Supporting Information for "Spatiotemporal distribution of hydrophobic organic contaminants in spiked-sediment toxicity tests: Measuring total and freely dissolved concentrations in pore and overlying water"

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List of materials

ADDITIONAL DESCRIPTIONS FOR MATERIALS AND METHODS

Determination of $K_{PDMS/water}$ for chlorpyrifos (CPS)

A 10-mL glass spitz tube received one piece of pre-cleaned 4-cm PDMS fiber and 10 mL of water (pH 4). pH was reduced to 4 with HCl in advance to minimize hydrolysis of CPS in water. The tube was spiked with 5 μL of 10 mg/L methanolic stock solution of CPS, closed with a glass stopper, and immediately shaken by hand a few times. Twenty replicate tubes were prepared this way and placed on a horizontal shaker (150 rpm) at 24°C. Five tubes each were retrieved 2, 4, 7, and 12 days after. Water (5 mL) was sampled and extracted with 2 mL of *n*-hexane solution containing 20 μg/L CPS-d_{10}. The PDMS fiber was taken with stainless-steel tweezers, blotted dry with a lint-free tissue, and extracted with 1 mL of the same *n*-hexane solution as above. The extracts were subjected to GC/MS analysis.

Semi flow-through water-renewal system setup

A semi flow-through water renewal system was developed according to Zumwalt et al. (1994) with slight modifications. The stainless tank (21 cm × 35 cm × 15 cm height) with eight 1-cm holes was used as a water delivery chamber. Eight holes were connected with tubes and needles (Fig. S2A). The length of the connecting tube and the needle gauge (i.e., needle diameter) can be changed to adjust water-renewal rates. In this study, a 26G needle (NN-2613S, Terumo Corporation) was used and the water drop rate from the needle was measured to be 680 ± 33 μL/min, which corresponds to the rate at which one volume water exchange (220 mL) can be completed in 5.3 h. Over the 10-day tests, 1.76 L of dechlorinated tap water (corresponding to one volume water exchange) was delivered to the stainless tank every morning and evening, with an exception that 7.04 L water (corresponding to four volumes) was delivered to each tank at Day -1.

A 300 mL tall glass beaker with a diameter of 67 mm and a height of 135 mm (82-0024, Iwaki & Co., Ltd.) was used as an exposure chamber. Each beaker has a 6.7-mm hole at the height of 275 mL (Figure S2B). And each hole was covered with a stainless mesh (mesh size: 500 μm) to prevent amphipods from escaping from the exposure beaker (Fig S2C).

Growth measurement

In Runs 3 and 4, the growth of surviving amphipods after 10 days of exposure was evaluated. At the start of exposure (Day 0), 30 additional juvenile amphipods (7-9 days old) were fixed with liquid nitrogen and used for measurement of body length and dry weight. Also, at Day 10, surviving amphipods were collected, transferred to a beaker containing overlying water for 1 h, and fixed with liquid nitrogen. The body length was measured by taking images with a stereomicroscope (M165C,

Leica) and a digital camera (MC190, Leica) and analyzing with Image J software (ver 1.52, National Institutes of Health). The analysis with Image J was repeated three times for the same individuals and the average values were used for further analysis. The body length was defined as the length along the mid-line between the tip of the rostrum and the end of the telson. After taking images, dry weight of amphipods per beaker was determined by weight measurements before and after 24-h drying at room temperature in a desiccator.

Bioaccumulation measurement

In Run 3, dried amphipods after weight measurement were used for the measurement of benzo[*a*]pyrene (BaP) accumulation. The amphipods were transferred to a 1.5 mL polypropylene tube containing 1.0 mL of acetonitrile and homogenized with glass beads and a homogenizer (μT-12, Taitec) at 3200 rpm for 3 min. The homogenates were filtered with a PTFE membrane (pore size: 0.45 μm), and used for the measurement of BaP concentration with HPLC. The recovery ratio of BaP by extraction was 106 ± 5% (n = 4) from spiked amphipods in the culture aquarium.

Bioconcentration factor (BCF, L/kg-wet) for BaP was calculated based on measured BaP concentration in the amphipods (C_{bio}, mg/kg-dry) and aqueous concentrations (C_{water} = C_{diss} or C_{free}, mg/L) according to the equation S1.

$$BCF = (C_{bio} \times 0.25)/C_{water} \quad \text{(equation S1)}$$

where 0.25 is the ratio of dry to wet weight of *H. azteca* (Othman and Pascoe 2001).

Instrumental analysis

HPLC: Either of the following two high performance liquid chromatography (HPLC) systems was used for the quantification of phenanthrene (Phe), pyrene (Pyr), and BaP.

(I) LC-VP series HPLC system (Shimadzu Corp.): The mobile phase was a mixture of acetonitrile and Milli-Q water (80:20 v/v) at a flow rate of 0.35 mL/min. The target chemicals were separated with a guard column (GVP-ODS, Shimadzu Corp.) and a silica-based C18 column (150 mm × 2.0 mm i.d., VP-ODS, Shimadzu Corp.) at 40°C, and quantified with a fluorescence detector (RF-10A XL, Shimadzu Corp.). The excitation/emission wavelengths used were 265/380 nm for Phe and Pyr, and 365/410 nm for BaP.

(II) Prominence HPLC system (Shimadzu Corp.): The mobile phase was a mixture of acetonitrile and Milli-Q water at a flow rate of 1 mL/min. The acetonitrile content was initially 85 % (v/v) (2.5 min hold), increased to 100% over 2 min (2.5 min hold), and set back to 85% over 0.5 min. An Eclipse PAH column (150 mm × 4.6 mm i.d., 3.5 μm particle size. Agilent Technologies) was used for

separation. The PAHs were detected by a fluorescence detector (RF-20A XS, Shimadzu Corp.). The excitation/emission wavelengths used were 260/362 nm for Phe, 260/390 nm for Pyr, and 365/450 nm for BaP. The instrumental detection limits of Phe, Pyr, and BaP were 0.02, 0.12, and 0.02 μg/L, respectively, as estimated from the residuals and slopes of regression lines of standard solutions.

GC/MS: Gas chromatography mass spectrometry (GC/MS) system (7890A/5975C, Agilent technologies) equipped with a HP-5ms UI column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies) was used for the CPS quantification. Ultra-pure helium gas was used as carrier gas at a flow rate of 1.2 mL/min. The ion source and transfer line temperatures were 230°C and 300°C, respectively. The oven temperature was programmed to increase from 70°C (held for 1 min) to 210°C at a rate of 30°C/min, to 240°C at a rate of 5°C/min, and then to 280°C (held for 1 min) at a rate of 30°C/min. The MS was operated in the selected ion monitoring mode, where two quantifier ions (m/z 314 and 324 for CPS and CPS-d_{10}, respectively) and one qualifier ion (m/z 197 for CPS) were monitored. The samples were injected into the GC with an MPS2 autosampler system (Gerstel) with an injection volume of 1 to 100 μL. The programable temperature vaporization injector (CIS4, Gerstel) was used to vent excess solvent. The sample was introduced into the injector at 3.2 μL/min while the injector temperature was kept at 70°C. When the sample introduction was completed, the vent valve was opened, and the injector was heated to 250°C at a rate of 12°C/min. The instrumental detection limits of CPS were 0.09 μg/L, as estimated from the residuals and slopes of regression lines of standard solutions.

TOC-L: Concentrations of dissolved organic carbon (DOC) in the filtered water samples were measured with a total organic carbon (TOC) analyzer (TOC-L, Shimadzu Corp.) using non-purgeable organic carbon (NPOC) method. TOC content of sediment samples was determined with TOC-L equipped with a solid sample module (SSM-5000A, Shimadzu Corp.), after freeze-drying.

96-h water-only test of BaP

Water-only toxicity tests of BaP were performed according to the standardized protocol (Environment and Climate Change Canada 2017). BaP solution was prepared by adding BaP stock solution dissolved in N,N'-dimethylformamide (DMF) to dechlorinated tap water, achieving the final DMF concentration below at 0.01% v/v. Ten juvenile amphipods (3–5 days old) were exposed to 200 mL of the prepared BaP solution in 300 mL tall glass beaker at five nominal concentrations (3.125, 6.25, 12.5, 25, 50 μg/L) with three replicates (totally 30 organisms). The DMF control treatment was

also performed. The exposure beakers were kept at 23°C under a photoperiod of 16 hours of light and 8 hours of darkness. To maintain the BaP concentration, substrate was not added. The amphipods were fed with 0.5 mL of yeast-cerophyl-trout chow (YCT) (Recentec) at the start and after 48 h of exposure. Water temperature and DO were checked every day, and pH and conductivity were checked at the start and end of exposure. Water samples were taken using a Pasteur pipette, diluted with acetonitrile, filtered with a PTFE membrane filter (pore size: 0.45 μm), and used for the measurement of total aqueous BaP concentration (C_{total}) with HPLC. The measured C_{total} was 78 ± 4% of the nominal concentrations at the start of exposure. Time-weighted average (TWA) concentrations (OECD 2012) were calculated based on the measured values (after 0 and 96 h of exposure) and used for the estimation of LC50. The number of survivals was recorded every 24 h and the dead individuals were removed.

Statistical analysis

The non-observed effect concentration (NOEC) was estimated using a one-tailed Dunnett's test implemented in the multcomp R package (ver.1.4-16) (Hothorn et al. 2008). For analysis of body length, a one-tailed Dunnett's test was applied after the fitting of the following linear mixed model (equation S2) using the nlme R package (ver.3.1-152) (Pinheiro et al. 2021) to separate the effects of differences in exposure beakers.

$$Body\ length = \beta_0 + \beta_{1,i} Concentration_i + r_j \quad \text{(equation S2)}$$

where β_0 and $\beta_{1,i}$ are regression coefficients and r_j is a random effect with mean of 0 and accounts for variability associated to exposure beakers (j). Concentration is a dummy variable taking a value of 0 or 1 and denotes nominal sediment concentration (C_{sed}).

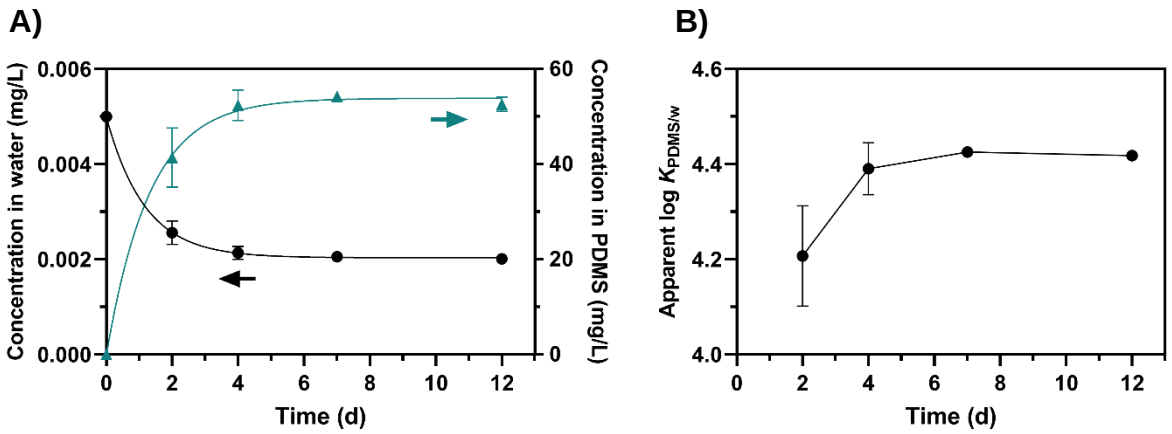

Figure S1. A) Sorption kinetics of CPS to PDMS fiber. Concentration in water (black circles) and concentration in PDMS fiber (green triangles) are shown. Dots and error bars represent the mean and the standard deviation (n =5), respectively. B) Time course of the partition coefficient between PDMS and water ($K_{PDMS/w}$). The mean of log $K_{PDMS/w}$ values from all replicates of Days 7 and 12 (4.42 ± 0.01) was used for derivation of C_{free}.

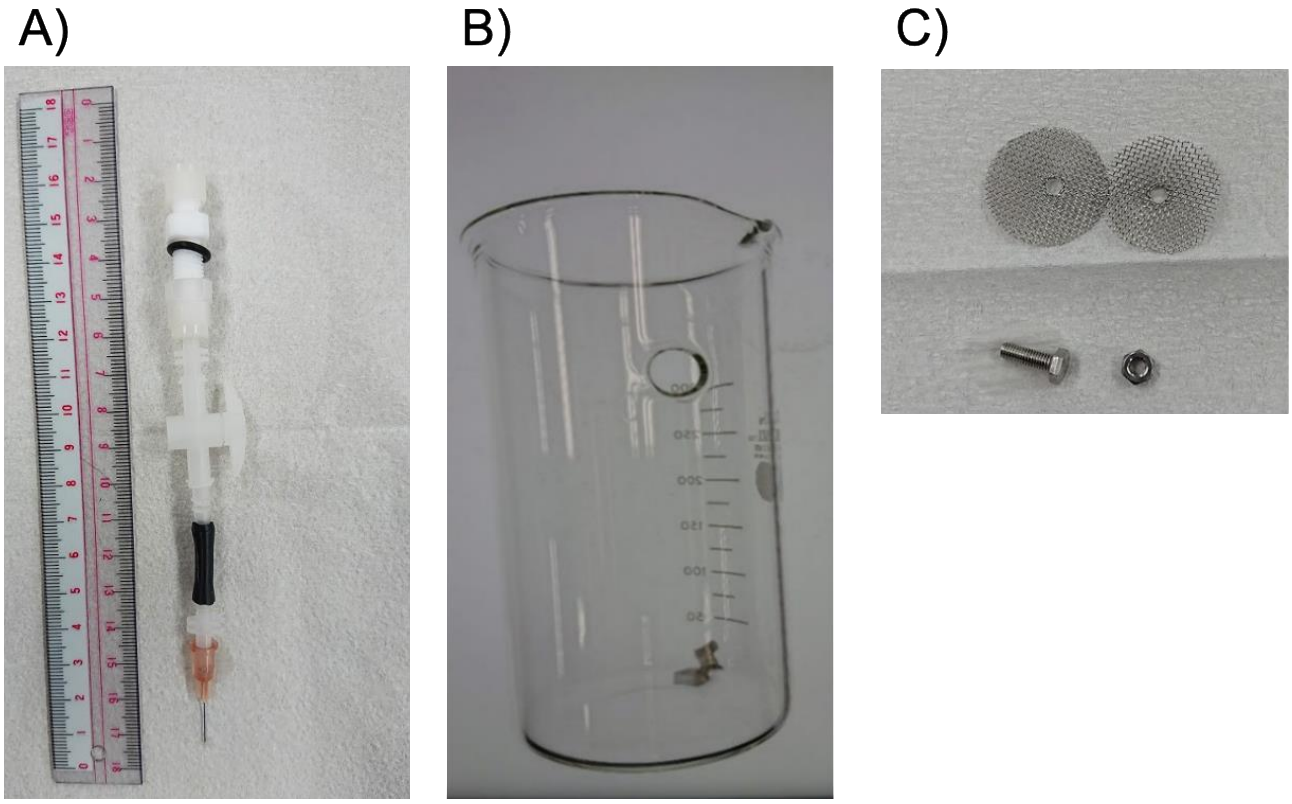

Figure S2. Apparatus used in semi flow-through water renewal system. A) Needle and connecting tube. B) Exposure glass beaker with a 6.7 mm (i.d.) hole. C) Stainless-steel mesh (opening size: 500 μm), screw, and nut to cover an overflow hole of glass beaker.

Table S1. Details of test conditions in each Run.

	Tested chemicals	Aging periods of spiked sediment	Aging temperature	Length and number of PDMS fibers per beaker		Nominal spiked concentrations (mg/kg-dry)	Number of beakers per concentration
				$C_{free,pore}$	$C_{free,over}$ or $C_{free,intf}$		
Run 1	Phe, Pyr, BaP	20 days	Room temperature (25°C)	4 cm × 3	7 cm × 2	5 and 50	2
Run 2	Phe, Pyr, BaP	14 days	6 to 8°C	3 cm × 2	3 cm × 2	5 and 50	3
Run 3	BaP	14 days	Room temperature	4 cm × 4	Not added	0 (control), 50, 100, 200, 400	6
Run 4	CPS	4 days	6 to 8°C	4 cm × 1	7 cm × 2	0 (control), 0.01, 0.032, 0.1, 0.32, 1	6

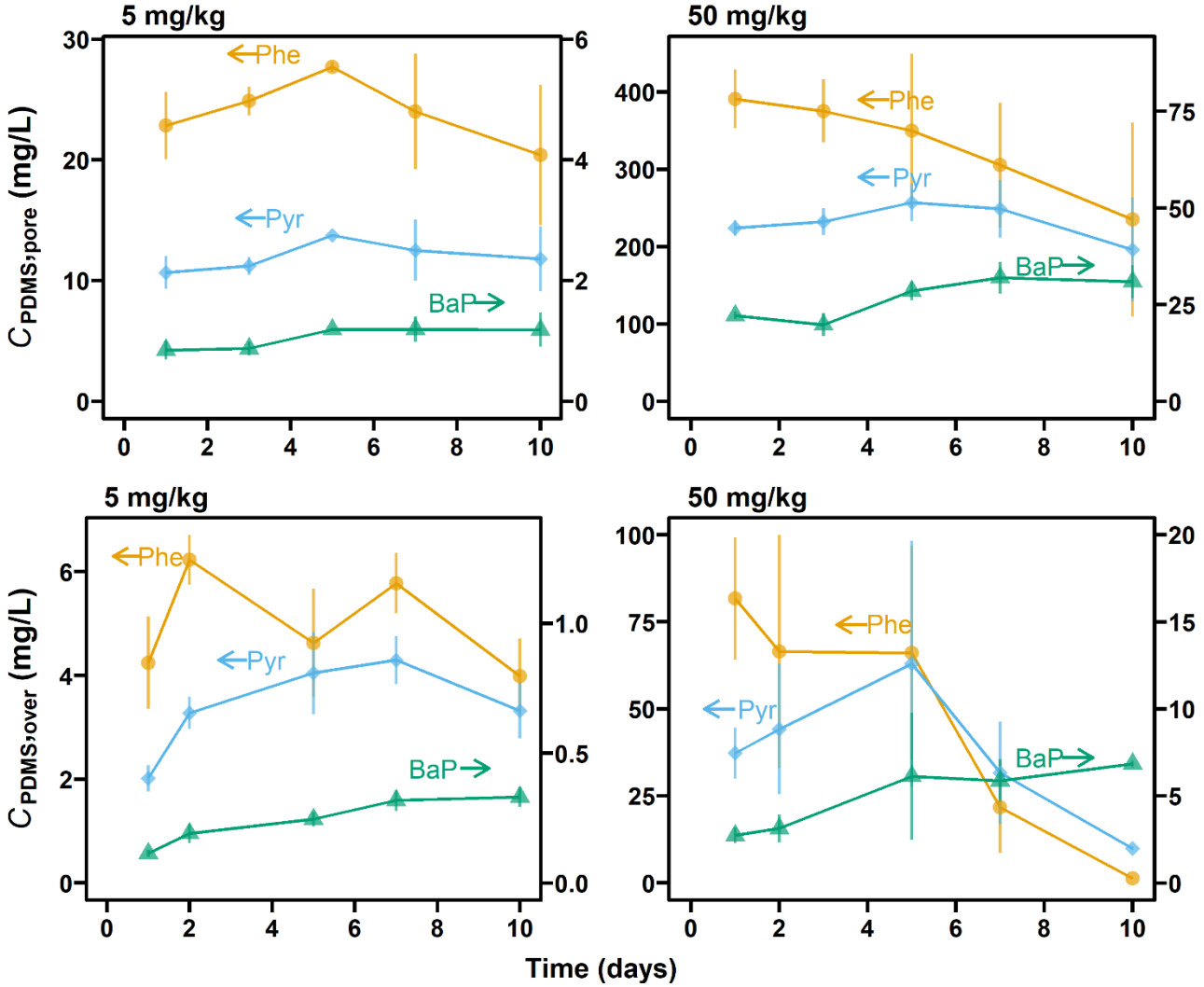

Figure S3. Concentration-time curves for Phe (yellow circles), Pyr (blue diamonds), and BaP (green triangles) in PDMS fiber (Run 1; nominal sediment concentration, 5 and 50 mg/kg-dry). Error bars represent standard deviations (n = 4–6). Top and bottom panels represent the data for pore and overlying water, respectively. Arrows indicate the axes that the data refer to. C_{PDMS} in pore water of Phe and Pyr were unexpectedly low at 50 mg/kg after 7 days for an unknown reason.

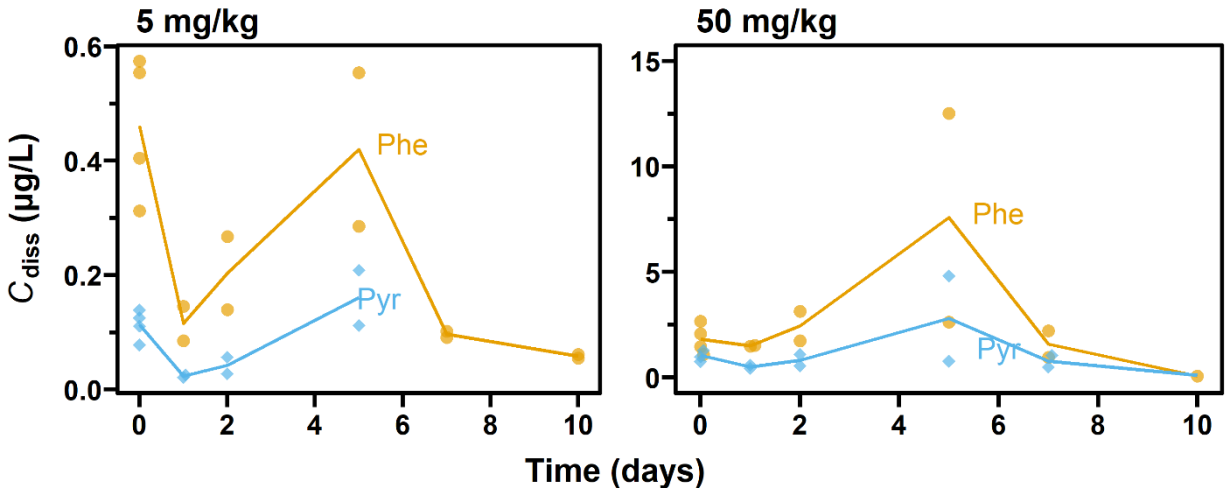

Figure S4. Temporal changes of total dissolved concentrations (C_{diss}) of Phe and Pyr in Run 1. BaP concentrations were mostly below the detection limits and thus are not shown. Lines represent the mean concentrations.

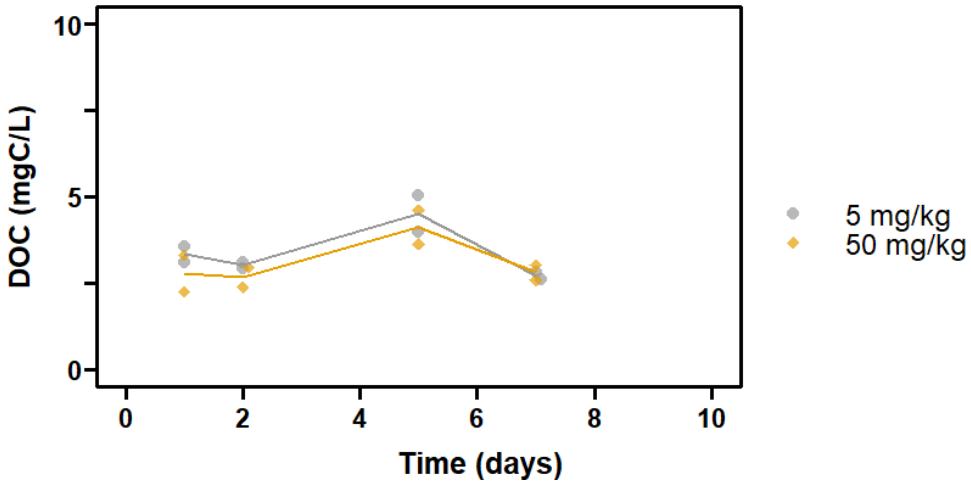

Figure S5. Measured dissolved organic carbon (DOC) concentrations in overlying water in Run 1. Lines represent the mean concentrations.

Table S2. Recovery ratio of three PAHs from spiked sediment in Run 1.

	Ratio of measured to nominal sediment concentration (%)					
	5 mg/kg-dry			50 mg/kg-dry		
	Day 1	Day 10	Average	Day 1	Day 10	Average
Phe	43 ± 10	50 ± 3	46 ± 5	44 ± 1	46 ± 3	45 ± 1
Pyr	45 ± 7	53 ± 4	49 ± 4	46 ± 0	57 ± 4	51 ± 1
BaP	54 ± 9	59 ± 3	57 ± 5	57 ± 1	62 ± 3	60 ± 1

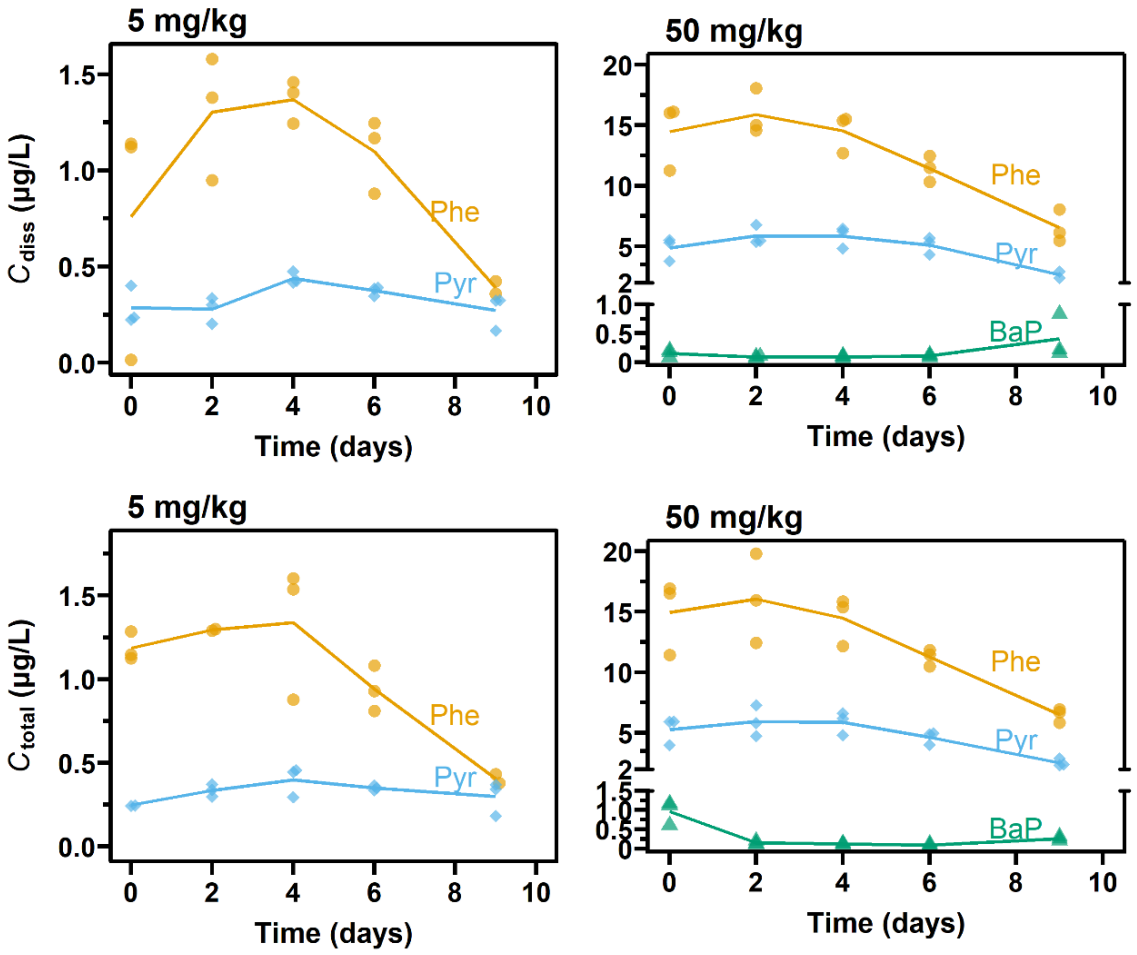

Figure S6. Temporal changes of total dissolved concentrations (C_{diss}, top panels) and total aqueous concentrations (C_{total}, bottom panels) of Phe (yellow circles), Pyr (blue diamonds), and BaP (green triangles) in Run 2. BaP concentrations were below the detection limits with the sediment prepared at 5 mg/kg-dry.

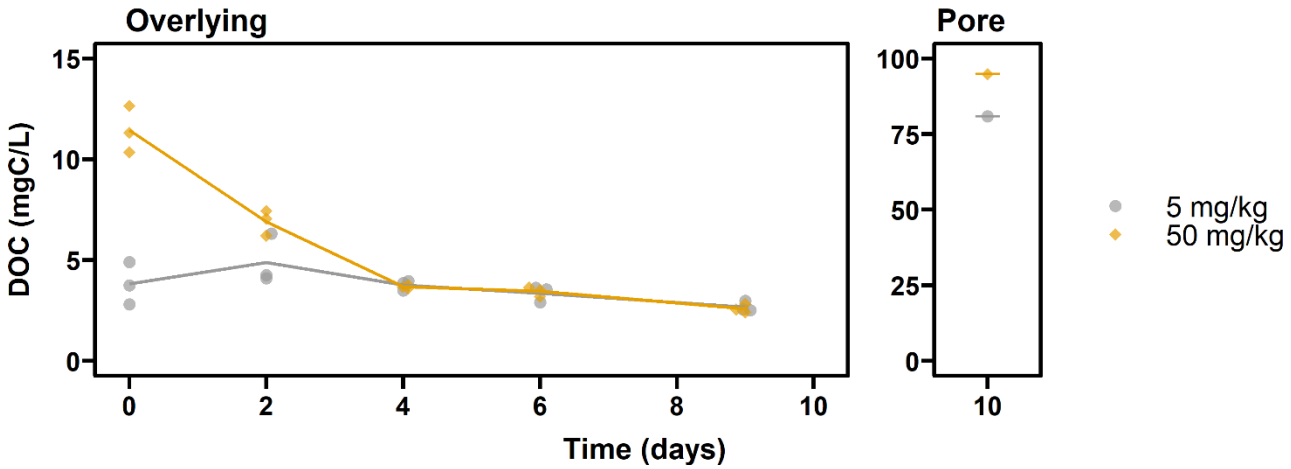

Figure S7. Measured dissolved organic carbon (DOC) concentrations in Run 2. Left and right panels represent the data for overlying and pore water, respectively. Dotted lines represent the mean concentrations.

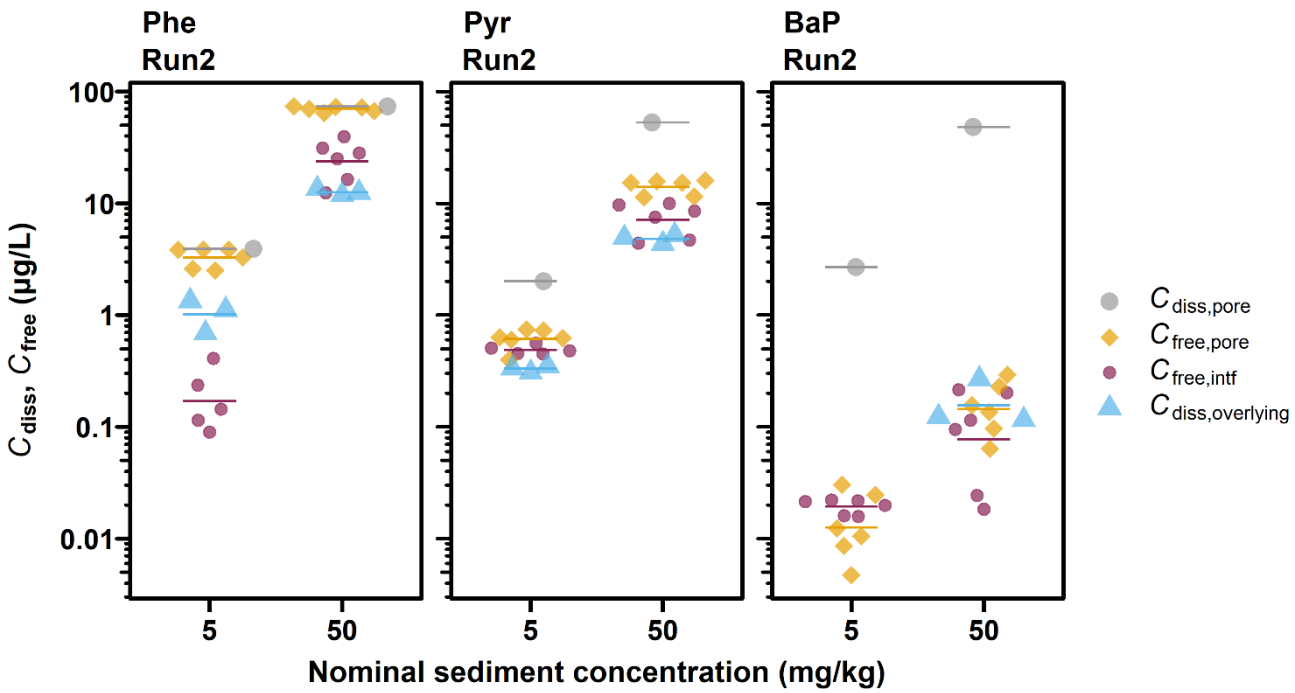

Figure S8. Measured $C_{diss,pore}$, $C_{diss,over}$, $C_{free,pore}$, and $C_{free,intf}$ of Phe, Pyr, and BaP in Run 2. $C_{diss,over}$ of BaP at 5 mg/kg was under the detection limits.

Table S3. Recovery ratio of PAHs from spiked sediment in Run 2.

	Ratio of measured to nominal sediment concentration (%)					
	5 mg/kg-dry			50 mg/kg-dry		
	Day -1	Day 10	Average	Day -1	Day 10	Average
Phe	42 ± 3	33 ± 3	38 ± 3	50 ± 2	35 ± 9	42 ± 5
Pyr	51 ± 2	39 ± 2	45 ± 2	57 ± 2	43 ± 10	50 ± 6
BaP	78 ± 3	63 ± 5	71 ± 4	71 ± 2	51 ± 4	61 ± 3

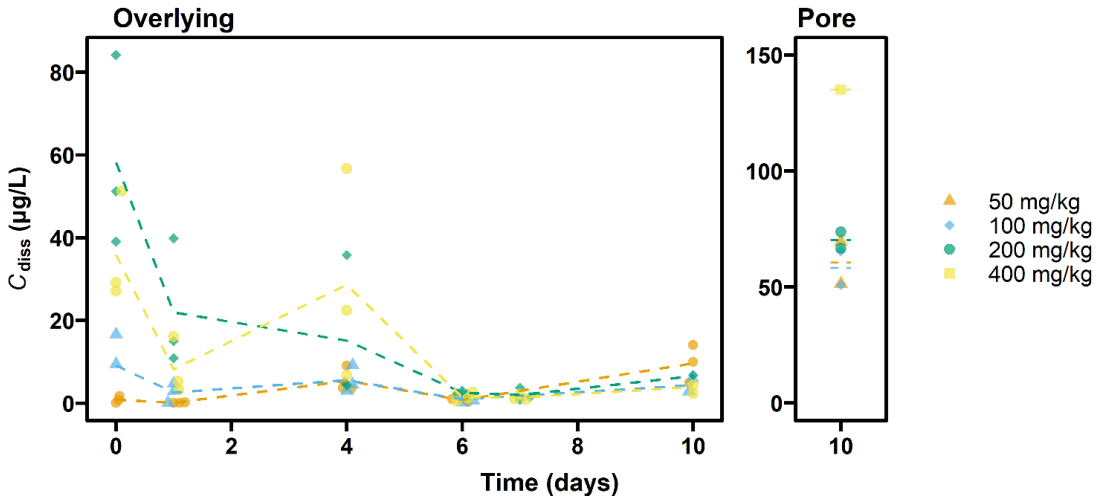

Figure S9. Measured total dissolved concentrations (C_{diss}) of BaP in Run 3. Left and right panels represent the data for overlying and pore water, respectively. Dotted lines represent the mean concentrations.

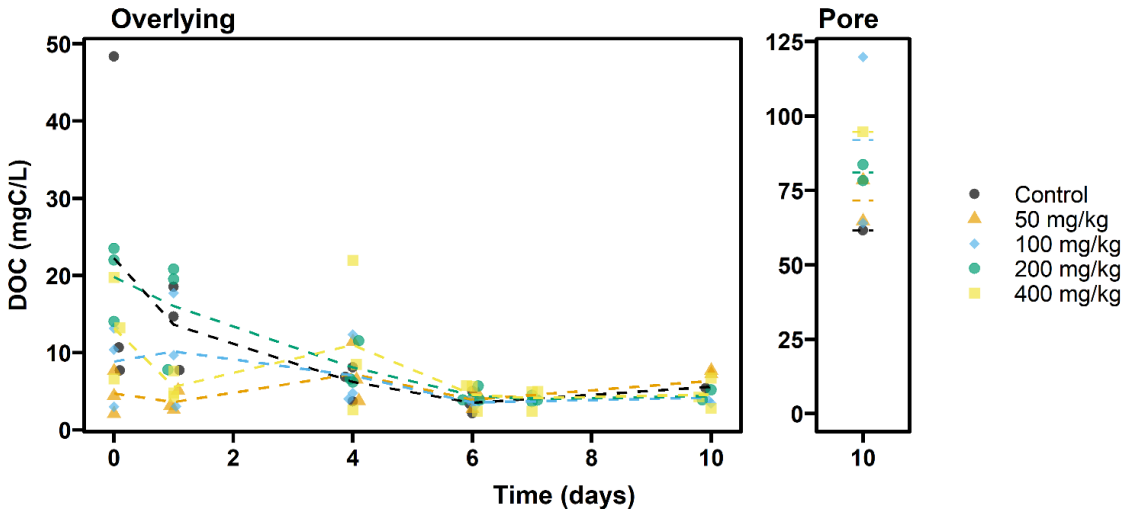

Figure S10. Measured dissolved organic carbon (DOC) concentrations in Run 3. Left and right panels represent the data for overlying and pore water, respectively. Dotted lines represent the mean concentrations.

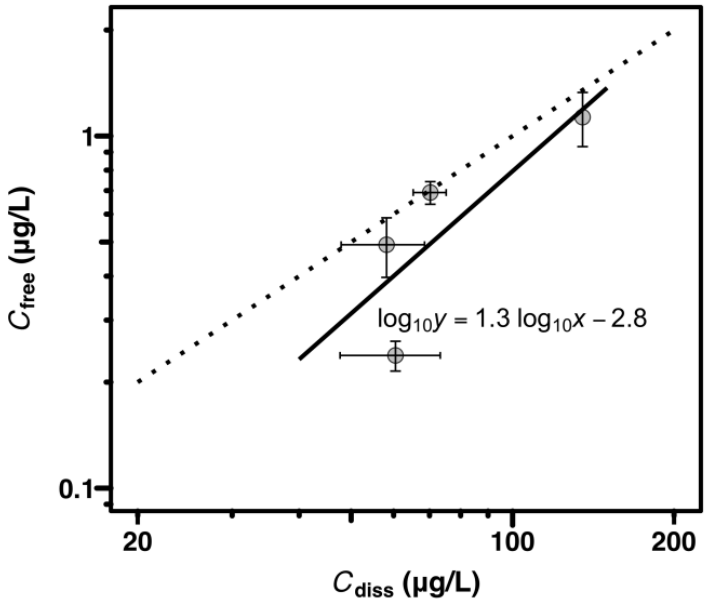

Figure S11. Relationship between total dissolved concentration (C_{diss}) and freely dissolved concentration (C_{free}) of BaP in pore water in Run 3. The solid line indicates the regression line and the dotted line indicates a ratio of 100:1.

Table S4. Recovery ratio of BaP from spiked sediment and measured total organic carbon content in Run 3.

	BaP at Day 10		Total organic carbon content (%)	
Nominal BaP concentration (mg/kg)	Measured BaP concentration (mg/kg)	Ratio of measured to nominal (%)	Day -1	Day 10
50	18 ± 8	36 ± 16	1.6	1.2 ± 0.4
100	54 ± 6	53 ± 6	2.2	1.8 ± 0.2
200	87 ± 12	44 ± 6	2.0	1.2 ± 0.2
400	228 ± 55	57 ± 14	1.5	1.6 ± 0.4

BaP measurement was done using sediment samples collected after 10 days of the toxicity experiment. Mean ± standard deviation (n = 3), except only mean value for total organic carbon content at Day -1 (n = 1).

Table S5. Measured water quality in overlying water in Run 3.

	Temperature (°C)	DO (mg/L)	pH	Conductivity (mS/m)	Total Ammonia concentration (mgN/L)
Control	22.8 ± 0.3	8.2 ± 0.7	8.0 ± 0.2	33.3 ± 2.2	0.91 ± 0.74
50 mg/kg	22.8 ± 0.4	8.3 ± 0.4	7.9 ± 0.2	34.7± 0.9	0.36 ± 0.16
100 mg/kg	22.8 ± 0.3	8.1 ± 0.8	7.9 ± 0.2	34.9 ± 0.8	0.84 ± 0.78
200 mg/kg	23.0 ± 0.2	8.2 ± 0.7	7.8 ± 0.2	35.1 ± 0.7	0.99 ± 0.88
400 mg/kg	23.2 ± 0.3	8.1 ± 0.7	7.9 ± 0.2	34.6 ± 1.2	0.47 ± 0.33

Mean ± standard deviation.

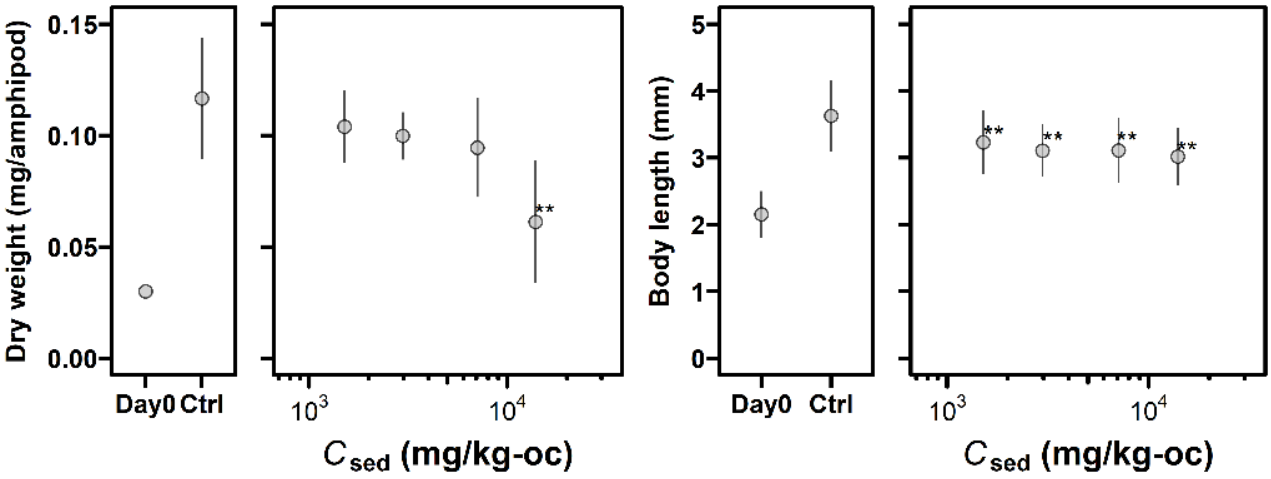

Figure S12. Measured sediment BaP concentration (C_{sed}) vs dry weight and body length in Run 3. Asterisks indicate statistically significant differences from the control (*: $p < 0.05$, **: $p < 0.01$, Dunnett's test).

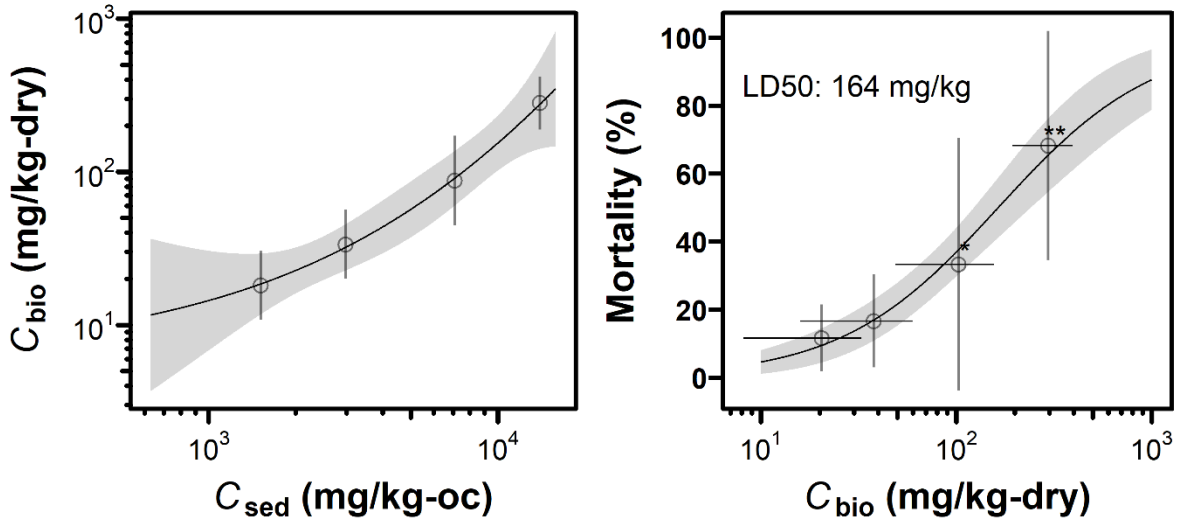

Figure S13. BaP concentration in surviving amphipods (C_{bio}) in comparison to sediment concentration (C_{sed}) and mortality in Run 3. Asterisks indicate statistically significantly different mortality from the control (*: $p < 0.05$, **: $p < 0.01$, Dunnett's test). Shaded areas are the 95% CI of regression curves.

Table S6. Comparison of bioconcentration factors (BCF) for BaP in spiked-sediment and water-only tests.

	Spiked-sediment test [a] (This study)			Water-only test [b] (Schlechtriem et al. 2019)
	$C_{diss,over}$	$C_{diss,pore}$	$C_{free,pore}$	C_{free}
log BCF	3.0 ± 0.5	2.2 ± 0.3	4.4 ± 0.1	3.5 ± 0.1 (Male) 3.5 ± 0.4 (Mixture of male and female)

Mean ± standard deviation. Unit: L/kg-wet. a) BCF in this study was estimated from measured total and freely dissolved concentration (C_{diss} and C_{free}) in Run 3. b) Kinetic BCF without lipid normalization is shown.

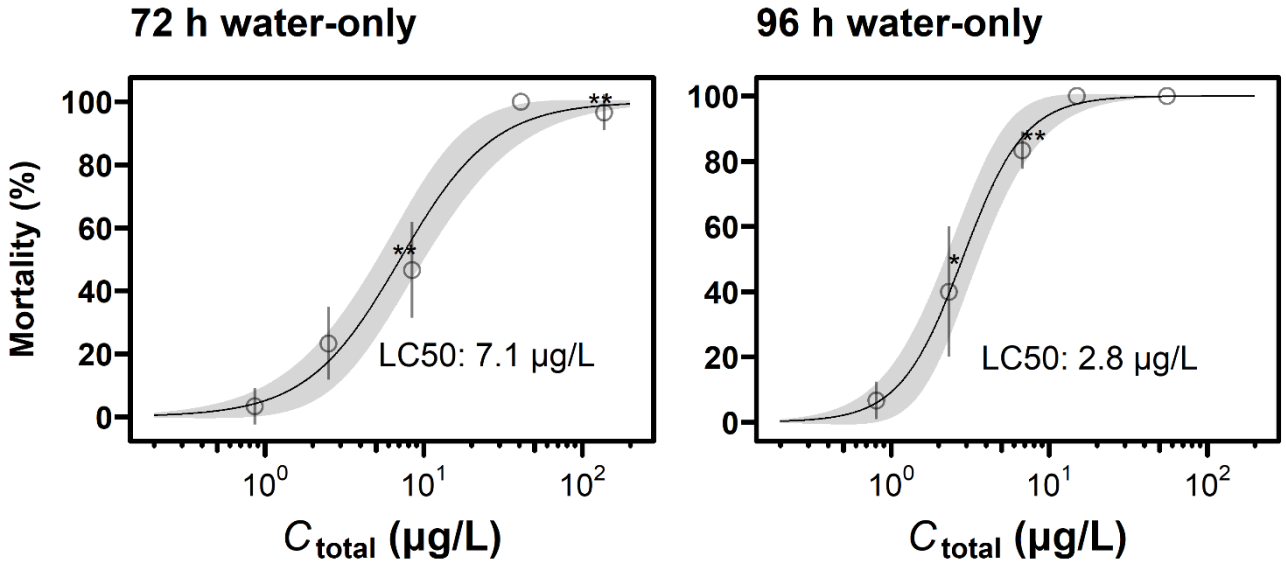

Figure S14. Results of 96-h water-only toxicity test of BaP using solvent spiking methods. Left and right panels represent the data at after 72 and 96 h of exposure, respectively. The X axis shows the time-weighted average (TWA) of total aqueous BaP concentrations (C_{total}). Asterisks indicate statistically significantly different mortality from the control (*: $p < 0.05$, **: $p < 0.01$, Dunnett's test). Shaded areas are the 95% CI of regression curves. The LC50 values were estimated to be 7.1 µg/L (95% CI: 4.7−9.5) and 2.8 µg/L (95% CI: 2.1−3.5) for 72 h and 96 h of exposure, respectively.

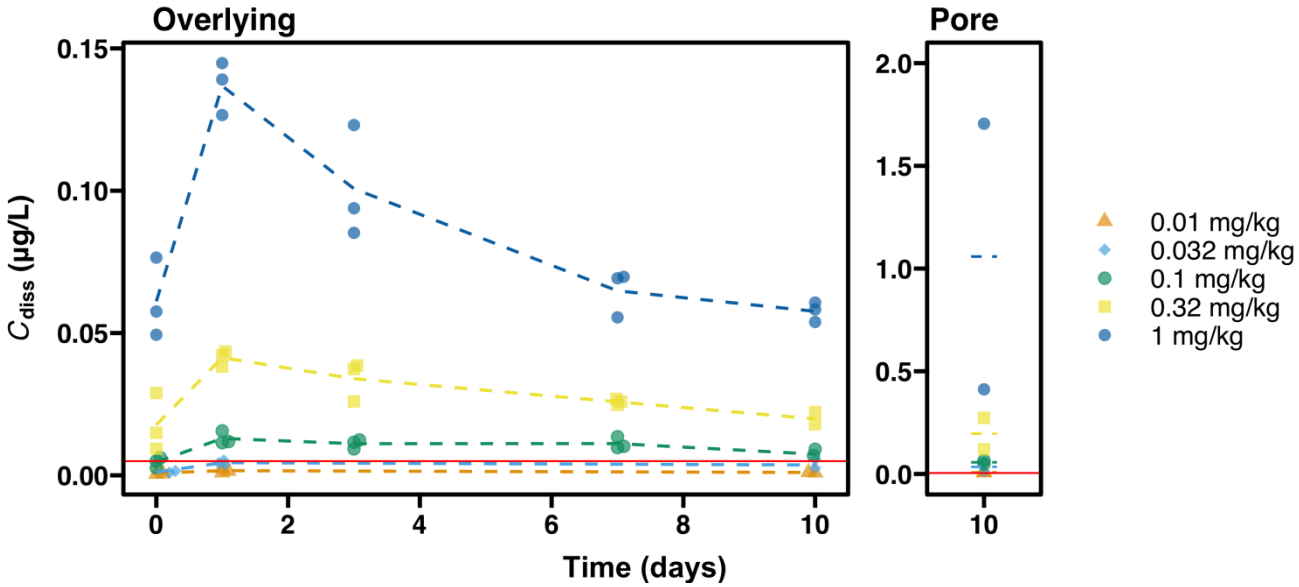

Figure S15. Measured total dissolved concentration (C_{diss}) of CPS in Run 4. Dotted lines represent the mean concentrations. The red solid line indicates the limit of quantification.

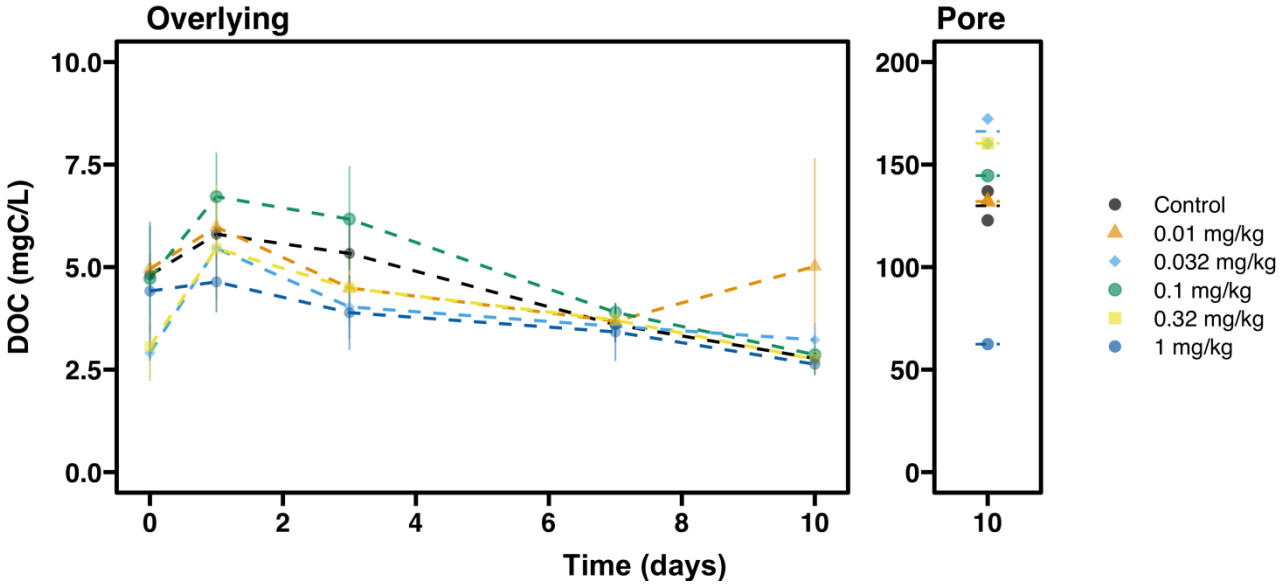

Figure S16. Measured dissolved organic carbon (DOC) concentrations in Run 4. Error bars represent standard deviations (n = 3).

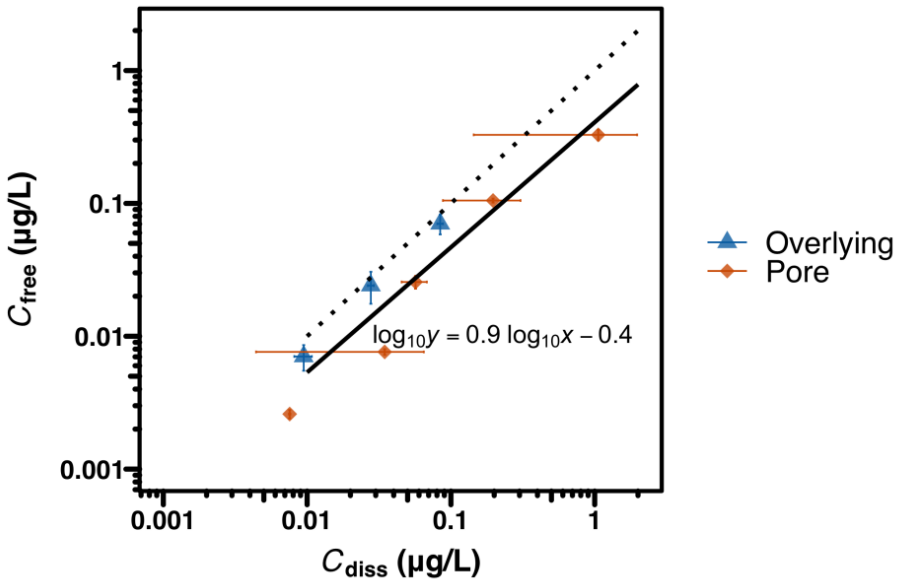

Figure S17. Relationship between total dissolved concentration (C_{diss}) and freely dissolved concentration (C_{free}) of CPS in overlying and pore water in Run 4. The solid line indicates the regression line for the data measured in pore water and the dotted line indicates a ratio of 1:1.

Table S7. Recovery ratio of CPS from spiked sediment in Run 4.

	Measured CPS concentration (mg/kg)			Ratio of measured to nominal (%)		
Nominal CPS concentration (mg/kg)	Day -1	Day 10	Average	Day -1	Day 10	Average
Control	Not detected	Not detected	Not detected	Not applicable	Not applicable	Not applicable
0.01	0.007 ± 0.000	0.006 ± 0.000	0.007 ± 0.000	71 ± 3	64 ± 3	68 ± 3
0.032	0.026 ± 0.004	0.020 ± 0.000	0.023 ± 0.002	82 ± 14	63 ± 0	75 ± 7
0.1	0.062 ± 0.003	0.053 ± 0.009	0.057 ± 0.006	62 ± 3	53 ± 9	58 ± 6
0.32	0.188 ± 0.030	0.192 ± 0.030	0.190 ± 0.030	59 ± 1	60 ± 1	59 ± 1
1.0	0.741 ± 0.072	0.610 ± 0.064	0.675 ± 0.068	74 ± 7	61 ± 6	68 ± 7

Mean ± standard deviation (n = 3).

Table S8. Measured total organic carbon content in Run 4.

	Measured total organic carbon content (%)		
Nominal CPS concentration (mg/kg)	Day -1	Day 10	Average
Control	2.2 ± 0.1	2.0 ± 0.2	2.1 ± 0.2
0.01	2.3 ± 0.1	2.3 ± 0.0	2.3 ± 0.0
0.032	1.5 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
0.32	2.1 ± 0.2	2.0 ± 0.0	2.0 ± 0.1
1.0	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1

Mean ± standard deviation (n = 3).

Table S9. Measured water quality in overlying water in Run 4.

	Temperature (°C)	DO (mg/L)	pH	Conductivity (mS/m)	Total Ammonia concentration (mgN/L)
Control	22.6 ± 0.3	8.3± 0.4	7.9 ± 0.1	28.5 ± 2.7	0.44 ± 0.20
0.01 mg/kg	22.4 ± 0.3	8.2 ± 0.5	7.9 ± 0.2	27.0 ± 0.3	0.27 ± 0.04
0.032 mg/kg	22.7 ± 0.4	8.2 ± 0.4	7.9 ± 0.2	27.3 ± 0.2	0.31 ± 0.04
0.1 mg/kg	22.7 ± 0.4	8.3 ± 0.4	7.9 ± 0.1	27.2 ± 0.3	0.26 ± 0.00
0.32 mg/kg	23.1 ± 0.4	8.2 ± 0.4	7.9 ± 0.1	27.0 ± 0.5	0.85 ± 0.69
1 mg/kg	22.9 ± 0.4	8.3 ± 0.3	7.8 ± 0.1	27.1 ± 0.2	0.21 ± 0.01

Mean ± standard deviation.

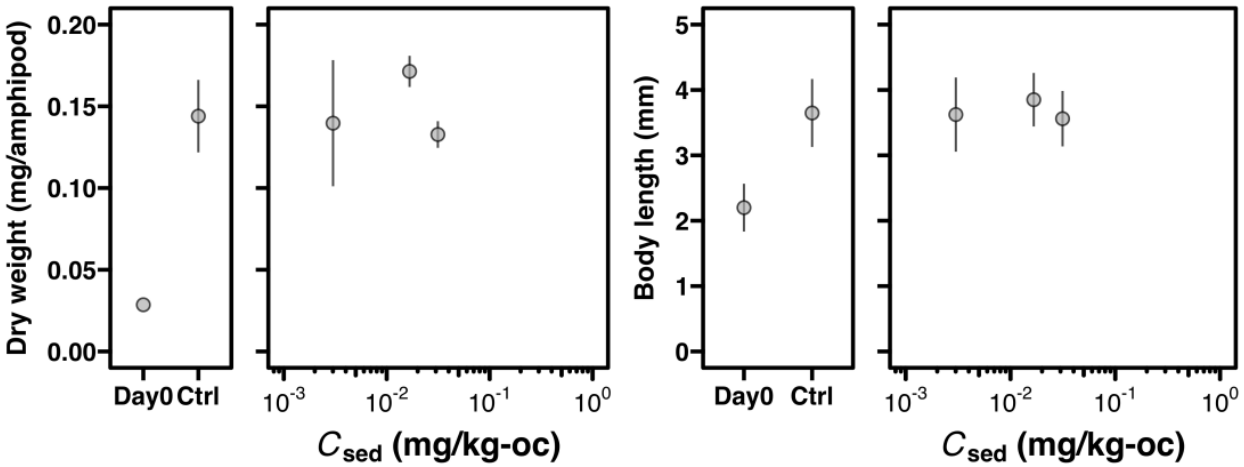

Figure S18. Measured sediment CPS concentration (C_{sed}) vs dry weight and body length in Run 4. Asterisks indicate statistically significant differences from the control (*: $p < 0.05$, **: $p < 0.01$, Dunnett's test).

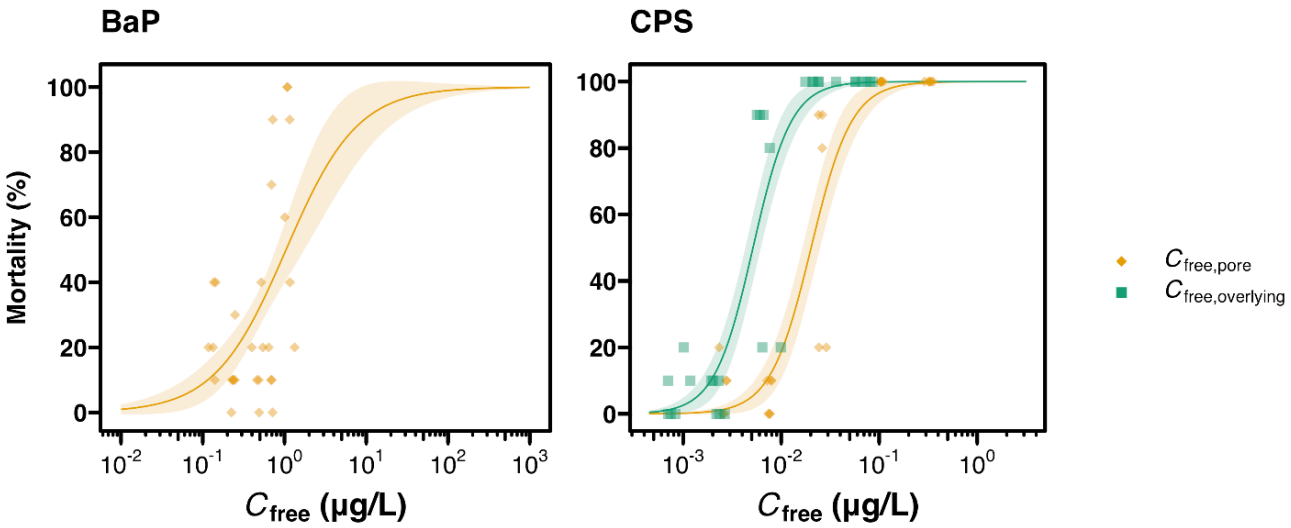

Figure S19. Relation between freely dissolved BaP and CPS concentrations ($C_{free,over}$ and $C_{free,pore}$) and 10-day amphipod mortality when C_{free} measured in each beaker was considered as an independent concentration. Shaded areas are the 95% confidence intervals (CI) of regression curves. The LC50 values were 1.04 µg/L (95% CI: 0.65−1.43 µg/L) for BaP, 5.2 ng/L (based on $C_{free,over}$, 95% CI: 4.2−6.1 ng/L) and 20 ng/L (based on $C_{free,pore}$, 95% CI: 16−24 ng/L) for CPS.

Table S10. Summary of spiked-sediment toxicity tests of BaP and CPS in Runs 3 and 4.

	LC10	LC50	NOEC
BaP	6.4×10^2 (1.7×10^2–1.1×10^3)	1.0×10^4 (5.7×10^3–1.5×10^4)	3.0×10^3
CPS	1.1 (0.8–1.4)	2.6 (2.2–3.0)	1.6

Effect concentrations were calculated based on the measured sediment concentrations (unit: mg/kg-$_{OC}$). Values in parentheses represent 95% confidence intervals.

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