur through air-borne fibres, the work environment, as well as plastic wear and equipment. Precaution measures against sample contamination are not yet applied by a standardised protocol. The necessity of a clean laboratory procedure was only recently addressed (A. Lusher et al., 2017). In order to provide consistent and representative results, the standardisation of laboratory procedures on biota samples is highly recommended. Choosing an appropriate digestion method is another factor that might influence the outcome of the laboratory analysis. Including a tissue digestion method to the protocol, accelerates and improves the inspection of the leftover material for anthropogenic particles. Previous studies applied different acidic or alkaline agents to digest the organic tissue of biota samples (A. Lusher et al., 2017). However, the commonly used digestive agents were shown to affect the surface structure or colour of the plastic polymers in the samples (Cole et al., 2014; Enders et al., 2017). Recent studies implemented less destructive enzymatic methods that proved to harmless to plastic (Cole et al., 2014; Karlsson et al., 2017; Löder et al., 2017). Thus, this study developed a laboratory protocol that takes precaution measures against contamination and utilises an enzymatic approach for tissue digestion.

The overall aim of this project was to evaluate the ingestion of marine debris in the two study species caught in the Swedish Skagerrak. Plastic particles were expected to occur more frequently in combination with ground-living prey. I suppose that particles on the sea floor were likely to be hidden between the benthic organisms and thus were ingested by accident. I also expected to encounter more plastic particles in individuals caught in coastal regions than offshore, as the main source of pollution is closer. In order to detect the driving forces of plastic ingestion, I tried to draw a connection to the diet composition. With the results of the study intended to support the global data base on the diet composition and the occurrence of plastic in marine species. Additionally, I aimed to find an interaction between the feeding behaviour and the ingested plastic particles in order to help understand the role of plastic in the food web.

2 Materials and Methods

2.1 Species

The two investigated fish species are frequently occurring and distributed throughout the Skagerrak. Thus, both fish act as key species in the different food webs, both as predators and prey, with large potential to have impact on the ecosystem dynamics (vattenmyndigheten, 2019).

Whiting (*Merlangius merlangus*)

The whiting (*Merlangius merlangus*) is a benthopelagic species living in the eastern part of the North Atlantic. It prefers softbottom habitats in 10 to 200 m depth. However, the species can occur in areas with sandy or rocky bottoms as well. The sizes range from 15 to 19 cm in one-year old until 30 to 34 cm in three-year old specimens. The breeding season lasts from February until June with a spawning peak in April (Bowers, 1954). Larvae and juveniles are pelagic and only become demersal when they reach a length of 5 to 10 cm. The diet primarily comprises fish, crustaceans, polychaetes, molluscs and cephalopods (Cohen, 1990). Wennhage and Pihl (2002) as well as Kihlman and Holm (1978) studied the diet content of whiting from the Swedish west coast. The examined diet samples were dominated by fish, consisting of gobies (*Gobiidae* spp.), herring (*Clupea harengus*), Norway pout (*Boreogradus esmaki*) and gadoids (*Gadidae* spp.).

The whiting is a common but "non-target" species in commercial fisheries, mainly caught by bottom trawls (Cohen, 1990). A significant proportion of the catches are by-catch (42% in 2017). Based on the results of the yearly international bottom-trawl surveys (IBTS), whiting catches decreased over the past 40 years (vattenmyndigheten, 2019). However, the results from the past three years revealed that whiting is one of the most abundant species in the Swedish North Sea (Bland & Hjelm, 2018; Hjelm & Bland, 2016, 2017). According to the latest IUCN report

on endangered species, the whiting received the status ‘least concern’ (Nieto et al., 2015).

Common dab (*Limanda limanda*)

The common Dab (*Limanda limanda*) is a benthic flatfish species that occurs mainly in sandy bottom habitats. It is distributed throughout the eastern North Atlantic in depths between a few meters up to 150 m (Bolle et al., 1994). The common dab is an opportunistic feeder. Although, the diet composition of dabs from the southern North Sea mainly consists of echinoderms of the family *Ophiuridea* (Hinz et al., 2005). This is supported by the study by Wennhage and Pihl (2002) conducted at the Swedish west coast. Furthermore, the diet composition contained polychaetes and crustaceans.

According to the international bottom trawl survey (IBTS) reports from the past three years, the common dab is a frequently occurring and widely distributed flatfish in the Swedish North Sea (Bland & Hjelm, 2018; Hjelm & Bland, 2016, 2017). In fisheries, dab mainly occurs as a by-catch species with extremely high discard rates of up to 90% (ICES, 2017). However, this seemed not to affect the populations wellbeing. The latest IUCN report on endangered species classified the status of the dab as ‘least concern’ (Nieto et al., 2015).

2.2 Sampling

The fish samples came from two different bottom-trawl surveys performed in Skagerrak, a region in the North Sea which borders the Swedish west coast, the Norwegian south coast and the northern tip of Denmark (see Fig. 1). The first batch of samples was collected during the coastal survey in September 2018 (Svensson et al., 2019). I analysed 125 dab and 153 whiting samples collected from several locations (see table 1). 12 dab samples from Älgöfjorden and ten whiting samples from Skår were analysed with a slightly different procedure as part of the pilot study (see 2.3.1.). The second batch came from the offshore survey taking place between 16th to 30th of January 2019 (J. Hjelm & Bland, 2019). I analysed 80 dab and 79 whiting samples.

Table 1. *Information on GPS position (latitude, longitude), depth and the number of sampled individuals per species for each location from the coastal IBTS in September 2018. If the catch allowed it, I sampled 30 individuals per species and location. Otherwise, I sampled all caught individuals. Not every haul contained both species, e.g. Slussen did not contain whitings and Skår and Torgestad did not contain dabs. The hauls at the locations Älgöfjorden and Kärso were taken on September 12th. The hauls at the other locations were collected on September 11th.*

haul location	N latitude	E longitude	depth [m]	whiting	dab
Älgöfjorden (SE Tjörn)	5754,85	1139,91	18,1	30	14

Askeröfjorden (Stenungsund)	5805,31	1147,56	15,8	18	40
Kärso (SE Tjörn)	5756,55	1137,88	18,5	30	33
Ljungskile	5815,24	1150,18	17	5	8
Skår (Gullmarsfjorden)	5817,31	1130,74	72,2	40	0
Slussen (Havstensfjord)	5817,66	1145,72	15,8	0	30
Torgestad (Gullmarsfjorden)	5820,52	1133,89	97,1	30	0
				153	125

Table 2. *Information on GPS position (latitude, longitude), depth and the number of sampled individuals per species for each location from the offshore IBTS in January 2019. Due to time restriction, I did not sample as many individuals as from the coastal samples. However, I processed a representative amount of fish from each species and location. The Hanstholm haul was collected January 25th, Skägga on January 27th and Hirtshals on January 29th.*

haul location	N latitude	E longitude	depth [m]	whiting	dab
20 N Hanstholm	5727,37	0835,42	54	30	30
NW Skägga	5829,99	1107,01	57	24	25
11 N Hirthals	5745,39	0947,48	38	25	25
				79	80

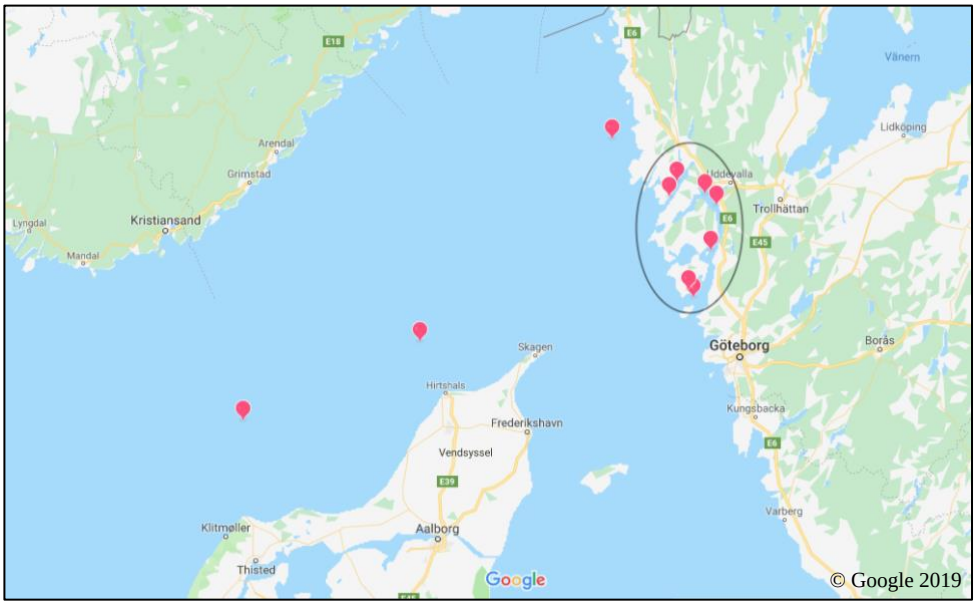


Figure 1. The map shows the seven haul locations of the coastal samples (inside black circle) and the three haul locations of the offshore samples in the Swedish Skagerrak.

2.3 Laboratory analysis

For sample processing I applied the method established by Winberg von Friesen et al. (2019). Since this study worked with a different organism, I conducted a pilot study to evaluate the fit of the method and adjusted it according to my needs.

2.3.1 Pilot study

I conducted a pilot study in order to design a reliable method for my study purpose. I evaluated whether performing a diet analysis before dissolving the organic tissue benefits the study design or causes an unacceptable contamination risk. Therefore, about ten fish of each species were analysed with each of the two compared method. First method: the fish were dissected, the gastro-intestinal tract (hereafter referred to as GIT) removed and transferred to a petri dish to inspect the gut content. Subsequently, the organic tissue of the samples was digested by an enzyme solution (see 2.3.4). After approximately 48 hours, the samples were filtered and all material bigger than 300µm were caught on a filter. For the alternative method, I dissected the fish and transferred the GIT directly into the glass bottle to digest the organic tissue. The diet analysis was conducted after the filtration step, by inspecting the leftover material on the filter under the microscope. Thus, I was able to perform a rough diet analysis, based on the digested prey remains. By applying the second method, I kept the sample in the flow chamber (see 2.3.7) and hence reduced the contamination risk. However, opening the gut enabled me to perform a more thorough diet analysis, based on the undigested prey remains. In addition, bigger prey items, e.g. bivalve shells, chitinous or skeletal parts, could be removed prior to the digestion. Since the enzyme set digests only organic tissue, these hard structures eventually ended up on the filter. Therefore, several filters were entirely covered with prey remains, which would have made identification of plastic particles more complicated. In order to enhance the possibility of finding anthropogenic particles on the filter, I decided to open the gut in advance to the digestion.

2.3.2 Dissection

Twelve hours prior dissection, I transferred the fish from the freezer (-20°C) to the fridge (5°C). When thawed, each the fish was measured from nose tip to tail tip and rinsed it with tap water before transferring it into the laminar air-flow cabinet (Kojair KR 125-safety). Before placing the fish onto the dissection tray, I weighed and rinsed it with Milli-Q water. Then, the GIT was removed, rinsed with Milli-Q and transferred to a petri dish. I protocolled the weight of the GIT. Whenever possible, the lid of the petri dish was kept closed to avoid contamination.

2.3.3 Diet analysis

For diet identification, the petri dish was placed under a stereomicroscope. Since this step could not be performed in the flow cabinet, the microscope was wrapped in a plastic cover to reduce the air flow (see Fig. 4) and thus the contamination risk (Torre et al., 2016). However, the cover had one opening on each side to handle the sample. Before opening the lid of the petri dish, he dissection equipment was cleaned with Milli-Q. Then, the GIT was opened and the content spread out in the dish. I examined it for maximum ten minutes and identified the prey remains to the lowest possible systematic level. For diet abundance I collected presence/absence data. If possible, the prey items were counted and quantity measures were protocolled as well. In cases when only parts or pieces of the prey were left, I protocolled the number of specimens that could clearly be identified as different individuals.

2.3.4 Digestion of organic matter

In order to visualise and detect the plastic debris in the GIT content, I used an enzyme set (Creon 40.000; extracted from pig pancreas) to dissolve the organic matter in the sample. Enzymatic digestion was tested to have no visible effect on the structure and surface of the plastic particles, in contract to acidic and alkaline digestion (ICES, 2017). In the flow cabinet, the sample was transferred from the petri dish into a glass bottle by means of a plastic funnel. If the diet contained any hard structures, such as fish skeletons, exoskeletons of crustaceans or mussel shells, I picked it out, rinsed it with Milli-Q into the glass bottle and transferred it to the biological waste. For the digestion of the sample, I required 10ml of enzyme solution (for 0 to 15g of sample volume). This was prepared a few hours beforehand. Wearing gloves, I added the required amount of enzymes and buffer solution in the specified composition (1 pill of Creon per 10ml of Tris hydrochloride buffer solution) to a glass bottle. The bottle was shaken intensely to dissolve the enzyme granules and homogenise the solution. Thereafter, the solution was kept in the incubator (Steri-Cult 200) at 37.5°C until it was needed later in the day. Then, the enzyme solution was added to the sample. I locked the bottle with a plastic lid and carefully mixed the content by hand. Afterwards, I kept the sample in the incubator at 37.5°C for 48 hours.

2.3.5 Filtration

After 48 hours, the more or less homogenous solution was filtered in the flow cabinet. For this, the sample was poured on a filter (300µm, 46mm diameter, nylon)

supported by a glass filter holder (Millipore Glass Filter Holder, 47 mm, 300 mL funnel) attached to a vacuum pump (see Fig.2). The glass funnel of the filter holder was rinsed thoroughly with Milli-Q water to remove leftovers of the sample. Whenever possible, the filter holder was covered with an aluminium lid to reduce contamination. After the sample was separated by the 300µm filter, the leftover fluid was saved and the filter carefully placed back into the petri dish. I sealed the petri dish with Parafilm to reduce airflow. The leftover fluid was poured on a second filter (100µm, 46mm diameter, nylon) to catch even smaller plastic particles. Again, the filter was transferred back into the petri dish right away. After a few days when the samples dried out, I sealed each petri dish with Parafilm. The samples were stored like this until further analysis. In this study, I only focused on plastic particles caught by the 300µm filter.

The filters were previously cut out of nylon screening. Every single filter was rinsed with tap water, placed in a petri dish and checked for particles under the microscope. If particles were spotted, I removed them carefully from the filter with fine forceps. Afterwards the filter was stored in the closed petri dish until used for filtration.

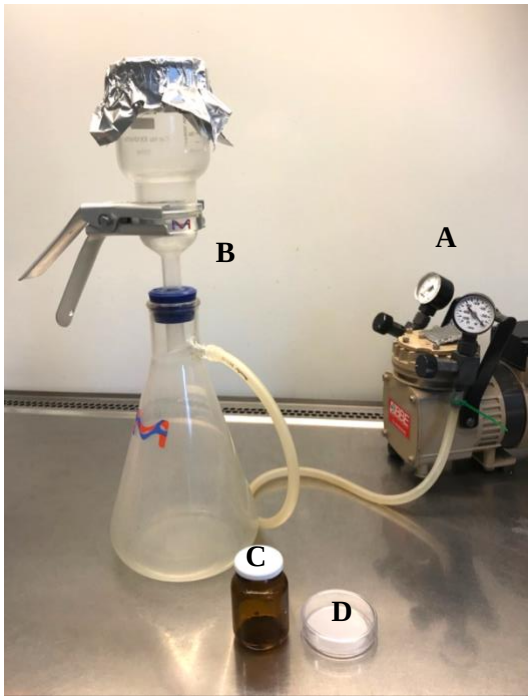


Figure 2. Filtration setup. The vacuum pump (A) was connected to the filtration unit (B), composing of a glass funnel and a filter mount, held together with a clamp, sitting on a glass flask. The filter funnel was covered with aluminium foil in order to prevent air-borne fibres to contaminate the sample. For the filtration, the digested sample (C) was poured into the filtration unit and the aluminium cover was put in place. After the sample ran through the filter, the clamp and funnel were removed and the filter was transferred from the mount into the petri dish (D).

2.3.6 Visual identification of plastic particles

The visual inspection for plastic particles was performed under a stereomicroscope (Leica M165C) attached to a camera (Lumenera, Infinity 3, application software: Infinity analyze 6.1). I examined the filter by screening from one side to the other, moving down one row and screening to the other side again. I proceeded in that manner across the entire filter. The dish was kept sealed. For each sample, I protocolled the sample ID and the filter load. Since the filters contained very different amounts of material, I specified the load on the filter (see Fig. 3). If I found a particle, I protocolled the various characteristics, measured length and width if possible and took a picture. I identified the plastic particles based on the criteria given by Norén (2007). All foreign anthropogenic material was protocolled, even if it did not fit the previously mentioned criteria, synthetic as well as natural. This was essential for estimating the degree of contamination and get an overview of the human impact in general, not only based on plastic. If a particle was suspected to be plastic or anthropogenic non-plastic, I took it aside and kept it on a separate stag to double check it. If after the second inspection the particle was still not certainly identified to be anthropogenic, it was excluded.
I took representative samples from the dust in the laboratory and from the lab coat being used.

Figure 3. The different amounts of leftover material from the samples, classified as four levels of filter load. From the left to the right: (1) the filter appeared empty or only contains a few items, (2) the filter contained sample material and was up to 50% covered, (3) the filter was more than 50% covered with leftover sample material, but some areas of the filter mesh very still visible , (4) the filter was at least 90% covered in leftover material or entirely full.

2.3.7 Contamination

In order to limit the contamination through the air and direct contact, I implemented several precaution steps.
Beforehand, the equipment, surfaces and hands were thoroughly cleaned and a lab coat (100% cotton, red) was worn at all times. I extracted fibres from the lab coat to use as reference material. The dissection and filtration steps were conducted in a laminar air-flow cabinet to limit air-borne contamination. The diet analysis was

performed under a stereomicroscope wrapped in a plastic cover (see Fig. 4). The plastic cover had openings for the hands to enter on both sides. This cover reduced the air flow under the microscope while the content of the GIT was emptied and inspected.
After the samples were filtered, the filters were transferred to a petri dish and the lid was closed. I sealed the dried samples with Parafilm after approx. two days.
I ran three blanks (5ml Milli-Q water + 10ml enzyme solution) per batch of samples. The blanks were treated in the same way as the samples to ensure representativeness. Additionally, I placed control dishes next to the samples. One dish was placed in the flow cabinet, the second one under the covered microscope. Both controls were checked for air-borne contamination after the batch of sample was processed.

Figur*e 4.* The setup for the diet analysis composing of a stereomicroscope wrapped in a plastic cover. The cover provided one opening on each side for the hands and the sample to enter. For the diet analysis, the sample was placed under the microscope. The lid of the sample was only removed while the sample was in the plastic cover. In order to monitor air-borne contamination in the plastic cover, an open petri dish filled with water was placed next to the sample during the analysis.

2.4 Data analysis

The datasets were prepared and to some extent analysed in Microsoft® Excel (Version 16.25). Further analysis was performed by the program RStudio (Version 1.0.153).

2.4.1 Condition and size

The size range of fish was explored for normal distribution (Shapiro-Wilk test). The data set was not normally distributed and attempts to transform the data set in order to achieve normal distribution were unsuccessful. Thus, I performed a Wilcoxon signed-rank test to compare the size range of the fish samples from the two regions of interest (coast and offshore) in both species. Furthermore, I applied a linear regression to investigate the correlation between length and weight of the fish. This correlation visualised the condition of the fish. The residuals of the linear model from each species were tested for differences by means of the Wilcoxon signed-rank test. I used the relationship between the variables 'fish length [mm]' and 'fish weight [g]', as well as 'fish weight [g]' and 'GIT weight [g]' to test for differences in the body condition. The data were log-transformed in order to express a linear distribution.

2.4.2 Diet analysis

Diet composition

During the examination of the GIT, I collected presence/absence as well as count data for the diet. Due to the late state of digestion or small size but numerous amounts of some of the prey items (e.g. skeletal parts of echinoderms) the count data were excluded from further analysis. Thus, the analysis on the diet composition was connected on presence/absence data. The collected data were sorted into prey groups, classified as follows: fish (*Pisces*), crabs (*Brachyura*), shrimps (*Dendrobranchiata* and *Caridea*), other crustaceans[1], bivalves (*Bivalvia*), gastropods (*Gastropoda*), other molluscs[2], polychaetes (*Polychaeta*), echinoderms (*Echinodermata*), nematodes (*Nematoda*), algae[3], others[4] and unidentified prey[5].
The previously mentioned prey groups were visualised in stacked bar plots comparing the diet between species and regions.

Non-metric multidimensional scaling (NMDS)

In order to properly analyse the dissimilarities between diet composition of different regions, I performed a two-dimensional non-metric multidimensional scaling

1 This group included all prey items from the Subphylum *Crustacea* that did not belong to the previously mentioned groups *Brachyura*, *Caridea* and *Dendrobranchiata* or could not be classified with certainty.

2 This group contained all prey items from the Phylum *Mollusca* that did not belong to the previously mentioned groups *Bivalvia* and *Gastropoda* or could not be classified with certainty.

3 This group included all plant-like structures that were part of the diet content.

4 Rare species from different groups than the ones previously mentioned were classified as 'others'.

5 Heavily digested and other unidentified diet content were listed in this group.

(NMDS)(Kruskal, 1964). NMDS computes a two-dimensional graph (or three-dimensional) out of a similarity matrix of multidimensional data. I used the *metaMDS* function on the presence/absence data set of the diet composition. The method visualises group differences in an ordination plot. For a clearer outcome, I excluded the prey groups 'others' and 'unidentified prey' from the data frame.
Additionally I included 'fish length' to the ordination with *envfit()* and *ordisurf()* to visualise the effect of the fish size on the diet composition.
Subsequently, I tested the dissimilarities of prey communities between the two regions for both species. Therefore, I applied a Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices with the *adonis* function (Anderson, 2001). 'fish length' and 'haul location' were included as co-variates. In addition, I explored the multivariate homogeneity of group dispersions with the *betadisper* function. This function tests whether the variances of the samples within each group are different between the groups. A significant output confirms the differences between the variances of the groups. The analysis was performed using the *vegan* package in R (Oksanen et al., 2013).

Similarity percentages
I performed an analysis of similarity percentages (SIMPER) (Clarke, 1993) to calculate the contribution of each prey group to the observed patterns shown in the NMDS ordination. It gives information about the influence of each prey group to the dietary differences between fish from different regions.

2.4.3 Plastic analysis

Pilot study
To compare contamination risk between the two methods, I focused on the contamination found in the three blanks per batch. The contamination from the controls was not taken into account, since direct air-borne contamination did not affect this part of the study design. Hence, I evaluated the contamination in the blank samples and compared the particle count between the two methods for both species. The number of particles in total was too low to apply statistical tests.
The samples used for the pilot study were included in the analysis of the main study.

Particle concentration
I calculated the amount of plastic and non-plastic particles (%) for both species and regions. The range of colours of the plastic and non-plastic particles were visualised in R. In addition, I calculated the percentage of occurrence for each colour. The differences in particle lengths were explored by means of a Student t-test on the log-

transformed data set. I compared the plastic particle lengths between species and the dab data between regions. For the comparison of whiting data between regions, applying a square-root-transformation achieved the best results of a normal distribution.

Link between prey and plastic

I investigated the connection between the diet composition and plastic ingestion. For this purpose, I counted the number of particles found together with a certain prey. For each prey group, I calculated the percentage of samples that contained anthropogenic particles as well as the prey, divided by the sum of inspected GIT samples. This analysis was performed on each species for both plastic and anthropogenic non-plastic polymers.

3 Results

3.1 Fish size

The size range (body length [mm]) of the dab samples differed greatly between the two regions (W=3240.5, p<0.0001) (Fig. 5A). The data set of the coast samples contained plenty of small specimens (min=81.0, max=250.0, med=130.0) compared to the captured conspecifics from offshore (min=114.0, max=256.0, med=170.5). Whiting samples from the coast region showed a significantly wider distribution in size compared to the offshore region (W=9551, p<0.0001) (Fig. 5B). The offshore individuals were very similar in size (min=116.0, max=194.0, med=141.5), whereas the body lengths of the conspecifics from the coast ranged much wider (min=76.0, max=230.0, med=169.0).

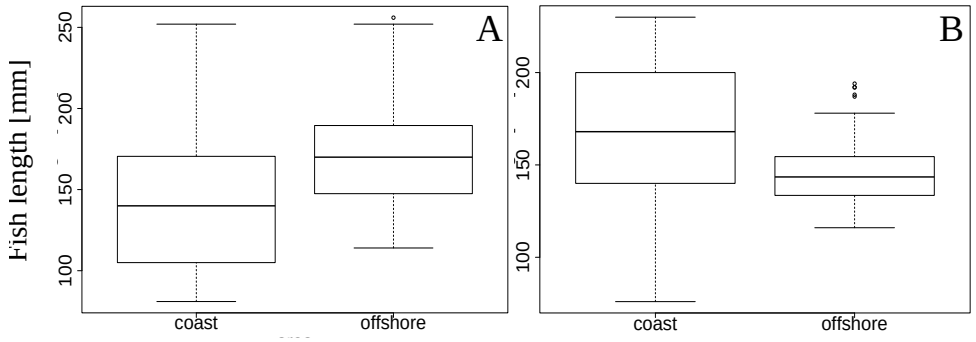


Figure 5. The graphs exhibit the size range of dab (A) and whiting samples (B). The fish length [mm] was compared between the two regions, coast (dab: N=125, whiting: N=153) and offshore (dab: N=80, whiting: N=79).

3.2 Condition

3.2.1 Condition ratio

As an indicator of the condition of the fish, I calculated two condition ratios as a correlation of (1) body length and body weight and (2) body weight and GIT weight. The comparison of the residuals from the linear regressions showed that the condition of fish from the coast and offshore region did not differ, neither in dabs nor in whitings (see appendix, Fig. 15 to 18).

3.2.2 Gut fullness as condition factor

According to the amount of diet content, fish from both species and regions seemed to be in a good condition. The GIT of dabs contained at least 50% food (gut fullness measure 3, 4 and 5 summed up) in 77.8% of coast and 70.0% of the offshore samples. In whitings, 69.3% of the coast and 61.5% of the offshore samples revealed an at least half-filled GIT.
In the dab samples, the gut fullness did not differ between individuals from the coast and offshore region ($t(5)=1.87$, $p=0.12$). In the whiting samples on the other hand, individuals from the coast included significantly more empty guts ($t(5)=3.94$, $p=0.01$).

3.3 Diet analysis

3.3.1 Abundance of prey groups

Overall, the diet of the collected dab samples was dominated by crustaceans, bivalves, polychaetes, echinoderms and algae (see Fig. 6). However, the diet of the offshore samples was dominated by echinoderms (41.7%). Whereas in the coast samples, only 19.4% of the diet contained echinoderms. Bivalves were conspicuously more present in coast (23.0%) than in offshore (9.6%) samples.

Table 3. *Counts (N) and numerical percentages (%) of prey types composing the diet of dab samples from the coast (N=125) and offshore (N=80) region in Skagerrak.*

	fish	other crustaceans	crabs	shrimps	other molluscs	bivalves	gastropods	polychaetes	echinoderms	nematodes	algae	others	unidentified
coast [N]	1	23	4	17	1	65	10	21	55	3	35	1	47
offshore [N]	2	15	4	1	1	15	4	19	65	0	11	2	17
sum [N]	3	38	8	18	2	80	14	40	120	3	46	3	64
coast [%]	0.4	8.1	1.4	6.0	0.4	23	3.5	7.4	19.4	1.1	12.4	0.4	16.6
offshore [%]	1.3	9.6	2.6	0.6	0.6	9.6	2.6	12.2	41.7	0	7.1	1.3	10.9
sum [%]	0.7	8.7	1.8	4.1	0.5	18.2	3.2	9.1	27.3	0.7	10.5	0.7	14.6

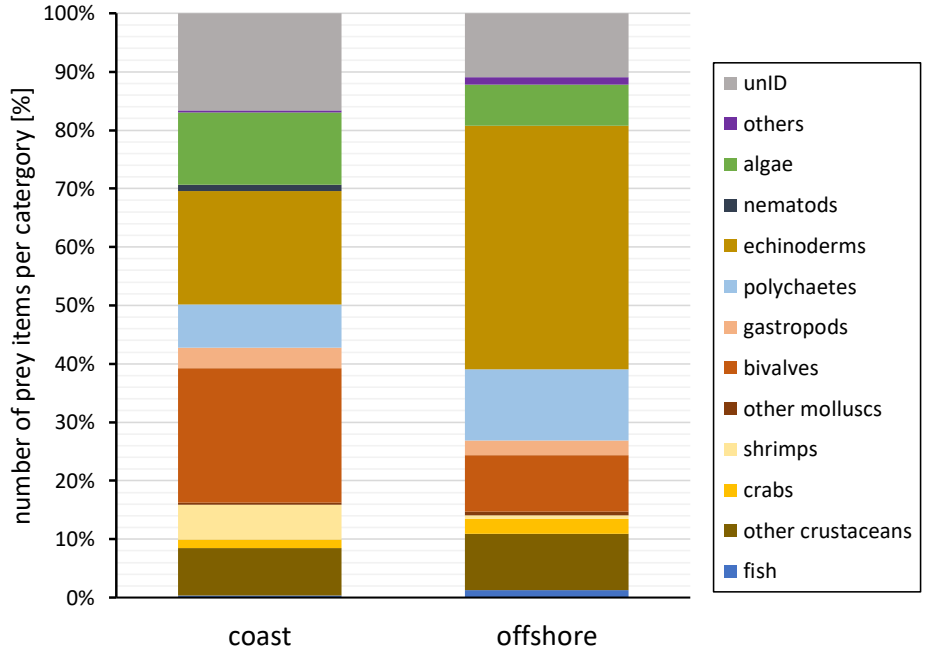


Figure 6. The bars represent the diet composition of dab samples from the coastal (N=125) and offshore (N=80) Skagerrak. The number of prey categories is shown as percentages of the pooled diet data and was calculated from a presence/absence analysis. 16.6% of the diet content from the coast samples and 10.9% from the offshore samples could not be identified (unID).

The diet composition of the whiting samples differed greatly in the two regions (see Fig. 7). While 34.5% of the coastal samples contained fish in the GITs, only 1.8%

of the whitings from the offshore ingested fish. Instead, the offshore samples were dominated by nematodes (21.5%) and polychaetes (19.0%). Crustaceans were represented in the diet of fish from both the coast (33.7%) and the offshore (27.6%) region. Within this prey group, shrimp were more abundant in the diet of coast (23.8%) than in offshore (11.0%) samples.

Tabell 4. *Counts and percentages of prey types composing the diet content of whiting samples from the coast (N=153) and offshore (N=79) region in Skagerrak.*

	fish	other crustaceans	crabs	shrimps	other molluscs	bivalves	gastropods	polychaetes	echinoderms	nematodes	algae	others	unidentified
coast [N]	87	24	1	60	0	5	0	21	0	3	2	0	49
offshore [N]	3	26	1	18	1	1	2	31	1	35	1	9	34
sum	90	50	2	78	1	6	2	52	1	38	3	9	83
coast [%]	34.5	9.5	0.4	23.8	0.0	2.0	0.0	8.3	0.0	1.2	0.8	0.0	19.4
offshore [%]	1.8	16.0	0.6	11.0	0.6	0.6	1.2	19.0	0.6	21.5	0.6	5.5	20.9
sum	21.7	12.0	0.5	18.8	0.2	1.4	0.5	12.5	0.2	9.2	0.7	2.2	20.0

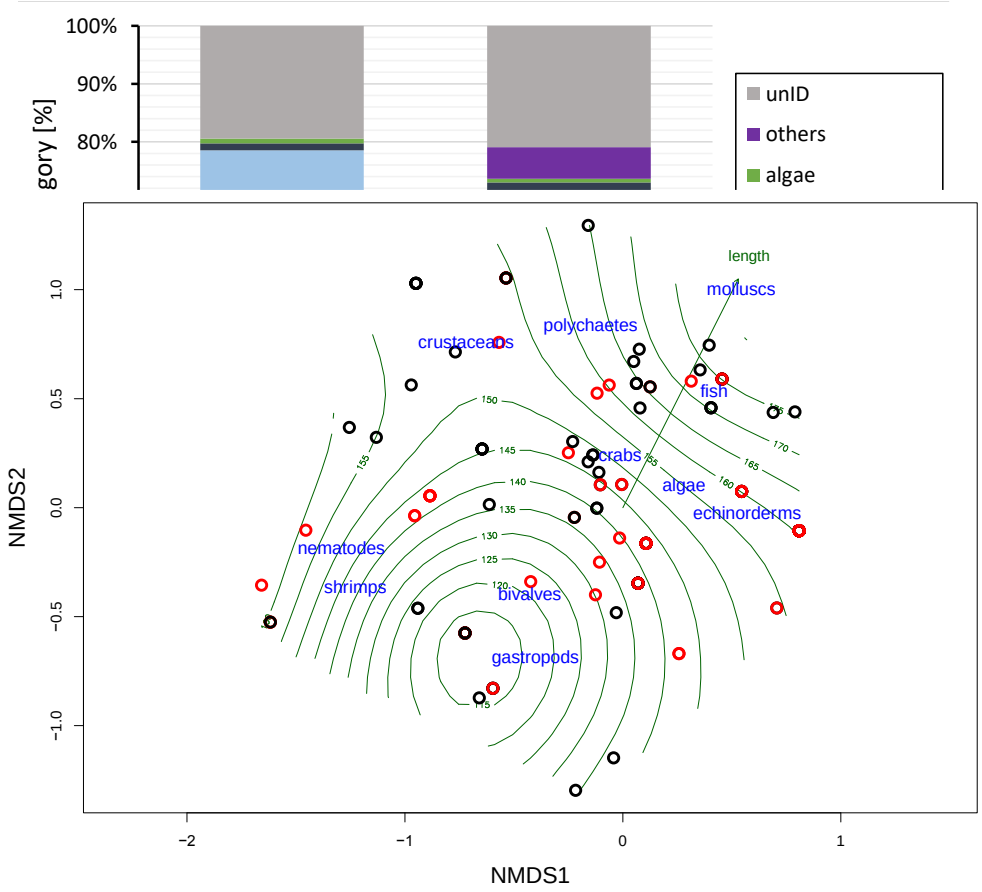


Figure 7. The bars represent the diet composition of whiting samples from the coastal (N=153) and offshore (N=79) Skagerrak. The amount of prey categories is shown as percentages of the pooled diet data and was calculated from a presence/absence analysis. 19.4% of the diet content from the coast samples and 20.9% from the offshore samples could not be identified (unID).

3.3.2 Diet composition

Dab

Both the coastal samples (black dots) and the offshore samples (red dots) are widely distributed across the ordination graph. The graph does not show a clear pattern of dissimilarities between the dab samples from the coast and offshore.

Figure 8. Two-dimensional non-metric multidimensional scaling plot of the diet composition in dab samples. The ordination is based on presence/absence data from samples collected from the coastal (black symbol) and offshore Skagerrak (red symbol). The graph shows the dissimilarities in diet

composition between samples from the two regions, where each dot represents an individual sample. The further apart in the plot, the more differs the diet composition of two samples. The different prey groups are listed in blue. In addition, the green surfplot represents the distribution of the body lengths in the fish samples. The arrow shows the increase in body length. The surfplot indicates the influence of the body length on the diet composition of the samples (Stress=0.11).

As implied by the graph (Fig. 8), the diet composition of the dab samples did not differ between the coast and the offshore region (ADONIS: R_2=0.002, p=0.521). However, the diet varied significantly between the various haul locations (ADONIS: R_2=0.391, p<0.001) and between fish with different body lengths (ADONIS: R_2=0.083, p<0.001). Since I did not find regional diet differences, the test for interactions between the region and the two other variables did not fall into account.

Table 5. *Permutational Multivariate Analysis of Variance using distance matrices on the effect of region, fish length and haul location on the diet composition of the dab samples. The effect of the region was additionally tested with fish length and haul location as co-variates. Terms were added sequentially.*

Predictor	Sum of squares	F-Model	R_2	p
region	0.1	0.79	0.002	0.521
fish length	3.46	26.81	0.083	<0.001
haul location	16.3	18.05	0.391	<0.001
region x fish length	0.26	1.98	0.006	0.133
region x haul location	1.03	1.33	0.025	0.155
region x fish length x haul location	1.89	1.14	0.045	0.282

According to the SIMPER analysis, bivalves, echinoderms, algae, other crustaceans and polychaetes represented the most influential prey groups in the dab samples. These five prey groups contributed 80.96% to the overall dissimilarity between coast and offshore samples. *Bivalvia* and *Echinodermata* as the most influential prey, contributed 21.22%, respectively 19.96% to the dissimilarity.

Whiting

The ordination shows dissimilarities in the prey community of whitings from offshore (red symbol) and coastal (black symbol) Skagerrak. Offshore samples seem to be located further right and coastal samples left in the plot (see Fig. 9). This suggests a dissimilarity pattern in diet composition between the samples of the two regions.

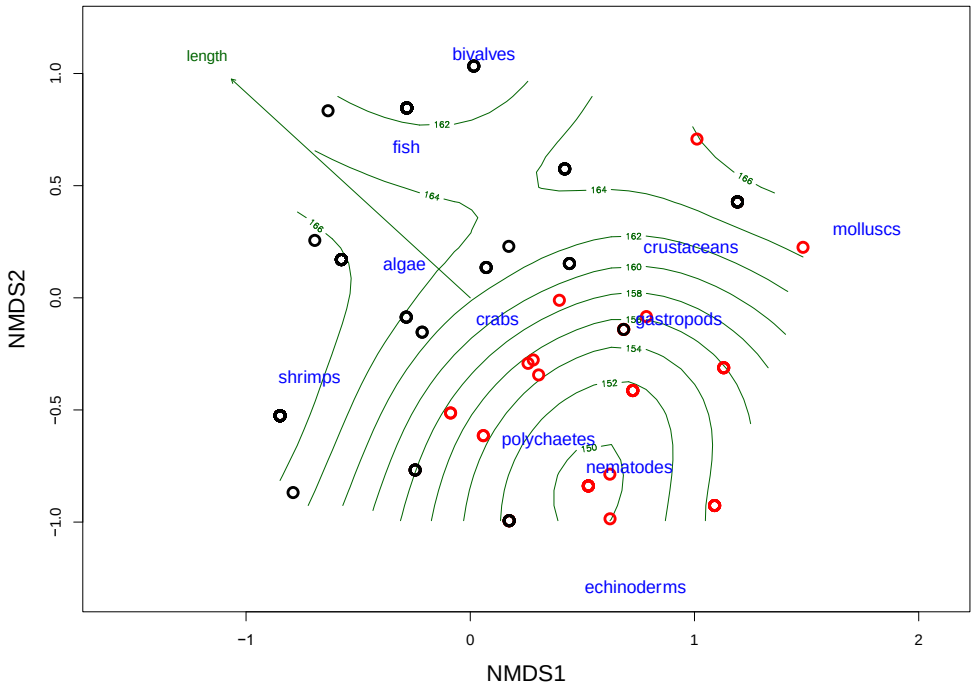


Figure 9. Two-dimensional non-metric multidimensional scaling plot of the prey community in the whiting samples (Stress=0.05). The ordination is based on presence/absence data from samples collected from the coastal (black symbol) and offshore Skagerrak (red symbol). The graph presents the dissimilarities in diet composition between samples from the two regions, where each dot represents an individual whiting sample. The further apart in the plot, the more differs the diet composition of two samples. In addition, the green surfplot represents the distribution of the body lengths in the fish samples. The arrow shows the increase in body length. The surfplot indicates the influence of the body length on the diet composition of the samples.

The diet composition between the whiting samples from the coastal and offshore region showed significant differences (ADONIS: R^2=0.203, p<0.001). However, in interaction with fish length the region did not affect the diet composition anymore (see table 6: region x fish length). Fish length had a slightly significant influence on the diet composition (ADONIS: R^2=0.010, p=0.047), which might have caused the dissimilarities between the two regions. The interaction with haul location resulted in NA values due to an unidentified technical problem. However, the interaction between region, fish length and haul location was found to be significant (ADONIS: R^2=0.053, p=0.008).

Table 6. *Permutational Multivariate Analysis of Variance using distance matrices of the effect the region and the fish length on the diet composition of the whiting samples.*

Predictor	Sum of squares	F-Model	R_2	p
region	11.92	54.69	0.203	<0.001
fish length	0.59	2.69	0.010	0.047
haul location	5.68	3.72	0.097	<0.001
region x fish length	0.08	0.38	0.001	0.721
region x haul location	NA	NA	NA	NA
region x fish length x haul location	3.08	2.10	0.053	0.008

The dissimilarities in species composition between coast and offshore whiting samples were caused by certain prey groups. According to the SIMPER analysis, the most influential prey groups were fish, nematodes, polychaetes and shrimps. Combined, these four groups caused 79.39% of the dietary differences between coast and offshore samples, whereas fish (24.29%) and nematodes (19.03%) contributed the most.

3.4 Plastic analysis

3.4.1 Pilot study

The blanks from the dab and whiting samples processed with the 'open' method were found to be empty. However, the blanks from the samples using the 'closed' method contained several non-plastic fibres. Three fibres, one in each sample, were found in the whiting blanks and another two cotton fibres of the lab coat were spotted in two of the whiting samples. The blanks of the dab samples contained four non-plastic fibres.

3.4.2 Contamination

I found 90 fibres in the samples, including both plastics and anthropogenic non-plastics. These fibres matched with the fibres recovered from the controls, blank samples or the lab coat fibres in colour, width and surface structure. The width was measured from the photograph by means of the application software 'Infinity analyze 6.1'. These fibres were extracted from 72 different samples. Thus, 16.51% of the samples contained contamination (for details see table 7).

Table 7. *Plastic and non-plastic contamination recovered from 72 samples according to the reference material found in the blank samples.*

	fibres [N]	contaminated fish [N]	contamination [%]	fish [N]
dab	51	39	19.12	204
coast	42	32	25.81	124
offshore	9	7	8.75	80
whiting	39	33	14.22	232
coast	33	29	18.95	153
offshore	6	4	5.06	79
sum	90	72	16.51	436

Filter load

Due to hard structures such as shells and skeletal parts that where not digested by the enzyme, leftover material ended up on the filter. This was mainly observed in dab samples. Category 3 contained 20.1% of the filters and even 4.41% were categorised to be in fully loaded (category 4). The rest of the dab samples had a low filter load, with 42.65% of the samples in category 2 and 32.84% in category 1.

The filters of the whiting samples contained only low amounts of leftovers. 95.69% of the samples were listed in category 1 (52.16%) and 2 (43.53%), while 4.31% were categorised with filter load 3. None of the whiting samples were fully loaded (category 4).

3.4.3 Visual identification

After accounting for the contamination, I resulted with a total of 60 plastic particles from the inspected samples, 36 particles in the dab and 24 in the whiting samples (see Fig. 10). Apart from one fragment, all other particles were identified as fibres. By calculating the percentage of plastic ingestion, I found that 10.8% of the sampled whitings contained at least one plastic particle. Offshore samples contained more plastic (11.8%) than samples from the coast (9.8%). I found 15 plastic particles in 14 of the examined coast samples and 9 plastic particles in 9 offshore samples. 17.6% of the processed dab samples contained plastic particles. The plastic ingestion rate was 18.5% in the coast samples and 16.3% in the offshore samples. This resulted from 23 plastic particles found in 19 coast samples, respectively 13 plastic particles in 12 offshore samples.

Additionally, I recovered 117 anthropogenic non-plastic particles from the total number of samples. Thus, 26.8% of the fish ingest anthropogenic non-plastic particles. Included were all natural, semi-synthetic and synthetic non-plastic materials mainly originating from the textile industry, such as cotton and rayon (recovered cellulose). Out of 117 particles, only 2 particles were classified as fragments, the rest were fibres. Ingestion of anthropogenic, non-plastic debris

accounted for 30.4% in the dab and 23.7% in the whiting samples. In dab samples, I found 35 particles in 28 individuals from the coast (29.0%, N=124) and 26 particles in 20 individuals from offshore (32.5%, N=80). 35 whiting samples from the coast contained 41 particles (25.5%, N=153). From the offshore samples, 12 individuals were found to contain 16 particles (20.3%, N=79).
The plastic ingestion rate between coastal and offshore samples differed slightly but not significantly in both species. As the common dab ingested more plastic and non-plastic particles than the whiting, there seemed to exist an inter-specific pattern.

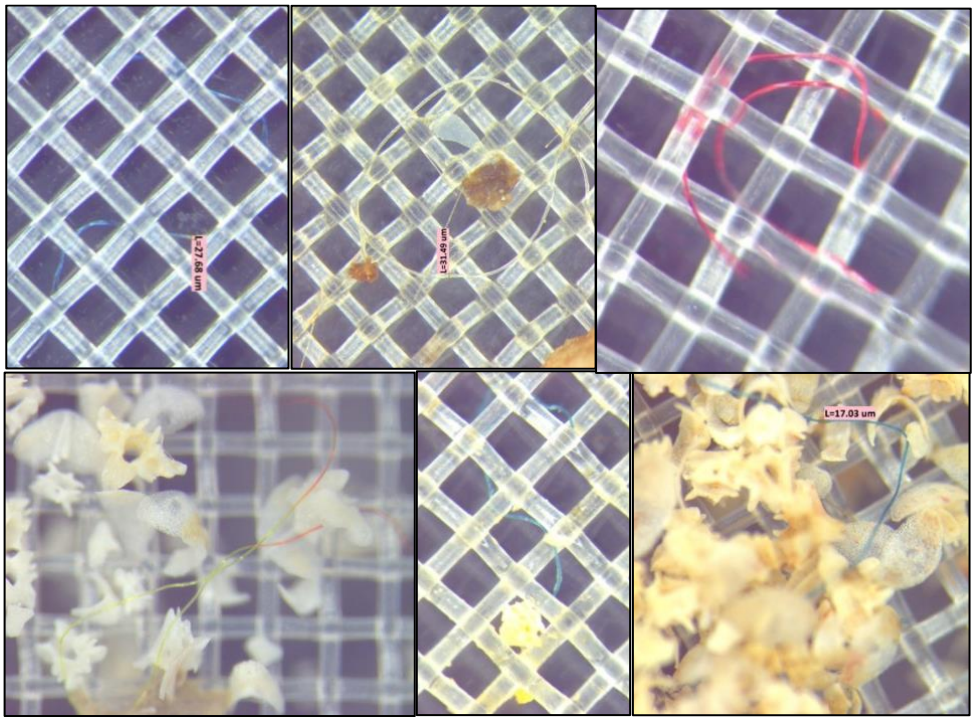


Figure 10. Example pictures of recovered plastic particles from the samples of the dab and the whiting. The mesh size of the filter (300µm) indicates the size range of the particles.

Colour

The identified plastic particles showed different colours (see table 9). However, translucent particles were dominating the samples in both species and regions (65.0%, N=39).

Table 8. *Number of recovered plastic particles per colour for both species and regions.*

	black	blue	heterogenous	red	translucent	turquoise	yellow	sum
dab	5	4	2	4	20	1	0	36
coast	4	3	1	3	12	0	0	23
offshore	1	1	1	1	8	1	0	13
whiting	1	3	0	0	19	0	1	24
coast	1	3	0	0	10	0	1	15
offshore	0	0	0	0	9	0	0	9
sum	6	7	2	4	39	1	1	60

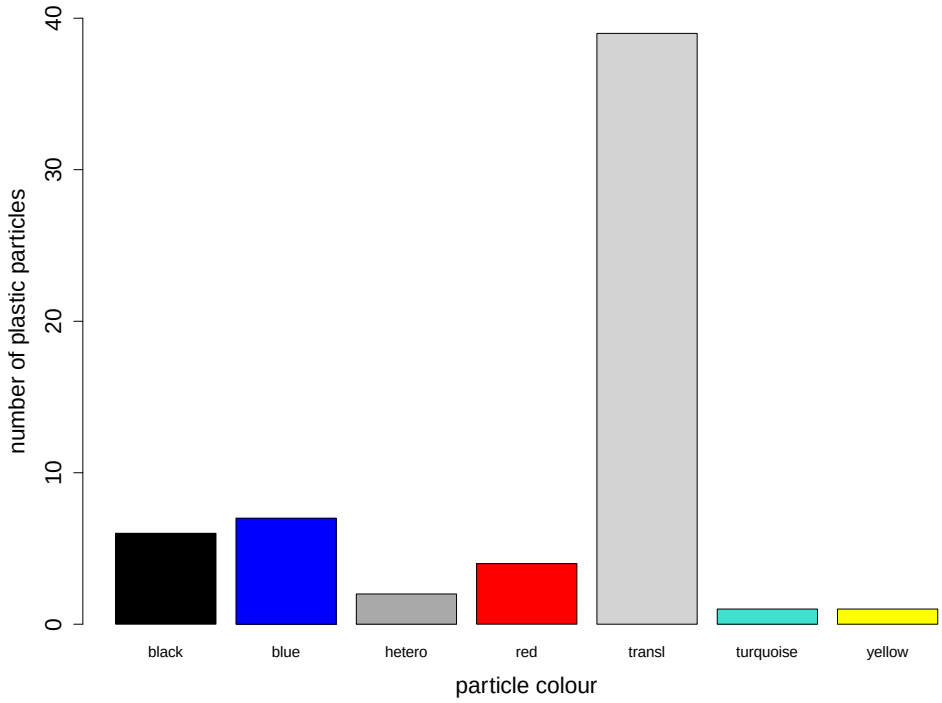


Figure 11. The colour spectrum of the recovered plastic particles from both species (N=60). The bars represent the different colours of the plastics (hetero = heterogenous, tranls = translucent).

The anthropogenic, non-plastic particles presented an even wider colour range than the plastic particles. Black particles occurred most frequently (27.35%, N=32). Additionally, blue (9.40%), red (19.0%), translucent (16.24%) and heterogenous particles (11.11%) were common findings (see table 10).

Table 9. *Number of recovered non-plastic particles per colour for both species and regions.*

	black	blue	green	heterogenous	red	translucent	turquoise
dab	18	4	2	8	11	11	2
coast	12	3	1	4	5	6	2
offshore	6	1	1	4	6	5	0
whiting	14	7	0	5	11	8	0
coast	9	7	0	3	6	4	0

offshore	5	0	0	2	5	4	0
sum	32	11	2	13	22	19	2

	violet	white	yellow	sum
dab	3	2	1	62
coast	1	1	1	36
offshore	2	1	0	26
whiting	5	1	4	55
coast	5	1	4	39
offshore	0	0	0	16
sum	8	3	5	117

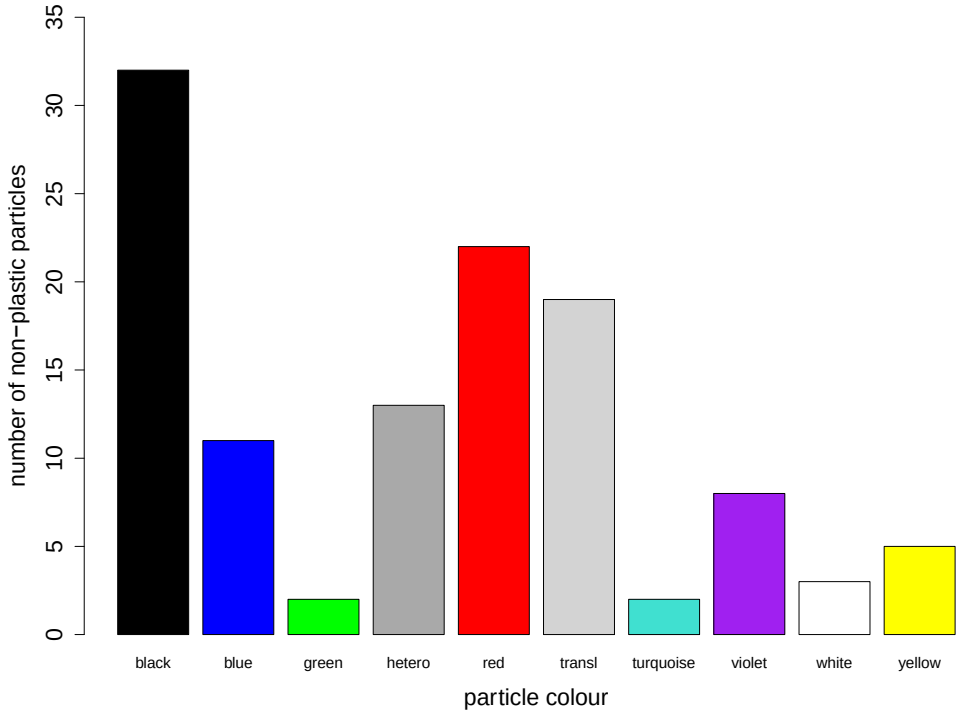


Figure 12. The colour spectrum of the recovered anthropogenic non-plastic particles (N=117). The bars represent the different colours of the non-plastics (hetero = heterogenous, tranls = translucent).

Length

The lengths of the plastic particles were ranging between 240µm and 25mm and differed significantly between the two species (t(56.17)=2.84, p=0.006). Dab samples contained longer particles than whiting samples (see Fig. 13). The particle lengths between regions did not differ, neither in the dab (t(32.23)=1.03, p=0.31) nor in the whiting samples (t(15.63)=-1.06, p=0.304).

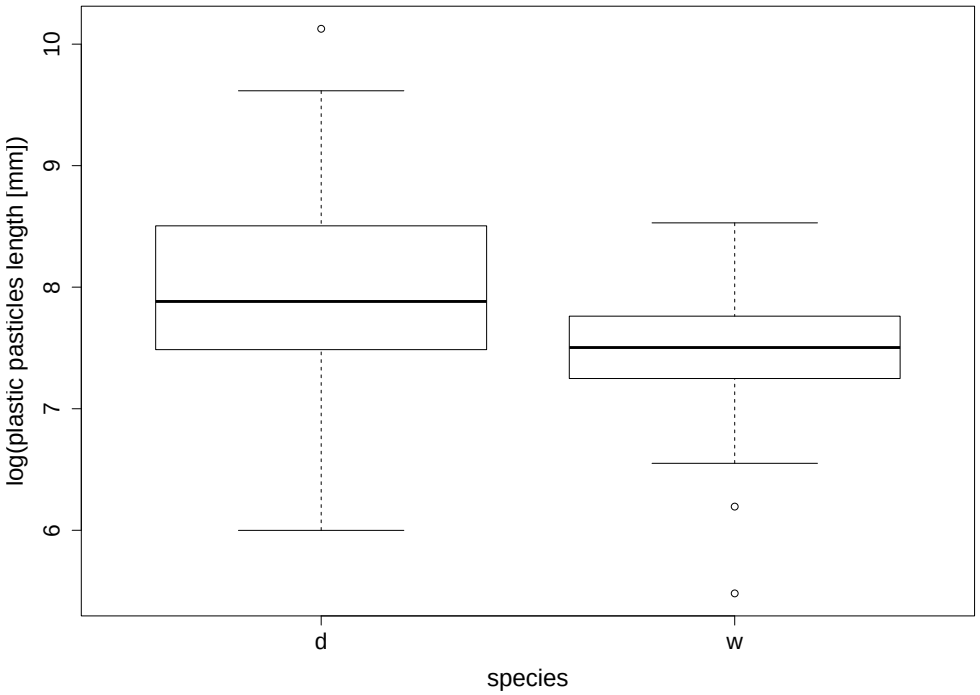
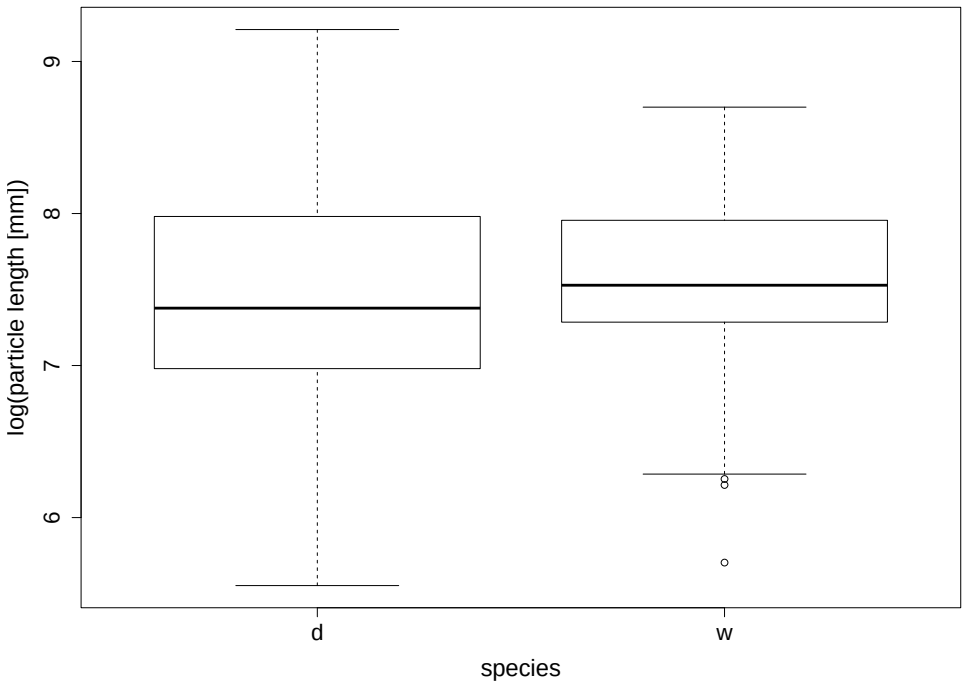


Figure 13. The size range of the plastic particles collected from the dab (d: med = 2650 mm, min = 403 mm, max = 25000 mm) and the whiting (w: med = 1815 mm, min = 240 mm, max = 5060 mm) samples.

Anthropogenic non-plastic particles did not differ in length between species (t(111.26)=-1.01, p=0.317). The size ranged between 258µm and 10mm (see Fig. 14). The particle lengths between regions were not significantly different in both dab (t(45.06)=-0.70, p=0.487) and whiting samples (t(28.0)=0.19, p=0.855).



Figur*e 14*. The size range of the anthropogenic non-plastic particles collected from the dab (d: med = 1600 mm, min = 258 mm, max = 10000 mm) and the whiting (w: med = 1860 mm, min = 300 mm, max = 6000 mm) samples.

3.4.4 Link between prey and plastic ingestion

The ingestion rate of anthropogenic particles might be influenced by the feeding strategy or the prey organisms of the examined fish. I analysed the data for a connection between each prey group and the ingested particles. The percentage of anthropogenic polymers occurring together with each prey group was calculated. The resulting percentage showed the proportion of samples that contained the investigated prey groups as well as anthropogenic polymers. The results indicated how often anthropogenic polymers were ingested in combination with a specific prey type.

In the GITs of the dab samples, some of the prey groups were found to occur 30 to 40% of the time together with anthropogenic particles. For instance, in 39.5% of the GITs that contained echinoderms, I also recovered anthropogenic particles. Similar proportions of occurrence were found in bivalves (38.0%), algae (37.8%), polychaetes (30.0%) and other crustaceans (36.8%). The proportions of the other

prey groups occurring together with ingested particles in the samples, were similarly high. According to these results, ingestion of anthropogenic particles did not show a clear link with a certain prey group.
In the whiting samples, some of the abundant prey groups were found more often together with anthropogenic particles. Hence, in 32.2 % of the samples that contained fish (N=90), I also found anthropogenic particles. The group 'other crustaceans' was recovered 26.0% of the time together with debris. On the other hand, in other abundant prey only 20.8% (shrimps, N=77) and 17.3% (polychaetes, N=52) of the samples included anthropogenic particles.
The prey groups represented in low numbers as well as the unidentified prey were neglected. The interpretation of these results would not be representative due to the small sample size or lacking information of the data.

All in all, a clear pattern could not be extracted from the given data. If the ingestion of anthropogenic material was linked to a certain prey groups, the results did represent it. However, in whitings ingestion of anthropogenic non-plastics might have been more prevalent in combination with fish.

Table 10. *Connection between ingested anthropogenic particles and the prey composition of the whiting and the dab samples. For this analysis, the data of the ingested plastic and anthropogenic non-plastic particles were pooled. prey = number of samples containing the prey group; prey+p = number of samples that contained the prey group together with anthropogenic particles; P% = percentage of samples containing both particles and the prey group divided by number of samples for both the whiting (N=232) and the dab samples (N=204).*

	fish	other crustaceans	crabs	shrimps	other molluscs	bivalves	gastropods	polychaetes	echinoderms	nematodes	algae	others	unidentified
Dab													
prey [N]	3	38	8	18	2	79	14	40	119	3	45	3	63
prey+p [N]	1	14	0	4	0	30	6	12	47	1	17	2	21
P [%]	33.3	36.8	0	22.2	0	38.0	42.9	30.0	39.5	33.3	37.8	66.7	33.3
Whiting													
prey [N]	90	50	2	77	1	6	2	52	1	38	3	9	83
prey+p [N]	29	13	0	16	1	3	1	9	0	7	0	1	27
P [%]	32.2	26.0	0	20.8	100	50.0	50.0	17.3	0	18.4	0	11.1	32.5

4 Discussion

Regarding the aim of the study, I successfully evaluated the amount of ingested plastic particles, documented the diet composition and analysed the body condition and size of the two study species. Plastic particles were expected to occur more frequently in combination with ground-living prey. Due to the high proportion of benthic prey in the diet composition of the common dab, the results would support this hypothesis. However, taking the number of occurrences of the prey groups into account, plastic particles might have been found often in combination with benthic prey due to its high occurrence rate. I also expected to encounter more plastic particles in individuals caught in coastal regions than offshore, as the main source of pollution is closer. In the common dab, a slightly higher percentage of individuals from the coast ingested particles than offshore individuals. This would support my hypothesis. In the whiting on the other hand, plastic ingestion was found to be slightly higher in individuals from the offshore region. Since the differences appeared to be rather inconspicuous, drawing a conclusion requires more significant results. Finally, I aimed to find an interaction between the feeding behaviour and the ingested plastic particles. With the giving results, this analysis did not reveal any significant interactions.

4.1 Condition and size

As presented, the body lengths of the samples of the common dab as well as the whiting differed significantly between the two regions. These differences can to a large extend be explained by seasonality, respectively the reproductive cycle. Based on studies by Van der Land (1991) and Bolle et al. (1994) on the common dab, the high abundance in small individuals in my coast samples suggests that the main spawning events take place between January and April in the coastal hatching grounds. This is followed by high densities of young dabs in fall. Reaching a certain size, dabs migrate to deeper regions. Here, high numbers of young adults occur

(Bolle et al. 1994). The pattern in size distribution of my dab samples between the regions agrees with this explanation.

In whitings, the body lengths from the coast showed a much wider size range compared to the offshore samples. Offshore whitings were very similar in size, showing a range between 11.6 and 19.4 cm. According to Cohen (1990), one-year old whitings range between 15 and 19 cm in length. After the first year, the young adults leave the shallow nursing grounds for the deeper offshore regions. Hamerlynck and Hostens (1993) confirmed this by reporting that most 0-group individuals monitored in coastal areas of south-west Netherland have left the nursing grounds by the year.

Apart from the seasonal effect on the species ecology, other environmental variables could have contributed to the differences in size ranges between the regions. The geographical distance between the haul locations might have led to varying food availability, predator abundance or water depth and temperature. For instance, water depth varied between the haul locations (see table 1 and 2), which indicates differing water temperatures. The water temperature is shown to influence egg hatching times and growth rate (Gerritsen et al., 2003; Henderson, 1998) and could hence have an impact on the observed differences in size range in both species.

Furthermore, fish depend on the availability of prey for their development and health. Thus, the growth and condition can strongly be affected. For instance, the wellbeing of the whiting population in the Bristol Channel was found to be constrained by the abundance of shrimps (*Crangon crangon*) (Henderson & Holmes, 1989). The feeding strategy of the dab on the other hand is very opportunistic. This might as well cause differences in body size, since the amount of energy intake highly depends on the type of consumed prey (Hinz et al., 2005). Thus, food availability influences the condition of dab populations.

According to the correlation between fish length and fish weight, the condition of the individuals from the coast and the offshore regions did not differ. The gut fullness, as an additional indicator of the condition, did not vary in the dab samples. In the whiting samples however, significantly more individuals revealed an empty gut compared to the dab. As nursery grounds, the coastal regions host high numbers of individuals during the breeding season. A high population density poses a certain pressure on the individual, such as competition for resources and prey availability (Hamerlynck & Hostens, 1993; Henderson & Holmes, 1989).

Finally, it is to mention that the sample size varied between the two regions. Despite the fact that I processed a representative number of samples from each species and region, the sample size from the coast doubled the number of offshore samples. A bigger data set is always better to draw conclusions and get closest to the real population measures.

4.2 Diet

The data were collected as presence/absence measure, which gives information about the frequency of occurrence. Using this measure, I missed out on quantitative information about the ingested prey. However, quantifying prey comes with difficulties. The proportion measures require precision and volumetric measures should be taken into consideration to account for the proportion of bulk and the size of the prey items. Additionally, some of the prey species, such as *Ophiuridea*, are extremely complicated to quantify due to fragmentation. Therefore, the simple presence/absence analysis represents a robust and effective measure (Baker et al., 2014). Even though, it is a simple approach, this analysis gives fast, representative and repeatable results of the diet composition (Buckland et al., 2017). For my purpose, the presence/absence measure was sufficient and achieved the expected results.

According to the NMDS analysis, the diet composition of the common dab did not vary between the two investigated regions. The dab is in general an opportunistic feeder. Thus, the prey availability in the two regions were supposedly similar. However, the diet of the offshore samples was dominated by echinoderms (41.9%), while conspecifics from the coast consumed a more diverse diet. Echinoderms were shown to be a dominant part of the diet in the offshore and frequent in the coast samples. The literature confirms the preferred food choice of the dab for *Echinodermata*. Wennhage and Pihl (2002) found echinoderms, respectively *Ophiuridea*, to represent 67% of the diet in the common dab from the Swedish West Coast. The results of Hinz et al. (2005) confirm this. A study by Ottosson (2008) on the by-catch rate of non-commercial invertebrates off the Swedish west coast revealed that echinoderms account for 95% of the total bycatch together with molluscs and arthropods. The documented differences between coast and offshore diet samples imply that *Echinodermata* represent one of the most dominant groups in the benthic community in the Skagerrak. In offshore regions (41.9%) more than at the coast (19.4%). Apart from echinoderms, bivalves (23.0%) and algae (12.4%) were frequently represented in the coastal samples. A study by Rees et al. (1999) compared the benthic biodiversity of the coastline and offshore around the United Kingdom. Their findings suggest that sediment coarseness and current speed contribute most to the differences in benthic communities between the investigated regions. The sediment size was positively correlated with the number of taxa. Hence, bigger sediment particles provide more structure and surface area for organisms to attach to. The water current speed on the other hand was found to be negatively correlated the number of taxa. A similar but weaker correlation occurred for the winter temperature. Furthermore, the study reflects that high densities of common species such as *Ophiura* spp. account for lower numbers of individuals.

Thus, a dominant species might act negative on the biodiversity in the area. The findings in the study might help to explain the observed differences in my data. Environmental factors such as sediment size, current speed, temperature and species interactions seem to play a crucial part in the interpretation of the differences in benthic communities.

The diet composition of the whiting between the coast and offshore region was significantly different. In combination with the body length of whitings however, the results were not significantly different anymore. This suggests, that the fish length accounted for the dissimilarities in the diet composition between the two regions. However, the interaction of region, haul location and fish length proved to be significant. Unfortunately, technical issues restricted the analysis of the interaction between region and location. Thus, it proofs difficult to draw conclusions from the given results. Nevertheless, Fish (34.5%) and crustaceans (33.7%) dominated the gut samples from the coast. Whereas the offshore samples were dominated by crustaceans (27.6%), nematodes (21.5%) and polychaetes (19.0%). Only 1.8% of the whitings from the offshore region were piscivorous. This number does not correspond with the literature. The diet of the whiting was reported to almost exclusively contain fish depending on the age. Reaching a certain size, the whiting diet might almost exclusively consist of fish (Hislop et al., 1991). Even cannibalism is common in whiting and was suggested to be a survival strategy. Bromley et al. (1997) proposed that additional spawning events of a female provide food resources for the early offspring. Thus, the survival and recruitment of the next generation is ensured. This might be one of the reasons for the high consumption rates of fish in the coastal compared to the offshore samples. Only the coastal region as a nursery ground provides large numbers of 0-group fish that can act as a food source for small or medium-sized whitings (Hislop et al., 1991). Since the main breeding events occur between February and June (Bowers, 1954), 0-group whitings are extremely abundant in the summer and autumn months. This suggest that the differences in species composition are based on the geographical distance of the data as well as on seasonal fluctuations in the abundance of whitings. The coastal survey of my study was performed in September while the offshore survey took place in January. Most species show seasonal fluctuations in abundance driven by their reproduction cycle. This is likely to affect the predator species, in this case the whiting. It is to be added that even though the diet samples contained whiting prey, the major proportion of fish prey consisted of *Gobiidae* (unpublished data). However, goby populations follow a seasonal pattern as well, which coincides with me results. Thus, the common goby (*Pomatoschistus microps*) and the sand goby (*Pomatoschistus minutus*) were found to be most abundant during the late summer and autumn months in the northern Baltic Sea (Nellbring, 1985). In the Dutch Wadden Sea and coastal North Sea, the sand goby population was observed to be

very abundant during the autumn. In winter, the numbers decreased and only increased again during spring (Fonds, 1973).

A high abundance in small fish would explain the high proportion of piscivores whitings in the coastal samples taken in September compared to the offshore samples collected in January. Whether seasonality alone explains the observed patterns or only in combination with the geographical distance, is unclear. In order to exclude this potential influence factor, coastal and offshore sampling needs to be conducted at the same time.

The differences in abundance of other prey taxa might have been caused by the availability of small fish as a resource. In the offshore region, where small fish are not as abundant as in coastal regions, the whiting is expected to shift its focus towards other prey to cover the energy loss. Thus, other taxa such as nematodes and polychaetes become a more frequent prey. On the other hand, the previously mentioned influence of environmental factors such as sediment size and current speed could have contributed to the observed patterns (Rees et al., 1999).

A popular prey species in both fish is the common shrimp (*Crangon crangon*). In the North Sea, the major breeding event takes place between February and June (Siegel et al., 2008). This leads to a peak in abundance during summer and autumn. Despite up to 20t of shrimps consumed annually by whiting and cod (*Gadus morhua*) in the German Wadden Sea, it never accounted for more than one-fifth of the shrimp production every month (Jansen, 2002). This indicates that the Common Shrimp represents an essential part of the whiting and most likely as well the dab diet throughout the year.

Focusing on both the whiting and the dab, the diet composition could have been affected by the diel feeding patterns of the fish. Mergardt and Temming (1997) showed in a laboratory experiment that whitings preferred to feed during night hours. However, a study on several fish species conducted on fish from a Scottish lake achieved different results. Whitings seemed to feed only in the morning between 3:00 and 10:00. Dabs were found to contain fresh food throughout the day, but preferably fed during sunrise and sunset (S. J. Hall et al., 1995). A different study conducted by Rindorf (2003) found the feeding behaviour of whitings to be influenced by the diel migration patterns of their prey. In conclusion, the various hauls during the sampling might have caught fish that contained different prey due to the capture time.

Finally, the proportion of unidentified prey in the dab samples accounted for 14.6% of the gut content and in the whiting samples for 20.0%. In several samples, the diet was strongly digested (bulk) and hence impossible to identify with my means. A lower proportion of bulk might have influenced the outcome of this study. In order to reduce the number of unidentified prey items, more preparation and detailed knowledge on the phylogeny of species is required from the staff.

4.3 Plastics

As a result of the visual inspection of the fish samples, I found 60 plastic particles and additionally 117 anthropogenic non-plastic particles. This accounted for an ingestion rate of 13.8% for plastic and 26.8% for anthropogenic non-plastic particles. I found more plastic ingested by the common dab than by the whiting. The plastic particle length was significantly longer in the dab than the whiting samples. The length of the anthropogenic non-plastic particles did not differ. The category of anthropogenic non-plastic particles included natural materials such as cotton, linen and wool, as well as semi-synthetic polymers such as rayon. Rayon is a fibre made from modified cellulose. Thus, it is artificial but technically not plastic. However, several studies on plastics in the environment considered rayon as plastic debris due to its intense modification process (Comnea-Stancu et al., 2016; Hartmann et al., 2019; A. Lusher et al., 2013; Neves et al., 2015). A. Lusher et al. (2013) even highlighted the importance of classifying rayon as marine debris, as it is commonly found in studies on microplastics. In myy study, I did not categorise rayon as plastic. Since rayon and other semi-synthetic and natural polymer are important and frequently occurring pollutants in the marine environment, I included a separate category to the study addressing anthropogenic non-plastic polymers. Whether pollution of anthropogenic non-plastics such as natural and synthetic textile fibres pose a relatable threat to the marine environment as plastic pollution is yet to be investigated. However, Ladewig et al. (2015) believed natural fibres to be underestimated in the discussion on chemical pollution of aquatic environments. As natural fibres are widely expected to naturally degrade, its threat to the aquatic environment was neglected in scientific studies. However, natural fibres have the ability to adsorb chemical pollutants and thus might pose a threat the aquatic environment (Ladewig et al., 2015). Therefore, the effect of anthropogenic non-plastic materials to the marine environment requires more attention in future research on chemical pollution in the aquatic environment.

Plastic has been frequently reported in the marine environment. Relating to this study, several studies reported plastic pollution in Swedish waters along the west coast. Plastic particles were recovered from the water (Norén, 2007), sediment (Karlsson et al., 2018), bivalves (Gustafsson, 2016) as well as in fish (Karlsson et al., 2017). In addition, the polyethylene production facility at Stenungsund was found to be a major contributor of plastic pellets in the nearby waters and beaches (Karlsson et al., 2018). Thus, recovering plastic particles from the fish samples in this study was not entirely surprising.
Relating my results to the exiting literature revealed very contrasting information about plastic ingestion in fish. Some studies found shockingly high amounts of

ingested plastic. For instance, 68.0% of individuals of the brown trout (*Salmo trutta*) from the Swedish Westcoast (Karlsson et al., 2017) and 80.0% of the common sole (*Solea solea*) from the Adriatic Sea contained plastic particles (Pellini et al., 2018). Whereas other studies only found very few plastic in the digestive tract of the fish (Foekema et al., 2013; Hermsen et al., 2017; Rummel et al., 2016). As my study, those studies were conducted on the whiting and/or the common dab collected in the North Sea. Hermsen et al. (2017) recovered only two particles from one sprat (*Sprattus sprattus*) out of 400 fish samples (0.25%) from five different species including the common dab and the whiting. Foekema et al. (2013) found plastic particles in 2.6% of the inspected fish (N=1203) from seven different species including whiting. Rummel et al. (2016) reported 5.5% plastic ingestion in the examined fish (N=290) including the whiting and the common dab. However, ingestion rates of plastics are not consistent. Numerous other studies found varying amounts of plastic in fish all over the world ranging from 9.2% to 36.5% (Bellas et al., 2016; Boerger et al., 2010; Davison & Asch, 2011; Lenz et al., 2016; A. Lusher et al., 2013; Morgana et al., 2018; Nelms et al., 2018; Neves et al., 2015). Nevertheless, fish from various species are frequently found to be contaminated with plastic. If fish are susceptible to take up plastic particles, what are the drivers? Plastic can be ingested actively or passively. Studies on different biota suggested the study species to accidently taken up plastic. For instance, the crustacean *Nephrops norvegicus* was expected to ingest plastic unintentionally through food scavenging and sediment uptake (Murray & Cowie, 2011). This hypothesis is supported by a study on four species of deposit- and suspension-feeding sea cucumbers (*Holothuroidea*) (Graham & Thompson, 2009). Additionally, filter-feeding zooplankton and fish were shown to be susceptible to ingest microplastics from the water column (Boerger et al., 2010; Setälä et al., 2014). If the feeding strategy makes fish more susceptible to plastic ingestion, this might explain the inter-specific differences between the whiting and the dab in this study. Additionally, it might explain the variation in particle lengths that were observed between the species. The particle size might differ according to the habitats.

Indirect plastic ingestion can not only result from certain feeding strategies, but as well be transferred through the food chain. In this case, the predator ingested a prey that previously fed on plastic. The so-called biomagnification was observed in fur seals (*Arctocephalus* spp.) on Macquarie Island, that were supposed to accumulate plastics through their preferred prey, the pelagic fish *Electrona subaspera* (Eriksson & Burton, 2003). In my case, shrimps as a popular prey group of the common dab and the whiting could have been a primary consumer of plastic polymers. According to a study by Devriese et al. (2015) synthetic fibres were recovered from 63.0% of the brown shrimp (*Crangon crangon*) samples from the North Sea. If a shrimp had ingested plastic and one of the sampled fish in this study would have consumed the

shrimp, the fish is a secondary consumer. This scenario is likely to be one of the contributing sources of plastic contamination in the sampled fish.

Active ingestion of marine debris can be caused by misidentification. Several studies assumed plastic particles to be confused for prey items. For instance, Boerger et al. (2010) detected high numbers of plastic particles in the fish guts that show similarities in colour with plankton. A similar study was published by Ory et al. (2017), in which blue microplastics were misidentified by the amberstripe scad (*Decapterus muroadsi*) for their copepod prey. This is supported by a laboratory study on juvenile gobies, that tested the prey selection capability and confusion with microplastics (de Sá et al., 2015). In my study, translucent plastic fibres were dominant. In addition, black was the most prevalent colour in anthropogenic non-plastic polymers, followed by frequently occurring colours such as red and translucent. Translucent particles are likely to be confused with small prey organisms of the whiting and the common dab such as larvae, small crustaceans or zooplankton. A study by Choy and Drazen (2013) reported that predatory pelagic fish in the North Pacific predominantly ingested white and clear particles, which may be associated with the gelatinous prey organisms of these species. A similar mechanism might have occurred in the whiting, which ingested high amounts of fish and shrimp. If the plastic polymer recovered from my fish samples were indeed ingested intentionally or accidently is yet to be found out.

Nonetheless, plastic ingestion is likely to be linked to the feeding strategy of the consumer. As all abundant prey groups in the dab samples were frequently found together with anthropogenic particles and occur in benthic habitats, certain feeding guilds seem to be more susceptible to plastic ingestion. Morgana et al. (2018) recovered more plastic polymers from the demersal bigeye sculpin (*Triglops nybelini*) than from the pelagic polar cod (*Boreogadus saida*). The study concluded that the feeding strategy and habitat is likely to cause the differences in plastic ingestion between the two investigated species. Similar conclusions can be drawn from my data, as the ground-living common dab ingested more plastic polymers compared to the rather pelagic whiting. Drawing further conclusion about the interaction between the diet composition and plastic ingestion in the study species appears to be difficult with the given data. It requires a broad knowledge on the trophic interactions in the food web (Machovsky-Capuska et al., 2019). Apart from that, which role does plastic play in the food web dynamics? It is yet to be found out how plastic effects the trophic interactions and if it shifts the energy transfer within the trophic cascade. Therefore, a more applied study design is required in order to investigate this research question.

4.4 Contamination

In order to prevent contamination as good as possible, I implemented several precaution steps and conducted a pilot study. The pilot study aimed to test for the appropriate dissection method. In one method, I opened the gut after the dissection to perform a diet analysis. In the other method, I kept the gut closed and transferred the GIT directly to the bottle with the enzyme solution. The diet composition was estimated from the leftover on the filter subsequently. Since the diet analysis provides important information that offers possibilities to draw a connection between the prey and the plastic ingestion, I decided for the more detailed open method. Additionally and most importantly, the contamination risk did not seem to increase during the procedure. Even though the results of the pilot study did not show an increase in contamination and I applied preparations to limit the contamination risk, the pilot study was only performed on a small sample size. Since contamination can lead to false interpretation of the results, taking measures to limit and protocol contamination is a crucial part of a study on microplastics.

Several recent studies on microplastics in fish are lacking or only partly applying precaution measures against contamination. Due to this, it is questionable whether the results in these studies are reliable. Contamination can originate from different sources such as clothing, equipment and the working environment. Special attention should be drawn to the amount of synthetic fibres in the air (Gasperi et al., 2018). Contamination through airborne fibres is a documented problem in microplastic research (Davison & Asch, 2011; Foekema et al., 2013). In order to control for air-borne as well as other sources of contamination, studies on microplastics in sediment and biota started to implement various precaution measures. The current literature covers a wide range of measures against contamination. Davison and Asch (2011) placed empty petri dishes next to the samples during the laboratory procedure that accounted for negative controls. A. Lusher et al. (2013) specifically mentioned the importance of a clean working environment. Hence, work surfaces, hands, dishes and instruments were always cleaned thoroughly, lab coat and gloves were worn and the samples were covered immediately after transfer to keep the exposure time to the open air as short as possible. Neves et al. (2015) avoided the use of plastic ware at any cost to eliminate potential contamination by the equipment itself. Karlsson et al. (2017) applied the previously mentioned steps and additionally rinsed the equipment and samples with Milli-Q water. Another important measure is the implementation of blank samples processed with every batch of samples (Rummel et al., 2016). Apart from clean working conditions, blank samples and negative controls, the usage of positive controls is another important measure to monitor contamination (Hermsen et al., 2017). However, one of the most important steps in terms of contamination precaution represents the implementation of the air-flow

chamber to conduct the laboratory under clean air conditions (Cannon et al., 2016; Foekema et al., 2013). The air-flow chamber extracts a significant number of air-borne fibres from the working environment. In my study, I almost exclusively performed the work in an air-flow chamber. Only during the diet analysis, which afforded the use a stereomicroscope, the samples were removed from the chamber. In order to maintain clean air conditions, I applied a method established by Torre et al. (2016). The microscopic work was performed under a microscope enclosed in a plastic cover with two openings for the hands. Even though this method does not reduce the contamination risk as effectively as the air-flow chamber, it offered me the opportunity to perform a diet analysis under sufficiently secured air conditions.
In order to implement a standard procedure for studies on microplastics, Hermsen et al. (2018) provided an assessment on quality criteria for a clean work environment. Applying the quality criteria suggested by this assessment in future studies reduces the contamination risk to a high extent and offers comparability between studies.
According to this assessment, the precaution measures in my study were sufficiently established, except for the measure of positive controls. Future studies are inquired to apply all suggested precaution measures provided by Hermsen et al. (2018).

4.5 Improvements and future research

Plastic is very heterogenous and comes in various shapes, colours and sizes. Thus, the visual inspection for and identification of plastic particles in the leftovers of gut samples is a challenging task. The particles are easy to miss in the sample as well as to mistake for something else. Since visual identification of plastic particles is very dependent on the person, the analysis requires the highest attention. Thus, every particle that was found in the samples was inspected thoroughly. If I were insecure about the particle type, it was not protocolled as plastic. The same accounted for non-plastic particles. Visual identification is sufficient to get an idea of the amount of plastic contamination. Several publications based their study results on visual identification (Bellas et al., 2016; Boerger et al., 2010; Davison & Asch, 2011; Peters & Bratton, 2016). However, an additional analysis of the polymer type is recommended in order to publish repeatable data (A. Lusher et al., 2017). For this purpose, methods such as FT-IR or Raman spectroscopy are popular and reliable.
A chemical inspection of the plastic particles was initially part of the workplan of my study, as it improves the study design and excludes misidentification. Eventually, I encountered issues with the access to the machine and it exceeded my time limits. Even though it was not part of this study, I are aiming to conduct the

chemical inspection of the sampled particles. A chemical inspection of the plastic polymers is a crucial part of nowadays research on marine debris. It provides better and more reliable information about the extracted particles and offers the possibility to publish representable and repeatable results. Several studies already applied FT-IR as part of the plastic analysis (Foekema et al., 2013; Hermsen et al., 2017; Karlsson et al., 2017; Löder et al., 2017; A. Lusher et al., 2013).

This study was the first to apply the tissue digestion protocol by Winberg von Friesen et al. (2019) on fish samples. Thus, an evaluation of the protocol is needed in terms of its efficiency and applicability for fish. The digestion of the gastro-intestinal tract is based on a pancreatic enzyme, which does not affect anthropogenic particles or the environment. It provides a harmless method to effectively digest organic tissue. In contrast to acidic and alkaline digestion methods, the enzymic method has even proven to be more efficient. Chemical digestion agents such as potassium hydroxide (Foekema et al., 2013) or sodium hydroxide (Cole et al., 2014) were found to be harmful for plastic polymers by affecting their surface structure or colour (Catarino et al., 2017; Cole et al., 2014; Enders et al., 2017). An evaluation of six different digestion protocols by Dehaut et al. (2016) showed that five of the tested chemical agents significantly affected the plastic polymer and/or insufficiently dissolved the organic tissue. Thus, enzymatic digestion technique provides an efficient and environmentally friendly approach. Several recent studies applied and proved enzymatic methods to be appropriate for tissue digestion, using a protease (Catarino et al., 2017), Proteinase-K (Cole et al., 2014; Karlsson et al., 2017), pancreatic enzymes (Granberg et al., 2019) and a commercial enzymatic mixture (Löder et al., 2017).
In general, the protocol by Granberg et al. (2019) was an appropriate choice for my study purpose. However, the original protocol was established on bivalves whereas my study organisms were fish. The digestion of the organic tissue seemed to be similarly efficient. The digestion of the diet content proved to be more complicated. As bivalves are filter feeder, the diet does not contain hard structures and skeletal parts. The diet content of fish on the other hand contains various hard structures, such as fish skeletons, chitinous exoskeletons of shrimps and bivalve shells. After digestion, these hard structures ended up on the filter as leftovers and complicate the visual inspection for plastic particles. Additionally, several of these skeletal parts are easy to mistake with plastic. Hence, this study could be improved by adding an enzymatic agent that is able to dissolve for instance chitin. Löder et al. (2017) used a combination of several enzymes that ensured the purification of the samples, composing amylase, protease, lipase, cellulase and chitinase. In order to achieve excellent results, the study established a basic enzymatic purification protocol composing of a series of detailed methods. Additionally, the study improved the

efficiency of the digestion by using technical grade enzymes, functioning on the optimum of their characteristics. The pancreatic enzyme used in my study (Creon 40.000) contains only amylase, protease and lipase extracted from pig pancreas. By adding at least chitinase to the enzyme set, a higher amount of skeletal parts in the digestive tract would be digested and less leftovers would end up on the filters. This is highly recommended for follow-up research on study organisms such as fish.
I applied several precaution steps in order to reduce the risk of contamination (see 2.3.7.). In addition to the established steps, future research is recommended to take samples of the gear (fishing net) and include positive controls. I suggest to follow the precaution steps for the analysis of microplastic assessed by Hermsen et al. (2018).

5 Conclusion

My results from two common fish species caught in the Swedish Skagerrak support the numerous publications in the literature that reported ingested plastics in fish. Plastic ingestion accounted for 10.8% in the whiting and 17.6% in the common dab. The recovered particles consisted almost exclusively of fibres. The sizes ranged between 240µm and 25mm and the colour spectrum was dominated by translucent particles. Next to plastic, my study recovered even higher numbers of anthropogenic non-plastic polymers (26.8%), respectively natural and synthetic fibres. According to the NMDS analysis, the diet composition in the whiting samples from the coast and offshore were significantly different. The varying diet composition were likely caused by seasonal and regional differences. The diet of the common dab did not differ between the two regions.

As for the reason of plastic ingestion, I suppose it to be linked to the feeding strategy of the fish. However, the data did not reveal a connection between a certain prey group and ingested particles. Anthropogenic particles were expected to be accidentally ingested by the common dab due to its feeding strategy. In the whiting, marine debris was suggested to be ingested secondarily through the prey organisms as well as by accident. In order to determine the drivers of plastic ingestion, the trophic and non-trophic interactions need to be understood and taken into account. Further research is required to help understand the role of ingested plastic in the food web dynamics.

Future research is recommended to conduct chemical analysis on the polymer type of the particles in order to support the results and apply the quality criteria against contamination provided by Hermsen et al. (2018). Furthermore, the usage of an enzymatic digestion method on the GIT of fish requires an additional enzyme set that ensures the digestion of hard prey structures. The filters were frequently covered with hard prey remains, which made the inspection for plastic particles more challenging. Nevertheless, this study successfully conducted an analysis on the diet composition and ingestion of plastics and anthropogenic non-plastics in the whiting and the common dab from the Swedish Skagerrak.

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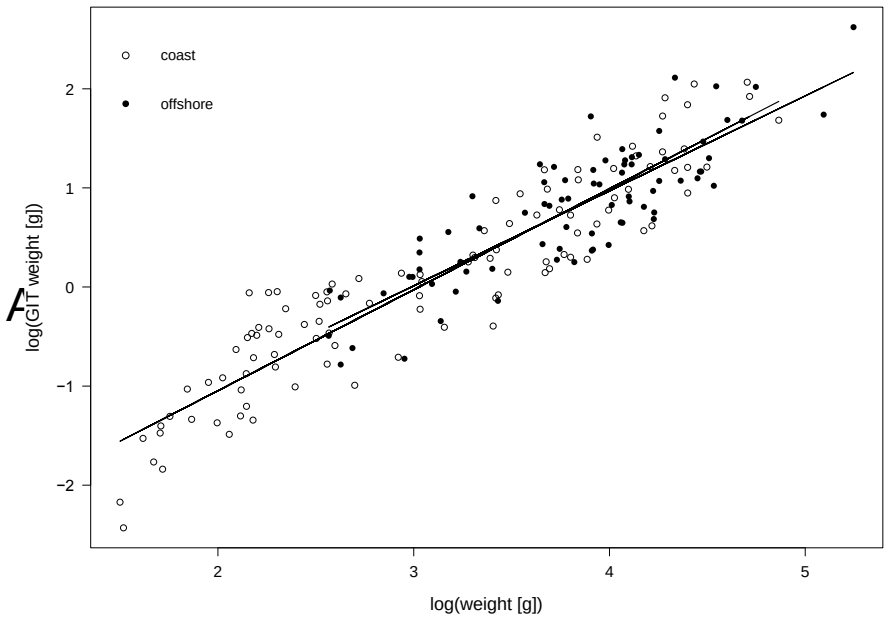

Figure 15. Linear regression of the GIT weight and the body weight of the dab. The graph shows the correlation between these two condition-dependent variables and compares this correlation between the two regions, coast (hollow dots) and offshore (filled dots).

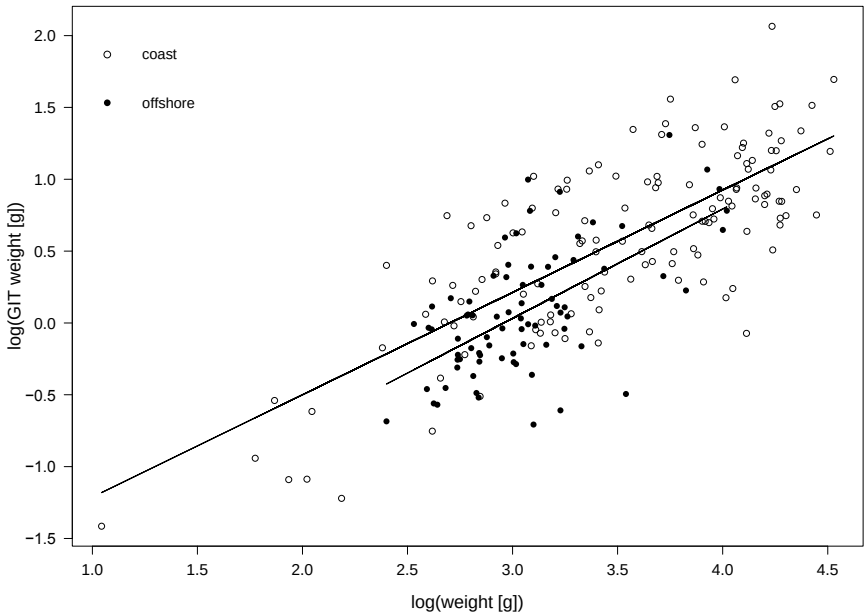

Figure 16. Linear regression of the GIT weight and the body weight of the whiting. The graph shows the correlation between these two condition-dependent variables and compares this correlation between the two regions, coast (hollow dots) and offshore (filled dots).

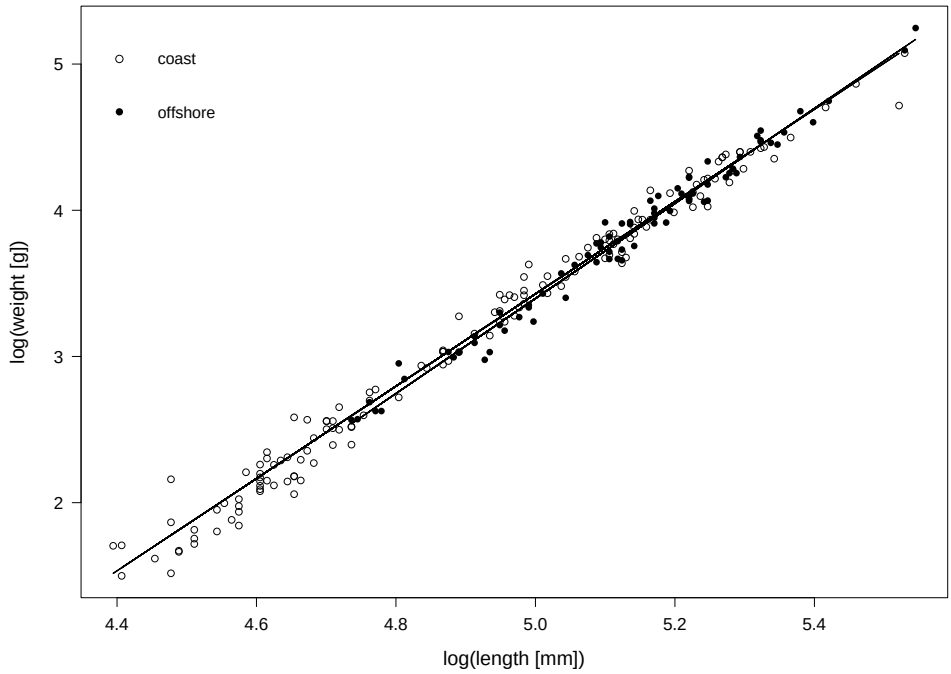

Figure 17. Linear regression of the body length and the body weight of the dab. The graph shows the correlation between these two condition-dependent variables and compares this correlation between the two regions, coast (hollow dots) and offshore (filled dots).

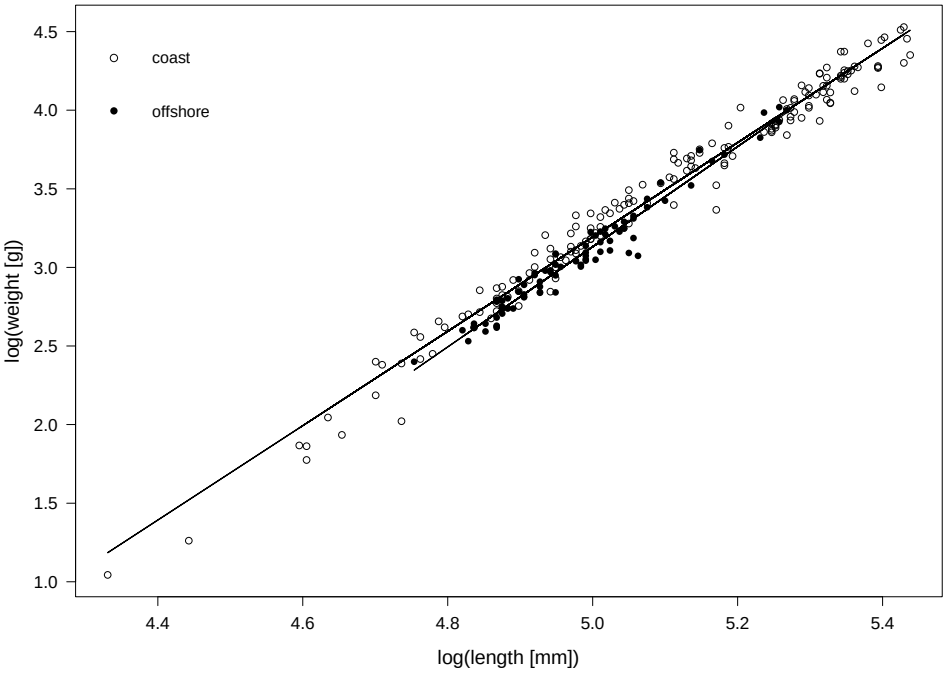

Figure 18. Linear regression of the body weight and the body length of the whiting. The graph shows the correlation between these two condition-dependent variables and compares this correlation between the two regions, coast (hollow dots) and offshore (filled dots).

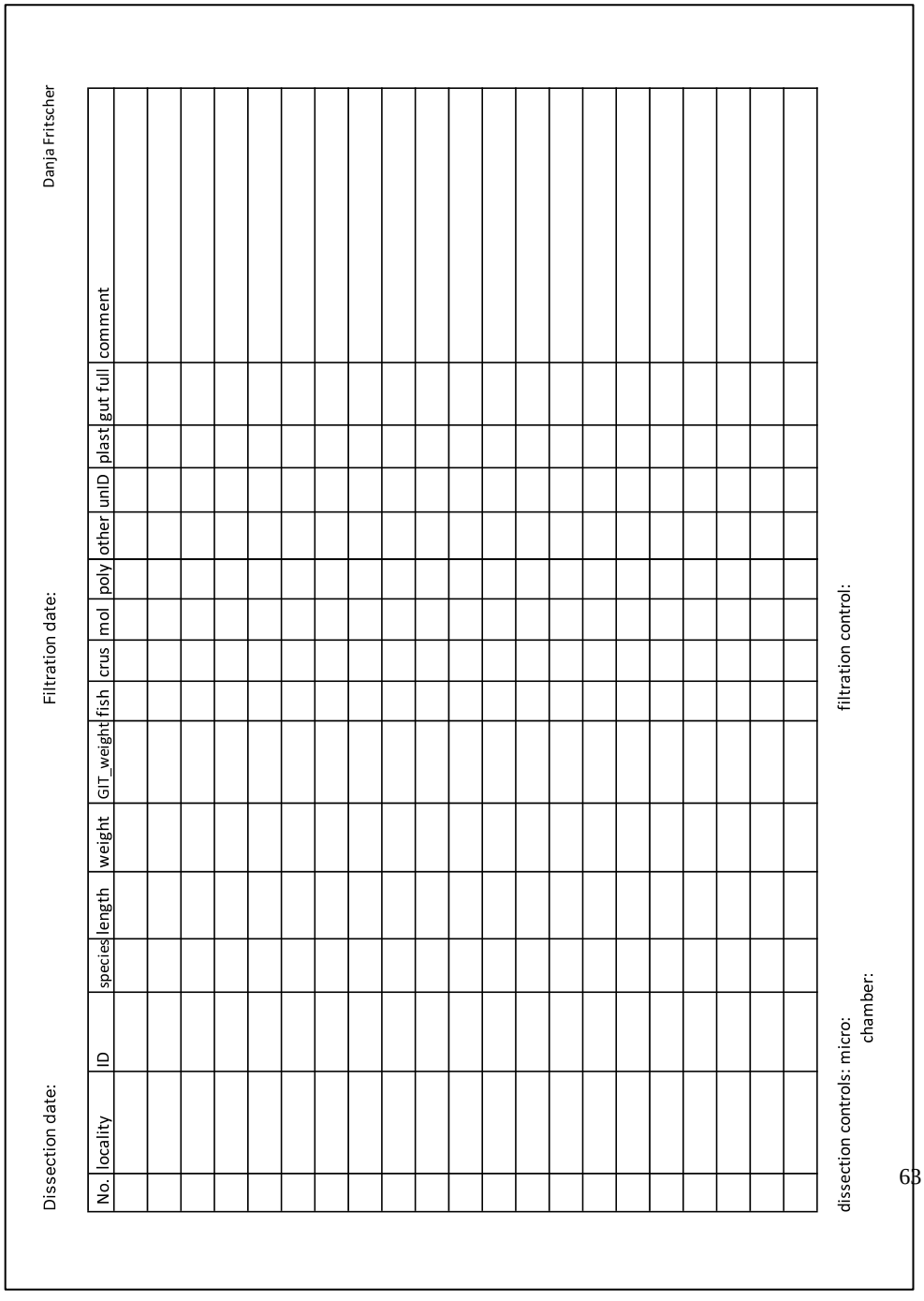

Figure 19. Laboratory protocol for the dissection, measurements and diet analysis of the samples.

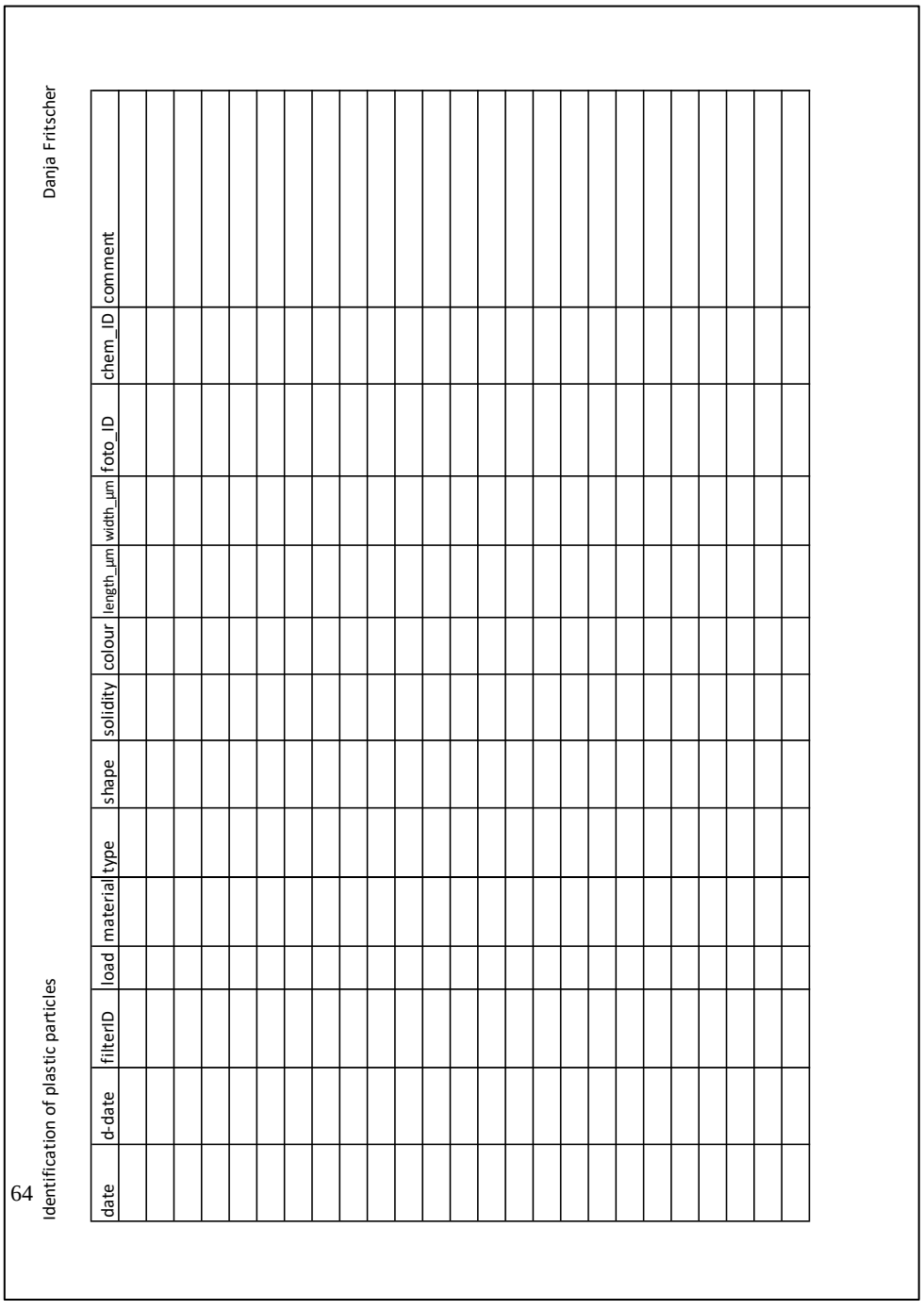

Figure 20. Laboratory protocol for the identification of plastic particles from the samples.