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Sources of Polycyclic Aromatic Hydrocarbons in Fecal Pellets of a *Marphysa* Species (Annelida: Eunicidae) in the Yoro Tidal Flat, Japan

Yuichiro Osaka¹, Satoshi Abe¹, Hirokazu Abe², Masaatsu Tanaka³, Mayu Onozato⁴, Kenji Okoshi¹, and Atsuko Nishigaki^{1*}

¹Department of Environmental Science, Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274-8510, Japan

²Department of Biological Sciences, Faculty of Science and Engineering, Ishinomaki Senshu University, 1 Shinmito, Minamisakai, Ishinomaki-shi, Miyagi 986-8580, Japan

³Department of Biology, Keio University, 4-1-1 Hiyoshi, Kohoku-ku, Yokohama-shi, Kanagawa 223-8521, Japan

⁴Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274-8510, Japan

The fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019) (Annelida, Eunicidae) living in the Yoro tidal flat (Ichihara, Chiba, Japan) contain high levels of polycyclic aromatic hydrocarbons (PAHs), and the concentrations rapidly decrease over time. To investigate the origin of the high-concentration PAHs in the fecal pellets and food sources of the worms, the PAH concentrations, carbon and nitrogen stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), total organic carbon, and total nitrogen for two types of sediment (sands and reduced muds), fecal pellets, and the body of the worms were determined. The PAH concentrations and chemical properties of the fecal pellets were similar to those of the reduced muds (20–30 cm sediment depth). The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C/N values of reduced muds were the same as the typical values of terrestrial C_3 plants, suggesting that reduced muds were derived from terrestrial plants. These data indicated that the worms selectively take up reduced muds containing high levels of PAHs. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the worm bodies indicated that the worms did not use the organic carbon derived from terrestrial C_3 plants as primary nutrition. Taking into consideration their selective uptake of reduced muds, excretion, and subsequent rapid decrease of PAHs in the fecal pellets, the worms could contribute to the remediation of chemical pollutants in the tidal flat sediments.

Key words: Annelida, *Marphysa*, fecal pellet, polycyclic aromatic hydrocarbons, tidal flat, stable isotope ratio, bioremediation

INTRODUCTION

Polychaetes of the genus *Marphysa* (Annelida, Eunicidae) usually inhabit tidal flats and rocky shores across various coasts of the world (Lavesque et al., 2022). Some *Marphysa* species inhabiting tidal flats are assumed to play an important role in the material cycle and movement in the coastal environment by forming deep burrows into the sediment (Lewis and Karageorgopoulos, 2008; Kara et al., 2020). Additionally, feeding habits of *Marphysa* vary by habitat and species, including surface-deposit feeders and omnivores (Jumars et al., 2015). Recently, we conducted DNA and morphological analysis of a Japanese species of *Marphysa* known as “Iwa-mushi”, and found that Iwa-mushi

did not consist of a single species, but is instead five genetically separate clades which were tentatively designated as five undetermined species (Abe et al., 2019). Among these, one species, *Marphysa* sp. E sensu Abe et al. (2019), was only found in muddy and sandy sediments of the inner part of Tokyo Bay.

Tokyo Bay, where *Marphysa* sp. E sensu Abe et al. (2019) was first discovered, is a typical enclosed inner bay area, surrounded by large cities. Although the environment of the bay has improved considerably compared to the 1960s, when eutrophication and/or chemical pollution was a serious problem (Yanagi, 2015), the water and habitat quality still have not recovered sufficiently, and red tide and bottom water hypoxia both occur annually (Ando et al., 2021). The loss of the majority of the tidal flats, which harbor a wide variety of organisms including polychaetes (Yanagi, 2015), as a result of land reclamation is one of the main causes for

* Corresponding author. E-mail: atsuko@env.sci.toho-u.ac.jp
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the loss of the environmental purification functions (Yanagi, 2015; Shan and Li, 2020).

Polycyclic aromatic hydrocarbons (PAHs) are released into the environment as a result of the incomplete combustion of fossil fuels, oil spills, and other anthropogenic/natural activities (Perra et al., 2009; Qiu et al., 2009). Some PAHs, such as benzo[a]pyrene, have toxic, mutagenic, and/or carcinogenic properties for humans (Hayakawa, 2016) and other animals (Honda and Suzuki, 2020), and therefore it is important to know their concentrations and behavior in the environment. PAHs emitted into the atmosphere can be introduced to surface water environments as a result of gravity and rainfall (Offenberg and Baker, 2002; Xia et al., 2022). Considering their hydrophobic and persistent nature, PAHs can easily be adsorbed onto suspended particulates in water environments, and subsequently swiftly settle to the bottom, and accumulate among bottom sediments (Yang et al., 2005). We previously reported that the concentrations of PAHs, cationic surfactants, and persistent organic environmental pollutants were approximately 10–100 times higher in the fecal pellets of one *Marphysa* species, which had originally been identified as *Marphysa sanguinea* and later reidentified as *Marphysa* sp. E sensu Abe et al. (2019), than in the sands of the Yoro tidal flat, Ichihara, Chiba Prefecture, Japan (Onozato et al., 2010). After excretion, PAHs concentrations in the fecal pellets rapidly decreased by approximately half within 2 h (Onozato et al., 2010, 2012). Although the high PAHs concentrations in the fecal pellets could be attributable to the diet of the worm, no such potential diets have been found in the Yoro tidal flat so far (Nishigaki et al., 2013). We recently found that the reductive, viscous reduced muds widely scattered throughout the Yoro tidal flat, contained 4–7 times greater concentrations of PAHs than the sands (Osaka et al., 2023). Since *Marphysa* sp. E sensu Abe et al. (2019) is a newly recognized species, its ecology, including feeding habits and food sources, is largely unknown.

In this study, to investigate the possibility of ingestion of the reduced muds by *Marphysa* sp. E sensu Abe et al. (2019), we measured and compared PAHs concentrations, stable isotope ratios of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), total organic carbon (TOC), total nitrogen (TN), and particle sizes in the fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019) and sediment samples (sands and reduced muds)

collected from the Yoro tidal flat. On the basis of these results, we discuss the feeding behavior of the worm, the origin of PAHs in its fecal pellets, and its contribution to the environmental remediation of tidal flats.

MATERIALS AND METHODS

Sample collection

Samples were collected in the Yoro tidal flat (Ichihara, Chiba, Japan: 35°32'33"N, 140°4'0.84"E) at ebb tide during the day in April to October in 2018 to 2021 (Fig. 1). The sediment on the Yoro tidal flat consists mainly of sand (Fig. 2A), while a black, highly viscous reduced mud (Fig. 2B) was scattered over a large area of the tidal flat, being present in a state of flux, in which the location of the "reduced mud" was dependent on the sampling date and was not always found at a fixed location. The reduced mud was mostly found within the deep sediment layers (> 30 cm depth), but was also often observed in the surface sediment (0–10 cm), particularly immediately after typhoons. Fragments of leaves, stems, and branches of terrestrial plants that had blackened due to lack of oxygen were often found in the reduced mud. Some benthic organisms such as bivalves (*Macra veneriformis*, *Meretrix* sp., and *Solen strictus*) and polychaetes (*Glycera* sp. and *Arenicola brasiliensis*), were commonly found in the sediments of the study area. *Marphysa* sp. E sensu Abe et al. (2019) was found to have been one of the most dominant infaunal species in terms of biomass in this area, with larger individuals measuring over 50 cm long and approximately 1 cm wide (Fig. 3). The highest density of the worms was roughly estimated to be 0.9 individuals m^{-2} using a quadrat method that counted the number of fecal mounds in 2022 (see Supplementary Text S1, Supplementary Figure S1, and Supplementary Table S1).

Marphysa sp. E sensu Abe et al. (2019) typically form branched burrows at least 50 cm deep in sediment. At the ebb tide, the posterior end of the body emerges from the burrow opening and excretes about 20–90 brown or black oval fecal pellets that form a mound on the surface of the sediment; a pellet is approximately 2 mm in major diameter (Fig. 2C). The weights of a fecal mound per worm were approximately 0.41 ± 0.24 g-wet (maximum weight: 0.92 g-wet, minimum weight: 0.10 g-wet, $n = 20$, measured in April 2023). When digging with a shovel in areas where fecal mounds of the worm have been found, burrows formed in sediment (Fig. 2D), and sometimes burrows passing through layers of reduced mud, were observed (Fig. 2E). Fecal pellets excreted within the burrows were infrequently observed (Fig. 2F).

Fecal pellets from *Marphysa* sp. E sensu Abe et al. (2019) were collected immediately after excretion, frozen on dry ice, and transported to the laboratory. The bodies of the worms were obtained by digging up the bottom sediment under the fecal pellets with a shovel

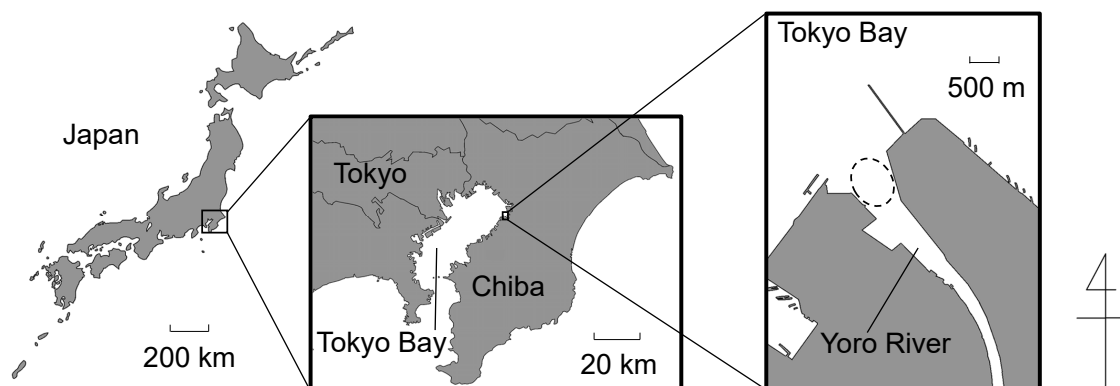


Fig. 1. Location of sampling sites in the Yoro tidal flat, Chiba Prefecture, Japan. Sampling sites are circled using dashed lines.

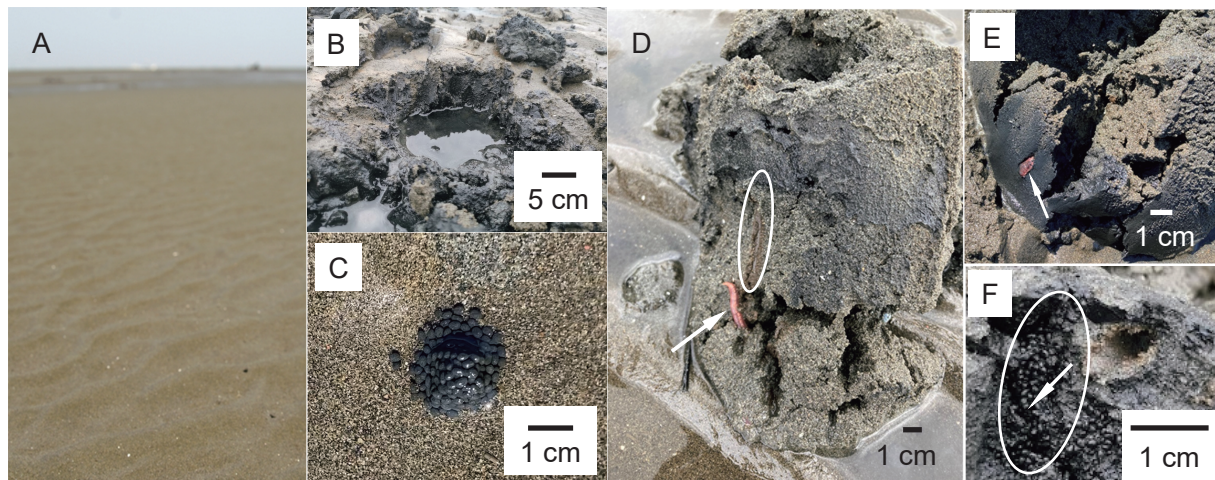


Fig. 2. Photographs of sediments (A, B), fecal mounds from *Marphysa* sp. E sensu Abe et al. (2019) (C), and burrows of worms in sediment (D–F) at Yoro tidal flat. (A) Surface condition of tidal flat. (B) Reduced mud, (C) Fecal mound of *Marphysa* sp. E on surface of sediment, (D) Burrow of *Marphysa* sp. E in sediment (white circle). Its posterior body is indicated by a white arrow. (E) Burrow of *Marphysa* sp. E in reduced mud with posterior body of the worm (white arrow). (F) Fecal pellets of *Marphysa* sp. E in burrow. Pellets and burrow are indicated by a white circle and white arrow, respectively.

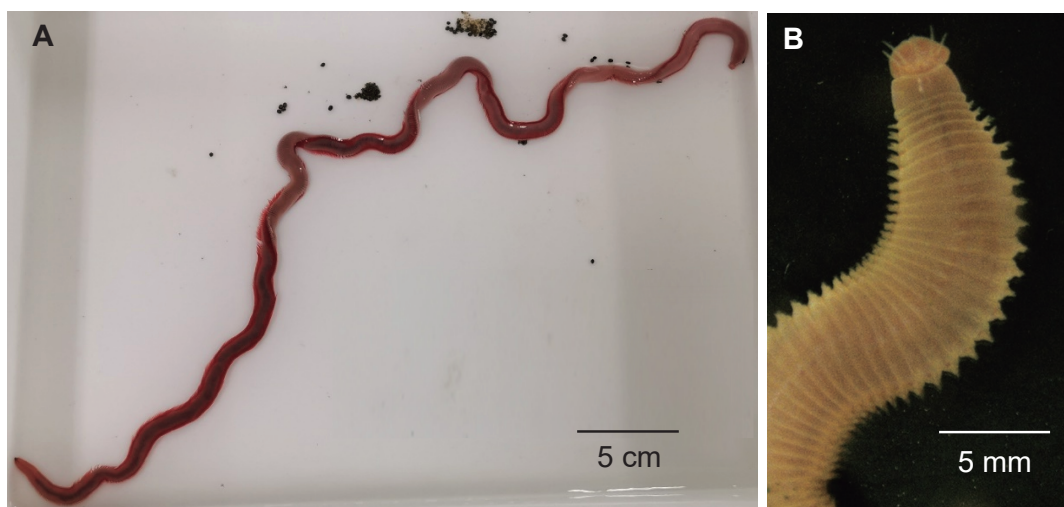


Fig. 3. Live coloration of *Marphysa* sp. E sensu Abe et al. (2019). (A) Entire body, with its head towards right corner (collected on 28 April 2021). (B) Magnification of anterior end (collected on 30 May 2022).

after collecting the pellets. Sediment samples were collected from the surface layer (depth of 0–5 cm) and the deep layer (depth of 30–40 cm) using a shovel, and then were sorted into two parts according to their color and texture: sand (brown, low viscosity) and reduced mud (black, high viscosity). Each sample was then stored in a freezer (–18°C) until analysis.

PAHs analysis

The PAHs investigated in this study were selected from 16 chemicals identified by the U.S. Environmental Protection Agency (USEPA) as hazardous pollutants which are frequently detected in sediment. The seven selected PAHs were phenanthrene (Phe), anthracene (Anth), fluoranthene (Flu), pyrene (Pyr), chrysene (Chry), benzo[b]fluoranthene ([b]flu), and benzo[a]pyrene ([a]pyr) (see Supplementary Figure S2 and Supplementary Table S2). Although perylene (Pery) was not included in the list of USEPA, Pery was added to the target compounds in this study, considering its high frequency of detection within the sediment. Each sample

was subjected to extraction, purification, and concentration, and then analyzed using gas chromatography-mass spectrometry (GC-MS). The conditions for sample preparation and GC-MS analysis were the same as in our previous study (Onozato et al., 2008; and see Supplementary Text S1).

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$, TOC, and TN analysis

Sediments (sand and reduced mud), fecal pellets, and the body tissue of *Marphysa* sp. E sensu Abe et al. (2019), approximately 1 g of each sample, were freeze-dried using an FDU-1200 (TOKYO RIKAKIKAI, Tokyo, Japan) for 24 h. Then, sand and body tissue were prepared as follows. Sand samples were decalcified in 5 mL of 1.0 M hydrochloric acid solution and then washed with 15 mL of ultrapure water produced by Direct-Q UV3 (Nihon Millipore, Tokyo, Japan). After washing, these samples were freeze-dried again. The body tissues were crushed using a mortar and pestle, and delipidated in Folch solution (methanol:chloroform = 1:2) (Folch et al., 1956; Bligh and Dyer, 1959). The delipidated samples

were subsequently freeze-dried again. Freeze-dried sediments, fecal pellets, and body tissue samples were stirred and powdered with a spatula (The Oceanographic Society of Japan, 2008; Ministry of the Environment, 2012). $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, TOC, and TN analyses for these samples were performed by Shoko Science Co. Ltd. (Saitama, Japan) using an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS; Flash EA1112-DELTA V ADVANTAGE, Thermo Fisher Scientific, MA).

Particle size analysis

Particle size analysis of the sediments and fecal pellet samples was conducted using the dry sieving method (The Oceanographic Society of Japan, 1986; Ministry of the Environment, 2012). After desalination and drying, samples were placed on a shaker (AS200, Retsch, Haan, Germany) and separated using sieves with mesh sizes of 0.063, 0.25, 0.5, and 1.0 mm. The particle size distributions were then determined as the composition of the particle weights remaining on each sieve.

RESULTS

PAHs concentrations of sediments and fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019)

Total PAHs concentrations for sand from the surface of the sediment (0–5 cm) around the fecal mound of the *Marphysa* sp. E sensu Abe et al. (2019), reduced mud from the deep sediment layer under the fecal mound (30–40 cm), and the fecal pellets of the worm collected on the same day (in May 2018) were 29 ± 5 , 610 ± 160 , and $500 \pm 80 \mu\text{g kg-dry}^{-1}$, respectively (Fig. 4, and see Supplementary Table S3). Furthermore, the total PAHs concentrations in reduced mud and fecal pellets were approximately 3–21 and 4–61 times higher, respectively, than those found in sand. The proportions of high molecular weight PAHs (Chry, [b]flu, [a]pyr, and Pery) were particularly high in reduced mud and fecal pellets, with concentrations being 31–48 times higher than those found in sand.

Carbon and nitrogen stable isotope ratios of sediment and fecal pellets and bodies of *Marphysa* sp. E sensu Abe et al. (2019)

The mean $\pm \sigma$ values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of sand ($n = 6$), reduced mud ($n = 5$), and the fecal pellets ($n = 5$) and body

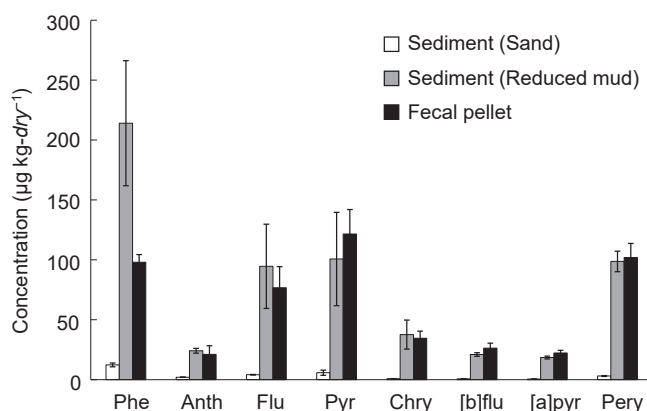


Fig. 4. PAH concentrations (mean $\pm \sigma$) of the sediments and the fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019) collected on 20 May 2018, as determined in triplicate ($n = 3$). The values of PAHs concentrations of each sample are shown in Supplementary Table S3.

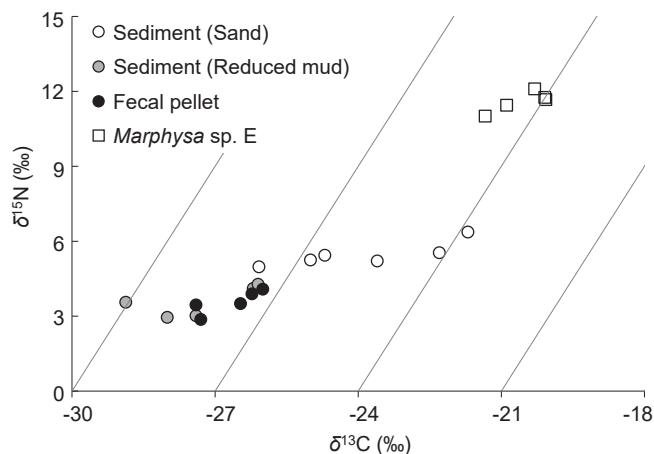


Fig. 5. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios for sand sediment (open circles), reduced mud sediment (gray circles), and fecal pellets (solid circles) and body (open squares) of *Marphysa* sp. E sensu Abe et al. (2019). The diagonal lines indicate the 1:3 relationships.

Table 1. Total organic carbon (TOC) and total nitrogen (TN) value and TOC/TN ratio (C/N) of sediments and fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019).

A. Sand				
		TOC (%)	TN (%)	C/N
Date	2018.7	0.058	0.009	6.4
	2019.8	0.17	0.016	10.6
	2019.9	0.061	0.008	7.6
	2019.9	0.17	0.017	10.0
	2021.4	0.058	0.007	8.3
	2021.9	0.22	0.020	10.8
	mean	0.12	0.013	9.0
	σ	0.07	0.006	1.8
B. Reduced mud				
		TOC (%)	TN (%)	C/N
Date	2018.7	1.5	0.12	11.7
	2018.7	1.6	0.13	12.2
	2019.8	2.9	0.22	13.0
	2021.6	1.7	0.11	16.2
	2021.9	1.7	0.15	11.6
	mean	1.9	0.15	12.9
	σ	0.6	0.04	1.9
C. Fecal pellet				
		TOC (%)	TN (%)	C/N
Date	2018.7	2.7	0.22	12.5
	2019.8	2.5	0.17	14.2
	2019.9	2.3	0.17	14.1
	2021.7	1.9	0.14	13.3
	2021.9	3.0	0.22	13.7
	mean	2.5	0.19	13.5
	σ	0.4	0.03	0.7

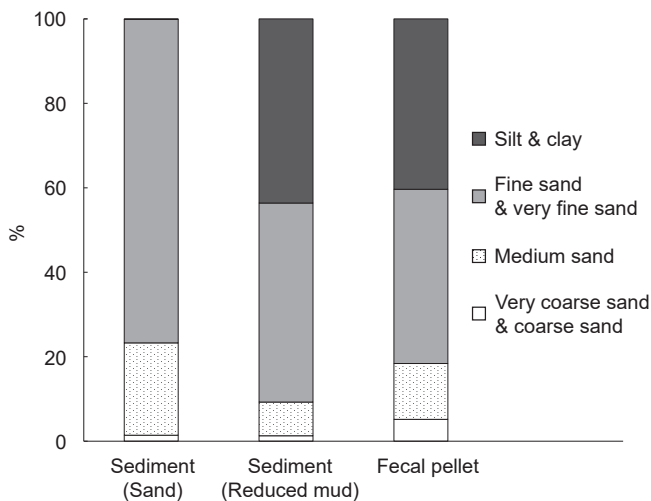


Fig. 6. Particle size compositions of sediments and fecal pellet of *Marphysa* sp. E sensu Abe et al. (2019). Particle sizes were classified as follows (Wentworth, 1922): very coarse sand & coarse sand (>0.5 mm), medium sand (0.25–0.5 mm), fine sand & very fine sand (0.063–0.25 mm), silt & clay (<0.063 mm).

tissue of *Marphysa* sp. E sensu Abe et al. (2019) ($n = 5$) were $-23.9 \pm 1.7\text{‰}$ and $5.5 \pm 0.5\text{‰}$, $-27.3 \pm 1.2\text{‰}$ and $3.6 \pm 0.6\text{‰}$, $-26.7 \pm 0.6\text{‰}$ and $3.6 \pm 0.5\text{‰}$, and $-20.1 \pm 1.2\text{‰}$ and $11.9 \pm 3.0\text{‰}$, respectively (Fig. 5, and see Supplementary Table S4). C/N ratios (TOC/TN) for sand, reduced mud, and fecal pellets were 9.0 ± 1.8 , 12.9 ± 1.9 , and 13.5 ± 0.7 , respectively (Table 1).

Particle size distribution of sand, reduced mud, and fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019)

Fine sand and very fine sand (0.25–0.063 mm) were the most abundant contents in all samples, comprising 77% of the sand, 47% of the reduced mud, and 41% of the fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019) (Fig. 6). However, the content of silt and clay (< 0.063 mm) was only 0.1% of the sand, and 44% and 40% in reduced mud and fecal pellets, respectively. Furthermore, very coarse sand fractions (1–2 mm) were very low, with a content of 0.03–0.5% in each sample (see Supplementary Table S5).

DISCUSSION

Source of the high concentrations of PAHs in the fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019)

Among the samples that were collected on the same day (in May 2018), total PAHs concentrations in the reduced muds were higher than those in the sands, while all PAHs concentrations except for Phe were found to have been similar in reduced mud and the fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019). The PAHs concentrations and distributions were also similar for reduced mud and fecal pellets collected on other days (Fig. 4, and see Supplementary Table S3). The fecal pellets and reduced muds were considered to be of the same quality in stable isotope analysis and particle size composition (Fig. 5, and see Supplementary Tables S4 and S5). The sediment of the Yoro tidal flat is generally composed of sands, although reduced mud is often observed to be scattered over large areas of the tidal

flat. Although the distribution of reduced muds is not uniform, the concentrations of total PAHs in fecal pellets excreted by different individuals on the different sampling dates were similar to those of reduced muds. These results suggested that the worm selectively ingested reduced muds containing high concentrations of PAHs and subsequently excreted them as fecal pellets. The concentrations of Phe varied significantly in each sediment and fecal pellet sample (see Supplementary Table S3). Since Phe is one of the most hydrophilic PAHs among the targeted PAHs in this study (see Supplementary Table S2), it can easily be separated from the surface of the sediment particles due to release into the aqueous system. High molecular weight PAHs (HMW-PAHs), Chry, [b]flu, [a]pyr, and Pery, were detected in reduced muds and fecal pellets, in significantly higher concentrations than in sands (see Supplementary Table S3). These HMW-PAHs were released into the coastal environment via fossil fuel combustion (Helfrich and Armstrong, 1986; Dhar et al., 2020), although they are hydrophobic (Mackay et al., 1992) and difficult to degrade in reductive bottom sediments (Lu et al., 2012), and can be easily adsorbed and persistent on organic carbon (Kafilzadeh et al., 2011). The TOC value of reduced muds ($1.9 \pm 0.6\%$) was approximately 10 times higher than that of sand ($0.12 \pm 0.07\%$) (Table 1), suggesting that reduced mud is likely to retain high concentrations of HMW-PAHs. Among these HMW-PAHs, Pery exhibited a high percentage in the reduced muds and fecal pellets. The percentage of Pery among Total PAHs in reduced muds and fecal pellets was notably higher than that in sand (5–10%), and ranged from 16–28% and 12–27%, respectively (Fig. 4 and see Supplementary Table S3). Pery has also been reported to be produced by reductive decomposition of terrestrial plants (Orr and Grady, 1967; Itoh et al., 2012). Recently, we reported that PAH concentrations, particle size analysis, and $\delta^{13}\text{C}$ measurements of reduced muds collected from the Yoro tidal flat indicated that reduced muds were produced from the decomposition of terrestrial plants, and that the percentage of Pery in the mud increased by 15–36% as plant decomposition progressed (Osaka et al., 2023). The $\delta^{13}\text{C}$ and C/N ratio of reduced muds were $-27.3 \pm 1.2\text{‰}$ and $12.9 \pm 1.9\text{‰}$, respectively (Table 1, and see Supplementary Table S4), which were in good agreement with those of terrestrial C_3 plants ($\delta^{13}\text{C}$ and C/N ratio around -27‰ and above 12, respectively) (Kang et al., 2003; Malet et al., 2008). Therefore, the reduced muds were attributable to terrestrial plants, and were presumably produced by the decomposition of terrestrial plants (deciduous leaves and branches) transported from the upper reaches to the estuary, thus resulting in the loading of Pery into the sediment. Additionally, since it has been reported that high concentrations of PAHs are attached to the leaves of plants alongside high-traffic roads (Yamamoto et al., 2004), some of the PAHs detected in the reduced mud may have originated from surface deposits on leaves transported to the estuary.

Contribution to the remediation of the tidal flat environment due to the uptake and excretion of reduced muds by *Marphysa* sp. E sensu Abe et al. (2019)

Although *Marphysa* sp. E sensu Abe et al. (2019) was considered to be consuming and subsequently excreting

reduced muds, the stable isotope ratios of fecal pellets ($\delta^{13}\text{C}$: -26.7‰ , $\delta^{15}\text{N}$: 3.6‰) and the body of the worm ($\delta^{13}\text{C}$: -20.1‰ , $\delta^{15}\text{N}$: 12.0‰) differed markedly. It is generally considered that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ increase by approximately 0–1‰ and 3–4‰, respectively, with an increase of one trophic level, and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are considered to be indicators of source and trophic level, respectively (Post, 2002; Kinoshita et al., 2021). It is also considered that the stable isotope ratios of fecal pellets generally correspond with those of the ingested targets (Gao et al., 2006; Landrum and Montoya, 2009). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the bodies of the worm were elevated by 5–10‰ and 7–10‰, respectively, relative to those of reduced muds and fecal pellets (Fig. 5), thus suggesting that, the organic matter of terrestrial plant origin in the reduced muds was not their primary source of nutrition although the worm ingested reduced muds. In general, marine organisms are reported to have difficulty decomposing materials derived from terrestrial plants (cellulose, lignin, etc.) and are therefore unable to utilize them as food resources (Simenstad and Wissmar, 1985). The reason for the uptake of reduced muds by the worm is not yet clear, but this could be for the ingestion of available nutritious materials (e.g., bacteria) (Fenchel, 1970) in reduced muds. The $\delta^{13}\text{C}$ values of the worms indicated that marine phytoplankton ($\delta^{13}\text{C}$: -22‰ or higher) (Middelburg and Herman, 2007) and benthic microalgae ($\delta^{13}\text{C}$: -20‰ or higher) (Sakamaki et al., 2010) are also possible food sources for the worm.

Our previous studies revealed that the concentrations of PAHs in the bodies of the worm were extremely low, as in the sand (Onozato et al., 2008), and the half-life of PAHs in the fecal pellets was approximately 2 h (Onozato et al., 2012). It was suggested that PAHs taken up with reduced muds were passed through and excreted without absorption into their bodies. It is generally reported that the half-life of PAHs in the sediment is between several weeks and months (Lu et al., 2012), and that HMW-PAHs with more than five rings were hardly degraded in reducing deep layer sediments (Karickhoff et al., 1979). The decrease found here in concentrations of PAHs in the fecal pellets was regarded as rapid compared to that in these reports, although the mechanism of the reduction of PAHs concentrations over a short period of time remains unclear. It has been reported that microorganisms (e.g., *Pseudomonas*, *Mycobacterium*, *Achromobacter*) in sediments degrade PAHs (Mrozik et al., 2003; Tiwari et al., 2010), and that microorganisms contribute to PAH degradation in the fecal pellets of a marine annelid, *Capitella* sp. (half-lives of Phe, Flu, Pyr, Chry, [b]flu, and [a]pyr were 1 to 2 months in Erlenmeyer flasks) (Hornig and Taghon, 2001). In addition, marine annelids such as *Nereis virens* (currently as *Alitta virens*) have been reported to possess an enzyme (cytochrome P450) capable of degrading PAHs (Rewitz et al., 2004; Jørgensen et al., 2008). Therefore, it is assumed that PAHs degradation was carried out by microbes and/or enzymes in the fecal pellets, similar to those in these annelid species. In the present study, it was found that the *Marphysa* sp. E sensu Abe et al. (2019) selectively consumed reduced muds containing high concentrations of persistent PAHs and then excreted them as fecal pellets. Therefore, these feeding and excretion behaviors of *Marphysa* sp. E sensu Abe et al. (2019) potentially make an important contribution to the purification of the intertidal flat

environment. Degradation of PAHs in the fecal pellets has only been studied for *Marphysa* sp. E sensu Abe et al. (2019), and the ability to degrade PAHs in other *Marphysa* species will need to be evaluated in future studies.

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COMPETING INTERESTS

We declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: AN, Formal analysis: YO and SA. Investigation: YO, SA, HA, MT, MO, KO, and AN. Writing—review and editing: YO, SA, HA, MT, MO, KO, and AN. All authors have read and agreed to the published version of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: <https://doi.org/10.2108/zs230020>)

Supplementary Text S1. Quadrat survey method. Chemicals and reagents. Sample preparation. Instruments and GC-MS conditions.

Supplementary Figure S1. The map of the quadrat survey area.

Supplementary Figure S2. Structures of the target PAHs in this study.

Supplementary Table S1. Number of fecal mounds in 5×5 m quadrats in the Yoro tidal flat.

Supplementary Table S2. Physicochemical properties of the target PAHs.

Supplementary Table S3. PAHs concentrations of sediments and fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019).

Supplementary Table S4. Stable isotope ratio of sediments, fecal pellets, and *Marphysa* sp. E sensu Abe et al. (2019).

Supplementary Table S5. Particle size composition of sediments and fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019).

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