OCS Study
MMS 2006-008

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Final Technical Summary

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FINAL TECHNICAL SUMMARY

STUDY TITLE: Use of Biological Endpoints in Flatfish to Establish Sediment Quality Criteria for Polyaromatic Hydrocarbon Residues and Assess Remediation Strategies.

REPORT TITLE: Use of Biological Endpoints in Flatfish to Establish Sediment Quality Criteria for Polyaromatic Hydrocarbon Residues and Assess Remediation Strategies.

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KEY WORDS: Polycyclic aromatic hydrocarbons, *Pleuronichthys verticalis*, Fluorescent aromatic compounds, DNA damage, PAHs, oil seep, CYP1A, FACs, GSI, sex steroids

BACKGROUND: Input of Polyaromatic hydrocarbons (PAHs) occurs through anthropogenic and natural mechanisms. Toxicities resulting from chronic exposure include immune suppression, reproductive dysfunction and carcinogenesis. As most PAHs tend to be lipophilic, there is a high propensity for accumulation in organisms and sediments with high organic content. While analytical measurement of specific compounds has been shown to be a relevant indicator of exposure within invertebrates, rapid biotransformation prevents accurate assessments of exposure in vertebrates such as fish.

OBJECTIVES: The following study attempted to utilize biochemical and physiological indicators in flatfish to estimate a threshold concentration which could be used in risk assessment paradigms to evaluate sediments contaminated with PAHs.

DESCRIPTION: Two species of flatfish were exposed to various dilutions of sediments collected from the natural oil seep off the coast of Santa Barbara, CA. In contrast to other studies carried out in anthropogenically contaminated areas, the predominant PAHs observed in the sediments were of low molecular weight. Hepatic cytochrome P450 1A (CYP1A), bilary fluorescent aromatic compounds (FACs), plasma steroid concentrations, gonadal somatic indices, and in some cases, hepatic DNA damage was utilized in each species exposed for either 7 (Hornyhead turbot) or 30 days (California Halibut) to diluted sediments. Attempts were made to generate dose-response curves which could be calibrated against reproductive function (GSI, sex steroids) for the estimation of sediment threshold concentrations.

SIGNIFICANT CONCLUSIONS: Of all of the endpoints measured, diminished plasma concentrations of 17β-estradiol was the most sensitive endpoint in either species. With the exception of hepatic DNA damage in Hornyhead turbot following the 7 day exposure, none of the endpoints, in particular, the reproductive endpoints, displayed dose-response relationships preventing comparisons between indicators. Comparisons of a potential threshold at the 1% COP sediment dilution for E2 diminishment with literature values for anthropogenic PAHs, demonstrated significant recalcitrance toward reproductive dysfuntion in California Halibut. Reasons for the discrepancy include a differing profile of specific PAHs, with low-molecular weight PAHs likely being less toxic than higher molecular weight PAHs.

STUDY PRODUCTS:

Abstract – Chapter 1:

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 4 treatments containing 0% (sediment from the Orange County Sanitation District's reference location), 33%, 66%, and 100% (COP) sediments. Sediment concentrations of twenty 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.

Abstract - Chapter 2:

Coal Oil Point (COP) is a natural oil seep off the coast of Santa Barbara, California. Although most studies examining the fate and effects of petroleum have focused upon urbanized or anthropogenic sources of inputs, few have examined the effects of PAHs derived from natural seeps. In order to evaluate the effects of polyaromatic hydrocarbons (PAHs) derived from COP on marine fish populations, hatchery-reared California Halibut (*Platichthys californicus*) were exposed for 30 days to seven dilutions of sediments collected from COP. Hepatic cytochrome P450 1A (CYP1A), biliary fluorescent aromatic compounds (FACs), gonadal somatic indices, and plasma steroid concentrations. Sixteen USEPA priority PAHs were targeted fro analysis in each sediment dilution. In general, biochemical responses were somewhat recalcitrant to dose-response relationships and were less sensitive than literature values established for the same indicators following exposure to urbanized PAHs. Trends toward reductions in plasma 17β–estradiol concentrations were observed, but reductions in gonadal somatic indices were not observed. FAC values for naphthalene, benzo(a)pyrene, phenanthrene-related compounds reached maximums at 33% COP sediment, but declined at higher concentrations. The resulting insensitivity may be unique for exposure to "natural" petroleum due to a higher concentration of lower molecular weight PAHs or uncharacterized inhibitors.

Publications:

Roy, L.A., S. Steinert, S. Bay, D. Greenstein, Y. Sapozhnikova, O. Bawardi, I. Leifer, and D. Schlenk. 2003. Biochemical effects of petroleum exposure in hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of sediments collected from a natural petroleum seep in CA, U.S.A. *Aquatic Toxicology* **65**:159-169.

Seruto, C., Y. Sapozhnikova, and D. Schlenk. 2004. Evaluation of the relationships between biochemical endpoints of PAH exposure and physiological endpoints of reproduction in male California halibut (*Paralichthys californicus*) exposed to sediments from a natural oil seep. *Marine Environmental Research* (submitted).

FINAL STUDY REPORT

Chapter 1.

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.
(Published in Aquatic Toxicology (2003), 65:159-169)

Luke A. Roy, Scott Steinert, Steve M. Bay, Darrin Greenstein, Yelena Sapozhnikova, Ola Bawardi, Ira Leifer, Daniel Schlenk.

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). 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 Coal Oil Point, has been estimated to have been occurring for thousands of years (Simoneit and Kaplan, 1980). An estimated 9500-19000 liters/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 (Straughan, 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 fluorescent aromatic compounds 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, California. The exposure system (Figure 1) consisted of eight 10-gallon 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 % $\pm$ 0.004. The composition was 73.8 % sand; 21.3% silt, and 4.90 % clay with a median grain size (phi) 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-gallon 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 standard deviation) and 116.7 grams (± 30.6 standard deviation), respectively. Gender was not differentiated prior to exposure. Water from the recirculating system was filtered through a 40 L carbon filter (Figure 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 hours of light and 8 hours of darkness.

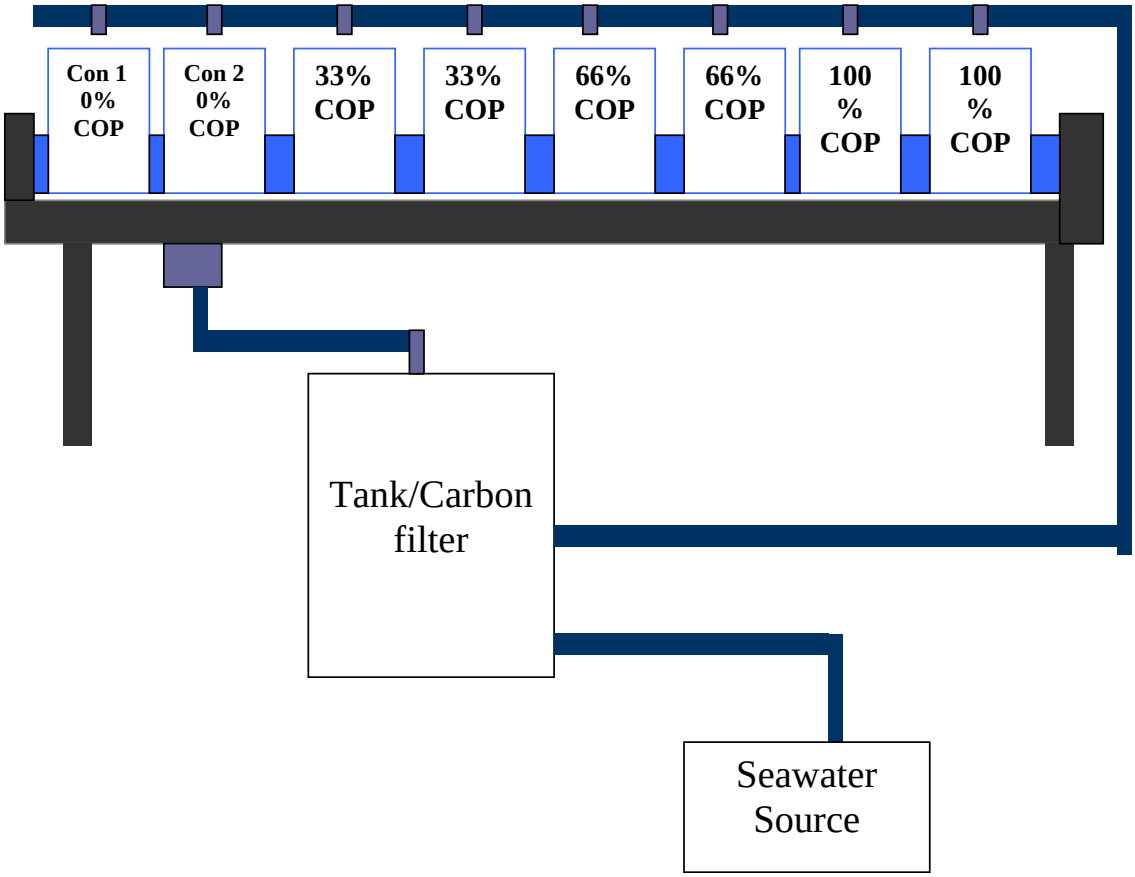

Figure 1. Recirculating system for exposing fish to sediments.

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 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 four 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 minutes 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°/min, and up to 300°C with 5°/min. The recoveries were 91-100% with a standard deviation of 4-13% and method detection limit (MDL) of 6-41 ng/g for water. In sediment, recoveries were 30-111% with a standard deviation of 2-15% and MDLs ranging from 11-53 ng/g sediments.

2.3 Biochemical endpoint measurements

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; Krahn et al., 1986). Fluorescence was measured with a Shimadzu fluorescence detector (RF-10 AXL) at 380/430 nm, 256/380 nm, and 290/335 nm, excitation/emission for BAP, PHN, and NAP, respectively.

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 (PBS)/10% DMSO. Small sections of liver were placed in 1 ml of ice-cold cryopreservation solution. Within 20 minutes, all samples were frozen in liquid nitrogen. Samples were transported to the Comet Analysis Laboratory and transferred to a -80°C freezer. Samples were evaluated for DNA damage using Comet analysis as previously described (Steinert et al. 1998).

CYP1A: Hepatic CYP1A levels were quantified using Western blotting techniques followed by semi-quantitative measurements of densitometry in optical density units (ODU) 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, USA) method with bovine serum albumin as the standard. Protein samples were normalized to contain 50 micrograms of protein per lane.

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 x g for 2 minutes at room temperature. Plasma/Serum was removed and stored in liquid nitrogen until processed for estradiol measurements. Estradiol was measued using enzyme-linked immunosorbant assay kits purchased from Cayman Chemical Co (Ann Arbor, MI, USA) following the manufactures 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).

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
phenanthrene	< 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

Table 2: Concentrations of low molecular weight PAHs (μg/g dry weight) in COP sediments diluted with reference sediments for fish exposures. ND = not determined.

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

High molecular weight PAHs increased linearly from the 0% COP treatment through the 100% treatment (Figure 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 (Figure 3). Analysis of water samples before and after the 7-day 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.

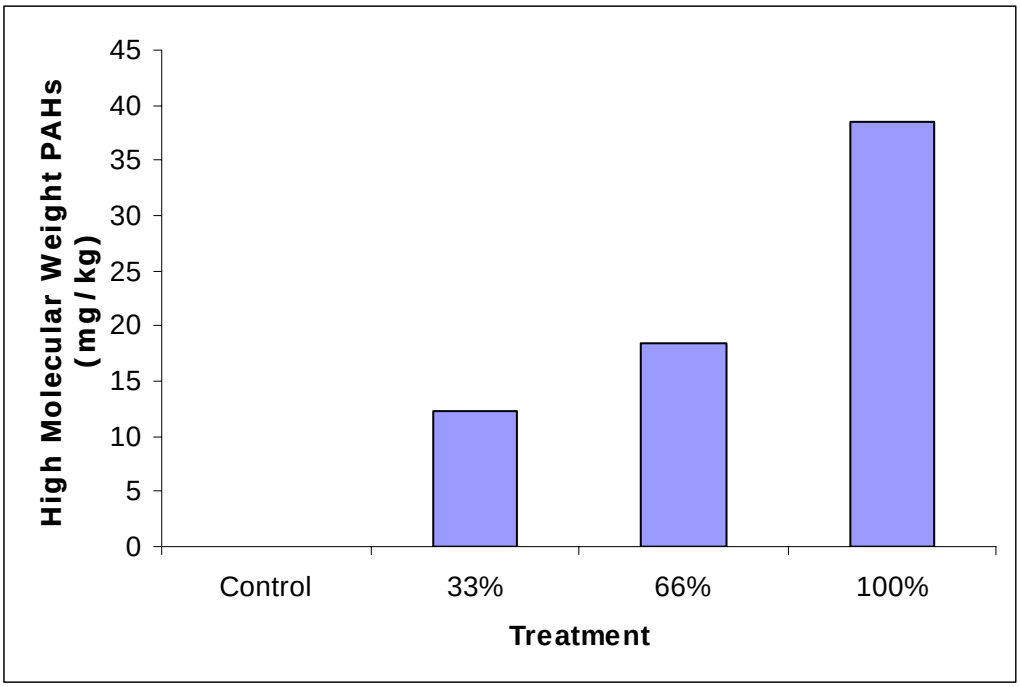

Figure 2. Total high molecular weight PAHs measured in sediments used in fish exposures. Control represents 0% COP.

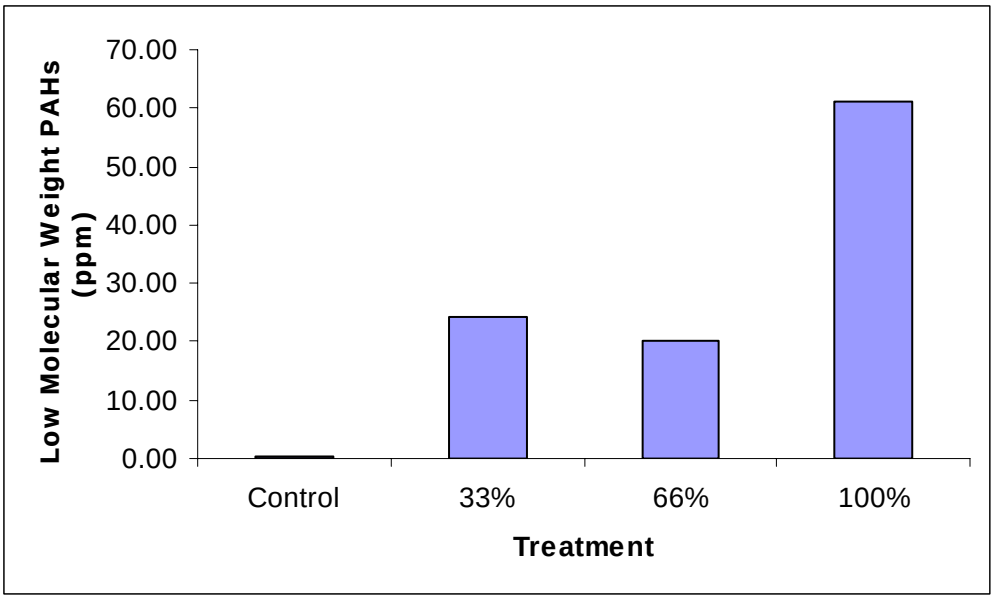

Figure 3. Total low molecular weight PAHs measured in sediments used in fish exposures.

3.2. Biochemical markers of exposure

Analysis of biliary FACs revealed increased levels of equivalents of naphthalene (NAP), phenanthrene (PHN), and benzo[a]pyrene (BAP) from 0% COP treatment through the 66% COP treatment and then a decrease at the 100% treatment groups (Figure 4). The 66% treatment group was statistically different from the control group. Equivalents of BAP ($p=0.015$), PHN (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 benzo[a]pyrene and phenanthrene, while mean low molecular weight PAH concentrations of 20.3 μg/g produced a significant increase in equivalents of naphthalene.

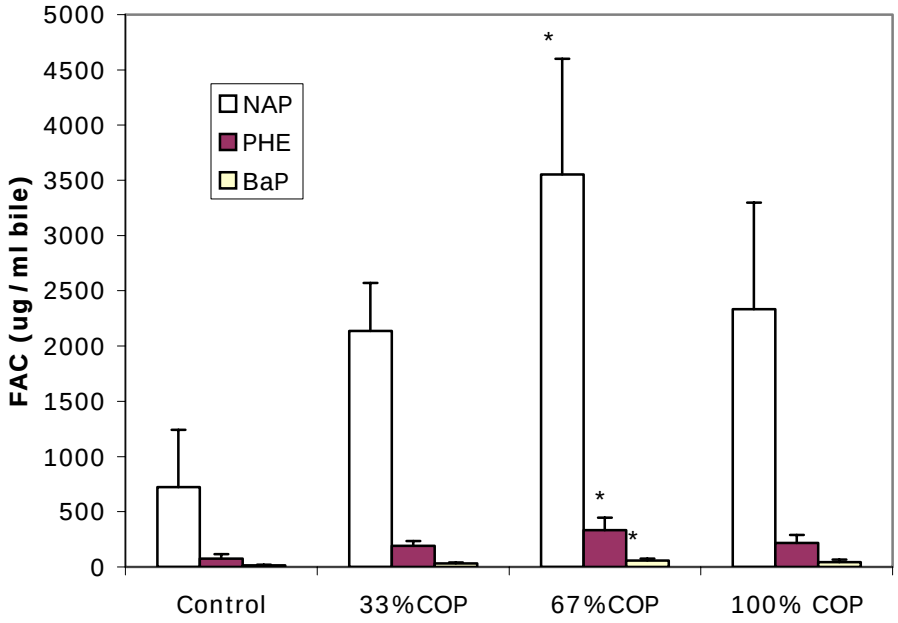

Figure 4. Equivalents of naphthalene, phenanthrene, and benzo(a)pyrene (µg/ml) in bile of hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of petroleum contaminated sediments for 7 days. Values represent mean and standard deviations of measurements. Asterisks (*) denote statistically significant difference from control ($p \leq 0.05$).

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

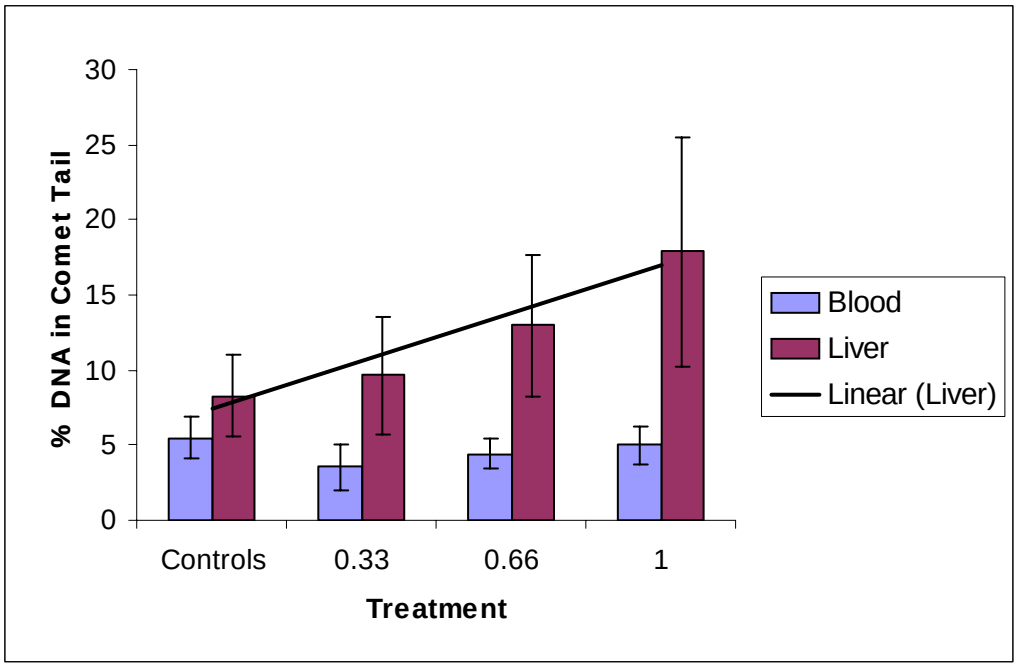

Figure 5. Percent DNA damage in comet tail for the blood and liver of hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of PAH contaminated sediments.

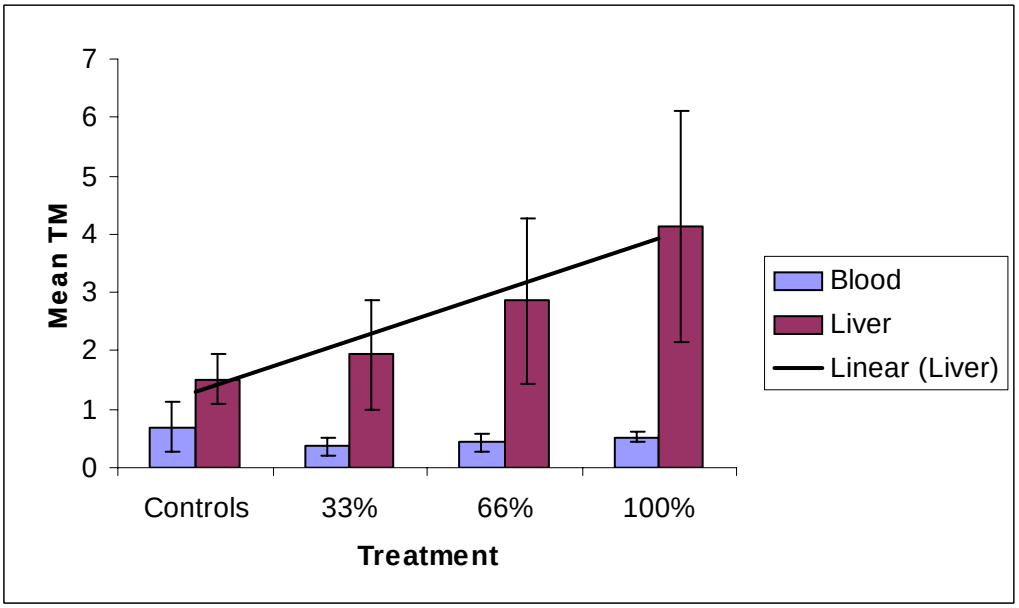

Figure 6. Tail Moment (TM) values for the blood and liver of hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of PAH contaminated sediment.

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. Plasma estradiol was observed 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).

Table 3. E2 Concentrations (pmol/ml) in Hornyhead turbots exposed to Coal Oil Point Sediments. Each value represents the mean of each value in parentheses $\pm$ SD.

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

Linear regression analyses revealed a significant relationship (r^2: 0.66; p = 0.014) between DNA damage (Tail Moment) and high molecular weight PAHs. A significant relationship was also observed between DNA damage (Tail Moment) 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 10-90 meters, but have been observed at depths of up to 201 meters (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 6 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 tend to have higher concentrations of lower molecular weight aromatic hydrocarbons (Spies et al., 1980).

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 the 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 naphthalene and phenanthrene 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 phenanthrene 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 benzo[a]pyrene. 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 as the least sensitive. These results contrast other studies which have demonstrated coordinate increases in CYP1A with FACs in field samples (Collier et al. 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 an 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 studies 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 stellatus*) 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.

Coal Oil Point 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 1970's 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 twenty 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.

Chapter 2.

Evaluation of the Relationships Between Biochemical Endpoints of PAH Exposure and Physiological Endpoints of Reproduction in Male California Halibut (Paralichthys californicus) Exposed to Sediments from a Natural Oil Seep. (Submitted to Marine Environmental Research)

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1. Introduction

Input of PAHs into the marine environment continue to increase (Brown, McCain et al. 1998; Matthiessen and Law 2002). Although anthropogenic sources are thought to dominate environmental input, natural oil seeps can also contribute a significant amount to local environments (Reed et al 1977, Blumer 1972, McKirdy 1986, Wijayarante and Means 1984). Seepage from the natural oil seep near Coal Oil Point, off the coast of Goleta/Santa Barbara, California has been estimated to have been occurring for thousands of years at 95000 to 19000 liters/day of petroleum (Simoneit and Kaplan 1980, Allen et al 1970). In contrast to urbanized PAHs, low molecular weight hydrocarbons tend to be the primary constituents of the seep material (Spies et al. 1980).

Indicators of PAH bioavailability in several studies around the world in various fish species include a variety of biochemical, histological and physiological endpoints (Petersen and Kristensen 1998; Arinc and Sen 1999; Kirby, Matthiessen et al. 1999; Miller, Mills et al. 1999; Aas, Baussant et al. 2000; Monteiro, Reis-Henriques et al. 2000; Teles, Pacheco et al. 2003). These indicators of exposure have been linked to various effects such as carcinogenicity, hepatic lesions, precocious and inhibited gonadal development, alterations in egg size and number, inhibited spawning, morphological abnormalities, and reduced egg and larval viability (Johnson et al 1999, 1997, 1993, 1988, Casillas et al 1991, Collier et al 1992, Incardona 2003). However, most of these studies evaluated urbanized multi-ring PAHs resulting from anthropogenic inputs.

Previous studies in feral fish collected from the COP area demonstrated enhanced hepatic cytochrome P4501A (CYP1A) expression and biliary fluorescent aromatic compounds, indicative of PAH bioavailbility (Spies et al. 1996; Roy et al. 2003). Seven day exposure studies by Roy et al. (2003) examining biochemical responses in naïve Hornyhead Turbot (*Pleuronichthys verticalis*) exposed to COP sediments indicated enhanced CYP1A and FAC concentrations, but at sediment PAH concentrations significantly higher than sediment thresholds suggested in urbanized areas (Collier et al. 2002). In contrast, serum estradiol concentrations were significantly reduced following sediment-only exposures at the lowest PAH dilution which was approximately 12 ug/g of 16 PAHs listed as USEPA priority pollutants (Roy et al. 2003).

To determine the potential effects of diminished steroid concentrations on higher level responses in flatfish, cultured male California halibut (*Paralichthys californicus*) were exposed to 7 concentrations of COP sediments for 30 days. Gonadal somatic indices, plasma steroid

concentrations, hepatic CYP1A and biliary FACs were measured to determine whether relationships existed between biochemical and physiological endpoints. California Halibut are benthic flatfish which inhabit estuaries and nearshore coastal areas as larvae and juveniles, then increase in spatial distribution offshore with increasing age. California Halibut are also an economically important species, being on average the 6th most valuable vertebrate catch off the California coast. Revenue for 2002 was $2,800,000 for California Halibut, with an average increase of about 10% per year for the past three years (http://www.st.nmfs.gov/; Personal communication from the National Marine Fisheries Service, Fisheries Statistics and Economics Division, Silver Spring, MD). Exposures were conducted with the aim of determining dose response concentrations which may be used in helping to set sediment thresholds for adverse effects and help in assessing locations undergoing remediation.

2. Materials and Methods

2.1 Exposure System

PAH contaminated sediment was collected from the natural marine oil seep Coal Oil Point (COP) in Goleta (Santa Barbara) (119.53.428 Longitutde; 34.24.370 Latitude), California as previously described (Roy et al. 2003). Exposure sediments contained 0, 0.33, 0.66, 1, 33, 66, and 100 percent COP sediments diluted to their respective nominal concentrations with reference sediment from Orange County, California (118.05.1999 Longitude; 33.36.055 Latitude). Due to consistency restraints, total organic carbon (TOC) and grain size could not accurately be determined from COP sediments. The TOC of the reference sediments were 0.42 $\pm$ 0.004 %. The composition was 73.8% sand; 21.3% silt and 4.9% clay with a median grain size of 3.68 (Roy et al. 2003). Exposures were conducted at The SEA Laboratory in Redondo Beach, California using filtered ambient seawater (18 $\pm$ 1 ^{o}C) from inflow pipes from the open ocean. Aquaria were housed in shaded outdoor areas receiving indirect sunlight in June of 2003 under natural light:dark conditions.

Male California halibut (56.5 $\pm$ 5.0 cm; 550 $\pm$ 52 g) were generously donated by Hubbs SeaWorld Research Institute (Carlsbad, CA). Fish were acclimated to the facilities at The SEA Laboratory for over two months in a circular tank of approximately 12 m^3. Prior to the addition of fish, approximately 2 L of sediment were transferred into 40 liter glass tanks under flow-through conditions and the sediment was allowed to settle. Two fish were placed in each aquaria, with two replicates per exposure. Fish were fed pellet food daily and exposures were conducted for thirty days.

Following 30 days, fish were transferred to containers having 10 L of seawater with 10g/L of MS 222. Following anesthesia, the fish were weighed and the lengths measured. Blood samples were collected from the spinal caudal artery with a syringe, and immediately centrifuged at 750 x g to separate serum/plasma. Serum/plasma samples were placed in separate tubes and frozen on dry ice. Length measurements were taken from the tip of the lower mouth to the end of the tailbone where the back fin begins to scale differently than the body scales. Backbones were severed with a knife, and liver and bile samples were removed, placed in cryogenic tubes, and immediately placed in dry ice for transport to a -80 freezer.

2.2 Chemical evaluation of PAHs in sediments

Chemical analyses of sediment were carried out as reported in Roy et al (2003). Samples were stored at 4 °C until analysis. Sixteen USEPA priority PAHs were measured as described in EPA method 8100 (USEPA, 1996). Sediment samples were extracted with hexane using an ultrasonic disruptor (550 Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA). Cleanup was performed with fully activated silica gel (8 g), conditioned 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 30–111% with a S.D. of 2–15% and MDLs ranging from 11–53 ng/g sediments. Selected PAHs were only a small representative of the total PAHs present.

2.3 Biochemical Endpoint Measurements

Blood steroid analysis- 17β–Estradiol (E2) and Testosterone (T) concentrations were analyzed spectrophotometrically using immunoassay kits from Cayman Chemical Company (Ann Arbor, MI) following the manufacturers instructions. Approximately 50 uL of serum/plasma was used for triplicate analyses. Testosterone measurements did not include 11-keto testosterone.

FAC analysis- Gall bladders were thawed on ice, and bile removed. Samples were diluted in 99% HPLC- grade methanol and fluorescence measured with a Shimadzu fluorescence detector (RF-10 AXL) at 380/430 nm, 256/380 nm, and 290/335 nm excitation/emission for benzo(a)pyrene (BAP), phenanthrene (PHN) and naphthalene (NAP) like compounds, respectively. Concentrations were calculated using standard curves developed from PHN, NAP, and BAP standards.

CYP1A analysis- Liver microsomal fractions were obtained by ultracentrifugation as previously described (Roy et al. 2003). Total protein concentrations for these fractions were attained by utilizing the Pierce kit BSA standards. CYP1A protein was measured using 50 ug of protein per sample and conducting western blots. Western blot detection was carried out using a primary anti-fish CYP1A monoclonal antibody, C10-7 from Biosense Laboratories (Bergen Norway). Proteins were initially separated in SDS PAGE, then transferred overnight to nitrocellulose. The nitrocellulose was incubated with blocking buffer (0.5 g nonfat dried milk in 50ml phosphate buffer solution) in a heat-sealed bag for one hour, then incubated with primary antibody followed by secondary anti-mouse alkaline phosphatase, each for one hour. The primary and secondary antibodies were each diluted 1000X in blocking buffer. Four fifteen minute rinses with PBS were completed in between each incubation. Following colorimetric detection, band optical densities were quantified using QuantityOne software from BIORAD (Hercules, CA). Proteins were measured using the Pierce reagent (Pierce Inc., Rockford, IL).

Statisical analysis- Bartletts's test of homogeneity was conducted to verify homogeneity of variance. If data was homogeneous, and treatment groups possessed an N of at least 3, one-way analysis of variance (ANOVA) was conducted to determine possible differences between groups. A Bonferonni post-hoc test was utilized with $p \leq 0.05$ employing GraphPad Prism 3 software (San Diego, CA).

3. Results

Concentrations of selected PAHs ranged from 0.180-99.8 ug/g (Table 1). In the 0.66 to 66 % COP sediments, acenapthene was the dominant PAH with an average concentration of 30 ug/g in the 100% COP treatment. Other PAHs with high relative concentrations included acenaphthylene as well as fluorine. Other notable concentrations included fluoranthene with an average concentration of 1.4 ug/g, followed by dibenzo(ah)anthracene at 7.2 ug/g, and phenanthrene at 4.4 ug/g in the 100% COP sediment.

Hepatic cytochrome P450 1A (CYP1A) expression was similar to the control values to the 33 % COP sediments, with significant induction at the 66 and 100% COP sediments (Figure 7). P-values were 0.05 for the 66% sediment, and 0.01 for the 100% sediment exposure.

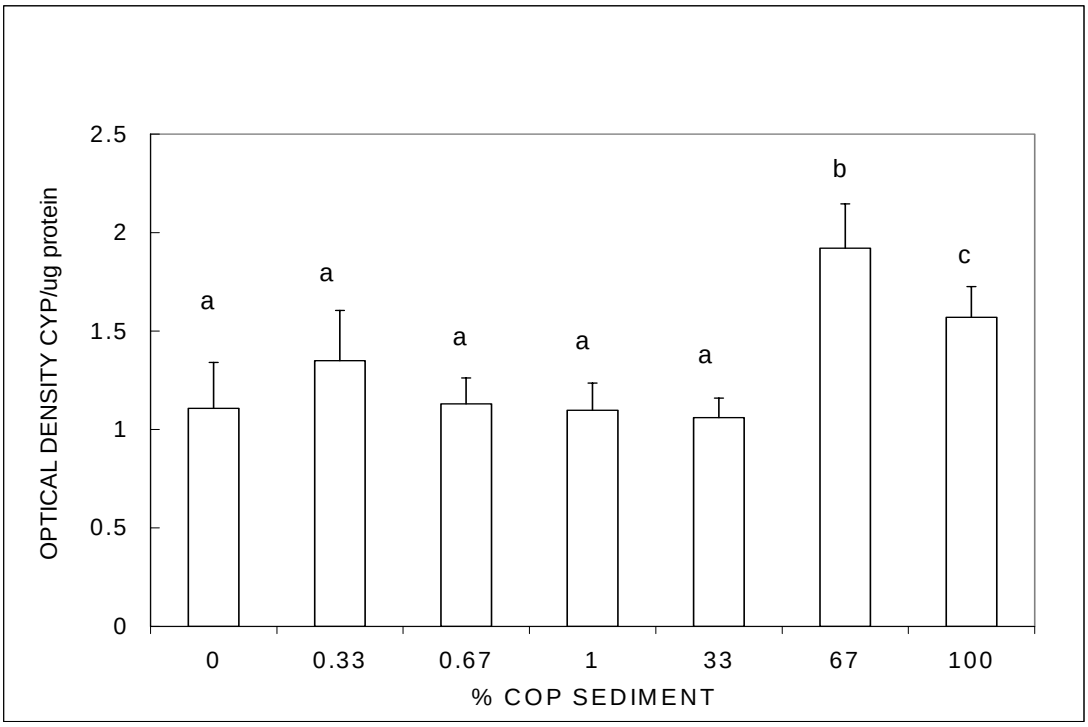

Figure 7. Hepatic cytochrome P450 1A (CYP 1A) expression in COP sediment.

FAC accumulation in bile was variable in the 33, 66 and 100% PAH sediment exposures, with a trend towards increasing FACs in the bile at the higher sediment concentrations (Figure 8).

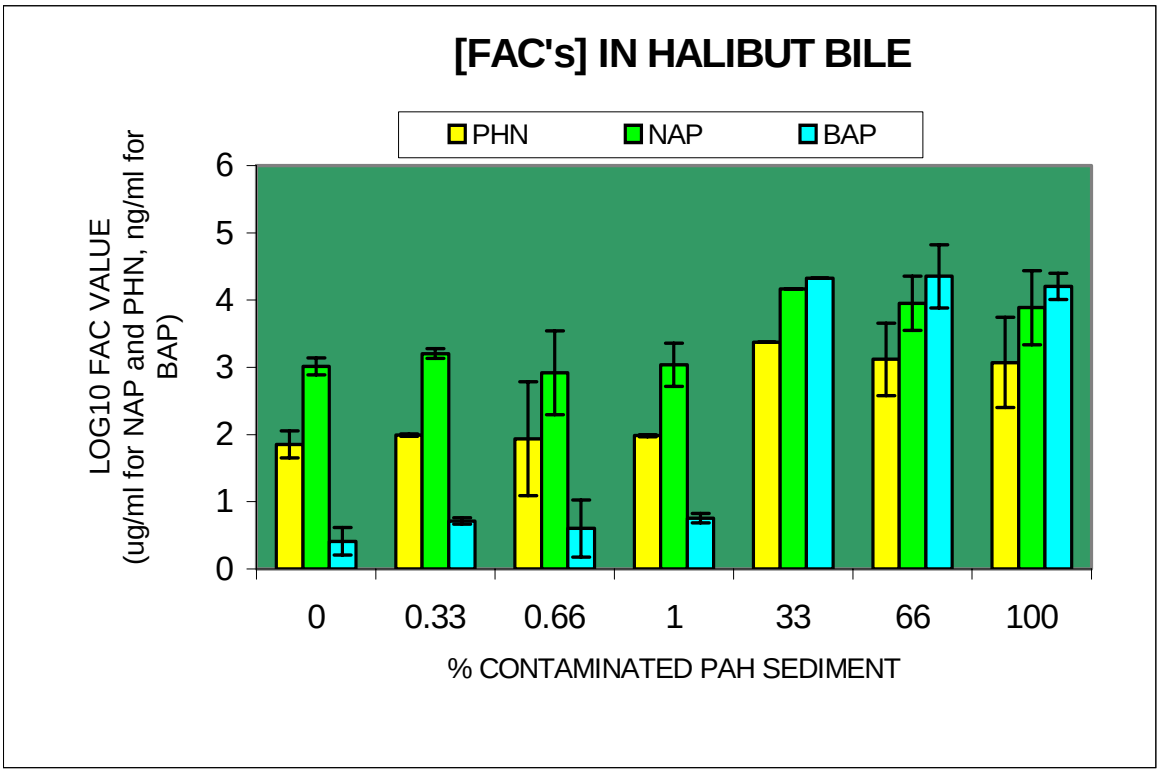

Figure 8. FAC's in halibut bile.

Values for BAP ranged from 5 to 30 ug/ml, PHE from 33 to 2,300ug/ml, and NAP from 500 to 14,000ug/ml. The lower concentrations of contaminated sediment (0.33%, 0.66%, and 1%) showed a slight trend for increasing PAHs with increases in sediment exposure. Increases in FACs did not correspond directly to increases in PAHs detected in the sediment particularly in the 33 and 66% COP sediments. Metabolites were highest following exposure to the 33% COP sediment, while PAHs were highest at the 100% sediment exposures. Much greater amounts of PAHs were found in the 100% sediments versus the 33% and 66%, and this did not result in a similar increase in FACs. FACs fluorescing at the PHN and NAP wavelengths were highly correlated, with an r^2 of 0.97. BAP values were less related to PHN and NAP, with r^2 values of 0.72 and 0.71 respectively.

No significant differences in plasma steroid concentrations between treatment groups and controls were observed (Figure 9). However, a trend toward reduction of estradiol levels was observed at the 1% COP treatment. E2 concentrations were between 347 to 849 pg/ml from the control to the 0.66% COP sediments, and from the 0.1 to the 100% COP sediments the values ranged from 21 to 494 pg/ml. T levels were lower than estradiol at all concentrations (including control). GSI values did not show any significant response to treatment.

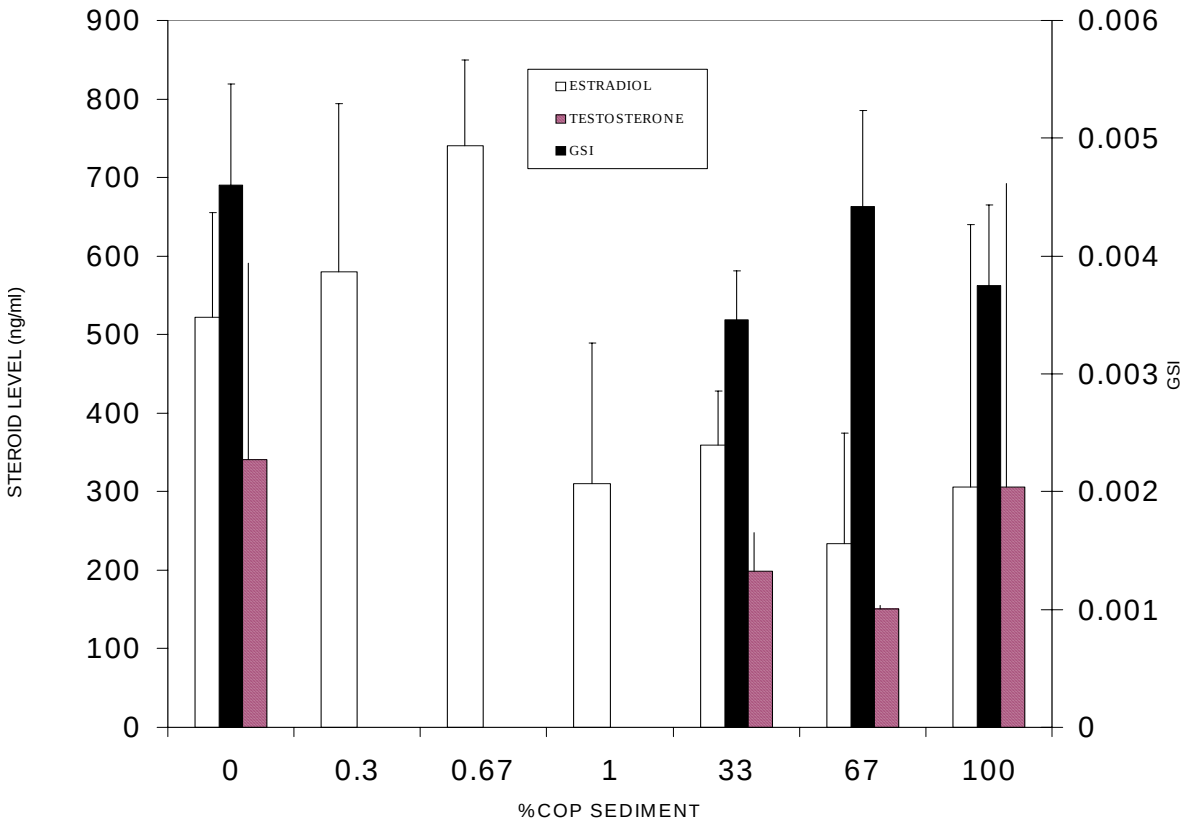

Figure 9. Plasma steroid concentrations between treatment groups.

4. Discussion

Benthic flatfish are included often in sediment contamination studies due to their continual exposure to sediments during their life histories. Threshold levels of contaminant exposure are needed in marine organisms to conduct accurate environmental risk assessments. California Halibut is an important marine fisheries resource economically, and they are also exposed to natural PAHs through seeps in the ocean floor along the California Coast as well as in their nurseries (estuaries). This study investigated the effects of natural PAH exposure to halibut by conducting lab exposures to several concentrations of natural PAH contaminated sediments from the oil seep Coal Oil Point in Goleta/Santa Barbara, California.

Attempts were made to measure 16 priority PAHs in the COP sediments and in treatment dilutions of COP sediments. The concentrations of PAHs of the 3 lowest dilutions (33, 67, and 100% COP) were similar to those found by Roy et al. (2003) who only evaluated 3 dilutions. The sediments for that study were collected more than a year prior to the sediments utilized for this study from the same location. Thus, there appears to be relatively little temporal variability in the concentrations of the 16 PAHs measured in sediments of the Coal Oil Point seep.

Although fish were exposed to sediments possessing 7 distinct PAH concentrations, no biochemical or physiological metric demonstrated a classic dose-response relationship. The

LOEC of the 16 compounds for enhanced FAC responses was approximately 36.3 ug/g and the NOEC was 1.9 ug/g. Since only targeted PAHs were measured, it is likely the NOEC and LOEC values of the total PAHs in the sediments are much greater than our estimates, and significantly higher than the 1 ug/g thresholds determined by Collier et al. (2002). Although FACs accumulated in the bile of Halibut following exposure to the PAH contaminated sediments, the response was not associated with PAH concentrations with increases only observed at the 33% COP treatment which were essentially unchanged in animals exposed to the 67 and 100% COP treatments. An 18 fold increase in the sediment PAH profiles was noted between the 1 and 33% treatments which corresponded to a 7-200-fold increase in various FACs. However, as PAH concentrations were approximately 3-fold higher in the 100% COP sediments, FAC concentrations remained unchanged. A similar "saturation" effect was noted previous in Hornyhead turbot exposed to 33, 67, and 100% COP sediments with saturation occurring at 67 rather than 33% (Roy et al. 2003). FACs have been used as indicators of PAH exposure in other species of demersal flatfish (Krahn et. al.1986, Ariese et. al. 1993, Huggett et al 2003). FACs in fish bile accumulate with longer durations of exposure, with saturation that may or may not be species or location specific (French et al 1996; Roy et al. 2003). Biliary FACs and CYP1A activity have been shown to be casually related with higher level effects such as neoplasia related liver lesions (Myers et al 1998; 2003). Contrasting studies showing direct relationships between FACs and PAH exposure, Aas et al. (2000) actually found an inverse relationship in flatfish between FACs and PAHs. Since FACs are highly dependent upon biotransformation and conjugation of PAHs to biliary metabolites, any alteration of biotransformation may have significant impacts on FAC formation. Numerous compounds have been shown to be substrates, inhibitors and/or inducers Phase I and Phase II enzymes. Fluoranthene has been shown to be a relatively strong inhibitor of CYP1A catalyzed oxidation of BaP (Willett et al. 2001). It is worthwhile to note that most previous studies have primarily focused their efforts on evaluating anthropogenic PAHs, whereas the PAHs in this study are un-urbanized. This difference in itself may be responsible for the lower than expected CYP induction. Consequently, the occurrence of complex mixtures and uncharacterized PAHs within sediments which may also act as substrates or alter metabolic processes within fish could conceivably be responsible for the lack of correlation between sediment PAH concentrations, biliary FACs and recalcitrant CYP1A induction.

Whereas FAC levels were relatively high at the 33% COP treatment, induction of CYP1A determined by protein quantification with western blots, was significant in the 66% and the 100% COP treatments. Several studies have shown marked increases in CYP1A induction in response to PAH exposure (for review see: Stegeman and Hahn 1994). This can typically be measured by optical density analysis of western blots (as completed in our study), or measurement of the catalytic activity of 7-ethoxyresorufin O-deethylase (EROD). Western blots carried out by Roy et al (2003) in Hornyhead Turbot (*Pleuronichthys verticalis*) showed similar recalcitrance with induction only in the 100% COP sediment group compared to the lower sediment exposures, which showed no presence of the protein. A study in Norway analyzing flounder (*Platichthys flesus*) and Atlantic cod (*Gadus morhua*) from various field locations found significant differences from reference and PAH-impacted areas in EROD as well as CYP activity (Beyer, Sandvik et al 1996). The insensitivity of each of these responses in two separate studies using two separate fish species suggests consistent reduction in the metabolic capabilities due to exposure to COP sediments. Since the chronic toxicity of PAHs has been suggested to be

mediated through bioactivation and metabolic conversion to reactive intermediates, the lack of response by naïve fish would indicate potentially lower levels of risk. However, other adverse effects, particularly targeting the endocrine and reproductive systems, may not be mediated through bioactivation. Consequently, gonadal indices and steroid concentrations were evaluated following treatment.

The gonad-somatic indexes (ratio of gonad weight to total body weight) were not associated with priority PAH concentrations or dilution treatments. Similarly, plasma steroid levels were unaltered by treatment, although a trend toward reduced E2 concentrations may be apparent. Previous studies in Hornyhead turbot observed significant reductions in circulating E2 concentrations following 7 days of treatment to 33% COP sediments (Roy et al. 2003). Response differences may be due to species sensitivities as concentrations of sex steroids in Hornyhead turbot are significantly different from California halibut as well as English Sole (Schlenk et al., submitted). For example, serum E2 concentrations were higher than testosterone concentrations in controls as well as treated animals. Whether this observation is an artifact of the method utilized to measure steroids (i.e. lack of specificity with regard to the E2 antibody) is unclear as there are no published reports of "normal" steroid concentrations in California Halibut in different developmental stages such as post-spawing. E2 concentrations in female flounder (*P. flesus*) have been shown to decrease with exposure to phenanthrene (Monteiro, Reis-Henriques et al. 2000), and similar steroid inhibition has been linked to inhibition in vivo of ovarian steroidogenesis in goldfish (Evanson and Van der Kraak 2001).

5. Conclusion

Biochemical and physiological metrics evaluated in the current study failed to demonstrate dose-response relationships preventing the estimation of accurate sediment thresholds for biomarker responses. With the possible exception of depressed E2 concentrations at 1% COP sediments, no significant adverse reproductive effects were noted. Significant induction of CYP1A and a trend toward enhanced biliary FACs was observed after 30 days of exposure to 67 and 33% COP sediments, respectively. In comparison to anthropogenic PAH values seen in other studies, halibut had diminished responses in regard to FAC accumulation in bile. CYP1A induction was also insensitive with regard to the amounts of PAHs in naturally contaminated sediment from oil seeps. This may be due to a species specific low induction response, or more likely due to unique mixtures of compounds which repress or inhibit Phase I or Phase II biotransformation pathways. Studies are currently underway to explore this possibility.

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.