



Distinct polymer-dependent sorption of persistent pollutants associated with Atlantic salmon farming to microplastics

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ABSTRACT

Interactions of microplastics and persistent organic pollutants (POPs) associated with Atlantic salmon farming were studied to assess the potential role of microplastics in relation to the environmental impact of aquaculture. HDPE, PP, PET and PVC microplastics placed for 3 months near fish farms sorbed POPs from aquafeeds. PET and PVC sorbed significantly higher levels of dioxins and PCBs compared to HDPE, while the levels sorbed to PP were intermediate and did not differ statistically from PET, PVC or HDPE. In addition, the composition of dioxins accumulated in caged blue mussels did not reflect the patterns observed on the microplastics, probably due to polymer-specific affinity of POPs. In conclusion, the results of this study show that microplastics occurring near fish farms can sorb aquafeed-associated POPs and, therefore, microplastics could potentially be vectors of such chemicals in the marine environment and increase the environmental impact of fish farming.

1. Introduction

Microplastics (MPs), commonly defined as plastic particles with size range from 1 µm to 5 mm (Hartmann et al., 2019), are ubiquitous and considered as emerging contaminants (Lambert and Wagner, 2018). In the marine environment, they have been reported in coastal waters, surface waters, the water column, deep-sea sediments, estuaries and fjords (Harris, 2020; Lusher, 2015), including in northern waters (AMAP, 2021). MPs can have a wide range of negative effects on biota, from reduced feeding to immune system alterations (Kögel et al., 2020; Lusher, 2015). Depending on size and shape, MPs can cross body barriers, e.g. be translocated from the digestive tract to other tissues and

organs, and be taken up into cells (Abihssira-García et al., 2020). MPs have been detected in liver and muscle tissue of both farmed and wild salmonids (Gomiero et al., 2020a). Plastic microfibers have also been detected in several organs from blue mussels (*Mytilus edulis*) exposed to concentrations of 2000 particles/L, including mantle and foot (Kolandhasamy et al., 2018). Accumulation of MPs in edible tissues might in turn constitute a health risk for human consumers (Danopoulos et al., 2020).

Negative effects of MPs on organisms may derive from plastic additives as well as the plastic polymer particles themselves (Campanale et al., 2020; Kögel et al., 2020). Additionally, persistent organic pollutants (POPs) can sorb to MPs due the hydrophobic nature these

Abbreviations: BTBPE, 1,2-bis(2,4,6-tribromophenoxy)ethane; DDT, Dichlorodiphenyltrichloroethane; EQS, Environmental Quality Standards; FF, Fish farm; GC-ECD, Gas Chromatography with Electron Capture Detector; GC-MS, Gas chromatography-mass spectrometry; HCH, Hexachlorocyclohexane; HDPE, High density polyethylene; HxCDD/F, Hexachlorodibenzo-p-dioxin/furan; HpCDD/F, Heptachlorodibenzo-para-dioxin/furan; HRGC/HRMS, High resolution gas chromatography/high resolution mass spectrometry; MP, Microplastic; LDPE, Low density polyethylene; LOD, Level of Detection; LOQ, Level of Quantification; OCDD/F, Octachlorodibenzodioxin/furan; PAH, Polycyclic Aromatic Hydrocarbon; PBCCH, Pentabromochlorocyclohexane; PBDE, Polybrominated diphenyl ethers; PBT, 2,3,4,5,6-pentabromotoluene; PCB, Polychlorinated biphenyl; PCDD/F, Polychlorinated dibenzodioxins/furans; PE, Polyethylene; PeCDD/F, Pentachlorodibenzo-P-dioxin/furan; PET, Polyethylene terephthalate; PP, Polypropylene; POP, Persistent organic pollutant; pTBX, 2,3,5,6-tetrabromo-p-xylene; PVC, Polyvinyl chloride; TBPhA, Tetrabromophthalic anhydride (also known as TBPA); TBoCT, Tetrabromo-o-chlorotoluene; TCDD/TCDF, Tetrachlorodibenzo-p-dioxin/furan; TEQ, Toxic Equivalency; TEF, Toxic Equivalency Factor; uPVC, Unplasticized polyvinyl chloride.

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chemicals, and act as vectors for POPs in the marine environment (Hirai et al., 2011). For example, several studies have documented sorption of PCBs, PAHs, pesticides and other POPs, on MPs collected from marine environments (Chen et al., 2019; Hirai et al., 2011; Rochman et al., 2013a; Rodrigues et al., 2019; Ziccardi et al., 2016). POPs sorbed to MPs may be transferred to the organisms after ingestion, increasing and complicating the impact of such particles and pollutants (Chua et al., 2014; Rochman et al., 2013b). The role of MPs as vectors of pollutants to organisms has been suggested to be irrelevant compared to dietary or environmental exposure. In addition, MPs could pass through the gastrointestinal tract without leaching chemicals (Koelmans, 2015; Lohmann, 2017). Nevertheless, translocation of small polluted MPs into cells, tissues and organs of organisms only exposed to low levels of POPs could have a significant impact in such individuals. In such cases, MPs might be a pathway for uptake and bioaccumulation of POPs in marine organisms (Bakir et al., 2016; Koelmans, 2015).

Dioxins are chemical substances formed as byproduct during combustion reactions in the presence of chlorine (Altarawneh et al., 2009). In vertebrates, dioxins are carcinogenic and can disrupt the immune, nervous and endocrine systems, and affect reproduction and development (Schechter et al., 2006). Considering the increasing numbers of wildfires as result of climate change (Calheiros et al., 2021; Higuera and Abatzoglou, 2021), and that dioxins tend to accumulate in polar regions due to slower degradation in cold areas (Kobusińska et al., 2020) and the grasshopper effect (Gouin et al., 2004), more attention should be paid to the impact of dioxins in the environment together with MPs. To our knowledge, there is no record of levels of dioxins adhered to MPs in the environment except for the levels found in charred MPs (Saliu et al., 2018). Therefore, interaction of MPs with certain POPs such as dioxins should be studied more in depth.

Atlantic salmon (*Salmo salar*) is one of the most consumed fish species in the world and the commercially most important farmed marine fish species (FAO, 2020). The levels of pollutants associated with Atlantic salmon farming have long been a concern. Salmon feed contains about 10% of fish oil (Ytrestøyl et al., 2015), which is the main source of POPs to farmed salmon. Analysis of 20 salmon feed samples randomly collected from salmon farms in Norway in 2017 showed concentrations of polychlorinated biphenyls (PCB₆) of 3 µg/kg, sum of dioxins (PCDD/PCDF) and dioxin-like PCBs of 0.6 ng TEQ/kg, and concentrations of polybrominated diphenyl ethers (PBDE₇) of 0.38 µg/kg (Sele et al., 2019). In addition, fish feed also contained traces of organochlorine pesticides such as DDT (and degradation products), toxaphene and endosulfan, and organophosphorus pesticides such as chlorpyrifos-methyl and pirimiphos-methyl. In recent years, replacement of fish oils with plant oils has led to reduced levels of POPs in farmed Atlantic salmon in Norway (Berntssen et al., 2010; Lundebye et al., 2017; Nøstbakken et al., 2015). However, the use of plant ingredients in aquafeeds has increased the levels of other organic pollutants in fish feed, such as PAHs and pesticides (Berntssen et al., 2010; Nacher-Mestre et al., 2014).

In Norway, about 1.3 million metric tons of Atlantic salmon is produced annually (Ytrestøyl et al., 2015). For each kg of salmon produced, about 0.5 kg of feces and unconsumed feed pellets are generated (Grefsrud et al., 2018). This waste slips through the open-cage net pens and spreads in the environment, depending on local physical, chemical and biological factors. Open fish farms thus represent local point-sources of pollution. Yet little is known about how MPs might contribute to the spreading of POPs near fish farms.

Fish farms benefit from the lightweight, strong and flexible plastic in permanent installations. Net pens, ropes, floats and pontoons are some of the framework structure made of plastic polymers such as HDPE, PP, PET or PVC (Lusher et al., 2017). Abrasion and loss of plastic items inevitably lead to the release of MPs into the environment. A recent study showed the presence of MP polymers such as PP, PE, PVC and PET in sediment, seawater and suspended matter around Atlantic salmon farms of Norway (Gomiero et al., 2020b). In Norway, about 1000

commercial salmonid farm facilities are placed in the marine environment (BarentsWatch, 2021). These are estimated to use a total of 191,799 tons of plastic materials, of which 35,571 tons are nets (PE and nylon), 17,201 tons are mooring ropes (PP), 4440 tons are feeding pipes, (HDPE) and 108,405 tons are cage floaters, handrails and walkways (HDPE) (Sundt et al., 2018). Based on the degradation rates of PE (0.45% mass loss/month), nylon (1.02% mass loss/month) and PP (0.39% mass loss/month) ropes in the marine environment calculated by Welden and Cowie (2017), the total amount of nets and ropes deployed by fish farms in Norway are estimated to release 3137 tons of MPs/year and 805 tons of MPs/year into the environment, respectively. Feeding pipes used in salmon farming have recently been shown to release an average of 0.25 g/m/day MPs along with the fish feed (Gomiero et al., 2020b). Based on this study, feeding pipes used in Norwegian aquaculture are estimated to release 225 tons of MPs/year in the water. In addition, cage floaters are estimated to release 5.4 tons of MPs/year based on the assumption that plastic degrades at an annual rate of 0.5% in the Norwegian marine environment (Booth et al., 2017). Based on all the above, fish farming in Norway might release more than 4172.4 tons of MPs annually into the marine environment. Thus, understanding the potential role of MPs as vectors of pollutants from fish farming is important for countries such as Norway.

The objective of this study was to evaluate whether MPs can sorb POPs released from fish feed significantly, and consequently act as potential vectors for these chemicals to the surrounding environment. As a step towards better understanding the impact of different MPs on the environment, we assessed the capacity of HDPE, PP, PET and PVC to sorb POPs. For this purpose, MPs of four different types of polymers commonly found in the environment and detected in the vicinity of salmon farming facilities (Bråte et al., 2017; Gomiero et al., 2020b; Suaria et al., 2016) were placed close to two salmon farms for three months. For comparison, MPs were additionally deployed in two sites not influenced by salmon farming; one low-polluted and one polluted location. Furthermore, a positive control for fish feed pollutants was set up under laboratory conditions. POPs sorbed to MPs were then qualitatively and quantitatively analysed. Blue mussels were collected from a reference site and placed next to the MPs at all stations, with the objective of documenting the pattern of POPs in the environment. This filter-feeder is often used in water monitoring studies as a bioindicator of water pollution (Beyer et al., 2017). Recently, mussels have also been suggested as bioindicator for marine MP pollution, since they are widely distributed and have been shown to indicate differences between sites with large differences in MP concentrations (Bråte et al., 2018; Li et al., 2019). Blue mussels are currently used in coastal environmental monitoring programs in Norway as bioindicators for heavy metals, organic pollutants and MPs (Beyer et al., 2017; Green et al., 2020).

2. Materials and methods

2.1. Blue mussels

Adult blue mussels (*Mytilus edulis*) were collected from an unpolluted location in Åselistraumen in Bodø Municipality, Norway (67°12'01"N 14°37'56"E) in October 2018. This site was also used as reference station (negative control) in the experiment. The mussels weighed on average 6.4 ± 2.1 g (including shells and epibionts) and had a length of 3.7 ± 0.2 cm ($n = 700$, mean \pm st.dev.). The mussels were transported to the laboratory in aerated water, divided into four groups and used in the field experiment as described below.

2.2. Plastic polymers

Commercial unplasticized and uncoloured MPs were purchased from GoodFellow (Huntingdon, UK) (Supplementary Table S1). PP (3 mm), HDPE (3 mm), uPVC (250 µm) and PET (3–5 mm) were selected for this study due to the high occurrence of these polymer types as MPs in the

marine environment. The three first mentioned polymers have been reported to be among the most abundant plastics in the sea (Morgana et al., 2018; Suaria et al., 2016), while the last one is the main polymer found in some demersal fish, such as Atlantic cod, probably due to its high density (Bråte et al., 2016; Morgana et al., 2018).

2.3. Field experiment

To assess the behaviour of POPs from salmon farming with respect to MPs, four stainless steel cages (40 × 40 cm) containing MPs and mussels were deployed in early October 2018 in four different sites of the Salten district of Nordland, Norway (Fig. 1): two at two different Atlantic salmon farms operated by the Salten Aqua AS Group, located in Morsdalsfjorden (FF1; 67°01'37.859N 14°07'26.76 E) and Saltaldsfjorden (FF2; 67°12'10.3"N 15°16'32.3"E); one in a polluted area, Bodø Harbour (67°17'46"N 14°23'43"E); and one in a non-polluted reference station situated in Åselistraumen (67°12'05"N 14°37'14"E).

Each cage contained 50 g of pristine MPs of each of the following four polymer types: HDPE, PP, PET and uPVC. Each MP polymer type was individually enclosed between two stainless steel sieves of 150 µm mesh size to prevent the polymer types from mixing, and were randomly placed in the cage (Fig. 2). Blue mussels ($n = 175$) were added to each cage to monitor chemicals present in the surrounding water. The four cages were deployed at 5 to 8 m depth at the fish farms, in approximately 5 m horizontal distance from the net pens. In January 2019, plastic polymers and mussels were collected after 3 months in the sea and stored at $-20\text{ }^{\circ}\text{C}$ for chemical analyses. Cages were deployed in autumn/winter, when the hours of light are at a minimum, to avoid excess biofouling growth on the cages, which would block the water flow to the MPs. Water temperatures during the 3 months experiment were

obtained from public data recorded at the fish farms. Average temperatures at FF1 and FF2 were $8.7 \pm 1.1\text{ }^{\circ}\text{C}$ and $7.3 \pm 1.0\text{ }^{\circ}\text{C}$, respectively, and decreased gradually from $10.7\text{ }^{\circ}\text{C}$ to $6.6\text{ }^{\circ}\text{C}$ at FF1 and from $8.3\text{ }^{\circ}\text{C}$ to $5.5\text{ }^{\circ}\text{C}$ at FF2 from October to January (BarentsWatch, 2021). Water salinity was measured during the experiment with a CTD deployed at 7 m depth in Saltfjorden and Saltaldsfjorden. The recorded salinities were 31.5 and 28.8 ppt, respectively.

2.4. Positive controls

To evaluate to which degree feed-derived POPs sorb to MPs placed close to fish farms, positive controls were prepared by mixing fish feed with each of the four types of MP polymers separately (Fig. 2). Twelve beakers of 1 L, three for each polymer ($n = 3$), were filled with 800 mL of seawater previously filtered through a 10 µm mesh and autoclaved. A concentration of 2 g/L of MPs and 4 g/L of fish feed, previously dried at $80\text{ }^{\circ}\text{C}$ for 19 h and mashed until powder with a mortar, were added to each flask. The mixtures of feed and MPs were kept in rotation for 3 days at room temperature on a magnetic stirrer at 600 rpm, a speed selected to ensure thorough mixing and to avoid the plastic to float. The MPs were then collected with small stainless steel spatula and stored at $-20\text{ }^{\circ}\text{C}$ until further analyses.

2.5. Chemical analysis

2.5.1. Mussel analysis

Chemical analyses of the soft tissue of whole blue mussels were carried out in the Chemistry and Undesirables Laboratory of the Institute of Marine Research, Bergen, Norway. The methods used to analyse polychlorinated dibenzo-para-dioxins (dioxins/PCDD), polychlorinated

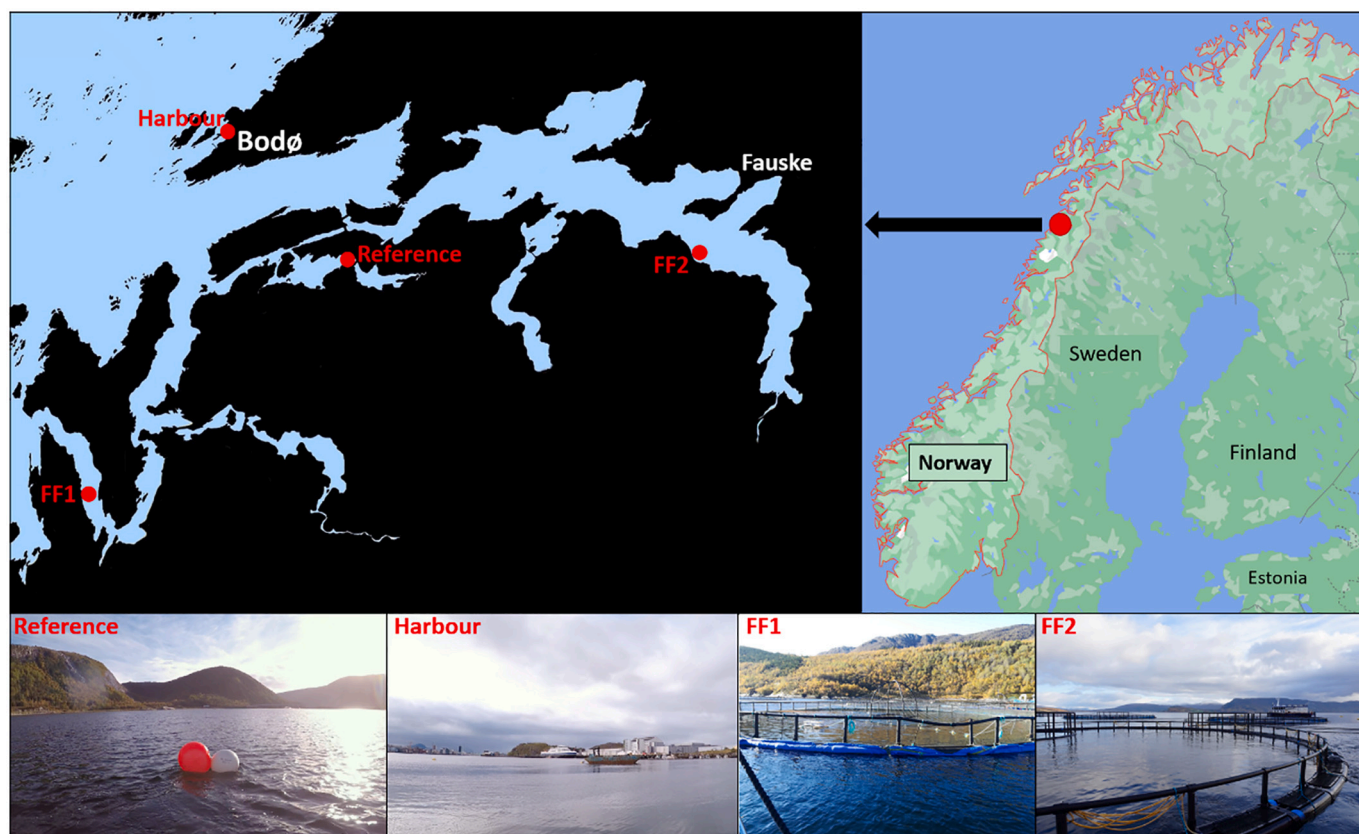


Fig. 1. Maps of the studied area (top). Cages were deployed in the Salten district of Nordland, Norway (top right, red point) in four different sites (top left, red points): Åselistraumen as non-polluted control (Reference), Bodø harbour as polluted control, and two fish farms located in Morsdalsfjorden and Saltaldsfjorden (FF1 and FF2, respectively). Bottom: The four studied sites. Pictures obtained on the day the cages were deployed. Maps were extracted from Google Maps; top left map was modified for better visualization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

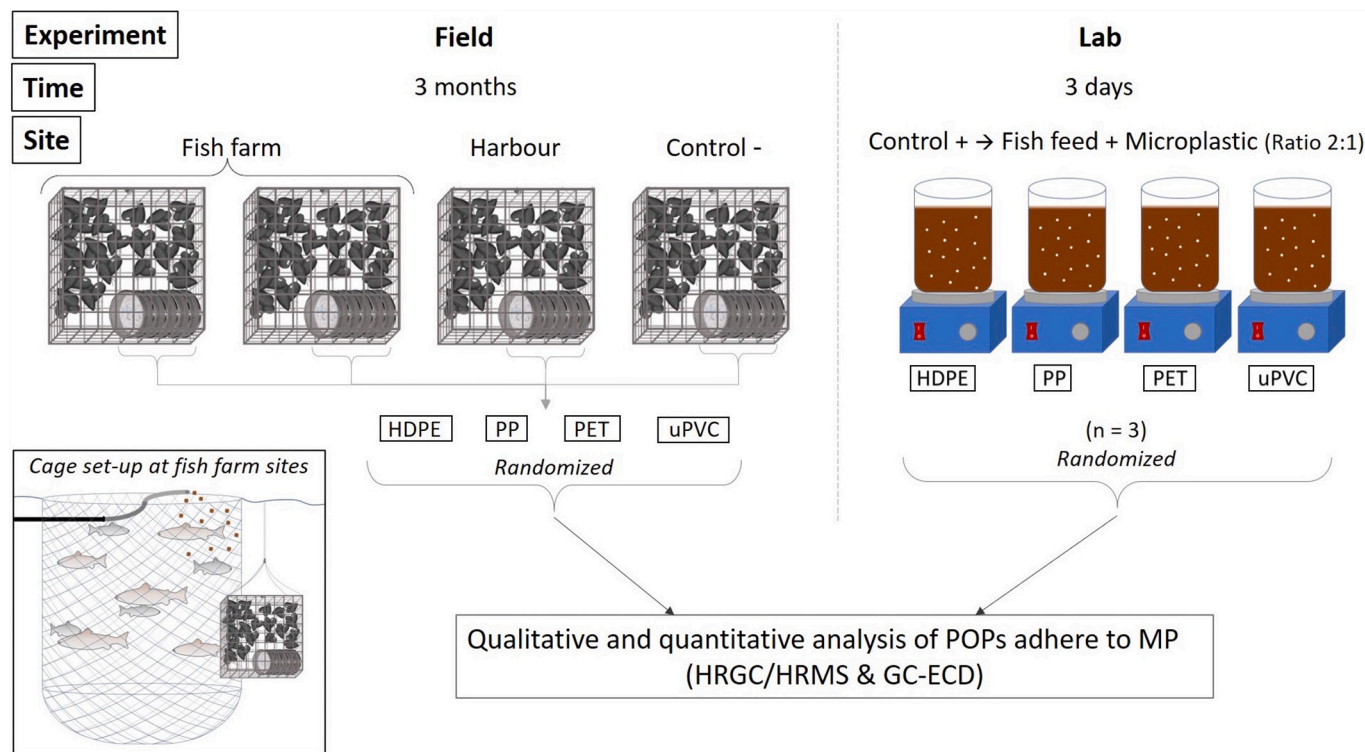


Fig. 2. Schematic overview of the experimental design. The field experiment is shown on the left, while the laboratory positive controls are shown on the right.

dibenzo- para- furans (furans/PCDF), dioxin-like polychlorinated biphenyls (dl- PCBs (non- ortho and mono-ortho PCBs)), non- dioxin-like polychlorinated biphenyls (ndl- PCBs) and polybrominated diphenyl ethers (PBDEs) are accredited for seafood. Wet or freeze-dried samples were mixed with hydromatrix, and internal standards (13C labelled EDF-8999 for PCDD/F, EC-5379 and EC-4937 for PCBs, EO-5100-10X, EO-5035, EO-5161, EO-5337, EO-5240, EO-5241 and EO-5003 for PBDEs; Cambridge Isotope Laboratories) for dioxins, furans, PCBs and PBDEs were added. The samples underwent extraction with hexane using an Accelerated Solvent Extractor (ASE; 350®, Dionex Corporation) instrument. The fat content in the sample was extracted by using sulfuric acid impregnated silica gel. The extract was further purified chromatographically using four consecutive columns, packed with AgNO₃-silica gel, H₂SO₄- silica gel, carbon and alumina, respectively, on a GO-HT (DSP-Systems, Ede, Netherlands) instrument. Two fractions were collected in the GO-HT process: fraction 1, which contains mono-ortho PCBs, ndl-PCBs and PBDEs, and fraction 2, which contains dioxins, furans and non-ortho PCBs. Further solvent exchange and adding of recovery standards (S-5117-ASS-NN and S-5118-ASS-NN, Chiron, Norway) were executed for both fractions by automated liquid handling (Microlab Starlet, Hamilton Nordic AB). Dioxins, furans and non-ortho PCBs were analysed using high-resolution gas chromatography/high resolution mass spectrometry (HRGC-HRMS; HRGC, Trace 2000 series; HRMS, DFS, Thermo Scientific) and quantified using an isotope dilution/internal standard method and relative response factors (RR) or response factors (RF). Mono-ortho PCBs, ndl-PCBs and PBDEs were analysed using gas chromatography/tandem mass spectrometry (GC-MS/MS; GC, 7890A; MS/MS, 7000B/C, Agilent Technologies) and were quantified using an isotope dilution/internal standard method and relative response factors (RR) or response factors (RF) (Supplementary Table S2). For dioxins, furans and dl-PCB the toxic equivalent values (TEQ) were calculated by multiplying the quantified concentrations of each congener with the toxic equivalent factor (TEF) of the associated congener. Further information on the QA/QC of the analyses is shown in Supplementary Table S3.

For pesticide analysis, all samples were weighed wet. Isotope

labelled internal standard (Cambridge Isotope Laboratories/Dr.Ehrenstorfer) was added to the sample material and followed by liquid-liquid extraction using the Citrate QuEChERS method by adding acetonitrile, water and citrate salts (Merck). Extraction was followed by low-temperature precipitation by freezing-out at -20 °C over night. For clean-up, two rounds of dispersive solid phase extraction (dSPE) were carried out by using Z-sep (Merck). The extracts were upconcentrated and solvent exchanged to isoctane before recovery standard (PCB-53, Dr. Ehrenstorfer) was added and analysis performed by gas chromatography/tandem mass spectrometry (GC-MS/MS; GC, Trace 1310; MS/MS, TSQ8000, Thermo Scientific). The area of the peaks in the chromatogram were used for quantification by the help of a multi-standard curve. The EURL criteria for pesticide analysis (described in the SANTE document 12682/2019) were followed.

The limit of quantification (LOQ) was set to three times the value of limit of detection (LOD). LOD was calculated by the software's noise calculation in addition to observations of the minimum in the calibration curve that is part of the linear area. The upperbound approach was used for samples <LOQ (EC, 2017), i.e. analysis results measured to be less than LOQ were given as <LOQ in numeral value and calculated as LOQ in sums.

2.5.2. Microplastic analysis

Two replicates of each of the PP, HDPE, PET and uPVC polymers incubated at each site were used for chemical analysis. Frozen samples were treated gently to remove the biofilm on the MPs through an enzymatically and strong alkali driven cleaning process. Each sample (1 g) was sequentially incubated at room temperature with a mixture 1:20 (v:v) of Celluclast and 1 mL Viscozyme enzymes (Sigma Aldrich) in 0.1 M PBS at pH 6.0 for 24 h and with 10% KOH for 6 h at RT. Plastic beads were gently flushed and rinsed with millipore water through a vacuum system to remove the degraded biofilm and were allowed to dry at room temperature for 1 h. Samples were then sonicated three times for 20 min with 5 mL of dichloromethane, HPLC grade, and the supernatant was collected after each sonication step. Extracts were preconcentrated by a Rotavapor system and analysed using the following HRGC/HRMS

methods: USEPA Method 1668B, USEPA Method 8290A and USEPA Method 1614A for polychlorinated biphenyls, dioxins and brominated flame retardants, respectively, using a GC–MS/MS (Shimadzu TQ8050); and the following GC-ECD method for chlorinated pesticides: USEPA Method 508.1 using a GC–MS (Shimadzu GC 2010) (Supplementary Table S4). Isotope labelled PCB mixture 77/101/141/178; 1,2,3,4,6,7,8-heptabromodibenzofuran and Aldrin 13C12 (Cambridge isotopes) were added as internal standards. The limit of quantification (LOQ) was set to three times the value of limit of detection (LOD) (Supplementary Table S5).

2.6. Statistical analysis

All the statistical analyses were carried out using R (1.1.463). To test our main hypothesis of whether MPs bind pollutants associated with fish farming, permutational multivariate analysis of variance (PERMANOVA) was carried out. PERMANOVA was applied to assess whether the composition of POPs sorbed to the MPs was similar in samples from the fish farms and positive control, and to assess whether the composition of POPs in MPs from the harbour was different to the other sites and positive control. Chemical measurements of dioxins, PCBs, pesticides and brominated flame retardants sorbed to the four polymers were used in this analysis. The homogeneity of multivariate dispersion was previously checked using betadisper and pairwise permutation MANOVA was used for *post-hoc* testing. The reference station was excluded from these analyses due to the excess of data below the detection limits.

To get a better understanding of the sorption capacity of the four studied MP polymers to POPs, Kruskal-Wallis test with Dunn's *post-hoc* test was carried out for each dioxin and PCB congener individually, using polymer type (HDPE, PET, PP, uPVC) as the independent variable. For this analysis, only dioxin and PCB data were used from the two fish farms and the positive control ($n = 6$), since the PERMANOVA test showed that compositions of POPs sorbed to MPs from these samples were statistically similar.

Correlation analysis was carried out to assess the relationship between the POPs sorbed to MPs and the bioaccumulated levels in mussels, the latter assumed to reflect the background level of POPs in the water. These analyses were used as a proxy to evaluate affinity and competitive binding of POPs to MPs. Spearman's rank correlation analysis was done for each polymer type individually using the levels of dioxins bound to MPs and bioaccumulated in mussels from all four stations. For this analysis, 2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD data

were used. For additional information, one way ANOVA, or Kruskal-Wallis test in case of non-parametric data, with Tukey or Dunn's *post-hoc* tests, respectively, were done to compare the levels of all POPs analysed in the mussels from the four different sites. Normality and homogeneity of variance were previously tested with Shapiro test and Bartlett test, respectively. These results are shown in Fig. 3 and Supplementary Table S6.

3. Results

3.1. MPs sorb POPs from fish farming

The levels of all analysed POPs were below the detection limit in the MP polymers placed at the reference station. For the harbour, the two fish farms and the positive control, the levels of the majority of pesticides (dieldrin, endrin, α -endosulfan, β -endosulfan, HCB and heptachlor) and half of the novel brominated flame retardants (PBCC, TBCT and pTBX) analysed were also below the detection limit in all polymer types. Hence, only data for dioxins and PCBs were used in the PERMANOVA analyses. The concentrations of all studied POPs sorbed onto the four MP polymer types are shown in Supplementary Table S7.

The levels of POPs sorbed to the MPs were quantitatively assessed to understand whether MPs can bind POPs associated with fish farming. PERMANOVA was used to compare the composition of POPs sorbed to MPs from the different sites and the positive control (Fig. 4). The results of the PERMANOVA analysis showed that the composition of POPs in MPs from the harbour was statistically different from the composition of POPs in MPs from FF1 and FF2, and the positive control, ($p < 0.05$). However, there were no significant differences in the composition of POPs sorbed to MPs from FF1, FF2 and the positive control.

3.2. Sorption capacity of POPs to MP polymers

To evaluate whether binding affinities of POPs differed among the studied MP polymers, a Kruskal-Wallis test was done for each dioxin congener and each PCB Aroclor (Figs. 5 and 6). Since this study focuses on the sorption of POPs from fish farming, only data of dioxins and PCBs sorbed to MPs from the two fish farms and the positive control were used. Positive control was included in this analysis because no differences were found in the previous analysis (PERMANOVA) in the composition of POPs of these MPs with the ones of the fish farms. Data of dioxins and PCBs sorbed onto the MPs placed at the harbour and the reference station were excluded because different cocktail of pollutants

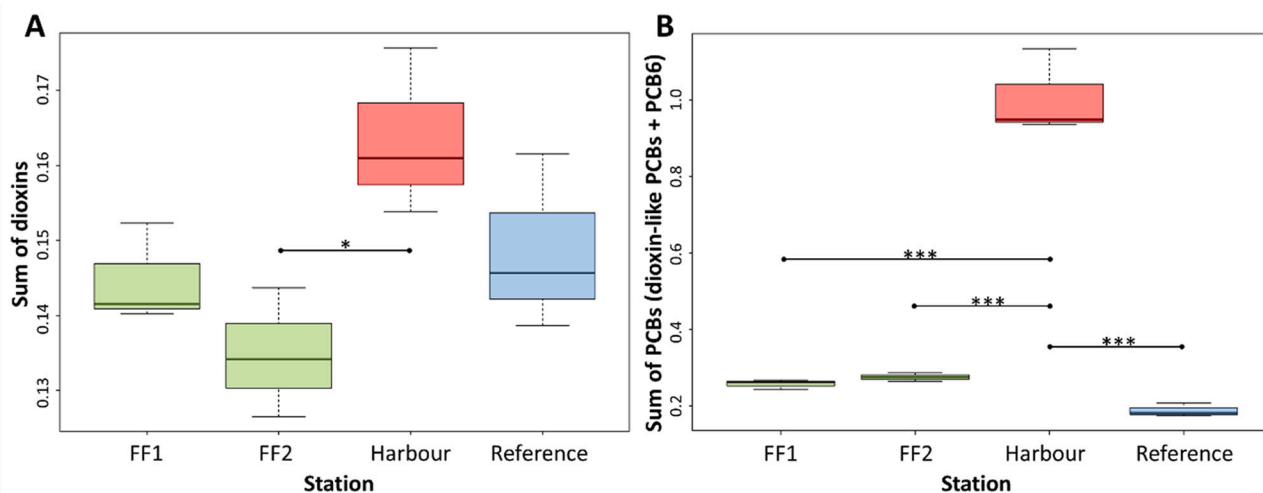


Fig. 3. Total levels of (A) dioxins and (B) PCBs in blue mussels placed for 3 months near Atlantic salmon fish farms (green), in a commercial harbour (red), and in a non-polluted reference station (blue). Statistical differences between sites are shown as * and *** (p -value < 0.05 and 0.001 , respectively). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

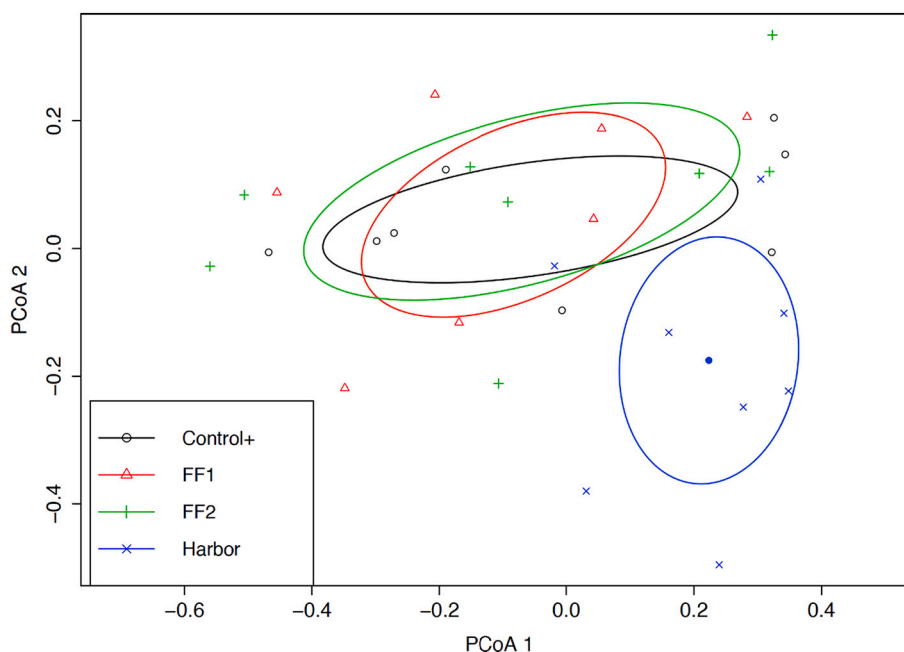


Fig. 4. Principal Coordinate Analysis (PCoA) ordination of quantitative data of POPs sorbed to MPs collected from the positive control (black), fish farm 1 (red), fish farm 2 (green) and Bodø harbour (blue). Overlapping circles show that the composition of POPs bound to the MPs from the two salmon farms and the positive control are statistically similar. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

might result in different sorption affinities. Chemical properties of the analysed dioxins and PCBs are shown in Supplementary Table S8.

Overall, dioxins and PCBs showed low affinity for HDPE and high affinity for PET and uPVC. The dioxin 2,3,7,8-TCDD (Fig. 5A) and the Aroclor 1248 (Fig. 6B) bound significantly more to uPVC MPs than to HDPE MPs ($p < 0.01$), while no statistical differences were detected for PP and PET MPs. The Aroclors 1016 and 1254 (Fig. 6A and C, respectively) bound significantly more to PET MPs than to HDPE MPs ($p < 0.01$ and 0.05 , respectively), and no differences were evident among the other polymer types. The dioxin congeners 1,2,3,4,7,8-HxCDD, 1,2,3,7,8-PeCDD, and the PCB Aroclor 1260 bound significantly more to both PET and uPVC MPs than to HDPE ($p < 0.05$ and 0.01) (Fig. 5B, E, and 6D, respectively). The levels of dioxins and PCBs sorbed to PP MPs were not statistically different to the levels found on the other three polymer types. The dioxins 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD and 1,2,3,4,6,7,8-HpCDD did not show a significant binding affinity towards any MP polymer.

3.3. Competitive behaviour of POPs to sorb MPs

The levels of dioxins bound to the MPs were compared to the levels of dioxins bioaccumulated in the blue mussels from the four sites to evaluate whether the composition of POPs sorbed to the MPs after three months in the sea reflected the levels of POPs in the surrounding water. No significant correlations were found between the dioxins sorbed to the four MP polymers and the dioxins bioaccumulated in the mussels (Fig. 7).

4. Discussion

This study suggests that MPs can sorb POPs associated with Atlantic salmon farming, and documents that such pollutants have polymer-specific binding affinities. Polymer type and pollutant type, in addition to background pollution in the water, are therefore determining factors that should be considered when assessing the potential role of MPs as vectors of pollutants from aquaculture in the marine environment.

In this study, four types of MP polymers (PE, PP, PET and uPVC) were

either incubated for three days with fish feed or placed in the sea for three months next to two marine fish farms, in an urban harbour and in a non-polluted fjord. MPs collected from the two fish farms, the harbour and the lab control sorbed dioxins, PCBs and some brominated flame retardants (TBPhA, BTBPE and PBT), whereas pesticides were barely detected. In contrast, MPs placed in the non-polluted fjord did not have quantifiable levels of any of the analysed POPs. It is well known that MPs can sorb pollutants in the environment. At the beginning of this century, plastic pellets sampled in waters from all five continents were reported to be polluted with PCBs and pesticides (Ogata et al., 2009). Since then, several studies have reported the presence of POPs bound to MPs in the marine environment (Rodrigues et al., 2019; Ziccardi et al., 2016). The main mechanisms by which MPs sorb POPs include hydrophobic and electrostatic interactions, although multiple mechanisms are often co-occurring in complex natural environments. Chemicals characterised by high hydrophobicity, which are defined by high octanol/water partition coefficients ($\log K_{ow}$), are expected to be more easily sorbed by plastic particles (Yu et al., 2019). Recent studies point out that the distribution coefficient of organic pollutants, such as PCBs, on MPs increases with hydrophobicity (Velzeboer et al., 2014). In our study, MPs had high levels of dioxins and PCBs while barely detectable levels of pesticides. Dioxins and PCBs have $\log K_{ow}$ values ranging from 7 to 8 (Edelbach and Lodge, 2000), while pesticides tend to be less hydrophobic. For example, aldrin and dieldrin have $\log K_{ow}$ values ranging from 5.68 to 7.4, and 4.32 to 6.2, respectively (EPA, 2003). Thus, hydrophobicity could partly explain the sorption patterns of POPs on MPs observed in this study.

PET and PVC MPs placed for 3 months close to salmon farms showed significantly higher levels of POPs than HDPE MPs. Sorption of POPs to MPs depends, in addition to the properties of the POPs mentioned previously, on the properties of the MPs and the water or other matrix surrounding the MPs. Such important properties are size, crystallinity degree, polarity, colour, occurrence of specific functional groups and surface area of the MPs as well as pH, salinity, temperature of the water and biofilm formation around the MPs (Rummel et al., 2017; Ziccardi et al., 2016). MPs used in this study were non-coloured. PE, PP and PET MPs were similar in size and shape, while uPVC MPs were smaller (1/6). Nevertheless, the levels of POPs sorbed to uPVC MPs were similar to the

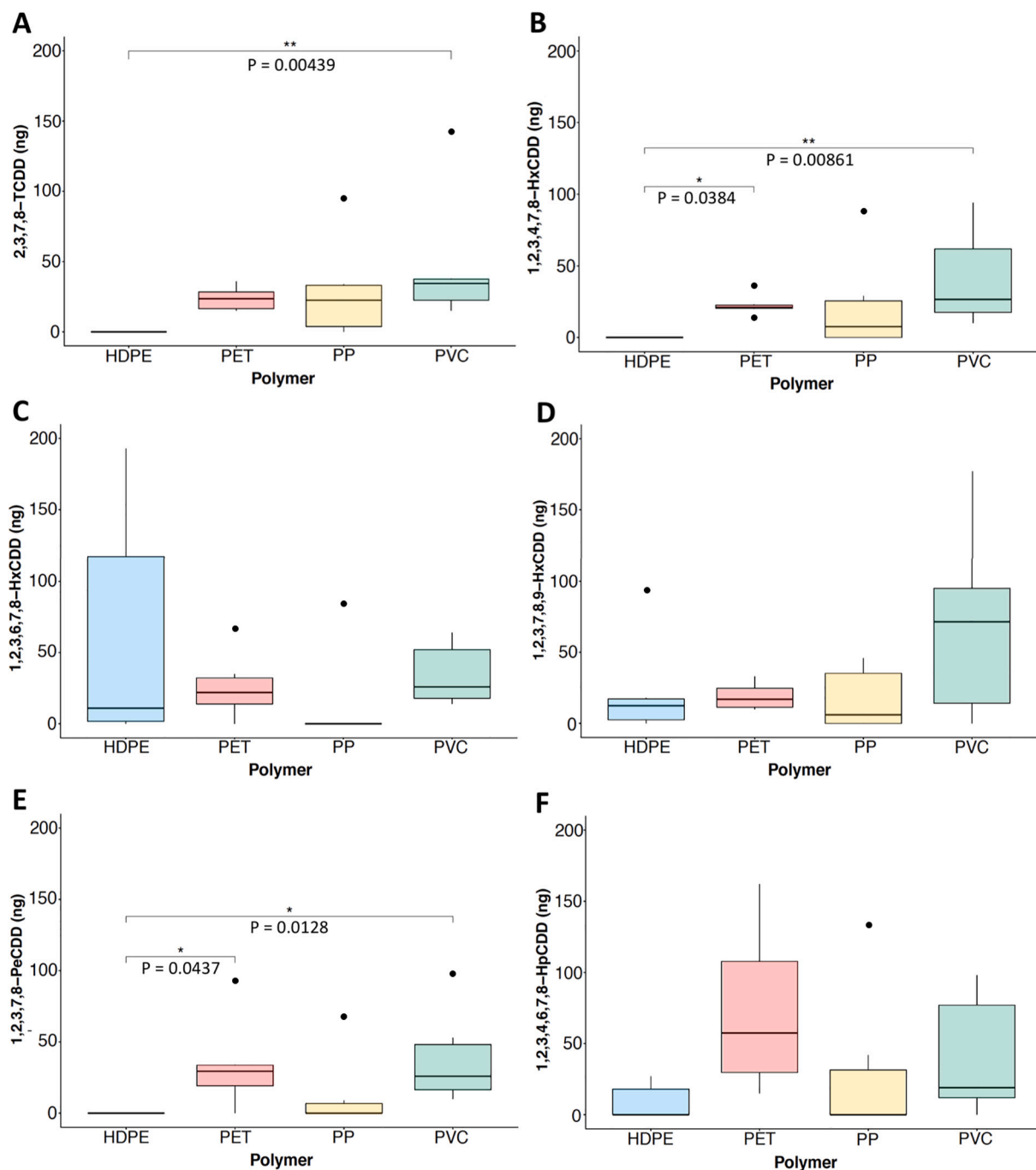


Fig. 5. Boxplot showing the levels of six different dioxin congeners associated with salmon farming sorbed to four MP polymer types: high density polyethylene (HDPE, blue), polyethylene terephthalate (PET, red), polypropylene (PP, yellow) and unplasticized polyvinyl chloride (PVC, green). Median ($n = 6$) represented by line in the box; top and bottom of the box are upper and lower quartiles; dots show outliers; * and ** show statistical differences between polymers ($p < 0.05$ and 0.01 , respectively); p -values written underneath. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels found on PET MPs, suggesting that the MP size was not the main factor influencing the amount of POPs sorbed to the different polymers. In addition, PET and PVC in presence of water tend to acquire positive and negative charges, respectively (Albrecht et al., 2009), suggesting that polarity might not play a significant role on the sorption of POPs in this study. The crystallinity of MPs, by contrast, varied among polymers. Crystallinity of polymers is an important factor that affects the sorption of POPs. Crystalline polymers have a well-ordered and firm structure that does not favor sorption of chemicals. Amorphous polymers have larger surface area that allows a higher sorption of POPs (Endo and

Koelmans, 2016; Rodrigues et al., 2019). HDPE is characterized by a relatively high crystallinity (70–80%), while PET and PP are considered semi-crystalline polymers, and PVC has amorphous structure. Thus, differences in the sorption of POPs to the four polymers studied may be explained by the degree of crystallinity. However, the level of crystallinity of a polymer can vary considerably as a result of the production process (Endo and Koelmans, 2016), which could explain differences observed between our study and earlier reports. A study carried out in California, USA, found that HDPE, LDPE and PP MPs deployed for several months in San Diego Bay had significantly higher levels of PCBs

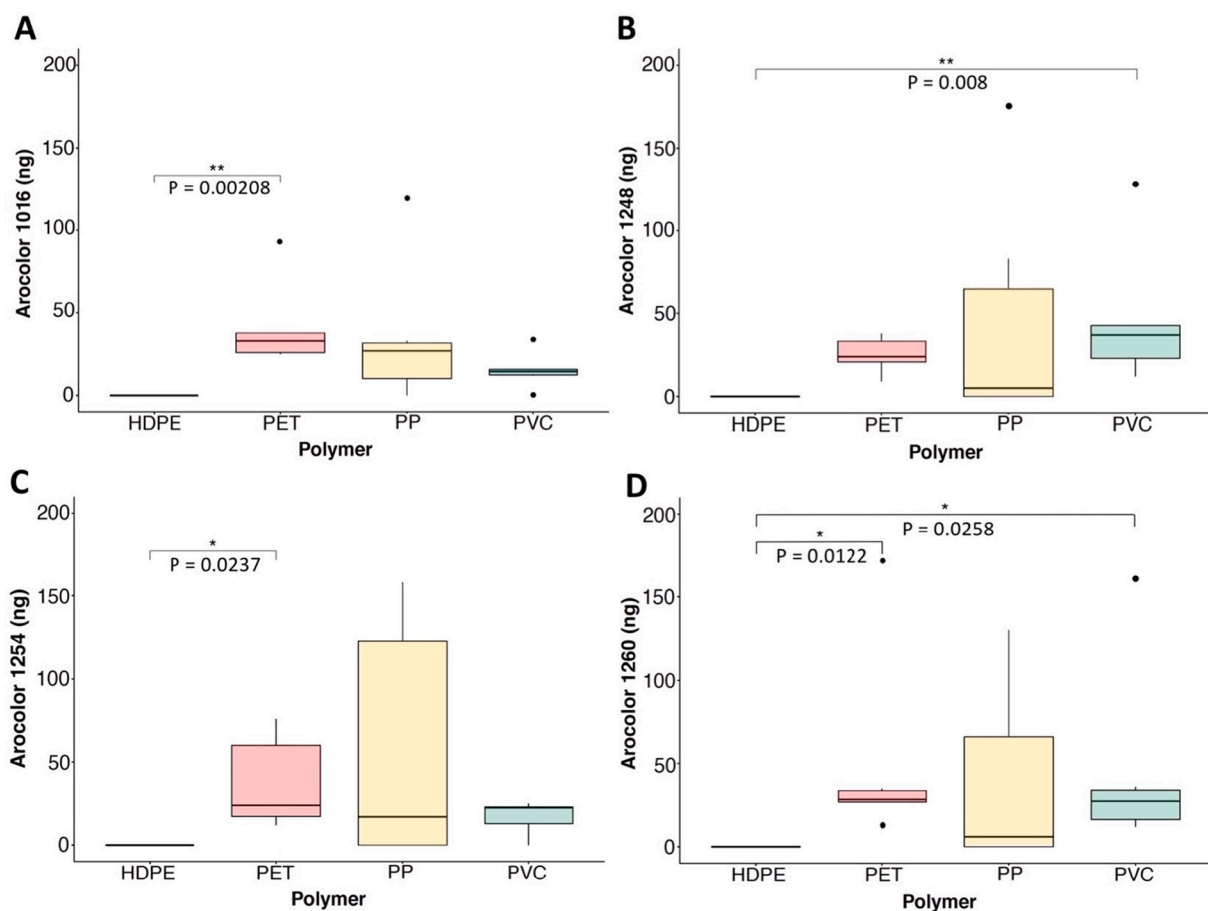


Fig. 6. Boxplot showing the levels of four PCB Aroclors associated to salmon farming sorbed to four MP polymer types: high density polyethylene (HDPE, blue), polyethylene terephthalate (PET, red), polypropylene (PP, yellow) and unplasticized polyvinyl chloride (PVC, green). Median ($n = 6$) represented by line in the box; top and bottom of the box are upper and lower quartiles; dots show outliers; * and ** show statistical differences between polymers ($p < 0.05$ and 0.01 , respectively); p-values written underneath. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and PAHs than PET and PVC MPs (Rochman et al., 2013a). In another study, PE MPs collected in Japanese coastal areas had higher amount of PCBs adsorbed than PP MPs, although the concentrations of PCBs in single pellets from same locations had a high variability (Endo et al., 2005). Differences in water temperature, salinity and biofilm formation could also explain the discrepancies with those studies, which were carried out at lower latitudes. Our study was carried out north of the Arctic Polar Circle during the winter, with low seawater temperatures and when the lack of light reduces biofilm growth (Aure and Skjoldal, 2003; NOAA, 2009). To our knowledge there are no previous studies that report sorption patterns of POPs in MPs in waters above the Arctic Polar Circles or in Arctic waters. Thus, comparison with previous studies is not straightforward. Furthermore, studies focused on the mechanisms of sorption of pollutants on MPs have primarily focused on laboratory-scale experiments, which can only heed limited known factors that might influence sorption behaviour. Such cannot sufficiently explain all the mechanisms by which MPs sorb organic pollutants under complex environmental conditions. Several types of pollutants that can exert synergistic or antagonistic effects on one another exist in the natural environment, and the interactions between MPs and pollutants become very complex with the constant changes in environmental conditions. The above highlights the need to further investigate interactions of chemicals and MPs in diverse regions of the planet to better understand the impact of MPs in the environment.

This study focused on the sorption of dioxins and PCBs to MPs and this is, to our knowledge, the first report to show that MPs can bind relatively high levels of dioxins close to salmon farms. The group of

POPs evaluated in this work might therefore be a relevant factor for the differences observed between this study and other reports. Previous studies have mainly analysed the sorption of PCBs, brominated flame retardants, pesticides and PAHs on MPs (Hirai et al., 2011; Rochman et al., 2013a; Rodrigues et al., 2019; Ziccardi et al., 2016). Very few reports are available on the levels of dioxins bound to MP polymers in the sea. To our knowledge, only one study has reported levels of PCDD/PCDFs on MPs and such pollutants were only detected in charred MPs collected at the coast of the Maldives (Saliu et al., 2018). Different types of pollutants can have different affinities to polymers, as shown, inter alia, in our study. For instance, PAHs and chlorinated benzenes were reported to sorb stronger to PE than PP, while PP had higher sorption capacity than PE for hexachlorocyclohexanes (HCHs) (Lee et al., 2014). Thus, pollutants with higher affinities to polymers may outcompete other pollutants. For example, in a mixture of DDT and phenanthrene, it was observed that the first chemical outcompeted the latter in terms of MP adsorption (Bakir et al., 2012). This process could potentially explain the non-correlation observed between the composition of POPs found in the mussels and the MPs. Bioaccumulated levels of dioxins in mussels placed next to the MPs for three months in the sea were different to those sorbed to the MPs. Mussels are sentinel species often used to biomonitor aquatic pollution since they are regarded to generally accumulate pollutants present in the water and have low biotransformation capacity (Beyer et al., 2017). Thus, pollutants found in their tissues tend to reflect those found in the surrounding environment. One possible explanation for the different levels of POPs in MPs and mussels in waters with a cocktail of pollutants could therefore be the competitive

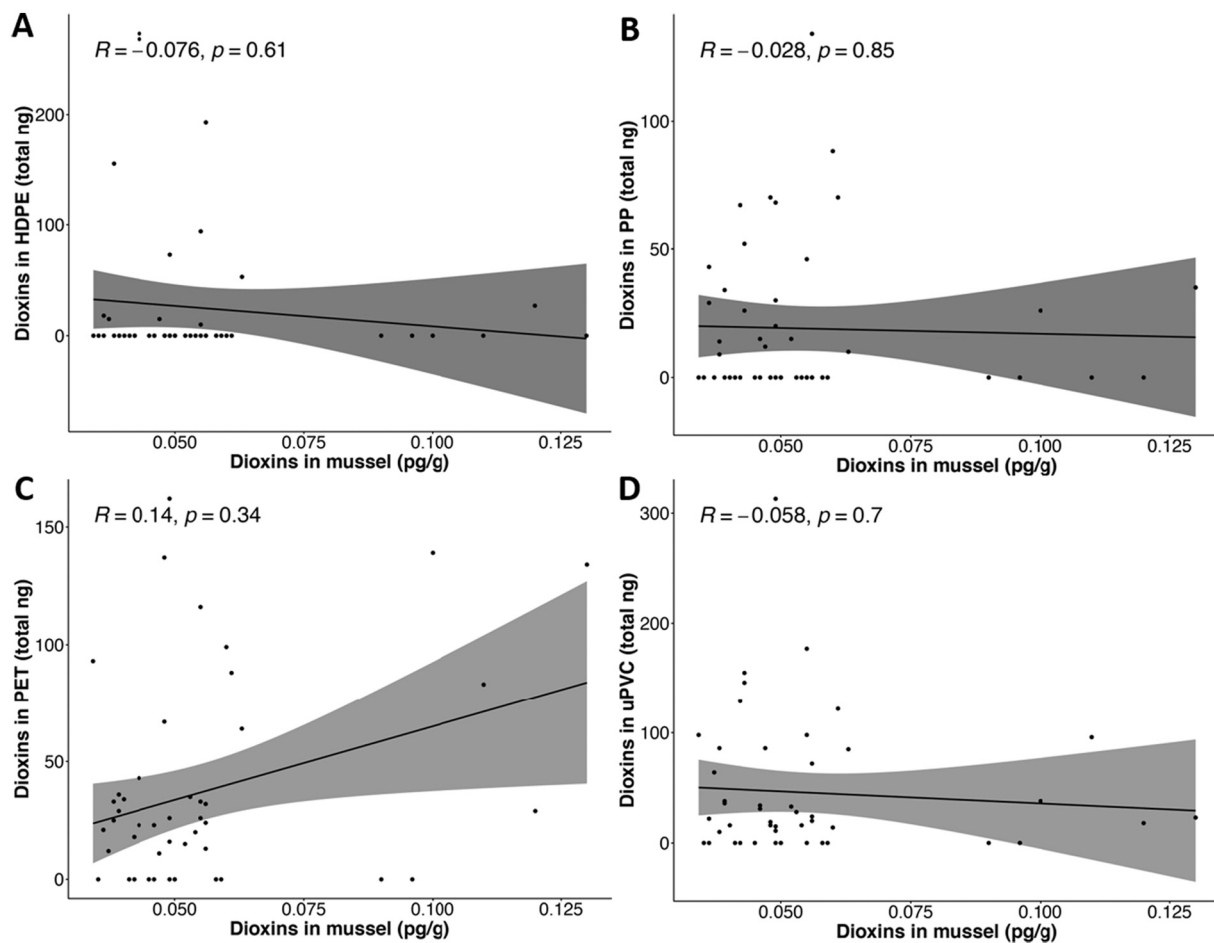


Fig. 7. Spearman's rank correlations comparing dioxin levels accumulated in blue mussels and dioxin levels sorbed to (A) HDPE MPs, (B) PP MPs, (C) PET MPs and (D) uPVC MPs from the reference station, Bodø harbour and both fish farms. Top left of each graph shows results of correlation analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

binding of pollutants to plastic.

In terms of fish farming, this study suggests that PET and PVC MPs could have a higher environmental impact than HDPE MPs. PET and PVC are high-density polymers (1.1 to 1.5 g/cm³). Since their densities are higher than seawater (1.02 g/cm³), these MP polymers tend to sink and accumulate in benthic sediments (Bråte et al., 2017; Gomiero et al., 2019), unless they are very small, in the nanoplastic scale, where floatability might vary (Ter Halle et al., 2017). Benthic areas beneath fish farms are usually enriched with organic waste, resulting from fish faeces and non-eaten feed pellets, and associated pollutants, which are likely fed on by wild benthic feeding biota. Sediment beneath fish farms could, therefore, be potential sources of polluted MPs to wild organisms. However, the impact of MPs derived pollutants on resident organisms is probably insignificant compared to the exposure to the same pollutants through other pathways (Koelmans, 2015), although polluted MPs could represent an environmental threat if carried to non-polluted areas by, for instance, ocean currents.

PP MPs also sorbed a significant amount of POPs associated to fish farming. PP has a lower density (0.9 g/cm³ approx.) than seawater and PP MPs will therefore remain for longer periods in the pelagic zone (Bråte et al., 2017). Thus, PP MPs could have higher capacity to transport POPs from aquaculture facilities to the surrounding areas than other MP polymer types (Koelmans, 2015). Furthermore, PP is used in fish farming materials such as mooring ropes, which eventually release MPs as a result of wear and tear (Lusher et al., 2017). Thus, the impact of this polymer in the environment and in relation to aquaculture could be more important than previously expected. Based on this study, HDPE

MPs might play a less important role as vector of POPs from aquaculture facilities to the environment. However, because feeding pipes in salmon farms are a known source of HDPE MPs to the environment (Gomiero et al., 2020b), and because the vast majority of materials used in fish farming are made of HDPE, this MP type might still play a significant role in spreading pollutants from fish farms. Moreover, weathering of plastic and changes in the degree of crystallinity of polymers in the environment could modify the sorption patterns observed in this study (Rodrigues et al., 2019). Considering all the above, the role of MPs as potential vectors of pollutants from aquaculture facilities should be studied more in depth and considered in future assessments of the environmental impact of fish farms with open-nets.

The role of MPs as vector of such pollutants to organisms or other environments is still a controversial matter (Koelmans et al., 2022). Some studies have shown that pollutants sorbed to MPs can be transferred to organisms under specific conditions. For instance, Murray River rainbow fish (*Melanotaenia fluviatilis*) exposed to MPs spiked with PBDEs bioaccumulated greater amount of such pollutants compared to individuals exposed to virgin MPs (Wardrop et al., 2016). Other studies have reported that exposure of organisms to pollutants sorbed to MPs is insignificant compared to exposure of pollutants through other pathways, such as diet or environmental exposure (Koelmans, 2015). Furthermore, it has been suggested that MPs might reduce bioavailability of such compounds in polluted environments by sorbing pollutants in the water (Koelmans et al., 2022; Sørensen et al., 2020). The aim of this study was to evaluate the potential of MPs to sorb POPs associated with fish farming and, consequently, to act as a possible vector of such

pollutants. Our results show that the composition of POPs sorbed to MPs placed for three months next to two fish farms were similar to that of MPs incubated with fish feed for three days, but were significantly different to MPs placed for three months in a harbour and the reference station. This suggests that MPs found in the surroundings of salmon farms can sorb POPs present in the fish feed. However, the ability of MPs to transfer POPs from fish farms to organisms will depend on several factors that were not addressed in this study. Pollution levels in the surrounding environments, ocean current dynamics in the area, species affected or even size and shape of MPs are some important factors to assess when studying the transfer of pollutants from MPs to organisms, which requires further investigation. Therefore, the role of MPs in transferring POPs from salmon farming remains uncertain based solely on our results.

5. Conclusion

To our knowledge, this is the first study to assess the potential role of MPs in the spreading of pollutants from marine aquaculture. Our results show that the potential of MPs to transport POPs depends on the type of polymer, and indicate that PET and PVC MPs might contribute to spreading pollutants from Atlantic salmon farming to a larger extent than HDPE per amount of MP. Overall, this study proposes that MPs could potentially act as vectors of POPs associated with fish feed and might play a part in the environmental impact of Atlantic salmon farming.

CRedit authorship contribution statement

Conceptualization and design: IA-G, PO and ToKr
 Experimental methodology: IA-G, MK, ToKr and PO
 Acquisition samples: IA-G and MK
 Analysis of samples and acquisition of data: IA-G, TaKo and AG
 Data Curation and Interpretation: IA-G
 Writing-Original Draft: IA-G (main)/TaKo and AG (part of Materials and Methods)
 Review and Editing: IA-G, TaKo, AG, ToKr, MK and PO

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2022.113794>.

[org/10.1016/j.marpolbul.2022.113794](https://doi.org/10.1016/j.marpolbul.2022.113794).

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