Abundance and properties of microplastics found in commercial fish meal and cultured Common carp (*Cyprinus carpio*)

Parichehr Hanachi[1], Samaneh Karbalaei[1*], Tony R. Walker[2], Matthew Cole[3], Seyed V. Hosseini[4]

[1] Department of Biology, Biochemistry Unit, Alzahra University, Tehran, Iran

[2] School for Resource and Environmental Studies, Dalhousie University, Halifax, NS, B3H 4R2, Canada

[3] Marine Ecology and Biodiversity group, Plymouth Marine Laboratory, Plymouth, PL1 3DH, United Kingdom

[4] Department of Fisheries, College of Agriculture & Natural Resources, Tehran University, Karaj, Iran.

* Corresponding author: Samaneh Karbalaei

Department of Biology, Biochemistry Unit, Alzahra University, Tehran, Iran

Phone: +989125457850

E-mail address: s.karbalaei@alzahra.ac.ir

Abstract

Microplastics (MPs) are environmental contaminants that are of increasing global concern. This study investigated presence of MPs in four varieties of marine derived commercial fish meal, followed by identification of their polymer composition using Fourier-Transform Infrared (FTIR) spectroscopy. Exposure experiments were conducted on cultured common carp (*Cyprinus carpio*) by feeding four varieties of commercially available fish meal to determine relationships between abundance and properties of MPs found both in meal and those transferred to cultured common carp. Mean particle sizes were 452±161 μm (±SD). Fragments were the predominant shape of MP found in fish meal (67%) and *C. carpio* gastrointestinal tract and gills (65%), and polypropylene and polystyrene were the most present plastic polymers found in fish meal (45% and 24%, respectively) and *C. carpio* (37% and 33%, respectively). Positive relationships were found between MP levels in fish meal and *C. carpio*. This study highlights that marine derived fish meal may be a source of MPs which can be transferred to cultured fish, thus posing a concern for aquaculture.

Keywords: Microplastics (MPs); Fish meal; Common carp (*Cyprinus carpio*); Gastrointestinal tract; Fourier Transform Infrared (FTIR) spectroscopy; Accumulation.

1. Introduction

Microplastics (MPs; defined as plastics <5 mm) have been recognized as a serious global environmental problem (Andrady 2011; Cole et al. 2011; Karbalaei et al. 2018; Schnurr et al. 2018; Xanthos and Walker 2017). MPs originate from breakdown of macroplastics (>5 mm) composed of synthetic polymers, known as secondary MPs or are industrially manufactured and used in many applications such as personal care products, also known as primary MPs (Andrady 2017; Auta et al. 2017). The origin of a polymer can use as a criterion to differentiate between natural and artificial (man-made, synthetic) polymers. Natural polymers (e.g., proteins, cellulose) are not considered as plastics while synthetic polymers commonly are. Modified natural polymers, for instance, rayon (an organic cellulose-based polymer) represent a special case. Synthetic additives have been added to the products of rayon. Therefore, rayon was classified as MPs (Hartmann et al. 2019).

MPs have been found in fish (Abbasi et al. 2018), birds (Provencher et al. 2018b; Trevail et al. 2015), freshwater aquatic ecosystems (Brennholt et al. 2018), sediments (Akhbarizadeh et al. 2017; Bergmann et al. 2017), and even in Arctic and Antarctic sea ice (Obbard et al. 2014). Adverse effects of MPs on organisms have been reported in their feeding activity, function, nutritional composition, behaviour and fecundity through investigating laboratory test organisms (Cole et al. 2015; Yin et al. 2018). Yin et al. (2018) found that polystyrene (PS) MPs reduced feeding activity, swimming and exploration ability, energy reserve, growth and nutritional quality of marine jacopever (*Sebastes schlegelii*) while shoaling behaviour increased. MPs have also been shown to be toxic in aquatic organisms, particularly when associated with persistent organic pollutants (Karami 2017). A recent study showed that low density polyethylene (LDPE) significantly increases toxic effects of polychlorinated biphenyl (PCB), brominated flame retardants (BFRs), perfluorinated compounds (PFCs), and methylmercury in zebrafish (*Danio rerio*) (Rainieri et al. 2018). MPs were also reported in popular products consumed by humans, including processed seafood products such as canned sardines and sprats (Karami et al. 2018), commercial salts (Karami et al. 2017a), drinking water (Kosuth et al. 2017), and fresh seafoods such as bivalves (Abbasi et al. 2018; Li et al. 2015). Thus, humans are potentially at risk due to consumption of these products. Rochman et al. (2015) found plastic debris in 55% of all sampled fish and shellfish directly sold for human consumption in Indonesia.

Millions of tonnes of fish meal are produced from raw marine derived fish, by-products of fish or seafood-processing industries for use as fertilizer and animal feed, especially for livestock, poultry, cultured fish and shrimps due to high-quality protein, essential amino acids and fatty acids (Macan et al. 2006). Approximately, 6-7 million tonnes of fish meal are produced globally annually (Rustad et al. 2011). In 2010, 73% of global fish meal production were used by the aquaculture industry (World Bank 2013). Most commercial fish meal is made from small pelagic oily fish such as blue whiting (*Micromesistius poutassou*), Peruvian anchovy (*Engraulis ringens*), and lesser sand eel (*Ammodytes tobianus*) (Salin et al. 2018). Studies have reported presence of MPs in fish tissues (Abbasi et al. 2018; Baalkhuyur et al. 2018; Rochman et al. 2015). For example, analysis of *A. tobianus* showed that 44.4% contained MPs in digestive tracts (Welden et al. 2018). In another study by Lusher et al. (2013), over 50% of *M. poutassou* and red gurnard (*Aspitrigla cuculus*) contained MPs in gastrointestinal tracts. Therefore, use of gastrointestinal tracts in fish meal production offers a potential pathway for contamination of fish meal by MPs.

This study investigated MP loads in four varieties of commercially available fish meal. All isolated particles were sampled based on their similar morphology and density to MPs. Fourier-Transform Infrared (FTIR) spectroscopy was used to identify polymer MP compositions. Relationships between abundance and properties of MPs in fish meal and cultured fish were assessed by feeding Common carp (*Cyprinus carpio*) with different varieties of fish meal. *C. carpio* were selected because they are a globally important aquaculture species (Haghi and Banaee 2017).

2. Materials and methods

A flow diagram of the experimental design is presented in Supplementary material, Appendix A.

2.1. Materials and chemicals

Fish meals were sourced from fish meal factories in Southern Iran, with the factories stating that fish meal was manufactured from salmon, sardine and kilka collected from the Persian Gulf and Caspian Sea. Chemical composition and fish species composition of fish meals are presented in Table 1. Sodium iodide (NaI) and potassium hydroxide (KOH) were purchased from Merck (Darmstadt, Germany). Ultrapure deionized water (purified by a Milli-Q Synergy UV system, Millipore, USA) was used for all solution preparations. Filter papers No. 540 and 541 (hardened ashless, pore size 8 μm and 22 μm, respectively) were purchased by Whatman and filter membranes (149 μm) were supplied from Spectrum Laboratories (USA).

2.2. MP particles in fish meals

2.2.1 Extraction of MPs from fish meals

MPs were extracted from fish meals according to Karami et al. (2017b). To avoid contamination of samples, experiments were performed in a pre-cleaned (with deionized water and 70% ethanol) closed laminar flow cabinet. Fish meal (10 g of each brand, *n*=30) was transferred into a 250 mL Schott Duran glass bottle, then 100 mL (1:10 w/v) of 10% KOH solution was added. Bottles were sealed with a premium cap and a pouring ring and incubated at 40°C for 72h. Digested samples clogged smaller pore size filter papers (8 and 22 µm) mainly due to presence of indigestible materials (i.e. tiny broken shells and bones) in fish meals. Therefore, all digestates were filtered through 149 µm filter papers using a vacuum system to extract particles larger >149 µm. Filter papers of each sample was immersed into 10-15 mL of 4.4 M NaI at a concentration of 1.5 g/mL and sonicated (50 Hz) by ultrasonic bath (Branson, 2510) for 5 min. Filters were removed and this process was repeated to ensure complete extraction of MPs. The solution was centrifuged at 500 × g for 2 min at room temperature, and supernatant containing MPs was filtered through No. 540, hardened ashless, pore size 8 µm, filter papers. To ensure complete isolation of plastic particles, this process was performed twice. Filters were stored in dry Petri dishes and airdried under laminar flow cabinet for visual identification of MPs.

2.2.2. Visual observation of the MPs

Filter papers were photographed using a Leica EZ4D Stereomicroscope (Leica, Germany). To measure particle sizes, digital images were examined using ImageJ software. A visual assessment was also used to identify suspected MPs according to their morphological characteristics such as colour, texture and shape (Karami et al. 2017a). Representative suspected particles that were visually identified as potential plastics were selected for corroboratory FTIR (Fourier Transform Infrared Spectroscopy) analysis.

2.2.3. Microplastic verification using FTIR

Suspected MPs were analyzed to identify polymer compositions of MPs using FTIR with a Vertex 70 spectrometer (Bruker) coupled with a Hyperion 2000 FTIR microscope (Bruker). Spectra were recorded as mean of 64 scans in the spectral wave range of 4000–600 cm^{-1} at a resolution of 4 cm^{-1}. Each sample spectrum was compared with a database from Bruker to identify polymer type. Samples which produced spectra with a match less than 60% were automatically excluded.

2.3 Laboratory uptake experiment

Three days post-hatching larvae (*C. carpio*) with a mean individual weight of 0.89 ±0.10 mg was purchased from a local agricultural market in Karaj, Iran and acclimatized in a laboratory tank for 6 d. Water temperature, dissolved oxygen, and pH were 24°C, 6.9 ± 1.0 mg/L, and 7.4 ± 0.2, respectively. Photoperiod was 12-hour light/12-hour dark. Initially, larvae were fed *ad libitum* with newly hatched *Artemia nauplii*, 3-5 times d^{-1} for two weeks. Experiments were carried out in 124 L aquarium (*n*=15 aquarium) stocked with 10 fish (mean weight±SD: 592.31±57.3 mg, mean total length: 34.32±2.92 mm) per aquarium with three replicates per treatment (*n*=30 fish per treatment, total fish=150) (see Supplementary material, Appendix B). Aquariums equipped with an aerating filter system. Four types of fish meals with different protein content were used: salmon (72 % protein), two varieties of sardine (55% and 65% protein, respectively), and kilka (60% protein) fish meal.

Each aquarium was provided with one type of fish meal. A control non-fish meal diet (soybean meal protein) was used. Soybean protein is the most available and economical plant protein source with relatively high digestible protein content and good amino acid composition (NRC 2011). Soybean meal were also analyzed for microplastic extraction according to Karami et al. (2017b). Fish meals were prepared under laminar flow cabinet by mixing with distilled water to form a dough. The prepared dough was passed through a hand pelletiser to make 2mm Pellets (Pradhan et al. 2019). Then, fish were fed at a rate of 5-10% of body weight three times d^{-1} for 4 weeks. To avoid contamination, any uneaten food was removed after 1 h. A half of aquarium water was siphoned daily and replaced with UV-treated and aerated water from a storage tank.

During the experimental period, the average ±SD water temperature, dissolved oxygen, pH and salinity were 25.5 ±1.1°C, 6.3 ±0.71 mg/L, 7.8 ±0.1, and <1, respectively. After 4 weeks, six individual fish (mean weight±SD: 55.21±9.10 g, mean total length: 14.10±2.18 cm; *n*=18) from each treatment were randomly euthanized by an overdose of Tricaine Methanesulfonate (MS222; Sigma, USA) washed twice with dechlorinated water, covered with foil and stored at -20˚C until MP extraction. MPs were extracted from gastrointestinal tracts (with digestive contents) and gills based on Karami et al. (2017b). Under laminar flow cabinet, gastrointestinal tracts and gills were placed separately into a 250 mL DURAN ® glass bottle sealed with a premium cap and pouring ring, and then KOH solution was added (1:10 w/v). Solutions were then incubated at 40°C for 72 h. Digestates were then filtered through 149 µm filter membrane using a vacuum pump. To separate potential plastic particles from other digestion resistant materials, the 149 µm filter membrane was soaked in 10-15 mL NaI solution (4.4 M, 1.5 g/mL) and sonicated at 50 Hz for 5 min., and eventually centrifuged at 500 × g for 2 min. Supernatant of the mixture containing plastic particles were filtered through another filter membrane with 8 μm pore size. Polymer compositions of MPs were identified by FTIR spectroscopy.

2.4 Quality control

To preclude potential contamination, glass bottles and instruments were washed using dishwashing liquid and tap water, then rinsed with deionized water and ethanol, and then dried in an oven at 50ºC for 5 h. All the solutions including deionized water (100 mL), 70% ethanol (10 mL), 10% KOH (100 mL), and 4.4 M NaI (10-15 mL) were filtered prior to use through a GF/D filter paper (pore size 2.7 μm). Cotton lab coats and gloves were worn during the experiment to reduce airborne contamination of clothing. Aquariums were covered with a glass plate to prevent airborne contamination into water. Fish body surfaces were rinsed twice with ultrapure deionized water and ethanol to remove any potential particle contamination. In the laboratory, procedural blanks were run to account for potential contamination, including 10% KOH extraction and NaI density separation.

2.5 Data analysis

Statistical analysis was conducted using SPSS software version 23 (SPSS, Inc., Chicago, IL, USA). Figures were generated using Microsoft Excel 2013, the Shapiro–Wilk test was performed to analyze the normality of data. Differences of MPs between four varieties of fish meals and treatments was determined by one-way analysis of variance (ANOVA). Concentrations of each polymer composition were compared among fish meals and treatments using a one-way ANOVA followed by Tukey's honestly significant difference (HSD) test to determine significant differences ($p < 0.05$). Pearson's coefficient was chosen with a significance level of 0.05.

3. Results

3.1 Identification of MPs in fish meals with FTIR

MPs were successfully extracted and identified from all types of fish meal. Sample contamination was prevented during MP extraction of fish meals and laboratory accumulation experiment, and no MPs were found in procedural blanks. A total of 226 MPs was isolated from four types of fish meal. Mean particle sizes were 452±161 μm (±SD). Smallest and largest particles were 158 μm and 810 μm, respectively (Fig. 1). Fragments were the most predominant morphology of MPs (67%) followed by films (19%), pellet (8%), and fiber (6%) (Fig. 2a). The most abundant plastic polymers in fish meals were PP (45%) followed by PS (24%), polyethylene (PE, 19%), polyethylene terephthalate (PET, 8%), and rayon (4%) (Fig. 2b). Fig. 3 are some of the captured images of extracted MP particles.

One-way ANOVA results showed statistically significant (p<0.05) differences in the number of extracted MPs among different types of fish meal. Salmon/sardine (65% protein) and sardine (55% protein) fish meals have significantly higher MPs (Tukey HSD, p<0.05) compared to kilka fish meal. However, no significant difference was found in the number of isolated MPs between salmon/ sardine (65% protein) and sardine (55% protein) fish meals (Fig. 4a). In each type of fish meal, the mean number of extracted MP polymers were comparable in PET, PE, PS, and rayon, except salmon fish meal which significant differences were observed in PE and rayon (Fig. 4b). As such, significant difference was found between PP and Rayon in fish meal types separately (Fig. 4b).

3.2 MP accumulation in C. carpio

Soybean meal and the control groups aquariums (fish fed by soybean meal) was free of MPs contamination. Accumulation of MPs was observed in all *C. carpio* fed by different types of fish meal. A total of 57 MPs were extracted from gastrointestinal tracts and gills of *C. carpio* fed by all fish meal types [salmon (72 % protein), two varieties of sardine (55% and 65% protein, respectively), and kilka (60% protein)]. Gastrointestinal tracts contained the highest level of MPs (72%) compared to gills (28%). Similar to morphology of MPs in fish meals, fragments were also the most predominant morphology of MPs (65%), followed by films (25%), pellet (7%), and fiber (3%) (Fig. 2c). The most abundant plastic polymers in fish were PP (37%) followed by PS (33%), PET (13%), PE (12%), and rayon (5%) (Fig. 2d). One-way ANOVA results showed significant differences between salmon (72 % protein), sardine (65% protein), and sardine (55% protein) compared to the control group (Fig. 4c). The mean number of some plastic polymers were significantly different in all fish meal types except Kilka (Fig. 4d). A positive linear correlation was observed between the concentration of MPs in different types of fish meals and accumulation of MPs in fish ($p<0.05$). However, the abundance of MPs in fish meal were much higher than MPs abundance found in fish (Fig. 5).

4. Discussion

Fish meal is obtained through cooking, pressing, drying and milling of whole fish or its by-product (Miles and Chapman 2006). Temperatures >90°C have been reported to reduce nutritional value of fish meal (FAO 1986), but cooking at 95–100°C for ~15–20 min. is commonly used to rapidly heat raw material. The purpose of the pressing section is to removed liquids from cooked materials to improve the quality of the fish meal. Furthermore, in the drying process, fish meal temperatures should not exceed 90°C in order not to impair nutritional value. Although it has been shown that high temperatures can impact integrity of plastic polymers and thus, might impede identification (Karami et al. 2017b), the lowest melting points among common LDPE plastic polymers are 110°C. Melting points in other common plastic polymers including PP, PS, PE, PET were 160, 240, 115-135, and 260°C, respectively. Therefore, it seems unlikely for MPs to change significantly their structure as a result of heat exposure during fish meal production (i.e. 95–100°C). It is possible that during fish meal processing, MPs might have been destroyed, contaminated or altered (e.g. morphological changes or fragmentation owing to grinding and heating). In the milling section, fish meals pass through a mesh screen ranging from 10 to over 100 mesh. Hence, nanoplastics (<100 nm) may also found in fish meals. Previous studies showed that nanoplastic particles are found in the aquatic environment (da Costa et al. 2016; Mattsson et al. 2018). As such the adverse effects of these nanoparticles on the molecular and biochemical biomarkers were observed on marine fish (*Dicentrarchus labrax*) (Brandts et al. 2018a) and mussel (*Mytilus galloprovincialis*) (Brandts et al. 2018b). Further studies are required to investigate the presence of nanoplastics in commercial fish meals.

Previous studies were employed a KOH solution to extract MPs from aquatic (Besseling et al. 2015; Foekema et al. 2013; Karami et al. 2017b; Rochman et al. 2015). According to results of this study, fish meals were fully digested in 10% KOH solution at 40 °C and digestion-resistant materials were successfully separated with NaI. Therefore, using KOH (10% w/v), filtration through 149 μm pore sized filter membrane, coupled with NaI solution suggests that most anthropogenic particles (>150 μm) in fish meal samples were efficiently extracted.

In the present study, the relatively high levels of MPs in different varieties of fish meal can be explained by the widespread presence of MPs in aquatic environments, and their ingestion by pelagic and demersal fish (Baalkhuyur et al. 2018; Lusher et al. 2013; Rummel et al. 2016). MPs of different shapes such as fragment, film, pellet and fiber were observed in fish meals. Fragment particles were the most abundant shape (67%) followed by film (19%), pellet (8%), and fiber (6%). Fragment values in this study were consistent with Phuong et al. (2016) who reported that MPs resembling filaments or fibers were mostly observed in lower trophic organisms (i.e. from zooplankton to Thaliacea) and fragments were mostly observed in higher trophic organisms (i.e. from fish to mammals).

Similarly, in a study by Digka et al. (2018) commercial mussels (*M. galloprovincialis*) and fish species (*Sardina pilchardus*, *Pagellus erythrinus*, *Mullus barbatus*) from waters in the Northern Ionian Sea (Mediterranean Sea), the majority of MPs were fragments both in mussels (77.8% fragments and 22.2% fibers) and fish (80% fragments and 20% fibers for *S. pilchardus*, 73.3% fragments and 26.7% fibers for *P. erythrinus* and 83.3% fragments and 17.7% fibers for *M. barbatus*). Another study conducted by Karami et al. (2017c), the presence of MPs was investigated in excised organs and eviscerated flesh of four commonly consumed dried fish species in Malaysia, and results showed the dominant type of anthropogenic particles (including plastic polymers, pigment particles, and non-plastic items) were fragments (85.7%), films (10.0%), and filaments (4.08%). According to a study by Akhbarizadeh et al. (2017) investigating the presence and location of MPs in commercially-important fish species from the Persian Gulf, a total of 828 MPs (filamentous fragments) were detected in gastrointestinal tracts, skin, muscle, gills and liver of demersal and pelagic fish (Akhbarizadeh et al. 2017).

PP, PS, and PE were the most common recovered plastic polymers in fish meals, which is consistent with their high-volume of production and widespread pollution in terrestrial and marine environments (Andrady and Neal 2009; Duis and Coors 2016). Recently, a study on the presence of MPs in the contents of the gastrointestinal tract of 26 commercial and non-commercial fish species in Saudi Arabian coast found PP (42%) and PE (42%) as the most abundant polymers in fish. Baalkhuyur et al. (2018) found MPs in the digestive tracts of 64 Japanese anchovy (*Engraulis japonicus*) which mostly were PE (52.0%) or PP (43.3%) plastic polymer (Tanaka and Takada 2016). Low-density MPs such as PP (0.90–0.91 g. cm^{3}) and PE (0.91–0.96 g. cm^{3}) are predominantly floating within the sea-surface microlayer. Over time, biofouling causes MPs to become less negatively buoyant leading to a more homogeneous distribution throughout the water column (Karami et al. 2017c; Muthukumar et al. 2011).

In this study, the dominant fish species used in production of fish meals were sardine, salmon, and common Kilka. Sardines and salmon inhabit both in coastal and open ocean waters (Chandrappa et al. 2011; Whitehead 1985). Persistent plastic pollution has been widely documented both in coastal and open oceans where degradation and weathering produces plastic fragments and MPs (Chae et al. 2015; Moore et al. 2011; Pettipas et al. 2016; Walker et al. 2006). Presence of MPs was observed in 20 varieties of canned sardines originating from 13 countries (Karami et al. 2018). Also, MP fibers and fragments were found in sardines (*Sardina pilchardus*) in the English Channel (Lusher et al., 2013). Three species of kilka (*Clupeonella* spp.) live in the Caspian Sea (Mamedov 2006) where industrial and municipal wastewaters and garbage are commonly discharged (Korshenko and Gul 2005). Disposal of municipal wastewaters contaminated with microfibers from washing of synthetic clothing has been reported as a major source of MPs to aquatic environments (McIlwraith et al. 2019; Ziajahromi et al. 2017), leading to accumulation of MPs in aquatic biota (including fish) (Provencher et al. 2018a). In a study by Naji et al. (2017) it was reported that PE, PET, and nylon were the most abundant polymer types along the beaches in the Persian Gulf. In Caspian Sea also, PS found as the most common items because of Tourism and recreational activities which are responsible for more than 90% of litter production (Sarafraz et al. 2016). Thus, in this study different percentages of plastic polymers in fish meals may be due to the ingestion of MPs by fish (e.g. salmon, kilka, sardine) living in the Persian Gulf and Caspian Sea, then production of fish meals from fish by-products.

Similar shape (fragment, film, pellet, and fibre) and polymer composition (PP, PS, PE, PET, and Rayon) of MPs in fish meals and excised organs and gills of *C. carpio* highlighted the uptake and ingestion of MPs in fish. In this study, the presence of MPs in fish gills despite the exposure of fish through food may because of ingestion of MPs via ventilation processes. That is the uptake of MPs into the gill chamber onto the gills by water movement and separated MPs from food pellets. Ingestion of MPs by *C. carpio* were similar to results reported previously for presence of

HDPE in the digestive system of blue mussel (*Mytilus edulis*) after 3 h of exposure (Von Moos et al. 2012).

Kashiwada (2006) found nanoparticles in liver, blood, gallbladder, and kidney of the See-through Medaka (*Oryzias latipes*) after 7 d of exposure to 10 mg. L^{-1} fluorescent particles, and suggested gills and gut epithelium as two translocation pathways. This study showed prevalence of smaller particles in fish meal samples, however, might be higher than the larger ones. Smaller sizes could help their translocation into other organs (e.g. liver) through two assumptions: (1) the agglomeration of smaller pieces, and/or (2) the gut lumen takes up directly these large particles by endocytosis, phagocytosis, or another mechanism, and allow particles to pass through the intestinal barrier (Collard et al. 2017), causing a higher level of toxicity. Several toxicological studies reported adverse effects of MPs on organisms (Anbumani and Kakkar 2018; Au et al. 2015; Choi et al. 2018; Deng et al. 2017). For example, physiological (swimming behaviours) and biochemical (enzymatic levels) toxicity of irregularly shaped and spherical MPs were observed in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*) (Choi et al., 2018). In another study, Espinosa et al. (2018) suggested exposure of fish to polyvinylchloride (PVC) or PE MPs could impair fish immune parameters. Laboratory studies showed several negative effects of the ingestion of plastic particles including trypsin and chymotrypsin activities in silver barb (*Barbodes gonionotus*) (Romano et al. 2018), superoxide dismutase, glutathione peroxidase and catalase activities in discus fish (*Symphysodon aequifasciatus*) (Wen et al. 2018), and head-kidney leucocyte activities in gilthead seabream (*Sparus aurata*) and European sea bass (*D. labrax*) (Espinosa et al. 2018). Therefore, this study highlights that presence of MPs in fish meals might pose a health risk to organisms consuming it including poultry, and cultured fish.

In this study, a positive relationship between MPs in fish meal and accumulation in fish was found. Thus, there is an urgent need to examine accumulation of MPs in aquatic organisms. Some laboratory studies have documented MPs uptake in fish, including *D. rerio* (Lu et al. 2016), red tilapia (*Oreochromis niloticus*) (Ding et al. 2018), and goldfish (*Carassius auratus*) (Grigorakis et al. 2017). However, the accumulation of MPs may be a variation of different factors, such as species, time, size, and exposure systems (Ding et al. 2018). MP shape and plastic polymers composition were similar in both fish meal and *C. carpio*. As such, PP were the dominant MPs in fish meals and fish. Oliveira et al. (2013) showed PP MPs significantly reduced acetylcholinesterase (AChE) activity in common goby (*Pomatoschistus microps*). Because humans consume livestock, poultry, and cultured fish, they are a direct route of exposure to MPs via diet and increase concerns related to MP-associated risk to humans. In addition to risks from posed by physical plastic debris, the hazardous hydrophobic organic chemicals bound to MPs may be transferred to humans (Rochman et al. 2013). Because there are few studies related to the potential health risks from MPs, more efforts to address interactions between MPs and biota are critical (Smith et al. 2018). Hazard and dietary exposure data for plastic particles, ingested by humans via the food chain are very scarce (Karbalaei et al. 2018). Due to present lack of knowledge, more studies are required to assess potential human health risks from MP ingestion.

5. Conclusion

This was the first study to investigate MP loads and their relationships in fish meals and their subsequent accumulation in fish. The presence of MPs in fish meals highlights that farmed organisms could be exposed to high levels of MPs. A correlation between MPs in fish meals and in *C. carpio* showed uptake and ingestion of MPs in fish. This study shows that *C. carpio* can be used as an effective bioindicator to reveal presence and transfer of MPs from the marine environment to the human food chain.

Partial or total replacement of fish meals by alternative protein sources might help to mitigate MP exposure to farmed organisms. However, the financial cost, ecological impact and dietary quality of such alternatives must also be considered. Also, greater attention and accuracy in the processing of fish meal production might help to obviate the presence of MPs inside these products. MPs pollution is an emerging area of concern related to their potential impacts of this plastic debris to human health. Recommendation for future research priorities is presented with a focus on the consequences of MPs for human health.

Acknowledgement

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Table 1. Summary of fish meal samples analyzed in this study.

Fish Meal type	Crude Protein %	Fat %	Moisture Content %
Salmon	72	9	4
Sardine	65	9	4
Kilka	60	6	4
Sardine	55	12	2

Figure Legends

Fig. 1. Histogram of number of isolated particles across different size categories (µm).

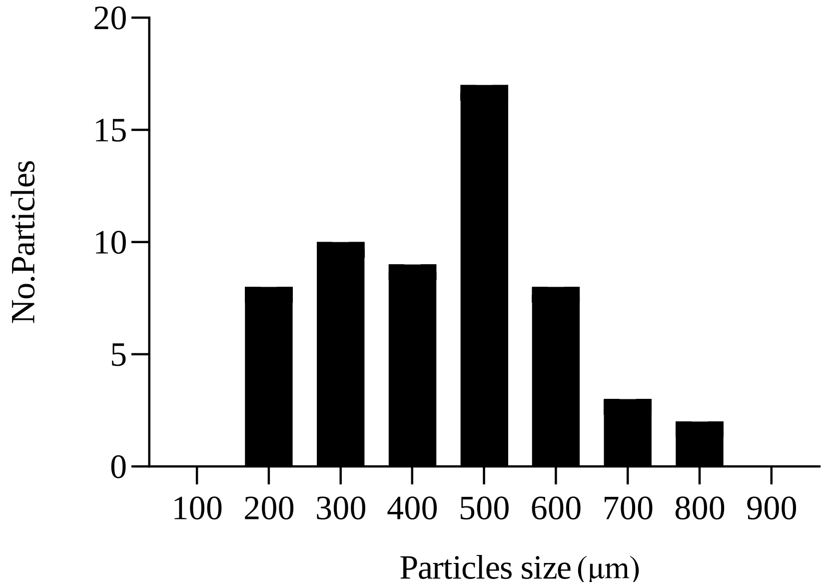
Fig. 2. Shapes (a, b) and polymers (c, d) of MPs in fish meals (n=30) and gastrointestinal tracts and gills of fish (*C. carpio*) (*n*=18).

Fig. 3. Microscopic images of MPs polymers from fish meal. Particles were identified as (a) Polypropylene (PP), (b) Polystyrene (PS), (c) Polyethylene (PE), (d) Polyethylene terephthalate (PET), and (e) Rayon.

Fig. 4. Total microplastics (a), isolated plastic polymers (b), from different types of fish meals (*n*=30), and total microplastics (c), isolated plastic polymers (d) from gastrointestinal tracts and gills of fish (*C. carpio*) (*n*=18). Bars surmounted with different letters are statistically ($P<0.05$, Tukey's multiple range test) different.

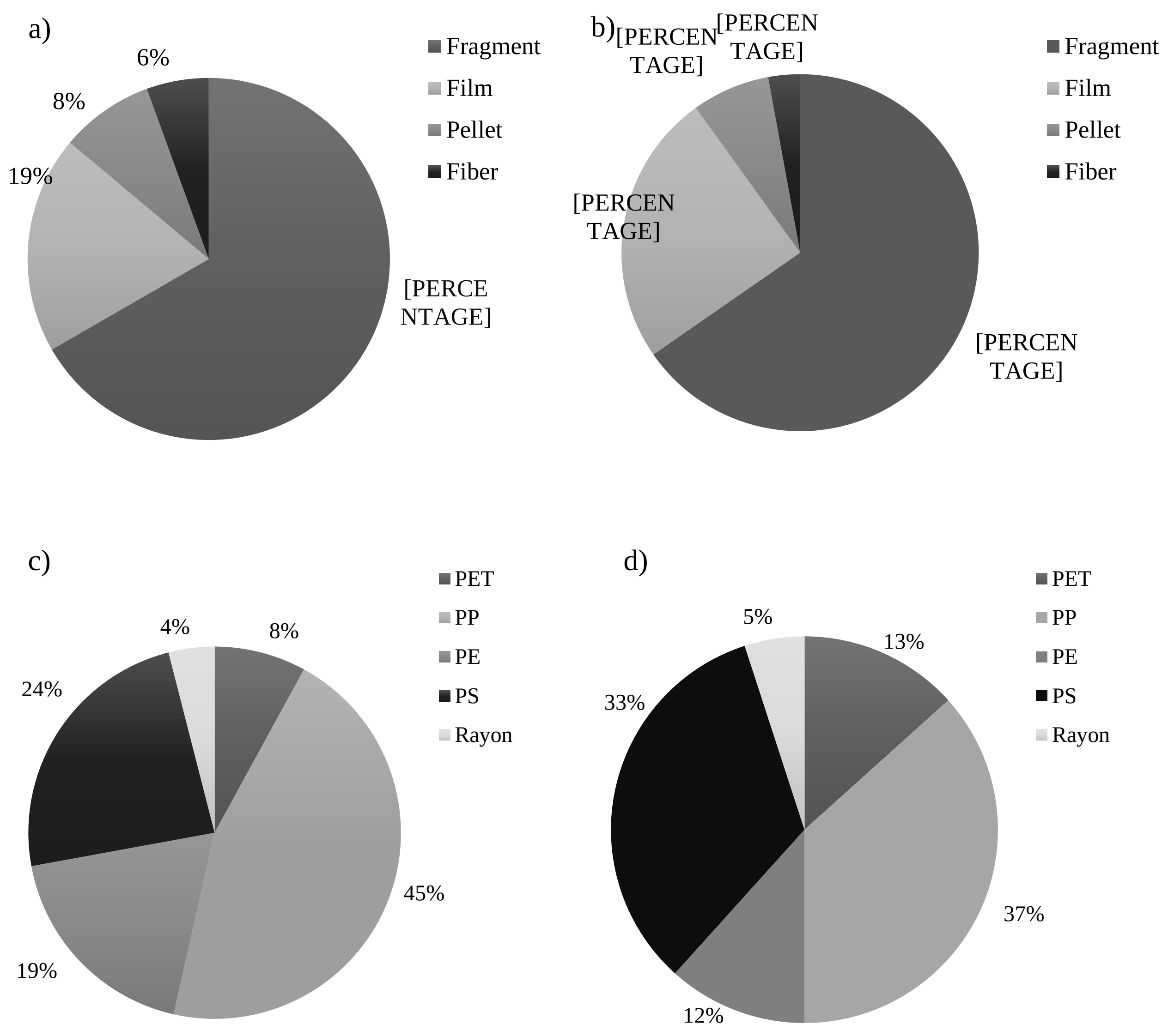
Fig. 5. Comparison of MP abundance in exposure experiment on cultured Common carp (*Cyprinus carpio*) (*n*=18) by feeding commercial fish meal (a) Salmon (b) Sardine (65%) (c) Kilka, and (d) Sardine (55%). Lines indicate upper quartile, median, and lower quartile, and dots show individual observations in box plots.

Fig. 1.



No.Particles
20
15
10
5
0
100 200 300 400 500 600 700 800 900
Particles size (μm)

Fig. 2.



In fish meals

In fish (*C. carpio*)

Fig. 3.

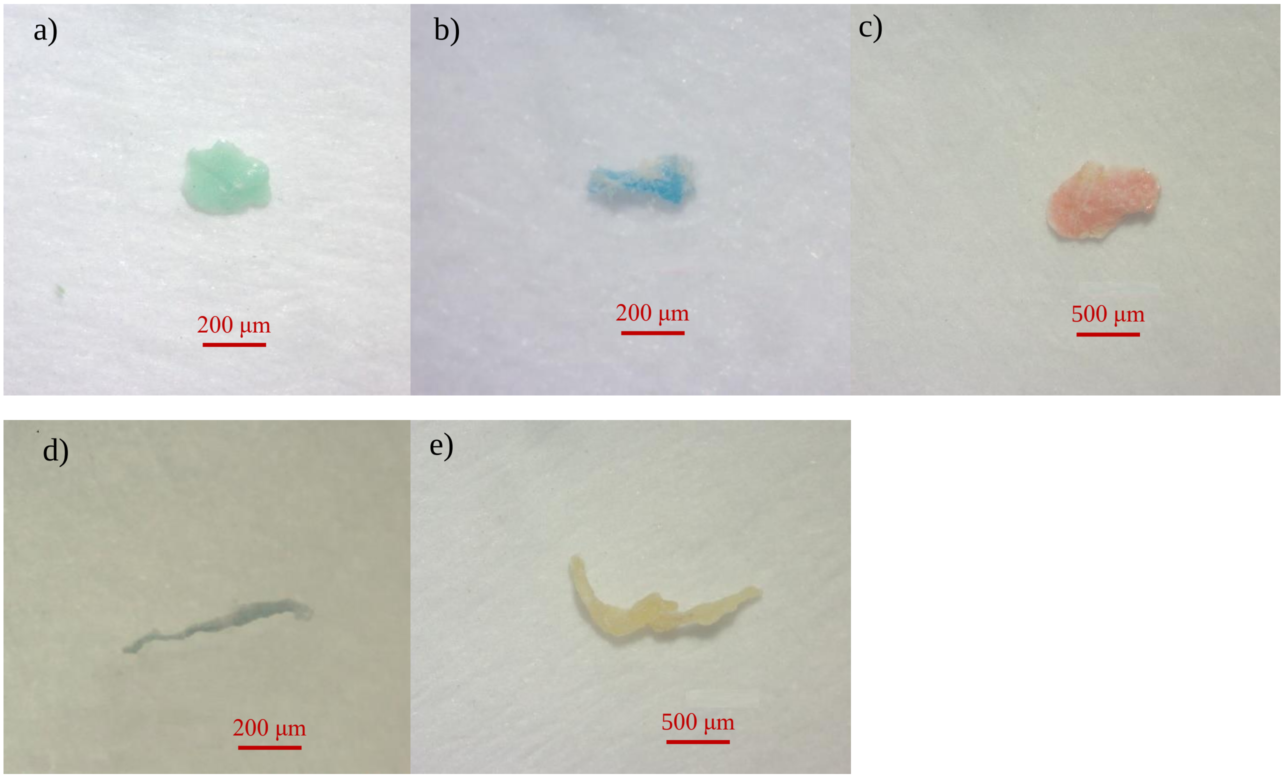
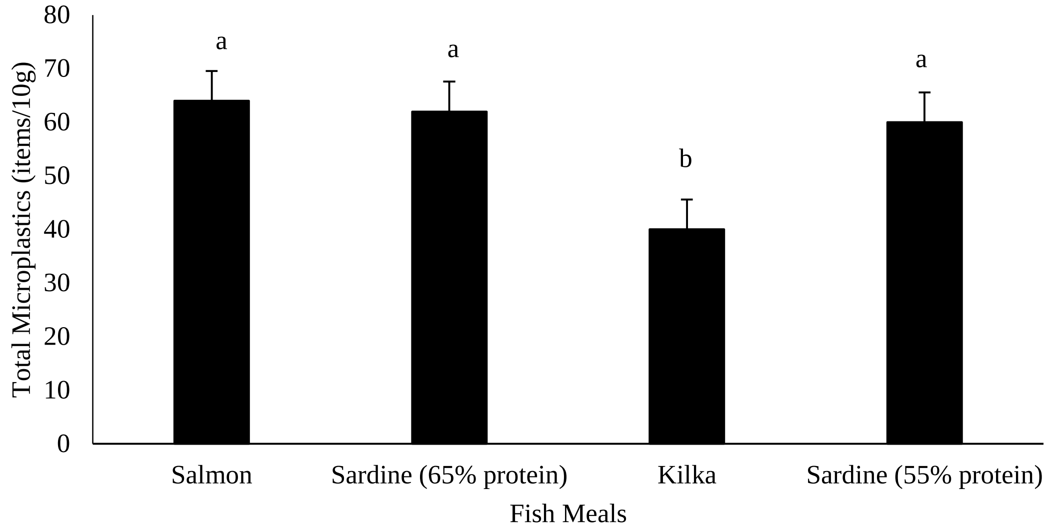
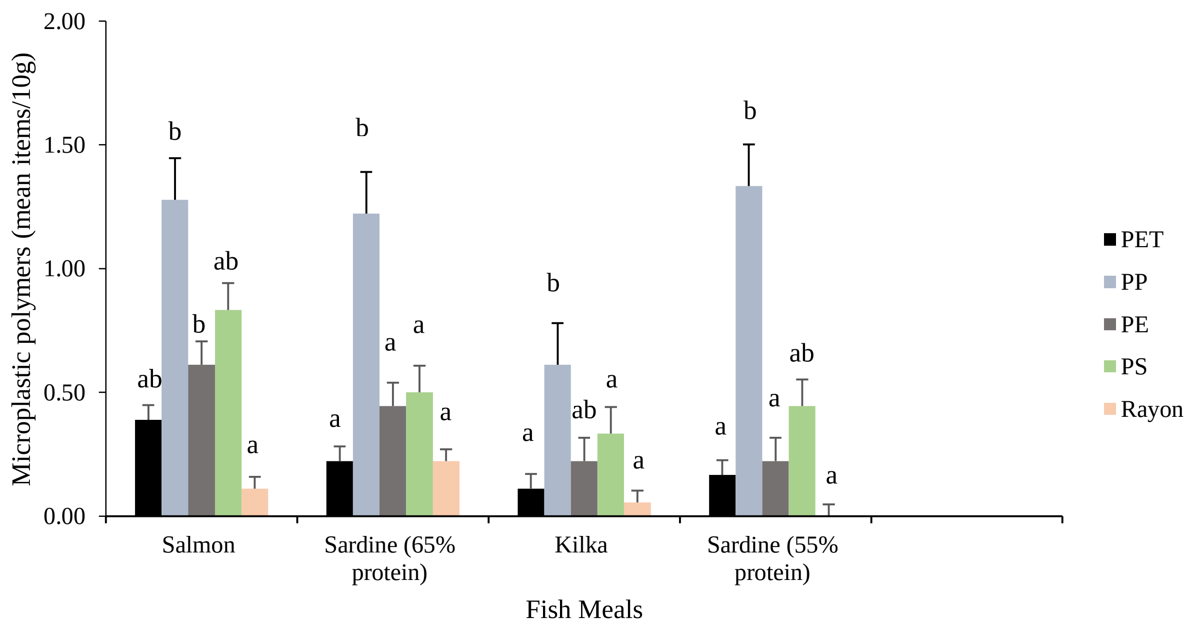


Fig. 4.

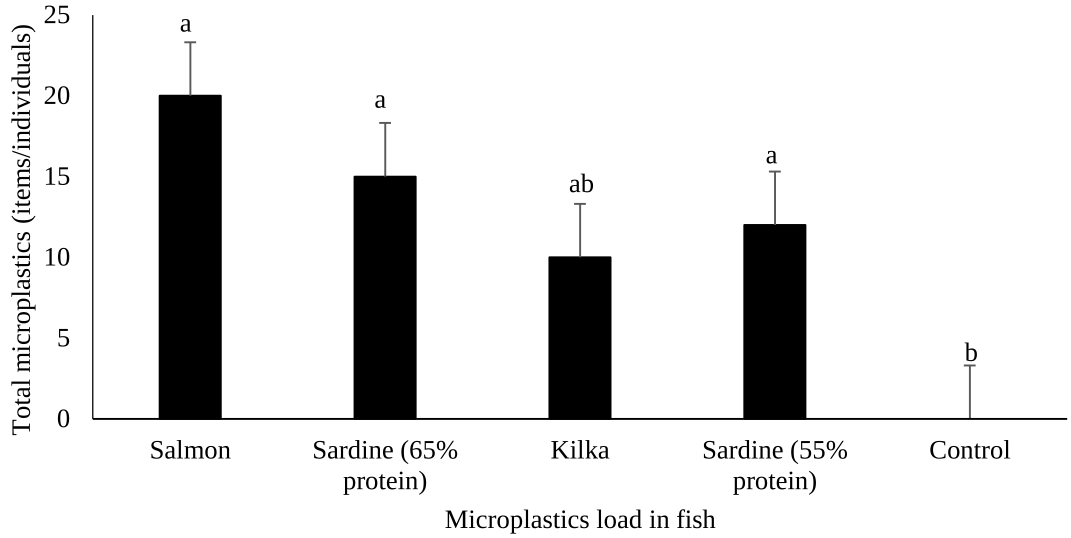
a)


80
70
60
50
40
30
20
10
0
Total Microplastics (items/10g)
a
a
b
a
Salmon
Sardine (65% protein)
Kilka
Sardine (55% protein)
Fish Meals

b)

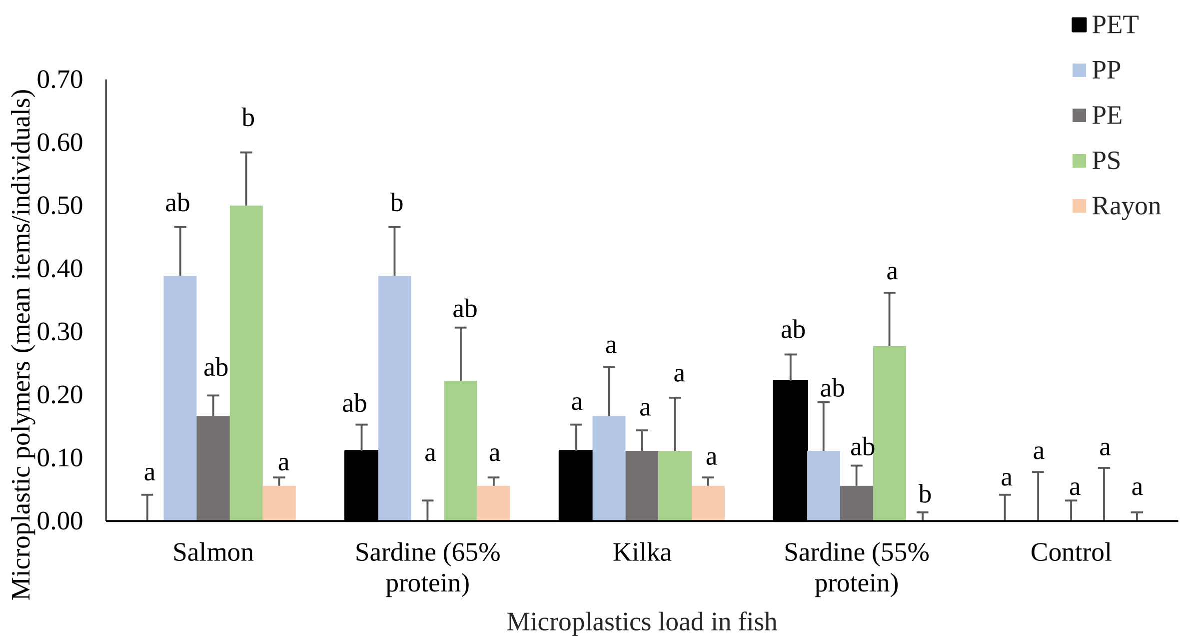

2.00
1.50
1.00
0.50
0.00
Microplastic polymers (mean items/10g)
ab
b
b
ab
a
a
b
a
a
a
a
b
ab
a
a
a
b
a
ab
a
Salmon
Sardine (65% protein)
Kilka
Sardine (55% protein)
Fish Meals
PET
PP
PE
PS
Rayon

c)



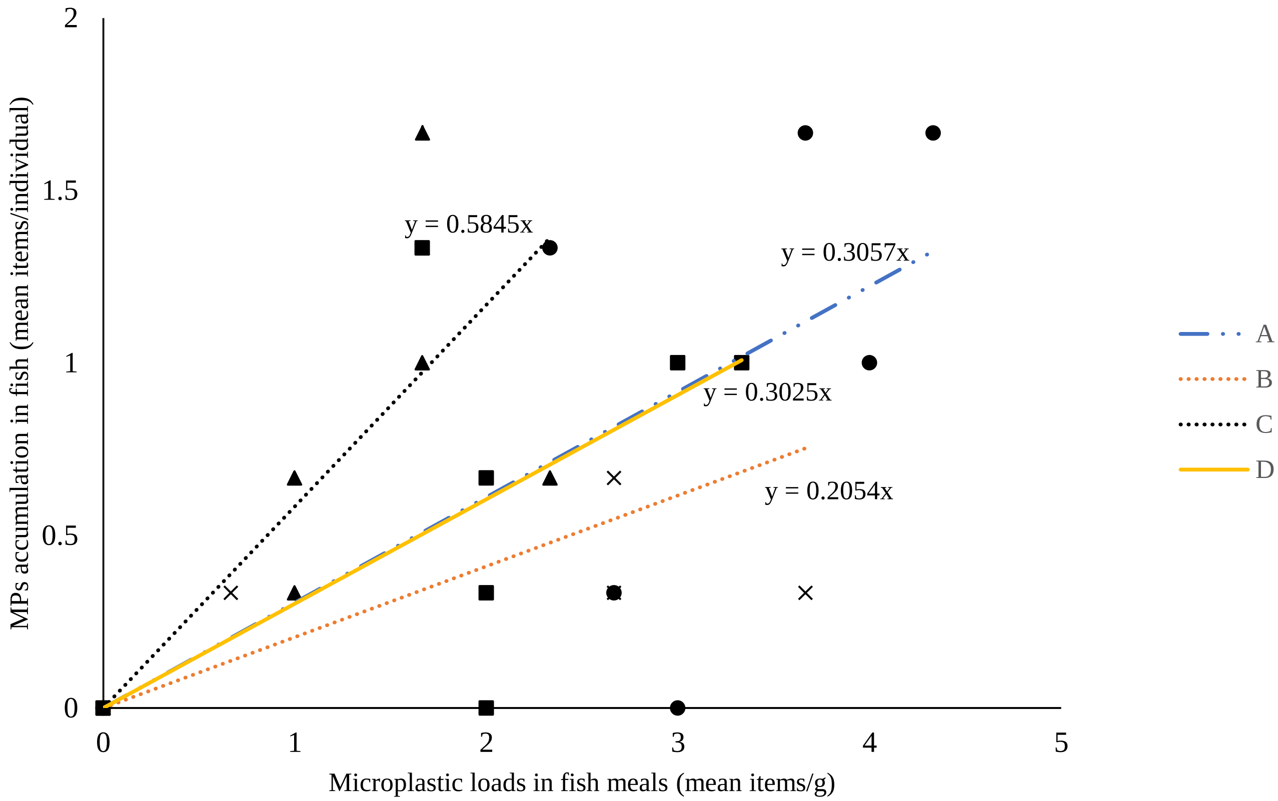
Total microplastics (items/individuals)
25
20
15
10
5
0
a
a
ab
a
b
Salmon
Sardine (65% protein)
Kilka
Sardine (55% protein)
Control
Microplastics load in fish

d)



PET
PP
PE
PS
Rayon
Microplastic polymers (mean items/individuals)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Salmon
Sardine (65% protein)
Kilka
Sardine (55% protein)
Control
Microplastics load in fish

Fig. 5.



MPs accumulation in fish (mean items/individual)
Microplastic loads in fish meals (mean items/g)
y = 0.5845x
y = 0.3057x
y = 0.3025x
y = 0.2054x
A
B
C
D
0
0.5
1
1.5
2
0
1
2
3
4
5

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