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Absence of microplastic bioaccumulation in cod fillets from plastic-polluted western Norwegian waters

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Abstract

Plastics are synthetic, persistent materials that are distributed worldwide. An important concern is whether microplastics (MP) can bioaccumulate in the food chain and pose a threat to human consumers. We studied MP in the fillet of resident coastal cod from a plastic-polluted area in Western Norway, where long-range transported marine litter accumulates and MP are generated on shore. We dissected the fish and processed the samples in an MP-free lab using gentle enzymatic treatment. Micro Fourier transform infrared spectroscopy (μFTIR) was used for identification of particles down to 10 μm. The fish were 40 to 74 cm, corresponding to 2-6 years old. 29 particles were observed in fillets from eight of the 23 individuals. The mean particle count was 1.55 ± 2.75 nMP/100 g, and the mean concentration was 2.81 ± 8.33 μg/kg wet weight. Six polymer groups were identified, where polypropylene (33%) and polyethylene (30%) were the most frequent. The majority (86%) of the particles were fragments ranging from 32-100 μm. Fibers and fragments over 200 μm were observed. The largest particle was a PP particle of 258.2 μm. Controls showed minimal contamination and the procedural blanks were negative. There was no significant correlation between age, body condition, time of capture, and MP concentrations, and no evidence of bioaccumulation of MP in the fillet of older fish after *in situ* exposure. MP in food is of concern for human consumption and emphasizes the importance of understanding MP distribution and fate as well as reducing and controlling plastic release into the environment.

Keywords: Microplastics, bioaccumulation, fish fillet, plastic pollution

INTRODUCTION

Human history has proven that man-made contaminants that are resistant to degradation are distributed far from their source of origin. These substances are often referred to as persistent organic pollutants (POPs). The last decade has documented that microplastics (MP) possess the same traits and have been detected in various environmental samples worldwide, testifying to a global distribution of synthetic polymer particles far from human activities[1-7]. Over the past decade, there has been extensive documentation of MP ingestion by marine organisms[8]. MP have been detected in the muscle and liver of fish and birds, as well as in human blood, placenta, and testes[9-14].

Studies have also provided experimental evidence of micro- and nanoplastic uptake and translocation in animals and humans[12,15-18]. This evidence of omnipresence and uptake into internal organs underscores the need to understand and describe MP kinetics and fate using traditional toxicological terms, such as understanding the routes of exposure and the movement and fate of MP in the food web. We need to understand the fate of MP in organisms and the food web to determine whether they share another common trait of POPs, the ability to bioaccumulate and biomagnify. Bioaccumulation refers to when a substance reaches significantly higher concentrations in an organism compared to its surroundings. Bioaccumulation often leads to biomagnification, which means organisms at higher trophic levels have higher concentrations of the substance due to their inability to excrete or metabolize it. This is of interest to human consumers, since we eat a wide range of foods from different trophic levels and live long lives.

Early experiments on mammals and birds in 1975 showed that MP can be absorbed through the intestines and distributed to different organs and body fluids within minutes of ingestion[16]. This was shown by the absorption of polyvinyl chloride (PVC) particles with an average size of 40 μm (ranging from 10 to 110 μm) in rats, mice, guinea pigs, rabbits, chickens, and dogs. Minutes after exposure, MP were found in the bile, and within the following 24 h, they were excreted via urine, lungs, milk, and placenta, indicating distribution throughout various organs[16]. Later experiments revealed that nanoplastic particles (< 1 μm) reached the brains of fish[15,19-21]. A recent study observed the uptake, distribution, and excretion of palladium-enriched polystyrene nanoparticles in fish and found that they were distributed throughout the body but were also excreted to levels below the detection limit after 48 h of exposure[22]. Similarly, the uptake, distribution, and elimination of ^{14}C-radiolabeled nano polystyrene were demonstrated in clams over a 24-day period, with larger particles (250 nm) still detectable after 48 days, indicating that particle size affects the kinetics[18]. Overall, these studies demonstrate the transfer of micro- and nanoplastics into tissues through barriers such as the intestinal wall and the blood-brain barrier. They also suggest that multiple body fluids and tissues may be affected by the ingestion or inhalation of MP. Furthermore, these findings indicate that micro- and nanoplastic particles may be present in many organs shortly after exposure, although their presence in tissues may be transient. Nonetheless, MP can have permanent deleterious effects, emphasizing the importance of studying chronic exposure to relevant concentrations and compositions of micro- and nanoplastic particles[22,23]. Understanding chronic exposure to relevant levels is thus of importance for understanding the consequences of MP pollution throughout the food web as well as in humans.

Food consumption is one of the most extensively studied routes of MP exposure in humans[24]. Previous studies have demonstrated the presence of MP in edible parts of fish[10,25,26], as well as exposure through seafood that may have become contaminated by plastics during processing, packaging, handling, and cooking[27-31]. The numerous potential sources of MP, differences in sampling methods, dissection techniques, analytical procedures and identification make comparisons between studies, species, and regions challenging[32] and make it hard to differentiate between the effects of trophic level or age and to be able to discern the effects of method uncertainties[33]. When evaluating the evidence for bioaccumulation

and the fate of MP in the food web, it is essential to eliminate contamination from sources of MP other than ingestion and trophic transfer, and it is vital to acknowledge limitations and uncertainties related to contamination[34].

This study aimed to elucidate the evidence for bioaccumulation in tissues of naturally exposed wild cod of various sizes and ages, strictly considering the MP that had translocated into tissues from direct or indirect uptake and ingestion of food and water or across respiratory epithelia, using methods sensitive enough to detect a single particle of MP. A high-tech plastic-free laboratory with a strong emphasis on contamination control was used to prevent external contamination. This approach gives the best possibility to investigate and differentiate between MP concentrations in individuals of different ages, and thus investigate the evidence of *in situ* bioaccumulation.

EXPERIMENTAL

The study was conducted using wild-caught cod from a highly plastic-polluted area located in the outer coastal region of southwestern Norway[35] [Figure 1]. The Norwegian Coastal current flows northward along the coastline, carrying marine litter from the North Sea, southern Europe, and even across the Atlantic on the Gulf Stream, as well as locally produced litter. Due to predominantly southwestern winds, floating items are pushed toward the shoreline, where geological formations with numerous islands and inlets act as a physical trap[35,36]. Within this plastic-polluted habitat, the coastal strain of the Atlantic cod (*Gadus morhua*) can be found. The coastal cod was selected for this study because it is a resident, non-migratory species living within a limited area[37,38]. It is a generalist feeder, consuming a wide range of species[39], which makes cod an ideal subject for investigating the uptake and potential bioaccumulation of MP from the environment. A recent study has documented frequent ingestion of plastics in cod from this region[40], and a pilot study identified measurable levels of MP in the muscle and liver of cod from this area[10]. The current study further expanded the sample size to examine the relationship between size (age), body condition, and microplastic concentration in cod. Additionally, updated methods were able to detect small microplastic particles and a plastic-free laboratory was employed to validate the previously observed concentrations of MP in cod from this plastic-polluted region.

Volunteers from the Norwegian Hunters and Anglers' Association captured cod at two locations during two periods between May 7, 2021, and November 2, 2021 [Figure 1 and Table 1]. The two locations were Vindkjeften (4,9771509°E; 60,3322916°N) and Nordre Hola (4,9547007°E; 60,2954264°N).

Only resident cod, which can be distinguished by their size and color, were collected for this study, and the migrant strain of the Atlantic cod was not present in these waters during the sampling period. The resident cod lives its whole life in the same region, and we therefore assume that it is has been exposed to plastics in water and food throughout its lifetime.

According to Norwegian regulations, fish below the minimum size of 40 cm were released back into the water, and thus, the youngest age groups are not represented. The fish collected from the sites were captured using fishing nets or fishing rods. The fish were euthanized by a blow to the head to avoid cutting the skin and potentially contaminating the tissue, wrapped in aluminum foil, and subsequently frozen at -20 °C until further dissection and chemical analysis at NORCE PlastLab.

Contamination control

Given the expected low microplastic concentration in tissues, controlling and recording any external contamination is crucial[41]. All samples were, therefore, processed inside the NORCE PlastLab.

Table 1. Characteristics of cod from two locations in western Norway (Nordre Hola and Vindkjeften), with bodyweight (g), length (cm) and Fulton's condition factor (k-factor), sample weight (g), number of MP by particle shape, MP count (nMP/100 g·ww) and calculated MP mass-concentration (μg/kg·ww)

Fish ID	Capture date	Weight	Length	k-factor	Sample weight	n-fibers/n-fragments	MP count	MP conc.
Location Nordre Hola								
#1	07.05.21	855	43	1.08	71.41	1/1	2.80	2.8
#2*	19.05.21	859	43	1.08	42.13	nd/nd	-	-
#3*	19.05.21	637	40	1.00	69.20	nd/nd	-	-
#4	25.05.21	698	40	1.09	67.96	3/4	10.30	38.9
#5*	30.05.21	863	42	1.17	83.53	nd/nd	-	-
#6	30.05.21	550	42	0.74	72.89	nd/nd	-	-
#7	30.05.21	570	44	0.67	85.02	nd/nd	-	-
#8	07.06.21	400	44	1.02	94.34	nd/nd	-	-
#9	27.10.21	708	42	0.96	78.46	3/nd	3.82	1.3
#10	27.10.21	1,334	44	1.57	91.23	nd/1	1.10	0.8
Location Vindkjeften								
#11	02.11.21	2,299	67	0.76	103.43	nd/4	3.87	11.4
#12	02.11.21	1,285	51	0.97	95.21	nd/nd	-	-
#13	02.11.21	2,059	58	1.06	108.59	nd/nd	-	-
#14	02.11.21	1,855	55	1.11	91.30	nd/nd	-	-
#15	02.11.21	4,639	73	1.19	152.78	nd/nd	-	-
#16	02.11.21	2,229	59	1.09	95.20	nd/nd	-	-
#17	02.11.21	1,459	53	0.98	97.90	nd/4	4.09	1.8
#18	02.11.21	1,823	54	1.16	108.13	nd/1	0.92	0.7
#19	02.11.21	2,023	56	1.15	107.64	nd/nd	-	-
#20	02.11.21	1,959	55	1.18	106.06	nd/nd	-	-
#21	02.11.21	1,356	51	1.02	103.47	nd/nd	-	-
#22	02.11.21	1,796	56	1.02	115.50	nd/5	4.33	7.1
#23	02.11.21	1,770	55	1.06	110.21	nd/nd	-	-
Mean ± SD	-	1,479.45 ± 917.52	50.3 ± 9.42	1.05 ± 0.18	93.50 ± 21.82	Sum: 10/26	1.89 ± 3.10	1.55 ± 2.75

*interference from the presence of protein in the analyses was observed, but samples met the QA/QC requirements of the laboratory. MP: Microplastic; nd: not detected; SD: standard deviation; QA/QC: quality assurance/quality control.

Contamination control, quality assurance/quality control (QA/QC) measures were implemented to minimize and account for any potential sources of microplastic contamination. The laboratory is specially designed to have plastic-free surfaces, including glass, steel, and non-plastic benches and floors. The Plastlab has high-efficiency ultra-low penetration HEPA-14H filtration with an efficiency > 99% for the most penetrating particle size (0.1-0.5 μm). Additional precautions, such as sticky floor mats and an air sluice, were in place to prevent dust from entering. Lab personnel wore clothing made of natural materials like cotton or wool. Glassware, steel filters, and other materials were covered in aluminum foil and burned in a muffle oven at 500 °C before use to remove any traces of MP. Steel filters, glass, and porcelain equipment were used for all procedures, with the only exceptions being silicon tubes, polytetrafluoroethylene (PTFE-Teflon) squeezy bottles, and computers.

Before and after fish dissection or sample preparation, surfaces were rinsed with Milli-Q water that was filtered once more over a Whatman GF/A- filter (1.6 μm) and wiped with tissue paper. Stainless steel filters, tweezers, and other equipment used were rinsed three times and sterilized using a gas burner (FLAMEBOY™, 1,350 °C) between samples. Scalpel blades were changed between samples.

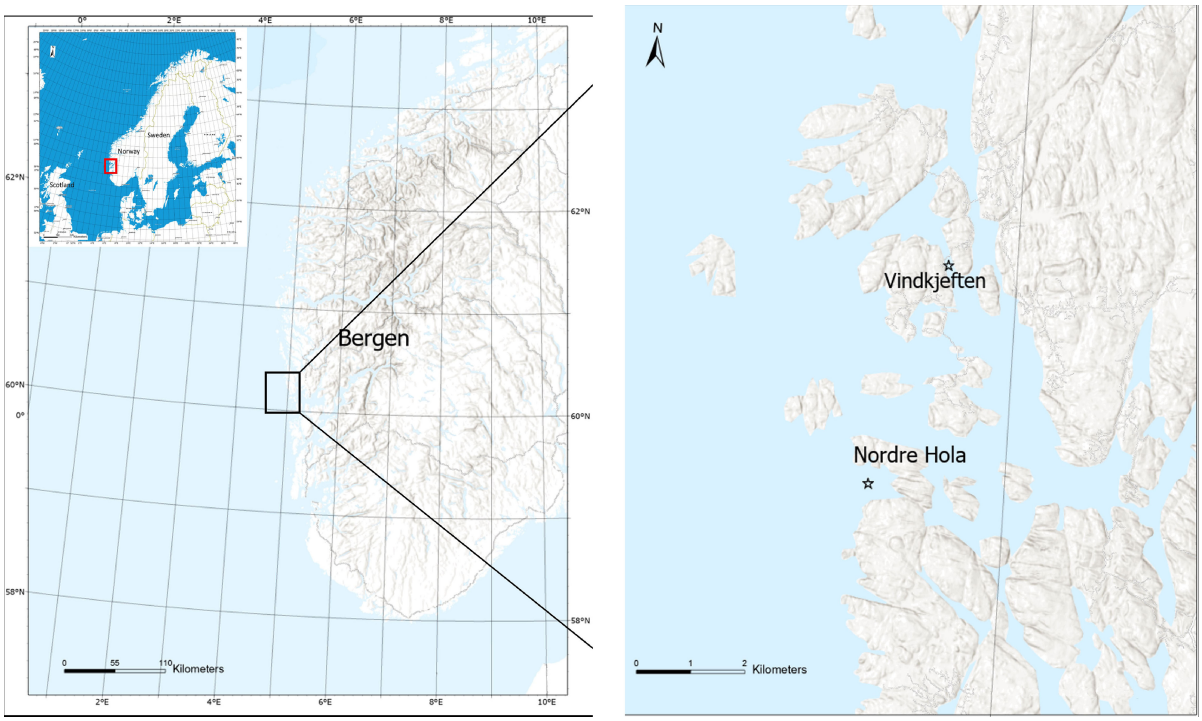

Figure 1. Sampling locations in Western Norway where cod was caught in the spring/summer and autumn of 2021.

To monitor and control for background contamination, open wet traps containing only Milli-Q water were used during the same period as the lab activities. One wet trap was deployed per sample dissection. When microplastic particles were identified in a tissue sample, the corresponding wet trap used during the processing of that sample was also analyzed. In case a plastic particle was detected in the wet trap, particles of the same polymer type were subtracted from the tissue sample results [Supplementary Table 1].

To prevent contamination from the solutions used during the procedure[41], all solutions used were pre-filtered using a glass fiber filter (Whatman GF/A, 1.6 µm) and stored in pre-burnt glass containers until use. Procedural blanks, which used the same solutions and equipment as for sample processing without any sample, were processed simultaneously with the samples.

All 23 fish were measured and weighed during dissection. Body length was measured from the snout to the tip of the tail (caudal length). Based on previously published growth rate curves for resident cod, the age of the cod in this study was estimated to be around two to six years old[37]. As the age of each fish is estimated based on their length, the length is used as a proxy for age. The estimated age of each fish is therefore not given.

Approximately 100 grams from the upper fillet, taken from the region behind the dorsal fin, were used for analysis. The samples were weighed, packed in a double layer of aluminum foil, and frozen at -20 °C until further processing and chemical analysis.

Purification and removal of organic content

To remove the tissue and leave the microplastic particles intact, a gentle enzymatic degradation was performed. The multi-enzymatic extraction protocol for microplastic particles is based on previously published methods[10,42-46]. The method has a recovery rate of 96%-99% and does not damage the plastic polymer[47].

In summary, the purification procedure involved the systematic removal of the muscle tissue, proteins, and fats through a stepwise process outlined in Figure 2. All incubations were done without agitation in a Termaks TS-4115 heating cabinet.

In detail, the cod muscle tissue (up to ~100 grams) was defrosted, cut into cubes, and placed in the sample beaker. First, to permeate cell membranes, 100 mL 5% sodium dodecyl sulphate (SDS) solution was added, and the sample was incubated at 50 °C for a minimum of 3 h [Figure 2].

After incubation in SDS, all the sample was transferred to a vacuum filtration assembly and passed through a 10 µm stainless steel filter. The sample container and filtration system funnel were rinsed with 50% ethanol from a PTFE squeezy bottle. The filter and sample were placed back into the same beaker to prevent loss of material, and 100 mL of 0.1 M glycine buffer at pH 10.0 was added. Ultrasonication was performed on the sample beaker for 10 minutes to release any particles attached to the filter before the filter was rinsed with 1 mL of Milli-Q water. In the second enzyme treatment, 1 mL of protease enzyme (P3111, Sigma Aldrich, Germany) was added, and the beaker was incubated at 50 °C for 48 h before repeating the filtration and ultrasonication steps. The third treatment used 100 mL phosphate buffer saline and 1 mL of lipase enzyme (L0777, Sigma Aldrich, Germany), incubated at pH 5.0 and 50 °C for 24 h.

For the final purification step, a strong oxidative digestion was performed using 50 mL of 30% hydrogen peroxide (H_2O_2) at 50 °C for 12 h to remove fats. The sample was now reduced to a clear liquid [Figure 2]. The sample was filtered to remove the oxidizing agent, sonicated to release any particles from the filter, and rinsed thoroughly with 50% ethanol. The sample was transferred to a tube and the ethanol was evaporated at 50 °C until 1 mL remained. The sample in the tube was transferred to a glass vial for storage, and the tube was washed with 4 × 1 mL of 50% ethanol to transfer all particles, resulting in a total volume of 5 mL in the sample vial. A list of enzymes and buffers can be found in Supplementary Tables 2 and 3.

Prior to identification of the remaining particles, the contents were filtered onto a 0.1 µm Anodisc filter (Whatman, Φ 10 mm) and dried at room temperature in a glass petri dish in the clean PlastLab with a lid until further examination.

Micro Fourier transform infrared spectroscopy identification of microplastics

Identification of polymer types and measurement of particle sizes were automatically performed using micro Fourier transform infrared spectroscopy (µFTIR) with a Thermo Fisher Nicolet iN10 MX Infrared Imaging Microscope. The instrument was equipped with a N_2-cooled 64 × 64 line array mapping detector and a quantum MCT (mercury cadmium telluride) detector. The linear array detector collected 64 scans per sample, and the IR spectra of each microplastic particle were recorded in the mid-IR range of 4,000-850 cm^{-1}, with a spectral resolution of 4 cm^{-1} in transmission mode. The linear array mapping covers the whole Anodisc filter, identifying and measuring the size of every particle on the filter. Currently, the nitrogen-cooled scanning µFTIR- method used in this study is capable of detecting particles down to a theoretical minimum of 6.25 µm according to the technical specifications by Thermo Fisher. As the steel filter mesh used for the pre-treatment is a minimum of 10 µm, we do not regard the method to be quantitative for particles under 10 µm, but smaller particles may stick to the mesh and may be retained nonetheless.

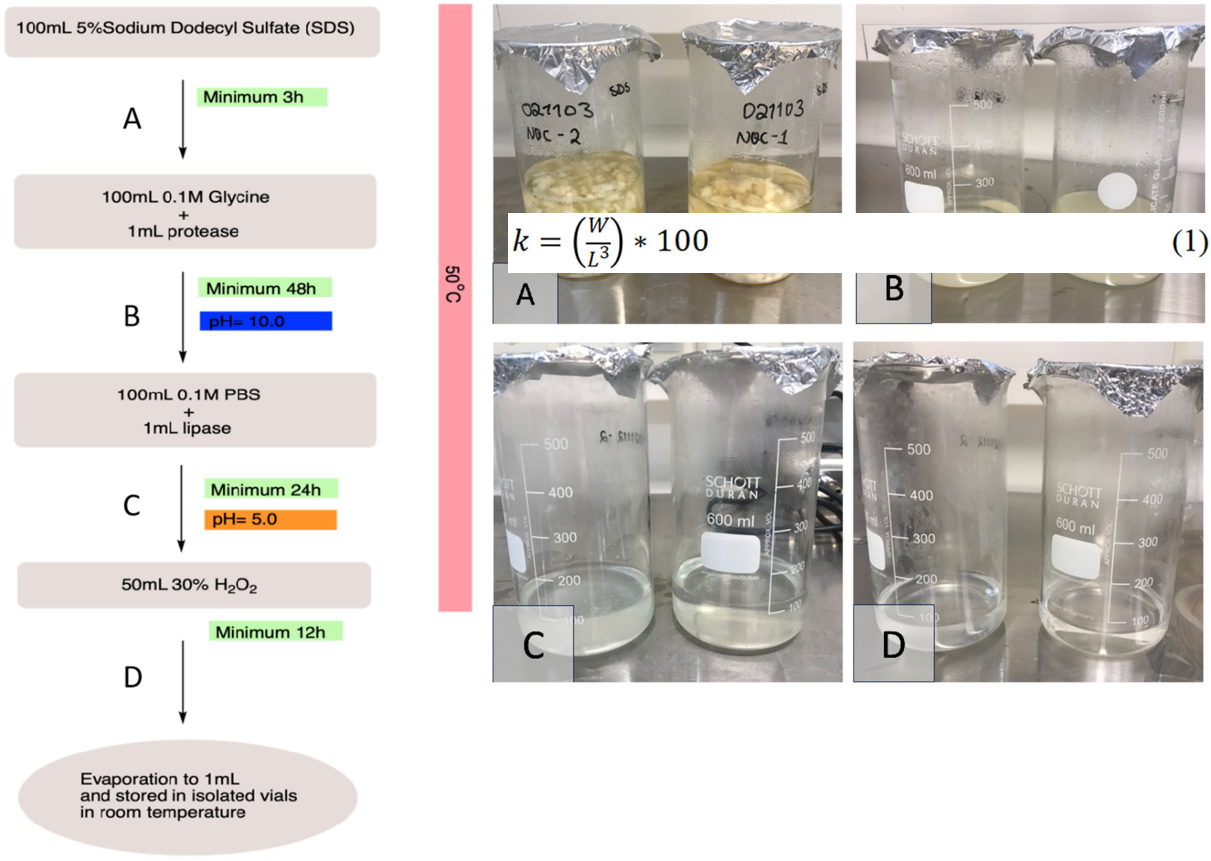

Figure 2. Detailed flowchart of the stepwise enzymatic treatment that ensured gentle and complete removal of proteins and fats in the cod muscle tissue. The progress of the treatment can be observed in the following stages: (A) the cod muscle tissue after 12 h in 5% SDS; (B) the same sample after 48 h in protease; (C) after 24 h in lipase; (D) and after 12 h in 30% hydrogen peroxide. SDS: Sodium dodecyl sulphate.

Polymer identification was performed automatically by the software by comparing the spectral match of the particles with a reference library (SiMPle, v1.3.1β)[48]. Polymers with a spectral match greater than 70% score were considered automatically positively identified. A spectral match above 65% would be accepted or rejected after manual inspection of the spectrum. The software used for identification and grouping of polymer types also facilitated automatic mass calculations per particle. The mass per particle was estimated by the software by multiplying the particle volume by the density of the polymer type[48]. The particle volume is automatically calculated by the software from the measured major and minor dimensions (length and width) and an estimate of the height (60% of the minor dimension). Particles on the filter that were not of synthetic origin (cellulose from tissue paper and any remaining proteins) were excluded from the results.

For shape classification of plastic particles, fibers were defined as having a length-to-width ratio above three, while fragments had a length-to-width ratio up to three.

Calculations and reporting

Body condition (k-factor)

The condition of the cod was described using Fulton's condition factor (k), as defined by Equation 1, where W represents the weight in grams and L represents the length in centimeters[49]. A healthy body condition factor, indicating that the fish is feeding and well, is typically around one.

$$k = \left(\frac{W}{L^3}\right) * 100 \quad (1)$$

Microplastic mass concentration

The mass concentration of MP was calculated in micrograms per kilogram wet weight (µg/kg·ww) using the estimated mass per particle obtained from the SiMPle software output, and the wet weight of the sample at the time of dissection (Equation 2).

$$MP\ Concentration = \frac{Particle\ mass\ (mg)}{Sample\ weight\ (kg\ ww)} \quad (2)$$

Standardized microplastic particle count

The number of detected MP (nMP) was standardized to 100 grams fillet (Equation 3).

$$\frac{nMP}{100g} = \frac{Sample\ weight\ (g)}{100} * n\ detected\ MP \quad (3)$$

Microplastic particle size classes

The MP were categorized into groups based on their size classes determined by their smallest dimension. The size classes included intervals of 10-30, 31-50, 51-100, 101-500, and 500-1,000 µm. In reporting, the focus is on the minor dimension since the smallest dimension is likely the determining factor for retention on the filter during processing.

Statistics

Data management, statistical analyses and calculations were completed using R, (version 2023.12.0+369), Microsoft Excel and IBM SPSS Statistics (version 26.0). To assess the normality of the data, the Shapiro-Wilk test was employed. The results indicated a non-normal distribution for most of the data. Consequently, non-parametric Mann-Whitney *U*-tests were employed to compare means between groups. Spearman's rho correlation test was conducted to examine the correlation between nMP and body length or k-factor. The statistical significance level was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Body condition (k-factor) of fish

The k-factor is often used instead of body weight for fish, as it is a parameter that combines both weight and length and indicates if the fish has been feeding and is healthy, which is of relevance to MP exposure and accumulation from the food web. Length and weight, however, are covariates, and are thus statistically dependent; therefore, examination of the correlation between nMP and one of the two variables, weight and length, suffices. Table 1 provides the weight, length, and k-factor for the cod. Based on growth rate curves for resident cod, the age of the cod in this study was estimated to be around two to six years old[37] . Cod from Nordre Hola (mean length: 41.4 ± 2.95 cm), primarily sampled in May, were significantly shorter compared to the cod from Vindkjeften (mean length: 51.37 ± 14.77 cm), sampled in November (Mann-Whitney *U*-test, $U = 0$, $P < 0.001$), which may in part be explained by higher age and growth during summer and early autumn. The fish from both sites were in generally good body condition, with k-factors ranging from 0.67 to 1.57, and a mean k-factor above 1, indicating that most of the fish were healthy and feeding at both sites and times. There was no significant difference in k-factor between sites (Mann-Whitney *U*-test, $U = 53$, $P = 0.475$).

Frequency of occurrence

Out of the 23 samples of cod muscle tissue, MP particles were detected in 8 cod from Nordre Hola (n = 4) and Vindkjeften (n = 4), resulting in a frequency of occurrence (FO) of 34.78%. As the FO is greatly influenced by the method of analysis, the mass of each sample, and the sensitivity of the detection method, it is subject to large variations among studies. FO up to 100% has been observed[50]. Comparison to previous studies is challenging due to variations in laboratory procedures and reporting, which highlights the importance of using sensitive, harmonized methods and the same format when reporting, for example, FO and size classes across studies. The current low FO in edible tissues of finfish is lower than the FO reported in other studies examining edible tissues and skin of finfish[10,30,50-54], where the FOs were frequently above 40%. Such previously published studies, using a range of different methods, have reported MP from sizes of approximately 100 to 1,000 µm in up to 100% of the samples. The lowest observed FO of 7%[27] used microscopy for manual selection and identified polymers by attenuated total reflectance FTIR (ATR-FTIR), but this method has limitations in detecting particles under 100 µm. In the current study, 93% of the particles were below 100 µm, which theoretically should increase the FO compared to studies that are limited to detection of larger particles. As analytical techniques become more sensitive and accurate, and if size- limits decrease in future studies, it is thus expected that the FO of MP in tissues may increase.

Characteristics of microplastics in cod muscle tissue

There were no significant differences in particle count or particle sizes of microplastic in cod from the two distinct sampling sites and capture periods. As the fish from the two sites were in the same body condition, all the fish were combined to represent the cod population in the local region.

Table 1 presents the microplastic concentration in two ways: particle count standardized to weight (nMP/100 g) and microplastic mass concentration (µg/kg·ww) for all 23 examined cod. The maximum particle concentration was 10.3 nMP/100 g, which equals 0.1 MP/g·ww muscle tissue (cod #4). This is similar to the observed particle concentrations in farmed salmon (*Salmo salar*) from two Norwegian salmon farms of 0.10 ± 0.09 and 0.11 ± 0.12 MP/g ww and in wild salmon with 0.10 ± 0.04 MP/g ww)[50]. The highest observed concentration in this study is lower than the mean concentration observed in the dorsal muscle of European seabass (*Dicentrachus labrax*), Atlantic Horse mackerel (*Trachurus trachurus*), and Atlantic Chub mackerel (*Scomber colias*) from the North East Atlantic, with 0.4 ± 0.7, 0.7 ± 1.3, and 0.6 ± 0.8 MP items/g, respectively[53]. Moreover, fifteen fish showed no detectable MP in the muscle tissue, contributing to a low mean concentration and a large standard deviation of the mean [Table 1]. The mean concentration, when including only the fish with detectable MP, was 4.47 ± 2.99 nMP/100 g, and the mean mass concentration among the same fish was 8.08 ± 13.01 µg/kg.

Polymer types

Six polymer groups were identified: polypropylene (PP), polyethylene (PE), polyester (PES), polystyrene (PS), polyamide (PA), and polyethylene terephthalate (PET). The dominant polymers were PP and PE, which were found in four out of eight positive samples. 38% of the particles in total were PP, and 31% of the particles in total were PE. The distribution of polymers in individual fish can be found in Figure 3, and polymer type, size, and shape per particle are shown in Supplementary Table 1.

MP shape and size

A total of 29 particles were identified as microplastic and passed quality assurance and quality control, of which 21 particles were classified as fragments and eight as fibers. The particle sizes [Figure 4] show that the majority (62%) were smaller than 50 µm. 93% of the particles were smaller than 100 µm in the minor dimension. Fibers and fragments over 200 µm were observed. The largest particle was a PP particle of 258.2 µm.

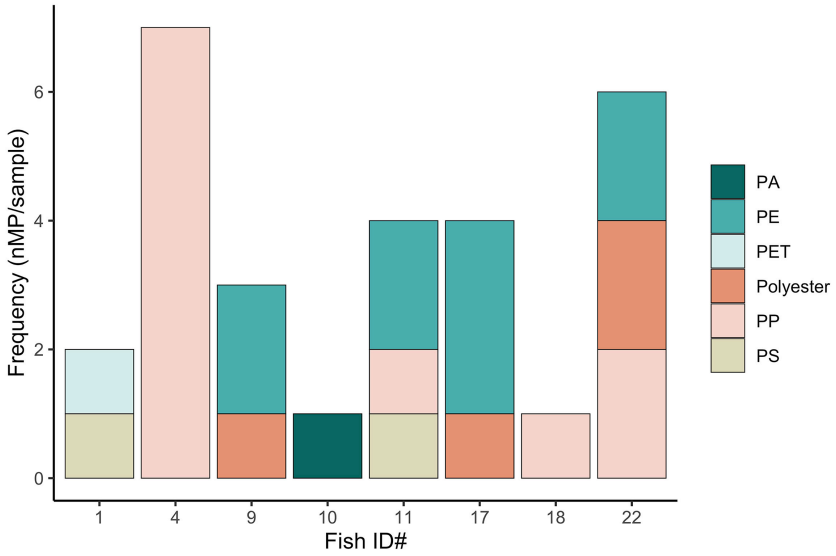

Figure 3. Microplastic concentration (nMP/sample) and polymer distribution in muscle tissue of wild-caught cod from a plastic-polluted area in western Norway 2021. PA: Polyamide; PE: polyethylene; PET: polyethylene terephthalate; PP: polypropylene; PS: polystyrene.

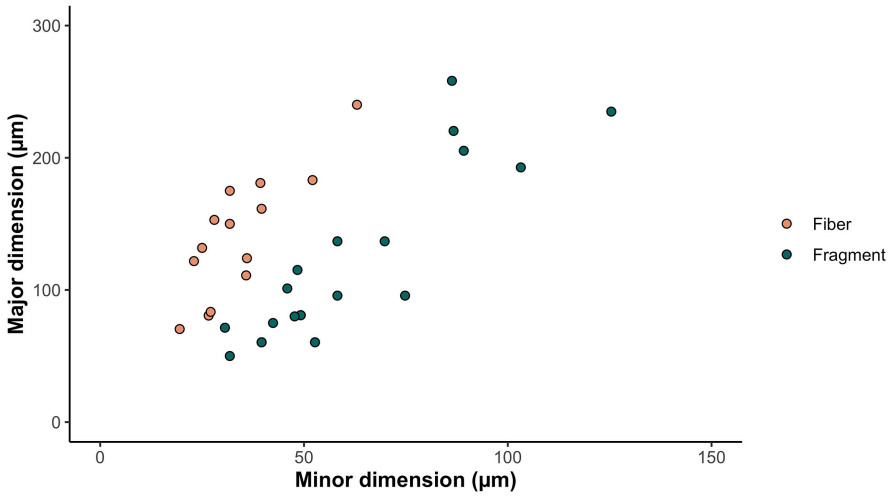

Figure 4. Size distribution of microplastic particles in cod muscle tissue. Major by minor dimension (µm).

Among the eight corresponding wet traps analyzed for quality control, only two contained microplastic particles, which corresponded to the processing of cod #1 and #4. Both wet traps had one PE fiber each, measuring 123 and 310 µm, respectively. Since the color of the PE fibers in the wet traps matched those detected in cod #1, the two PE polymers detected in cod #1 were excluded from the analysis.

Cod #4 exhibited large fragments of polypropylene (PP) up to 235 µm but did not contain any PE which was the polymer type observed in the corresponding wet trap; thus, no subtraction of PE or particles was performed in cod #4. The fishnets used were made of orange PP, but the color did not match the observed particles for cod #4.

No further MP were detected in the wet traps corresponding to fish with observed microplastic particles; thus, the MP are trusted to be from the fish samples, and not contamination from the external lab environment. After subtracting the particles corresponding to wet trap controls, a total of 29 out of 31 particles were included in the results (All particles identified as microplastic are shown in Supplementary Table 1). The procedural blanks did not exhibit any contamination, and therefore, no subtraction of particles was required to account for procedural contamination.

Mass and concentration

MP concentrations in cod muscle tissue (µg/kg·ww) varied greatly, with mean concentrations of 8.08 ± 13.01 µg/kg in the eight cod where MP particles were detected. The highest single concentration of 38.9 µg/kg was found in a 40 cm long fish (cod #4), which had a notable presence of large PP fragments and fibers that significantly impacted the calculated MP mass. However, this concentration, which is considerably higher than the mean for this study, is much lower than the previously reported concentration in the small pilot study of three cod from the same region which observed mean concentrations of 670 ± 470 µg/kg ww in cod muscle tissue[11]. This may be the result of improved analytical methods and the use of a plastic-controlled environment in this study. Cod with both high and low MP concentrations and of different lengths had k-factors above 1, indicating good condition and feeding status irrespective of the MP concentrations observed.

We examined statistically which factors could contribute to the levels and variation of microplastic in the cod muscle tissue. Due to the low number of cod with detected MP particles in the muscle tissue, and the non-normal data distribution, regression analyses could not be performed.

Non-parametric correlation tests were performed on the cod to investigate the correlation between MP occurrence and other likely influencing factors [length (age), body size (weight) and k-factor], combining the cod from the two sites. There were no significant correlations between particle counts (nMP/100 g) and fish body length, weight, or k-factor [Figure 5]. Likewise, there was no correlation between MP mass-concentration (µg/kg·ww) and length (Spearman's rho, r = -0.092, $P = 0.68$) MP mass-concentration and weight (Spearman's rho, r = -0.014, $P = 0.95$) or MP mass-concentration and k-factor (Spearman's rho, r = -0.1, $P = 0.65$). Interestingly, both large and small cod were observed to have zero MP in their muscle tissue [Figure 5]. The results suggest that length (age), body weight, and k-factor are not determining factors for the MP concentration in muscle tissue of cod in plastic-polluted areas. However, the sample size is small, and the low number of cod with positive observations of MP in the fillet hampers statistical analyses. On the other hand, one would expect that if accumulation occurred throughout the lifetime of fish up to six years of age, it would be observable with the sensitive methods used.

Limitations of the study

In this study, we observed a low FO and low concentration of MP in the muscle tissue of wild-caught cod from a highly plastic-polluted area in Western Norway. One potential reason for the low concentrations can be the strict contamination control and good laboratory practice that prevented external contamination from influencing the results[41]. Other influencing factors may theoretically be potential particle losses during the analyses. The use of strong reagents during tissue removal may damage the particle surface, which is why this study instead uses a gentle enzymatic degradation method that has observed a 96%-99% recovery rate. Maintaining the integrity of the polymer particles is important because, if particles close to 10 µm in one dimension experience a small reduction in size, they may be lost through the 10 µm filter mesh.

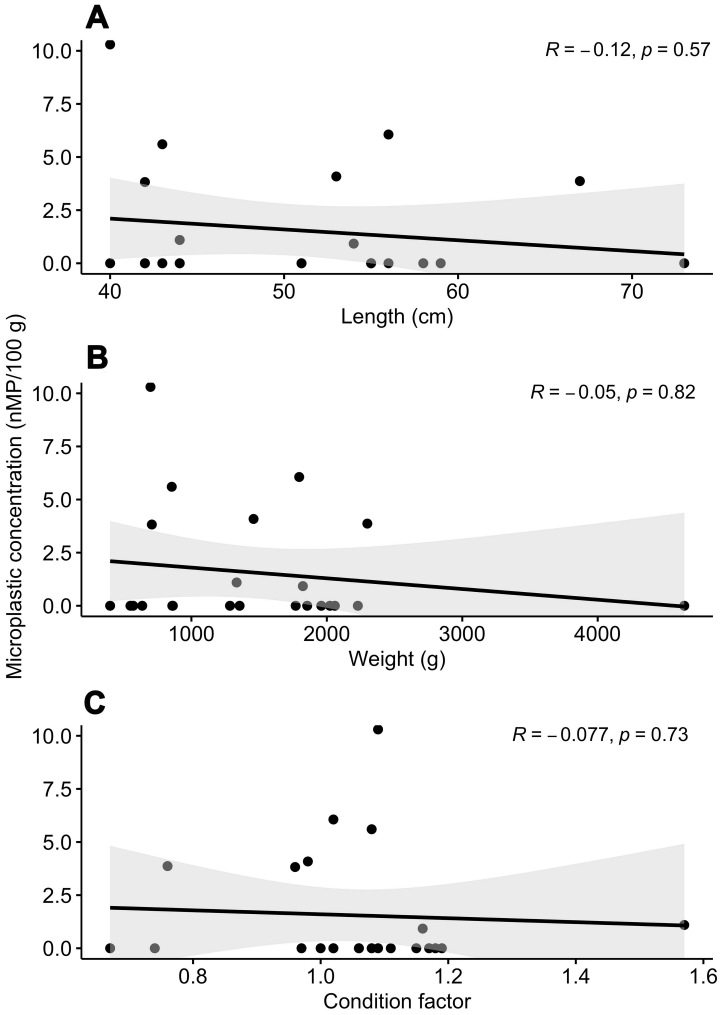

Figure 5. The relationship between nMP/100 g and (A) body length, (B) body weight, and (C) k-factor in resident cod from two locations in Western Norway 2021.

Regarding potential contamination of the samples, which would increase the observed concentrations, strict precautions were taken in the NORCE Plastlab to minimize the introduction of microplastic particles to the samples, in order to strictly quantify the particles that were present due to ingestion in the wild and translocation into fish tissues. The observation of somewhat larger PP fibers and fragments raised some concerns. It is possible that particles larger than 200 μm may have become attached to the fish externally during handling, despite measures taken to minimize contamination. The source of these fibers and fragments remains unidentified, and they do not correspond to the equipment used. This shows how challenging it is to entirely avoid plastic contamination in a plastic-dependent society. Various potential sources of contamination during all procedures, such as fishing gear, clothing, ropes, buckets, and transportation, need to be considered and the use of plastic minimized throughout the procedure. However, removing the larger particles (> 200 μm) from the dataset did not impact the results.

The lack of standardized methods and variation in contamination control measures pose limitations to building knowledge from studies conducted across different regions[32,55]. Previous studies have reported the occurrence of MP in edible parts of finfish[10,25,28,30,51,56,57]. However, due to differences in methods, reported

size classes, and variation in contamination control, comparing concentrations and distribution patterns of MP among these studies is challenging.

In terms of replicate analyses, working with a larger number of samples with fewer negative findings is ideal for increased statistical power and understanding method uncertainties. However, microplastic analysis with these sensitive and gentle methods is time-consuming and expensive, making it financially very challenging to expand the sample size to increase data resolution and reduce uncertainties.

The microplastic burden in the water column and sediments of the plastic-polluted region where the cod was caught is assumed to mirror the macroplastic pollution observed on many beaches in the region that are known to receive large volumes of marine debris[35], and it is assumed that the cod had been exposed to macroplastic and MP throughout its lifetime. Cod from this region have been observed to have a higher rate of plastic in their stomach than cod from other areas in Norway[40]. However, direct measurements of the concentrations of MP in these waters and sediments where the fish were caught were not performed, due to funding limitations. A study of MP in sediments from a nearby fjord demonstrated high concentrations of microplastic particles, with the majority being under 100 μm[42], and the polymer composition suggesting both terrestrial and marine sources.

Implications for human consumers

Previous studies have shown that the highest number of microplastic particles are in the lower size classes when using similar methods of analyses[1,42,58]. However, the presence of a high number of small MP in the environment does not necessarily indicate high uptake by organisms or high prevalence in tissues, as studies have demonstrated a low absorption rate of nanoplastics in finfish[22,59].

It has been shown experimentally that fragments over 100 μm can be taken up by organisms[16]. MP have been found to pass through biological tissue either between enterocytes or through phagocytosis by organs such as the gills or gastrointestinal tract[16,60]. The absorption process appears to be influenced by the size of the MP[16,61], but is not fully understood, and a number of studies report larger particles in tissues than what is believed to be able to pass through or between cells. This highlights the need for studies that elucidate the uptake and translocation mechanisms of MP and may provide answers to the most likely size classes to be translocated into, and potentially retained in, tissues.

For human consumers, the presence of MP in our food is of concern, especially if MP are found to accumulate throughout the life of an organism. Our current observations that MP do not increase with age in cod may indicate that the muscle tissue in cod is not the target organ where MP will accumulate, and thus that consumption of old and large fish does not imply a higher exposure to MP. This is in contrast to the known bioaccumulation of several persistent contaminants with the age of the organism, where consumption of older or larger organisms represents a greater risk of exposure, which is therefore advised against.

The Minderoo Monaco Commission has referred to MP as POPs due to their common characteristics[62]. Even without proof of bioaccumulation, persistence and mobility alone are criteria for the POP classification, meaning MP may indeed be classified as POPs. However, the need to thoroughly assess the physico-chemical properties of MP and their bioaccumulation and biomagnification throughout the food web remains. If MP do bioaccumulate, as has been indicated by recent studies of mammalian testis[14], it would warrant increased funding for research into human microplastic exposure and kinetics and support toward the ongoing work to negotiate the international binding treaty on plastic pollution (UNEP-INC),

aimed at curbing the production and unregulated release of plastics, as well as monitoring of the generation and distribution of MP in the same way as for the well-known legacy POPs, such as polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethane (DDT) and tetrachlorodibenzodioxins (TCDDs) (dioxins).

Little evidence for bioaccumulation *in situ*

Bioaccumulation of a substance implies that the concentration increases significantly above that of the surrounding environment and can be observed to increase with age due to inefficient excretion or metabolism. This study cannot conclude with evidence of bioaccumulation in the muscle tissue of up to six-year-old cod of increasing length and body mass. Instead, this study adds to a growing number of publications that do not find evidence for microplastic bioaccumulation *in situ*. Similarly, no correlation was found between fish mass and microplastic concentrations in Smallmouth bass (*Micropterus dolomieu*), Lake whitefish (*Coregonus clupeaformis*), and Northern pike (*Esox lucius*), and the study observed no trend of increasing levels in organs over time. A recent study of marine animals at different trophic levels concluded with no evidence of bioaccumulation of MP > 100 μm in the digestive tracts and liver[63].

Similarly, a review of 226 field studies across various fish and seafood species also found no increase in microplastic concentrations per individual with trophic level, challenging the notion of bioaccumulation of plastic[55]. However, the mentioned review cautioned against using the presence of MP in the gastrointestinal tract as an indicator of bioaccumulation, as the transient presence of MP in the gut may simply indicate recent ingestion and could vary over short periods of time due to egestion.

In contrast, another systematic review[33] concluded that bioaccumulation of MP did, in fact, occur within trophic levels, when focusing on the gastrointestinal tract of finfish rather than muscle tissue, which was criticized[55]. The criticized study, however, also concluded with no evidence of biomagnification at higher trophic levels and acknowledged the challenges in identifying such processes over several decades and across the development of different methods for microplastic quantification.

To definitively answer the question of microplastic bioaccumulation, carefully designed *in vivo* experiments or larger datasets from *in situ* observations are needed. For now, the weight of the *in situ* evidence is leaning toward no evidence of bioaccumulation. However, the absence of evidence is not the same as evidence of absence; thus, carefully designed studies and sensitive methods are necessary to settle this question of bioaccumulation of microplastic.

Body distribution of microplastic

The current study focuses on microplastic concentrations in cod muscle tissue. Studies of nanoparticle uptake and depuration in fish do not indicate any specific target organ where nanoplastic particles accumulate[22]. Observations of nanoplastics being taken up, distributed, and eventually eliminated from the liver and other organs may also suggest that the presence of the larger MP in livers is transient and not persistent[17]. In a previous report on MP in salmonids, no significant difference was observed between muscle and liver tissue concentrations, indicating that muscle tissue could serve as equally suitable for monitoring[50]. Similarly, a recent study[63] observed no bioaccumulation in the liver after analyses that included lean fish. Observations confirming the occurrence of MP in the livers of wild fish do not necessarily imply that the liver is a primary target organ for MP accumulation, and studies have shown that MP can be excreted through bile in vertebrates[16], further supporting the likelihood of a transient presence of MP in the liver.

For future studies on cod or fatty fishes, it would be feasible to examine several tissues to determine if there are differences in microplastic distribution after a lifetime of exposure. Furthermore, it is advisable to collect samples from fresh, unfrozen tissue, as freezing and thawing can cause particularly the liver to disintegrate, potentially leading to the loss of microplastic particles. Obtaining sufficiently large liver samples for microplastic analyses may moreover be challenging in small cod.

Although the current evidence suggests that bioaccumulation of MP is not observed using existing analytical methods, it does not imply that exposure to plastic and MP is safe or that plastic pollution is not an environmental concern. The production of plastic involves the use of numerous chemicals, and the uncontrolled release of plastic waste results in the dispersal of unknown quantities of these chemicals into the environment, with increasing and chronic MP exposure to animals and humans. This may be harmful even if the presence in tissues is transient.

Rather than relying on the demonstration of bioaccumulation and biomagnification of MP throughout the food chain, before we take measures against MP pollution, we should consider whether we need more evidence that plastic behaves exactly like the legacy POPs to stop plastic pollution and implement better management practices for plastic materials.

CONCLUSIONS

In conclusion, this study of coastal cod from a plastic-polluted region in Western Norway supports previous observations of the presence of microplastic in the edible tissues of finfish. The study did not find evidence of bioaccumulation in the muscle tissue of two- to six-year-old cod of good body condition. These findings align with previous studies that conclude with no bioaccumulation or biomagnification of MP across different species and trophic levels. The analyses were performed in a state-of-the-art plastic-free laboratory, ensuring minimal external contamination. The study highlights the importance of accurate and sensitive methods and the need for the development of efficient techniques to process larger sample volumes without potentially damaging small microplastic particles. The study found no correlation between fish size, age, body condition, and microplastic concentration; thus, this study provides no support for bioaccumulation with age, but the sample size is small, and the results should be interpreted with care. No bioaccumulation indicates no increased risk for humans of MP ingestion from consuming fillets of large fish. The intake of MP from cod from this region is, based on this study, projected to be low, with a FO of 34.78 % and an average concentration of 2.81 ± 8.33 µg/kg·ww. Future studies could include controlled laboratory experiments to assess the dose-uptake relationship of MP using environmentally relevant exposures. Moreover, humanity already possesses sufficient knowledge about the negative effects of plastic and associated chemicals to justify better management practices and efforts to stop plastic pollution.

DECLARATIONS

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Authors' contributions

Idea, concept, study design, data treatment, graphics, writing, and editing: Haave M
Field work, laboratory work and analysis, data treatment, and proofreading: Hæggernes E
Methodology, data analysis, laboratory supervision, FTIR analyses, QA/QC, and proofreading: Gomiero A

Availability of data and materials

Original FTIR raw data from all cod and the two wet traps with observed MP are available at the Supplementary raw data.

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Conflicts of interest

Haave M is currently employed by SALT Lofoten AS, which declares no conflicts of interest regarding the study results. Other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable. The fish used in the experiment were caught as part of teaching for the Norwegian Hunters and Anglers Association in the school project TAM and were subsequently donated for research aimed at MP analyses. The sampled fish were caught from the wild in accordance with Norwegian fishing and hunting regulations. Coastal cod recreational fishing is permitted without requiring ethical approval, provided that minimum size regulations are followed. Rules for recreational fishing and the minimum permissible size can be found at https://lovdata.no/dokument/SF/forskrift/2021-12-23-3910.

Consent for publication:

Not applicable.

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