Zoological Science

Supplementary Materials

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

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Supplementary Text S1.

1. Quadrat survey method

The density of *Marphysa* sp. E sensu Abe et al. (2019) was roughly estimated using a quadrat method that counted the number of fecal mounds within a 300 × 200 m area of the Yoro tidal flat in 2022. Surveys were conducted on four separate days: 30 May, 17 June, 27 August, and 8 October in 2022, during the 1 to 3 hours of exposed bottom sediment around low tide time. Each survey employed a 50 m square section (250 m^2: A1−D6 in Supplementary Figure S1) with a 5 × 5 m quadrat set at the top-left corner and the number of fecal mounds within each quadrat was counted. Two to 14 quadrats were investigated per survey; quadrats not investigated were either under water or left out due to time limitations. The results are shown in Supplementary Table S1.

2. Chemicals and reagents

Environmental analysis grade-PAH, phenanthrene (Phe), fluoranthene (Flu), pyrene (Pyr), chrysene (Chry), benzo[*b*]fluoranthene ([b]flu), benzo[*a*]pyrene ([a]pyr), Phe-d_{10}, anthracene (Anth)-d_{10}, Flu-d_{10}, Pyr-d_{10}, Chry-d_{12}, [a]pyr-d_{12}, perylene (Pery)-d_{12}, *p*-terphenyl-d_{14} and practical grade-Pery, pesticide residue and polychlorinated biphenyl tests grade- acetone, hexane, and ethanol, guaranteed reagents potassium hydroxide and sodium sulfate (anhydrous), environment analysis grade- 5% water-impregnated silica

gel were all purchased from FUJIFILM Wako Pure Chemicals Corporation (Osaka, Japan). Anth for analytical standard by Sigma-Aldrich (St. Louis, MO, U.S.A.).

3. Sample preparation

Sediment and fecal pellets of *Marphysa* sp. E sensu Abe et al. (2019) were sieved through a 2 mm mesh. The sieved samples were placed in centrifuge bottles (5–20 g of sediment and 2.5 g of fecal pellets), and 50 mL of acetone and 100 μL of surrogate substance mixture solution (1.0 ppm) were added. The samples were then shaken (160 rpm; MMS-3010, TOKYO RIKAKIKAI, Tokyo, Japan) and sonicated (US-2R, AS ONE, Osaka, Japan) for 10 min respectively, and then centrifuged (himac ct6E, HITACHI, Tokyo, Japan) at 1630 ×*g* for 10 min. The same procedure was performed again, followed by concentration to approximately 20 mL with a rotary evaporator (N-1000, TOKYO RIKAKIKAI, Tokyo, Japan). The concentrated solution was supplemented with 50 mL of 1.0 M KOH/ethanol solution and shaken at 36 rpm for 15 h for the sediment and 20 h for the fecal pellet for alkaline degradation. After alkaline degradation, the solution was subjected to liquid-liquid extraction with 50 mL of hexane, and the obtained hexane layer was washed with 50 mL of H_2O. Then the hexane extracted solution was dehydrated with anhydrous sodium sulfate. The solution was concentrated to 1 mL using a rotary evaporator and gentle nitrogen gas blowing. Subsequently, the solution was loaded onto

a 5% water-impregnated silica gel column (10 × 350 mm i.d.), and eluted with 100 mL of 1.0% acetone/hexane solution. The eluent was then concentrated to less than 1 mL with a rotary evaporator and nitrogen gas, 10 μL of *p*-ter (10 ppm) was added, and hexane was added to a volume of 1.0 mL.

4. Instruments and GC-MS conditions

PAHs analysis used a GC-MS (QP-2010, SHIMADZU, Kyoto, Japan) with the following conditions:

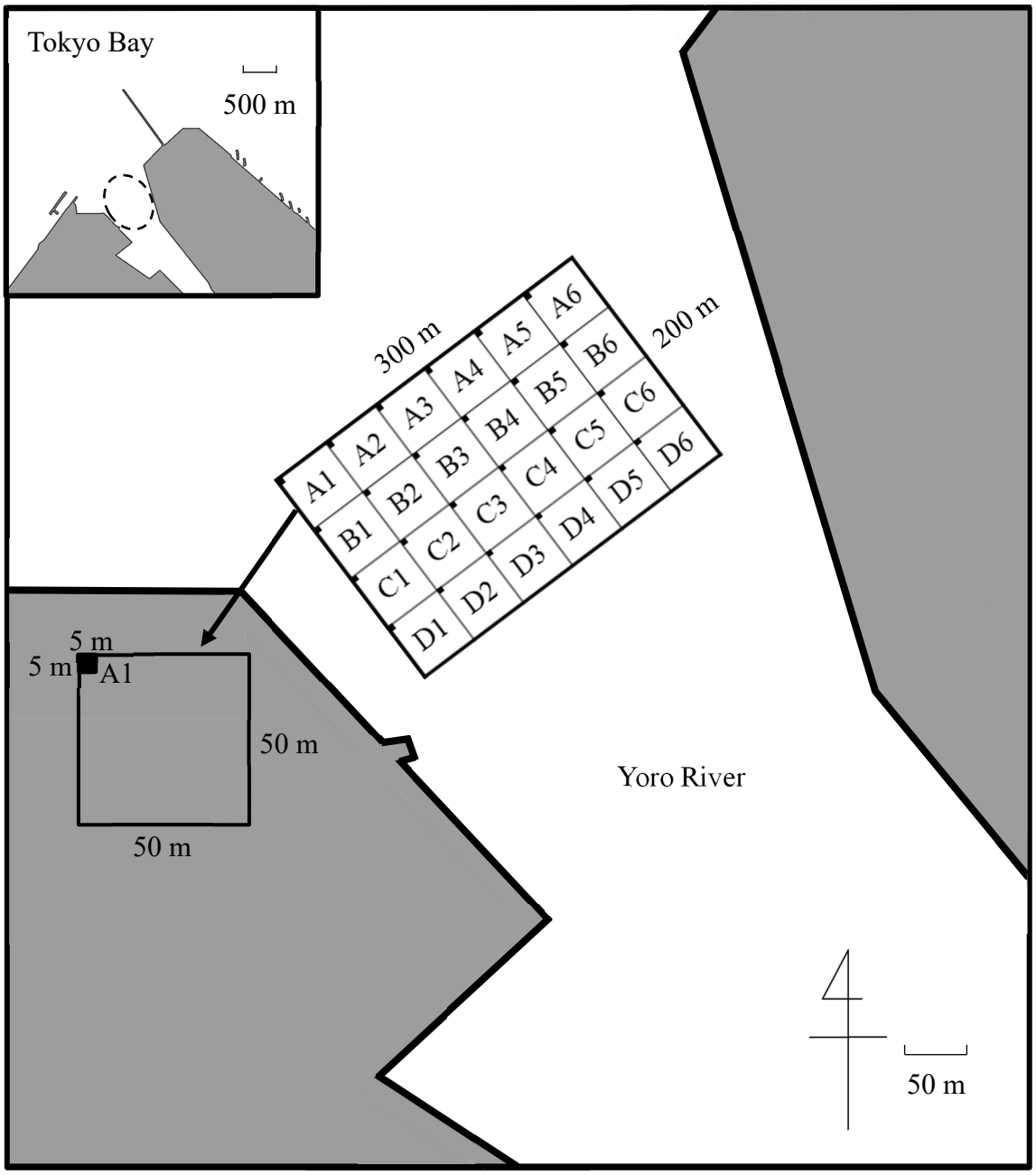
A fused silica capillary column (Rtx®-5MS: 30 m, 0.25 mm i.d., 0.25 μm film thickness, RESTEK, Pennsylvania, USA) was used for the analysis. Helium (99.995% purity) was used as the carrier gas. To ionize the samples, electron ionization (EI) was used with ionization voltage of 70 eV. The sample vaporization chamber, ionization source, and interface temperatures were 320°C, 200°C, and 250°C, respectively. The column temperature was maintained at 50°C for 2 min, then increased to 310°C at a rate of 7°C min^{-1}, and maintained at 310°C for 10 min. Sample injection was in splitless mode with an injection volume of 2.0 μL. Monitoring was performed in selected ion monitoring (SIM) mode to detect the *m*/*z* of the molecular ions of each of the PAHs and PAHs-*d* as the quantitation ion (Onozato et al., 2008).

The quantitative results for each of the PAHs were obtained by considering the dry weight loss of the sample and the recovery rate of PAHs-*d*. The samples (approximately 3 g) were dried using an oven (NDO-400, TOKYO RIKAKIKAI, Tokyo, Japan) at 100°C for 2 h. After drying, the weights of the samples were measured and the dry weight loss of the samples was calculated using the following formula:

Loss of drying (%) = [Wet weight (g-*wet*) – Dry weight (g-*dry*)] / Wet weight (g-*wet*) × 100

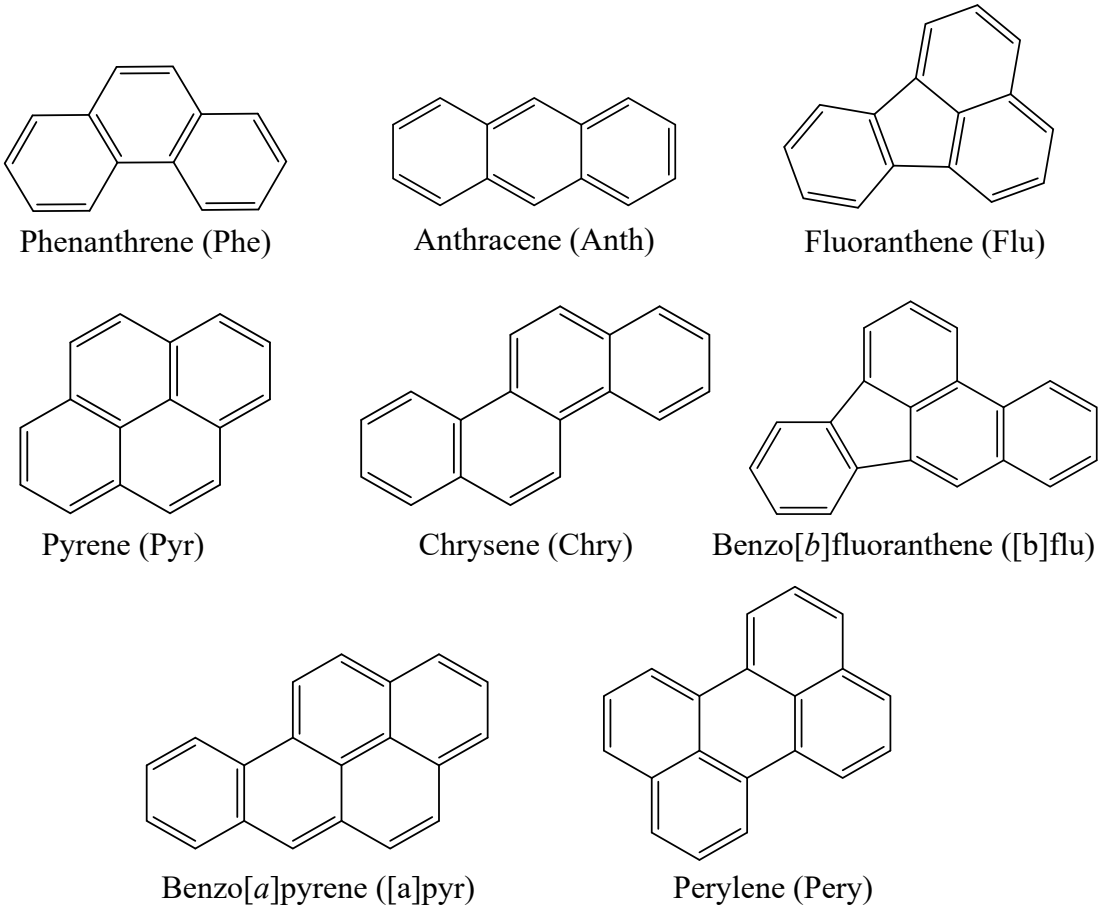
5. Supplementary Figures

5.1



Supplementary Figure S1. The map of quadrat survey area.
The magnification of each section is shown on the left side. One section was 50 m square, and a 5 × 5 m quadrat at top-left corner in the section, shown by the black square, was examined.

5.2



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

6. Supplementary tables

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

	Date of survey			
Code	30-May-22	17-Jun-22	27-Aug-22	8-Oct-22
A1	1	-	-	-
A2	3	-	-	-
A3	6	-	-	-
A4	3	-	-	-
A5	8	-	-	-
A6	10	-	-	-
B1	-	-	-	-
B2	4	1	-	22
B3	2	5	-	7
B4	5	8	-	-
B5	12	20	-	-
B6	11	7	-	-
C1	-	-	-	-
C2	6	0	-	-
C3	5	3	-	-
C4	17	7	4	-
C5	-	4	3	-
C6	-	12	1	-
D1	-	-	-	-
D2	-	-	0	-
D3	-	-	0	-
D4	-	-	1	-
D5	-	-	1	-
D6	-	-	2	-
Total	93	67	12	29
Number of investigated quadrats	14	10	8	2
Maximum	17	20	4	22
Minimum	1	0	0	7
Mean	6.6	6.7	1.5	14.5
Standard deviation	4.5	5.9	1.4	10.6
Maximum density (m^{-2})	0.68	0.8	0.16	0.88

-: not surveyed

6.2 Supplementary Table S2. Physicochemical properties of the target PAHs

PAHs	Chemical formula	Molecular weight	Ring number	Solubility in water (mg / L)	*Log* K_{ow}
Phe	$C_{14}H_{10}$	178	3	1.3 [a]	4.53
Anth	$C_{14}H_{10}$	178	3	0.073 [a]	4.53
Flu	$C_{16}H_{10}$	202	4	0.26 [a]	5.24
Pyr	$C_{16}H_{10}$	202	4	0.14 [a]	5.07
Chry	$C_{18}H_{12}$	228	4	0.0020 [a]	5.77
[b]flu	$C_{20}H_{12}$	252	5	0.0015 [b]	6.52
[a]pyr	$C_{20}H_{12}$	252	5	0.0038 [a]	6.23
Pery	$C_{20}H_{12}$	252	5	0.00040 [a]	6.39

Meador et al. (1995)

[a]Mackay and Shiu (1977)

[b]Dhar et al. (2020)

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

Date.	Samples	Concentrations (mean ± σ)/μg kg-*dry*$^{-1}$								
		Phe	Anth	Flu	Pyr	Chry	[b]flu	[a]pyr	Pery	Total PAHs
2018. 5	Sand (0–5 cm)	12.3 ± 1.6	2.05 ± 0.19	4.08 ± 0.26	5.8 ± 2.2	0.79 ± 0.09	0.67 ± 0.06	0.53 ± 0.09	3.0 ± 0.4	29 ± 5
2019. 8	Sand (30–40 cm)	59 ± 5	3.2 ± 0.3	15.1 ± 1.0	25 ± 5	2.8 ± 0.5	2.6 ± 0.4	2.2 ± 0.4	7.9 ± 0.7	118 ± 13
2019. 9	Sand (0–10 cm)	70 ± 22	6.1 ± 0.8	19 ± 3	17.8 ± 2.1	4.0 ± 2.1	2.3 ± 1.2	2.7 ± 1.1	6.5 ± 0.9	130 ± 30
2018. 5	Reduced mud (30–40 cm)	210 ± 50	24.1 ± 2.0	100 ± 40	100 ± 40	38 ± 12	21.0 ± 1.5	18.5 ± 1.2	99 ± 9	610 ± 160
2018. 7	Reduced mud (30–40 cm)	80 ± 7	16 ± 5	56 ± 5	62 ± 7	36 ± 9	26 ± 7	26 ± 10	108 ± 13	410 ± 60
2019. 8	Reduced mud (0–10 cm)	156 ± 24	28 ± 15	69 ± 8	105 ± 25	27 ± 4	15.6 ± 2.4	13.0 ± 1.7	160 ± 3	570 ± 80
2018. 5	Fecal pellet	98 ± 6	21 ± 7	77 ± 18	122 ± 20	35 ± 6	26 ± 4	22.3 ± 2.2	102 ± 12	500 ± 80
2018. 7	Fecal pellet	137 ± 10	14 ± 4	94 ± 22	95 ± 27	47 ± 8	31 ± 6	29 ± 7	128 ± 8	580 ± 90
2019. 8	Fecal pellet	960 ± 120	58 ± 7	270 ± 50	218 ± 10	49 ± 4	27.9 ± 1.0	28.3 ± 2.6	212 ± 8	1820 ± 200
2019. 9	Fecal pellet	320 ± 40	9.2 ± 1.2	85 ± 7	150 ± 40	23 ± 4	20.8 ± 1.3	21.1 ± 1.4	228 ± 7	860 ± 100

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

A. Sand

	Date						mean	σ
	2018.7	2019.8	2019.9	2019.9	2021.4	2021.9		
$\delta^{13}C$ (‰)	−21.7	−25.0	−23.6	−24.7	−22.3	−26.1	−23.9	1.7
$\delta^{15}N$ (‰)	6.4	5.3	5.2	5.4	5.5	5.0	5.5	0.5

B. Reduced mud

	Date					mean	σ
	2018. 7	2018. 7	2019. 8	2021. 6	2021. 9		
$\delta^{13}C$ (‰)	−26.2	−26.1	−27.4	−28.0	−28.9	−27.3	1.2
$\delta^{15}N$ (‰)	4.1	4.3	3.0	3.0	3.6	3.6	0.6

C. Fecal pellets

	Date					mean	σ
	2018. 7	2019. 8	2019. 9	2021. 7	2021. 9		
$\delta^{13}C$ (‰)	−26.0	−27.3	−27.4	−26.2	−26.5	−26.7	0.6
$\delta^{15}N$ (‰)	4.1	2.9	3.5	3.9	3.5	3.6	0.5

D. *Marphysa* sp. E

	Date						mean	σ
	2018. 7	2019. 9	2021. 10	2021. 10	2022. 8	2022. 8		
$\delta^{13}C$ (‰)	−20.3	−17.9	−21.3	−20.9	−20.1	−20.1	−20.1	1.2
$\delta^{15}N$ (‰)	12.1	13.3	11.0	11.4	11.7	11.8	11.9	0.8

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

	Percentage (%)				
	Very coarse sand (> 1 mm)	Coarse sand (1–0.5 mm)	Medium sand (0.5–0.25 mm)	Fine sand & Very fine sand (0.25–0.063 mm)	Silt & Clay (< 0.063 mm)
Sand	0.03	1	22	77	0.1
Reduced mud	0.05	1	8	47	44
Fecal pellet	0.5	5	13	41	40

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