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PAH Exposure in Gulf of Mexico Demersal Fishes, Post-*Deepwater Horizon*

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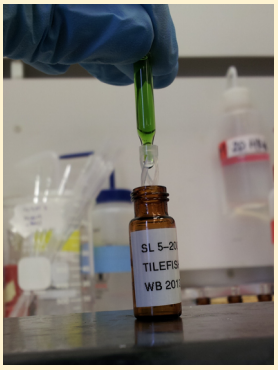
Supporting Information

ABSTRACT: Following the 2010 *Deepwater Horizon* (DWH) blowout, we surveyed offshore demersal fishes in the northern Gulf of Mexico (GoM) in 2011−2013, to assess polycyclic aromatic hydrocarbon (PAH) exposure. Biliary PAH metabolites were estimated in 271 samples of golden tilefish (*Lopholatilus chamaeleonticeps*), king snake eel (*Ophichthus rex*), and red snapper (*Lutjanus campechanus*), using high performance liquid chromatography with fluorescence detection. Mean concentration of naphthalene metabolites in golden tilefish (240 μg g^{-1}) was significantly higher (p = 0.001) than in red snapper (61 μg g^{-1}) or king snake eel (38 μg g^{-1}). Biliary naphthalene metabolite concentration decreased over the study period in red snapper (58%) and king snake eel (37%), indicating likely episodic exposure, while concentrations were persistently high in golden tilefish. Naphthalene metabolite levels measured in golden tilefish are among the highest concentrations measured in fishes globally, while concentrations for red snapper and king snake eel are similar to pre-DWH levels measured in GoM species. In contrast, concentrations of benzo[a]pyrene metabolites were similar for all three species (p = 0.265, mean 220 ng g^{-1}) and relatively low when compared to GoM, global data and previous oil spills. These data support previous findings that fish life history and physiology play significant roles in exposure and uptake of PAH pollution.



INTRODUCTION

The *Deepwater Horizon* (DWH) blowout in the Gulf of Mexico (GoM) released 4.9 million barrels of crude oil between April 20th and July 15th, 2010.[1] Through multiple mechanisms, 4−31% of the oil residue settled on the northern GoM seafloor and its effects are apparent in sediment cores taken at sites polluted by the spill with negative impacts on the benthos.[2−5] Given the persistence of DWH oil in the environment, there is a need to understand the interactions with, and impacts on fish and other biota, particularly demersal fishes living in contact with sediments that may contain residual DWH oil.[2,6−8]

Polycyclic aromatic hydrocarbons (PAHs) are considered the most toxic component of crude oil to marine life and are ubiquitous pollutants in the marine environment.[9,10] Hydrocarbon analysis of DWH oil collected from the wellhead documented 3.8−4.0% PAHs by weight.[11,12] With 4.9 million barrels of oil released during the DWH blowout, there was a large episodic pulse of PAHs associated with DWH into the GoM in 2010, with the potential to negatively impact fishes. Exposure to PAHs has been linked with a variety of sublethal effects in fish, including DNA damage, hepatic lesions and neoplasia, epidermal lesions, immunosuppression, cardiotoxicity, reduced adult fitness, altered and reduced growth, "toxicant-induced starvation", disrupted cell membranes, gill abnormalities, osmoregulatory imbalance, endocrine disruption, decreased fecundity and reduced survival to maturity.[10,13−17]

Routes of exposure to PAHs in demersal fishes include ingestion, ventilation over the gills and dermal uptake and may act simultaneously.[10,18,19] However, the relative importance of each route of exposure will change with physicochemical properties of each PAH, such as the octanol−water partition coefficient (K_{ow}).[20] Direct exposure to PAH-contaminated sediment and a diet of benthic prey is suspected to be an important source of exposure for demersal organisms.[21−24] Following uptake, the hepato-biliary system in fishes works to metabolize and eliminate PAHs.[25] The metabolites are accumulated in the bile which is stored in the gall bladder. In fish, metabolism rapidly converts up to 99% of PAH molecules into a more hydrophilic PAH metabolite leading to rapid excretion. Therefore, the concentration of parent PAHs in routinely monitored tissues, such as muscle and liver, often reveal only trace levels of contamination and are not necessarily good quantitative indicators of PAH exposure.[20,26−30] The presence of biliary PAH metabolites represents an early marker of relatively short-term exposure to PAHs from all routes of exposure and has been associated with known sublethal effects in fish.[22,28,31,32]

We studied three demersal fish species, the burrow-forming golden tilefish (*Lopholatilus chamaeleonticeps*), the mud-dwelling king snake eel (*Ophichthus rex*) and the reef fish, red snapper (*Lutjanus campechanus*). These species represent a gradient of likely sediment associations, with golden tilefish being heavily associated with sediments, king snake eel likely

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being moderately/heavily associated with the sediments, and red snapper being more distantly associated with sediments.[33−35] Studying differential PAH exposure between fish with varying levels of association with the sediment, including fishes that ingest sediment, has shown that the level of direct contact with contaminated sediment leads to differential body burdens of PAHs.[36]

Golden tilefish are demersal, nonmigratory and relatively long-lived fish that are commercially important in the GoM.[37−39] Golden tilefish have been observed living in a variety of shelter habitats, however, their primary habitat is a large funnel-shaped burrow.[38,40] Living in depositional environments, the filling-in of a burrow is rapid, therefore, substantial maintenance of a burrow is required, consequently, golden tilefish frequently bioturbate sediments with their mouths and bodies.[34,37,40] This incidental sediment ingestion could potentially be a key route of exposure to PAH pollution for the species. In addition, golden tilefish are a species of interest due to the highest frequency of external skin lesions (7%), observed during a 2011 disease survey.[13] While cause and effect have not been established, it has neither been rejected.[13]

Little information is available about king snake eel life history. They are described as "mud eels" and "obligate mud dwellers" that are associated with soft-bottom habitat and are often concentrated near oil rigs.[35,41] The limited literature does not suggest king snake eel form permanent burrows comparable to those of golden tilefish but they may burrow into sediments to hide or to search for food as is similar to the behavior of other *Ophichthid* eels.[42] Additionally, 82% of king snake eel habitat overlaps with the area that received some oil following DWH blowout, making it a species of high interest relative to oil pollution effects.[43] Red snapper are a commercially and recreationally important demersal reef fish associated more with vertical structure (e.g., natural and artificial reefs, offshore oil infrastructure) than the bottom sediments.[33,44] All three study species are known to eat benthic prey during parts of their life cycle.[41,44,45]

The objective of this study was to estimate equivalent concentrations of metabolites of three common PAHs found in DWH crude oil, naphthalene (NPH), phenanthrene (PHN), and benzo[*a*]pyrene (B*a*P) as a biomarker of recent PAH exposure in three demersal fish species in the GoM following the DWH blowout and primarily designed to monitor relative concentrations over time. This research is part of a much larger study of fish disease and contamination in the GoM and ongoing analyses include quantifying PAHs and alkylated homologues in muscle and liver tissue, exposure studies to assess sublethal effects, sediment analysis, and risk assessment. Knowing the relative level of PAH exposure is important in understanding the link between PAH exposure, accumulation in edible tissues and resulting sublethal effects measured, particularly since PAHs exert their toxic effects after metabolism.[22,46,47] In addition, this study provides ample baseline data, which can be used to measure future environmental impacts in the GoM.

In three years following the DWH blowout (2011−2013), we collected bile from the three species to screen bile samples for relative concentrations of biliary PAH metabolites and fluorescent aromatic compounds (FACs). This study used a widely accepted semiquantitative method, high performance liquid chromatography with fluorescence detection (HPLC-F), to estimate concentrations of biliary PAH metabolites.[29,48,49] This methodology estimates the concentration of parent PAHs, their metabolites, alkylated derivatives of PAHs, their metabolites and N-, S-, and O-containing compounds with the same aromatic structure.[29] The HPLC-F bile screening method has been validated by comparison to quantitative GC/MS methodology following oil spills and in environmental monitoring, demonstrating strong correlation between the two methods for NPH equivalents (r = 0.94, p < 0.0001), PHN equivalents (r = 0.93, p < 0.0001) and B*a*P equivalents.[29,30,50,51] The method is routinely used in a number of biomonitoring studies to estimate PAH exposure in fish and is frequently the preferred method over the more rigorous analytical approaches following an oil spill where there is a need to test large numbers of samples for exposure in a timely and cost-effective manner.[29,30,47,49,51−53]

MATERIALS AND METHODS

Collection of Samples. During 2011−2013, extensive demersal longline surveys evaluating fish disease were conducted in the GoM, from the West Florida Shelf (WFS) to west of the Mississippi River.[13] In 2011, fish were caught via demersal longlining on chartered commercial fishing vessels between June and August at depths ranging from 38−180 m (Figure 1). From 2012, all fish were caught via demersal longlining in the month of August, onboard the R/V *Weatherbird II*, at depths ranging from 34−389 m. Additionally, red snapper samples were obtained from the Madison-Swanson fishery closed area, by rod and reel in June of 2013 and 2014, at a depth of 85 m (Figure 1).

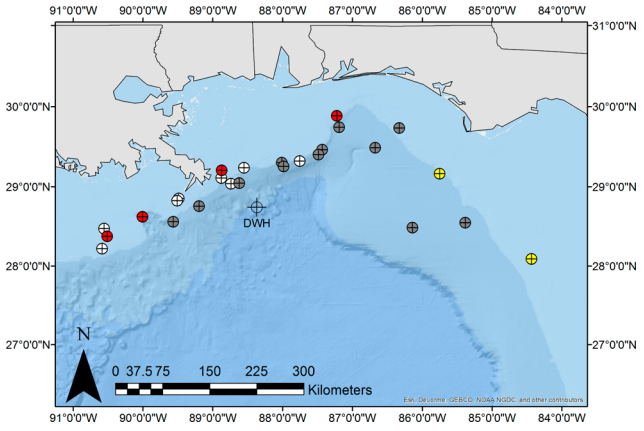

Figure 1. Location of sampling stations conducted in the northern Gulf of Mexico in 2011 (white), 2012−2013 (red, gray, yellow) and the *Deepwater Horizon* (DWH) blowout. Red markers denote red snapper stations grouped as northern Gulf of Mexico (nGoM) stations. Yellow markers denote red snapper stations grouped as West Florida Shelf (WFS) stations. Adapted by permission. Copyright © 2015 Esri, DeLorme, GEBCO, NOAA, NGDC. All rights reserved.

At each longline sampling station, an average of 495 baited #13 circle hooks were attached to 91-kg-test leaders and to 3.2 mm galvanized steel (2011, 2012), or 544-kg-test monofilament main line (2013). Bait used was cut fish (Atlantic mackerel (*Scomber scombrus*)) or various squid. Temperature−time-depth recorders (Star: Oddi CDST Centi) were deployed at the beginning and end of each longline set. Latitude, longitude, depth, and weather conditions were also recorded at the beginning and end of each set. Average soak time was 2 h.

Target fish for the longline surveys included the three species analyzed in this study. The target fish species were processed at

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Table 1. Summary Statistics for Biliary Naphthalene (μg g^{-1}) and Benz[*a*]pyrene (ng g^{-1}) Metabolite Equivalent Concentrations for Red Snapper, Golden Tilefish and King Snake Eel Caught in the Northern Gulf of Mexico, Separated by Year of Catch[a]

year		naphthalene equivalents (μg g^{-1})						
	species	mean	median	range	SD	*n*	mean length (cm)	% female : % male
2011	red snapper	120	110	41−470	78	30	65	60:40
2012	red snapper	61	54	20−130	27	15	58	60:40
	golden tilefish	240	230	110−340	61	24	67	n/a
	king snake eel	38	24	11−88	27	23	151	n/a
2013	red snapper	51	48	13−140	27	63	65	47:53
	golden tilefish	220	230	22−480	110	72	65	63:37
	king snake eel	24	16	3.6−210	34	44	140	n/a

year		benzo[*a*]pyrene equivalents (ng g^{-1})				
2011	species	mean	median	range	SD	*n*
	red snapper	280	260	94−590	140	30
2012	red snapper	220	170	68−540	150	15
	golden tilefish	170	140	51−470	110	24
	king snake eel	260	160	46−850	150	23
2013	red snapper	380	300	310−1500	330	63
	golden tilefish	370	220	71−3030	450	72
	king snake eel	160	130	34−880	150	44

[a]SD, standard deviation, *n*, number of samples analyzed. Mean length (cm) and sex ratio (% female: % male) is also provided, and are the same for individuals measured for naphthalene and benzo[*a*]pyrene equivalents. Sex ratio is not provided for king snake eel (n/a = not applicable), as sex is difficult to determine, and sex is not provided for 2012 golden tilefish catch.

time of capture for standard and total lengths, weight, sex and a subsample of the catch were selected to be sampled for bile, liver, muscle, otoliths and other tissues. Bile was collected by dissecting the gall bladder away from the liver, cutting the bile duct, and draining the fluid via the duct into 8 mL combusted amber vials. The samples were immediately frozen. In the laboratory, bile samples were stored at −40 °C until analysis.

Laboratory Analysis. All 2011 bile samples (n = 30) were analyzed at the Northwest Fisheries Science Center (NWFSC), Seattle, WA. The 2012 (n = 62) and 2013 (n = 179) bile samples were analyzed at Mote Marine Laboratory (MML), Sarasota, FL. Prior to analysis of the 2012 and 2013 samples, an interlaboratory comparison was completed to validate methods, precision and accuracy between the two laboratories. The interlab comparison used three bile samples from 2011, from different species (cobia (*Rachycentron canadum*), greater amberjack (*Seriola dumerili*), red snapper) and over a wide range of concentrations (for NPH: 41−240 μg g^{-1}; for PHN: 6.3−46 μg g^{-1}, for B*a*P: 97−310 ng g^{-1}). Prior to the interlab comparison, accuracy was monitored as part of the quality assurance plan at the NWFSC using a fish bile control sample (bile of Atlantic salmon (*Salmo salar*) exposed to 25 μg mL^{-1} of Monterey Crude oil for 48 h). There was successful interlaboratory agreement for the three bile samples, with a CV of less than 15%, for PAH equivalents for NPH, PHN, and B*a*P. Bile samples were then analyzed using the semiquantitative bile screening HPLC-F method following NWFSC Environmental Chemistry program protocols described below.[29,48,49]

Untreated bile samples (3 μL) were injected directly onto the HPLC-F system (MML: Agilent Technologies, Series 1100, Santa Clara, CA; NWFSC: Waters, Milford, MA) equipped with a C-18 reverse-phase column (Synergi 4 μm Hydro-RP 80A, Phenomenex, Torrence, CA), with the column temperature held at 50 °C. Fluorescent aromatic compounds were eluted at 1 mL/min using a linear gradient from 100% solvent A (0.5% acetic acid in water) to 100% solvent B (methanol). Chromatograms were recorded at representative wavelength pairs of 292/335 nm for the NPH equivalents (2−3 ring FACs), 260/380 nm for the PHN equivalents (3−4 ring FACs) and 380/430 nm for the B*a*P equivalents (4−5 ring FACs). All peaks within the portion of the chromatogram where metabolites elute (6−19 min), were integrated for each wavelength pair, summed and FACs were calculated using external standards of the respective parent compounds, NPH, PHN, and B*a*P, to convert sample area (fluorescence response) to PAH equivalents (ng g^{-1}) bile wet weight using the following calculation:[48]

$$\frac{\text{standard concentration}}{\text{standard mean area}} \times \frac{\text{integrated sample area}(6-19\text{min})}{\text{density of bile}} \times \frac{\text{uL of standard injected}}{\text{uL of sample injected}}$$

where the density of bile is 1.03 g mL^{-1}.[21] All equivalent concentrations were reported to two significant figures.

Quality Assurance/Control. Quality assurance was monitored in four ways. (1) An interlaboratory comparison of three 2011 bile samples was performed between MML and the NWFSC discussed above. (2) A methanol solvent blank was run prior to every field sample. The area of the methanol blank was subtracted from the area of the field sample that was subsequently analyzed. (3) Each field sample was run in duplicate, with a CV of less than 15%. If the CV between duplicates was greater than 15%, the sample was run again in triplicate until the CV reached less than 15%. (4) A continuing calibration was used to monitor instrument stability throughout the entire analysis by running the quantifying standards of parent PAHs of NPH (2.5 μg mL^{-1}), PHN (1 μg mL^{-1}) and B*a*P (250 ng mL^{-1}) every 12 field samples, making sure the CV remained less than 15%.

Data Analysis. Nonparametric, or permutation-based, hypothesis tests were run as it is unrealistic that biological data meet the assumptions (e.g., normal distribution, random sampling) of traditional parametric analyses, such as analysis of variance.[54,55] If a potential explanatory variable was categorical (e.g., year), a nonparametric multivariate analysis of variance

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(npMANOVA), also known as a permutation-based analysis of variance when used on univariate data, was run using $\alpha = 0.05$ to reject the null hypothesis. Between-group dispersion was checked for homogeneity prior to each npMANOVA and if dispersions were nonhomogenous, the data were transformed to minimize heterogeneity. If the npMANOVA was significant, a pairwise npMANOVA was run to assess subset's significant differences. An adjusted p-value, using the Holms−Bonferroni transformation, was used to test the null hypothesis for a pairwise npMANOVA, using $\alpha = 0.05$.

RESULTS AND DISCUSSION

Biliary PAH Metabolite Concentrations. This study examined 271 bile samples, from 26 longline stations, collected over the three years following the DWH blowout, including 96 golden tilefish, 67 king snake eel, and 108 red snapper (Table 1). The concentrations of biliary NPH and PHN equivalents varied linearly within individuals ($r = 0.92$, $p = 0.001$), for all three species, and within sites, therefore, only results for NPH equivalents are analyzed and discussed to avoid redundant information. The strong correlation between NPH and PHN metabolite concentration has been found in other studies suggesting a common petrogenic PAH source.[51,56,57] The correlation between NPH and B*a*P metabolites was also significant ($r = 0.30$, $p = 0.001$), although much weaker than the correlation between NPH and PHN, therefore, results for B*a*P metabolite concentration are analyzed and presented separately. The low molecular weight (LMW) PAHs, specifically NPH and PHN, are present in relatively high concentrations in DWH crude oil while B*a*P is found in much smaller concentrations, or not quantified above the detection limits of the instrument.[11,12]

Species-Specific Differences in Biliary PAH Metabolite Concentration. For both 2012 and 2013 data, there is a significant difference in NPH metabolite concentrations between all three study organisms, with golden tilefish having significantly higher concentrations of biliary NPH metabolites than king snake eel or red snapper (Figure 2; $p = 0.001$, $p = 0.001$ respectively). Mean NPH metabolite concentration in golden tilefish was, on average, four times higher than red snapper for both 2012 ($p = 0.003$) and 2013 ($p = 0.003$), six times higher than king snake eel in 2012 ($p = 0.003$) and nine times higher than king snake eel in 2013 ($p = 0.003$). This difference persists overall and at the four longline stations where golden tilefish and king snake eel co-occurred. Golden tilefish and red snapper have never been caught at the same station on our longlining cruises due to differences in habitat and depth range. Red snapper mean NPH metabolite concentration is consistently higher than king snake eel concentrations, with mean concentrations five times higher in 2012 ($p = 0.012$) and two times higher in 2013 ($p = 0.003$). This pattern also occurred at two of three longline stations where red snapper and king snake eel co-occurred.

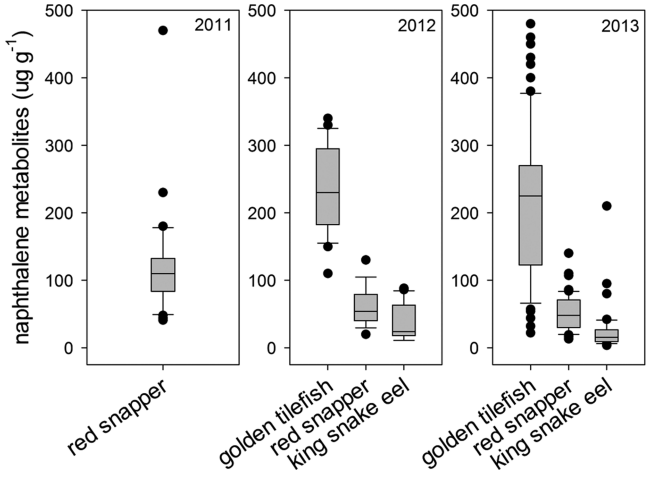

Figure 2. Biliary naphthalene metabolite concentrations (μg g^{-1}) for golden tilefish (2012: $n = 24$; 2013: $n = 72$), red snapper (2011: $n = 30$; 2012: $n = 15$; 2013: $n = 63$) and king snake eel (2012: $n = 23$; 2013: $n = 44$), sampled in 2011, 2012, and 2013 in the northern Gulf of Mexico.

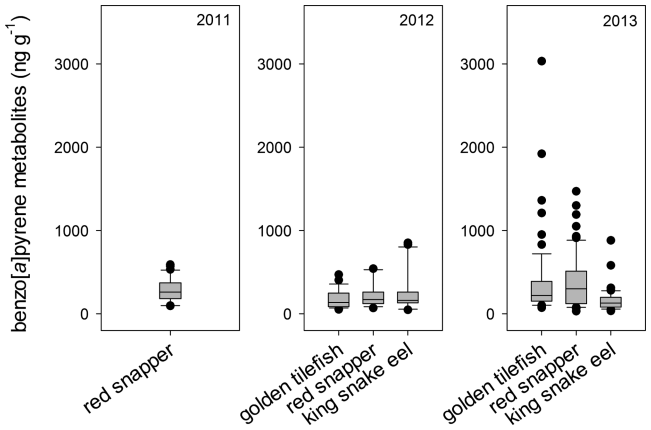

Figure 3. Biliary benzo[*a*]pyrene metabolite concentrations (ng g^{-1}) for golden tilefish (2012: $n = 24$; 2013: $n = 72$), red snapper (2011: $n = 30$; 2012: $n = 15$; 2013: $n = 63$) and king snake eel (2012: $n = 23$; 2013: $n = 44$), sampled in 2011, 2012, and 2013 in the northern Gulf of Mexico.

In contrast to results for NPH, no significant differences in B*a*P metabolite concentration were found among the three species for 2012 (Figure 3; $p = 0.265$). However, for 2013, average B*a*P metabolite concentration is two times higher in golden tilefish and red snapper compared to king snake eel (Figure 3; $p = 0.003$, $p = 0.003$ respectively), while golden tilefish and red snapper concentrations are statistically similar ($p = 0.751$).

The uptake of PAHs by organisms is generally governed by a number of complex mechanisms including chemical bioavailability, species-specific metabolism, and biotransformation, diet, trophic level, and habitat use. Bioavailability is a function of both species-specific metabolic processes and uptake, as well as physicochemical properties. Molecular weight and K_{ow} have been found to be negatively correlated with bioavailability of individual PAHs.[58] The less hydrophobic LMW PAHs (log $K_{ow} < 4$) tend to dissolve more rapidly than high molecular weight (HMW) compounds (low $K_{ow} > 4$), thereby increasing their bioavailability to marine organisms. Preferential uptake of LMW PAHs, by two species of benthic fish, has been documented and attributed to both the bioavailability of the compounds, as well as biotransformation mechanisms, such as higher biotransformation rates of HMW compounds compared to LMW PAHs.[59−61] However, in contrast, higher rate of metabolism of LMW PAHs has also been found and attributed to differences in K_{ow}.[62,63] In addition, research suggests that the use of chemical dispersants, such as Corexit 9500, increases the uptake, bioavailability and bioconcentration of PAHs by exposed fish.[64−66] The use of chemical dispersants following

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the DWH blowout, therefore, could have influenced both the bioavailability and potentially the toxicity of PAH to exposed fishes.

The significant difference in NPH metabolite concentrations among the three fish species, which persists even when species are sampled at the same station, indicates these differences are not a sampling artifact or due to spatial heterogeneity of exposure. The much higher concentrations of NPH metabolites in golden tilefish as compared to the other two species is likely due to their burrowing lifestyle, incidental sediment ingestion, and their diet of benthic prey (e.g., benthic invertebrates and demersal fishes), which may result in additional routes of exposure to sedimented PAH contamination. As the solubility of PAHs decreases with increasing molecular weight, the bioaccumulation potential for LMW compounds from sediment tends to be greater than the HMW compounds.[59] Previous studies on organic contaminants also demonstrated negative correlations between biota-sediment accumulation factors (BSAF) and log K_{ow}, suggesting decreasing bioavailability with increasing log K_{ow}.[67] Furthermore, HMW compounds can be physically bulky limiting their ability to pass through lipid membrane barriers, resulting in lower body burdens. The more hydrophobic HMW PAHs bind more readily with particulates, dissolved organic material (DOM) and oil droplets, also hindering their bioavailability to marine organisms. Recent studies of the DWH blowout document a hydrocarbon-contaminated marine snow event and found evidence of rapid deposition of associated oil-particle aggregates and degraded oil to the seabed.[3,5,68] When dissecting golden tilefish, it is evident that they ingest large volumes of sediment, seen in the digestive tract, buccal cavity and gills, while obvious sediment ingestion is not observed in the king snake eel or red snapper digestive tracts (personal observation). Together the observed hydrocarbon-contaminated marine snow and degraded oil deposition on the seafloor, differences in bioavailability, and species-specific metabolism and uptake help to explain the higher levels of NPH observed in golden tilefish.

We hypothesize king snake eel may have lower biliary PAH metabolite concentrations than the other species analyzed due to their inordinate epidermal mucus production, inefficiency of metabolism or peculiarities of their life history. King snake eel may eliminate LMW PAHs through mucus, as they produce prodigious amounts relative to the other species (personal observation). Exposure studies have documented high levels of NPH and metabolites in the epidermal mucus of both rainbow trout (*Oncorhynchus mykiss*) and starry flounder (*Platichthys stellatus*), exposed to [^{3}H]naphthalene, suggesting epidermal mucus is an important pathway of LMW PAH excretion from the body.[69,70] Alternatively, mucus may provide a physical barrier against dermal uptake. King snake eel may also be less efficient at metabolizing PAHs, although, hepatic ethoxyresorufin-*O*-deethylase (EROD) activity, a biomarker of exposure to organic contaminants, in eel of another family, the European eel (*Anguilla anguilla*), was found to be comparable to a teleost, European flounder (*Platichthys flesus*), suggesting that eels can metabolize PAHs.[71] King snake eel life history, although unknown, may play a role in contaminant exposure and metabolism, as other *Ophichthid* eels (*Myrophis punctatus*) have complex life history and sexual maturation, which may influence enzymatic activity.[71,72]

Fish length, weight, and sex have been previously correlated with biliary PAH concentrations.[52,53] In this study, fish sex and length were examined as factors influencing biliary PAH metabolite concentration. Male red snapper were found to have higher biliary NPH metabolite concentrations than females (p = 0.021) with the mean concentrations of 61 μg g^{-1} and 44 μg g^{-1}, respectively. Differences in biliary PAH metabolite concentration between sex has been documented and hypothesized to be due to sex hormone differences in PAH metabolism (e.g., enzyme induction and activity), and regulation of monooxygenase activity by estrogens, leading to male fish having higher concentrations of biliary FACs.[10,53,73] Larger king snake eel were found to have lower concentrations of both biliary NPH and B*a*P metabolites (NPH: r = −0.40, p = 0.001; B*a*P: r = −0.60, p = 0.002). A similar trend with length was seen for golden tilefish for B*a*P metabolites (r = −0.33, p = 0.004). The weak but significant negative relationship between biliary B*a*P and fish length for golden tilefish suggests diet may play a larger role in exposure. Golden tilefish exhibit an ontogenetic shift in diet, from benthic invertebrates, to a higher proportion of fish, which may be a mechanism of changing PAH exposure with fish length, as invertebrates have lower metabolism and elimination efficiencies for PAHs compared to vertebrates. Very little is known about king snake eel, therefore, their life history, physiology or possibly the same ontogenetic shift in diet may explain the similar trend, however, no diet studies have been published for the species.

Temporal Variation in Biliary PAH Metabolite Concentration. Between 2012 and 2013, there was no significant change in golden tilefish NPH metabolite concentration (Figure 2; p = 0.367), with the mean concentration declining 8% from 240 μg g^{-1} to 220 μg g^{-1}. However, there was a significant increase in golden tilefish B*a*P metabolite concentration over the two years (Figure 3; p = 0.025), with the mean concentration increasing from 170 ng g^{-1} to 370 ng g^{-1}. Between 2012 and 2013, the mean concentration of NPH metabolites in king snake eel declined by 37%, from 38 μg g^{-1} to 24 μg g^{-1}, although this difference was not significant (Figure 2; p = 0.063). There was, however, a significant decrease in king snake eel B*a*P metabolite concentration over the two years (Figure 3; p = 0.025), decreasing 38% (260 ng g^{-1} to 160 ng g^{-1}).

Over the three-year period following the DWH event, there was a significant decrease in mean red snapper NPH metabolite concentration (Figure 2; p = 0.001). Between 2011 and 2012, there was a significant 49% decrease from a mean concentration of 120 μg g^{-1} to 61 μg g^{-1} (p = 0.003). Between 2012 and 2013, there was a continued decrease in the mean concentration, from 61 μg g^{-1} to 51 μg g^{-1}, although the difference between 2012 and 2013 was nonsignificant (p = 0.194). Red snapper B*a*P metabolites remained at similar concentrations over the three years (Figure 3; p = 0.282).

The significant declines in NPH metabolite concentration in red snapper is indicative of an episodic exposure event to NPH prior to 2011.[13] A similar, although nonsignificant, decrease in king snake eel NPH metabolite concentration between 2012 and 2013 also suggests episodic exposure to elevated PAHs. For both species, these data support a scenario of increased NPH contamination in the environment coincident with the DWH blowout and a decrease in exposure reflected in decreasing biliary NPH metabolite concentration in following years. In comparison, the persistent, and significantly higher, concentrations of biliary NPH metabolites in golden tilefish suggests that the source of NPH for golden tilefish did not decrease over time, since biliary PAH metabolites indicate short-term exposure to PAHs.[28,31] If PAHs exist in sediments associated

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with golden tilefish burrows and are being sequestered by continued sedimentation, the digging behavior of golden tilefish may re-expose these animals to PAHs, while other co-occurring species may not be exposed to the same routes and thus the same effective exposure levels. Crescent gunnel (*Pholis laeta*), a demersal fish, collected from sites polluted by the *Exxon Valdez* oil spill, showed elevated concentrations of LMW biliary PAH metabolites 10 years after the event, indicating that oil can persist in the environment resulting in persistently elevated levels of biliary PAH metabolites.[74] The combination of sediment enrichment from the hydrocarbon-contaminated marine snow event following the DWH and the slow dissolution rates of HMW PAHs from oil droplets, particulates and DOM, help to explain the increasing concentration of biliary B*a*P metabolites for golden tilefish, as the bioavailability of PAHs in oil droplets or DOM increases over time.[75]

Spatial Variation in Biliary PAH Metabolite Concentration. Spatial variability in biliary PAH metabolite concentration was evaluated by comparing results for the northern GoM to the WFS for red snapper sampled in 2013 (Figure 1). Red snapper caught on the WFS had significantly lower biliary PAH metabolites, for both NPH (p = 0.001) and B*a*P (p = 0.001), compared to red snapper caught in the northern GoM, closer to the Mississippi River, extant oil infrastructure and the DWH event (Figure 4). Additionally, the

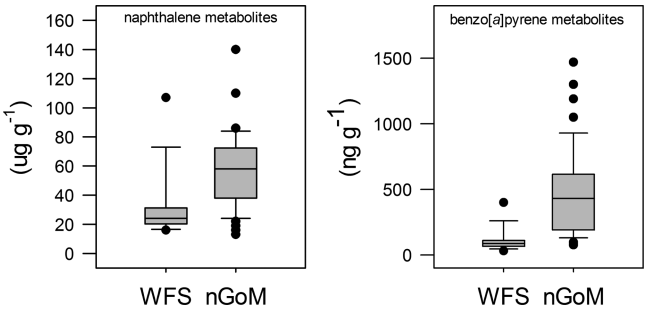

Figure 4. Comparison of red snapper biliary polycyclic aromatic hydrocarbon metabolite concentration for naphthalene (μg g^{-1}) and benzo[*a*]pyrene (ng g^{-1}) from two regional groups sampled in 2013, West Florida Shelf (WFS, n = 14) and northern Gulf of Mexico (nGoM, n = 49). For both NPH and B*a*P metabolite concentrations, p = 0.001.

northern GoM stations showed a decrease in biliary PAH concentration over time, for both NPH and B*a*P metabolites, suggesting episodic exposure to an elevated source of PAHs, whereas biliary PAH concentrations from the Madison−Swanson fishery closed area on the WFS have not decreased between years. Therefore, these stations on the WFS may represent baseline biliary PAH metabolite concentration for red snapper in the GoM. No significant spatial trends existed for golden tilefish and king snake eel biliary PAH data, as these species were not caught in large numbers on the WFS.

Comparison to Historical Biliary PAH Data. We conducted a metadata analysis of previous studies that used similar HPLC-F methods to quantify biliary PAH metabolite concentrations in fish, including studies of three other oil spills, three previous GoM studies, six polluted estuaries, and one "pristine" site upstream of the 1984 Columbia River oil spill[51,56,57,76−80] (Figure 5). In comparison to all of these, golden tilefish biliary NPH metabolite concentrations (240 μg g^{-1}), collected two years hence of the DWH blowout, were among the most contaminated fish, only falling below pink salmon (*Oncorhynchus gorbuscha*, 480 μg g^{-1}) sampled immediately after the *Exxon Valdez* oil spill and an assortment of species from a polluted channel (São Sebastião channel, 290 μg g^{-1}) in São Paulo, Brazil, that serves as the largest petroleum terminal in that country, frequently experiencing oil spills and discharge.[56,76] Golden tilefish NPH metabolite concentrations are about 1.5 times higher than those reported from inshore fish (primarily Atlantic croaker (*Micropogonias undulatus*)) caught in the northern GoM both before and after Hurricane Katrina (190 μg g^{-1}, 150 μg g^{-1}, respectively) and 6.5 times higher than white sturgeon (*Ancipenser transmontanus*, 32 μg g^{-1}) caught downstream of the Columbia River oil spill.[76,77] Red snapper and king snake eel biliary NPH metabolite concentrations (120 μg g^{-1} (from 2011), 38 μg g^{-1} (from 2012), respectively) comparatively rank much lower, closer to previous GoM data sampled from an assortment of species offshore of Texas in 1993 (110 μg g^{-1}), with king snake eel concentrations being similar to the relatively unpolluted site in the Columbia River.[77,80] For biliary B*a*P metabolites, all three species have comparatively low concentrations (170−280 ng g^{-1}), which are very similar to the 1993 GoM data (200 ng g^{-1}), and much lower than the highly polluted estuaries (580−2900 ng g^{-1}) and inshore fish samples taken before and after Hurricane Katrina (1400−1600 ng g^{-1}).[76,77,79,80]

Comparisons of PAH data from these three species post-DWH reveal that the level of LMW PAH exposure was extensive, particularly for golden tilefish, even two years and more following the event. While the levels of LMW PAHs in golden tilefish prior to the DWH are unknown, the 1993 study off of Texas, which included anchor tilefish (*Caulolatilus intermedius*), had a much lower average of LMW PAHs (116 μg g^{-1} for NPH), and perhaps indicated the residual pollution levels in areas where oil and gas rigs are located.[80] In contrast, the B*a*P metabolite concentrations in our study were lower than most other studies. Benzo[*a*]pyrene is primarily derived of from combusted hydrocarbons (e.g., from urban and industrial pollution), thus, the relatively high levels of B*a*P in studies of polluted estuaries, and inshore GoM, serve more a function of depth and distance from shore, compared to the offshore locations sampled in this study.[76,78] While large quantities of DWH oil were burned at sea, it is unknown what portion of the oil resulted in B*a*P contamination, although the quantity of B*a*P in DWH source oil was very low, if present at all.[11,12]

The data synthesized in this study constitute one of the largest biliary PAH data sets for fishes and the largest for the GoM. Nearly 300 bile samples were analyzed from three GoM demersal fish species over three years following the DWH blowout. Significant interspecies differences exist between the three species in concentrations of LMW biliary PAH metabolites. Golden tilefish exhibit the highest known concentrations of biliary NPH metabolites of data available on GoM fishes, and were among the highest concentrations in comparable studies globally. Differences in habitat, physiology, and diet most likely account for the observed differences in LMW PAH exposure, however, differences in bioavailability, and species-specific rates of uptake, metabolism, and elimination, all play a role. While king snake eel may directly encounter polluted sediments as well, their physiology may result in avoidance (e.g., mucus barrier) and elimination of ingested PAHs, although, this is conjecture. Red snapper, in contrast, are not as intimately coupled with sediments, leading to respiration and diet as the more likely exposure routes, with

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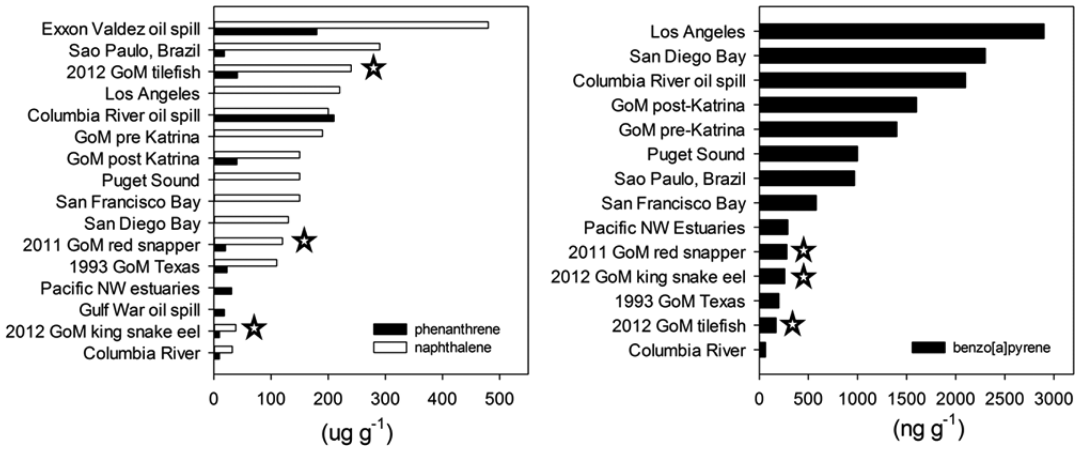

Figure 5. Comparison of biliary naphthalene (left, thin white bars), phenanthrene (left, thin black bars) and benzo[*a*]pyrene (right) metabolite concentration between post-*Deepwater Horizon* golden tilefish (n = 24) sampled in the northern Gulf of Mexico (GoM) in 2012, red snapper (n = 30) sampled in 2011 and king snake eel (n = 23) sampled in 2012, to other oil spills, polluted estuaries and one pristine site on the Columbia River. Data from this study are denoted with stars.

species-specific metabolism and excretion most likely lending to the lower biliary PAH concentrations. To definitively understand these species-specific differences, further analysis of muscle and liver tissue from the field-caught individuals in this study, will be analyzed for PAHs and alkylated homologues to better understand the distribution of PAHs between bile, liver, and muscle, and to better understand PAH source. Controlled exposure studies could also assist in understanding these mechanisms.

Temporal trends were observed for interspecies variation of biliary NPH metabolite concentrations. The high concentrations of biliary NPH persisted over time in golden tilefish, whereas they declined in red snapper and king snake eel. The statistically significant, exponential decrease over time of biliary NPH metabolites in red snapper suggests exposure to LMW PAH pollution from an episodic event likely occurred in the GoM prior to 2011.[13] The same, albeit lower, decrease over time was seen in NPH and B*a*P biliary PAH metabolite concentration for king snake eel samples. It is possible (likely) that the episodic event was the DWH blowout, since it is unlikely that the other sources of PAHs to the GoM would decrease substantially (e.g., 50%) in the same region at the same time.[13] Concentrations of biliary PAH metabolites were significant higher in the northern GoM, closer to the DWH event and Mississippi River, compared to the WFS, for 2013 red snapper samples. The declining concentrations of PAHs with distance from the DWH well site is consistent with this event being the source of elevated PAHs in red snapper observed.

While the collection and analysis of biliary PAHs in fish to evaluate temporal trends in contamination reveals much about the source and magnitude of pollution in the GoM, these studies would have been aided by the availability of pre-DWH baseline data. The lack of such baseline complicates but does not obviate the assessment of PAH pollution source and magnitude. Spatially relevant, precise and replicated baseline data would have been useful in detecting the levels of exposure of GoM fishes. Nevertheless, temporal changes in contaminant levels reveal much about the ephemeral and more persistent levels of contamination, as suggested by long-term studies of the *Exxon Valdez* oil spill. Continued monitoring of GoM fishes should document return to baseline conditions in areas not subjected to persistent levels of PAH contamination.

■ ASSOCIATED CONTENT

Supporting Information

A summary table of the information conveyed in Figure 5. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01870.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

DWH	Deepwater Horizon
GoM	Gulf of Mexico
PAH	polycyclic aromatic hydrocarbons
NPH	naphthalene
PHN	phenanthrene
B*a*P	benzo[*a*]pyrene
FAC	fluorescent aromatic hydrocarbons

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HPLC-F	high performance liquid chromatography with fluorescence detection
WFS	West Florida Shelf
NWFSC	Northwest Fisheries Science Center
MML	Mote Marine Laboratory
LMW	low molecular weight
HMW	high molecular weight
BSAF	biota-sediment accumulation factor
K_{ow}	octanol–water partition coefficient
DOM	dissolved organic matter

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