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Biochemical effects of petroleum exposure in hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of sediments collected from a natural petroleum seep in CA, USA

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Abstract

Concentrations of serum/plasma estradiol, biliary fluorescent aromatic compounds (FACs), levels of hepatic CYP1A expression, and DNA damage were measured in sexually mature hornyhead turbot (*Pleuronichthys verticalis*) exposed in the laboratory for 7 days to a gradient of sediments collected from a natural petroleum seep in the Santa Barbara Channel. Coal oil point (COP) sediments were homogenized and divided into four treatments containing 0 (sediment from the Orange County Sanitation District's reference location), 33, 66, and 100% (COP) sediments. Sediment concentrations of 20 PAHs ranged from below the detection limit for the 0% COP sediment treatments to 105 μg/g in the 100% treatments with lower molecular weight compounds predominating. Concentrations of biliary FACs were not linear with COP treatment but levels of hepatic DNA damage increased linearly with increasing concentrations of high molecular weight PAHs. Hepatic CYP1A expression was elevated only in the 100% treatments. A reduction of plasma estradiol in male and female fish was observed in all COP exposures. These results demonstrate that acute sediment-only exposure of flatfish to naturally-derived PAHs elicits alterations in biochemical endpoints indicative of PAH bioavailability and adverse effects with different sensitivities.

Keywords: Polycyclic aromatic hydrocarbons; *Pleuronichthys verticalis*; Fluorescent aromatic compounds; DNA damage

1. Introduction

The area surrounding the coastal region of Santa Barbara, California is well known for its natural sources of petroleum (Spies et al., 1980).

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The natural petroleum seeps in the Santa Barbara Channel, such as coal oil point (COP), represent a continuous source of hydrocarbon exposure and contamination independent of effects caused by urban areas (Spies et al., 1996), Seepage of petroleum at the Isla Vista seep, one of the seeps around COP, has been estimated to have been occurring for thousands of years (Simoneit and Kaplan, 1980). An estimated 9500–19 000 l/day of petroleum are released from the Isla Vista seep (Allen et al., 1970). Seep sediment hydrocarbon concentrations have been reported as high as 1000 μg/g (Straughn, 1976; Reed et al., 1977; Stuermer et al., 1982).

Since fish tend to degrade PAHs rapidly, biochemical markers of exposure have been used extensively to evaluate biological effects in feral fish exposed to sediment PAHs (Stein et al., 1992; Myers et al., 1994; Collier et al., 1995; French et al., 1996; Spies et al., 1996). Biliary fluorescent aromatic compounds (FACs) have been used extensively to monitor the metabolites of PAHs in feral fish (Krahn et al., 1984; Malins et al., 1987; Collier and Varanasi, 1991; Krahn et al., 1991; Beyer et al., 1996; Lin et al., 1996; Spies et al., 1996; Aas et al., 2000b) and have proven one of the most direct indicators of PAH exposure. DNA modifications including strand breaks, DNA base modifications, cross-linkages, and depurination have been observed to be associated with exposure to a number of contaminants, including PAHs (Padrangi et al., 1995; Steinert, 1996; Steinert et al., 1998).

In this study, hornyhead turbot (*Pleuronichthys verticalis*) were collected from a reference station and exposed to sediments collected from COP. The primary objective of this study was to determine chemical concentrations of selected PAHs in sediment capable of eliciting biochemical responses in organisms following sediment-only exposure. A gradient of exposure regimes (0, 33, 66, and 100% COP sediments) were utilized to determine exposure using biochemical markers. Hepatic CYP1A, serum estradiol, biliary FACs and DNA damage in blood and hepatic cells were used to compare organism responses to sediment PAH concentrations.

2. Materials and methods

2.1. Recirculating exposure system

The experiment was conducted in the Aquatic Toxicology laboratory at the Southern California Coastal Water Research Project (SCCWRP) facility in Westminster, CA. The exposure system (Fig. 1) consisted of eight 10-gal glass aquaria, divided into four separate treatment groups. The first treatment group, consisting of two separate tanks, contained 0% COP sediment and was comprised of sediment collected from the reference station (118 05.199′ Longitude; 33 36.055′ Latitude) used by Orange County Sanitation District (OCSD) (OCSD, 1999). The total organic carbon of the sediments were 0.42% ±0.004. The composition was 73.8% sand; 21.3% silt, and 4.90% clay with a median grain size (φ) of 3.68 (see OCSD 1999).

The second, third, and fourth treatments contained 33, 66, and 100% COP sediments, respectively (119. 53.428 Longitude; 34. 24.370 Latitude). Because of consistency restraints and logistics, no measures of TOC, composition or grain size were available for these sediments. The tanks containing 33 and 66% COP contained 67 and 34% reference sediments, respectively. Sediments were homogenized and divided prior to their introduction into the exposure system. Two liters of sediment were placed in each of the 10-gal aquaria. Two sexually mature hornyhead turbot were placed in each tank.

Fish were collected from Santa Monica Bay (118.5008 Longitude; 33.9039 Latitude) and held for 1 month prior to sediment exposure. Average length and weight of turbot were 20.2 cm (±2.1 S.D.) and 116.7 g (±30.6 S.D.), respectively. Gender was not differentiated prior to exposure. Water from the recirculating system was filtered through a 40 L carbon filter (Fig. 1), Flow rates were set at 300 ml/min for all tanks and water temperature was maintained at 13.8 °C. Light–dark cycles were set at 16 h of light and 8 h of darkness.

For quality control purposes, water samples for PAH analyses were collected twice throughout the 7-day experiment (day 0 and day 7) to monitor the effectiveness of the filter. Ammonia levels were

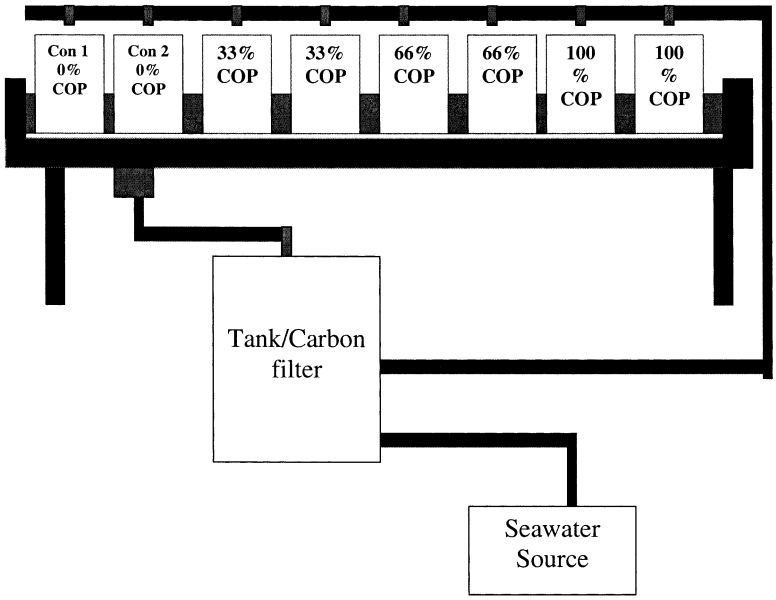

Fig. 1. Recirculating system for exposing fish to sediments.

also monitored throughout the experiment and revealed no impacts on the fish. After the sediments were introduced into the tanks, the water was permitted to circulate through the system for 4 days before the hornyhead turbot were introduced into the tanks to allow for complete settling of the sediments. Prior to and during the experiment hornyhead turbot were fed lug worms (*Neanthes* sp.) obtained from a local bait store in Seal Beach, CA. These animals possessed no detectable levels of the 20 PAHs that were analyzed and were fed to the fish twice a week. Feeding organisms throughout the course of the experiment has been suggested to portray more actual environmental conditions (Aas et al., 2000a). In a similar study, English sole (*Pleuronectes vetulus*) not fed during exposure had increased retention of PAH metabolites in the bile, up to seven times higher, than reference organisms fed throughout the experiment (Collier and Varanasi, 1991).

At the end of the experiment, hornyhead turbot were sacrificed by severing the backbone of the fish. Length, weight and gender were recorded for each fish. Liver and gall bladder samples were removed and stored in dry ice until transport to a −80 °C freezer. Samples for DNA damage were preserved and transported in liquid nitrogen to the laboratory.

2.2. Sediment chemical and water analysis

Sediment and water samples were stored in a refrigerator (4 °C) until analysis. PAHs were analyzed as described in EPA method 8100 (USEPA, 1996). For water samples separatory funnel liquid–liquid extraction was employed. Water samples were extracted with dichloromethane three times for 2 min and then passed through sodium sulfate with subsequent evaporation of solvent. Sediment samples were extracted with hexane. An ultrasonic disruptor was used and extracts were passed through sodium sulfate, combined and solvent evaporated. Cleanup was performed with fully activated silica gel (8 g), preluted with hexane, and PAHs were collected from the column with 25 ml of methylene chloride/hexane (2:3, v:v) A GC-FID (flame ionization detector) with a capillary column (DB-5) was used for analysis and quantification. The oven temperature was 40 °C, ramped to 160 °C with 40 °C/min, and up to 300 °C with 5 °C/min. The recoveries were 91–100% with a S.D. of 4–13% and method

detection limit (MDL) of 6–41 ng/g for water. In sediment, recoveries were 30–111% with a S.D. of 2–15% and MDLs ranging from 11–53 ng/g sediments.

2.3. Biochemical endpoint measurements

2.3.1. FACs

Florescent biliary metabolites of benzo[a]pyrene (BAP), naphthalene (NAP), and phenanthrene (PHN) were analyzed in fish bile using fluorescence detection. The assays were conducted using a variation of previously reported methods (Krahn et al., 1984, 1986). Fluorescence was measured with a Shimadzu fluorescence detector (RF-10 AXL) at 380/430, 256/380 and 290/335 nm, excitation/emission for BAP, PHN and NAP, respectively.

2.3.2. DNA damage

Preservation of blood was achieved by gently mixing and freezing a small volume (<100 μl) in 1 ml of ice-cold cryopreservation solution, phosphate buffered saline/10% DMSO. Small sections of liver were placed in 1 ml of ice-cold cryopreservation solution. Within 20 min, all samples were frozen in liquid nitrogen. Samples were transported to the Comet Analysis Laboratory and transferred to a $-80\,^{\circ}C$ freezer. Samples were evaluated for DNA damage using Comet analysis as previously described (Steinert et al., 1998).

2.3.3. CYP1A

Hepatic CYP1A levels were quantified using Western blotting techniques followed by semi-quantitative measurements of densitometry in optical density units as previously described (Schlenk et al., 1996). The CYP1A protein was assayed utilizing monoclonal mouse anti-peptide CYP1A IgG as the primary antibody (Rice et al., 1998). Microsomal protein was measured using the Pierce kit (Pierce Inc., Rockford, IL) method with bovine serum albumin as the standard. Protein samples were normalized to contain 50 μg of protein per lane.

2.3.4. Serum/plasma estradiol

Approximately 0.5 ml of blood was collected from the dorsal aorta of the fish prior to euthanasia. Blood was immediately centrifuged at $750 \times g$ for 2 min at room temperature. Plasma/serum was removed and stored in liquid nitrogen until processed for estradiol measurements. Estradiol was measured using enzyme-linked immunosorbant assay kits purchased from Cayman Chemical Co. (Ann Arbor, MI) following the manufacturer's guidelines.

2.4. Statistical analysis

Analysis of variance (ANOVA) was conducted to determine possible difference between treatment groups. Bartlett's test of homogeneity was conducted to verify homogeneity of variance. If homogeneity of variance was not obtained values were log transformed prior to ANOVA analysis, When significance ($P < 0.05$) was obtained, Tukey's Post Hoc test was used to single out treatments responsible for the statistical differences. Linear regressions were performed to determine relationships between biochemical effects and sediment concentrations. Pearson correlations were used to explore relationships between biomarkers.

3. Results

3.1. Sediment PAHs

Sediment PAHs ranged from below detection limits in the control treatments, to a mean of 31.9 μg/g in the 100% COP treatment groups for high molecular weight PAHs (Table 1). Low molecular weight PAHs ranged from 0.22 μg/g in the control groups to a mean of 61.19 μg/g in the 100% COP treatment groups (Table 2), High molecular weight PAHs increased linearly from the 0% COP treatment through the 100% treatment (Fig. 2). Low molecular weight PAHs increased from 0.22 μg/g in the 0% COP group to the 24.2 μg/g in the 33% COP treatments, then were diminished for the 66% treatments (22.43 μg/g), and were elevated again in the 100% (61.2 μg/g) treatment groups (Fig. 3).

Table 1
Concentrations of high molecular weight PAHs (μg/g dry weight) in COP sediments diluted with reference sediments for fish exposures

HMW PAHs	Control 1	Control 2	33% 1	33% 2	66% 1	66% 2	100%1	100% 2
Fluorene	< 0.029	< 0.029	2.9	3.2	4.05	7.2	9.08	12.4
PHN	< 0.042	< 0.042	1.48	2.09	1.69	1.06	4.64	4.23
Anthracene	< 0.048	< 0.048	0.34	0.59	3.1	1.4	1.74	2.14
Fluoranthene	< 0.038	< 0.038	0.77	1.13	0.8	1.5	2.01	0.78
Pyrene	< 0.048	< 0.048	0.25	0.27	0.54	0.4	1.7	1.94
Benz(a)anthracene	< 0.030	< 0.030	0.56	0.36	0.33	0.3	1.54	1.16
Chrysene	< 0.032	< 0.032	0.15	ND	0.21	0.14	3.98	4.35
Benzo(ghi)perylene	< 0.025	< 0.025	0.52	0.24	3	2.8	0.9	0.8
Benzo(b)fluoranthene	< 0.028	< 0.028	0.39	0.32	0.26	0.3	0.5	0.9
Benzo(k)fluoranthene	< 0.022	< 0.022	< 0.022	< 0.022	< 0.022	< 0.022	< 0.022	< 0.022
Benzo(e)pyrene	< 0.043	< 0.043	0.34	0.36	0.21	0.73	3.72	3.35
Benzo(a)pyrene	< 0.021	< 0.021	0.78	0.58	0.79	2.2	0.28	0.45
Dibenz(ah)anthracene	< 0.020	< 0.020	1.88	4.76	2.3	1.4	8.35	5.98
Benzo(j)fluoranthene	< 0.011	< 0.011	0.09	0.07	0.11	0.22	0.14	0.06
Total	< DL	< DL	10.46	13.97	17.38	19.64	38.58	38.54

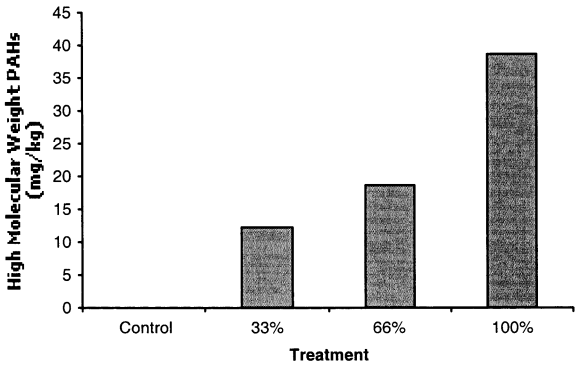

Fig. 2. Total high molecular weight PAHs measured in sediments used in fish exposures. Control represents 0% COP.

Analysis of water samples before and after the 7-days exposure indicated the presence of only indene at detectable levels. Aqueous indene concentrations in the exposure tanks and returning from the carbon filter ranged from 0.35–0.62 μg/g. All other PAHs were beneath the detection limit.

3.2. Biochemical markers of exposure

Analysis of biliary FACs revealed increased levels of equivalents of NAP, PHN and BAP from 0% COP treatment through the 66% COP treatment and then a decrease at the 100% treatment groups (Fig. 4). The 66% treatment group was statistically different from the control group. Equivalents of BAP ($P = 0.015$), PHN

Table 2
Concentrations of low molecular weight PAHs (μg/g dry weight) in COP sediments diluted with reference sediments for fish exposures

Low molecular weight PAHs	Control 1	Control 2	33% 1	33% 2	66% 1	66% 2	100% 1	100% 2
Indene	0.1	< 0.053	0.76	0.65	0.28	0.2	0.89	0.81
Naphthalene	0.08	< 0.027	1.25	0.8	0.19	0.2	4.46	4.4
Acenaphthylene	0.1	< 0.038	4.42	6.6	7.93	9.16	26.18	21.47
Acenaphthene	0.06	0.1	11.8	17.88	6.3	8.76	36.61	23.15
Perylene	< 0.028	< 0.028	0.05	ND	0.05	0.02	0.15	0.33
Indeno(123cd)pyrene	< 0.030	< 0.030	2.23	1.89	2.28	5.24	0.76	3.15
Total	0.34	0.1	20.52	27.82	17.03	23.58	69.05	53.33

ND, not determined.

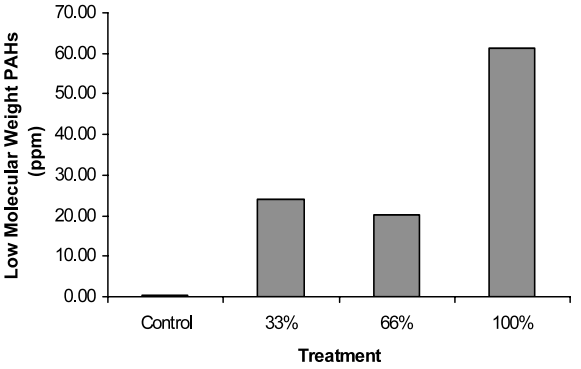

Fig. 3. Total low molecular weight PAHs measured in sediments used in fish exposures.

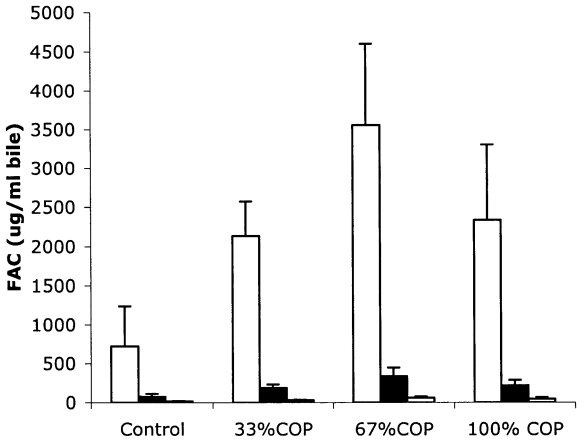

Fig. 4. Equivalents of NAP, PHN and BAP (μg/ml) in bile of hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of petroleum contaminated sediments for 7 days. Values represent mean and S.D. of measurements. Asterisks (*) denote statistically significant difference from control ($P \leq 0.05$).

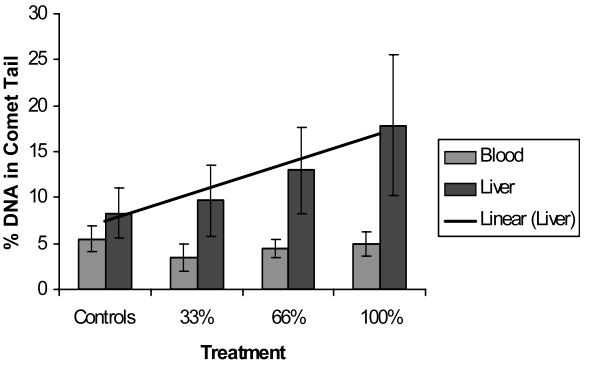

Fig. 5. Percent DNA damage in comet tail for the blood and liver of hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of PAH contaminated sediments.

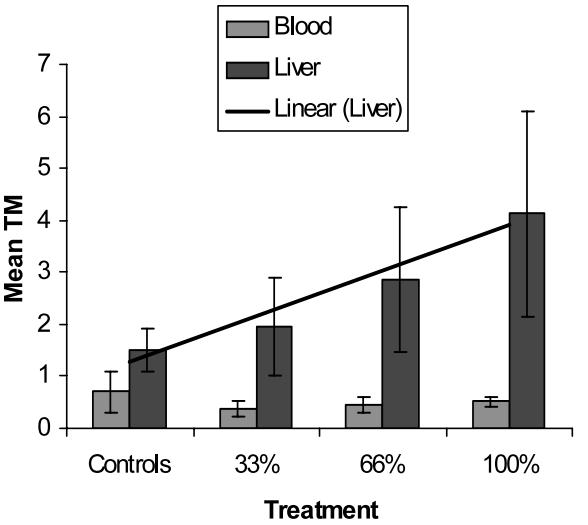

Fig. 6. TM values for the blood and liver of hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of PAH contaminated sediment.

(0.0068), and NAP (0.011) were log transformed prior to ANOVA analysis. The 66% treatment group was approximately 400, 350 and 300% higher than the 0% COP treatment group for NAP, PHN and BAP, respectively. Mean high molecular weight sediment PAH concentrations of 18.5 μg/g produced a statistically significant increase in biliary FACs for equivalents of BAP and PHN, while mean low molecular weight PAH concentrations of 20.3 μg/g produced a significant increase in equivalents of NAP.

Levels of hepatic DNA damage increased from the 0% COP sediments to the 100% COP sediments (Figs. 5 and 6). The mean tail moment (TM) was 26, 93 and 187% higher than the control for the 33, 66 and 100% treatments, respectively (Fig. 6). DNA damage in the blood revealed no significant relationships with any other metric.

Hepatic CYP1A expression was only observed in fish receiving the 100% COP treatment and was not detected in fish from any of the other treatments (Fig. 7). Plasma estradiol was observed

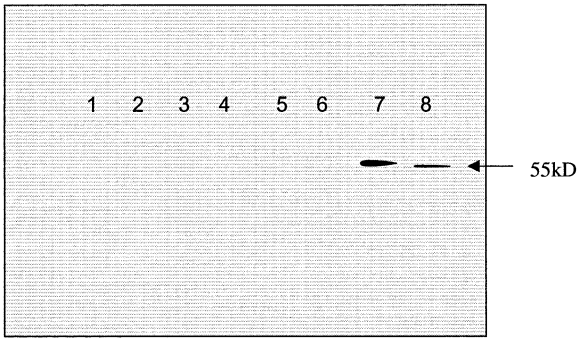

Fig. 7. CYP1A immunoblot of hepatic microsomes from hornyhead turbot exposed to a gradient of petroleum-contaminated sediments for 7 days. Lanes 1–2 Control (0% COP sediments); Lanes 3–4 (33% COP sediments); Lanes 5–6 (67% COP sediments); Lanes 7–8 (100% COP sediments).

slightly above detection in male and female fish exposed to all sediment exposures having PAHs. Because of the low sample number of males and females ($n = 1$–2) no statistical comparisons could be carried out between gender. However, when males and females were combined, a significant reduction in serum/plasma estradiol was observed following exposure to all of the COP dilutions (Table 3).

Linear regression analyses revealed a significant relationship (r^2: 0.66; $P = 0.014$) between DNA damage (TM) and high molecular weight PAHs. A significant relationship was also observed between DNA damage (TM) and low molecular weight PAHs (r^2: 0.56; $P = 0.032$), Significant relationships were observed between sediment BAP concentrations and biliary PHN equivalents (r: 0.99; $P = 0.0084$), sediment BAP and biliary NAP equivalents (r: 0.99; $P = 0.011$), as well as sediment PHN and NAP equivalents (r: 0.99; $P = 0.0022$).

4. Discussion

Previous studies have indicated the value of biochemical markers in exploring effects due to PAH contamination in marine systems (Collier et al., 1995; Spies et al., 1996). The use of multiple biomarkers in a laboratory setting provides insight into the processes and mechanisms that govern field responses of organisms exposed to PAH contaminated sediments. This study sought to explore biochemical effects and dose–response relationships using hornyhead turbot (*Pleuronichthys verticalis*). Hornyhead turbot are generally associated with soft mud and sand bottoms and are found throughout southern California coastal waters (Kramer et al., 1995; Love, 1996) at depths ranging from 10 to 90 m, but have been observed at depths of up to 201 m (Allen, 1982; Love, 1996). Their diet consists almost entirely of worms, although they also feed on clam siphons (Allen, 1982; Love, 1996). The demersal ecology of this flatfish species and its resilience in captivity make it a sound experimental model for biochemical endpoint studies.

Only six low molecular weight hydrocarbons were measured and compared to 14 high molecular weight PAHs. Earlier studies have demonstrated the occurrence of numerous alkylated PAHs as well as branched and cyclic alkanes (Spies et al., 1980; Stuermer et al., 1982). Concentrations of low molecular weight compounds in COP sediments were higher (61.2 μg/g at the 100% COP treatment) than the high molecular compounds (38.6 μg/g in the 100% COP treatment). This is consistent with previous studies showing that natural petroleum seeps lend to have higher concentrations of lower molecular weight aromatic hydrocarbons (Spies et al., 1980).

Table 3
E2 concentrations (pmol/ml) in hornyhead turbots exposed to COP sediments

	Reference	33%	67%	100%
Female	1750+900 (3)	104±12 (2)	102±8 (2)	116±60 (2)
Male	17.6 (1)	ND (2)	ND (2)	ND (2)

Each value represents the mean of each value in parentheses ±S.D.

The use of biliary FACs as a biochemical endpoint has proved an effective method of determining exposure to xenobiotic compounds (Krahn et al., 1987; Beyer et al., 1996; Aas et al., 2000a). Our results indicate a clear increase in BAP, PHN, and NAP equivalents in bile with increasing sediment PAH concentrations up to 100% COP treatment group, which possessed reduced levels of PAH equivalents when compared to the 66% treatment group. Other studies have demonstrated that increasing levels of PAH contaminants in sediments, diet and water result in higher levels of biliary FACs in fish exposed to these contaminants. Aas et al. (2000a) reported an increase in biliary PAH metabolites over a 30-day period in a laboratory experiment with Atlantic cod (*Gadus morhua*) exposed to crude oil. Hellou and Upshall (1995) exposed winter flounder (*Pleuronectes americanus*) to PAH contaminated sediments and found a strong relationship between PAH metabolites in bile and sediment PAH contaminants. Spies et al. (1996) observed compounds fluorescing at NAP and PHN wavelengths in rainbow surfperch (*Hypsurus caryi*) to be significantly higher from a petroleum seep in close proximity to COP with high levels of PAH contaminants compared to a reference location. In the same study, a significant difference between compounds fluorescing at PHN wavelengths from the petroleum seep when compared to the reference for rubberlip surfperch (*Rachochilus toxodes*) was also observed. An earlier study by Collier and Varanasi (1991) observed an excellent dose–response relationship for FAC levels in bile of English sole (*Pleuronectes vetulus*) exposed to an increasing concentrations of sediment BAP. The lower FAC response at 100% COP was intriguing and warrants further studies to determine the potential mechanism(s) for this phenonmenon.

Hepatic CYP1A induction was only observed in animals receiving the 100% COP treatment. Compared to the serum estradiol, or FAC responses, the CYP1A response was the least sensitive. These results contrast other studies which have demonstrated coordinate increases in CYP1A with FACs in field samples (Collier and Varanasi, 1991; Spies et al., 1996). Reasons for the insensitivity of CYP1A in hornyhead turbot include the short duration of a sediment-only exposure (7 days), the predominance of low molecular weight PAHs (limited Ah-receptor binding), and/or unique species-specific peculiarities in CYP1A expression. Little is known regarding the CYP1A response in hornyhead turbot, so further calibration studies are necessary to determine whether unique species effects are present.

Although other indicators of PAH uptake were only induced in fish receiving 67 and 100% COP treatments, serum/plasma estradiol concentrations were reduced by all treatments in both genders. Reductions in circulating steroid concentrations in fish following exposure to PAHs have been observed in earlier studies. Female flounder (*Platichthys flesus*) exposed to dietary PAHs chronically for 12 weeks demonstrated significant reductions in plasma steroid levels (Monteiro et al., 2000a). Atlantic croaker dietarily exposed to BAP (Thomas, 1988) and English sole (*Parophrys vetulus*) captured in areas contaminated with PAHs and PCBs (Johnson et al., 1988) also possessed lower levels of serum/plasma estradiol. Many field studies are often confounded by the occurrence of other reproductive toxicants in 'contaminated sediments'. However, in the current study, PAHs are the predominant, if not exclusive, chemicals in the sediments that were used for the fish exposures. The mechanism(s) for PAH-mediated antiestrogenicity are complex and appear to involve not only reductions in estrogen receptor levels (Chaloupka et al., 1992), but also inhibition of steroidigenic enzymes (Monteiro et al., 2000b). Since FAC or CYP1A values failed to correlate with either group of PAHs, it is possible that other hydrocarbons or PAHs not measured analytically may be involved in the repression of estradiol in the exposed fish. As it was not possible to differentiate gender prior to exposure, future studies implementing greater numbers of organisms are necessary to obtain adequate sample size for each gender to determine threshold responses for steroid reduction and other reproductive anomalies due to PAH exposure.

Johnson et al. (1993, 2002) observed relationships between levels of FACs indicative of PAH exposure and inhibition of ovarian development in English sole females from sediment contaminated

sites in Eagle Harbor and the Duwamish Waterway in Puget Sound, WA. The National Marine Fisheries Program of the National Oceanic and Atmospheric Administration has recommended a sediment quality criteria of 1 μ/g of total PAH for adverse reproductive and hepatic lesions in English sole (Johnson et al., 2002). Although obtained from a non-urban source, the concentrations observed in this study significantly exceed these recommendations. Further study on organisms residing in this location are necessary to better understand the adaptive mechanisms that allow these animals to survive in this ecosystem.

The assessment of DNA damage has proven effective in monitoring the effects of xenobiotic chemicals in sediment (Padrangi et al., 1995; Steinert et al., 1998; Aas et al., 2000a). Our results indicate a clear dose–response relationship in hepatic DNA damage from the 0% COP treatment up to the 100% COP treatment, indicating that increased concentrations of sediment PAHs may be responsible for an increase in levels of hepatic DNA damage in hornyhead turbot. Levels of DNA damage in the control fish were identical to those from fish obtained at the OCSD reference site where sediments were collected (Roy et al., 2003). French et al. (1996) also reported a linear increase in DNA damage (hepatic DNA adducts) in English sole with increasing concentrations of sediment PAHs. Levels of DNA damage in bullheads (*Ameriurus nebulosus*) collected at PAH contaminated sites (Big Creek, Hamilton Harbour, and the Detroit River) were elevated when compared to reference locations (Padrangi et al., 1995). Stein et al. (1992) reported increased levels of DNA damage (PAH adducts) in English sole and starry flounder (*Platichthys stellatux*) sampled from the PAH contaminated Duwamish Waterway when compared to a reference station near Puget Sound, WA. Levels of DNA damage (hepatic DNA adducts) were also found to be significantly elevated in Atlantic cod and corkwing wrasse (*Symphodus melops*) at a PAH contaminated site in the Karmsund Straight, Norway (Aas et al., 2001). As with FAC and CYP1A analyses, failure of DNA damage to inversely correlate with estradiol diminishment indicates other compounds or mechanisms may be involved in steroid reduction and warrants further study on uncharacterized hydrocarbons in petroleum.

COP seep sediment PAH concentrations were much higher than concentrations typically found in most other areas of southern California coastal waters (Anderson et al., 1999; Schiff et al., 2000). It is generally accepted that anthropogenic sources and emissions of PAHs into the southern California marine environment have been steadily decreasing since 1970 (Schiff et al., 2000). As a result, concentrations of PAHs in sediments have also been declining (Schiff et al., 2000). Improved wastewater treatment since the 1970s has allowed a recovery of the benthic infaunal community and demersal fish populations surrounding outfall areas, including hornyhead turbot and California tonguefish (*Symphurus atricauda*) that were impacted by chemical contamination several decades ago (Schiff et al., 2000). Although the sediment PAH concentrations in this study were higher than in other areas of southern California that are recovering from historical impacts, this information could prove useful in monitoring areas immediately surrounding oil platforms where concentrations of PAHs tend to be higher or to verify remediation efforts.

5. Conclusion

The most sensitive sublethal response observed in a sediment-only exposure scenario was serum/plasma estradiol which was reduced in male and female animals at the lowest concentration of COP sediments (approximately 36 μg/g of the 20 PAHs measured) after 7-days of exposure. Significant increases in biliary FACs and hepatic CYP1A were only observed in fish receiving the 67 and 100% COP treatments. Although no significant increases were observed, the relationship between hepatic DNA damage and the increasing concentration gradient of PAH contaminated sediments indicated reliability of this biochemical endpoint in demonstrating PAH uptake and bioavailability exposure in an acute exposure scenario. This study also demonstrates that a dietary component for PAH contamination may not be necessary to impair reproduction in flatfish species. Although

a proposed threshold for hepatic damage related to total PAHs in sediment has been suggested, further studies are necessary to determine threshold PAH concentrations for 'natural' seep sediments that impair plasma/serum steroid concentrations and, likely, reproduction in flatfish.

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