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Polycyclic aromatic hydrocarbons in oysters and sediments from the Yatsushiro Sea, Japan: Comparison of potential risks among PAHs, dioxins and dioxin-like compounds in benthic organisms

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) were analyzed in oysters collected from 18 stations in the Yatsushiro Sea, western Japan. PAHs were detected in all samples analyzed, and the highest concentration (mean 230 ng/g wet weight) was found in oysters from Tanoura Bay. The high molecular weight PAHs to low molecular weight PAHs ratios in oysters from Tanoura Bay were higher than at other stations. Sediment samples collected from 42 stations in Tanoura Bay were analyzed for PAHs to understand their concentrations and distribution. Higher concentrations were found in sediment samples at two stations in the southern inner bay (mean 30,200 ng/g dry weight), which were approximately two orders of magnitude higher than at a reference site. These observations strongly suggest severe contamination and significant sources of PAHs in Tanoura Bay. Dioxins and dioxin-like compounds (PCDFs, and non- and mono-*ortho* coplanar PCBs) were analyzed in sediments from eight stations in Tanoura Bay. The concentrations were comparable to, or lower than, at the reference sites, suggesting that there are no specific sources of these compounds in this bay. PAH, dioxins and dioxin-like compounds DR-CALUX relative potencies (REP) were applied to the sediment concentrations to evaluate the potential for toxicological effects on benthic organisms. PAHs made the highest contribution to the total REP concentration, supplying 99% of the total REP, followed by PCDDs (0.18%), PCDFs (0.04%), and PCBs (< 0.001%). In this area, PAHs appear to be the most important Ah receptor binding chemicals for potential toxicity to benthic species.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants that have been detected in a wide variety of environmental matrices, including air, water, sediment, and organisms. High PAH concentrations (of tens to hundreds of μg/g dry weight) have been found in bivalves and sediments from heavily industrialized and urbanized regions, including Boston Harbor, USA (Wang et al., 2007), Lazaret Bay and Ajaccio Harbor, France (Baumard et al., 1998), and the Pearl River Delta, China (Mai et al., 2002). 'Mussel Watch Projects', conducted in the US (Sericano et al., 1995) and Asian coastal waters (Isobe et al., 2007), have provided information on the distribution of PAH contamination on a global scale. PAHs

have been found to accumulate in benthic organisms, such as lugworms, bivalves, and tidal flat fish, in lower trophic ecosystems in coastal waters (Nakata et al., 2003). In recent years, naphthalene, phenanthrene, and other low molecular weight PAHs have been detected in marine mammals (Kannan and Perrotta, 2008; Moon et al., 2011) and human adipose tissue (Guerranti et al., 2009; Moon et al., 2012), which implies high exposure and moderate PAH persistency in higher trophic species.

PAHs are released into the environment from pyrogenic, petrogenic, and biogenic sources. Pyrogenic PAHs are produced by pyrolysis or incomplete combustion processes, resulting in the production of high molecular weight PAHs. Petrogenic PAHs, in crude oils, are characterized by a predominance of low molecular weight (2–3 rings) PAHs. Low molecular weight PAHs and alkylated PAHs can be used as indicators to evaluate oil spills and discharge, from routine tanker movements or storage tanks, into the aquatic environment. Perylene is known to have a biogenic

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source, being produced by diagenesis processes on natural pre- cursors (Hites et al., 1980). PAH compositions in environmental matrices have been investigated extensively, to evaluate the occurrence and input of PAHs derived from anthropogenic sources (Yunker et al., 2002).

PAHs have toxic properties, especially carcinogenicity and mutagenicity to wildlife and humans. Several guidelines to eval- uate the toxic potencies of PAHs to benthic organisms against PAH concentrations in sediment are available. Long et al. (1995) proposed sediment quality guidelines (SQGs) for PAHs, including the ERL (effect range low; identifying the 10th percentile of the effect data) and the ERM (effect range medium; identifying the 50th percentile of the effect data). Baumard et al. (1998) suggested that PAH concentrations in mussels and sediments are categorized into four groups, low (0–100 ng/g), moderate (100–1000 ng/g), high (1000–5000 ng/g), and very high (>5000 ng/g), on a dry weight basis. These guideline concentrations have been used, in many previous studies, to estimate the potential risk and effects of PAHs in marine ecosystems.

Some PAH congeners bind to the aryl hydrocarbon receptor (AhR), like dioxins and dioxin-like compounds. AhR binding potencies of individual PAHs relative to 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD) have been reported, so that the toxico- logical implications of their presence in environmental matrices can be evaluated (Machala et al., 2001; Villeneuve et al., 2002; Willett et al., 1997). Although PAHs are not persistent and are generally less toxic than dioxins and dioxin-like compounds, PAH concentrations in sediments are generally several orders of mag- nitude higher than dioxin concentrations. Therefore, benthic organisms are likely to have higher exposure to, and possibly a higher toxic contribution from, PAHs. Nakata et al. (2003) com- pared the 2,3,7,8-TCDD toxic equivalent (TEQs) values of PAHs and coplanar PCBs in sediment and tidal flat organisms, and suggested that PAHs may play an important role in adverse effects on clams and crabs, because of a high contribution of PAHs to the total TEQs. However, little information is available on the comparison of toxic contributions from PAHs, PCDDs, PCDFs, and PCBs in environ- mental matrices.

In recent years, the DR-CALUX (Dioxin-Responsive-Chemical Activated Luciferase gene eXpression) bioassay, which consists of a genetically modified rat hepatoma H4IIE cell line, has been used to screen the potential risk from dioxins and dioxin-like compounds. The DR-CALUX- relative effective potencies (REP) of eight PAHs relative to 2,3,7,8-TCDD ranged from 1.3×10^{-6} (phenenthrene) to 4.9×10^{-3} (dibenz-[a,h]anthracene) (Behnisch et al., 2003).

The REP values of dibenz[a,h]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, and indeno[1,2,3-cd]pyrene were compar- able with the REP values of the non-$ortho$ coplanar PCBs, PCB77 (1.5×10^{-3}) and PCB169 (4.3×10^{-3}). Bekki et al. (2010) analyzed PAHs, PCDDs, PCDFs, and coplanar PCBs in airborne particles collected in China and Japan, and the sample extracts were applied to the DR-CALUX assay. They suggested that the PAH contribution to the total DR-CALUX-TEQ was higher than the dioxin and dioxin- like compounds contributions in the samples from both countries.

The Yatsushiro Sea is an enclosed bay which is located in Kyushu Island, western Japan. The population density of the Yatsushiro Sea region is 400 persons/km^2, which is approximately the one-fiftieth of that in the Tokyo Bay cosmopolitan area (Ministry of Environment, Japan-a). High PAH concentrations (140 ng/g wet weight) in a bivalve sample from the Yatsushiro Sea was recently reported by Tanaka and Onduka (2010), who analyzed bivalves collected from 64 stations in Japanese coastal waters. This concentration was approximately seven times greater than the mean PAH concentration (19 ng/g wet weight) in their samples. However, in that study only one bivalve sample from the Yatsushiro Sea was analyzed, and little information is available on the general contamination by, and spatial distribution of, PAHs in the Yatsushiro Sea.

The objectives of this study were to understand the present level of PAH pollution and its spatial distribution in the Yatsushiro Sea by analyzing bivalves and surface sediments collected from polluted and reference sites. A range of PAHs was analyzed to estimate their potential sources in this area, and the toxicological effects of PAHs to benthic organisms were evaluated by comparing PAH concentrations in sediments with the Sediment Quality Guidelines (SQGs) and threshold levels proposed by Long et al. (1995) and Baumard et al. (1998), respectively. We also analyzed PCDDs, PCDFs, and coplanar PCBs in 10 sediment samples from polluted and unpolluted areas of the Yatsushiro Sea to evaluate their toxicological contributions for benthic organisms.

2. Materials and methods

2.1. Chemicals analyzed

Twenty PAHs, including the 16 EPA priority PAHs, were analyzed, which were 2-methylnaphthalene (2-MN), 1-methylnaphthalene (1-MN), 1,2-dimethyl- naphthalene (1,2-DMN), acenaphthylene (ANTHY), acenaphthene (ANTN), fluorene (FLU), phenanthrene (PHE), anthracene (AN), fluoranthene (FLR), pyrene (PY), chrysene (CHRY), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]
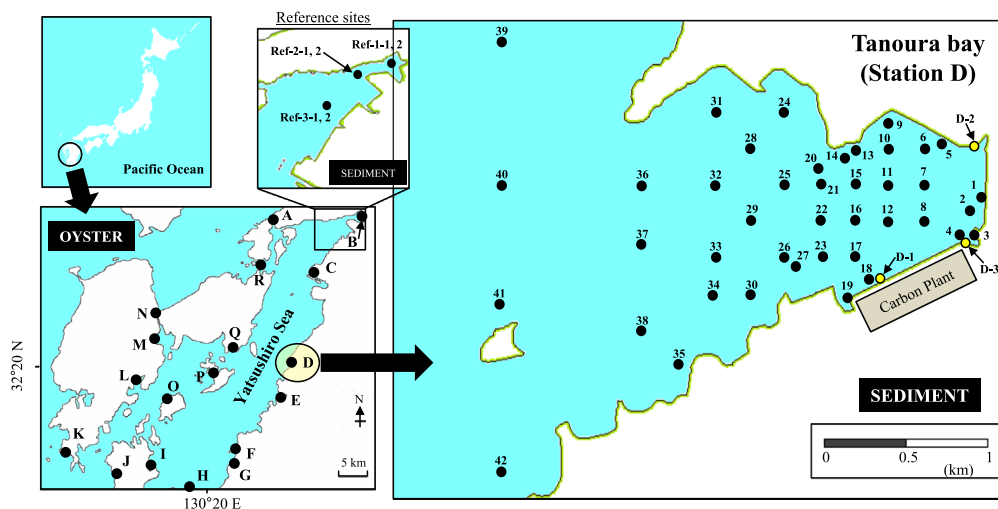

Fig. 1. Map showing the oyster and sediment sampling sites in the Yatsushiro Sea, Japan, including Tanoura Bay.

Table 1
Concentrations of PAHs (ng/g wet wt.) in bivalve samples collected from 18 sites in Yatsushiro Sea, western Japan.

Station ID Name of station	A Misumi	B Hikawa	C Yatsushiro	D-1 Tanoura	D-2 Tanoura	D-3 Tanoura	E Sashiki	F Minamata	G Fukuro	H Izumi	I Wakisaki	J Karakuma	K Ushibuka	L Nakata	M Funazu	N Hondo	O Shishi-jima	P Goshoura-jima	Q Oomichi	R Aizu
Lipid (%)	1.9	2.5	1.7	1.9	1.1	1.4	2.1	1.7	2.6	2.2	2.1	2.2	1.7	2.3	1.6	1.6	2.4	2.0	1.5	1.8
2-MN	0.17	0.14	0.33	0.36	ND	ND	0.67	0.56	0.50	0.65	0.25	0.19	0.61	0.32	0.23	0.22	0.31	0.31	0.20	ND
1-MN	0.17	0.52	0.41	0.76	ND	ND	1.2	1.0	0.83	1.0	0.66	0.70	0.93	0.24	0.18	0.20	0.53	0.56	0.23	0.19
1,2-DMN	0.088	0.050	0.034	0.194	0.11	0.17	1.9	1.0	0.16	0.77	0.90	0.15	0.38	0.12	0.12	0.069	0.34	0.12	0.29	0.33
ANTHY	0.060	0.070	0.029	0.161	0.057	0.12	0.21	0.20	0.12	0.20	0.14	0.11	0.16	0.09	0.068	ND	0.18	0.052	0.18	0.85
ANTN	0.31	0.24	0.21	0.36	0.35	1.6	0.84	0.44	0.61	0.35	0.60	0.37	0.22	0.10	0.77	0.15	0.30	0.18	0.29	ND
FLU	0.37	0.37	0.17	0.86	0.36	1.2	6.8	4.6	0.92	3.3	2.0	0.80	3.9	0.44	1.1	0.22	1.0	0.40	1.7	0.39
PHE	2.1	1.2	1.2	7.8	4.5	17	13	12	2.8	8.8	4.4	2.0	3.7	1.6	1.7	1.0	4.4	2.0	5.1	1.7
AN	ND	ND	ND	ND	0.12	1.4	ND	ND	ND	ND	ND	ND	ND	0.11	0.20	0.13	ND	ND	0.61	0.073
FLR	2.5	2.4	1.6	57	19	110	7.4	4.5	3.9	3.9	1.4	3.6	2.5	1.9	3.2	1.6	3.5	2.3	5.9	2.9
PY	1.4	2.0	0.86	31	10	74	7.7	8.3	1.7	4.2	1.6	3.5	3.2	0.84	3.5	1.2	4.3	1.9	5.3	1.7
BaA	0.19	0.34	0.17	23	2.8	23	1.7	1.0	0.33	0.76	0.36	0.68	0.56	0.20	0.92	0.23	0.84	0.53	1.2	0.28
CHRY	0.38	0.39	0.39	33	3.3	32	2.6	1.9	1.0	1.5	0.57	1.8	1.1	0.48	1.1	0.48	3.1	1.4	1.9	0.45
BbF	0.56	0.94	0.31	39	7.2	38	2.2	1.8	1.0	1.5	1.6	1.5	1.1	0.58	2.4	0.81	1.9	1.3	1.9	0.64
BkF	0.16	0.23	0.04	10	1.7	8.4	0.72	0.56	0.27	0.34	0.54	0.31	0.31	0.14	0.93	0.24	0.43	0.46	0.60	0.20
BeP	0.23	0.44	0.16	12	3.0	14	0.77	0.73	0.34	0.52	0.56	0.63	0.44	0.21	0.82	0.33	0.80	0.52	0.66	0.25
BaP	0.08	0.17	0.052	6.5	1.5	9.5	0.28	0.49	0.090	0.19	0.30	0.23	0.15	0.080	0.55	0.11	0.34	0.12	0.40	0.10
PERY	0.22	0.61	0.40	1.8	0.45	2.4	0.26	0.10	0.065	0.36	0.086	0.16	0.051	ND	0.19	0.10	0.088	0.058	0.13	0.13
IcdP	0.06	0.13	ND	2.6	0.83	6.6	0.24	0.58	0.067	0.16	0.26	0.15	0.16	0.081	0.45	0.11	0.30	0.14	0.25	0.069
BghiP	0.10	0.21	0.076	2.9	1.2	6.9	0.32	0.73	0.11	0.27	0.37	0.41	0.30	0.10	0.47	0.18	0.57	0.20	0.47	0.12
DahA	ND	ND	ND	0.89	0.27	1.8	ND	0.08	ND	ND	ND	ND	ND	ND	0.11	ND	ND	ND	ND	ND
∑PAHs (ng/g wet wt.)	9.2	10	6.5	230	57	350	49	40	15	29	17	17	20	7.6	19	7.4	23	13	27	10
Selected ∑PAHs (ng/g dry wt.)[a]	40	45	26	1100	280	1700	190	160	58	110	60	76	68	31	83	32	100	55	120	44
HM-PAHs/LM-PAHs (H/L)	0.31	0.58	0.42	1.36	0.65	0.70	0.26	0.27	0.35	0.28	0.49	0.59	0.32	0.39	0.83	0.63	0.63	0.71	0.40	0.33

Moisture content of bivalves was assigned as 80%.

[a] Sum of concentrations of PHE, AN, FLR, PY, BaA, CHRY, BbF, BkF, BeP, BaP, PERY, IcdP, BghiP, and DahA.

fluoranthene (BkF), benzo[e]pyrene (BeP), benzo[a]pyrene (BaP), perylene (PERY), indeno[1,2,3-cd]pyrene (IcdP), benzo[ghi]perylene (BghiP), and dibenz-[a,h]anthracene (DahA). Polychlorinated dibenzo-p-dioxins (2,3,7,8-T$_4$CDD, 1,2,3,7,8-P$_5$CDD, 1,2,3,4,7,8-H$_6$CDD, 1,2,3,6,7,8-H$_6$CDD, 1,2,3,7,8,9-H$_6$CDD, 1,2,3,4,6,7,8-H$_7$CDD, and OCDD), polychlorinated dibenzofurans (2,3,7,8-T$_4$CDF, 1,2,3,7,8-P$_5$CDF, 2,3,4,7,8-P$_5$CDF, 1,2,3,4,7,8-H$_6$CDF, 1,2,3,4,6,7,8-H$_6$CDF, 1,2,3,7,8,9-H$_6$CDF, 2,3,4,6,7,8-H$_6$CDF, 1,2,3,4,6,7,8-H$_7$CDF, 1,2,3,4,7,8,9-H$_7$CDF, and OCDF), non-$ortho$ substituted PCBs (PCBs 77, 81, 126, and 169) and mono-$ortho$ substituted coplanar PCBs (PCBs 123, 118, 114, 105, 167, 156, 157, and 189) were analyzed in eight sediments collected in Tanoura Bay and two sediments from the reference site in the northern Yatsushiro Sea.

2.2. Sample collection

Oyster samples were collected from 18 coastal sites, including Misumi (station A), Hikawa (B), Yatsushiro (C), Tanoura (D), Sashiki (E), Minamata (F), Fukuro (G), Izumi (H), Wakisaki (I), Karakuma (J), Ushibuka (K), Nakata (L), Funazu (M), Hondo (N), Shishij-jima (O), Goshoura-jima (P), Omichi (Q), and Aizu (R), in the Yatsushiro Sea in July and October, 2008 (Fig. 1 and Table 1). A pooled sample of oysters (n: 4–9, shell length: approx. 2–6 cm) from each location, except station D, was analyzed for PAHs. Oysters were collected from three different positions at station D and they were analyzed individually to obtain detailed information on the PAH distribution in that area. All oysters did not depurated before the tissue was dissected from the shell.

Surface sediments (depth 0–5 cm) were collected from 42 locations in Tanoura Bay in September 2010 using the Ekman Berge or Smith–McIntyre grab samplers (Fig. 1). Sediments were also collected from three reference site stations (Ref-1 to 3) in the northern Yatsushiro Sea. All oyster and sediment samples analyzed in this study were packed into clean plastic bags and stored at −20 °C until chemical analysis. Recently, we analyzed ^{210}Pb and Cs in a sediment core sample (number of layer: 18), collected from Tanoura Bay. The sedimentation rate was 0.22 cm/year (Kondo et al., 2013). This indicates that two years difference between the sampling date for sediment and oyster have not influenced in this study.

2.3. Analytical procedures

PAHs were analyzed following a previously published method (Nakata et al., 2003) with some modifications. Approximately 3–5 g of oyster tissue was homogenized with anhydrous sodium sulfate and then extracted for five hours with a dichloromethane:hexane (8:1) mixture in a Soxhlet apparatus. The extract was concentrated to 10 mL, and an aliquot of the extract was analyzed for extractable lipid. Deuterated PAH surrogate standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{14}) were then added to the sample extract before it was fractionated using gel permeation chromatography (with 1:1 dichloromethane:hexane as the mobile phase) and silica gel column chromatography. The eluate was concentrated to 20–30 μL, and ^{13}C PCB105 was added as an internal standard. PAHs were determined by gas chromatography (Agilent 6980, Agilent Technologies, USA) coupled with mass spectrometry (Agilent 5973) in SIM mode. The GC column used was a BPX-5 fused silica capillary column (30 m × 0.25 mm id., S&G Scientific Inc., Australia). The oven temperature program was: 70–150 °C at 30 °C/min, then 4 °C/min to 300 °C, held for 10 min. The injector and detector temperatures were 270 °C and 300 °C, respectively. The carrier gas was helium. A procedural blank was analyzed with every set of six samples. The detection limit was defined as three times the value of the blanks.

Sediment samples were freeze dried, homogenized, and sieved (0.5 mm mesh) before extraction. Approximately 2 g of sample was analyzed for PAHs using the same extraction, pretreatment, and instrumental procedures as described above. The total organic carbon content of the sediments was determined using a CHN elemental analyzer.

PCDD/Fs and coplanar PCBs were analyzed using a previously published method (Japan Environment Agency, 2008) with some modifications. Briefly, approximately 10 g dried sediment was extracted with 400 mL toluene in an accelerated solvent extractor for 15 min (Dionex ASE-100, Thermo Fisher Scientific Inc., USA). The extract was spiked with internal standards (PCDD/Fs ^{13}C-labeled at the 2, 3, 7, and 8 positions, and ^{13}C-labeled non- and mono-$ortho$ coplanar PCBs), and it was cleaned using multilayer chromatography (silica gel with 2% KOH (0.5 g), silica gel (0.5 g), sulfuric acid silica gel (3 g), silica gel (0.5 g), 10% silver nitrate silica gel (6 g) and activated carbon dispersed silica gel). The eluent was concentrated and analyzed on a gas chromatograph (Agilent 6980) coupled with high resolution mass spectrometry (Autospec Ultima, Micromass Ltd., UK), using capillary GC columns of SP-2331 (60 m × 0.25 mm id, Supelco, USA), and a BPX-DXN (60 m × 0.25 mm id, Kanto Chemical, Japan) for PCDDs/DFs and a HT8-PCB (60 m × 0.25 mm id, Kanto Chemical, Japan) for coplanar PCBs.

2.4. Statistics

Principal components analysis (PCA) was conducted to investigate differences between PAH mixtures found in the oyster samples using the Excel Statistics version 5.0 program (Esumi, Tokyo, Japan).

2.5. Quality assurance

Deuterated surrogate standards were used to compensate for PAH losses during the analytical procedure. The mean recoveries of acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{14} were 52 ± 11%, 75 ± 10%, 114 ± 14%, and 130 ± 19% ($n=48$), respectively. The recoveries of ^{13}C PCDDs, PCDFs, and coplanar PCBs were 63%, 85%, and 75%, respectively. The limit of detection (LODs) of PAHs in oyster and sediments was 0.04 ng/g and 0.02 ng/g, respectively.

3. Results and discussion

3.1. Concentrations, distribution and compositions of PAHs in oysters

PAHs were detected in all of the oyster samples analyzed (Table 1). The mean total PAH concentration in the oysters, excluding samples from station D (Tanoura Bay), was 19 ± 12 ng/g (wet weight) (mean ± standard deviation), which is comparable with reported background PAH concentrations in bivalves from Japanese coastal waters (mean: 17 ± 19 ng/g wet weight, $n=169$; Tanaka and Ontsuka, 2010). PHE, FLU, and PY dominated the PAH profiles, supplying 19 ± 6.3%, 19 ± 6.1%, and 16 ± 3.3% of the total PAH concentrations, respectively. PAH concentrations in oyster samples from station D were relatively high, ranging from 57 to 350 ng/g wet weight (mean 210 ng/g; Table 1), approximately an order of magnitude higher than in samples from the other stations. FLU, PY and BbF were major components in station D oyster samples, contributing 30 ± 4.6%, 18 ± 3.9%, 14 ± 3.1% to the total PAH concentration, respectively. Higher contributions from high molecular weight (HM-)PAHs, such as BaP (2.7 ± 0.13%) and BghiP (1.8 ± 0.43%), to the total PAH concentrations were found in oysters from station D than from other stations.

PCA was performed on the PAH concentrations in the oyster samples to investigate the PAH profiles. The first two principal components, PC1 and PC2, explained 41.2% and 12.8% of the total variability between samples, respectively (SI-1). Interestingly, oysters collected from station D (Tanoura Bay) and station M (Funazu) were grouped together, with negative PC1 eigenvectors and relatively high contributions of HM-PAHs. In these samples, BaP (−0.323), BbF (−0.316), BeP (−0.305), CHRY (−0.290), DahA (−0.267), BkF (−0.265), and IcdP (−0.244) all showed negative PC1 loadings (SI-2, values shown in brackets). The high PAH concentrations and their profiles in the oysters from Tanoura Bay strongly suggest the existence of a point source of PAHs in this bay.

There are two major sources of PAHs, pyrogenic and petrogenic activities, and the ratios of HM- and low molecular weight (LM-) PAHs have been used to identify their potential sources in the environment. The predominance of HM-PAHs (with four or more aromatic rings) and LM-PAHs (with two or three aromatic rings) in samples implies pyrogenic and petrogenic sources, respectively. The mean HM/LM-PAH ratio in oysters in this study was 0.52 ± 0.26 (range 0.26–1.36), and higher values were found in Tanoura Bay (0.90) and Funazu (0.83) (Table 1). Zakaria et al. (2002) analyzed PAHs in different types of crude oils, and reported HM/LM-PAH ratios ranging from 0.02 to 0.13 (mean 0.06 ± 0.03). Isobe et al. (2007) reported higher HM/LM-PAH ratios in mussels from Asian countries than in crude oil, and the ratios we found were comparable with that study. Although there are many, and mixed, sources of PAHs in the environment, it is likely that the major source in the Yatsushiro Sea is pyrogenic.

We compared the PAH concentrations in oysters with PAH 'guidelines' proposed by Baumard et al. (1998) (who categorized PAH concentrations in mussels and sediments into four groups, see introduction). In our samples, PAH concentrations in oysters at 12 out of 18 stations were categorized as 'low', with total PAH concentrations ranging from 26 to 83 ng/g dry weight (mean 51 ± 18 ng/g; Table 1), comparable to concentrations in oysters

from the Ariake Sea, Japan (56 ± 29 ng/g dry weight; Nakata et al., 2004) and mussels from Cambodia and Vietnam (Isobe et al., 2007), areas that are generally less polluted than many Asian coasts. PAH concentrations in oysters at six stations (E, F, H, O, Q, and D-2) were in the 'moderate' category (100–280 ng/g dry weight; Table 1). However, two samples from Tanoura Bay were in the 'high' category (1100 and 1700 ng/g dry wt.; Table 1), and were comparable with samples from polluted sites, including in India (1133 ng/g) and Tokyo Bay (1269 ng/g) (Isobe et al., 2007).

A factory that produces high quality carbon and graphite materials, such as fine carbon, and graphite electrodes is located on the coast near station D. The production of such carbon materials often includes processes that involve heating a carbonaceous gas to a high temperature in a reducing or inert atmosphere, and high PAH concentrations, especially HM-PAHs, have been detected in air in graphite and electrode plants (Petry et al., 1996). It is possible that the carbon plant may discharge wastewater containing high PAH concentrations into Tanoura Bay, resulting in contamination.

3.2. Risk assessment of the oysters as seafood

The EU Scientific Committee on Food assessed 33 PAHs and identified 15 PAHs that possess both carcinogenic and genotoxic properties (Wenzl et al., 2006). Maximum allowed concentrations (MACs) for BaP in various food products, such as oils, infant foods, meat, and seafood have been set in EU legislation. The MAC of BaP in bivalve mollusks is 10 ng/g wet weight, which is more than 10 times higher than the BaP concentrations in most of the samples we collected. However, BaP concentrations in oysters from station D-3 (9.5 ng/g wet weight) were comparable to the MAC.

Dietary exposure to BaP in the Japanese population was calculated using the concentrations found in oysters from station D. The typical seafood consumption of the Japanese population is 82.2 g/day (Ministry of Agriculture, Japan), so the estimated dietary intake of BaP using our oyster concentrations ranged from 2.5 to 15.5 ng kg^{-1} BW day^{-1}. The BaP NOAEL (no-observable adverse effect level) has been set at 0.21 mg kg^{-1} day^{-1} in Japan (Ministry of Environment, Japan-b), and, because the 'unknown factor' is usually set to 100, the acceptable daily intake (ADI) of BaP

can be calculated as 2.1 µg kg^{-1} day^{-1}. Using these values, the hazard ratio (H/Q) of BaP in oysters ranged from 1.2×10^{-3} to 8.0×10^{-3}, more than three orders of magnitude lower than the ADI. These results suggest that, even though oysters from station D contained BaP concentrations close to the MAC, the risk to human health is likely to be small. However, there is a lack of information on PAH concentrations in other seafood, such as fish and crabs, in this area, so further investigations of the accumulation profiles of PAHs and human exposure risks are required in Tanoura Bay.

3.3. PAHs in sediments in Tanoura Bay

As described earlier, the highest concentrations of PAHs were detected in oysters from Tanoura Bay. To understand the detail concentrations and spatial distribution of PAHs at station D, we collected 42 sediment samples from Tanoura Bay and three samples from reference sites in the northern Yatsushiro Sea (Fig. 1). The spatial distribution of PAH and TOC concentrations is shown in Fig. 2 and SI-3. The mean total PAH concentration in sediments was 5240 ng/g dry weight, and the highest concentration was found at station 26, in the southern inner bay (32,700 ng/g), followed by stations 17 (27,700 ng/g), and 23 (10,100 ng/g). The mean concentration in Tanoura Bay was approximately 20 times higher than the reference site concentrations (mean 273 ng/g dry weight, $n=6$), and was comparable with concentrations at heavily polluted sites in Tokyo Bay (mean 9546 ng/g; Zakaria et al., 2002). According to sediment guideline concentrations (Baumard et al., 1998), PAH concentrations at 12 out of the 42 Tanoura Bay locations were classified as 'very high' (>5000 ng/g). Furthermore, sediments from 25 stations were in the 'high' category (1000–5000 ng/g), and station 18 and the outer bay samples (stations 39–42) were classified as 'moderate' (100–1000 ng/g).

The sediment PAH concentrations in Tanoura Bay decreased with increasing distance from St. 26, where showed highest concentration of PAHs in the inner bay, to the middle and outer bay (Fig. 3). The mean PAH concentration at stations 28, 32, and 34 (0.5 km from St. 26) was 3120 ± 466 ng/g, approximately an order of magnitude lower than at station 26 (32,700 ng/g). The mean PAH concentration in sediments at stations 36, 37, and 38 (1.0 km from St. 26) was 1650 ± 240 ng/g, and the concentrations were
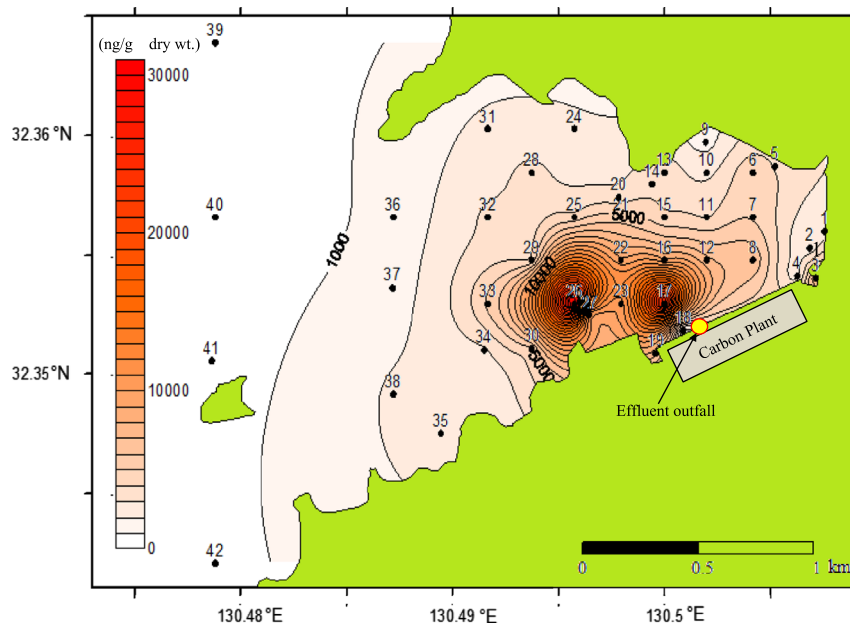

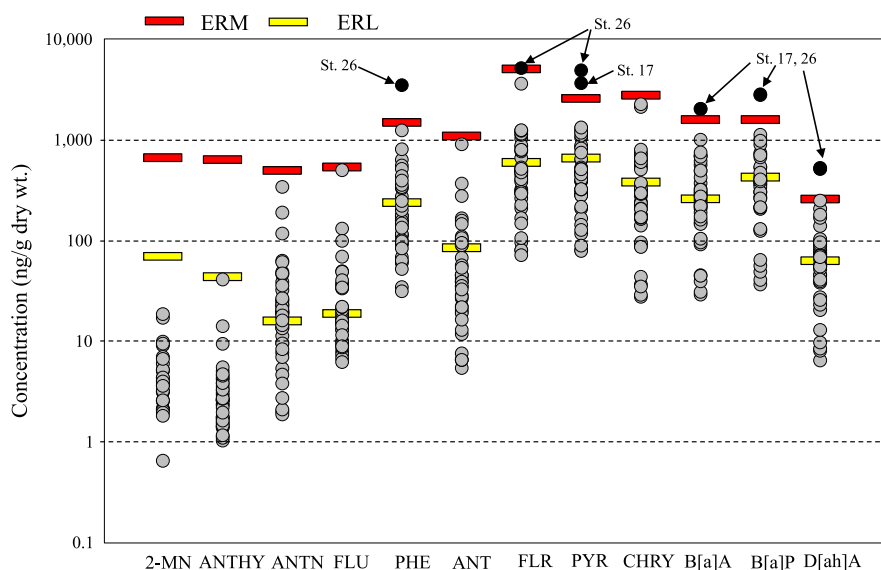Fig. 2. Spatial distribution of PAH concentrations in sediments in Tanoura Bay.

Fig. 3. Comparison of ERM and ERL values with PAH concentrations in sediment samples from Tanoura Bay.

lower still at stations 39, 40, 41, and 42, >1.5 km from St. 26 (mean 632 ± 104 ng/g). These results suggest the existence of a PAH discharge site in Tanoura Bay, and PAHs are transported and dispersed from St. 26 and its surrounding area in the inner bay to the middle and outer bay.

3.4. PCDDs, PCDFs, and coplanar PCBs in Tanoura Bay sediments

PCDDs, PCDFs, and non- and mono-*ortho* coplanar PCBs were detected in eight Tanoura Bay sediments (stations 8, 17, 23, 26, 33, 37, 40, and 41) and two sediments from the reference sites (stations 1-1 and 3-1) (Fig. 1). Because Tanoura Bay has big tide (approximately 3–4 m), PAHs are expected to transfer from east to west in the bay. The mean concentrations of PCDDs, PCDFs, and PCBs were 1700 ± 1200, 150 ± 180, and 110 ± 38 pg/g dry weight, respectively (Table 2; SI-4). OCDD, 1,2,3,4,6,7,8-HpCDD, and OCDF were dominant in most samples analyzed in this study. In contrast to the PAH concentrations, OCDD and 1,2,3,4,6,7,8-HpCDD concentrations in the reference site sediments were 2–3 times higher than in Tanoura Bay sediments. Comparable PCDF and PCB concentrations were found in sediments from Tanoura Bay and the reference sites, which suggests an absence of a point source of PCDDs, PCDFs, and coplanar PCBs in this bay. Masunaga et al. (2001) reported that OCDD, OCDF, 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8-HpCDF are impurities in agrochemicals, from the production process. The dominant, highly chlorinated PCDDs and PCDFs, in our sediment samples probably came from past applications of organochlorine pesticides, such as pentachlorophenol, in the Yatsushiro Sea region.

The PCDD, PCDF, and PCB TEQs in the sediment samples were 3.4, 1.5, and 0.0083 pg/g TEQ, respectively (SI-4). 1,2,3,4,6,7,8-HpCDD (mean TEQ 1.1 pg/g), 1,2,3,7,8-PeCDD (TEQ 1.0 pg/g), OCDD (TEQ 0.47 pg/g), and 1,2,3,4,7,8-HxCDF (TEQ 0.46 pg/g) dominated, and contributed 25%, 15%, 12%, and 11% of the total TEQ, respectively. The total sediment TEQs ranged from 0.75 to 8.5 pg/g (mean TEQ 5.0 pg/g), more than an order of magnitude below the benchmark TEQ value, 150 pg/g in sediment, set by the Japanese government.

3.5. Toxic potencies of PAHs in sediment to benthic organisms using sediment quality guidelines

To evaluate the toxic potencies of sediment to benthic organisms, PAH concentrations in sediments were compared with the SQGs (which, as described in the Introduction, have ERL and ERM values). Concentrations equal to and above the ERL but below the ERM indicate possible adverse effects, and concentrations equal to and above the ERM value indicate probable adverse effects (Long et al., 1995).

Twelve PAH concentrations in sediments from Tanoura Bay were compared with the ERL and ERM values (Fig. 3). More than 50% of the sediment samples analyzed (n > 22) exceeded the ERL values for ANTN, B*a*A, and D*ah*A. FLU, PHE, AN, FLR, PY, CHRY, and B*a*P concentrations were also high, with more than 29% of samples exceeding the ERLs. The concentrations of PHE, FLR, PY, B*a*A, B*a*P, and D*ah*A at station 26 were higher than the ERM values. Station 17, the second most polluted site, had concentrations higher than the ERM for PY, B*a*A, B*a*P, and D*ah*A. These findings imply that adverse effects on benthic organisms may be observed at more than 50% of the sampling stations investigated, especially at stations 17 and 26, which were heavily contaminated with PAHs.

3.6. Toxicological implications of co-exposure to PAHs, dioxins and dioxin-like compounds

The toxicological implications of co-exposure to, and the separate contributions from, PAHs, PCDDs, PCDFs, and coplanar PCBs in sediment were evaluated using the published REPs of individual compounds (Behnisch et al., 2003). The REP of a chemical refers to its activity in the DR-CALUX in vitro bioassay relative to TCDD (2,3,7,8-tetrachlorodibenzodioxin), as a way of estimating the compound's binding capacity to the AhR (and causing dioxin-like effects). The total REP concentration for each compound was calculated as follows:
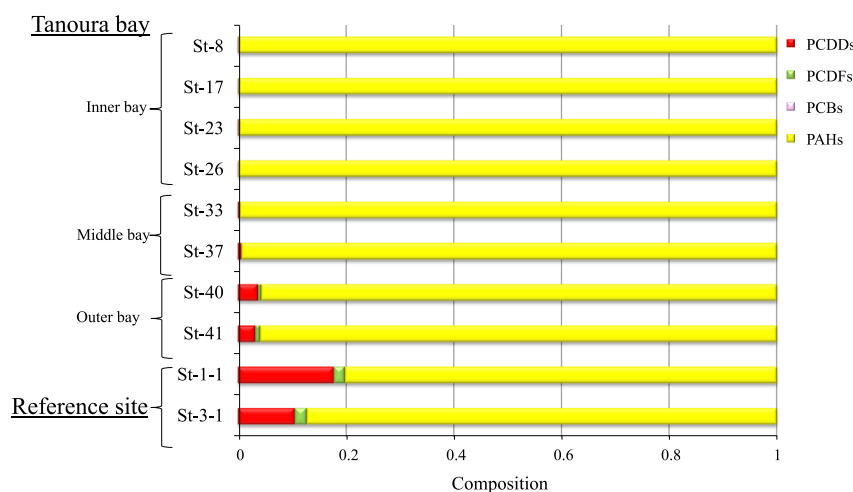
Total REP concentration $= \sum Ci \times REPi$,

where Ci is the concentration of an individual PCDD, PCDF, coplanar PCB, or PAH, and $REPi$ is the REP value of the chemical relative to TCDD (Behnisch et al., 2003).

The total REP concentration in all sediments ranged from 5.1 to 46 pg REP/g dry weight for PCDDs (mean 20 pg REP/g), 0.52 to 23 pg REP/g for PCDFs (mean 6.4 pg REP/g), and 0.01 to 0.16 pg REP/g for coplanar PCBs (mean 0.07 pg REP/g) (SI-5). 1,2,3,4,6,7,8-HpCDD dominated the dioxin-like POP REP concentrations, and OCDD gave the next highest contribution. The total PCDD REP concentrations in samples from the reference sites (46 and 40 pg

Table 2
PCDDs, PCDFs, PCBs, and PAHs concentrations (ng/g dry wt.) in sediments from the Yatsushiro Sea.

Name of station	St.-8	St.-17	St.-23	St.-26	St.-33	St.-37	St.-40	St.-41	Ref-1-1	Ref. 3-1
T_4CDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P_5CDD	ND	ND	0.0016	ND	ND	ND	0.0019	0.0024	0.0022	0.0023
H_6CDD	ND	ND	0.0062	0.0056	0.0063	0.0051	0.011	0.012	0.016	0.013
H_7CDD	0.029	0.063	0.08	0.063	0.070	0.051	0.12	0.13	0.24	0.21
OCDD	0.58	1.2	1.7	1.1	1.1	0.78	2.1	0.23	3.8	3.1
T_4CDF	ND	ND	0.0024	0.0015	0.0011	ND	ND	0.0017	ND	0.012
P_5CDF	ND	ND	0.0044	0.0027	0.0012	ND	0.0014	0.0026	0.0010	ND
H_6CDF	0.002	ND	0.011	0.0035	0.0026	ND	0.0058	0.0073	0.0079	0.0032
H_7CDF	0.0068	ND	0.035	0.015	0.014	0.0077	0.021	0.027	0.037	0.025
OCDF	0.037	0.51	0.18	0.062	0.047	0.019	0.048	0.059	0.11	0.067
Non-*ortho* PCB	0.0051	0.010	0.0094	0.0059	0.0073	0.0059	0.012	0.015	0.011	0.013
Mono-*ortho* PCB	0.090	0.16	0.11	0.067	0.073	0.043	0.093	0.11	0.15	0.12
PCDDs	0.61	1.3	1.8	1.2	1.1	0.84	2.2	0.37	4.1	3.3
PCDFs	0.046	0.51	0.23	0.085	0.066	0.027	0.077	0.097	0.16	0.11
PCBs	0.095	0.17	0.12	0.073	0.080	0.049	0.11	0.12	0.16	0.14
PAHs	7100	28,000	10,200	32,000	5300	1500	650	600	210	330

TEFs were cited from Van den Berg et al. (2006) for the calculation of TEQs in this study.

Fig. 4. Contributions of PCDD, PCDF, PCB, and PAH REP concentrations to the total REP concentrations in sediments from Tanoura Bay and reference sites in the Yatsushiro Sea.

REP/g) were higher than the Tanoura Bay ones (15 pg REP/g), and there was little difference in the total PCDF and PCB REP concentrations of the polluted and reference sites. The mean PAH REP concentration in Tanoura Bay sediments was 11,500 pg REP/g, more than 30 times higher than for sediments from the reference sites. Furthermore, the PAH REP concentrations were 2–5 orders of magnitude higher than the dioxin-like POP REP concentrations in Tanoura Bay (SI-5).

The highest contribution to the total REP concentration in sediments came from PAHs, followed by PCDDs, PCDFs, and PCBs (SI-4, Fig. 4), in that order. PAHs contributed more than 99% of the total REP concentrations in sediments from polluted sites (stations 8, 17, 23, 26, 33, and 37) in Tanoura Bay, and also made a high contribution (95%) at locations in the outer bay (stations 40 and 41). Contributions to the total REP concentrations at the reference sites decreased in the order PAHs (80–87%), PCDDs (10–18%), PCDFs (2%), and PCBs (< 0.05%). These results suggest that PAHs in Yatsushiro Sea sediments may pose more risk than PCDDs, PCDFs, and PCBs, particularly in Tanoura Bay. Higher risk contributions from PAHs than from PCBs have also been reported for sediments and biota collected from the Ariake Sea, Japan, where PAHs are reported to pose potential risks to tidal flat organisms (Nakata et al., 2003). PAHs also contributed more to the toxicity of airborne particles than PCDDs, PCDFs, and coplanar

PCBs in samples taken in Japan and China (Bekki et al., 2010). A detailed investigation and evaluation of the potential risks of PAHs in sediments to benthic organisms in Tanoura Bay are required.

4. Conclusions

The following conclusions were made from this study:

1. High PAH concentrations were detected in oysters and sediment in Tanoura Bay, Yatsushiro Sea, Japan, suggesting the existence of a point source.
2. Sediment samples from Tanoura Bay exceeded the ERL values for ANTN, B*a*A, and D*ah*A and the ERMs for PY, B*a*A, and B*a*P (plus PHE and FLR in one sample), implying that adverse effects in benthic organisms are likely in that area.
3. PCDD, PCDF, and coplanar PCB concentrations in sediments from Tanoura Bay were lower than, or similar to, concentrations in reference site sediments, suggesting that there are no significant sources of these compounds in the Yatsushiro Sea.
4. The total PAH REP concentrations in the sediments from Tanoura Bay were several orders of magnitude higher than the PCDD, PCDF, and PCB REP concentrations. A detailed

investigation and risk assessment of PAHs in benthic organisms is necessary in this bay.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2013.10.005.

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