Estimation of the uptake of polycyclic aromatic hydrocarbons desorbed from polyethylene microplastics in the digestive tract of the red seabream (Pagrus major) and mummichog (Fundulus heteroclitus)

メタデータ	言語: English 出版者: 公開日: 2025-02-06 キーワード (Ja): キーワード (En): Vector effect; Scorption; Desorption; Log Kow; In vitro 作成者: 河野, 久美子, 伊藤, 真奈, 羽野, 健志, 大久保, 信幸 メールアドレス: 所属: 水産研究・教育機構, 水産研究・教育機構, 水産研究・教育機構, 水産研究・教育機構
URL	https://fra.repo.nii.ac.jp/records/2013225

Estimation of the uptake of polycyclic aromatic hydrocarbons desorbed from polyethylene microplastics in the digestive tract of the red seabream (*Pagrus major*) and mummichog (*Fundulus heteroclitus*)

Kumiko Kono*, Mana Ito, Takeshi Hano, Nobuyuki Ohkubo

Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Maruishi 2-17-5, Hatsukaichi, Hiroshima, 739-0452, Japan

* Corresponding author.
E-mail addresses: kono_kumiko51@fra.go.jp

Abstract

The vector effects of microplastics (MPs) in natural marine environments and their contribution to PAH bioaccumulation in fish are still unknown. This study, therefore, aimed to evaluate the effects of MPs on carrying PAHs under conditions close to the actual marine environments. The PAH content sorbed onto MPs from seawater positively correlated with the octanol/water partition coefficient (log K_{OW}) in both polyethylene and polystyrene MPs. The desorption rate constant (k_2) of polyaromatic hydrocarbons (PAHs) sorbed onto MPs from seawater in the digestive tract was calculated using two marine teleost fish in an *in vitro* desorption experiment. A negative correlation was observed between the log K_{OW} and k_2 of the PAHs. The PAHs with larger log K_{OW} and lower water solubility are easier to sorb onto MPs and less likely to desorb in the digestive tract of marine fish species. The estimation of PAH uptake into the fish body using the k_2 of three selected PAHs (phenanthrene, pyrene, and chrysene) indicated that more than 95% of the chemicals were uptaken from seawater via gills. It was presumed that there was an additional uptake of PAHs into the fish body (vector effect) due to desorption from MPs in the digestive tract when marine fish ingested MP that was sorbed and concentrated with PAHs. The degree of uptake was, however, much lower than that from seawater via gills. The vector effect of PAHs by MPs in the actual sea may be negligible, and the risk is presently considered to be low.

Keywords: Vector effect, Sorption, Desorption, Log K_{OW}, *In vitro*

1. Introduction

Microplastics (MPs) are ubiquitous in the global environment. (Ohno and Iizuka, 2023; Zhao et al., 2024), with the abundance of pelagic MPs estimated to increase and the maximum MP concentration expected to be 1 mg/L by the 2060s (Isobe et al., 2019). The bioaccumulation of MPs has recently become one of the most important environmental problems today (Wang et al., 2021; Gedik et al., 2023; Valencia-Castañeda et al., 2024).

Tanaka and Takada (2016) reported the ingestion of MPs by 77% of Japanese anchovies (*Engraulis japonicus*) captured in urban coastal waters around Japan. A median of 60% of fish, belonging to 198 species captured in 24 countries, contained MPs in their organs (Sequeira et al., 2020); MPs were detected approximately 86% in the gastrointestinal tract and 92% in the gills of examined fish (Jaafar et al., 2021); particularly high MP abundance (99%) was observed in fish living in urban estuaries (Parker et al., 2020). Small environmental MPs in the liver were reported in adult benthopelagic fish (Zitouni et al., 2022).

Laboratory experiments have, however, demonstrated that the retention time of MPs ingested by fish is comparable to that of digesta (Grigorakis et al., 2017), and more than 90% of MPs ingested is excreted from the body of the fish within approximately 24 h, resulting in minimal accumulation in the digestive tract (Jovanović et al., 2018; Ohkubo et al., 2020; Ohkubo et al., 2022).

The ingestion of MPs may induce adverse effects by transporting sorbed and accumulated hydrophobic organic contaminants (HOCs) from the seawater to marine organisms, in a process known as the MP vector effect (Bakir et al., 2016; Qiu et al., 2020; Fred-Ahmadu et al., 2020). MPs adsorb and accumulate HOCs, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls, and polybrominated diphenyl ethers (Yeo et al., 2020; Arias et al., 2023).

PAHs are widely distributed in the marine environment (Barhoumi et al., 2016; Wallace et al., 2020; Shi et al., 2022; Wu et al., 2022; Liu et al., 2023) because they result from not only oil spills but also the incomplete combustion of fossil fuels. They are regulated in both the environment and food by the United States Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) because of their toxicity, carcinogenicity, potential for human exposure, and frequency of occurrence (WHO, 1998). A strong interaction was reported between PAHs and MPs where it was noted that MPs accumulate PAHs up to 106 times higher than the surrounding water (Teuten et al., 2007) and mean concentrations of total sorbed PAHs on polyethylene (PE) MPs were 722 ng/g (low-density PE, LDPE) and 797 ng/g (high-density PE, HDPE) (Rochman 2013) and 108.76 ± 12.88 ng/g (Arias et al., 2023). Mai et al. (2018) measured the concentrations of 16 priority EPA PAHs ranging from 3,400 to 119,000 ng/g in MPs collected from coastal areas associated with human activities.

It was reported that an increase of benzo(a)pyrene (B(a)P) was detected in mussels after a three-day exposure, particularly when mixed with MPs (Romdhani et al., 2022). Abouda et al. (2024) reported that time-dependent bioaccumulation and increased acidic mucus were observed when seaworms were exposed to environmental MPs and B(a)P, alone and mixed, and their co-exposure induced oxidative stress and neurotransmission disorders (Abouda et al., 2022).

In vivo experiments have demonstrated the role of MPs as vectors when Japanese medaka (*Oryzias latipes*) were exposed to both anthracene (Ant), a type of PAH, and 50 μm spherical PE-MP (Qiu et al., 2020). It has, however, been suggested that the ingestion of MPs may not be an important pathway for the transportation of contaminants to aquatic organisms in model analyses (Bakir et al., 2016; Koelmans et al., 2016). The role of MPs as a vector is dependent on their size (Takai et al., 2023); however, that phenomenon in an actual marine environment is still unknown. A recent study indicated that PAHs sorbed on MPs were desorbed in the guts of fish (Ito et al., 2022) using 250–300 μm PE-MP typically found in fish gut contents (Garcia-Garin et al., 2019; Tanaka and Takada, 2016). The contribution of PAH uptake from the ingestion of PAH-sorbed MPs, typically found in fish, is necessary to evaluate the vector effect of MPs.

Although it has been reported that the bioaccumulation of PAH and the vector effects of MPs for PAHs depend on the concentrations of PAHs in seawater (OECD, 2012) and the size of MPs (Takai et al., 2023), respectively, these effects have been evaluated using extremely high PAH concentrations and very small-sized MPs and have not been reported in actual marine environments to date. The vector effects of MPs in actual marine environments and their contribution to PAH bioaccumulation in fish are, therefore, still unknown. This study, therefore, aimed to evaluate the vector effects of MPs for PAHs under conditions close to actual marine environments.

This study first determined the sorption properties of PAHs from seawater on 250–300 μm MP and further compared its sorption properties between different materials (PE and polystyrene; PS). The desorption rate constant of PAHs sorbed on 250–300 μm PE from seawater in the digestive tract of two marine teleost fish, red seabream (*Pagrus major*) and mummichog (*Fundulus heteroclitus*), was then calculated using the *in vitro* desorption experiment. The study finally investigated the contribution of the uptake of PAHs desorbed from MPs in the digestive tract relative to waterborne uptake, considering it is another key pathway of contaminant bioaccumulation, and further estimated its potential risks to human health. The description of the present study is presented in Fig. 1.

2. Materials and methods

2.1. Reagents

Clear microsphere PE beads of particle size 250–300μm (Cospheric, Santa Barbara, CA, USA), PS beads of particle size 200–300μm (PolyScience, Niles, Illinois, USA), and 16 priority PAHs classified by the Environmental Protection Agency (16 EPA PAHs) (AccuStandard, New Haven, CT, USA) were used as the MPs and PAHs in this study. Throughout the experiment, 16 d-PAHs (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) in acetone were used as internal standards (Table S1). Analytical-grade acetone and *n*-hexane were obtained from FUJIFILM Wako Pure Chemical Corp., Osaka, Japan.

2.2. Experiment 1. Sorption of PAHs on MP

2.2.1. Calculation of sorption rate constant

The sorption of PAHs on MPs was based on the OECD test guideline 106 (OECD, 2000) and was achieved by mixing 1 L of artificial seawater with 16 PAHs and PE (100 mg/L) for 1, 3, 6, 24, and 48 h at 20°C while stirring in a light-shielding and tightly capped triplicate glass bottle. Each PAH concentration was adjusted to 2 μg/L, corresponding to that occurring in the aquatic environment (Anyakora et al., 2005; Maskaoui et al., 2002; Zhang et al., 2016). Twenty milliliters of the test solution were subjected to PAH analysis at the beginning and end of the sorption test immediately after collection. The amount of PAHs sorbed on the MP was calculated as the difference between the amount of the test substance initially present in the solution and the amount remaining at the end of the experiment (indirect method).

The sorption rate constant (*k*) was calculated from the first-order approach to the equilibrium model (Allan 2009) using the exponential rise-to-maximum equation (1):

$$C_t = C_{eq}\,(1 - e^{-kt}), \qquad (1)$$

where C_t is the PAH content on MP at time t (μg/g); k is the sorption rate constant (/h); and C_{eq} is the predicted equilibrium PAH content on MP (μg/g). The time required to reach the 80 percent steady state (t_{80}) was calculated using equation (2) (OECD, 2012):

$$t_{80} = -\ln(0.20)/k = 1.6/k \qquad (2)$$

2.2.2. Comparison of PAH sorption properties between PE and PS

The PAH concentrations of the test solutions were adjusted to 0.05, 0.5, 1, and 2 μg/L. At the beginning of the sorption test, 100 mg of the MP (PE or PS) was added to 1 L of the test solution. After stirring for 48 h, the PAH concentration in the test solution and that on the MP was measured. The MP was collected via glass fiber filtration (Whatman GF/B, Whatman International Ltd., Maidstone, UK) and stored at -20°C until further analysis or use.

2.2.3. Comparison of PAH sorption index between PE and PS

To characterize an index of sorption to MPs, the Freundlich adsorption coefficient was calculated based on the OECD Test Guideline 106 (OECD, 2000).

The Freundlich adsorption isothermal formula is followed by equation (3) or (4), where V is the PAH content on the MPs (μg/g), C is the PAH concentration in the test solution (μg/mL), a is the sorption coefficient, and n is the sorption index.

$$V = aC^{(1/n)} \quad (3)$$

$$\log V = \log a + (1/n) \log C \quad (4)$$

2.3. Experiment 2. Calculation of desorption rate constant in vitro desorption experiment

2.3.1. Animals

Red seabream fish were purchased from a commercial fish farm (Amarine Kindai, Wakayama, Japan) and kept in a 500-L fiberglass-reinforced plastic tank with flow-through seawater at 20°C under a 14 h light: 10 h dark cycle of illumination. The Arasaki strain of the mummichog (Shimizu, 1997), which has been bred in our laboratory for several decades, was used in this study. The mummichogs were kept in an indoor aquarium with flow-through seawater and natural light illumination before the experiments. The salinity (30 psu) and dissolved oxygen (> 80%) were maintained for their acclimation in the laboratory. The fish were fed twice daily with commercial fish food (Otohime EP 2; Marubeni Nisshin Feed, Tokyo, Japan) until saturation. Red seabream (OECD, 2019) and mummichog (Shimizu, 1997) have been widely used as experimental fish. Using these two fish species, we have already clarified the MP uptake and gut retention time (Ohkubo et al., 2020) and established the *in vitro* desorption experiment (Ito et al., 2022). All animal care and use procedures were performed in accordance with the guidelines for animal experimentation proposed by the Fisheries Technology Institute of the Japan Fisheries Research and Education Agency.

2.3.2. In vitro desorption experiment of PAHs from MPs

The digestive tract was obtained from two marine teleost fish—red seabream (n = 10, total length (TL) 250 ± 8.6 mm, BW 280 ± 20 g) and mummichog (n = 30, TL 120 ± 5.9 mm, BW 34 ± 5.0 g)—that had been fed until saturation. After feeding, the fish were narcotized in ice seawater and then dissected, and the gut content was collected from the midgut (pyloric ceca and foregut) in red seabream and foregut in mummichogs, considering these parts efficiently desorb PAHs from MP, based on a previous study (Ito et al., 2022). The content samples were frozen in liquid nitrogen and stored at -80°C until further use.

The gut contents were homogenized using a sonicator (Sonics Vibra-Cell VCX 130; Sonics and Materials Inc., Newtown, CT, USA) in 0.25 M sucrose (1:5 w/v), then centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant (gut content extract) was used for subsequent desorption experiments of PAHs from MPs. PE was used in the experiments.

For the desorption experiment, 1 ml of the gut content extract was mixed with 50 mg of PAH-sorbed PE and 9 ml of 0.1M phosphate buffer (pH 7.0, red seabream, and pH 6.0, mummichog, the optimum pH in their digestive tracts) (Ito et al., 2022). The PAHs-sorbed MP was prepared by stirring the PE (100 mg/L) in the artificial seawater containing 16 PAHs (2 μg/L) for 48 h as described in Exp. 1. The present study was a part of our ongoing research on MP's vector effects; the MP concentration (100 mg/L) was, therefore, selected in line with our previous study (Ito et al., 2022). The concentration is within the environmentally relevant levels that will occur by the 2060s (1 mg/L) (Isobe et al., 2019) and convergence zone MP levels that were 300 times higher than in non-convergence zones (Nakakuni et al., 2024). In terms of each PAH, 2 μg/L was selected considering some PAHs occur in seawater on some coasts and estuaries with high human activity at this level (Anyakora et al., 2005; Maskaoui et al., 2002; Zang et al., 2016). The concentrations of PAHs sorbed on MP used in the desorption experiments were reported by Ito et al. (2022). The mixture in the glass tube (n = 25) was then incubated under 20°C under dark conditions with horizontal shaking at 100 rpm and sampling (five replicates) was performed at five different intervals (30 min, 1,3,6, and 24 h).

Two types of blanks were prepared according to Ito et al. (2022). Blank 1 was used to determine the PAH desorption without gut contents extract, and Blank 2 to define the PAH concentration derived from the gut contents and PAHs-free PE. After incubation, the PE was separately collected using a glass filter (GF/C; Whatman), and the PAH concentration in the filtrate was analyzed according to a previously reported procedure (Ito et al., 2022). The PAH content of the MP was calculated by subtracting the filtrate at the beginning and end of the desorption experiment.

The desorption rate constant (k_2) was calculated from the first-order model of the following equation (5), which is based on OECD test guideline 305 (OECD, 2012):

$$C_p = C_{p0} * e^{-k_2 t}, \qquad (5)$$

where C_p is the PAH content of the MP at time t (ng/g), k_2 is the desorption rate constant (/d), and C_{p0} is the PAH content of the MP at the beginning of the desorption experiment (ng/g).

2.4. Experiment 3. Contribution of the uptake of PAHs desorbed from MP in the digestive tract

Together with the present findings and previous research, the PAH uptake into the fish body via MPs in the marine environment was estimated using the formulae listed in Table 1. A total of three PAHs were selected for subsequent estimation—phenanthrene (Phe), pyrene (Pyr), and chrysene (Chr)—because they are more frequently detected in the environment and are highly bioaccumulated in living organisms (Koyama et al., 2004; Cheikyula et al., 2008; Wallace et al., 2020; Shi et al., 2022; Wu et al., 2022). The concentration of PAHs in seawater was calculated from the maximum MP concentration (1 mg/L) expected by the 2060s (Isobe et al., 2019), the concentration of PAHs contained in MP collected from actual sea areas (Mai et al., 2018), and the desorption ratios from MP to seawater (Phe; 42%, Pyr; 26%, and Chr; 38%) that was preliminarily obtained when the PAHs-sorbed MP used in Exp. 2 was added to seawater (5 L) in a glass aquarium at a concentration of 100 mg MP/L and stirred for 24 h. The bioconcentration factor (BCF) of PAHs in teleost fish (Gerhart and Carlson, 1978; Baussant et al., 2001) was thereafter used to estimate the PAH concentrations in fish derived from seawater. The PAH content desorbed from the MP in the digestive tract was additionally determined using k_2 obtained in Exp. 2. The PAH desorbed content from MP in the fish digestive tract at 3 h was calculated when more than 80% of the PAH content in the steady state was desorbed from the MPs in Exp. 2. The PAH concentrations in fish were then estimated from the MPs in the digestive tract, assuming that all the PAHs desorbed from MP in the digestive tract would be taken up into the fish body.

2.5. Chemical analysis

2.5.1. Extraction of PAHs in the test solution and filtrate

Liquid-liquid extraction was employed to extract PAHs from the test solution in Exp. 1 and filtrate in Exp. 2 based on a previous study (Ito et al., 2022). The samples were mixed with the internal standard and hexane, and shaken thoroughly for 10 min. After centrifugation, the hexane layer was subjected to gas chromatography-mass spectrometry (GC/MS) analysis.

2.5.2. Extraction of PAHs sorbed on MPs

The PAHs sorbed on the MPs were extracted based on the PAH analysis (Ito et al., 2022). PAHs sorbed on MP were desorbed using 1N KOH/EtOH at 80°C for 1 h. The desorbed PAH solution was passed through a glass filter (GF/B; Whatman, Maidstone, UK). The filtrate was mixed with distilled water and hexane for liquid-liquid extraction. After extraction, the hexane layer was separated, rinsed thrice with distilled water, concentrated, and applied to a silica gel cartridge column (Supelclean LC-Si: 1 g, 6 ml; SUPELCO Inc. St. Louis, MO, USA) that had been preconditioned with 10 ml of acetone and hexane before use. The cartridge column was eluted with 10 ml of hexane. The eluate was concentrated to 0.5 ml using nitrogen gas and subjected to GC/MS.

2.5.3. GC/MS analysis

Gas chromatographic separation was performed using the Agilent 7890A gas chromatograph equipped with a 5975C mass spectrometer (Agilent Technologies, Tokyo, Japan) (Ito et al., 2022). The monitored SIM ion, retention time, and quantification limit of the PAHs are presented in Tables S1 and S2. For the statistical analysis, PAH concentrations below the quantitation limit were set to half of the quantitation limit (OECD, 2004). The desorbed PAH concentration in the gut content groups, which was defined as the PAH desorption concentration enhanced by adding gut contents, was obtained by excluding the desorbed PAH concentrations in Blank 1.

2.6. Statistical analysis

Correlations between PAH content on MP and their physiochemical properties (octanol/water partition coefficient (log K_{OW}), water solubility; WHO, 1998) and between the k_2 of PAHs desorbed from the MPs in gut contents and log K_{OW} were analyzed using Pearson's correlation analysis. All the computations were performed using the R software (R Development Core Team, 2007).

3. Results and Discussion

3.1. Experiment 1. Sorption of PAHs on MP

3.1.1. Calculation of PAH sorption rate constant

The k tended to decrease with an increase in the number of rings (Table 2). The value of t_{80} tended to increase as the number of rings increased. The time required to reach equilibrium between the PE debris and seawater is longer for more hydrophobic and higher-molecular-weight PAHs (Lee et al., 2017). The findings of this study are consistent with Rochman et al. (2013), who reported that the time to reach an equilibrium of PE in the marine environment for benzo(ghi)perylene (six rings) was longer than that for fluoranthene (four rings). It was inferred that PAHs with a larger number of rings required a longer time to reach equilibrium; however, 48 h was sufficient to reach equilibrium in Exp. 1.

3.1.2. Comparison of PAHs sorption properties between PE and PS

The PAH content sorbed onto the MPs is presented in Fig. 2. PAHs with a larger number of rings tended to sorb more on the MP, and the PAH content on the MPs tended to increase with increasing concentration in the test solution, irrespective of the MP material. In particular, the sorption enhanced from 1,2benzanthracene (B(a)A), and higher molecular weight PAHs.

The total PAH contents on PE were found to be 3,600, 21,000, 42,000, and 91,000 ng/g, and those on PS were 1,800, 38,000, 59,000, and 120,000 ng/g in the test solutions of 0.05, 0.5, 1, and 2 μg/L, respectively. These results correspond to those reported by Mai et al. (2018) (3,400–119,000 ng/g). The highest adsorption amount to MP was dibenz(a,h)anthracene for both PE and PS, and the sorption amount in the test solution of 2 μg/L was approximately 7,000 and 10,000 times the test solution concentration for PE and PS, respectively. The concentration factor of Phe, Pyr, and Chr calculated from the test solution of 2 μg/L to MP was 230, 1,000, and 4,800 for PE and 420, 390, and 5,400 for PS. Phe has a lower concentration factor than the BCF (940), and Pyr and Chr have higher concentration factors than the BCF (Pyr: 72, Chr: 54) (Gerhart and Carlson, 1978; Baussant et al., 2001).

As presented in Fig. 3, the PAH content of MP positively correlated (PE: $r = 0.89$, $p < 0.01$; PS: $r = 0.90$, $p < 0.01$) with the log K_{OW}, an index of water solubility and lipophilicity. A negative relationship (PE: $r = -0.85$, $p < 0.01$; PS: $r = -0.82$, $p < 0.01$) was observed between the log-normalized water solubility and the PAH content on MP. It was inferred that PAHs with larger log K_{OW} and lower water solubility were more easily sorbed onto MPs. The Phe, Pyr, and Chr contents of MPs collected from coastal areas have been reported to be 15,000, 5,000, and 2,500 ng/g, respectively (Mai et al., 2018), which decreased with an increase in log K_{OW}. This reflects the PAH composition in seawater, in which the main compounds among the 16 PAHs are 2–3-ring PAHs (Zhang et al., 2016; Tong et al., 2019; Shi et al., 2022).

3.1.3. Comparison of PAHs sorption index between PE and PS

As an index of sorption characteristics on MP, the Freundlich sorption coefficient was calculated based on the OECD Test Guideline 106 from the results of the sorption properties on MPs.

The adsorption coefficient (a) demonstrated a tendency to be larger for the PAHs, with higher log K_{OW} values for both PE and PS (Fig. S1). The coefficient was particularly enhanced for B(a)A (log K_{OW}; 5.6) and higher-molecular-weight PAHs in both the MPs. Campos et al. (2023) also reported that PAH concentrations in the LDPE were higher in the higher-molecular-weight PAHs than those in the lower-molecular-weight PAHs after the MP spiking procedure in the PAH working water solution. The sorption properties of PAHs from seawater were comparable, suggesting that the PAH sorption properties of PS from seawater may be estimated to some extent using PE.

3.2. Experiment 2. Calculation of desorption rate constant in vitro desorption experiment

The desorption of PAHs from MPs reached almost a steady state 24 h after the initiation of the test. More than 80% of the PAH concentration in the steady-state test water was desorbed within 3 h after the initiation of the test in both the fish species (Fig. 4). The desorption of PAHs from MP can, therefore, be divided into two phases: 0–3 h and 3–24 h. This is because the k_2 of each of the 16 PAHs were consistently higher for 0–3 h than those for 3–24 h. The k_2 at 0–3 h tended to be large in the three rings (k_2 = 0.75 /d and 0.86 /d) and small (k_2 = 0.42 /d and 0.34 /d) in the 5–6 rings depending on the type of PAHs in red sea bream and mummichog, respectively (Table 3). In addition, there were significant negative relationships ($r = -0.82$, $p < 0.01$) between k_2 for 0–3h and log K_{OW} of PAHs in both the fish species (Fig. 5). It was, therefore, inferred that the PAHs with larger log K_{OW} and lower water solubility were easier to sorb on MP and less likely to desorb from MP in the digestive tract of marine fish species. These results were consistent with the study that reported that bioconcentration factors of one major low-ring PAH (phenanthrene) in fishes demonstrated a significant correlation with MP abundance, implying that they potentially participated in the selective bioaccumulation of PAHs (Li et al., 2023).

3.3. Experiment 3. Contribution of the uptake of PAHs desorbed from MPs in the digestive tract

The contributions of Phe, Pyr, and Chr derived from the MPs in the digestive tract were calculated to be 1.8, 24, and 14% and 3.1, 35, and 22% of the total concentration in fish derived from seawater and MPs in the digestive tracts of the red seabream and mummichog, respectively (Table 1). More than 95% of the three PAHs accumulated in fish were derived from seawater, with most of them observed to be Phe in both the fish species (Fig. 6). These results were strongly supported by the study which reported that zebrafish were exposed to PS MPs with sorbed B(a)P or a realistic mixture of oil PAHs from the water accommodated fraction of a naphthenic crude oil. However, the contaminated MPs did not cause PAH accumulation in zebrafish (Martínez-Álvarez et al., 2024). Qiu et al. (2020) reported that the amounts of Ant via waterborne pathways or those sorbed on the 50 μm PE-MPs account for approximately 67% and 33% of the Ant concentrations in medaka. The contribution of Ant derived from MP ingestion (33%) was much higher than the current results of Phe (1.8% for red seabream and 3.1% for mummichog) despite the similar log K_{OW} of Phe (4.6) and Ant (4.5). There are two possible reasons for this discrepancy. First is that the concentrations of PAH in water media used for the sorption to MP were 2 μg/L for the present study and 100 μg/L for Qiu et al. (2020), which may remarkably affect the PAH bioaccumulation in fish from seawater. The second reason is the difference in the MP size used for the experiment. Takai et al. (2023) demonstrated that the size of MPs is a critical determinant of their ability to serve as vectors for anthropogenic chemicals. The authors reported that the concentration of Ant in the muscle of Java medaka exposed to both Ant and 2-μm PS-MPs was higher as compared with those of any other two groups; both Ant and 10-μm PS-MPs or Ant alone. It should additionally be noted that the estimation in this study is based on the assumption that assimilation efficiency is the maximum value of 100% due to its uncertainty. Future research is necessary to clarify these issues (PAH concentration in the test media and MP size) and the uncertainties (assimilation efficiency of PAHs). Generally, assimilation efficiency is lower than 60%

for highly bioaccumulated contaminants, such as polychlorinated biphenyls and organotin compounds (Opperhuizen and Schrap, 1988; Ikeda and Yamada, 2003). Therefore, the actual concentration of PAHs taken up into the fish body derived from MP in the digestive tract was lower than the estimated value.

These estimates were finally compared to the carcinogenic levels of concern set by the U.S. Food and Drug Administration (FDA), especially for Phe (490 μg/g), Pyr (49 μg/g), and Chr (35 μg/g). Consequently, the concentration of PAHs in the fish body derived from MP was approximately 1/4.5 million to 1/1 million of the above values. It was, therefore, inferred that the risk as food was extremely low, even if the PAH concentration in seawater was relatively higher (2 μg/L), corresponding to that occurring in the aquatic environment (Anyakora et al., 2005; Maskaoui et al., 2002; Zhang et al., 2016; Shi et al., 2022) and if the assimilation efficiency of the PAHs was 100%.

4. Conclusion

Based on the results of the *in vitro* desorption test conducted in this study and the estimation using MP size, expected MP concentration in seawater, and PAH concentration in MP reported in an actual marine environment, it was presumed that there was an additional uptake of PAHs into the fish body (vector effect) due to desorption from MPs in the digestive tract when marine fish ingested MP that was sorbed and concentrated with PAHs. The degree of uptake was, however, much lower than that from seawater via gills. The PAHs with larger log K_{OW} and lower water solubility are easier to sorb onto MPs and less likely to desorb in the digestive tract of marine fish species. Collectively, the vector effect of PAHs by MPs in the actual sea may be negligible, and the risk is considered to be low at present. Our findings reveal that the degree of PAH uptake from MP ingestion was much lower than that from seawater via gills on the PAH bioaccumulation in fish. Future research is necessary to clarify the effects of PAH concentration in the test media and MP size, and the assimilation efficiency of PAHs.

Funding

This work was supported by a grant-in-aid from the Fisheries Agency of Japan.

CRediT authorship contribution statement

Kumiko Kono: Conceptualization, Methodology, Investigation, Formal analysis, Writing- Original draft preparation. **Mana Ito**: Investigation, Writing- Original draft preparation. **Takeshi Hano**: Investigation, Writing- Original draft preparation. **Nobuyuki Ohkubo**: Investigation, Writing- Original draft preparation.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

Acknowledgments

We are grateful to Dr. Kazuhiko Mochida (National Research Institute of Fisheries Technology) for his advice on academic English and Mrs. Miki Shoda, Yuko Makimoto, and Mika Momosaki (National Research Institute of Fisheries Technology) for their kind assistance.

References

Abouda, S., Galati, M., Oliveri Conti, G., Cappello, T., Abelouah, M.R., Romdhani, I., Ait Alla, A., Ferrante, M., Maisano, M., Banni, M., 2024. Metabolomic and biochemical disorders reveal the toxicity of environmental microplastics and benzo[a]pyrene in the marine polychaete Hediste diversicolor. J. Hazard. Mater. 477, 135404 https://doi.org/10.1016/j.jhazmat.2024.135404.

Abouda, S., Missawi, O., Cappello, T., Boughattas, I., De Marco, G., Maisano, M., Banni, M., 2022. Toxicological impact of environmental microplastics and benzo[a]pyrene in the seaworm Hediste diversicolor under environmentally relevant exposure conditions. Environ. Pollut. 310, 119856 https://doi.org/10.1016/j.envpol.2022.119856.

Allan, I.J., Booij, K., Paschke, A., Vrana, B., Mills, G.A., Greenwood, R., 2009. Field Performance of Seven Passive Sampling Devices for Monitoring of Hydrophobic Substances. Environ. Sci. Technol. 43, 5383–5390. https://doi.org/10.1021/es900608w.

Anyakora, C., Ogbeche, A., Palmer, P., Coker, H., 2005. Determination of polynuclear aromatic hydrocarbons in marine samples of Siokolo Fishing Settlement. J. Chromatogr. A 1073, 323–330. https://doi.org/10.1016/j.chroma.2004.10.014.

Arias, A.H., Alvarez, G., Pozo, K., Pribylova, P., Klanova, J., Rodríguez Pirani, L.S., Picone, A.L., Alvarez, M., Tombesi, N., 2023. Beached microplastics at the Bahia Blanca Estuary (Argentina): Plastic pellets as potential vectors of environmental pollution by POPs. Mar. Pollut. Bull. 187, 114520 https://doi.org/10.1016/j.marpolbul.2022.114520.

Bakir, A., O'Connor, I.A., Rowland, S.J., Hendriks, A.J., Thompson, R.C., 2016. Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environ. Pollut. 219, 56–65. https://doi.org/10.1016/j.envpol.2016.09.046.

Barhoumi, B., El Megdiche, Y., Clérandeau, C., Ameur, W.B., Mekni, S., Bouabdallah, S., Derouiche, A., Touil, S., Cachot, J., Driss, M.R., 2016. Occurrence of polycyclic aromatic hydrocarbons (PAHs) in mussel (*Mytilus galloprovincialis*) and eel (*Anguilla anguilla*) from Bizerte lagoon, Tunisia, and associated human health risk assessment. Cont. Shelf Res. 124, 104–116. https://doi.org/10.1016/j.csr.2016.05.012.

Baussant, T., Sanni, S., Skadsheim, A., Jonsson, G., Børseth, J.F., Gaudebert, B., 2001. Bioaccumulation of polycyclic aromatic compounds: 2. Modeling bioaccumulation in marine organisms chronically exposed to dispersed oil. Environ. Toxicol. Chem. 20, 1185–1195. https://doi.org/10.1002/etc.5620200607.

Campos, J.Md., Wintruff, L.T.T., Souza-Bastos, L.Rd., Dal Pont, G., Dolatto, R.G., Westphal, G.G.C., Grassi, M.T., Ostrensky, A., Sadauskas-Henrique, H., 2023. Osmoregulatory responses in the neotropical fish species Astyanax lacustris, exposed to single and combined microplastics, polycyclic aromatic hydrocarbons, and their mixture. Aquat. Toxicol. 263, 106693 https://doi.org/10.1016/j.aquatox.2023.106693.

Cheikyula, J.O., Koyama, J., Uno, S., 2008. Comparative study of bioconcentration and EROD activity induction in the Japanese flounder, red sea bream, and Java medaka exposed to polycyclic aromatic hydrocarbons. Environ. Toxicol. 23, 354–362. https://doi.org/10.1002/tox.20352.

Fred-Ahmadu, O.H., Bhagwat, G., Oluyoye, I., Benson, N.U., Ayejuyo, O.O., Palanisami, T., 2020. Interaction of chemical contaminants with microplastics: Principles and perspectives. Sci. Total Environ. 706, 135978 https://doi.org/10.1016/j.scitotenv.2019.135978.

Garcia-Garin, O., Vighi, M., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., Borrell, A., 2019. Boops boops as a bioindicator of microplastic pollution along the Spanish Catalan coast. Mar. Pollut. Bull. 149, 110648 https://doi.org/10.1016/j.marpolbul.2019.110648.

Gedik, K., Eryaşar, A.R., Emanet, M., Şahin, C., Ceylan, Y., 2023. Monthly microplastics change in European anchovy's (*Engraulis encrasicolus*) gastrointestinal tract in the Black Sea. Mar. Pollut. Bull. 194, 115303 https://doi.org/10.1016/j.marpolbul.2023.115303.

Gerhart, E.H., Carlson, R.M., 1978. Hepatic mixed-function oxidase activity in rainbow trout exposed to several polycyclic aromatic compounds. Environ. Res. 17, 284–295. https://doi.org/10.1016/0013-9351(78)90031-2.

Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*). Chemosphere 169, 233–238. https://doi.org/10.1016/j.chemosphere.2016.11.055.

Ikeda, K., Yamada, H., 2003. Changes in dietary bioaccumulation of tributyltin chloride (TBTCl) in red sea bream (*Pagrus major*) with the concentration in feed. Water Res. 37, 1497–1504. https://doi.org/10.1016/S0043-1354(02)00521-3.

Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10, 417 https://doi.org/10.1038/s41467-019-08316-9.

Ito, M., Hano, T., Kono, K., Ohkubo, N., 2022. Desorption of polycyclic aromatic hydrocarbons from polyethylene microplastics in two morphologically different digestive tracts of marine teleosts: Gastric red seabream (*Pagrus major*) and agastric mummichog (*Fundulus heteroclitus*). Environ. Pollut. 308, 119589 https://doi.org/10.1016/j.envpol.2022.119589.

Jaafar, N., Azfaralariff, A., Musa, S.M., Mohamed, M., Yusoff, A.H., Lazim, A.M., 2021. Occurrence, distribution and characteristics of microplastics in gastrointestinal tract and gills of commercial marine fish from Malaysia. Sci. Total Environ. 799, 149457 https://doi.org/10.1016/j.scitotenv.2021.149457.

Jovanović, B., Gökdağ, K., Güven, O., Emre, Y., Whitley, E.M., Kideys, A.E., 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Mar. Pollut. Bull. 130, 123–131. https://doi.org/10.1016/j.marpolbul.2018.03.016.

Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environ. Sci. Technol. 50, 3315–3326. https://doi.org/10.1021/acs.est.5b06069.

Koyama, J., Uno, S., Kohno, K., 2004. Polycyclic aromatic hydrocarbon contamination and recovery characteristics in some organisms after the Nakhodka oil spill. Mar. Pollut. Bull. 49, 1054–1061. https://doi.org/10.1016/j.marpolbul.2004.07.010.

Lee, H., Chang, S., Kim, S.-K., Kwon, J.-H., 2017. Fugacity analysis of polycyclic aromatic hydrocarbons between microplastics and seawater. Ocean Sci. J. 52, 43–55. https://doi.org/10.1007/s12601-017-0004-9.

Li, H., Wang, X., Mai, Y., Lai, Z., Zeng, Y., 2023. Potential of microplastics participate in selective bioaccumulation of low-ring polycyclic aromatic hydrocarbons depending on the biological habits of fishes. Sci. Total Environ. 858, 159939 https://doi.org/10.1016/j.scitotenv.2022.159939.

Liu, B., Gao, L., Ding, L., Lv, L., Yu, Y., 2023. Trophodynamics and bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in marine food web from Laizhou Bay, China. Mar. Pollut. Bull. 194, 115307 https://doi.org/10.1016/j.marpolbul.2023.115307.

Mai, L., Bao, L.-J., Shi, L., Liu, L.-Y., Zeng, E.Y., 2018. Polycyclic aromatic hydrocarbons affiliated with microplastics in surface waters of Bohai and Huanghai Seas, China. Environ. Pollut. 241, 834–840. https://doi.org/10.1016/j.envpol.2018.06.012.

Martínez-Álvarez, I., Le Menach, K., Cajaraville, M.P., Budzinski, H., Orbea, A., 2024. Effects of polystyrene nano- and microplastics and of microplastics with sorbed polycyclic aromatic hydrocarbons in adult zebrafish. Sci. Total Environ. 927, 172380 https://doi.org/10.1016/j.scitotenv.2024.172380.

Maskaoui, K., Zhou, J.L., Hong, H.S., Zhang, Z.L., 2002. Contamination by polycyclic aromatic hydrocarbons in the Jiulong River Estuary and Western Xiamen Sea, China. Environ. Pollut. 118, 109–122. https://doi.org/10.1016/S0269-7491(01)00208-1.

Nakakuni, M., Nishida, M., Nishibata, R., Kishimoto, K., Yamaguchi, H., Ichimi, K., Ishizuka, M., Suenaga, Y., Tada, K., 2024. Convergence zones of coastal waters as hotspots for floating microplastic accumulation. Mar. Pollut. Bull. 206, 116691 https://doi.org/10.1016/j.marpolbul.2024.116691.

OECD, 2000. Test No. 106: Adsorption – Desorption Using a Batch Equilibrium Method, OECD Guidelines for the Testing of Chemicals, Section 1. OECD Publishing, Paris, France.

OECD, 2004. OECD Environment Health and Safety Publications. Series on Testing and Assessment. Organization for Economic Co-Operation and Development, Paris, France. No. 46.

OECD, 2012. Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure, OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing, Paris, France.

OECD, 2019. Test No. 203: Fish, Acute Toxicity Testing, OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris, France.

Ohkubo, N., Ito, M., Hano, T., Kono, K., Mochida, K., 2020. Estimation of the uptake and gut retention of microplastics in juvenile marine fish: Mummichogs (*Fundulus heteroclitus*) and red seabreams (*Pagrus major*). Mar. Pollut. Bull. 160, 111630 https://doi.org/10.1016/j.marpolbul.2020.111630.

Ohkubo, N., Yoneda, M., Ito, M., Hano, T., Kono, K., 2022. Microplastic uptake and gut retention time in Japanese anchovy (*Engraulis japonicus*) under laboratory conditions. Mar. Pollut. Bull. 176, 113433 https://doi.org/10.1016/j.marpolbul.2022.113433.

Ohno, H., Iizuka, Y., 2023. Microplastics in snow from protected areas in Hokkaido, the northern island of Japan. Sci. Rep. 13, 9942 https://doi.org/10.1038/s41598-023-37049-5.

Opperhuizen, A., Schrap, S.M., 1988. Uptake efficiencies of two polychlorobiphenyls in fish after dietary exposure to five different concentrations. Chemosphere 17, 253–262. https://doi.org/10.1016/0045-6535(88)90218-4.

Parker, B.W., Beckingham, B.A., Ingram, B.C., Ballenger, J.C., Weinstein, J.E., Sancho, G., 2020. Microplastic and tire wear particle occurrence in fishes from an urban estuary: Influence of feeding characteristics on exposure risk. Mar. Pollut. Bull. 160, 111539 https://doi.org/10.1016/j.marpolbul.2020.111539.

Qiu, X., Saovany, S., Takai, Y., Akasaka, A., Inoue, Y., Yakata, N., Liu, Y., Waseda, M., Shimasaki, Y., Oshima, Y., 2020. Quantifying the vector effects of polyethylene microplastics on the accumulation of anthracene to Japanese medaka (*Oryzias latipes*). Aquat. Toxicol. 228, 105643 https://doi.org/10.1016/j.aquatox.2020.105643.

R Development Core Team, 2007. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna.

Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013. Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environ. Sci. Technol. 47, 1646–1654 https://doi.org/10.1021/es303700s.

Romdhani, I., De Marco, G., Cappello, T., Ibala, S., Zitouni, N., Boughattas, I., Banni, M., 2022. Impact of environmental microplastics alone and mixed with benzo[a]pyrene on cellular and molecular responses of *Mytilus galloprovincialis*. J. Hazard. Mater. 435, 128952 https://doi.org/10.1016/j.jhazmat.2022.128952.

Sequeira, I.F., Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2020. Worldwide contamination of fish with microplastics: A brief global overview. Mar. Pollut. Bull. 160, 111681 https://doi.org/10.1016/j.marpolbul.2020.111681.

Shi, W., Xu, M., Liu, Q., Xie, S., 2022. Polycyclic aromatic hydrocarbons in seawater, surface sediment, and marine organisms of Haizhou Bay in Yellow Sea, China: Distribution, source apportionment, and health risk assessment. Mar. Pollut. Bull. 174, 113280 https://doi.org/10.1016/j.marpolbul.2021.113280.

Shimizu, A., 1997. Reproductive cycles in a reared strain of the mummichog, a daily spawner. J. Fish Biol. 51, 724–737. https://doi.org/10.1111/j.1095-8649.1997.tb01994.x.

Takai, Y., Tominaga, A., Uchida, Y., Honda, M., Qiu, X., Shimasaki, Y., Oshima, Y., 2023. Size effect of polystyrene microplastics on the accumulation of anthracene for Java medaka (*Oryzias javanicus*). Chemosphere 338, 139543 https://doi.org/10.1016/j.chemosphere.2023.139543.

Tanaka, K., Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci. Rep. 6, 34351 https://doi.org/10.1038/srep34351.

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for Plastics to Transport Hydrophobic Contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s.

Tong, Y., Chen, L., Liu, Y., Wang, Y., Tian, S., 2019. Distribution, sources and ecological risk assessment of PAHs in surface seawater from coastal Bohai Bay, China. Mar. Pollut. Bull. 142, 520–524. https://doi.org/10.1016/j.marpolbul.2019.04.004.

Valencia-Castañeda, G., Medina-López, J.A., Frías-Espericueta, M.G., Páez-Osuna, F., 2024. Farmed stage (age)-dependent accumulation and size of microplastics in Litopenaeus vannamei shrimp reared in a super-intensive controlled system. Sci. Total Environ. 918, 170575 https://doi.org/10.1016/j.scitotenv.2024.170575.

Wallace, S.J., de Solla, S.R., Head, J.A., Hodson, P.V., Parrott, J.L., Thomas, P.J., Berthiaume, A., Langlois, V.S., 2020. Polycyclic aromatic compounds (PACs) in the Canadian environment: Exposure and effects on wildlife. Environ. Pollut. 265, 114863 https://doi.org/10.1016/j.envpol.2020.114863.

Wang, S., Hu, M., Zheng, J., Huang, W., Shang, Y., Kar-Hei Fang, J., Shi, H., Wang, Y., 2021. Ingestion of nano/micro plastic particles by the mussel *Mytilus coruscus* is size dependent. Chemosphere 263, 127957 https://doi.org/10.1016/j.chemosphere.2020.127957.

WHO, 1998. Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria 202. World Health Organization, Geneva.

Wu, J., Wang, Z., Zhang, Y., Tian, J., Song, L., Han, J., Yu, J., Zhang, Y., 2022. Spatial distribution and ecological risks of polycyclic aromatic hydrocarbons in sea ice and seawater from northern Liaodong Bay, China. Mar. Pollut. Bull. 174, 113319 https://doi.org/10.1016/j.marpolbul.2022.113319.

Yeo, B.G., Takada, H., Yamashita, R., Okazaki, Y., Uchida, K., Tokai, T., Tanaka, K., Trenholm, N., 2020. PCBs and PBDEs in microplastic particles and zooplankton in open water in the Pacific Ocean and around the coast of Japan. Mar. Pollut. Bull. 151, 110806 https://doi.org/10.1016/j.marpolbul.2019.110806.

Zhang, A., Zhao, S., Wang, L., Yang, X., Zhao, Q., Fan, J., Yuan, X., 2016. Polycyclic aromatic hydrocarbons (PAHs) in seawater and sediments from the northern Liaodong Bay, China. Mar. Pollut. Bull. 113, 592–599. https://doi.org/10.1016/j.marpolbul.2016.09.005.

Zhao, W., Li, J., Liu, M., Wang, R., Zhang, B., Meng, X.-Z., Zhang, S., 2024. Seasonal variations of microplastics in surface water and sediment in an inland river drinking water source in southern China. Sci. Total Environ. 908, 168241 https://doi.org/10.1016/j.scitotenv.2023.168241.

Zitouni, N., Cappello, T., Missawi, O., Boughattas, I., De Marco, G., Belbekhouche, S., Mokni, M., Alphonse, V., Guerbej, H., Bousserrhine, N., Banni, M., 2022. Metabolomic disorders unveil hepatotoxicity of environmental microplastics in wild fish *Serranus scriba* (Linnaeus 1758). Sci. Total Environ. 838, 155872 https://doi.org/10.1016/j.scitotenv.2022.155872.

Figure captions

Fig.1. Description of the present study

Fig. 2. The PAH content on MP at the end of the sorption experiment (PE(a) PS(b) (the legend presents the concentration of the test solution)

Fig. 3. Relationship between log K_{OW} (a) or water solubility (b) and PAH content on MP at the end of the sorption experiment using the test solution of 2 μg/L (PE ● PS ○). Log K_{OW} and water solubility values are from WHO (1998)

Fig. 4. Percentage of the desorbed PAH in the test solution containing the gut contents extract when the desorption amount is 100% at 24 h (the legend presents the number of aroma rings in PAH).

Fig. 5. Relationship between log K_{OW} of PAHs and desorption rate constant at 0–3 h, a) red seabream and b) mummichog. Statistical significance was based on Pearson's correlation coefficient (r) at $p <$ 0.01. Log K_{OW} values are from WHO (1998)

Fig. 6. The estimated uptake ratio of PAHs from seawater and MP which was ingested and desorbed in the digestive tract of the a) red sea bream and b) mummichog. (SW: seawater, Phe: phenanthrene, Pyr: pyrene, Chr: chrysene)

Fig. S1. Relationship between log K_{OW} and adsorption coefficient (a) of PAH (PE ● PS ○)

Table 1. Parameters and formulas used for the estimation of PAH concentrations in fish.

			Red sea bream			Mummichog			Reference
		Calculation formula	Phe	Pyr	Chr	Phe	Pyr	Chr	
	MP concentration in seawater (mg/L)	a	1						Isobe et al., 2019
	MP uptake ratio in fish (%)	b	0.010			0.019			Ohkubo et al., 2020
	PAH desorption time in digestive tract (h)	c	3			3			Ohkubo et al., 2020, This study
	PAH content on MP (ng/g MP)	d	15000	5000	2500	15000	5000	2500	Mai et al., 2018
Uptake from seawater	PAH desorption ratio from MP to seawater (%)	e	42	26	38	42	26	38	This study
	PAH concentration in seawater (ng/L)	f=a*d*(e/100))/1000	6.3	1.3	0.95	6.3	1.3	0.95	
	BCF	g	940	72	54	940	72	54	Gerhart & Carlson, 1978, Baussant et al., 2001
	PAH concentration in fish (ng/g)	h=f*g/1000	5.9	0.094	0.051	5.9	0.094	0.051	
Uptake from MP ingestion	PAH residue content on MP (ng/g MP)	i=d*(1-(e/100))	8700	3700	1600	8700	3700	1600	
	PAH desorption rate constant of MP (k_2) (/d)	j	1.1	0.66	0.42	1.0	0.59	0.37	This study
	PAH content desorbed from MP (ng/g MP)	k=h*(1-exp(-j*c/24)	1100	290	82	1000	260	72	
	PAH concentration in fish (ng/g)	l=k*b/100	0.11	0.029	0.008	0.19	0.050	0.014	
	Contribution of MP ingestion to total uptake (%)	l*100/(h+l)	1.8	24	14	3.1	35	22	

Table 2. The sorption rate constant (k) of PAHs to MP from the test solution and the time required to reach the 80% steady state (t_{80}).

PAH	Abbreviation	Ring	k (/h)	t_{80} (h)
Naphthalene	Nap	2	0.29	5.5
Acenaphthylene	Acel	3	0.49	3.3
Acenaphthene	Ace		0.65	2.4
Fluorene	Flu		0.43	3.7
Phenanthrene	Phe		0.84	1.9
Anthracene	Ant		0.55	2.9
Fluoranthene	Flut	4	0.52	3.1
Pyrene	Pyr		0.41	3.9
1,2benzanthracene	B(a)A		0.14	11
Chrysene	Chr		0.12	13
1,2benzo(b)fluoranthene	B(b)F	5	0.07	25
1.2benzo(k)fluoranthene	B(k)F		0.07	23
Benzo(a)pyrene	B(a)P		0.04	44
Dibenz(a,h)anthracene	D(ah)A		0.11	14
Indeno(1,2,3-cd)pyrene	Inden	6	0.04	36
Benzo(g,h,i)perylene	B(ghi)P		0.10	16

Table 3. The desorption rate constant of PAHs from MP in the fish digestive tract at 0–3 h and 3–24 h, respectively.

		Red seabream		Mummichog	
No. of rings	Chemicals	0-3 hr	3-24 hr	0-3 hr	3-24 hr
2 rings	Naphthalene	-	-	-	-
3 rings	Acenaphthylene	1.2 ± 0.14	0.027 ± 0.035	2.0 ± 0.46	0.080 ± 0.073
	Acenaphthene	1.1 ± 0.15	0.025 ± 0.032	1.3 ± 0.30	0.050 ± 0.039
	Fluorene	1.4 ± 0.21	0.025 ± 0.034	1.4 ± 0.31	0.044 ± 0.035
	Phenanthrene	1.1 ± 0.19	0.029 ± 0.029	1.0 ± 0.18	0.039 ± 0.014
	Anthracene	1.2 ± 0.28	0.015 ± 0.028	0.87 ± 0.18	0.032 ± 0.014
4 rings	Fluoranthene	0.77 ± 0.18	0.031 ± 0.027	0.63 ± 0.11	0.033 ± 0.010
	Pyrene	0.66 ± 0.15	0.029 ± 0.026	0.59 ± 0.10	0.032 ± 0.010
	1,2benzanthracene	0.58 ± 0.17	0.014 ± 0.020	0.48 ± 0.085	0.017 ± 0.0087
	Chrysene	0.42 ± 0.12	0.011 ± 0.016	0.37 ± 0.071	0.011 ± 0.0067
5 rings	1,2benzo(b)fluoranthene	0.47 ± 0.12	0.012 ± 0.021	0.43 ± 0.063	0.0090 ± 0.0058
	1.2benzo(k)fluoranthene	0.40 ± 0.13	0.0051 ± 0.014	0.33 ± 0.064	0.0065 ± 0.0051
	Benzo(a)pyrene	0.29 ± 0.079	0.0048 ± 0.010	0.35 ± 0.064	0.0063 ± 0.0044
	Dibenz(a,h)anthracene	0.37 ± 0.11	0.010 ± 0.022	0.22 ± 0.029	0.0053 ± 0.0039
6 rings	Indeno(1,2,3-cd)pyrene	0.38 ± 0.10	0.0042 ± 0.018	0.31 ± 0.045	0.0065 ± 0.0069
	Benzo(g,h,i)perylene	0.66 ± 0.13	0.018 ± 0.029	0.39 ± 0.054	0.012 ± 0.0080

Table S1 Internal standard PAHs and analytical parameters determined using gas chromatography.

	Internal standard PAHs	RT[a]	SIM ion	Native PAHs	RT	SIM ion
2 rings	Naphthalene-D8	6.993	136	Naphthalene	7.022	128
3 rings	Acenaphthylene-D8	10.634	160	Acenaphthylene	10.672	152
	Acenaphthene-D10	11.213	164	Acenaphthene	11.32	154
	Fluorene-D10	13.223	176	Fluorene	13.345	166
	Phenanthrene-D10	17.767	188	Phenanthrene	17.858	178
	Anthracene-D10	17.97	188	Anthracene	18.041	178
4 rings	Fluoranthene-D10	21.95	212	Fluoranthene	21.997	202
	Pyrene-D10	22.566	212	Pyrene	22.611	202
	1,2-Benzanthracene-D12	25.823	240	1,2-Benzanthracene	25.874	228
	Chrysene-D12	25.912	240	Chrysene	25.97	228
5 rings	1,2-Benzo(b)fluoranthene-D12	28.335	264	1,2-Benzo(b)fluoranthene	28.384	252
	1,2-Benzo(k)fluoranthene-D12	28.395	264	1,2-Benzo(k)fluoranthene	28.435	252
	Benzo(a)pyrene-D12	28.993	264	Benzo(a)pyrene	29.038	252
	Dibenz(a,h)anthracene-D14	31.695	292	Dibenz(a,h)anthracene	31.779	278
6 rings	Indeno(1,2,3-cd)pyrene-D12	31.626	288	Indeno(1,2,3-cd)pyrene	31.687	276
	Benzo(g,h,i)perylene-D12	32.316	288	Benzo(g,h,i)perylene	32.39	276

[a] RT, retention time

Table S2 Quantification limit of PAHs in MP (ng/g) and red seabream and mummichog (ng/ml).

	MP	**Red seabream**	**Mummichog**
Naphthalene	0.19	1.05	0.21
Acenaphthylene	0.01	0.07	0.09
Acenaphthene	0.01	0.56	0.53
Fluorene	0.24	0.13	0.09
Phenanthrene	0.26	0.72	0.13
Anthracene	0.14	0.18	0.09
Fluoranthene	0.13	0.77	0.13
Pyrene	0.27	0.43	0.14
1,2benzanthracene	0.32	0.09	0.10
Chrysene	0.27	0.46	0.07
1,2benzo(b)fluoranthene	1.00	0.38	0.72
1.2benzo(k)fluoranthene	0.35	0.32	0.18
Benzo(a)pyrene	0.40	0.11	0.18
Dibenz(a,h)anthracene	1.05	0.27	0.10
Indeno(1,2,3-cd)pyrene	0.63	0.24	0.29
Benzo(g,h,i)perylene	0.51	0.10	0.16