TRACKing of PLASTtic emissions from aquaculture industry (TrackPlast)

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Acronyms used

ACy	Acrylates
CMC	Cellulose chemically modified
DW	Dry Weight
EVA	Ethylene vinyl acetate
FTIR	Fourier Transform Infrared Spectroscopy
FPA	Focal Plane Array
GI	Gastro Intestinal
HDPE	High density polyethylene
LDPE	Low density polyethylene
LLDPE	Linear low-density polyethylene
m b.s.l.	meters below sea level
NQC	Norwegian quality cut
PA	Polyamide (nylon)
PAN	Polyacrylonitrile,
PC	Polycarbonate
PE	Polyethylene
PE	Polyethylene
PEEK	Polyetheretherketone
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
POM	Polyoxymethylene
PP	Polypropylene
PS	Polystyrene
PSUL	Polysulfone
PVC	Polyvinyl Chloride
Pyr- GCMS	Pyrolysis gas chromatography mass spectrometry
RU	Rubber
SAWS	Semi-Automatic Water Sampling device
SDS	Sodium dodecyl sulfate
SOP	Standard Operating Procedure

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Sammendrag

Målsetningene med dette studiet har vært: 1) å framskaffe kunnskap om akvakulturrelaterte utslipp av plastikk og mikroplast (MP) til det marine miljøet; 2) å identifisere og bestemme relative mengder av spesifikk mikroplast i vannsøyle, suspendert materiale og sjøbunn i umiddelbar nærhet av et oppdrettsanlegg; 3) å evaluere hvilke akvakulturprosesser som kan være potensielle kilder til identifisert mikroplast i miljøet. studiet, Studiet vil gi en vitenskapelig bakgrunn for å utvikle en handlingsplan for å redusere plastutslipp fra akvakultur.

For å nå disse målene ble råstoff og ingredienser som brukes i nåværende fiskefôrproduksjon samt det ferdige produktet innsamlet sammen med miljøprøver av sjøvann, marine sedimenter og suspendert materiale nær en lakseoppdrettslokalitet. Prøver av gjeller og fordøyelsessystem i oppdretts- og villlaks ble innsamlet for å estimere potensiell eksponering av akvatiske organismer til plastpartikler med opphav i akvakultur. Videre ble slipeeffekten indusert i fôrledninger ved fordeling av fôrpellets eksperimentelt simulert. Dette bidro til økt forståelse både av rollen aldring av plastledninger kan spille som en relevant faktor i fragmenteringsmønsteret, og til foreløpig karakterisering av størrelsesfordeling av partikler som potensielt blir frigitt fra fôrledninger under en normal oppdrettssituasjon.

Massespektrometrianalyser indikerte MP-kontaminering i noen av de analyserte råstoffene brukt i forproduksjon og i produsert fôr. Mengden av MP ble funnet i størrelsesorden noen få µg/g av polyetylen (PE) og polyamida (PA) i fiskeråstoff og polypropylen (PP) i det ferdige produktet. Undersøkelse av produksjonslinjen for hvetegluten bidro til å identifisere en primærkilde til PP-frigjøring, og tiltak er foreslått for å eliminere denne kontamineringskilden. Ved partikkelanalyse av det samme materialet ble noen PE-, PA- og polyetylen-tereftalat- (PET) partikler (21-38 µm) identifisert som et betydelig bidrag til MP-kontamineringen. Et stort fragment av PP (0.8-1,0 mm) og mindre forekomster av andre polymertyper, slik som PA, ble også funnet. Totalt 10 polymertyper utgjorde 95% av polymersammensetningen i fôret. Sediment prøver hadde MP total mengder fra 38 til 920 partikler pr. kg tørrvekt med hovedtyngden i intervallet 10-300 µm. PE og polystyren (PS) viste høyere konsentrasjoner i lokalitetene nær merdene, mens alle de øvrige undersøkte polymertypene hadde ingen klar områdefordeling ift akvakulturaktivitet, dvs. studiets referanselokalitet viste liknende sammensetning av polymerer, ofte med liknende akkumulasjonsnivåer. I suspendert materiale var det totale partikkelantallet 220 000 - 360 000 partikler/kg tørrvekt, omtrent tusen ganger konsentrasjonen i prøver av bunnsediment. PET, PP og PA var dominerende polymertyper. I vannprøver ble konsentrasjonen av partikler større enn 10µm analysert vha. pyrolyse GC/MS (Pyr-GCMS). PE, PS og PET var de dominerende polymertypene fra 0,021 µg/L for PET til 0,180 µg/L for PE. PE viste høyere konsentrasjoner ved prøvetakingslokalitetene nær merdene. Kompleksiteten av MP-fordelingen i akvatiske kystøkosystemer krever videre undersøkelser med større antall prøver

og flere prøvetakingstidspunkt med formål å skille mellom akvakulturbidraget i ulike produksjonsfaser.

De kvalitative resultatene av histologiske analyser i gjellene til oppdrettslaks viste tilstedeværelsen av MP (5 til 25 µm partikler) i gjellelamellene hos noe mer enn halvparten av undersøkte fisk, og massespektrometrianalysene identifiserte tilstedeværelsen av PE i de samme prøvene. Som simulert ift. en eksperimentell aktivitet I dette studiet, kan det antydes at slipeeffekten på de PE-inneholdende fôrledningene i oppdrettet med påfølgende frigjøring av mikrometerstørrelse MP er en kilde til den identifiserte PE mikroplasten. Ingen tidligere data finnes på tilstedeværelsen av MP i gjeller hos hverken vill- eller oppdrettslaks. I fordøyelsessystemet ble det ikke detektert MP over kvantifikasjonsgrensen i oppdrettslaks, mens det var mulig å detektere MP i fordøyelsessystemet hos villaks.

Generelt vil best strategi vedr. prøvetakings- og analysemetoder avhenge av om framtidig fokus vil være å overvåke endringer eller å utføre MP-screening i utpekte områder for undersøkelse av mulig akvakulturproduksjon. Kombinasjon av prøvetaking og analyser av suspendert MP i vannsøylen ved bruk av sedimentasjonsfeller og sediment ved bruk av van Veen grabb vil muliggjøre samtidig overvåking av korttidsflukser og langtidstrender.

De til nå oppnådde resultatene bør tolkes som foreløpige indikasjoner i den komplekse vurderingen av utslipp av MP fra akvakultur.

Abstract

The objectives of this study were: 1) to acquire knowledge about aquaculture related release of plastic and microplastic to the marine environment; 2) to identify and determine relative amounts of specific microplastics in the water column, suspended matter and seabed, in the immediate vicinity of an aquaculture farm; 3) to evaluate which aquaculture processes are the potential sources of identified microplastics in the environment. This study will provide a scientific basis for the development of an action plan to reduce plastic emissions from the seafood industry.

To achieve these goals, raw materials and ingredients currently used for fish feed production as well as the finished product were collected along with environmental samples of seawater, marine sediments and suspended matter near a salmon production site. Gills and GI-tracts of farmed and wild salmon were collected, to estimate the potential exposure of aquatic life to plastic particles originating from aquaculture activities. Furthermore, the abrasion effect induced in the feeding pipes during the distribution of pelleted fish feed was experimentally simulated. This contributed to the understanding of both the role of the aging of the plastics pipes as a relevant factor in the fragmentation pattern, as well as to preliminarily characterize the grain size distribution of the particles potentially released from the feed pipes, during normal aquaculture production.

Mass spectrometry analyses indicated microplastic (MP) contamination in some of the analysed raw materials used for feed production and finished feed. Amounts of MP were in the range of a few µg/g of polyethylene (PE) and polyamide (PA) in fish meal and polypropylene (PP) in the finished product. Investigation of the wheat gluten production line helped to identify a primary source of the PP release and actions are suggested to eliminate this source of contamination. Particle analysis of the same material identified a few PE, PA and polyethylene terephthalate (PET) particles (21-38 µm) as the major contribution of the MP contamination. A large fragment of PP (0.8-1.0 mm), and minor occurrences of other polymer types such as PA were also found. In total 10 polymer types accounted for 95% of the polymer composition in feed. Sediment samples had a total amount of MP ranging from 38 to 920 particles/kg of dry weight (DW) with the majority in the 10-300 µm range. PE and polystyrene (PS) displayed higher concentrations at the sites close to the cages, while all the remaining investigated polymer types had no clear area related distribution relative to aquaculture activity, i.e. the reference site in the study showed a similar pool of polymers, often with similar levels of accumulation. In suspended matter, the total amount of particles was 220 000-360 000 particles/kg of dry weight, around 1000 times the concentration of the bottom sediment samples. PET, PP and PA were the dominant polymer types. In water samples the concentration of particles over 10µm were analysed using pyrolysis GCMS (Pyr-GCMS). PE, PS and PET were the dominant polymer types, ranging from 0.021 µg/L for PET to 0.180 µg/L for PE. PE displayed higher concentrations at the sampling sites close to the cages. The complexity of the MP distribution in aquatic coastal ecosystems calls for further investigations with a higher number of samples and several time points, aiming at discerning

the contribution from aquaculture in relation to the production phases. The obtained results should be interpreted as preliminary indications in the complex assessment of emissions of MP from aquaculture activities.

The qualitative results of histological analyses in the gills of farmed salmon showed the presence of MP (5 to 25 µm particles) in the lamellae of gills of slightly more than half of the sampled fish, and the mass spectrometry analysis identified the presence of PE in the same samples. As simulated during an experimental activity within this study, the abrasion of the PE containing feed pipes during the aquaculture production and the consequent release of microns sized MP may suggest that the pipes are a source of the identified PE microplastic. No previous data exists on the occurrence of MPs in gills of either wild or farmed salmon. In the GI-tract, no MP above the limit of quantification was detected in farmed salmon, while it was possible to detect MPs in the GI-Tract of wild salmon.

Overall, the best strategy regarding sampling methods and analyses, depends on if the future focus will be to monitor changes or to make a MPs screening of the investigated area designed for aquaculture production. Combining sampling and analysis of suspended MP in the water column using sedimentation traps and of sediments using van Veen grabs would allow for simultaneous monitoring of short-term fluxes and long-term trends.

1 Introduction

Norway has the ambition to intensify aquaculture production to fulfil a growing demand. Farmed salmon has become a significant source of national income with an excess of 1 million tonnes of salmonids produced every year in Norway (Marine Harvest, 2017). As the demand has grown, the number of aquaculture facilities has increased, and existing locations have expanded. For the farms and production lines, the aquaculture industry benefits from a diversity of synthetic materials. Synthetic ropes offer lower weight and greater strength and durability than natural fibres, and are easier to handle, compared to their natural counterparts. Most modern aquaculture activities use plastic-based lines, cages or nets suspended from buoyant or submergible structures (in part made of plastic) as well as nanotech plastic-based anti-biofouling agents and paints (Lusher et al., 2017). Tanks, pens, nets, floats and pontoons as well as the pipes of the fish feed suppling systems are made of plastic material. Plastic materials within aquaculture sites are maintained and controlled for chemical degradation, biofouling and corrosion, with regular inspections to ensure strength and stability. In Norway, farming equipment is certified according to the NYTEK standard ensuring that the equipment is fit for purpose. In the context of ocean plastic pollution, the aquaculture industry has been reported as a potential significant contributor (Hinojosa et al., 2009; SALT, 2019). Lost gear, broken and fragmented equipment, and release of MP debris because of intense use have been suggested as sources of both macro and microplastic emissions from aquaculture at both the global and local level (Astudillo et al., 2009). The level of contribution from direct release of MPs during production procedures remains a knowledge gap that needs to be filled (SINTEF, 2017; Miljødirektoratet, 2018). Europe and Norway are responsible to counteract marine waste and agreed on implementing the UN sustainability goals, especially SDG 14 “Conserve and sustainably use the oceans, seas and marine resources for sustainable development”. SDG 14 is also repeated in the Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment (https://eur-lex.europa.eu/eli/dir/2019/904/oj). Furthermore, the Marine Strategy Framework Directive requests that the amount and composition of marine waste does not cause harm to marine and coastal environments.

However, neither amounts of plastics nor the hazards posed by plastics and microplastics in the environment are fully understood. Standardized methods for sample preparation, analysis and quantification of MPs do not yet exist, hampering the comparison of results between studies. Standardised methods are urgently needed and should be based on direct comparison of different sampling and analytical methods. Visual identification approaches using morphological criteria alone have often led to significant errors, which underlines the importance of using chemical identification (Löder and Gerdts 2015). The present study documents the utility and sensitivity of current methods, and as such contributes to the background and knowledge base needed for the establishment of national monitoring programmes for microplastic.

MPs have been found to be omnipresent (Lusher, 2017; Rochman, 2018) with potential negative effects from plastic additives as well as the plastic polymers (VKM, 2019; Kögel, 2019). Knowledge about levels of potentially harmful MPs in feed and the finished products is necessary as well as knowledge about environmental release of potentially harmful substances during production. The precautionary principle as well as the existing strict requirements for food safety may also be applied to microplastics in food and the environment. In this respect it should be noted that a recent risk assessment done in Norway (VKM, 2019) concluded that at present the available information on MP does not provide a sufficient basis to characterize potential toxicity in humans. According to FAO (Lusher et al., 2017), the risk of MP ingestion for humans is reduced by the removal of the gastrointestinal tract (GI-tract) in most species of seafood consumed.

Regulations on environmental threshold levels must be complied to. Such regulations must therefore be based on scientific knowledge and documentation of tolerance. In the case of aquaculture, quantification of MPs in feed ingredients, production lines and finished products is advocated. Quality control in the feed industry involves the verification of quality standards established for each feed ingredient prior to use and during processing. Quality control continues as ingredients are mixed and finally stored as final compound feed (FAO, 1980). The purpose of quality control of raw materials is to ensure that minimum requirements are met. It provides knowledge concerning the composition of raw materials, nutrient quality and the levels of potential toxic substances so that the final feed is safe and of the required nutritive value. However, documenting the occurrence and composition of MPs in the feed ingredients and product is not currently required, and methods for monitoring are not standardised.

Abrasion of feeding pipes has been speculated to be a significant source of MPs in the aquatic environment. A preliminary attempt to estimate the total amount of released MP was performed by Naturvernforbundet (Naturvernforbundet, 2018). A rough calculation based on the loss of weight in worn feed pipes indicate releases in the range of 0.25-5.0 tonnes per aquaculture site during the theoretical lifespan of 5 years per pipe, and a total loss of 300 tonnes per year in Norway. However, a more recent study has demonstrated lower global emissions, from 10 to 100 tonnes per year, than those estimated by Naturvernforbundet (SALT, 2019 - FHF HAVPLAST project).

Pellets that pass-through feed pipes under high pressure cause abrasion in the pipes and wear the plastic from the inside, causing the formation of an unknown number of plastic fragments. Plastic from the feed pipes enters the salmon pens together with the pellets, and is spread into the sea, where it may be taken up by biota. The long-term effects of MP ingestion are unknown (VKM 2019). Little is also known about the number or the grain size distribution of the plastic particles formed during normal feeding, as well as how these parameters vary in time as consequence of the aging of the feed pipes. These circumstances call for empiric analysis of the situation.

This project was initiated by FHF in order to fill knowledge gaps on MPs released from fish farms and the fate and distribution in the vicinity of such farms.

The objectives of this study were: 1) to acquire knowledge about aquaculture related release of plastic and microplastic to the marine environment; 2) to identify and determine relative amounts of specific microplastics in the water column, suspended matter and seabed,

in the immediate vicinity of an aquaculture farm; 3) to evaluate which aquaculture processes are the potential sources of identified microplastics in the environment and 4) to develop a draft action plan for reducing plastic emissions from the seafood industry.

2 Materials and methods

2.1 Study sites

2.1.1 The feed manufacturing facility

Skretting is world-leading within feed production for aquaculture, producing over 2 million tonnes of feed globally, each year. The Skretting fish feed facility that participated in this study is located in Stavanger (Figure 2.1).

Figure 2.1 – The Skretting fish feed production facility that participated in this case study, with the distinctive white 80-ton silos to the right (Photo: Kristian Førland Steinsland).

The production lines for fish feed receive many different products. The raw material used in various products is shown in Table 2.2, and includes soy protein concentrate, fishmeal, wheat, wheat gluten, *Faba* beans, sunflower meal, fish oil, rapeseed oil, rapeseed lecithin and SPAR oil. These are mainly transported to the facilities by boat in large plastic bags and stored in oil tanks and material silos by mechanical transport systems such as redlers, elevators and gravimetric transports.

For each product, the various raw materials needed are transported from the on-site silos to begin the milling process. After being milled into low particle size, the mass enters the mixer where vitamins and nutrients are added. The semi-finished meal mix product is stored in pre-

batch silos ready for on-demand production. The meal mix enters the extruder process where it is first mixed with oil, hot water and steam in the preconditioner. The output of the extruder is the processed mass which is cut to pellet size at the end of the machine. The pellets are transported to a dryer and water is extracted in order to enable oil to enter. The next step is a coater which uses a vacuum to make the oil enter the pores of the dry pellet. After this process, the product is cooled, shaken to remove excess particles, weighed and packed into 750 kg bags for storage and transportation to the customer. The main critical production steps are summarized in Figure 2.2.

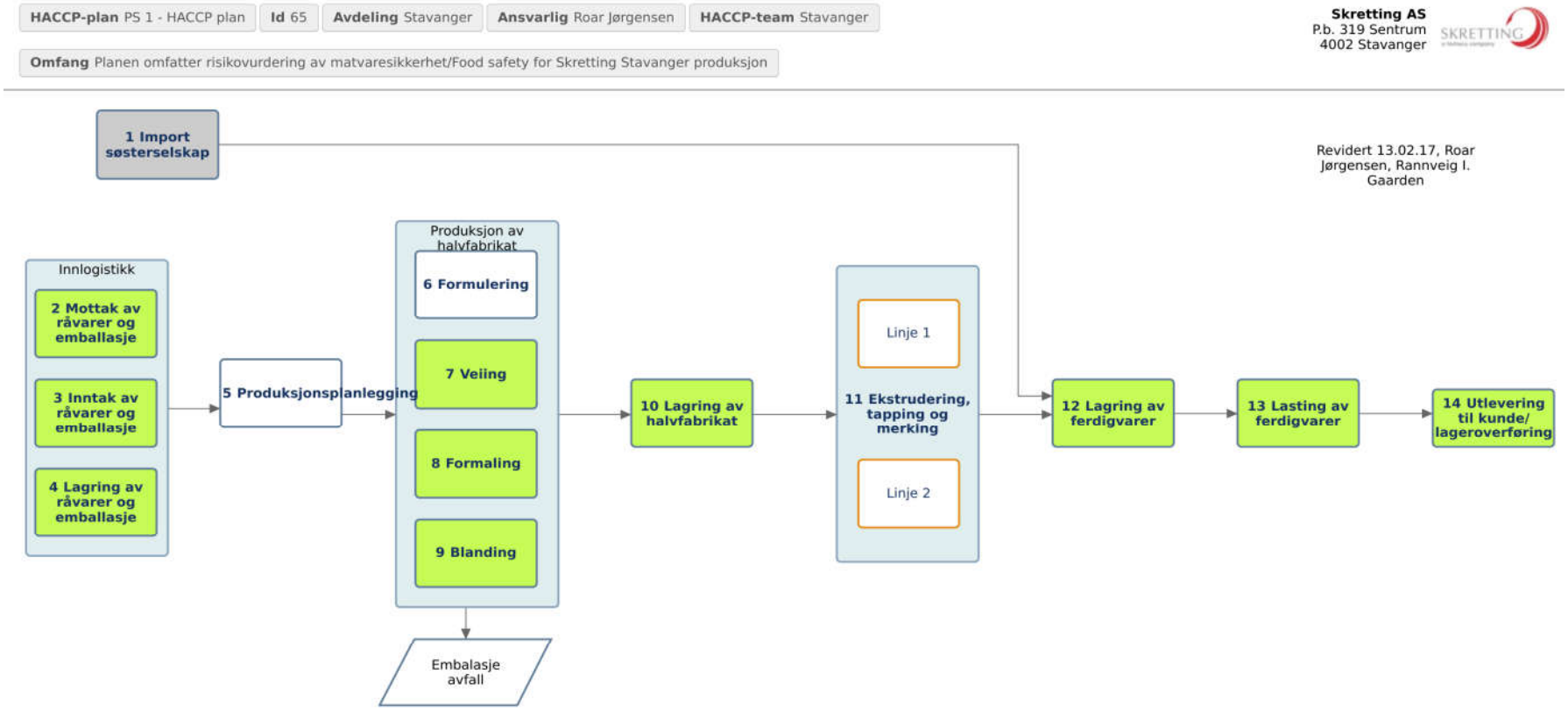

Figure 2.2 – Summary of the critical production steps at Skretting's production facility located in Hillevåg, Stavanger.

2.1.2 The MOWI aquaculture production facility

For the investigation of MP levels in environmental samples at a fish farm, we selected the Kjeahola facility at Ombo in Finnøy municipality. The location is northeast of the island of Ombo, where the Austre Ombofjord meets the inner parts of the Jelsafjord (Figure 2.3). The facility is located about 200 m from land (Figure 2.4). The fjord bottom under the facility slopes down towards the northeast, linking to the deep trench that extends into the Jelsafjord. Under the northern part of the site the depth is about 110-180 m, while it is about 120-170 m in the southern part. The main current direction in the area is towards the southeast, thus water enters the Jelsafjord and exits through the Austre Ombofjord.

Where the Jelsafjord meets the Nedstrandfjord west of Ombo, the fjord is narrow and has a shallower threshold of about 177 m depth before it widens and connects to the very exposed Boknafjord which opens to the ocean in the west. South of Ombo, the rather shallow Austre Ombofjord (30-100 m) meets the deeper Hjelmelandsfjord via a threshold of about 72 m depth and extends further into the Gardsundfjord south of Ombo (Figure 2.5).

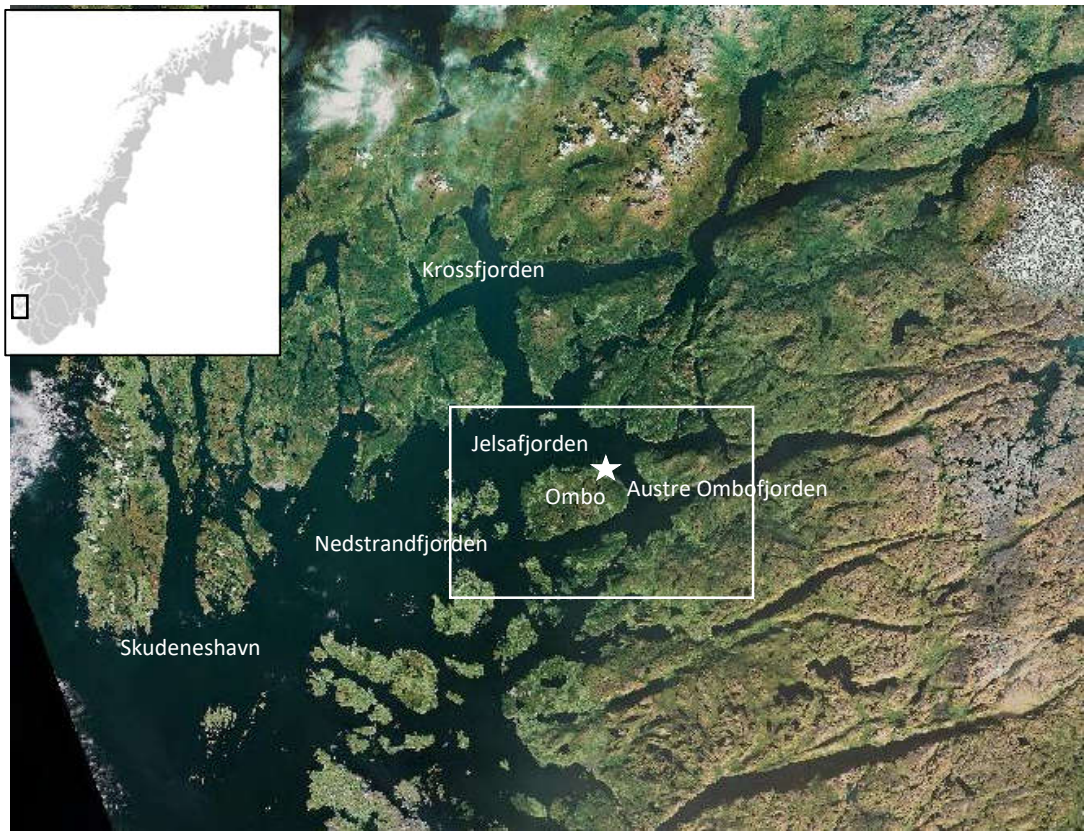

Figure 2.3 - Placement of the Kjeahola facility on the west coast of Norway.

Figure 2.4 - Map of the region in South-Western Norway, and placement of the production facility Kjeahola (star) at Ombo in Rogaland county.

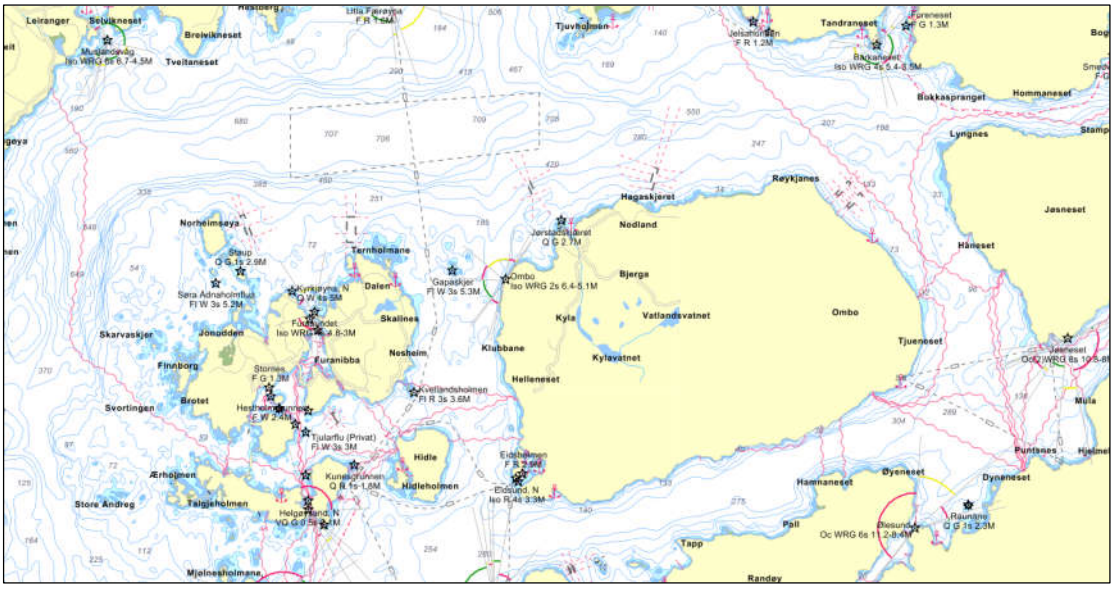

Figure 2.5 - Detailed map of the bathymetry of the fjords around the Kjeahola facility.

Figure 2.6 – MOWI production facility at Kjeahola.

The Kjeahola facility (Figure 2.6) has been in use since 2001 and is approved for a production of 7020 tonnes. The facility consists of 10 rings with a circumference of 160 m and 38 m deep net pens. The volume per cage is 35 020 m^3 (calculated to a depth of 22 m with a circumference of 122 m). The plant's operating history is summarized in Table 2.1. To note, the feed used at Kjeahola was not produced by Skretting.

Table 2.1- Main figures of feeding and production at Kjeahola since 2011. (Source: MOWI).

BBD-Kjeahola /year	2011	2012	2013	2014	2015	2016	2017	2018
Feed weight	31 963	1 306 574	7 082 166	1 673 631	5 987 025	3 454 065	7 074 672	1 617 305
Growth	950 154	1 300 174	5 972 633	1 709 026	5 131 253	3 004 509	5 794 595	1 406 947

2.2 Sampling activity

2.2.1 Sampling of raw materials and finished feed

A total of 30 samples of raw material for fish feed from different steps in the production process were collected, together with the information on the products (i.e. origin, date of production and supplier; Table 2.2, Figure 2.7). When possible, raw materials were homogenized before collection. When a large volume was available, a subsample was collected. Materials were collected using stainless-steel spoons into stainless steel cans. Sampling operators were dressed in cotton clothes and no plastic gloves were used during the sampling sessions. Equipment was burned at 500°C before use, to remove any plastic contamination. Samples were transported in stainless steel cans and stored in a cold dark room prior to analysis.

Table 2.2 – List of collected raw fish feed materials for analyses.

Feed ingredient	Number of samples analyzed	Meal or oil	Ingredient group
Soy protein concentrate	5	Meal	Vegetable protein
Wheat gluten	5	Meal	Vegetable protein
Fishmeal (different batches)	5	Meal	Marine protein
Wheat	5	Meal	Carbohydrates
Fava beans	5	Meal	Vegetable protein
Sunflower meal	5	Meal	Vegetable protein
Rapeseed oil	5	Oil	Vegetable oils
Fish oil	5	Oil	Marine oils
Fish oil from farmed fish	5	Oil	Marine oils
Total	45		
Product		**Type**	
Feed	5	Finished feed before fat coating	
Feed	5	Finished feed fat coated	
Total	10		

Figure 2.7 - Pictures of the sampling activity at the Skretting facility. A, D = soya protein line; C = wheat gluten line; B, E = plastic bags used for raw material shipment.

2.2.2 Mapping of polymer types used at the aquaculture facility

Frequently used plastic items and equipment (Figure 2.8, Table 2.3) from different steps of the production at Kjeahola were collected and chemically characterized using mass spectrometry Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GCMS). The occurrence of the polymers PE, PA, PP, PVC, PS, PC and PMMA was investigated. Of those polymers, the investigated equipment contained PE, PA and PP.

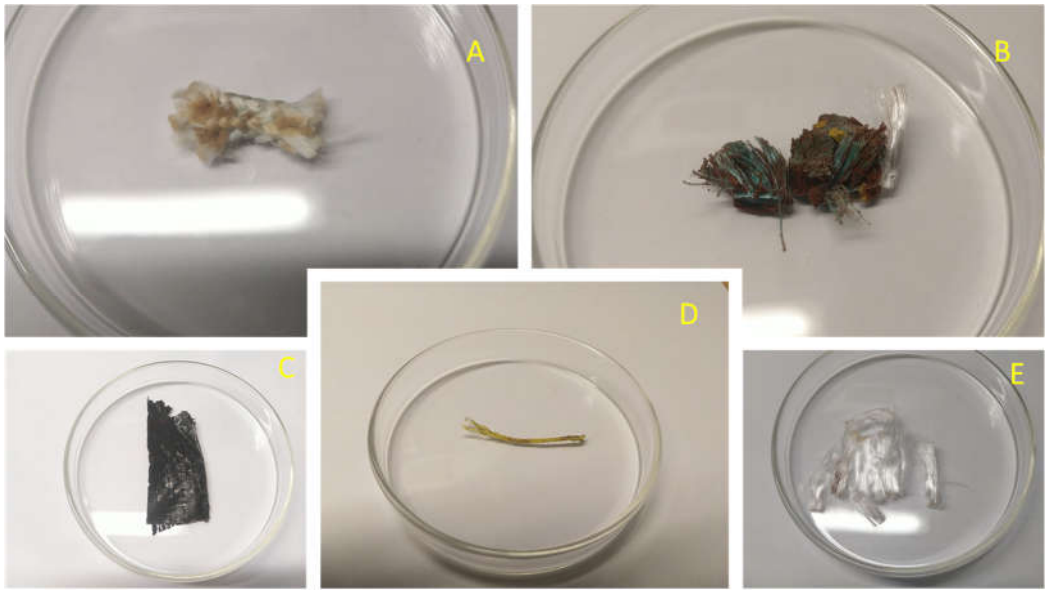

Figure 2.8- Sample of the plastic material collected from the MOWI Kjeahola production site. A, E = rope for net enclosures; B = rope for mooring systems, with antifouling paint, C = artificial kelp; D = rope for anti-predator nets.

Table 2.3 - Chemical characterization of plastic materials in use at the Kjeahola facility.

Item and function	Identified polymer type
Floating collars	PE
Buoys (in mooring systems)	PE
Ropes (in mooring systems)	PA+PP
Antifouling paint	PE
Net enclosures	PP
Anti- predator nets	PA
Feeding pipe	PE
Floating pontoon	PP
Artificial kelp (shelter for cleaner fishes)	PP

2.2.3 Sampling of marine sediments

The coordinates of the planned sampling sites are shown in Table 2.4 and Figure 2.9. Field activities took place on the 27th of March 2019. Marine sediments were collected from eight sites positioned downstream of the facility with increasing distances from the platform (Kje0, Figure 2.9), and one reference station 1 km upstream (Ref). The top 5 cm of sediments were collected through the four top openings of a Van Veen grab using a customized stainless-steel spoon (Figure 2.10). Samples were collected into pre-cleaned stainless-steel cans and stored

in a cold room prior to analyses. The spoon was thoroughly rinsed with seawater and paper between each station. Sediments were collected from nine of the eleven planned stations. Due to unfavourable seafloor conditions (steep slope and rocky bottom) samples were not obtained from stations NE500 and SE150. The sediments were generally fine to medium, and fine sand of dark/light grey colour with some occurring rocks according to NS ISO 16665 (2013) guidelines. No smell was reported, and benthic fauna was observed at the Kje0, NE50, SE50, NE250 and reference (REF) stations. The location has previously had scores of 1 in the most recent B- investigations based on the NS9410: 2016 standard.

Table 2.4 - Station names, coordinates (Coordinate system WGS84) and description of the collected sediment samples.

Site name	Latitude (N)	Longitude (E)	Comments **Weather conditions: Partially cloudy, no precipitation, wind: 1m/s NNE, light waves on the day of sampling.**
Kje0	59°17.568'	6°04.694'	Closest to the facility. Benthic fauna observed, no hydrogen sulfide (H_2S) smell, light grey colored sediment, medium and fine sand
SE50	59°17.557'	6°04.747'	
SE150	59°17.517'	6°04.827'	*Steep and rocky bottom, no successful sample obtained*
SE480	59°17.521'	6°05.193'	No H_2S smell, light grey colored sediment, medium and fine sand
SE750	59°17.405'	6°05.432'	No H_2S smell, light grey colored sediment, medium and fine sand
S250	59°17.441'	6°04.715'	No H_2S smell, light grey colored sediment, medium and fine sand
S500	59°17.306'	6°04.794'	No H_2S smell, light grey colored sediment, medium and fine sand
NE50	59°17.615'	6°04.755'	Benthic fauna, no H_2S smell, light grey colored sediment, medium and fine sand
NE250	59°17.687'	6°04.911'	
NE500	59°17.761'	6°05.092'	*Steep and rocky bottom, no successful sample obtained*
REF	59°18.039'	6°03.698'	Reference station. Benthic fauna observed, no H_2S smell, light grey colored sediment, medium and fine sand

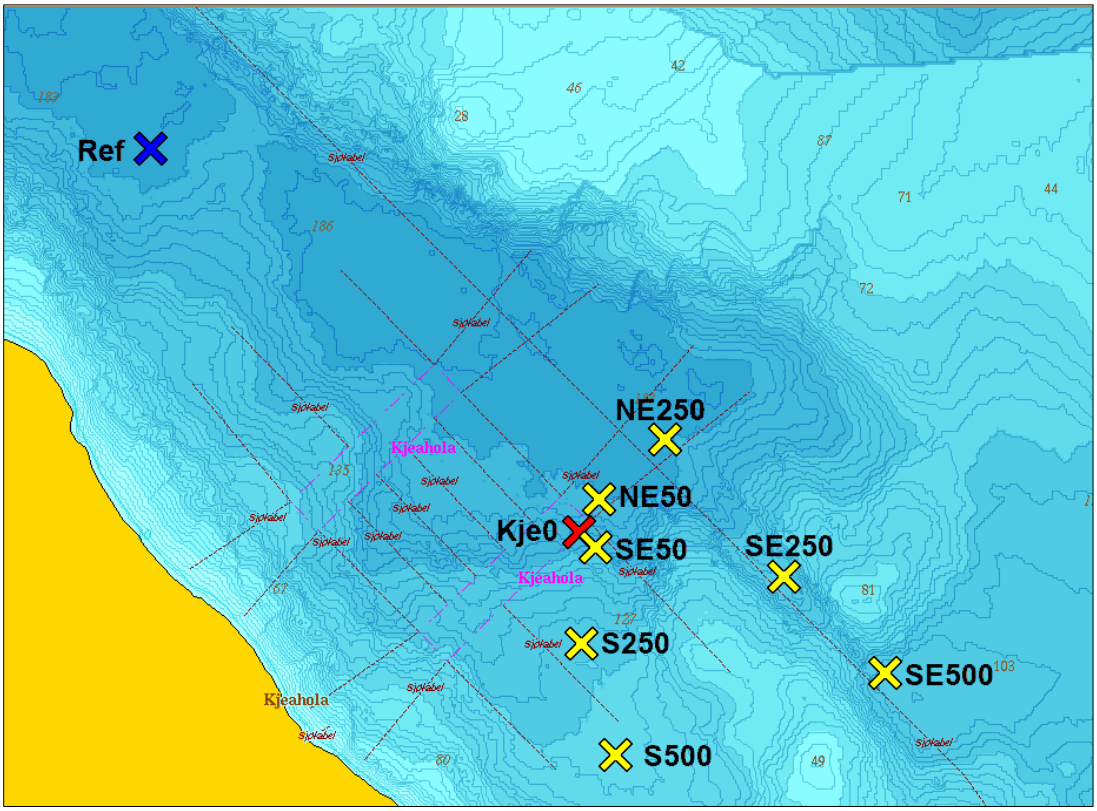

Figure 2.9 – Overview of the location Kjeahola with sampling stations. The station names indicate direction and distance in meters from the centre of the facility (Kje0).

Figure 2.10 - Photos of sediments collected from a van Veen grab using a flat stainless steel sampling spoon. The top 0-5 cm of the sediment were collected in a metal container.

2.2.4 Sampling of seawater near the aquaculture site

Water samples were collected during normal operation of the facility on the 27th of March 2019 from 9.00 to 16.00 (CET). At the time of sampling the facility was in the mid-term stages of production and the fish weighed approximately 2-3 kg. The samples represent times of intermediate feeding and biomass production. Replicates (n=3) of water samples were collected at Kje0 and at the reference site using a Semi-Automatic Water Sampling device (SAWS). The submergible stainless-steel pump delivers 8 L/min and was lowered to approximately 1.5 m below the surface (Figure 2.11) and ran for approximately 15 min, delivering approximately 100 L of seawater. Precise volumes of water are provided in Table 2.5. The water was pumped through the customized, stainless steel multi-layered sieving system.

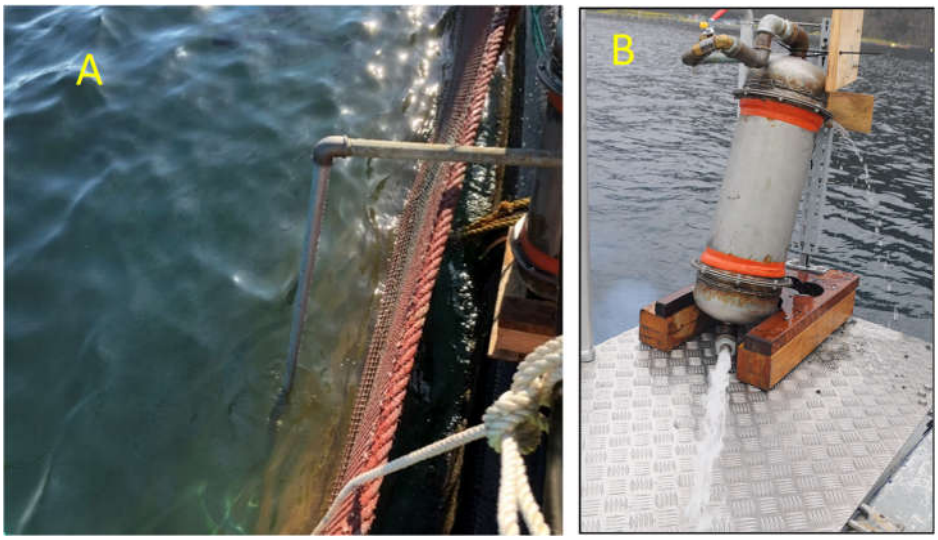

Figure 2.11 - Pictures of the Semi-Automatic Water Sampling device (SAWS) used for water sampling. (A) collection point inside the cage; (B) collection point at the reference site, SAWS system placed on the open deck of the Ognøysjefen R/V.

Table 2.5 – Recorded volumes of seawater for each of the collected replicates in the two sampling stations. Kje0 = cage site; Ref = reference.

Site name and replicate #	Collected volume (L)
Kje0 - 1	102
Kje0 - 1	108
Kje0 - 1	112
Ref - 1	105
Ref - 2	101
Ref - 3	110

2.2.5 Sampling of suspended matter near the aquaculture site

Two sediment traps at two different depths (-5 m and -20 m) were deployed both at the Kje0 site beside a net pen and at a reference site 1NM upstream of the main current (Figure 2.12; Figure 2.13). Sediment trap were loaded with a dense saline solution and left for seven days. Sedimentation chambers were then recovered, and the content emptied into pre-cleaned stainless-steel cans and rinsed twice with filtered MilliQ water to help transfer all sedimented material. Samples were stored at 4°C prior to analyses.

Figure 2.12 – Deployment of sediment traps (two chambers for each depth) at the reference site.

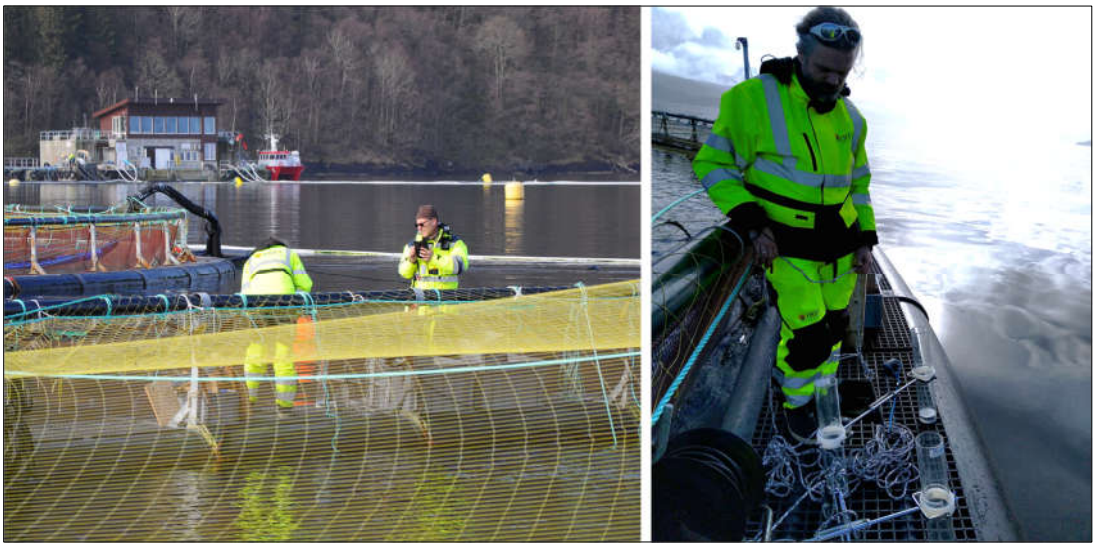

Figure 2.13 – Deployment of sediment traps at Kje0 next to a net pen.

2.2.6 Fish tissue collection

To compare the occurrence and polymer composition of MPs in some target organs of farmed and wild salmons, 20 individuals of approximately 2.5 kg where collected at the Kjeahola facility (Figure 2.14), located in the Boknafjord. Additionally, 20 wild salmon individuals of approximately 2.3 kg were delivered by the VOSSO scientific program run by NORCE and taken from the Sørfjord.

Figure 2.14 – Farmed salmon sample provided by MOWI Kjeahola.

The following samples were prepared under plastic clean laboratory conditions at the Institute of Marine Research. Approximately 2 mL subsamples of gills (2-3 g) and 20 g of the GI-tract were snap frozen in liquid nitrogen, stored in aluminium foil in glass jars and sent to the Veterinary Institute. Furthermore, subsamples of gill (as above), gut (5 g) and muscle (10 g) were fixed in “Carnoy” fixative (methanol 60%, glacial acetic acid 10%, chloroform 30%) in a ratio of 1/5 for sample/fixative, and stored in glass vials as back up biological material for histological studies. Within the sampling session, gill racks and the GI-tract were collected from 15 individuals, frozen and stored in food grade glass jars. Furthermore, muscle, Norwegian Quality Cut (NQC; Figure 2.15) and kidney samples were collected for the FHF project # 901521“Salmodetect”.

Figure 2.15 – Farmed salmon. Tissue dissection and NQC collection.

2.2.7 Feed pipe abrasion test

The formation and the size distribution of MPs produced from feeding pipes, as well as the influence of the feed pipe shape and age was investigated using 5-meter pieces of two new and two aged HDPE feed pipes. To simulate different naturally occurring shapes of the pipes at a coastal aquaculture site, one of each of the new and aged pipes were placed on a plane testing table and curved off with a horizontal plane to reach a 10˚angle (Figure 2.16). The remaining two pipes were kept straight. Pipes were weighed before and after the experiment. Pellets were pushed through the pipes twice a day (6h + 6h) for one week under the conditions reported in Table 2.6. Approximately a total of 2 tonnes per day of artificial, uneven spheroid pellets made of clay, very fine sand, agarose and sunflower oil were used to simulate pellets. The artificial pellets mimicked the physical properties of real feeding pellets, such as density, weight, dimension, fragmentation behaviour (according to ASTM - C131, 2006) and abrasion properties (according to ASTM F735, 2017).

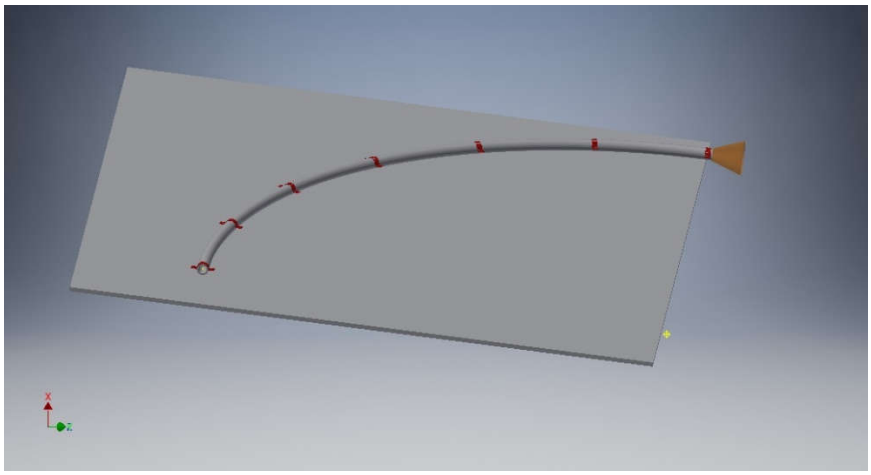

Figure 2.16 – Illustration of the curved feed pipe used within the experiment.

Table 2.6 –Conditions applied during the feed pipe abrasion experiment.

Parameter	Value
Air speed	20 m/s
Pellet speed	15 m/s
Pressure	0.5 bar
Temperature	70 ˚C
Blower system - Air volume	200 m^3/h
Blower system - pressure	1 bar

After the experiment, the pellets and the resulting dust were collected in pre-cleaned stainless-steel barrels for chemical-physical characterization.

2.3 Method development – extraction and purification protocols

The main aim of the method development part of this project was to extract MPs from the investigated samples, and to apply a gentle and efficient purification step prior to chemical identification in a way that allows for a quantitative analysis. The main interferents for a reliable quantification are the organic components. In this project this would be a complex mixture of proteins and fats (natural esters of glycerol, as well as fatty acids) that may trap and aggregate MPs. Fishmeal and wheat gluten represent protein-rich raw materials and sunflower meal represents esters and fatty acid rich raw materials. The liquid samples, such as rapeseed oil, fish oil and oil from farmed fish, are characterized by a high fraction of hydrophobic natural esters of glycerol and a fraction of various fatty acids that accounts for

more than 95% of the total mass. The fats in the dry feed materials and viscosity of the liquids present a problem for extraction and purification of samples, i.e. for the separation of the MP fragments from the matter. These are factors that can reduce the efficiency of the extraction process, as well as interfere with the chemical analysis and quantification process, causing an increase in the background signal and reduce the signal-to noise ratio.

2.3.1 Optimization of protocol for raw materials and fish feed

For all matrices, optimization of the purification steps was performed to minimize the organic content during the chemical identification of polymers by µFTIR (micro Fourier-Transform Infrared Microscopy) and Pyr-GCMS (see subchapter 2.4). As a starting point, to remove proteins and fats, a multi-step sequence of dispersants, enzymes and oxidizing treatments were tested. The selection of reagents was based on our previous experience and successful applications for other complex matrices such as sewage sludge (RFF Vest project # 260053/2016). Different combinations of enzyme concentrations, oxidizing agent concentrations, reaction times and reaction temperatures, were tested before an optimum protocol for removal of interfering organic compounds was identified. The protocol was used for the further sample processing.

Ten replicates of 10 g from each of the raw materials and fish feed were used for each trial. Commercially available batches of protease, lipase and lignin oxidase were obtained from a professional supplier (Sigma, Darmstadt, Germany). The results of the optimization are seen in Figure 2.17, showing the final flow chart for sample preparation of the solid samples.

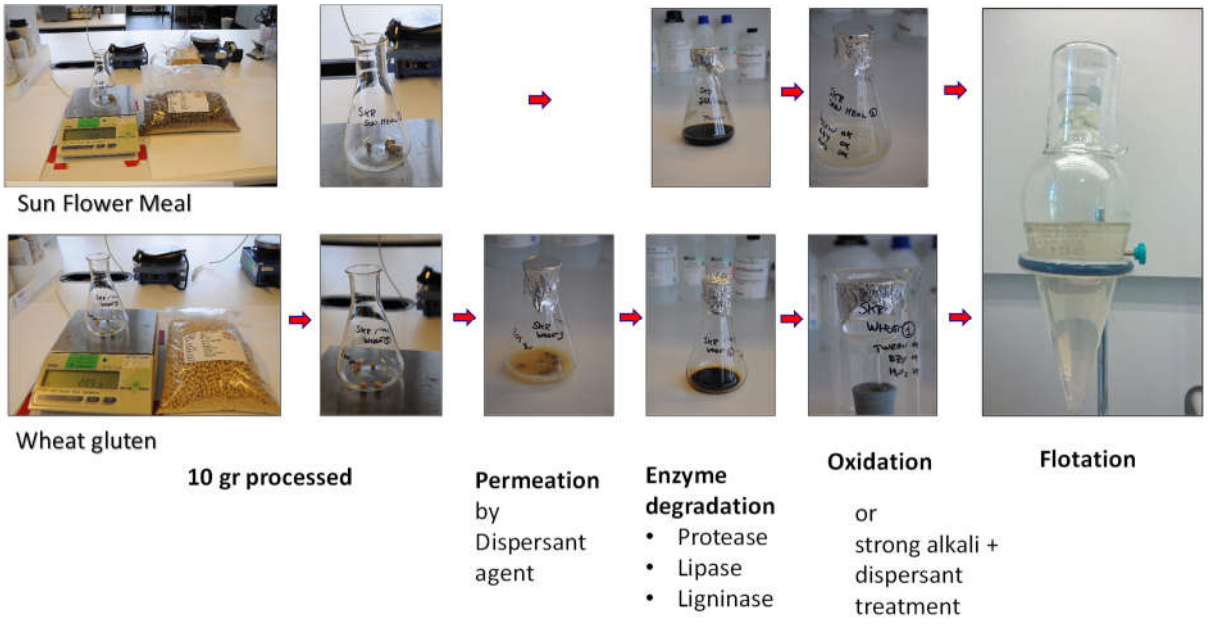

Figure 2.17 - Visual flow chart of sample preparation for solid samples.

Success criteria for the evaluation of fat removal were a) minimal clogging; b) efficiency of the filtration step and c) signal-to-noise ratio calculated during the quantification steps. The method of choice became a chemical-physical driven extraction/purification, to increase the water solubility and decrease the viscosity of the oily samples. A surfactant chemical, polysorbate 20, in combination with a 10% aqueous solution of potassium hydroxide (KOH), was used. The processed samples were filtered through 10 µm mesh stainless steel filters and washed twice with 30 mL of pre-filtered Milli-Q water to clean the filter, prior to a final density separation step using zinc chloride (1.70–1.75 g/cm^3) for 96h in a glass separator funnel. The supernatant was collected, filtered, washed and stored in 50% ethanol at room temperature for analysis.

Recovery tests for polymers during treatments were performed to ensure that MPs were not degraded during the treatment conditions. Tests were performed on microbeads of PE (100 µm), PP (20 µm) and PVC (250 µm) for the optimized protocols for both dry and liquid raw materials. Five replicates were run using enzymatic, oxidizing and/or alkali treatments, and two controls encompassing the same polymers treated with Milli-Q water. Using Pyr-GCMS the loss rate was determined to range between 0 and 3% loss for treatments, and between 1 and 4 % for controls (treated with Milli-Q). We conclude that the treatment did not induce significant polymer degradation or loss for the tested MP types. The results of the recovery test are reported in Table 2.7.

Table 2.7- Results of the degradation test in the selected polymer types using oxidizing and alkali reagents as treatments and Milli-Q as control. Values are reported in µg ± standard deviation.

Treatment	Before treatment	After treatment	Loss rate
(n=5)	µg		
PE	100.0 ± 10.0	98.1 ± 2.1	≈1 %
PP	3.0 ± 0.5	3.0 ± 0.6	0 %
PVC	500.0 ± 6.0	490.0± 4.0	≈ 2 %
Control	**Before treatment**	**After treatment**	**Loss rate**
(n=2)	µg		
PE	99.0 ± 11.0	97.1 ± 4.1	≈ 1%
PP	3.0 ± 0.3	2.9 ± 0.3	≈ 1 %
PVC	500.0 ± 6.0	470.1 ± 13.0	≈ 4%

Several recent publications have pinpointed that under strong oxidizing or alkali conditions, under high concentrations, high temperatures >60˚C and long incubation times >48h irreversibly damage some polymers, hampering their detection by current analytical methods. Therefore, the temperature was limited to 50˚C and incubation time to 36h. As an alternative, the iron catalysed hydrogen peroxide oxidation (Fenton's reaction) was tested to optimize the degradation of organic matter with reduced sample preparation duration. However, the high reactivity of some types of materials leads to sudden increases in temperature with bubble formation which are hard to control. Therefore, the final set up for the Fenton's reaction (duration, incubation temperature, hydrogen peroxide concentration) should be evaluated for each sample.

2.3.2 Method used for marine sediments, seawater, suspended matter and tissue of marine biota

For sediment analysis, bulk samples from each of the sampled sites were homogenized with a standard stainless-steel orbital feed mixer with a K-beater knife. The dry weight (DW) was estimated and 1 kg (DW) of sediments were processed. MPs were extracted from sediment samples by density separation using zinc chloride (specific gravity of 1.70 g/ cm^3) in a Micro-Plastic Sediment Separator (MPSS, HYDROBIOS, Germany; Figure 2.18) following procedures described in Imhof et al (2012) and Haave et al. (2019).

Figure 2.18 - Micro-Plastic Sediment Separator (Hydrobios, Germany) used to extract microplastics from the collected sediments (Left). Detail of the top chamber with the extracted sample (Right, photo: NORCE).

The extracted sample was collected from the MPSS top chamber (Figure 2.18, right side) and size fractionated (Hidalgo-Ruz et al. 2012) using a 300 µm stainless steel sieve. All potential MP particles in the 300 µm-5 mm fraction were manually isolated by visual investigation under a WILD MZ8 binocular microscope, photo-documented by a MC190 HD camera (both Leika, Germany) and characterized by ATR-FTIR analysis. All potential MP in the 10–300 µm fraction were analysed by both µFTIR and Pyr-GCMS after purification and pre-concentration steps using combined enzymatic and oxidizing treatments. Samples were first treated with a surfactant, sodium dodecyl sulfate (SDS), followed by enzyme treatments, i.e. protease and cellulase, then oxidized with Fenton's reagent and density separated according to Löder et al. (2017).

For the MP content in suspended matter, samples were dried and gently homogenized with a stainless-steel spatula. The total initial dry weight was recorded before starting the sample preparation. Samples were treated with cellulase, protease and further oxidized with Fenton's reaction to reduce the interference of organic matter. Plastic particles in the digested samples were extracted with a final density separation step in zinc chloride solution by adding $ZnCl_2$ powder to reach a final density of 1.70 g/cm^3, size fractionated and chemically identified by Pyr-GCMS and µFTIR.

For the estimation of the MP content in seawater samples, the 300 µm-5 mm fraction was treated with SDS, followed by Fenton's reaction, prior to manual isolation and recording through stereomicroscopy and ATR-FTIR analysis. MPs in the 10–300 µm fraction were first treated with SDS, followed by enzymes, protease and cellulase, oxidized with Fenton's reagent and density separated by means of a solution of zinc chloride (density: 1.70 g/cm^3) in glass separator funnels.

Biological samples such as fish gills and GI-tracts were weighed, the inside of the GI-tract rinsed in Milli-Q water, treated with 5% SDS overnight, followed by protease, cellulase, lipase

enzymes, and finally oxidized with Fenton's reagent. The obtained extract was density separated by a zinc chloride solution (density 1.70 g/cm^3) following a modified protocol from Löder et al. (2017). The supernatant was filtered through a 10 µm stainless steel mesh, washed with ethanol:water (50:50) and concentrated in 5 mL ethanol:water (50:50) prior to chemical characterization by µFTIR and Pyr-GCMS analysis.

For histological analyses, 5 µm thick cryosections of gills from both farmed and wild salmon samples were made, air-dried for 10 min and stained with hematoxylin and eosin according to Pittura et al. (2018). The sections were inspected at the Veterinary Institute facility located in Oslo using a Leika DM 5000H microscope coupled to a Nikon DS-Ri2 camera using polarized light (Figure 2.19).

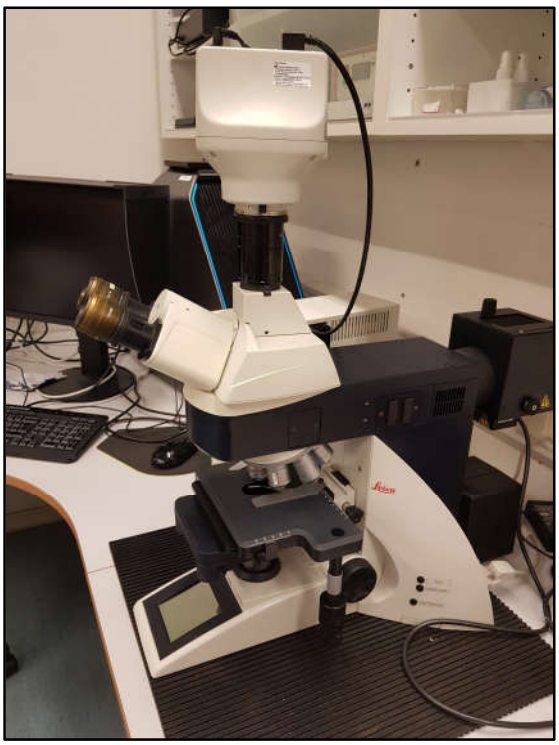

Figure 2.19 - Microscope (Leica) coupled to a Nikon DS-Ri2 camera with polarized lens used to localize plastic particles in cryosections of samples salmon's gills at the Veterinary Institute, Oslo.

2.3.3 Method used for material from the abrasion experiment

Sub-samples of approximately 20 kg of pellets and dust per tested treatment were submitted to analysis. Five replicates of each of the testing conditions were performed. Samples were gently mixed in hot (50 ˚C) saturated NaCl solution (density: 1.25 g/cm^3) for 3h to density separate MPs from the pellet surface and incubated for 5 days. After flotation, the obtained supernatant (≈ 500 mL) was collected and particle size was analysed with a Multisizer 3 Coulter Counter (Beckmann Coulter Counter, Germany) with a 100 µm capillary aperture. 5 mL aliquots were dissolved in 95 mL of Isoton II™ diluent for the analyses. 10 technical replicates were measured from each of the testing conditions. A limited number of sub-

aliquots were further analysed by µFTIR microscopy for chemical characterization. Analysed feeding pipes were weighed before and after the abrasion simulation experiments to record the loss of weight.

2.3.4 Plastic free laboratory and contamination control

Tissue dissection was performed at the Institute of Marine Research (IMR, Bergen). The MP laboratory at IMR is equipped with high efficiency ultra-low penetration HEPA filtration with an efficiency of 99.995% for the most penetrating particle size (0.3-0.5 µm particles). The laboratory has overpressure and the entrance has an airlock (sluice) with a sticky floor mat to avoid dust entry. The laboratory is entered with dedicated low-abrasion shoes and a cotton laboratory coat. Clothing with loosely weaved artificial polymer fibres is avoided. Either no gloves or Nitrile gloves are worn. Wherever possible, non-plastic equipment is employed. Samples are handled under a laminar flow bench (Class II biological safety, Thermo Scientific SAFE 2020). Tissue samples are prepared with parallel procedural controls, i.e. duplicates of open glass jars of filtered Milli-Q water are placed in the working area in the laboratory and in the LAF bench each working day.

The preparation of the raw materials, fish feed, sediments, suspended matter, seawater samples and the material obtained from the abrasion experiment was performed at the NORCE facility in Mekjarvik. All glassware used for sample preparation and analysis was pre-burned at 500 ˚C to remove traces of plastic contamination. All solutions and reagents used within the analysis were pre-filtered on pre-burned GF/F fiberglass filters. During the sample preparation phases, dust trap collectors (Pyrex crystallising dishes filled with 500 mL GF/F filtered Milli-Q water) were used to evaluate possible contamination from airborne particles. Daily, water from the crystallising dishes was collected and analysed for MP contamination. Additionally, a procedural blank was run together with the processed samples following the same treatment steps to estimate contamination through the reagents.

2.4 Identification of MPs by vibrational spectroscopy: µFTIR

Fourier Transform Infrared spectroscopy (FTIR) is a vibrational spectroscopy. Light with different wavelengths (energy) cause different vibrational pattern in the molecules in the polymers. The result can be seen as spectra with typical peaks or “fingerprint areas”, which can be used to chemically identify materials and plastic types, by comparison with reference libraries.

FTIR was performed in two ways depending on particle size. A qualitative analysis of selected potential plastic particles over 300 µm was assessed using Attenuated Total Reflectance FTIR (ATR-FTIR), while a quantitative analysis of MP from 10-300 µm was done by µFTIR imaging. Due to the analysis of chemical identity through the transmission/reflection of infrared light, MPs containing large amounts of carbon black, such as in car tyres, were not detected by FTIR analysis.

2.4.1 Analysis by ATR-FTIR

Very few particles were larger than 300 µm. The particles were picked out using tweezers under a stereo microscope, measured, weighed and analysed by ATR-FTIR. If possible, three spectra were acquired for each particle. The obtained spectra were then compared to an openly available spectral library (https://simpleplastics.eu/download.html). The identification was accepted if the similarity score was more than 70%. If the match was between 60 and 70% expert judgement of the spectra was applied to approve or reject the results. Below 60% the results were rejected.

2.4.2 µFTIR imaging

µFTIR imaging was performed using an Agilent Cary 620 FTIR microscope coupled to a Cary 670 FTIR spectrometer (Figure 2.20) at IMR. The system is equipped with a liquid nitrogen cooled 128x128 Focal Plane Array (FPA) detector, allowing for imaging of 128x128 pixels in a single measurement, a MIR Source with a spectral range of 9000-20/cm, purged enclosure, 15x IR/Vis reflective objective (NA 0.62, WD: 21mm), 4x Vis glass objective (NA 0.2, WD: 38mm), motorized sample stage, 0.1x0.1 MCT as well as GladiATR for single particle analysis of larger MPs. Extracted environmental samples were distributed on Anodisc ceramic filters, which were then imaged. Each pixel is imaged for the whole spectrometric range (Figure 2.21).

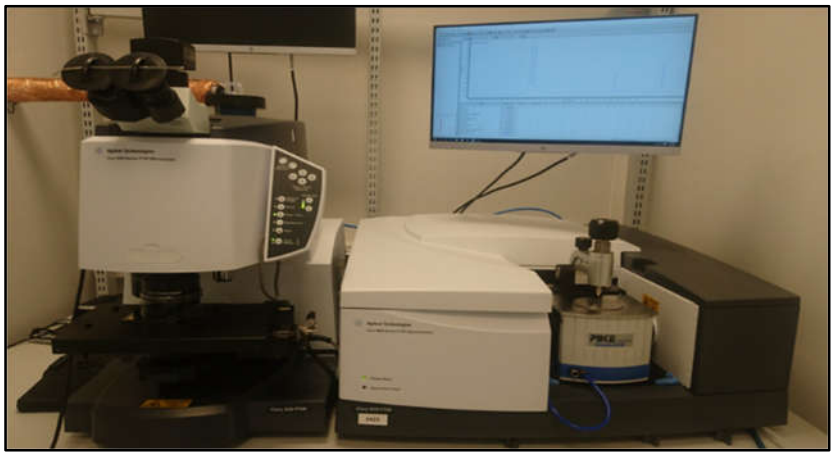

Figure 2.20 - µFTIR equipment at the IMR microplastic laboratory (Photo: Ørjan Bjorøy, IMR).

Simultaneous optical images allow for the determination of the size of the particles in two dimensions. Usually, these two dimensions are the larger dimensions, as the filtration process cause the particles to “lie down”. Automatic image processing smoothed the edges of the determined MPs and assigned a false color coding for chemical identity (polymer groups) to the particles (Figure 2.22). These data can be statistically analyzed according to number of particles per size and polymer group. With this system, both polymers and particle size distribution (>11 µm) of an extracted sample can be determined. For dataset analysis, data

was processed by siMPLE (Systematic Identification of MicroPLastics in the Environment, Primke et al., 2018) and spectra were compared to libraries from Bio-Rad and Agilent, the Alfred-Wegener Institute Helgoland and IMR's own additions. Since the analysis method is non-destructive, the same samples can subsequently be analyzed by Pyr-GCMS, thus providing information about the total mass per polymer group in the same sample. Pyrolysis adds the possibility to measure MPs below 11 µm, if there is enough mass to exceed the limit of quantification.

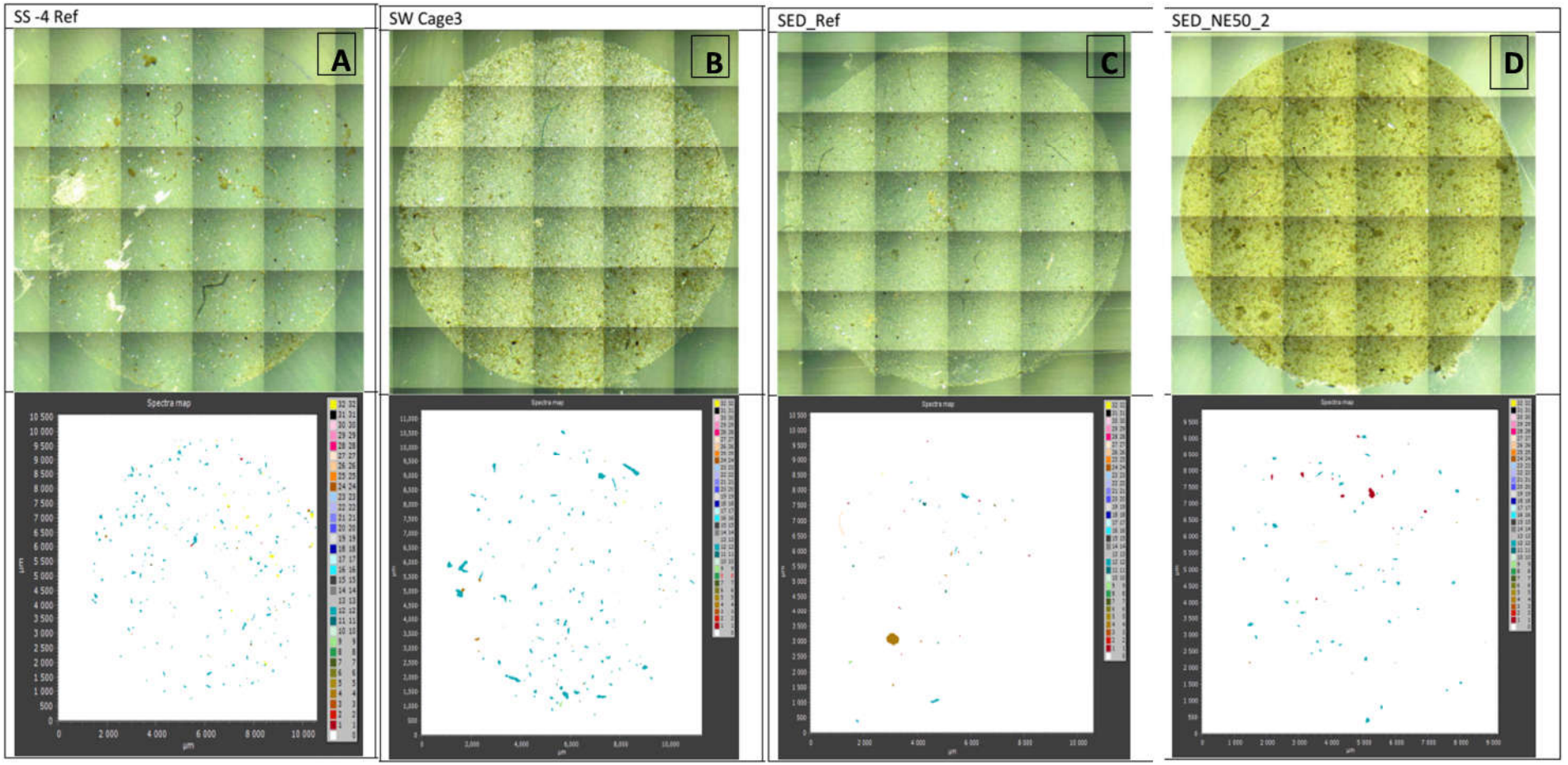

Figure 2.21 – Visual images of the filters (upper part) and false color plots showing different plastic polymers detected by FTIR imaging (bottom) of the same filters. Color codes for chemical identity groups. A: from suspended solid matter collected at the reference site, B: from seawater sample at Kje0, C: from sediments sample at the reference station and D: from sediment collected at NE50.

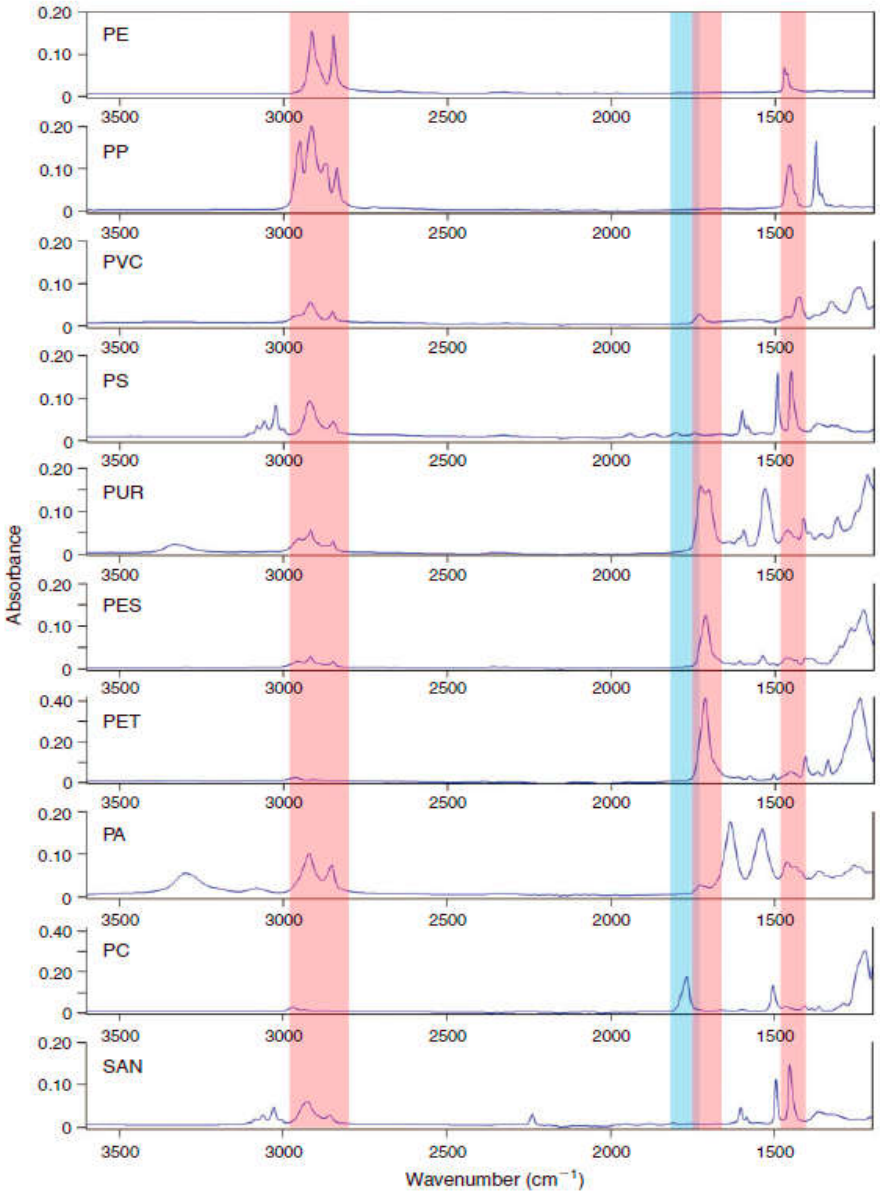

Fig. 10. Identification scheme for the marking of potential microplastics of different polymers by the band regions 1480–1400, 1760–1670 and 2980–2780 cm^{-1} (red, for polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PUR), polyesters (PES), polyethylene terephtalate (PET), polyamide (PA) and styrene acrylonitrile (SAN)) and 1800–1740 cm^{-1} (blue, for polycarbonate (PC)) by focal plane array (FPA) detector-based chemical imaging.

Figure 2.22 - Examples of fingerprint spectra used for polymer identification by FTIR, from Mintenig et al. (2017).

2.5 Thermal degradation analysis: Pyr-GCMS

Pyrolysis Gas Chromatography Mass Spectrometry (Pyr-GCMS) is a thermal decomposition of materials at elevated temperatures in an inert (low-oxygen) atmosphere, avoiding burning that involves oxygen. Large molecules break at their weakest bonds, producing smaller, more volatile fragments. These fragments can be separated by gas chromatography and detected by a mass spectrometer. The output data can either be used as a fingerprint to identify material, or the GCMS data can be used to identify individual fragments to obtain structural information. The obtained pyrograms, with peaks of ions appearing at different retention times, are compared with a customized database and cross-checked with literature to identify the chemical composition of the material using recommendations and selecting criteria from Fischer and Scholz-Böttcher (2017) and Gomiero et al. (2019). Standard curves with known concentrations are used to calculate the concentrations of materials present in the sample. In contrast to FTIR, Pyr-GCMS is a destructive method that irreversibly degrades the polymers and does not produce an image of the material, however it provides the mass of the identified polymers independent of the particle size. The methods FTIR and Pyr-GCMS are therefore complementary and increase the information gained from an extracted sample. Pyr-GCMS analyses were performed by NORCE (Stavanger) with a Shimadzu Optima 2010C GCMS controlled by GCMS solution V 4.45, equipped with a Rxi-5ms column (RESTEC, Bellefonte, PA) and coupled with Frontiers lab's Multi-Shot Pyrolizer EGA/PY-3030D with auto-shot sampler (BioNordika, Norway, Figure 2.23).

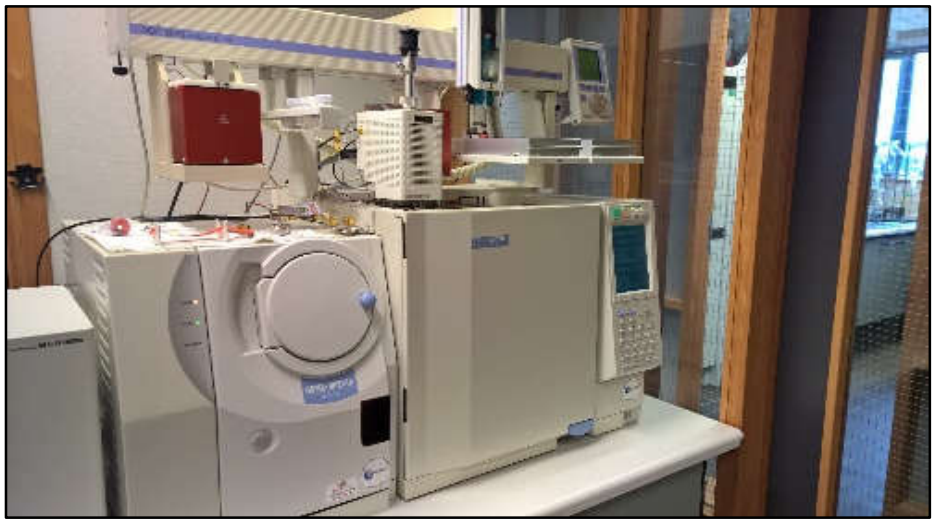

Figure 2.23 - Pyr-GCMS equipment at NORCE PlastLab (Photo: Alessio Gomiero, NORCE).

2.6 Statistical analyses

Data were analysed using Statistica 12 (StatSoft) statistical software. Data distribution was tested for normality by means of Shapiro-Wilk's W test and for homogeneity of variance with Levene's test. Data showing a normal distribution were tested with ANOVA, otherwise the

non-parametric test for statistical dependence based on the Kendall's rank correlation coefficient (tau) was performed to measure the strength of dependence between independent and dependent variables and to assess significant correlations. Alternatively, the Correlation matrix analysis was performed. Significance level was set to $p<0.05$.

3 Results

3.1 Occurrence and concentrations of MPs in raw material and finished fish feed products

In fish feed, 10 large plastic fibres (ranging from 0.8 to 1.2 mm length) were identified as PP in the feed pellet. In the remaining solid and liquid fish feed ingredients no MPs >300 µm were observed. Occurrence of MP (in the 10-300 µm range), was found in all three investigated fish meal batches. An average of 1, 0.1 and 1.3 particles (21- 38 µm /g material) were observed for batch #1, #2 and #3, respectively. PA accounted for the most abundant polymer type in fishmeal batch #1 (80%) while a single PE particle was detected in fishmeal batch #2. PET (62%) followed by PA (31%) and PE (7%) were detected in the fishmeal batch #3 (Figure 3.1).

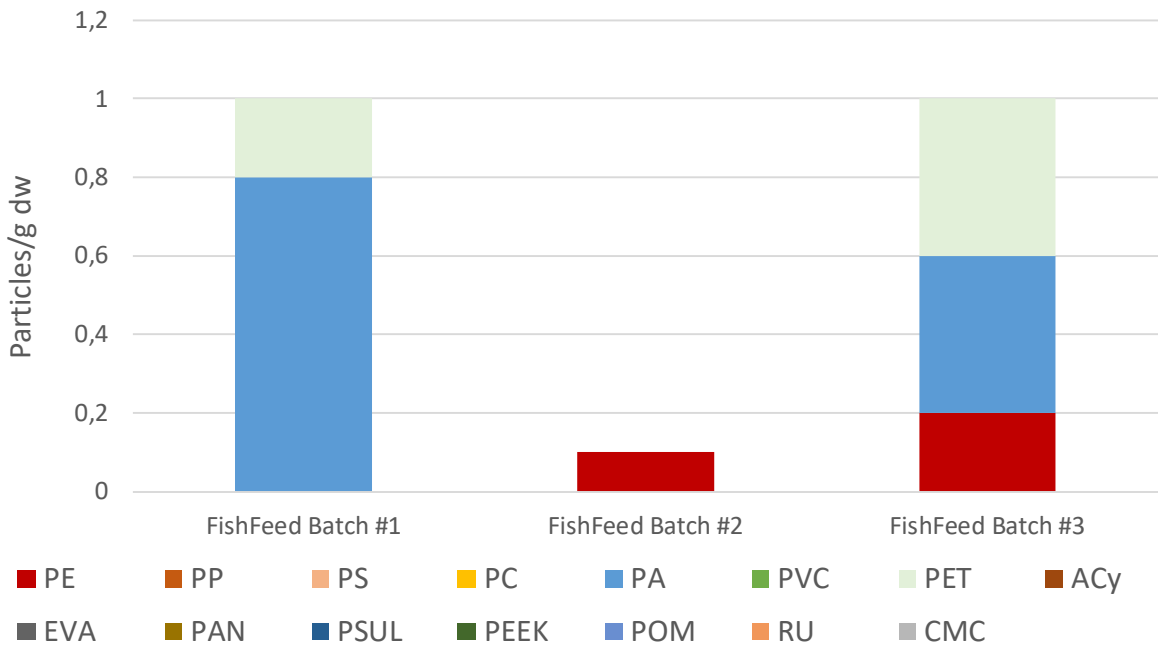

Figure 3.1 - Polymer composition of MP (10-300 µm, identified by µFTIR) in fish meal batches #1, #2 and #3.

The same extracts were further analysed by Pyr-GCMS. The analysis confirmed the occurrence of PE, PA and PET with concentrations ranging from < LOQ (LOQ = 1 µg/kg DW) to 8.2 ±1.0 µg/kg DW (Table 3.1).

The occurrence of the large PP particles in the final fish feed, both before and after fat coating, was further investigated. After the examination of the feed production process, the industrial process responsible for the contamination was identified as being the mechanical system which opens the wheat gluten ingredient produces large PP particles from bags by cutting the bottom. For the remaining ingredients, no MPs were observed.

Table 3.1 – Result of the chemical quantification of plastic polymers in investigated raw materials and finished feed product. Concentrations given as <1 and <2 µg/kg DW indicates that concentrations were below the Limit of Quantification (LOQ).

Feed ingredient	µg/Kg DW							
	PE	PP	PS	PVC	PA	PMMA	PC	PET
Soy protein concentrate	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Wheat gluten	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Fishmeal batch #1	6.0 ± 1.0	< 1	< 1	< 1	8.0 ± 1.0	< 1	< 2	< 1
Fishmeal batch #2	4.3 ± 1.0	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Fishmeal batch #3	< 1	< 1	< 1	< 1	6.2 ±1.0	< 1	< 2	8.2 ± 1.0
Wheat	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Fava beans	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Sunflower meal	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Rapeseed	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Fish oil crude low	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Fish oil from farmed fish	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Crushed beans	< 1	< 1	< 1	< 1	< 1	< 1	< 2	< 1
Finished feed before fat coating	9.2 ± 4.3	16.0 ±5.1	< 1	< 1	< 1	< 1	< 2	< 1

3.1.1 Characterization of the abrasion effect in the feed pipes

An abrasion test was performed on new curved and new straight pipes (NCP and NSP, respectively) as well as aged curved (ACP) and aged straight (ASP) feed pipes. Figure 3.2 shows an example of one of the pipe sections used.

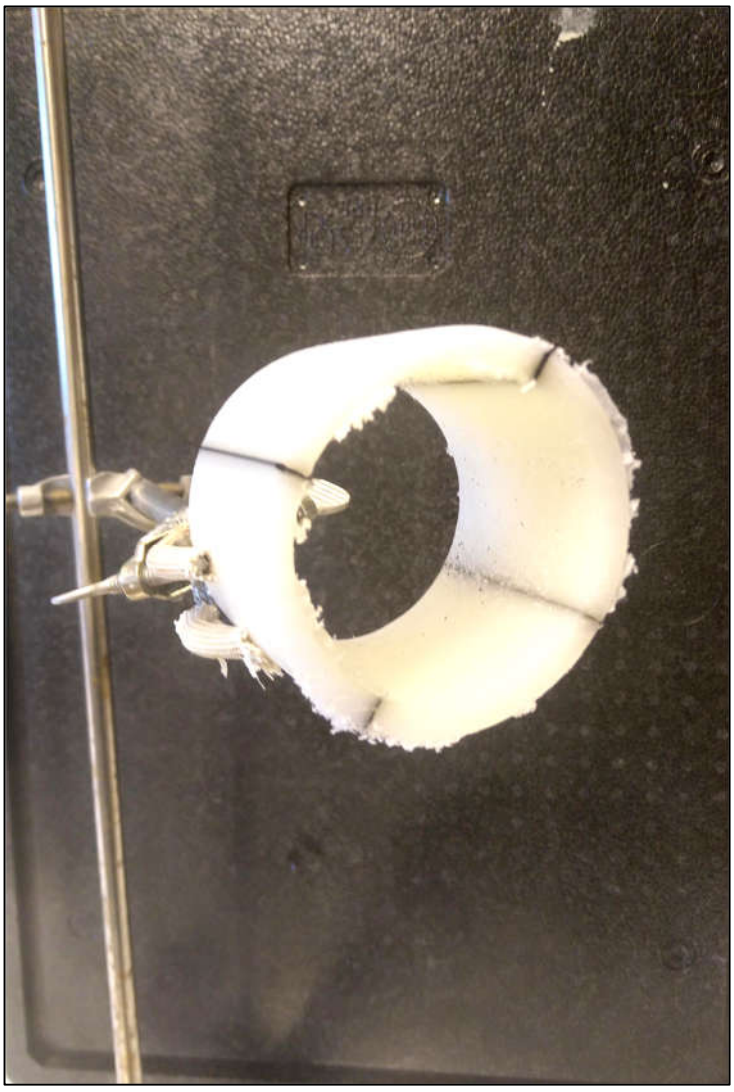

Figure 3.2 - Section of the feeding pipe used for the abrasion simulation phase.

The µFTIR microscopy analysis confirmed that the fragments were 100% polyethylene from the HDPE pipes. The analysis of the grain size showed that the different testing conditions induced a different particle size distribution. The flow of artificial pellets in the NCP created a

bimodal distributed fragmentation with two main peaks of median average particle size of 2.2 and 6.7 µm (Figure 3.3- A). While, under the same experimental conditions, the flow of artificial pellets in the ACP feeding pipe produced a fragmentation pattern with particles having a normal distribution centred on 2.8 µm (median value; Figure 3.3-B). The straight feeding pipes showed different behaviour under the tested conditions. The flux of pellets through NSP produced fragments between 3 and 15 µm, with a median value of 5.9 µm (Figure 3.3 C), which is similar to the secondary peak observed in the fragments from NCP (Figure 3.3-C). For the ACP the grain size distribution tended to shift toward smaller particles (Figure 3.3-D). Note that the instrument's 95% confidence range is in the range of 2.1–80 µm for the given capillary aperture (100 µm), thus the software applied a cut off calculation for particle sizes below the trustable detection area (< 2.1 µm, Figure 3.3). This indicates that particles smaller than 2.1 µm may be produced but not detected by this method.

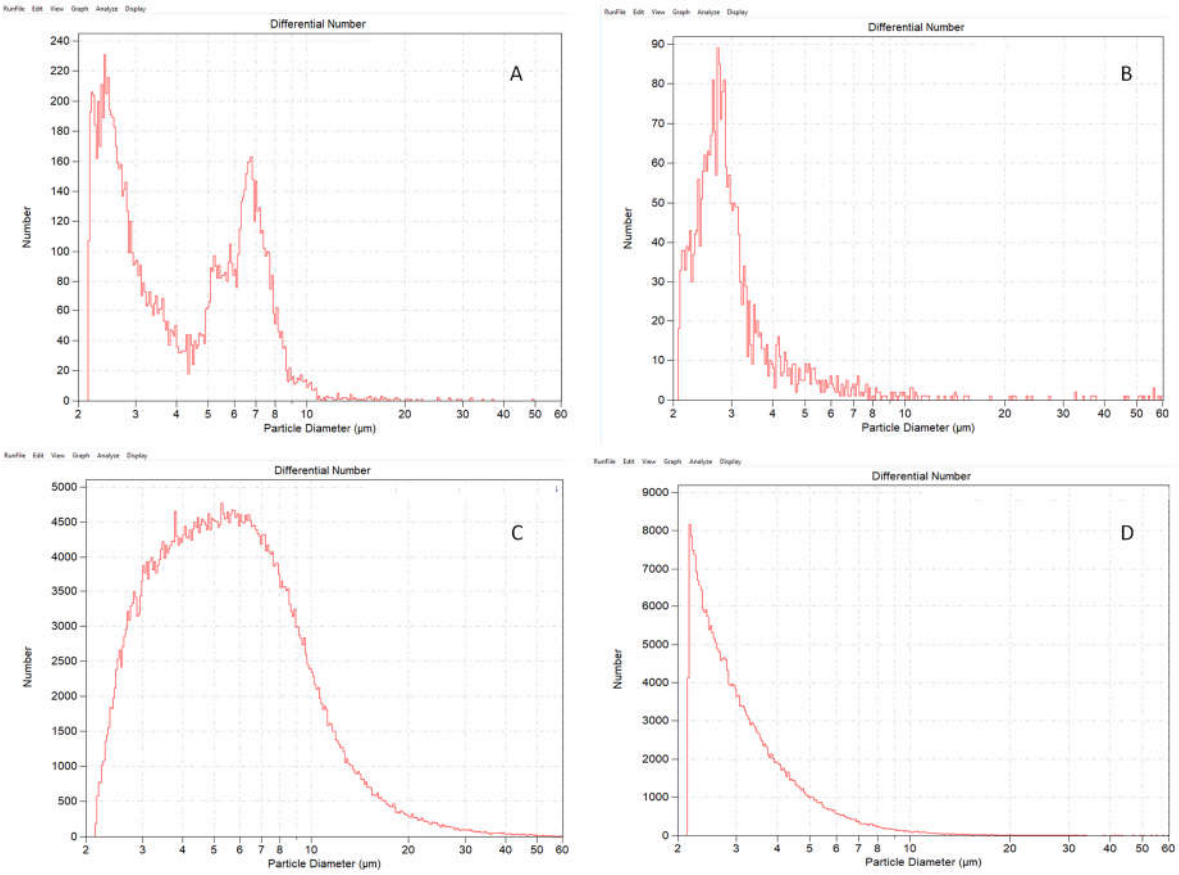

Figure 3.3 - Size distribution of fragments resulting from abrasion test in feeding pipes. A: New curved pipe, B: Aged curved pipe, C: new straight pipe and D: Aged straight pipe. Note lower size cut-off of 2.1 µm.

The weight of the 5-meter feeding pipes was measured and recorded before and after the experimental activities. Data are reported in Table 3.2. The weight loss after abrasion was higher in aged pipes than in new ones, which may be due to plastic turning more brittle in time due to the leaching of plasticizers and additives. The curved shape also caused a significantly higher weight loss (Mann-Whitney, $p<0.05$). This could be expected, as pellets would hit a limited area of the inner surface more frequently in a curved pipe. During the one week experiment a loss of 5-14 g of pipe material was recorded, meaning an average loss of 0.10-0.40 g/meter/day at the given experimental conditions. This adds up to a theoretical release from 150 to 569 kg/year of MPs per aquaculture site, assuming a production set up with 8 cages and an average length of the feed pipes of approximately 500 m. Lower levels of release are reported by the model calculation developed under the FHF granted project "Havplast" with estimated global emissions in Norway ranging from 10 to 100 tonnes/year (SALT, 2019).

Table 3.2- Weights values of feeding pipes before and after the abrasion test and estimation of the weight loss (gr/meter/day).

Feed pipe status -tested condition	Length (mm)	Initial weight (gr)	Final weight (gr)	Loss (gr/meter/day)
New Pipe – Straight shape	511.0±2.0	9249.1±0.2	9245.1±0.1	0.11
New Pipe Curved shape	501.0±3.0	9068.1±0.4	9062.4±0.2	0.16
Aged pipe – Straight shape	499.0±1.0	6636.7±0.1	6625.8±0.2	0.31
Aged pipe Curved shape	515.0±3.0	6849.5±0.3	6835.4±0.3	0.39

The aim of the study was to assess the possible input of MPs to aquatic environments associated with the feed pipes with special focus on the contribution of plastic aging in the abrasion pattern, as well as to provide a preliminary characterization of the produced particle sizes. Despite the limits of the simulation and the applied experimental set up such as short length of pipes, static shape, flow set up parameter, the study documents the potential formation of plastic particles in the low µm size range. The current method does not detect potential nanoscale plastic particles that are likely to form. In a real life scenario there will be a large variability of key parameters, such as the length of the pipes, their age, the occurrence of wind and waves resulting in different shapes when applied, the feeding schedule and the feeding set up (pressure and flow, the amount of pellets per hour, dimension, shape and characteristics of the pellets). These all influence the final wearing processes. Therefore,

extrapolating the values obtained by this experiment to model the overall input of MPs from feeding tubes into the aquatic environment may not be representative of the situation at a farm. The obtained results indicate a potential significant loss of pipe material due to abrasion, and may be an area for mitigating actions, following further studies.

3.2 Occurrence and concentrations of MPs in the environmental samples

3.2.1 MPs in marine sediments

In all sediments collected, the plastic fraction > 300 µm contained very few black, green and orange coloured plastic particles and fibres. These were found in the sampling sites close to the cage (NE50 and SE50). Black particles are consistent with the fact that some structures of fish farms are made of black plastic. Several constituents of fish farms, such as feed pipes, which can have white and black parts, are made of PE. Green or orange coloured PA particles are consistent with ropes and nets used in the farms and were observed in sampling sites close to the net pen, but not at the reference station.

The 10-300 µm fraction of MP ranged from 27 to 287 particles/kg DW at stations NE 250 and SE480, respectively. These values could be even higher as the current method has difficulties in recognizing and quantifying acrylates, polyurethanes and varnish from natural non-plastic materials. Matrix effects may have hampered the detection of acrylates, polyurethanes and varnish in these samples as the spectral signals of these polymer groups were not significantly different from the modified fatty substances expected to be found in these sediments. In the end, the data for acrylates, polyurethanes and varnish had to be removed. To better distinguish acrylates from the fatty substances, methods need to be developed, specifically focussing on sample extraction, instrumental set-up, library development and characterization of the fatty substances from the sediments located around aquaculture installations.

In general, the highest particle concentrations were found in sediments in the dominating direction of currents (the South East; SE transect), followed by sediments collected from the North East (NE) and South (S) transects.

The size distribution of MPs showed similar patterns for all sampling sites including the reference site. PE and PP were found in all sediment samples and together accounted for over half (54%) of the identified MPs. On average, PE contributed from 8 to 72% in the analysed samples. PP was the second most frequent polymer encountered, showing a contribution ranging from 10 to 41% in the sediment samples. Polyesters including PET (13%) and chemically modified cellulose (12%), followed by polyamide-nylon (8%) and PS (7%) accounted for most of the remaining identified MP. Not all polymers were found in all samples.

At the site closest to the net pen (Kje0), the dominant polymer was PE (72%). PP and PE showed a gradient-like distribution in the sediments of the investigated sites with high levels characterized near the cage (Kendall's tau correlation test, $p< 0.05$). The relative contribution per polymer and the estimated total number of MPs from µFTIR are given in Figure 3.4.

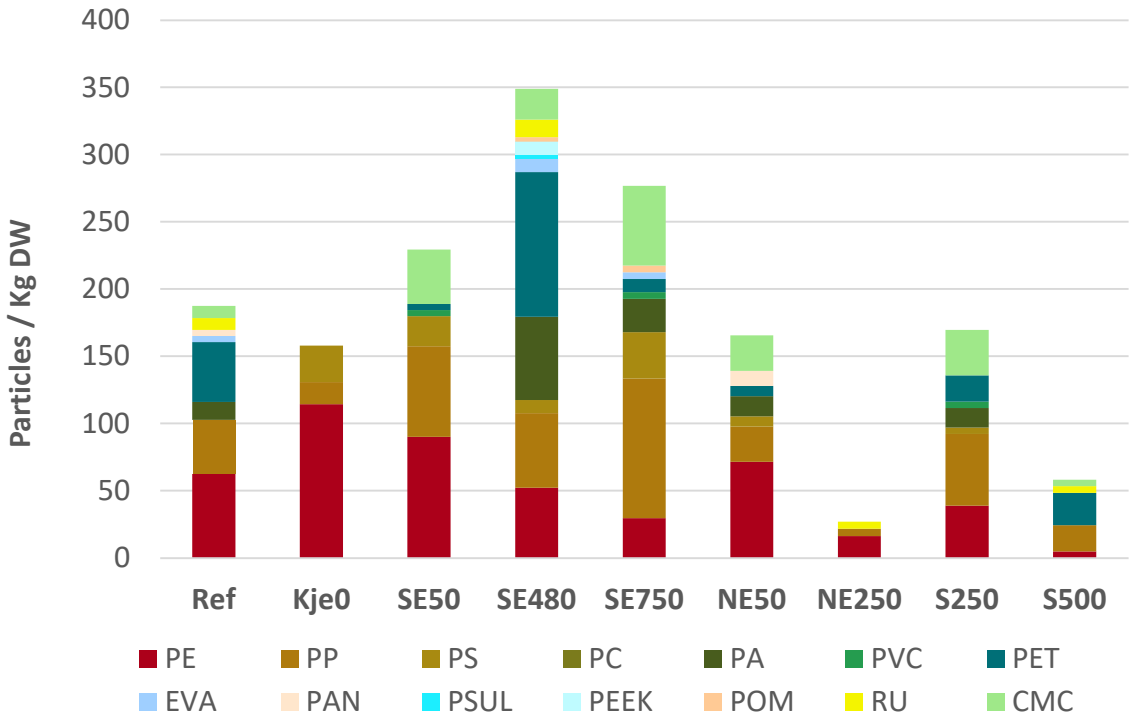

Figure 3.4 – µFTIR analysis: polymer distribution and MP particle concentration kg^{-1} DW for particles in the 10-300 µm fraction in sediments sampled in the North East (NE), South (S) and South East (SE) transects as well as the reference site (Ref). Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC), Polyamide-Nylon (PA), Polyvinyl chloride (PVC), Polyester and Polyethylene Terephthalate (PET), Ethylene vinyl acetate (EVA), Polyacrylonitrile (PAN), Polysulfone (PSUL), Polyetheretherketone (PEEK), Polyoxymethylene (POM); Rubber (RU), chemically modified cellulose (CMC).

The same samples were subsequently analysed by Pyr-GCMS to estimate the mass distribution of a selected group of environmentally relevant polymer types (PP, PS, PE, PVC, PET, PMMA, PA and PC; Figure 3.5) and to verify the results of the µFTIR analysis (Figure 3.4) Total polymer concentrations ranged from 38.5 to 110.2 µg/kg DW observed at stations S500 and Kje0, respectively. According to Pyr-GCMS analysis, PP, PE, PET and PA were the most accumulated polymers in the top layer of the investigated sediments, similarly to the FTIR-results. The concentrations varied among the different transects with PP ranging from < 1 (SE750) to 12 µg/kg DW at Kje0 (average 10% of total), PE ranging from 5 µg/kg DW at the reference site (2% of total) to 22 µg/kg DW reported in sediments collected at the Kje0 site (18% of total); PET ranging from 9 µg/kg DW at S500, corresponding to 20% of the total, up to 22 µg/kg DW at NE250 representing 32% of the total; PMMA ranging from < 1 at SE50 to 6 µg/Kg DW at SE480 (10% of the total); PS ranging from 2 to 20 µg/kg DW, found at S250 and Kje0, respectively, PA ranging from 11 µg/kg DW at site SE750 (19% contribution in the total

estimated polymer's mass in the site) to 38 µg/kg DW at NE50 (49% of the total estimated polymer's mass in the site) and PVC ranging from 0.3 µg/kg at Kje0 to 1.9 µg/kg at NE250. PC was not detected in any of the investigated samples. A statistically significant space related distribution with higher values observed close to the pens was observed only for PE and PA polymer types in both the SE and the NE transects (ANOVA, $p<0.05$). Such distribution might have been influenced by a combination of the main current's orientation as well as the seafloor shape. In contrast, the remaining polymers such as PS, PET, PMMA and PS showed a homogeneous distribution among all investigated sites. This may be explained by detection limits, by a nonsignificant contribution of the aquaculture sites and other local sources such as discharges of sewage treatment plants, urban runoff, occurrence of other industrial activities contributing to the pollution with these plastic types, or a different distribution behaviour of the particles that were not detected by this assessment design.

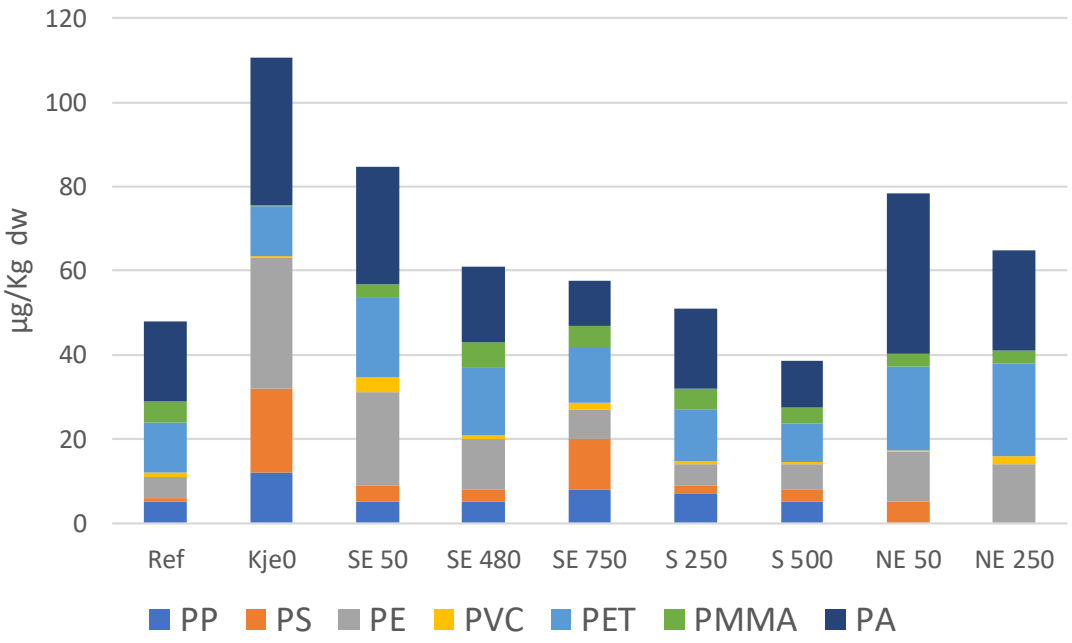

Figure 3.5 – Pyr-GCMS analysis: polymers mass distribution kg^{-1} DW for particles in the size fraction 10–300 µm. Sediments sampled in the North East (NE), South (S) and South East (SE) transects and at the reference site (Ref). Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC), Polyamide-Nylon (PA), Polyvinyl chloride (PVC), Polyethylene Terephthalate (PET).

3.2.2 MPs in suspended matter

For the analysis of suspended matter, two replicates for each of the depths (-4 and -20 m below sea level) were processed. Between 1.1 and 1.5 g (DW) of sediment material were analysed (Table 3.3).

Table 3.3 -Dry weight for each of the suspended matter samples.

Site	Depth and replicates	Dry weight (g)
Kje0	-4m _R_1	1.2
	-4m _R_2	1.3
	-20m _R_1	1.4
	-20m _R_2	1.5
Ref	-4m _R_1	1.1
	-4m _R_2	1.2
	-20m _R_1	1.2
	-20m _R_2	1.3

No plastic particles > 300 µm were found in the sedimentation traps. However, for the 10-300 µm size fraction the number of MPs ranged from 220 at the reference site at -20m to 360 particles/g dry weight at the reference site at -4 m (Figure 3.6). At the reference site at -4m the dominant particle was Rubber (RU; 31%), followed by PP (18%) and PA (12%). The reference site also had measurable amounts of polyvinylchloride (PVC), Ethylene vinyl acetate (EVA) and chemically modified cellulose (CMC). A lower total particle number was observed at the net-pen (Kje0). FTIR analyses showed that the dominant polymers at Kje0 at -20 m were PET (31%), PP (23%) and PA (20%). Only minor amounts of PE (3% of all identified particles) were found at Kje0. This station also had measurable amounts of EVA, RU and CMC. At Kje0 -4m the most abundant polymer types were PET (27%) and PA (21%), followed by RU (18%), PP (15%) and PE (14%). An attempt was made to extrapolate the mass of the MP identified in the smaller size fraction (10-300 µm) based on the volume of the particles, assuming an ellipsoid shape, and the density of the polymer type. Such theoretical calculations show an increase in polymer mass for PET at -20m at the cage site relative to the reference site, and increased polymer mass for PE and PP at -4m in the cage site relative to the reference site.

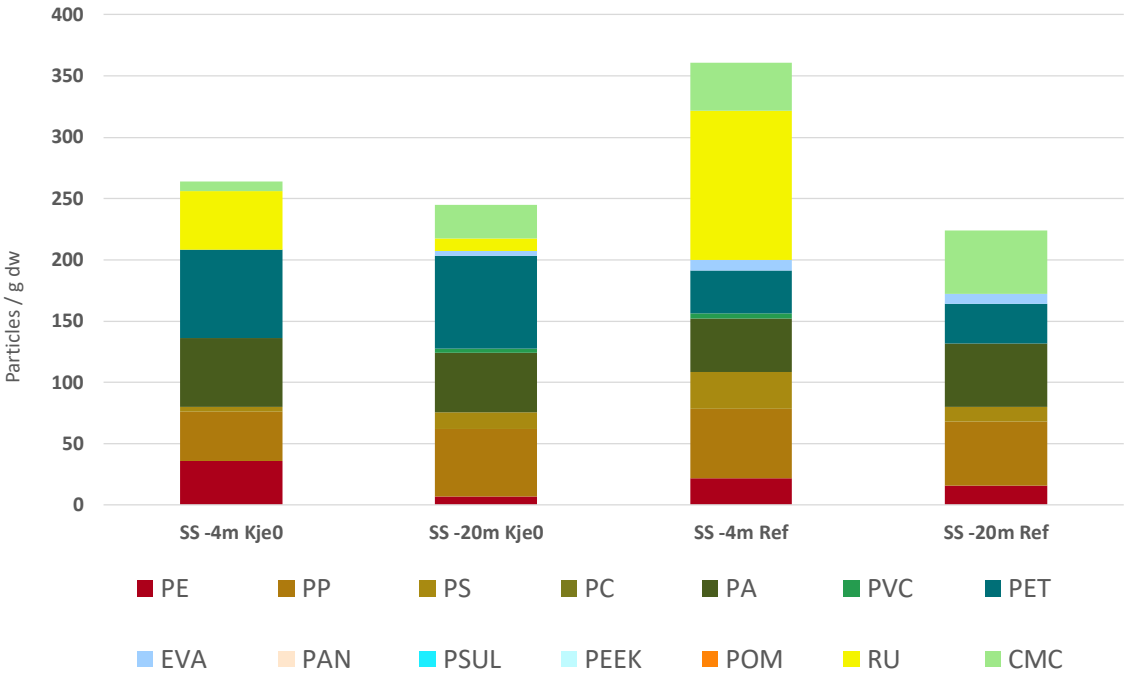

Figure 3.6 – µFTIR analysis: polymer composition of MPs (10-300 µm) in suspended matter collected at the net-pen (Kje0) and at the reference site. Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC), Polyamide -Nylon (PA), Polyvinyl chloride (PVC), Polyethylene Terephthalate (PET), Acrylates (ACy), Ethylene vinyl acetate (EVA), Polyacrylonitrile (PAN), Polysulfone (PSUL), Polyetheretherketone (PEEK), Polyoxymethylene (POM); Rubber (RU), Cellulose chemically modified (CMC).

The results are roughly comparable to the mass estimation by Pyr-GCMS analysis (Figure 3.7). PA (6-17 µg/g; 20-30% contribution to the total calculated polymer mass) was the most abundant polymer type at both sites followed by PP (6-16 µg/g; 14-29%) PET (5-12 µg/g; 12-31%), PE (4-12 µg/g; 15-21%) and PS (1-4 µg/g; 4-10%).

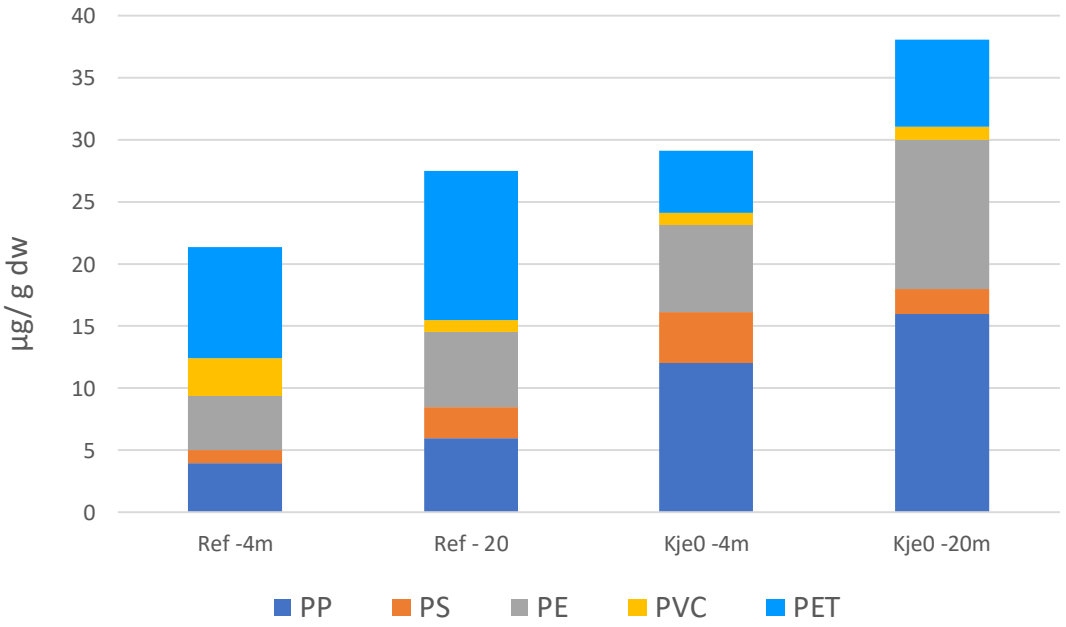

Figure 3.7 - Pyr-GCMS analysis: polymer mass distribution kg^{-1} DW of MP (10–300 µm) in suspended matter collected at the net pen (Kje0) and at the reference site (Ref). Polyethylene (PE), Polypropylene (PP), Polystyrene (PS) and Polyethylene Terephthalate (PET).

The overall differences in mass were relatively small between the net pen (Kje0) and the reference site. We measured roughly 1000 times the MP particles in suspended matter sediment traps as compared to bottom sediments. This may indicate a fast distribution of MPs by ocean currents away from the net pens, and that the particles are not reaching the bottom sediments in the vicinity. Therefore, trap analysis may be better suited than sediment analysis for local source MP surveillance. However, the data is too scarce for a firm conclusion.

3.2.3 Results of filtered seawater samples

Three replicates of 100 L seawater from the reference site and Station Kje0 were taken for analysis. There were very few MPs over one mm (1-5 mm), identified as PP, PA and occasionally as PS (Figure 3.8).

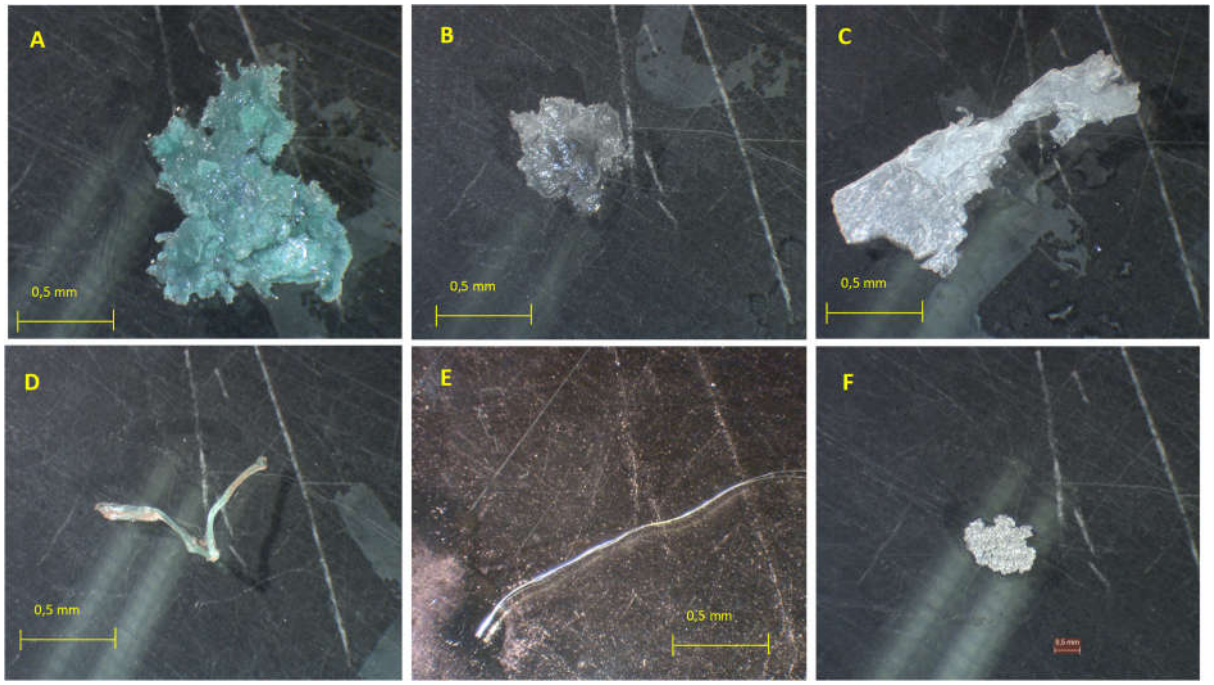

Figure 3.8 - Examples of particles > 300 µm detected in the seawater samples. A: PS_{1_Kje0}, B: PP_{1_Kje0}, C: PP_{2_Kje0}, D: PA_{1_Kje0}, E: PA_{1_ref} and F: PP_{1_ref}.

For the larger size MP particles in water the mass of the single PS and the three PP particles from Kje0 (mass in µg: PS_{1_Kje0} = 298; PP_{1_Kje0} = 441; PP_{2_Kje0} = 380; PA_{1_Kje0} = 118) were similar to the reference site (PA_{1_ref} = 141 µg; PP_{1_ref} = 378 µg; Figure 3.8).

The 10-300 µm fraction showed a mean MP abundance ranging from 0.6 particles/L to 2.3 particles/L. At Kje0, PE particles dominated (29%) followed by PP (25%), PA (11%), PC (10%) and PET (7%). The highest number was found for PE (72%) in one of the samples. Polyvinyl acetate (PVC) and Polycarbonate (PC) were only found at Kje0, however in small numbers. At the reference site PP accounted for 35% of the total, followed by PET (23%), RU (17%) and PE (11%; Figure 3.9). The highest number found in one sample was for PP (67%). Only PE showed significantly higher levels in Kje0 compared to the reference site (Correlation matrix analysis, $p<0.05$).

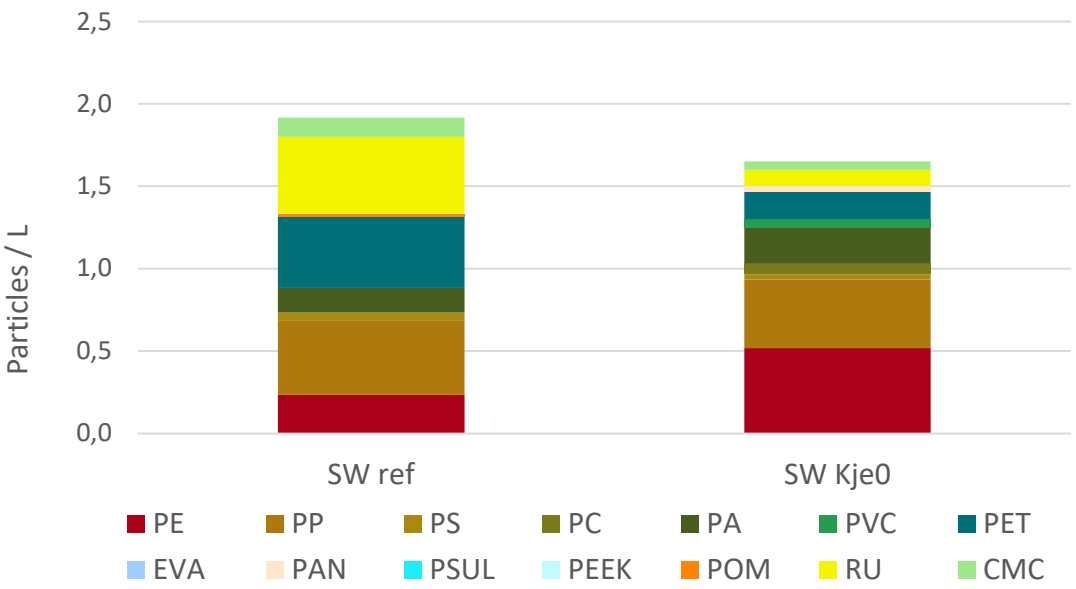

Figure 3.9 – µFTIR analysis: polymer composition of microplastic particles (10-300 µm) in seawater samples collected at the reference site and near the netpen (Kje0). Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC), Polyamide-Nylon (PA), Polyvinyl chloride (PVC), Polyethylene Terephthalate (PET), Acrylates (ACy), Ethylene vinyl acetate (EVA), Polyacrylonitrile (PAN), Polysulfone (PSUL), Polyetheretherketone (PEEK), Polyoxymethylene (POM); Rubber (RU), Cellulose chemically modified (CMC).

The results of the Pyr-GCMS analysis support the dominant contribution of PE, PS and PET in the investigated samples (Figure 3.10). In detail, the concentration and distribution were as follows at the reference site and Kje0, respectively: PE dominated with 76 and 180 ng/L (47 and 72%) followed by PS with 30 and 41 ng/L (19-16%), and PET 33 and 21 ng/L (20 and 5%). Overall, comparing the distribution across the two sampling sites, most polymers showed a homogeneous distribution and sometimes higher values in the reference compared to the net pen. Only PE was significantly higher at Kje0 than at the reference site with respect to number of particles and total estimated mass (ANOVA, $p < 0.05$). In the procedural blanks, which were treated simultaneously with the seawater samples, no signs of contamination were recorded.

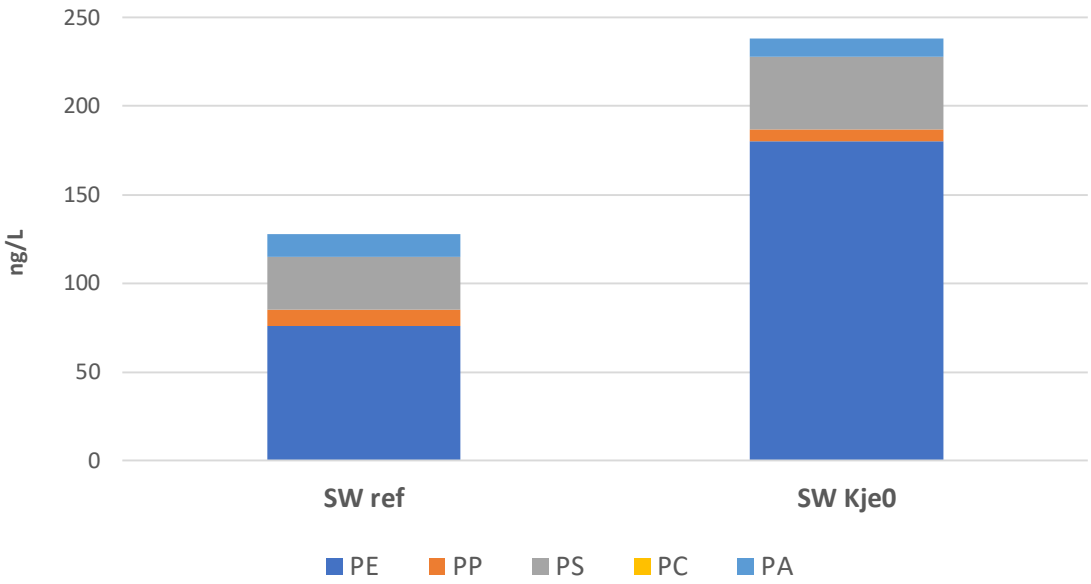

Figure 3.10 - Pyr-GCMS analysis: polymers mass distribution kg^{-1} DW for microplastic particles (10–300 µm) in sea water samples collected at the reference site (SW ref) and near the net pen (SW Kje0). Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC) and Polyamide-Nylon (PA).

3.2.4 Results of chemical characterization and histological analysis of biological samples

Purified extracts from gills and GI-tracts were analysed for MP concentration by Pyr-GCMS. Approximately 3 g of fresh gill arches tissue per individual were submitted to chemical analysis while the remaining tissue (2 gr) was used for histological analyses. PE was the only identified polymer in 11 out of the 20 analysed farmed salmon samples, with concentrations from 1.0 to 1.2 µg/g. In the remaining 9 individuals, the levels of MPs were below the limit of quantification (LOQ). None of the investigated polymers were found above the LOQ in gills from wild salmon.

The presence and localization of MPs was evaluated in 10 µm thick cryostatic sections of gills. After staining with haematoxylin and eosin (De Castro et al., 2019), slides were observed by polarized light microscopy (Pittura et al., 2018). The assessment was not quantitative, thus results on MP in tissues are descriptive and qualitative.

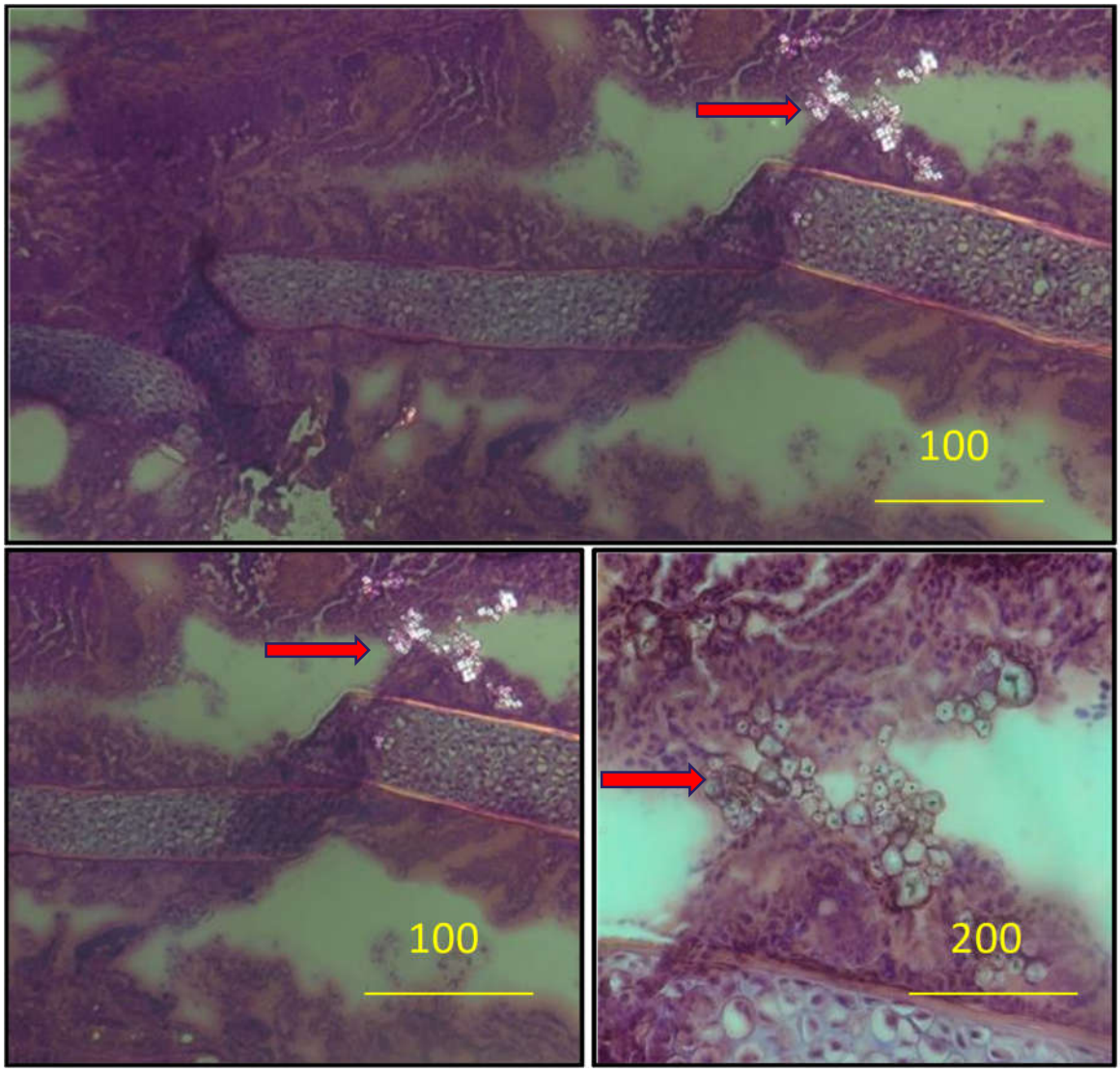

Figure 3.11 – Polarized-light microscopy images showing the presence of MP in gills of farmed salmons (red arrows). Yellow numbers indicate the length of the scale bar.

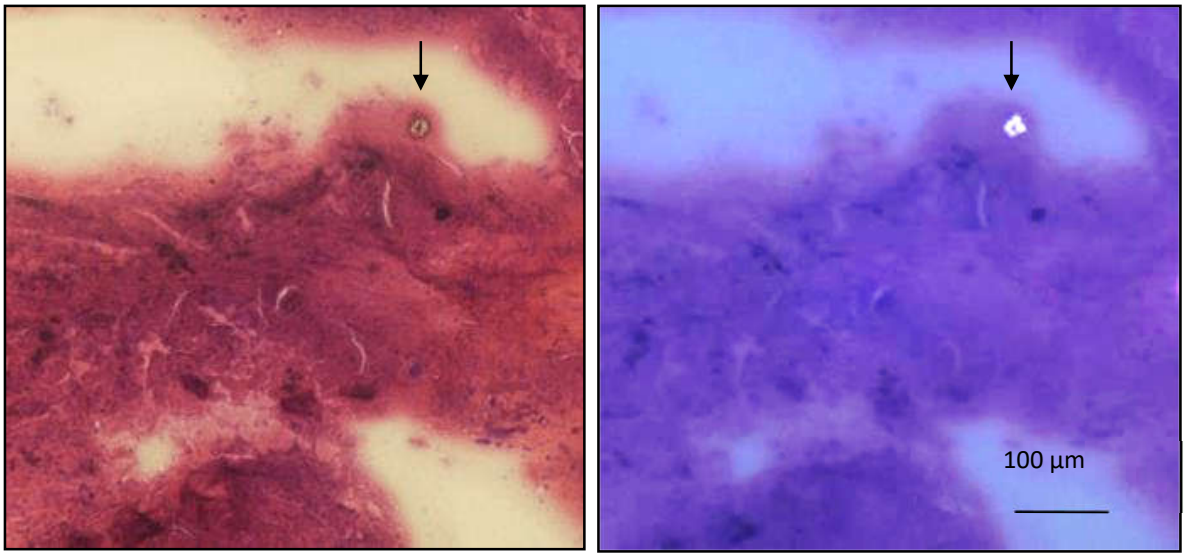

Figure 3.12 - Polarized-light microscopy images showing the presence of microplastic particles in gills of wild salmons (black arrow).

Histological analyses revealed the presence of MPs in the gill lamellae of farmed salmons (Figure 3.11), while a lower number of MPs was observed in gills collected from wild salmon (Figure 3.12). The chemical characterization of the same samples by Pyr-GCMS found PE concentrations from 1.0 to 1.2 µg/g WW. Image analysis showed MP particles in the size range 5 to 30 µm. Taken together, the particle size distribution, together with the chemical characterization in the investigated tissues as well as the results presented in section 3.1.1 and the observed particle size distribution in the cryosections, this suggests that the source of these particles may be the abrasion of feed pipes. Furthermore, PE is one of the most common polymer types used worldwide, with a range of applications and products. Thus, further investigations of material in gills of farmed fish is needed to confirm the source. The released MPs are concentrated enough in the aquatic environment near the net pens to increase the risk of exposure in farmed salmon. Nevertheless, no sign of ultrastructural alterations such as swelling, necrosis or cell infiltration was observed in farmed salmon samples.

Three replicates of 20 g of GI-tract per individual of farmed (n= 20) and wild salmon (n = 20) were submitted to quantification of MP by Pyr- GCMS. Results of Pyr-GCMS analysis indicated levels below the LOQ in all but two of the analysed wild salmon individuals, where PP (1.2 and 3.0 µg/g) and PE (2.1 and 2.3 µg/g) were observed. Analyses of the GI-tracts of farmed salmon showed in general levels of MP below the LOQ. In a single individual, PA was detected (2.1 ± 1.0 µg/g). Results of the Pyr-GCMS were in good agreement with the results of the FTIR analysis. No particles > 300 µm were found in the GI-tracts of either wild or farmed salmons. In the smaller size classes, 3 and 4 PP particles (60 - 110 µm) as well as 9 and 12 PE particles (27-46 µm) were observed in the same two wild salmon individuals as previously described. Furthermore, PA and 3 PP particles (25-38 µm) were detected in the GI-tract of the farmed

salmon. However, all the detection methods applied have a lower detection limit of 10 µm due to sieving. Therefore, the bulk number of MPs produced in the abrasion experiment, around 2-7µm, would not have been detected. Considerable method development is necessary to lower this detection level for quantitative results.

4 Evaluation of collected data and conclusion

To our knowledge, there is no previous systematic data collection on the contribution of aquaculture to the marine plastic load. Microplastic losses may vary from farm to farm depending on the waste management protocols applied at individual facilities. This is the first study to attempt a quantitative assessment of microplastic release from a selection of relevant sources within the fish farm. The results of the present study show the complexity of MP distribution assessment in aquatic coastal ecosystems, as these environments are dominated by multiple input sources and heavily affected by several different anthropogenic activities. For polymer types such as PE and in some cases PA, higher concentrations are detected in sediments close to the net pens compared to the reference area, while for most of the other polymer types investigated the concentrations appeared more homogeneously distributed between the investigated sites, with some polymer types occurring with higher concentration in the reference site.

PE and PP are dominant polymers in aquaculture and fishing, used for ropes and fishing nets, as buoyancy materials or to construct feed pipes (Lusher et al., 2017). Their dominance is, however, not unique to the aquaculture industry, as these polymers are versatile and are dominant in production and use, covering nearly 60% of the European and Norwegian market demand for plastics.

The high number of black (PE and PP) particles found in the sampling sites near the pen is consistent with the fact that the ring-structure of most fish farms is made of black plastic. Additionally, ropes and nets used in the farms are usually of green (PA) or orange colour, which were represented in the SE and NE transects but not in the reference zones, suggesting that these MPs could have derived from the installations. A visual inspection tracking and recording the colour of the extracted MPs while collecting samples near an aquaculture site may be an additional simple tool in helping to understand the fluxes and the distribution of plastics in the investigated areas.

Large fibres of PA were found in seawater samples and may come from fishing lines and ropes. Several studies have directly related nylon fibres found in the water column (Suaria et al., 2016), marine sediments (Claessens et al., 2011) and animal guts (Possatto et al., 2011; Pellini et al., 2018) to fishery activities. On the other hand, the occurrence of PS and PP particles observed within the study can be associated to different diffuse sources commonly used in consumer products (e.g. plastic bags, bottles, caps, films, containers, etc.) and possibly originate from the breakdown of larger macro debris not directly associated with aquaculture activities, which could explain the more homogeneous distribution between sites. It also cannot be excluded that some contamination might have entered the samples during

collection as procedural contamination from the boat, although care was taken to avoid exposure of samples to the open air.

Furthermore, the general distribution of PE and PP in the sediment sites far from the pens observed in the present study is consistent with other studies on MP identification in marine sediments, including in the investigated area (Boknafjord). Previous studies have observed that PE and PP were present or dominant but with lower MP concentrations compared to what was reported in the current study (Frias et al., 2016; Vianello et al., 2013; Gomiero et al., 2019, Haave et al. 2019). However, direct comparisons among studies are difficult due to a lack of method harmonization.

Based on both the observed gradient of polymers in sediments, the results of the polymer distribution in seawater and the outcomes of the simulated abrasion in the feed pipes, the present study indicates a potential emission of PE microparticles from aquaculture activities. This could be linked with the possible continuous abrasion of the feed pipes during feed distribution. In this context, it is worth advising a cautionary approach while attempting to calculate the overall PE contribution of the aquaculture industry based on the presented experimental and field results. The actual feed pipe abrasion effect can vary significantly from case to case. Factors such as the age and replacement rate of the feed pipes, their shape in the operational phase and the settings and working conditions of the feed distribution equipment (blower unit) and cleaning procedure, may influence the actual abrasive forces greatly, thus influencing the final number, dimension and shape of the released particles. Connected to the feed pipe abrasion phenomenon, there is also concern about the potential formation of a nanoplastics (1-900 nm) that have the potential to be absorbed across cell membranes, including gut epithelia (Mattson et al 2017). Nanoplastics can bioaccumulate in tissues after crossing the cell membrane (Kashiwada et al., 2006; Mattson et al 2017). This may lead to transfer across trophic levels, although transfer studies and fate in the food web is not within the scope of this study. Due to the current technological limitations in sampling and detection capabilities, the nano fraction of plastic particles is still not quantified in the environment but has been detected qualitatively (Ter Halle et al 2017).

MPs located in the gills of farmed fish have been documented in this study. Although this is a potential threat for farmed fish, the histological assessment in gills of farmed salmon does not show any significant ultrastructural or histological changes in the tissues. Ecotoxicity assessment of PE has been evaluated in relation to particle size, exposure levels, bioaccumulation, additive leaching, organic pollutant absorption and release in several species, resulting in inconclusive outcomes (VKM, 2019), and a need for better data has been indicated. Also, from a salmon welfare perspective, more research is needed to better understand the physiological effects of chronic exposure to MPs during production and the interaction with other chemicals and pharmaceuticals used during production. It should also be noted that MPs were below LOQ in the guts of farmed salmon although above LOQ in a small number of wild salmon, indicating that wild salmon are also exposed to MPs.

Many species of edible demersal, pelagic and reef fish, sampled from across the globe, have been found to ingest MPs (Bellas et al., 2016; Rummel et al., 2016; Rochman et al., 2015; Lusher et al., 2017; Pellini et al., 2018). Furthermore, it has been previously reported that the number of particles reaching and taken up in several tissues increases with decreasing particle

size (Jani et al. 1992, Kashiwada 2006, Browne et al. 2008, Jeong et al. 2016, Critchell and Hoogenboom 2018). Although MPs can be detected in several species, FAO (2017) evaluated that a worst-case scenario of exposure to microplastics after consumption of a portion of mussels (225 g) would lead to ingestion of 7 micrograms of plastic. Apart from the particles, this would have a negligible effect (less than 0.1 percent of total dietary intake) of chemical exposure to certain persistent organic pollutants and plastic additives.

While parameters such as colour, flavour, total amount of protein, fats, amino acids, metals, and pharmaceuticals content is regulated and routinely monitored, no guidelines and regulations have so far been set at the national or international level for MP contamination. This study addressed the characterization of MP content in ingredients used for feed production. The occurrence of MP contamination in the finished feed products may also affect its quality, as in the potential of chemicals adsorbed to MPs from the raw material processing.

In the present study, previously developed MP analysis methods were optimized and the detection of MP contamination in both the raw materials and in the finished feed product was performed. This represents the starting point for further method development and research, as new knowledge, experience and improved technologies will become available in the future. The presented results are difficult to compare to other studies due to the very limited available data in literature, and constantly improving methodology.

The research activities and good cooperation with the industrial partners in the project has helped to identify the production process responsible for some of the contamination and to suggest actions that can be taken to eliminate this source of contamination. Based on this experience, more integrated projects linking the research and industry communities may promote industry competitiveness and sustainability.

Furthermore, the study indicates that global plastic use has led to contamination of some of the raw materials used in fish feed production. In this case concrete actions can be suggested, such as establishing a combination of contamination mitigation routines and monitoring using validated analytical methods based on the best available competence and technology. Baseline levels of MPs in the different raw materials should be established, and deviations from the baselines detected, investigated and mitigated. Regulations and guidelines should be set in the future, with the aim of identifying the highest admissible levels of MPs in raw and finished product, such as those already defined for other contaminants. Such regulations must be based on risk assessments and knowledge of threshold levels, thus a scientific basis for the evaluation is needed. We are still a long way away from a well-documented knowledge base for risk assessments in order to document seafood safety with respect to microplastic content.

5 Main findings and conclusions

- Microplastic particles of various polymers were observed and quantified in fish feed, sea water, suspended matter, sediments and on fish gills.
- Microplastics were detected at the reference site as well as the production facility.
- Some of the raw ingredients for fish feed had measurable levels of PA, PE and PET. PP in the feed production line was caused by contamination from packaging.
- For PE and in some cases PA, higher concentrations are detected in sediments close to the fish pens compared to the reference area.
- Gradient-like distribution in sediments, seawater samples and the outcomes of the simulated abrasion in the feed pipes indicates a potential emission of PE MPs from aquaculture activities.
- The abrasion experiments indicated that the majority of MPs generated were smaller than what could be analysed with the current methods used
- Several plastic polymers such as PS, PET, PMMA and PS showed a homogeneous distribution among all sampling areas without a clear pattern of distribution in relation to the aquaculture facility.
- Ropes and nets used in the farms are usually of green (PA) or orange colour, which were represented in the SE and NE transects but not in the reference zones, suggesting that these particles could have derived from the facility.
- The observed PE and PP general distribution in the sediment sites far from the net-pens observed in the present study is consistent with other studies (Frias et al., 2016; Vianello et al., 2013; Gomiero et al., 2019, Haave et al., 2019) on MP identification in marine sediments, including in the investigated area (Boknafjord), where PE and PP were dominant.
- MPs were identified in the gills of farmed fish, and in a small number of wild fish, indicating a source related to the fish farm.
- The histological assessment in gills of farmed salmon did not show any significant ultrastructural or histological changes in the tissues.
- No MPs were detected in the GI-tracts of farmed salmon, and only in two out of the twenty analysed wild salmons.
- MP counts in sediment traps were roughly 1000 times higher than in bottom sediments. This warrants further investigation to identify the distribution patterns of MP.

In general, the findings of this study warrant follow-up studies to quantify the MP contamination, including smaller MPs below 10 µm. The results show that there is a likely contamination of MP above background levels at aquaculture sites and indicates the major polymer types. This study is insufficient to estimate the extent of the contamination, nor conclude if there is a resulting impact. In order to clarify the latter, uptake in and effect on salmon needs to be quantified by applying the observed concentrations of MP in long term exposure experiments, considering polymers and particle shape and size.

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Annex 1 - statistical analyses

Table AN1 - Results of two-way analysis of variance for polymer levels in sediments collected near the aquaculture site as estimated by Pyr-GCMS.

mass concentration	Marine Sediments						
	p						
	PE	PP	PS	PC	PA	PVC	PET
sites SE	0.0245	0.1241	0.2444	nd	0.0443	0	0.0661
sites NE	0.0325	0.1901	0.3114	nd	0.0303	0	0.0761
sites E	0.0617	0.2281	0.3736	nd	0.0363	0	0.0911
two-way analysis of variance							

Table AN2 - Results of two-way analysis of variance for polymer levels in seawater samples collected near the aquaculture site as estimated by Pyr-GCMS.

mass concentration	Seawater samples						
	p						
	PE	PP	PS	PC	PA	PVC	PET
site	0.024	0.124	0.2447	nd	0.0502	0.157	0.156
two-way analysis of variance							

Table AN3 - Results of two-way analysis of variance for polymer levels in suspended matter samples collected at two depths in both near the aquaculture and in a reference site as estimated by Pyr-GCMS.

mass concentration	Suspended matter						
	p						
	PE	PP	PS	PC	PA	PVC	PET
site	0.125	0.0326	0.249	nd	0.0326	0.226	0.0601
depth	0.072	0.289	0.126	nd	0.0446	0.138	0.0841
site X depth	0.227	0.319	0.0614	nd	0.051	0.185	0.0741
two-way analysis of variance							

Table AN4 - Kendall's tau (τ) correlation matrix analysis of µFTIR results on marine sediments. Values interpretation: exactly +1, perfect downhill (positive) linear relationship; +0.70, strong downhill (positive) linear relationship; +0.40, moderate downhill (positive) relationship; +0.10, weak downhill (positive) linear relationship; 0, no linear relationship; -0.10, weak uphill (negative) linear relationship; -0.40, moderate uphill (negative) relationship; -0.70, strong uphill (negative) linear relationship; exactly -1, perfect uphill (negative) linear relationship. Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC), Polyamide -Nylon (PA), Polyvinyl chloride (PVC), Polyethylene Terephthalate (PET), Acrylates (ACy), Ethylene vinyl acetate (EVA), Polyacrylonitrile (PAN), Polysulfone (PSUL), Polyetheretherketone (PEEK), Polyoxymethylene (POM); Rubber (RU), chemically modified cellulose (CMC).

Kendell's tau (τ) correlation coefficient		
	Distance	
Polymer	tau	Correlation
PE	-0.64	strong
PP	-0.710	strong
PS	-0.373	ns
PA	-0.250	ns
PVC	-0.092	ns
PET	-0.110	ns
Acy	-0.381	ns
EVA	-0.138	ns
PAN	-0.402	ns
PSUL	0.085	ns
PEEK	0.085	ns
POM	0.278	ns
RU	0.092	ns
CMC	-0.330	ns

Table AN5 – Correlation matrix analysis of µFTIR results on suspended matter samples. Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC), Polyamide -Nylon (PA), Polyvinyl chloride (PVC), Polyethylene Terephthalate (PET), Acrylates (ACy), Ethylene vinyl acetate (EVA), Polyacrylonitrile (PAN), Polysulfone (PSUL), Polyetheretherketone (PEEK), Polyoxymethylene (POM); Rubber (RU), chemically modified cellulose (CMC).

Correlation Table									
		Pearson			**Spearman**			**Kendall**	
parameter	**polymer**	**r**	**p**		**rho**	**p**		**tau B**	**p**
Distance	PE	-0.017	0.983		0	1		0	1
Distance	PP	-0.408	0.592		0	1		0	1
Distance	PS	0.514	0.486		0.447	0.553		0.408	0.439
Distance	PC	NaN	NaN		NaN	NaN		NaN	NaN
Distance	PA	0.9	0.1		0.894	0.106		0.816	0.121
Distance	PVC	-0.075	0.925		-0.236	0.764		-0.224	0.683
Distance	PET	-0.853	0.147		-0.894	0.106		-0.816	0.121
Distance	Acy	0.717	0.283		0.894	0.106		0.816	0.121
Distance	PVA	0.507	0.493		0.447	0.553		0.408	0.439
Distance	PEEK	NC	NC		NC	NC		NC	NC
Distance	PSUL	NC	NC		NC	NC		NC	NC
Distance	RU	0.107	0.893		0	1		0	1
Distance	CMC	0.509	0.491		0.447	0.553		0.408	0.439

Table AN6 - Correlation matrix analysis of µFTIR results on seawater samples. Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polycarbonate (PC), Polyamide -Nylon (PA), Polyvinyl chloride (PVC), Polyethylene Terephthalate (PET), Acrylates (ACy), Ethylene vinyl acetate (EVA), Polyacrylonitrile (PAN), Polysulfone (PSUL), Polyetheretherketone (PEEK), Polyoxymethylene (POM); Rubber (RU), chemically modified cellulose (CMC).

		Correlation Table							
		Pearson			**Spearman**			**Kendall**	
testing parameter	**polymer**	**r**	**p**		**rho**	**p**		**tau B**	**p**
Distance	PE	0.661	0.02		0.648	0.03		0.63	0.02
Distance	PP	0.231	0.66		-0.29	0.57		-0.26	0.51
Distance	PS	0.291	0.58		0	1		0	1
Distance	PC	0.447	0.37		-0.45	0.37		-0.45	0.32
Distance	PA	-0.06	0.91		-0.29	0.57		-0.26	0.51
Distance	PVC	0.447	0.37		-0.45	0.37		-0.45	0.32
Distance	PET	0.757	0.08		0.693	0.13		0.624	0.12
Distance	Acy	0.695	0.13		0.683	0.14		0.602	0.13
Distance	PVA	NC	NC		NC	NC		NC	NC
Distance	PEEK	0.447	0.37		-0.45	0.37		-0.45	0.32
Distance	PSUL	0.447	0.37		0.447	0.37		0.447	0.32
Distance	RU	0.299	0.56		-0.1	0.85		-0.1	0.82
Distance	CMC	0.20	0.70		0	1		0	1


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