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Abundance and size of microplastics in a coastal sea: Comparison among bottom sediment, beach sediment, and surface water



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ABSTRACT

Microplastics have adverse effects on marine life. This study examined the abundance and size of microplastics as well as their polymer types in the surface water and the bottom and beach sediments of Hiroshima Bay. The fragmentation process and sinking factors of foamed polystyrene (FPS) microplastics were also examined. Serious FPS pollution spread out not only in the beach sediments but also in the bottom sediments. The average size of FPS particles in the bottom sediments was significantly smaller than that of beached FPS particles. Field emission scanning electron microscopy images suggest that large amounts of microsized or nanosized FPS fragments are likely to be generated from the margins of beached FPS microplastics. X-ray computed tomography images show that FPS microplastics from the bottom sediments had tunnel-like structures inside the particle. Based on these images, FPS microplastics in the bottom sediments were susceptible to biofouling and soil deposition.

1. Introduction

Annual plastics production has increased, reaching 322 million tons in 2015 (Plastics Europe, 2016). Some plastics slip through refuse dumps and enter the ocean. According to Jambeck et al. (2015), the mass of land-based plastic waste entering the ocean in 2010 is estimated at 4.8-12.7 million metric tons. Plastic waste entering the ocean will drift on the sea surface, wash ashore or sink to the sea bottom. In this process, plastic waste, especially on beaches, is exposed to UV radiation and fragmented into small pieces (Andrady, 2011). Fragments of 0.3-5 mm in size are called microplastics and their impact on the marine environment including the ecosystem has become a major concern. For example, microplastics absorb toxic compounds while drifting in the sea and then they are ingested by marine organisms (Hirai et al., 2011). Besseling et al. (2013) examined the effects of plastics on benthic organisms including the transfer of persistent organic pollutants (POPs), reporting a positive relationship between microplastic concentration in the sediment and both uptake of plastic particles and weight loss by the lugworm Arenicola marina. They also observed a reduction in feeding activity at a polystyrene (PS) dose of 7.4% dry weight. Lu et al. (2016) investigated the toxic effects of PS microplastics on the liver of zebrafish (Danio rerio) and reported a significant increase in the activity of superoxide dismutase and catalase, indicating induced oxidative stress.

Microplastic ingestion has been demonstrated in several marine

organisms. Desforges et al. (2015) found the first evidence of microplastic ingestion by marine zooplankton in the Northeast Pacific Ocean and estimated that the consumption of microplastic-containing zooplankton will lead to the ingestion of 2–7 microplastic particles/day by individual juvenile salmon in coastal British Columbia. Neves et al. (2015) examined the digestive tract contents of 263 individuals from 26 species of commercial fish for the presence of microplastics. They found microplastics in 17 species, corresponding to 19.8% of the fish of which 32.7% had ingested more than one fiber or microplastic particle. Considering the more abundant ecosystem in coastal seas than in the open ocean, microplastics are more likely to enter the ecosystem in coastal seas than in the open ocean. To determine the extent of microplastic pollution in the coastal marine environment, it is necessary to understand the distribution of microplastics in the reservoirs of the coastal marine system, namely, the sea surface, water column, sea bottom, beaches and marine ecosystem. In addition, microplastic fluxes among the reservoirs should be estimated to assess and predict the adverse effects of microplastics on the ecosystem (Hardesty et al., 2017).

Understanding the abundance and size of microplastics in a coastal sea is a key factor in determining the spatial distribution and fluxes, because their behaviors in coastal seas will depend on their sizes. Isobe et al. (2014) investigated the quantity and size of microplastics and mesoplastics (approximately > 5 mm) in the surface water in a coastal sea by means of field surveys and a numerical particle-tracking model to interpret the size-dependent distribution of plastics and the possible

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transport process in the surface water. Recently, Hinata et al. (2017) revealed the size dependence of the residence times of beached plastics due to the size-dependent responses to turbulence generated by swash waves on the beach. Although the abundance of microplastics in the reservoirs of the coastal marine system has been investigated worldwide (e.g., Claessens et al., 2011; Lee et al., 2013; Vianello et al., 2013; Matsuguma et al., 2017; Tsang et al., 2017), these studies examined microplastic concentrations in only one or two reservoirs of the coastal marine system. In addition, the size of microplastics and its comparison among reservoirs have not been discussed in previous studies. Microplastic distribution in the reservoirs and microplastic fluxes between the reservoirs are still unknown.

This study examined the abundance and size of microplastics as well as their polymer types in the surface water and the bottom and beach sediments of Hiroshima Bay to infer the behaviors of microplastics in the region. Moreover, this study aimed to examine the shape characteristics, surface states and internal structures of FPS microplastics in the beach and bottom sediments by using digital microscopy, field emission scanning electron microscopy (FE-SEM) and X-ray computed tomography (X-ray CT) to deduce the sinking and fragmentation process of FPS microplastics. This study is the first step toward a comprehensive understanding of the behaviors of microplastics including the fragmentation process in the region.

2. Materials and methods

2.1. Study site

Hiroshima Prefecture is the leading center of oyster aquaculture in Japan by production quantity (Ministry of Agriculture, Forestry and Fisheries, 2016). There are 11,284 oyster aquaculture rafts in Hiroshima Bay (Fig. 1), covering about 2.2 km2 (Ministry of Agriculture, Forestry and Fisheries, 2016). Foamed polystyrene (FPS) is often used for floats to keep the oyster aquaculture facilities on the sea surface. The specific gravity of FPS is 0.01-0.02 due to the inclusion of pores, which is much less than that of seawater. In the marine environment, FPS is likely to be transported by surface residual currents, Stokes drift and winds. The surface residual current in the study region flows southward from the head of Hiroshima Bay toward Suo-Oshima Island (Lee et al., 2001), and the bottom residual current returns to the bay head (Yanagi, 2008) mainly due to the inflow of freshwater from the mouth of the Ohta River (see Fig. 1). FPS floats without covers (i.e. protective surfaces) are directly exposed to the environment and easily break down into small spherules (Lee et al., 2013). Microplastics originating from FPS floats have been spread throughout the beaches of the bay by residual currents, Stokes drift and winds, causing serious pollution on the beaches (Fig. 2; Fujieda and Sasaki, 2005).

Fig. 1 shows an overview of the sampling sites. Sampling was performed at a total of 16 sites; beach and bottom sediments were collected at six sites, and surface water was collected at four sites. Considering the structure of the residual currents, the sampling sites were located from the upstream (bay head) to downstream (off the north coast of Suo-Oshima Island) of the surface residual current. The water depth at the sites ranged from 18 to 35 m. Straight beaches pointing in various directions were selected as sampling sites. Sampling of beach sediments and surface water was conducted in August and September 2016. Bottom sediment sampling was conducted in January and April 2017.

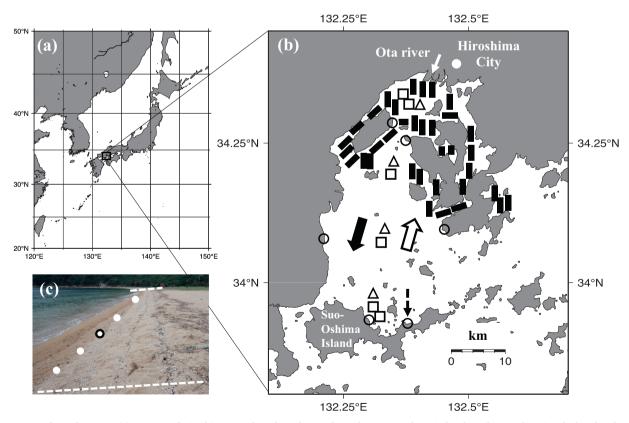


Fig. 1. Overview of sampling sites. (a) Location of Hiroshima Bay. (b) Enlarged map of Hiroshima Bay. White circles show the sampling sites for beach sediments and white triangles show the sampling sites for surface water. White squares show the sampling sites for bottom sediments. At the head of Hiroshima Bay, black rectangles show the positions of oyster aquaculture facilities, but the number or size of the rectangles may be different from that of the actual oyster aquaculture facilities. The black arrow and the white arrow show the direction of residual currents in the surface layer and bottom layer, respectively. The broken arrow represents the location of the beach in Fig. 2. (c) Sample collection on beaches; ends of the beach (dotted lines), center of the beach (outlined circle) and five sampling points per beach (outlined circle and white circles).





Fig. 2. Photos of FPS pollution on a beach and stacked oyster aquaculture rafts. (a) A beach on Suo-Oshima Island (see Fig. 1). In the photo, almost all white sediments were FPS particles. (b) Stacked FPS floats on a beach at the head of Hiroshima Bay.

2.2. Sample collection

At the six beach sites, beach sediments were collected at the high tide line of the observation day, which was determined by leveling. Then both alongshore ends of the beach were determined and the center of the beach was measured. Next, both the right side and left side of the beach were divided into three equal parts in the alongshore direction. In this way, five sampling points per beach were determined (Fig. 1c). A quadrate method proposed by Ogi and Fukumoto (2000) was applied. By using a quadrate of $40 \times 40 \times 2$ cm, beach sediments within 1 cm of the surface layer (about 1.6 L in volume) were collected at the five sampling points per beach. Beach sediment samples were placed in sealable bags and transported to our laboratory.

At the four surface water sites, samples were collected once per site by towing a neuston net (5552; Rigo Co., Ltd., Tokyo, Japan) for $10{\text -}15\,\text{min}$ at a speed of $2{\text -}3\,\text{kn}$. The net mouth was $75\times75\,\text{cm}$, with a length of $300\,\text{cm}$ and mesh size of $350\,\mu\text{m}$. A flow meter (5571A; Rigo Co., Ltd.) was equipped at the mouth of the net to measure the water volume passing through the net during the sampling. For sampling, particular attention was paid to two points: adjusting the positions of the buoys attached to the mouth frame of the net and the speed of the vessel to keep the height of the mouth relative to the sea surface; and setting up the net on the side of the vessel to avoid the effects from stirring of the surface water by the propeller of the vessel. Samples were transferred to $2\,\text{L}$ bottles (wide-mouth T-type AS ONE bottles) and transported to our laboratory.

At the six sites for bottom sediment sampling, samples (about 4.2 L) were collected from the 0–11 cm layer once per site by using a Smith-McIntyre bottom sediment sampler (Rigo Co., Ltd.). Samples were placed in metal buckets and transported to our laboratory.

2.3. Extraction of microplastics

The 1.6 L of beach sediment collected per sampling point was reduced to 100 ml by a quartering method (e.g., Mediavilla et al., 2009), in which a sample was fully mixed, divided into quarters, and two opposite quarters of the sample were kept for mixing. Precise details of the method are described in Appendix A.1. Next, microplastics were extracted from the 100 ml of beach sediment. First, 200 ml of a saline

solution of 1.14 specific gravity was added to the $100\,\mathrm{ml}$ of beach sediment and stirred for $30\,\mathrm{s}$. The beach sediment was then left to settle for 2 min before the supernatant was poured through a $355\,\mu\mathrm{m}$ mesh sieve (Tokyo Screen Co., Ltd.). Residue on the mesh sieve was washed with distilled water and suction filtration (Glass Microfiber Filters GF/A; Whatman) was conducted. For each beach sediment sample, this extraction process was performed three times to minimize the remainders. Precise details of the extraction are described in Appendix A.2.

Surface water samples were poured through $5.6\,\mathrm{mm}$ and $355\,\mathrm{\mu m}$ mesh sieves (Tokyo Screen Co., Ltd.). Residue on the $355\,\mathrm{\mu m}$ mesh sieve was washed with distilled water and suction filtration was conducted.

The bottom sediments were sieved with a 5.6 mm and a 355 µm mesh sieve using tap water from our laboratory. The tap water included no microplastics (except fibers) from the sieving tests in which 10-13 L of water was sieved with a 90 µm mesh sieve (Tokyo Screen Co., Ltd.) once in the morning and once in the afternoon on weekdays. Detected fibers would have been from our clothes or the air. In this study, fibers were excluded from the analysis because it was difficult to prevent sample contamination from the air during the laboratory processing. After that, microplastics were extracted from the reduced-volume samples by using a similar method to that for the beach sediment samples. First, 200 ml of a sodium solution of 1.7 specific gravity was added to the reduced-volume sample and then stirred for 2 min. The sample was then left to stand for 1 h before the supernatant was poured through the 355 µm mesh sieve. Residue on the mesh sieve was washed with distilled water and suction filtration was conducted. For each reduced-volume sample, this extraction process was performed five times to minimize the remainders. In addition, all residual sediments were checked with the naked eye after the fifth extraction. One 2.367 mm polyethylene (PE) fragment was found in the residual sediments. Thus, almost all the microplastics were extracted from the bottom sediment samples by performing the five-time extraction procedure.

Microplastic concentrations were expressed as the number of microplastics per kg of dry sediment (pieces/kg dry sediment) and the number of microplastics per unit sampling area (pieces/m²).

2.4. Measurement of microplastics

After extraction from all samples, microplastics and suspicious objects were collected by visual observation and with a stereoscopic microscope (S9 D; Leica Microsystems, Tokyo, Japan). The size of these microplastics was measured by the longest length of each irregularly shaped fragment (Isobe et al., 2014) using a USB camera (MC170 HD and Application Suite V4; Leica Microsystems). Microplastic particles were categorized into different polymer types: PE (specific gravity 0.91–0.97), polypropylene (PP; 0.90–0.91), FPS (0.01–0.02), PS (1.04–1.09; FPS is made from PS by inflating) and other polymer types. Other polymer types include polyamide (PA), ethylene-vinyl acetate (EVA), polyacrylates (PAK), polyvinyl chloride (PVC), polycaprolactone (PCL) and polymethyl methacrylate (PMMA). Particles of each type were identified and non-plastic particles were excluded by using Fourier-transform infrared spectroscopy (FTIR; Model ALPHA T; Bruker Optics, Tokyo, Japan).

2.5. Digital microscopy, FE-SEM and X-ray CT

Digital microscopy (DVM6; Leica Microsystems, Tokyo, Japan) and LAS X Life Science software (Leica Microsystems) were used to obtain 3D images of microplastics from the beach and bottom sediment samples. FPS microplastics in the bottom sediments had complex shapes and the beached FPS microplastics were even thicker due to the inclusion of pores, but digital microscopy provided clear images (see Fig. 5). FE-SEM (JSM-7500F; Jeol Ltd., Tokyo, Japan), which can observe samples at higher magnifications than generic scanning electron microscopy (SEM), was used to examine the surface states of the microplastics.

The pore structure of FPS microplastics was examined by X-ray CT using a microscopic CT scanner (TDM1000; Yamato Scientific, Tokyo, Japan). The analysis was conducted to develop a better understanding of the porous network in the microplastics from the beach and bottom sediments. The imaging conditions for the samples included X-ray energy of 90 kV and 1200 projections. A 16-bit image comprising 2¹⁶ levels of brightness ranging from 0 (black) to 65,535 (white) was used. The threshold value in determining the pore/solid fractions was consistently set as 16,508 for all the samples tested, which is an important factor for visualizing the porous structure based on the gray scale value.

Two FPS microplastic particles were selected from the beach and bottom sediment samples with representative shapes and sizes (4.137 and 1.774 mm, respectively), and their images were obtained by digital microscopy, FE-SEM and X-ray CT.

3. Results and discussion

3.1. Relative abundances of polymer types

In the beach sediments, FPS was overwhelmingly numerically dominant (90%), followed by PE (5%), PP (3%), PS (0%) and others (2%; Fig. 3). FPS sank to the sea bottom in Hiroshima Bay, where it was still numerically dominant (79%), followed by PE (13%), PP (6%), PS (1%) and others (2%; Fig. 3). Tsang et al. (2017) measured microplastics in the surface water and bottom sediments of Hong Kong and 'PE and PP' (total of high-density polyethylene and low-density polyethylene) accounted for 95.5%. Vianello et al. (2013) measured microplastic concentrations in the bottom sediments of the Lagoon of Venice and found that all samples contained PE and PP, which accounted for > 80% (Fig. 3). Along the Belgian coast, Claessens et al. (2011) reported that no FPS was found in the beach and bottom sediments. In the canal in Tokyo Bay, Matsuguma et al. (2017) detected microplastics primarily composed of heavy polymers (others 73%; Fig. 3). In most studies, FPS was numerically less dominant than other polymers including PE and PP. In contrast, Lee et al. (2013) reported the dominance of FPS (96-99%; Fig. 3), similar to this study, on

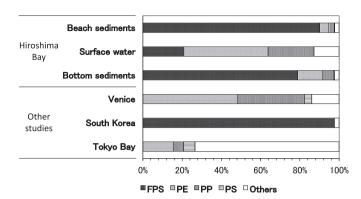


Fig. 3. Ratio of polymer types in the present study and other studies; FPS (foamed polystyrene), PE (polyethylene), PP (polypropylene), PS (polystyrene) and others, which includes PA (polyamide), EVA (ethylene-vinyl acetate), PAK (polyacrylates), PVC (polyvinyl chloride), PCL (polycaprolactone) and PMMA (polymethyl methacrylate) in this study, and includes polymers except for FPS, PE, PP and PS in the other studies. References: In Venice (Italy), Vianello et al. (2013) examined microplastics from bottom sediments, and in South Korea, Lee et al. (2013) examined microplastics from beach sediments. Microplastics from the bottom sediments in Tokyo Bay (Japan) were examined by Matsuguma et al. (2017).

beaches in South Korea near oyster aquaculture facilities. In areas where aquaculture is widespread, FPS pollution is likely to be serious and FPS microplastic pollution should be considered. In these regions, identifiable sources of FPS would allow us to elucidate the traffic channels of FPS including the fragmentation process.

Interestingly, microplastics in the surface water of Hiroshima Bay formed a different ratio of polymers from the beach and bottom sediment samples; PE was numerically dominant (43%), followed by PP (23%), FPS (21%), PS (0%) and others (13%; Fig. 3). This would be caused by differences in their specific gravities. Isobe et al. (2014) used a numerical model to explain the selective onshore transport of small plastic fragments drifting in coastal waters. They found that relatively large mesoplastics that have greater buoyancy are likely to be carried onshore faster than microplastics by Stokes drift. The specific gravity of FPS is much smaller than that of PE and PP, so FPS is likely to be washed ashore immediately by Stokes drift, surface residual currents and winds.

Hinata et al. (2017) examined the dependence of macroplastics and mesoplastics beach residence times on their upward terminal velocities and estimated the residence times of microplastics from the dependency by conducting mark-recapture experiments. The results revealed that plastics with larger upward terminal velocities are likely to have longer residence times on the beach. The much smaller specific gravity of FPS causes much larger upward terminal velocities than those of PE and PP, so FPS particles have much longer residence times on the beaches in the study region than PE and PP. Based on the above, FPS microplastics have much shorter residence times on the sea surface and much longer residence times on the beaches, resulting in the overwhelming dominance of FPS on the beaches and PE/PP dominance on the sea surface.

3.2. Abundances of microplastics in sediments and surface water

Minimum and maximum values of microplastic concentrations in Hiroshima Bay and their comparison with other studies are shown in Table 1. Two types of microplastic concentrations (pieces/kg dry sediment and pieces/m²) were calculated for this comparison. FPS and PE, PP are separately shown in Table 1 because of their different behaviors in the marine environment mainly due to their difference in specific gravities (3.1).

In Hiroshima Bay, FPS microplastic concentrations in the bottom sediments were 552–9128 pieces/m² and they were four to six orders of magnitude larger than in the surface water (0.004–0.06 pieces/m²)

Table 1

Microplastic concentrations (min. to max.) in the beach sediments, surface water and bottom sediments in Hiroshima Bay and their comparison with other studies. Microplastic concentrations are expressed as the number of microplastics per kg dry sediment (pieces/kg dry sediment) and the number of microplastics per sampling area (pieces/m²). In Hiroshima Bay, the number of microplastics per sampling area (pieces/m²) in the beach and bottom sediments shows microplastic concentrations in the surface layer of 0–1 cm and 0–11 cm, respectively. Sampling areas of the surface water were estimated by dividing the water volume passing through the net by the height of the water at the mouth of the net (three-quarters of the mouth of net; 0.56 m). In Belgium, PE, PP, PS, nylon and polyvinyl alcohol were found in sediments, but FPS was not found. In Tokyo Bay, PE and PP microplastic concentrations in the core (0–30 cm) were found by Matsuguma et al. (2017). In South Korea, FPS microplastics in beach sediments were estimated from the total microplastic concentrations and the ratio of FPS (96–99% of the total). Lee et al. (2013) also showed the microplastic concentrations as the average of ten quadrats (depth of 0–5 cm) at each beach.

			Defined size of microplastics (mm)	Beach sediments	Surface water	Bottom sediments	Reference
pieces/kg dry sediment	Hiroshima Bay, Japan	Total	0.3 to < 5	5 to 1245	_	24 to 253	Present study
		FPS	0.3 to < 5	5 to 1206	_	13 to 221	Present study
		PE, PP	0.3 to < 5	0 to 200	_	5 to 29	Present study
	Venice, Italy	Total	< 1	_	_	672 to 2175	Vianello et al., 2013
		PE, PP	< 1	_	_	579 to 1780	Vianello et al., 2013
	Belgium	Total	< 1	49 to 156	_	67 to 391	Claessens et al., 2011
	Tokyo Bay, Japan	Total	< 5	_	_	1845 to 5385	Matsuguma et al., 2017
		PE, PP	< 5	-	_	582 to 1196	Matsuguma et al., 2017
pieces/m ²	Hiroshima Bay, Japan	Total	0.3 to < 5	80 to 18,060	0.03 to 0.24	1000 to 10,444	Present study
_		FPS	0.3 to < 5	80 to 17,500	0.004 to 0.06	552 to 9128	Present study
		PE, PP	0.3 to < 5	0 to 1640	0.03 to 0.17	210 to 1210	Present study
	Tokyo Bay, Japan	PE, PP	< 5	-	0.7 to 3.2	20,000 to 60,000	Matsuguma et al., 2017
	Beaches, South Korea	Total	1 to < 5	1.6 to 92,217	_	_	Lee et al., 2013
		FPS	1 to < 5	1.6 to 88,528	-	-	Lee et al., 2013

(Table 1). This study is the first report of the dominance of FPS microplastics in bottom sediments. The maximum value of microplastic concentrations in the beach sediments (17,500 pieces/m²) was 10 times larger than that in the bottom sediments (Table 1). The relative abundances of polymer types (3.1) and the numerical densities demonstrate that serious pollution by FPS microplastics spreads out not only in the beach sediments as reported by Fujieda and Sasaki (2005) but also in the bottom sediments in Hiroshima Bay. In South Korea, FPS microplastic concentrations in the beach sediments (0–5 cm) were 1.6–88,528 (pieces/m²) (Table 1). The order of maximum beached FPS microplastic concentrations (0–1 cm) in South Korea was similar to that of the beaches in Hiroshima Bay. The even smaller difference in abundances (one order of magnitude) between the beach and bottom sediments in Hiroshima Bay suggests that FPS microplastics would also be abundant in the bottom sediments in South Korea.

For PE and PP particles, microplastic concentrations were $0{\text -}1640\,\text{pieces/m}^2$ in the beach sediments, $210{\text -}1210\,\text{pieces/m}^2$ in the bottom sediments and $0.03{\text -}0.17\,\text{pieces/m}^2$ in the surface water (Table 1). PE and PP microplastic concentrations in the bottom sediments were three to five orders of magnitude larger than that in the surface water. Similar to this study, in Tokyo Bay, Matsuguma et al. (2017) sampled microplastics in the bottom sediment cores and in the surface water, reporting that the abundance of PE and PP microplastics (pieces/m²) in the bottom sediments was 1×10^4 times higher than in the surface water. From this result, they concluded that the bottom sediment is a significant sink for microplastics. Therefore, the sea bottom will become a sink for microplastics regardless of their polymer type.

In Hiroshima Bay, FPS or 'PE and PP' microplastic concentrations in the beach sediments were similar to or 10 times larger than that in the bottom sediments. The area of Hiroshima Bay is 1043 km² and the length of the beach is 598 km (Chugoku Regional Development Bureau, 2018; Biodiversity Center of Japan, 1998). Assuming a 5-m equivalent uniform width of the beach along the coast of Hiroshima Bay, the total beach area is estimated at 2.99 km². Assuming uniform microplastic concentrations in the beach sediments, the surface water and the bottom sediments (see Table 1), microplastics will accumulate in the order of bottom sediments, beach sediments and surface water in the Hiroshima Bay environment. This may be caused by differences in the microplastic residence times in the bottom sediments, beach sediments and surface water. In the future, it will be necessary to estimate the residence times to elucidate the behaviors of microplastics in coastal

marine environments.

It is difficult to compare microplastic concentrations among different studies because of the lack of standardized procedures for sampling, separation, identification and size measurement. However, in Tokyo Bay, PE and PP microplastic concentrations in the bottom sediments were predominantly larger than that in Hiroshima Bay (Table 1). The microplastic concentrations in Tokyo Bay (Table 1; 20,000-60,000 pieces/m²) were converted to concentrations in the layer with a thickness of 0–11 cm, 7333–22,000 pieces/m² as a result. PE and PP microplastic concentrations in the bottom sediments in Tokyo Bay were 7-105 times larger than that in Hiroshima Bay. PE and PP were assumed to be from landbased sources. The watershed populations of Tokyo Bay and Hiroshima Bay were about 31 million people in 2014 and about 1.8 million people in 2009, respectively, and the area of Tokyo Bay is similar to that of Hiroshima Bay (Tokyo Bay: 1380 km²; Hiroshima Bay: 1043 km²) (Tokyo Bay Environmental Information Center, 2018; Chugoku Regional Development Bureau, 2018). The population for Tokyo Bay was 17 times larger than that for Hiroshima Bay. The difference in watershed populations could not account for the difference in PE and PP microplastic concentrations between Tokyo Bay and Hiroshima Bay. Microplastic concentrations would be related to the population densities or the proportion of urban areas within the watersheds (Yonkos et al., 2014).

3.3. Presumed behaviors of microplastics

Microplastic distributions were significantly affected by the size of microplastics regardless of the polymer type. Namely, larger microplastics are likely to be on the beaches and are unlikely to be on the sea bottoms (Fig. 4). The amount ratio of larger microplastics ($> 2\,\mathrm{mm}$) was highest in the beach sediments and decreased in the order of the surface water and bottom sediments for both FPS and 'PE and PP' (Fig. 4). By contrast, the ratio of smaller microplastics ($< 1\,\mathrm{mm}$) was highest in the bottom sediments and decreased in the order of the surface water and beach sediments for both FPS and 'PE and PP' (Fig. 4). Also, significant differences in the average size were found between the microplastics in the beach and bottom sediments for both polymer types. Therefore, microplastics must become smaller particles in order to sink to the sea bottom.

In the bottom sediments, the smaller-microplastic ratio of FPS microplastics was higher than that of 'PE and PP', and the average size of

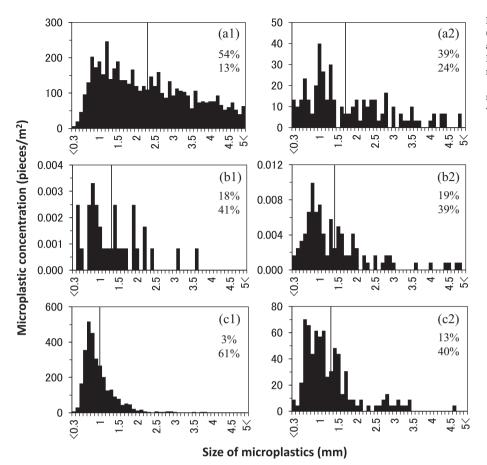


Fig. 4. Size of microplastics in the beach sediments (FPS: a1; PE and PP: a2), surface water (FPS: b1; PE and PP: b2) and bottom sediments (FPS: c1; PE and PP: c2). In each graph, straight lines represent each mean size, and two values represent the ratio of > 2 mm and < 1 mm microplastics from above. Care must be taken with the values on the vertical axis, which are different from each other.

FPS was significantly smaller than that of 'PE and PP' (Fig. 4 (c1) and (c2)). In contrast, in the beach sediments, the larger-microplastic ratio of FPS was higher than that of 'PE and PP', and there was a significant difference between the average sizes of FPS and 'PE and PP' (Fig. 4 (a1) and (a2)). These results show that FPS microplastics must become even smaller than 'PE and PP' microplastics to sink to the sea bottom and, in contrast, the larger FPS microplastics are likely to be washed ashore and stay there for longer because of their even smaller specific gravity (Hinata et al., 2017).

Assuming the ideal fragmentation process in which one microplastic particle is fragmented into two particles of equal size, the number of microplastics will increase exponentially as the size becomes smaller. However, the number of smaller microplastics (< 0.8 mm for the beach sediments and the surface water) decreased as the sizes became smaller (Fig. 4 (a) and (b)). The smaller microplastics are considered to sink (Cózar et al., 2014); however, the same qualitative results were obtained from the bottom sediments for both FPS and 'PE and PP'. This may be caused by smaller microplastics being overlooked or passing through the neuston net or sieves.

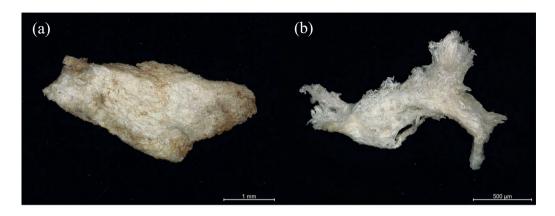
3.4. Fragmentation process and sinking factors of FPS microplastics

The fragmentation process and sinking factors of FPS microplastics were investigated based on the differences in their shapes in the beach and bottom sediments (Fig. 5). The majority of FPS microplastics in the beach sediments were thick and had a rounded shape (Fig. 5a); however, the majority in the bottom sediments were thin and had a rather complex shape (Fig. 5b). Based on the shape, the pores inside the FPS microplastics from the bottom sediments were considered to have collapsed and their specific gravity possibly approached that of PS (i.e. 1.04–1.09) due to the pores collapsing.

The surface state of FPS microplastics was examined in detail by FE-

SEM (Fig. 6). FPS microplastics in the bottom sediments had cracks over the entire surface and a complex surface (Fig. 6 (b1)) that could not be identified from the microscope image (Fig. 5b). In contrast, cracks were seen mainly on the margin of FPS microplastics from the beach sediments (Fig. 6 (a1)), and the shape of the margin was similar to that of FPS microplastics from the bottom sediments (Fig. 6 (a2) and (b2)). This suggests that smaller FPS particles are likely generated from the margins of larger FPS particles. Moreover, in Fig. 6 (a3) and (b3), diatoms were found in the cracks of FPS microplastics from both the beach and bottom sediments (although the diatoms may have become trapped after FPS microplastics reached the sea bottom). The specific surface area of FPS microplastics would be larger as the cracks increased. Therefore, FPS microplastics with complex surfaces would be susceptible to biofouling and deposition of soil particles because of their larger specific surface area.

In a review assessing the relevance of plastics characteristics to their role as a pollutant with potentially serious ecological impacts, Andrady (2017) suggested that fragmentation leading to smaller microplastics may involve a surface-ablation mechanism that is likely to result in large numbers of much smaller microplastics (several microns in size). Also, in Fig. 6 (a2) and (b2), FPS microplastics had scaly surfaces and had started to flake, so large amounts of nano- or microsized FPS fragments are likely to be generated from one microplastic-size FPS particle. Shim et al. (2014) analyzed FPS particles stained with Nile red using a fluorescent microscope and SEM with energy dispersive spectroscopy, suggesting that a number of nanosized FPS fragments were produced during the accelerated mechanical abrasion experiment. These results demonstrate that a future study needs to consider a new fragmentation process in which the number of plastic particles increases dramatically by the production of nano- and microsized fragments from the surface. At least, the fragmentation process presumed from the FE-SEM images is not as simple as one microplastic being



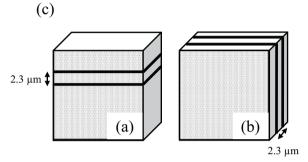


Fig. 5. Images of FPS microplastics using digital microscopy. (a) FPS particle extracted from the beach sediment (4.137 mm). (b) FPS particle extracted from the bottom sediment (1.774 mm). These particles were observed by FE-SEM (Fig. 6) and X-ray CT (Fig. 7). (c) The directions of the slices of FPS microplastics for X-ray CT analysis. The dotted squares correspond to Fig. 5 (a) and (b). X-ray CT sliced FPS microplastics in the directions of the planes shown by black lines.

fragmented into two equal microplastics.

The interior structures of FPS microplastics were examined in detail by X-ray CT (Fig. 7). Fig. 7 shows examples of images of FPS microplastics in 2.3 µm slices. The directions of the slices are shown in Fig. 5. X-ray CT whitens the comparatively higher density parts; in contrast, it blackens the lower density parts. That is, the pores of FPS microplastics become black. Fig. 7 (a) shows that black dots (pores) spread out inside the FPS microplastics from the beach sediments. In contrast, elongated pores extend into the FPS microplastics from the bottom sediments (see Fig. 7 (c)). This shows that FPS microplastics in the beach sediments had individual pores inside and maintained a lower density; in contrast, FPS microplastics in the bottom sediments had tunnel-like structures extending from the surface to the inside, which resulted from the merging of pores (Fig. 7). Therefore, the specific surface area of FPS microplastics in the bottom sediments would be much larger than that of FPS microplastics in the beach sediments. FPS microplastics in the bottom sediments were susceptible to biofouling and soil deposition, as inferred from the FE-SEM images (Fig. 6).

4. Conclusions and future studies

In Hiroshima Bay, where oyster aquaculture is widespread, FPS was numerically dominant and serious pollution by FPS microplastics spread out not only in the beach sediments as reported by Fujieda and Sasaki (2005), but also in the bottom sediments. This study is the first report of the dominance of FPS microplastics in bottom sediments. The average size of FPS microplastics in the bottom sediments was significantly smaller than that of beached FPS particles. In the surface water, microplastics formed a different ratio of polymers from the beach and bottom sediments; PE and PP microplastics were more abundant. This suggests that FPS microplastics are likely washed ashore immediately by Stokes drift, surface residual currents and winds because of their much smaller specific gravity (Isobe et al., 2014). The smaller specific gravity of FPS causes much larger upward terminal velocities in the sea water compared to that of PE and PP, so FPS

particles have much longer residence times on the beaches than PE and PP (Hinata et al., 2017), resulting in the overwhelming dominance of FPS on the beaches.

The fragmentation process and sinking factors of FPS microplastics were examined. FE-SEM images suggest that large amounts of micro- or nanosized FPS fragments are likely to be generated from the margin of one microplastic-size FPS particle. Also, the specific surface area of smaller FPS microplastics with a complex shape is much greater than that of larger FPS particles. X-ray CT images show that FPS microplastics from the bottom sediments had tunnel-like structures, with pores extending from the surface to the inside. Therefore, based on the FE-SEM and X-ray CT images, FPS microplastics in the bottom sediments were found to be susceptible to biofouling and soil deposition.

This study examined the abundance, polymer type, size and shape of microplastics in three reservoirs, that is, the beach and bottom sediments and the surface water of Hiroshima Bay. In this study, the spatial distributions of the abundance and size of microplastics among the sampling sites were not discussed, because significant relationships between the residual current structure (estuarine circulation) and the spatial distributions among the sites have not yet been found, probably due to the relatively coarse resolution of sampling sites and an insufficient understanding of the current structures and their variabilities in the region. In the future, three-dimensional numerical simulations including the sinking and beaching processes as well as the Stokes drift velocity, density- and wind-driven currents will help us understand the microplastic residence times, the fluxes between reservoirs, and the adverse effects of microplastics on the regional ecosystem.

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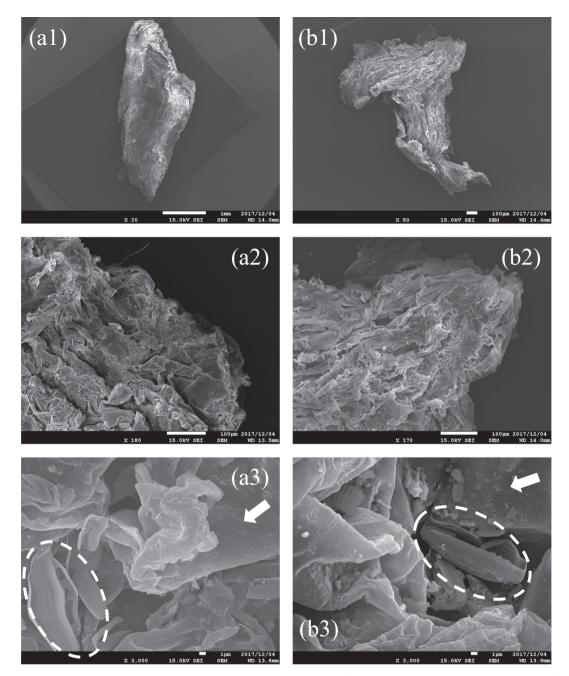


Fig. 6. FE-SEM images of the surface state of FPS microplastics. (a) FPS microplastics from the beach sediments. (b) FPS microplastics from the bottom sediments. Complete pictures of each microplastic are shown in (a1) and (b1). Also, the margins of each microplastic are shown in (a2) and (b2), and more detailed surface states of each microplastic are shown in (a3) and (b3). In (a3) and (b3), the arrows indicate the parts of FPS microplastics that started to flake, and the dotted-line circles indicate diatoms. These samples were the same as the microplastics shown in Figs. 5 and 7.

Appendix A. Verification of precision

A.1. Quartering

An 800 ml sediment sample (sand sample) was divided into eight 100 ml sediment samples by a quartering method. The method was performed as follows: the 800 ml sediment sample was fully mixed, divided into quarters, and two opposite quarters of the sample (both diagonal lines) were mixed. At this time, the initial samples were each divided into two samples (in half). This procedure was performed repeatedly until eight 100 ml sediment samples were obtained. For the eight 100 ml sediment samples, the extraction process described in Section 2.3 was performed three times, and then the number and size of microplastics extracted from each sample were measured.

The average number of microplastics and the standard deviation was 325 (pieces/100 ml) and 41 (pieces/100 ml), respectively. Fig. A1 shows the ratio of each size of microplastics for the eight samples. The dispersion of < 1 mm microplastics was comparatively large.

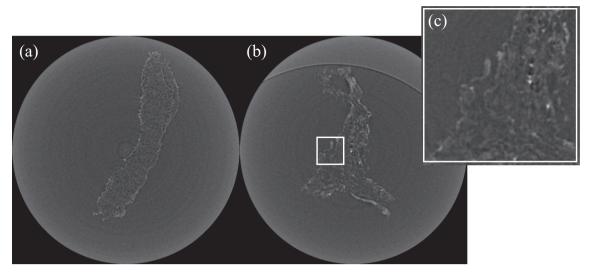


Fig. 7. Examples of X-ray CT images of 2.3 μm slices of FPS microplastics. The images show FPS microplastics from the beach sediments (a) and bottom sediments (b). (c) Enlarged view of the white square in (b), representing the tunnel-like structures extending from the surface to the inside. The directions of the slices of the FPS microplastics are shown in Fig. 5 (c). X-ray CT whitens the comparatively higher density parts and blackens the lower density parts. Pores of FPS microplastics become black. These samples were the same as the microplastics shown in Figs. 5 and 6.

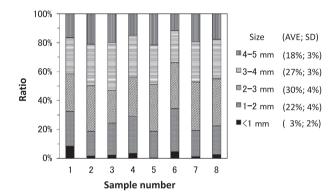


Fig. A.1. Ratio of each size of microplastics from eight samples. The legend shows the pattern used for the size of microplastics (AVE: average ratio of eight samples; SD: standard deviation of ratio).

A.2. Microplastics recovery efficiency from the beach sediment samples

Seven 100 ml beach sediment samples were each spiked with 15 pieces of PE and PP, respectively (< 1 mm, 1–2 mm, 2–3 mm, 3–4 mm, 4–5 mm; three pieces in each size) and 15 pieces of FPS (≤ 1 mm, 2–3 mm, 3–5 mm; five pieces in each size). Then, extraction was performed five times and the polymer type, size and number of microplastics were measured for every extraction.

For the seven samples, after the five-time extraction, almost all the remaining microplastics were white or yellow PP and < 2 mm (Table A.1). This shows that small PP microplastics may be comparatively difficult to detect because of their color. In the future, the number for verification should be increased considering the color of microplastics. In contrast, microplastics $\geq 2 \text{ mm}$ could almost be completely recovered (Fig. A.2). The upward terminal velocities of microplastics $\leq 2 \text{ mm}$ were greater than that of the microplastics < 2 mm. To extract the microplastics < 2 mm, it is necessary to use a heavier solution or allow the samples to settle for a longer time in the extraction process.

Table A.1
Remaining microplastics after five-time extraction: number of pieces, polymer type and size of remainders. 'y' and 'w' indicate yellow and white PP, respectively.

Sample number	Number of pieces	Polymer type	Size (mm)			
1	1	PP	0.801 (y)			
2	3	PP	1.170 (y)	1.262 (y)	4.318 (y)	
3	3	PP	0.752	0.976 (w)	1.131 (y)	
4	1	PP	0.803			
5	4	PP	0.822 (w)	1.615 (y)	3.373 (y)	
		PE	0.924	*.	•	
6	1	PP	0.847			
7	2	PP	0.642	0.869		

To estimate the actual amount of microplastics in the beach sediment samples of Hiroshima Bay and for comparison with other studies, in which the number of extraction processes was different, the approximate curve was calculated and integrated with the number of extraction processes. Fig. A.3 shows the relationship between the number of extraction processes and the number of microplastics extracted from each sample. Exponential approximation of the number of extracted microplastics was performed, assuming the same extraction efficiency of microplastics for each process. The approximate curve was calculated by a weighted least squares method. By integrating the approximate curve with the number of extraction processes, it was estimated that 93% (99%) of the microplastics could be retrieved by the three-time (five-time) extraction (cumulative ratio in Fig. A.3). This estimation suggests that at least the three-time extraction process should be performed; many smaller microplastics cannot be retrieved by only a one-time extraction (Fig. A.2).

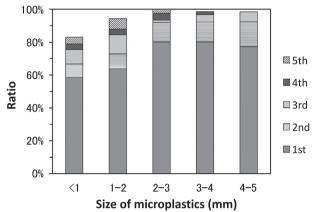


Fig. A.2. Average ratio of extracted microplastics at each size. The classification shows the number of extraction processes.

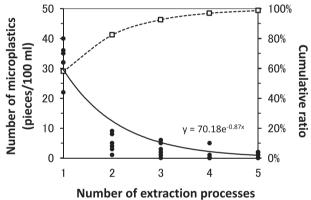


Fig. A.3. Relationship between the number of extraction processes (horizontal axis) and the number of microplastics extracted from each sample, which is shown on the first axis. Exponential approximation of the number of extracted microplastics was performed, assuming the same extraction efficiency of microplastics for each process. The solid line is the approximate curve calculated by a weighted least squares method. The dotted line is the cumulative ratio of extracted microplastics by integrating the approximate curve with the number of extraction processes, which is shown on the second axis.

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