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Hepatobiliary Analyses Suggest Chronic PAH Exposure in Hakes (*Urophycis* spp.) Following the *Deepwater Horizon* Oil Spill

Rachel E. Struch,[a] Erin L. Pulster,[b,*] Andrea D. Schreier,[a] and Steven A. Murawski[b]

[a]Department of Animal Science, University of California Davis, Davis, California, USA
[b]College of Marine Science, University of South Florida, St. Petersburg, Florida, USA

Abstract: Prior to the *Deepwater Horizon* oil spill, we lacked a comprehensive baseline of oil contamination in the Gulf of Mexico's sediments, water column, and biota. Gaps in prespill knowledge limit our ability to determine the aftereffects of the *Deepwater Horizon* blowout or prepare to mitigate similar impacts during future oil spill disasters. We examined spatiotemporal differences in exposure to and metabolism of polycyclic aromatic hydrocarbons (PAHs) in 2 hake species (*Urophycis* spp.) to establish a current baseline for these ecologically important, abundant, and at-risk demersal fishes. Gulf hake (*Urophycis cirrata*) and southern hake (*Urophycis floridana*) were collected throughout the Gulf of Mexico during extensive longline surveys from 2012 to 2015. Analyses of biliary PAH metabolites and liver PAH concentrations provided evidence of exposures to di- and tricyclic compounds, with the highest concentrations measured in the northern Gulf of Mexico. Species-specific differences were not detected, but temporal trends observed in biliary PAHs suggest a decrease in acute exposures, whereas increasing liver PAHs suggest chronic exposures marked by greater assimilation than metabolism rates. To our knowledge, the present study provides the first multitissue contaminant analyses, as well as the most exhaustive biometric analyses, for both gulf and southern hakes. Though sources of exposure are complex because of multiple natural and anthropogenic PAH inputs, these results will facilitate the development of much needed health metrics for Gulf of Mexico benthos. *Environ Toxicol Chem* 2019;38:2740–2749.

Keywords: Oil spill; Polycyclic aromatic hydrocarbons; Metabolites; Fish; *Urophycis*

INTRODUCTION

The *Deepwater Horizon* oil rig blowout released approximately 4.9 million barrels of Louisiana light sweet crude into the northern Gulf of Mexico from 20 April to 15 July 2010 (Lubchenco et al. 2012). This volume was approximately 7 times the normal annual oil budget of the entire Gulf of Mexico and resulted in an estimated 160-fold increase in total hydrocarbon concentrations measured in the water column over the 3-mo period (Murawski et al. 2016). Although some of the oil was recovered or washed onshore, numerous studies have indicated that a substantial proportion (2–14%) was transported to the seafloor in a mass sinking event referred to as a marine oil snow sedimentation and flocculent accumulation (MOSSFA; Valentine et al. 2014; Brooks et al. 2015; Romero et al. 2015). A similar event was also observed following the *Ixtoc 1* blowout in 1979 and was potentially caused by an aggregation of bacterial-mucous webs, zooplankton, phytoplankton, fecal matter, detritus, and oily particulate matter. Evidence of MOSSFA is still visible in cores taken at sites impacted by these spills because crude oil can remain in sediments for decades (Schwing et al. 2019).

When these polluted sediments are disturbed by storms, currents, or dredging activities, demersal organisms may be repeatedly exposed to oil and its harmful degradation products (Brown-Peterson et al. 2015). In addition, as organisms manipulate sediments, create burrows, or utilize burrows created by other organisms (i.e., bioturbation), they may be chronically exposed to higher concentrations of sediment-associated contaminants, including polycyclic aromatic hydrocarbons (PAHs; Granneman et al. 2017). Because of their persistence in the environment and their ability to cause toxic, mutagenic, and/or carcinogenic effects to biota, PAHs are widely considered the most toxic components of crude oil (Tuvikene 1995). Once PAHs are bioavailable, they can bioaccumulate (Meador et al. 1995), bioconcentrate (Baussant et al. 2001), and, in rare cases, biomagnify (Dadamo et al. 1997) in animal tissues.

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* Address correspondence to epulster@mail.usf.edu
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Demersal fishes are exposed to PAHs from multiple routes including direct contact with contaminated water through the gills, consumption of contaminated prey, ingestion of contaminated sediment, and, potentially, through transdermal exposure to contaminated sediments (Law and Hellou 1999). However, parent PAHs are rapidly transformed by the fish hepatobiliary system during phase I and phase II metabolism (Pampanin and Sydnes 2013). These phases work together to make PAHs more water soluble and easier to eliminate in urine and bile. Several studies have demonstrated that biliary PAH metabolites are a sensitive biomarker because they are an indication of recent (e.g., days or weeks) and ongoing exposure to PAHs (Beyer et al. 2010). In contrast, hydrophobic compounds found within animal tissues such as liver and muscle indicate exposure and accumulation within weeks or months (Pampanin and Sydnes 2013).

With residual oil from the *Deepwater Horizon* spill still found in Gulf of Mexico sediments (Adhikari et al. 2016; Romero et al. 2017) and serious gaps in baseline information for vulnerable demersal communities, researchers have a unique opportunity to investigate long-term ecosystem impacts. A study published by Snyder et al. (2015) found high concentrations of naphthalene (NPH) PAH metabolites in the bile of burrowing golden tilefish (*Lopholatilus chamaeleonticeps*) 3 yr after the *Deepwater Horizon* spill. Murawski et al. (2014) also reported the elevated presence of skin lesions, bile metabolites, and tissue PAHs in several deep-water species including golden tilefish, red snapper (*Lutjanus campechanus*), yellowedge grouper (*Hyporthodus flavolimbatus*), and southern hake (*Urophycis floridana*). Notably, southern hake liver and muscle samples were dominated by high proportions of low molecular weight (LMW) parent PAHs, and a decrease in southern hake abundance was observed between 2011 and 2017 (Murawski et al. 2018). Although this decrease could be related to fisheries bycatch, these results are concerning because they seem consistent with reports of lesions in southern hake in the 1980s. Grizzle (1986) observed a higher prevalence of hepatic lipid changes in hakes in close proximity to oil and gas platforms in the Gulf of Mexico and attributed these findings to toxicant exposure from drilling-fluid components. European hakes (*Merluccius merluccius*) evaluated after exposure to the *Prestige* oil spill have also shown indications of stress including inflammatory responses, presence of nematode parasites, and hepatocellular nuclear polymorphism (Marigomez et al. 2006).

We investigated PAH exposures in southern and gulf hakes (*Urophycis cirrata*) given their potential sensitivity to oil, commercial and ecological importance in the Gulf of Mexico (Pulver 2015), and elevated exposure risks because of their close association with sediments and the use of burrows (Brooks et al. 2011). These species belong to the taxonomic order Gadiformes and the family Phycidae and are related to other ray-finned fishes including cod and haddock. They are deep-water, benthopelagic, marine fishes found abundantly distributed at the continental shelf break (Cohen et al. 1990; Murawski et al. 2014). Whereas gulf hake prefer mud bottoms, generally between 300 and 400 m, southern hake are most abundant on sand and mud bottoms between 200 and 300 m (McEachran and Fechhelm 1998; Murawski et al. 2018). Like most gadids, they are thought to feed on crustaceans, worms, fish, and squid; but, to our knowledge, no stomach content data are available for these species (Pequegnat et al. 1983).

The main objectives of the present study were to 1) establish baseline biometric and PAH contaminant data for *U. cirrata* and *U. floridana* in the Gulf of Mexico, 2) examine relationships between biometrics and metabolism of PAHs in these *Urophycis* spp., and 3) investigate spatiotemporal trends for biliary PAH metabolites compared with parent PAHs and alkyl PAH concentrations in hake livers. Given the slow degradation of PAHs expected in cold water (Fisher et al. 2016), the broad distribution of these species in polluted habitats, and their suspected sensitivity to crude oil, we hypothesized that *Urophycis* spp. in the Gulf of Mexico exhibit signs of chronic PAH exposure and may be experiencing a relatively slow period of recovery following the *Deepwater Horizon* spill.

METHODS

Sample acquisition and laboratory facilities

Between 2012 and 2015, bile and liver samples were collected from gulf hake and southern hake during extensive demersal longline surveys aboard the R/V *Weatherbird II*. Samples were collected in the northern Gulf of Mexico during summer surveys from 2012 through 2015, with additional sampling in the southern Gulf of Mexico during 2015 (Figure 1). Hooks were baited with fresh-cut mackerel or squid, and fish were caught with 13/0 circle hooks and processed at the time of capture following protocols described by Snyder et al. (2015) and Murawski et al. (2018). In brief, fish were inspected for lesions, and total length, weight, and sex were obtained for most individuals. Temperature–time–depth recorders (Star-Oddi; CDST Centi-TD) were deployed at the beginning and end of each longline set, and latitude, longitude, and weather conditions were also recorded at these times. The average soak period was 2 h, with sampling depths ranging from 109 to 500 m. Samples were immediately frozen at sea and then transferred to the laboratory at the University of South Florida, College of Marine Science, in St. Petersburg, Florida, USA. Bile was stored at –40 °C, and livers were stored at –80 °C until analysis from June to September 2016. In total, 164 individuals (*U. cirrata*, $n = 124$; *U. floridana*, $n = 40$) from 18 sites (north central Gulf of Mexico, 11 sites, $n = 110$; west Florida shelf, 5 sites, $n = 41$; and southwest Gulf of Mexico, 2 sites, $n = 13$) were analyzed for PAHs. The bile analysis ($n = 141$) and liver tissue analysis ($n = 126$) were not performed for every individual because of sample collection limitations (e.g., empty gallbladder) and/or insufficient sample.

Analysis of biliary metabolites

Bile samples were analyzed for PAH metabolites following a semiquantitative protocol for high-performance liquid chromatography with fluorescence detection (HPLC-F; Krahn et al. 1986). Briefly, untreated bile samples (3 µL) were injected directly into the HPLC-F system (Hitachi High

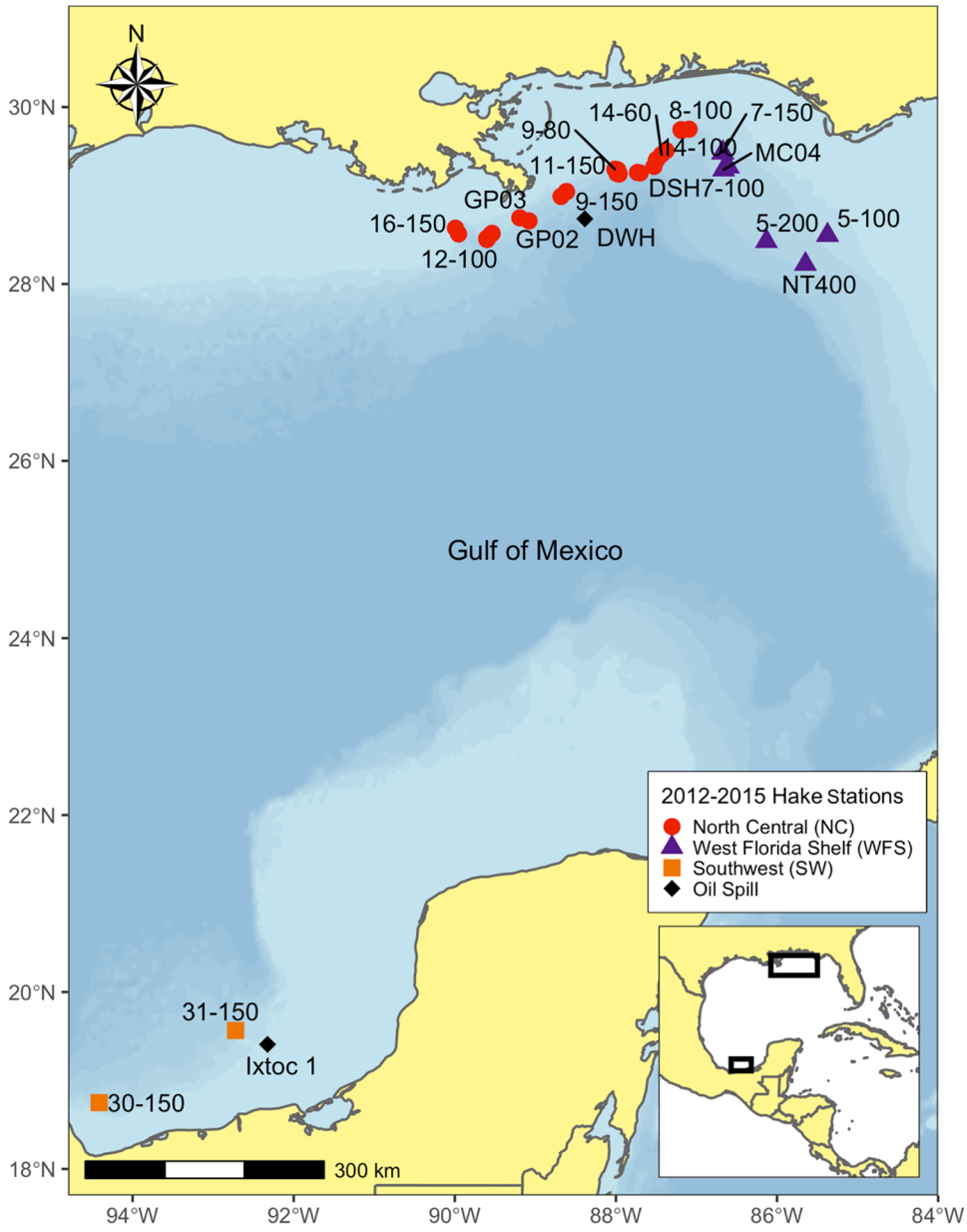

FIGURE 1: Gulf hake and southern hake longline stations from 2012 to 2015. North central, west Florida shelf, and southwest regional groups are indicated by the red circles, purple triangles, and orange squares, respectively. Black diamonds mark the locations of the *Deepwater Horizon* (DWH) and *Ixtoc 1* oil spills.

Technologies America; Hitachi LaChrom Elite), with the column temperature held at 50 °C. Fluorescent aromatic compounds (FACs) 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 NPH equivalents (FACs with 2–3 rings), 260/380 nm for phenanthrene (PHN) equivalents (FACs with 3–4 rings), and 380/430 nm for benzo[*a*]pyrene (BaP) equivalents (FACs with 4–5 rings). During the time that metabolites elute (5–19 min), all of the chromatogram peaks were integrated for each wavelength pair and summed with Agilent's EZChrom SI software. External standards of NPH, PHN, and BaP (10-µL injections) were used to convert sample area (fluorescence response) to wet weight PAH equivalents (ng/g bile) with the following calculation:

$$\text{PAH equivalents}\left(\frac{\text{ng}}{\text{g bile}}\right) = \frac{\text{standard concentration}}{\text{standard mean area}} \times \frac{\text{integrated sample area}}{\text{density of bile}} \times \frac{\mu\text{L of standard injected}}{\mu\text{L of sample injected}}$$

where the density of bile is 1.03 g/mL (Krahn et al. 1987). All equivalent concentrations were reported to 2 significant figures.

Liver extractions and PAH analysis

Liver samples were extracted using a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method (Forsberg et al. 2011) optimized specifically for these study species. Details of the QuEChERS protocol are provided in the Supplemental Data. Extracts were analyzed using Agilent's 7890B gas chromatograph (GC) coupled to a 7010 tandem mass spectrometer (MS/MS) operating in multiple reactions mode (MRM). Two-layer sandwich injections drew 2 μL of sample or standard, along with 0.2 μL of analyte protectant (20 mg/mL L-gulonolactone and 10 mg/mL D-sorbitol in acetonitrile) for enhanced peak signal quality (Maštovská et al. 2005). A total of 19 parent PAHs and 25 alkylated homologs (Supplemental Data, Tables S1 and S2) were targeted because of their known presence in crude oil and/or their status as a US Environmental Protection Agency (USEPA) priority pollutant (Keith 2015). Commercial PAH standards and a British Petroleum crude oil surrogate served as references to optimize the instrument parameters and collision energies for each MRM transition. A detailed description of the GC/MS/MS instrument parameters is provided in the Supplemental Data. Agilent's MassHunter software (Ver. B.07.00) was used for instrument control and to identify compounds based on their retention times and precursor, quantitative, and qualitative ions. Compounds were measured relative to 19 deuterated standards (Supplemental Data, Table S1) and by calculating relative response and dilution factors. All liver PAH concentrations were reported to 3 significant figures in ng/g wet weight.

Quality assurance and quality control

A performance-based quality assurance and quality control program was implemented to ensure acceptable levels of analytical accuracy and precision following the USEPA's Method 8270D (US Environmental Protection Agency 2014) and the National Oceanic and Atmospheric Administration's quality assurance and quality control plan for *Deepwater Horizon* Natural Resource Damage Assessment (National Oceanic and Atmospheric Administration 2012). The program included analysis of solvent blanks, procedural blanks, sample replicates, matrix spikes, blank spikes, and matrix-matched standards. Solvent blanks were analyzed before and after each field sample to monitor laboratory contamination. Procedural blanks were also prepared, processed, and analyzed with each analytical batch of liver samples. Biliary PAHs were analyzed in triplicate for each individual sample, maintaining a coefficient of variation (CV) <20%. If the CV between sample batches was >20%, the sample was reanalyzed until the quality control criteria were met. Continuing calibration standards (NPH, PHN, and BaP) were analyzed every 12 field samples, maintaining a CV <20% to monitor instrument stability throughout the analysis and to calculate the biliary PAH equivalent concentrations (see formula in section *Analysis of biliary metabolites*).

Matrix spikes and blank spikes were analyzed in duplicate on the GC/MS/MS at the beginning and end of the project to assess liver matrix effects and determine method accuracy and precision. For matrix spikes, mean recoveries of the 19 deuterated standards ranged from 70 to 102%, with a mean and standard deviation of 81 ± 12%. For blank spikes, mean recoveries ranged from 63 to 100%, with a mean standard recovery of 81 ± 13%. Matrix-matched standards were prepared with all field sample extraction batches in order to calculate recoveries. For all field samples run on the GC/MS/MS, the mean standard recoveries ranged from 60 to 84%, with an overall mean of 74 ± 13%. The mean CV for all internal standard runs analyzed on the GC/MS/MS was 6%, indicating strong instrument stability.

Statistical analyses

Nonparametric tests were performed because the data did not fulfill the normality and homogeneity assumptions for parametric analyses. The Wilcoxon-Mann-Whitney test was used to determine differences in fish biometrics (e.g., total length and wt), biliary FAC concentrations, and total liver PAH concentrations between the 2 species groups. Spatial and temporal differences were examined using Kruskal-Wallis one-way analysis of variance followed by Dunn's post hoc test with the Bonferroni correction for adjusted p values. Spearman's rank correlation was also used to determine the strength of associations between biliary FAC concentrations or total liver PAH concentrations and biometrics. All statistical testing was conducted using R, Ver. 3.5.1 (R Development Core Team 2018).

RESULTS AND DISCUSSION

Overall findings

Gulf hake and southern hake generally had comparable total lengths, total weights, liver weights, and gonad weights. The gonadosomatic index, hepatosomatic index (HSI), gastrosomatic index, and Fulton's condition factor (K) also suggest similar and relatively stable body conditions for both species (Table 1), though it is difficult to draw conclusions regarding health status given the lack of pre-*Deepwater Horizon* baseline biometric data. We did not observe a high prevalence of lesions, as reported in 2011 hakes (Murawski et al. 2014), but abnormalities including a spleen tumor and gut nodules were identified via general microscopy in 2 north central Gulf of Mexico hakes. When compared with results from a 1980s study which reported the last known biometrics data for southern hake near oil platforms, the mean HSI of southern hake sampled in the present study (0.041) was 78% higher than that of oil platform–associated hake and 141% higher than that of controls (Grizzle 1986). Grizzle hypothesized that the significantly greater HSI in fishes near platforms could be related to changes in food supply or to toxicant-related increases in liver weight attributable to hepatic enzyme induction. If hepatomegaly was the direct action of toxicants, the larger livers measured in the present study could also be linked with increased exposures to petroleum compounds.

TABLE 1: Summary statistics for species biometrics

Year	Species	*n*	Depth (nm)[a]	Sex (M:F:U)	TL (cm)	Wt (kg)	Liver Wt (kg)	Gonad Wt (kg)	GSI	HSI	GaSI	K
2012	GH	14	199	0:13:1	43	0.61	0.021	0.014	0.016	0.029	0.038	0.73
	SH	1	80	0:1:0	37	0.50						0.99
2013	GH	14	150	0:14:0	43	0.59	0.015	0.009	0.014	0.025	0.053	0.71
	SH	11	109	0:10:1	46	0.75	0.021	0.007	0.011	0.031	0.055	0.74
2014	GH	36	155	2:32:2	41	0.51	0.029	0.016	0.029	0.058	0.076	0.71
	SH	11	94	2:9:0	45	0.79	0.038	0.023	0.026	0.048	0.045	0.85
2015	GH	60	142	4:56:0	47	0.85	0.033	0.011	0.014	0.037	0.060	0.71
	SH	17	122	2:15:0	39	0.45	0.015	0.005	0.011	0.033	0.063	0.74

[a]Mean.
n = sample size; M:F:U = male:female:undetermined; TL = total length; Wt = weight; GSI = gonadosomatic index; HSI = hepatosomatic index; GaSI = gastrosomatic index; K = Fulton's condition factor; GH = gulf hake; SH = southern hake.

We discovered an unexpectedly high skewed sex ratio in these species, with 150 females, 10 males, and 4 unidentified individuals caught during the study period. Median biliary PAH concentrations for females, males, and unidentified hakes were 26 000, 46 000, and 35 000 ng/g bile, respectively. Median liver PAH concentrations for females, males, and unidentified hakes were 91.3, 102, and 71.6 ng/g, respectively. No significant differences were observed between the sexes for biliary FACs ($p = 0.100$) and liver PAHs ($p = 0.570$), so all sexes were combined for spatiotemporal analyses.

Granneman et al. (2017) also reported a female-biased sex ratio in southern hake collected in the Gulf of Mexico in 2011, though the sample size was small and dominated by lesioned fish. The extremely skewed sex ratio in *U. cirrata* and *U. floridana* may indicate an unknown catch bias for larger females using our longline fishing methods because differences in sex proportions have previously been explained by gear selectivity for female spotted hake (*U. regia*) and red hake (*U. chuss*; Eklund and Targett 1990). Alternatively, the sex skew could signal an increase in male mortality or involve a currently unknown life history parameter. Differences in biliary PAH metabolite concentrations and sex have previously been documented in fish, with males typically carrying higher contaminant burdens thought to be linked to sex hormones (Tuvikene 1995). Some PAHs are also known to induce estrogenic responses in fishes which can lead to the feminization of genetic males (Collier et al. 2013). Protandrous hermaphrodism is another possible explanation, whereby hakes may begin life as males and transition to the complete female gonadal type during a later life stage. At least one case of hermaphroditism has been documented in Pacific hake (*Merluccius productus*; Millikan and Pattie 1970), though other demersal fishes in the Gulf of Mexico, including most groupers (Parker and Mays 1998), employ this reproductive strategy.

Correlations with fish health indices

Strong positive correlations were detected between biliary NPH and PHN ($\rho = 0.80$, $p < 0.001$) and between these 2 compounds and total biliary metabolites ($\rho = 0.96$, $p < 0.001$ and $\rho = 0.88$, $p < 0.001$, respectively). The strong relationship between NPH and PHN metabolite concentrations has been found in other studies and is often indicative of a common petrogenic PAH source (Krahn et al. 1993; Da Silva et al. 2006). Biliary NPH, PHN, and BaP concentrations were not closely linked with the fish health indices, though BaP was negatively correlated with total fish length ($\rho = -0.26$, $p = 0.002$). Snyder et al. (2015) also found a weak, but significant, negative relationship between biliary BaP and fish length for golden tilefish, which may suggest that diet plays a significant role in the exposure for these species. Positive correlations were also detected between total liver PAHs and the following biometrics: total fish weight ($\rho = 0.23$, $p = 0.010$), liver weight ($\rho = 0.23$, $p = 0.011$), and Fulton's K ($\rho = 0.19$, $p = 0.035$). These relationships are not surprising given the hydrophobic nature of PAHs, which accumulate in fatty tissues, including livers, and the length to weight relationship used to calculate K (Pampanin and Sydnes 2013).

Species differences

Biliary PAH metabolite concentrations did not differ significantly between species when all years and stations were combined for analysis. Because these data were not normally distributed, median concentrations are reported to more accurately describe the central tendencies of each sample population. Gulf hake had slightly higher and more variable concentrations than southern hake for all 3 FACs (Figure 2), but the total median FACs were similar for both species with 29 000 and 27 000 ng/g bile, respectively. NPH metabolite equivalents were the highest in both species (*U. cirrata* = 23 000 ng/g, *U. floridana* = 21 000 ng/g), followed by PHN metabolites (*U. cirrata* = 6300 ng/g, *U. floridana* = 6000 ng/g). Levels of BaP were approximately 2 orders of magnitude lower than NPH and PHN metabolites (*U. cirrata* = 180 ng/g, *U. floridana* = 160 ng/g), and these biliary concentrations support the NPH > PHN > BaP trend reported for other GoM demersal fish species following the DWH spill (Snyder et al. 2015). These results are also consistent with the higher proportions of LMW PAHs (e.g., NPH and PHN) measured in the Gulf of Mexico's post-*Deepwater Horizon* sediments and deep water column (Reddy et al. 2012; Adhikari et al. 2016), compared to the less bioavailable and smaller concentrations of high molecular weight (HMW) PAHs (e.g., BaP).

Our findings differ slightly, however, from those previously reported for gulf and southern hakes in the northern Gulf of Mexico. Between 2011 and 2014, southern hake sampled in oiled and reference sites had higher biliary metabolite concentrations and CYP1A activity than gulf hake, though these species

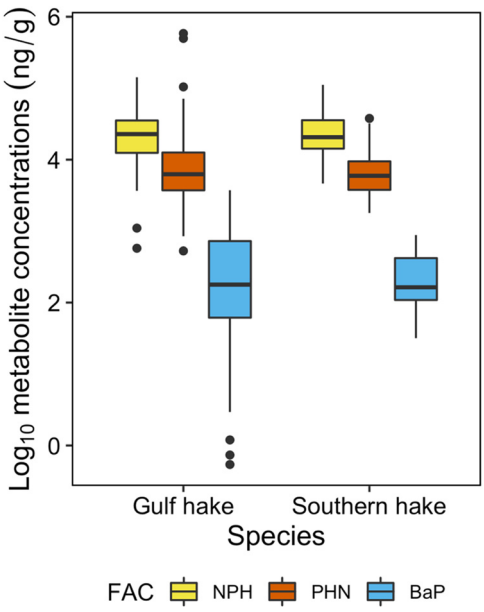

FIGURE 2: Biliary naphthalene (NPH), phenanthrene (PHN), and benzo [*a*]pyrene (B*a*P) metabolite concentrations for gulf hake and southern hake sampled in the Gulf of Mexico between 2012 and 2015. Horizontal lines denote the medians, colored boxes show the interquartile ranges (IQRs), vertical black lines represent 0.25 to 1.5 IQR/0.75+IQR, and black circles are sample outliers. BaP = benzo[*a*]pyrene; NPH = naphthalene; PHN = phenanthrene.

differences were also statistically insignificant (Leary 2015). Leary also reported lower levels of mean NPH metabolites (*U. cirrata* = 14 624 ng/mL, *U. floridana* = 28 106 ng/mL), with higher biliary concentrations of PHN than NPH measured in gulf hake. These contrasts are likely attributed to individual differences as well as variations in sample sizes, locations, depth distributions, and years because PAHs co-occur in dynamic compositions and concentrations that depend on their source and degradation (Pampanin and Sydnes 2013). When compared with other demersal fish species sampled in the northern Gulf of Mexico in 2012, median biliary NPH concentrations in king snake eel (*Ophichthus rex*) were similar to those measured in gulf and southern hakes, whereas median concentrations in red snapper and golden tilefish were markedly greater (Snyder et al. 2015).

Total liver PAH concentrations were also similar between the 2 species, though species comparisons were limited by the smaller sample size of *U. floridana* livers ($n = 31$). Both species exhibited a broad range of concentrations, with *U. cirrata* liver PAHs ranging from 7.66 to 404 ng/g and *U. floridana* liver PAHs ranging from 7.65 to 144 ng/g. Median PAH levels and standard deviations for gulf hake and southern hake equaled 85.3 ± 55.7 and 88.9 ± 37.8 ng/g, respectively. The PAH profiles for the 2 species were dominated by LMW compounds spanning NPH to anthracene (2–3 ring PAHs), suggesting that these species were exposed to a petrogenic source such as fresh crude or lightly weathered oil. Naphthalene and C1-C2 naphthalene homologs (N1-N2) contributed to >90% of the total PAHs (Supplemental Data, Table S4), with a median NPH concentration of 7.74 ng/g. This concentration is slightly higher than the median NPH level reported for fish muscle tissue (4.74 ng/g) in a seafood study conducted 1 mo post–*Deepwater Horizon* spill (Xia et al. 2012). However, all PAHs detected in hake livers were below the limit of concern (US Food and Drug Administration 2010), and it is unknown if this tissue is edible or used for human consumption. The median N1 and N2 concentrations were 51.0 and 19.4 ng/g, respectively. These homolog levels are consistent with other studies that have shown alkylated PAH concentrations are much higher than their corresponding parent PAHs in crude oil, oil mousse, and oiled sediments (Liu et al. 2012).

Following the NPHs, the next most abundant compounds measured in hake livers were fluorene, C1-fluorenes, PHN, and C1-phenanthrenes/anthracenes. Nearly all of the HMW compounds targeted in the present study were not detected in any of the liver samples (Supplemental Data, Table S4). The preferential uptake of LMW PAHs has been documented in other benthic fishes in the Gulf of Mexico (Snyder et al. 2019; Pulster et al. 2020) and is generally attributed to the bioavailability of these compounds and the higher biotransformation rates of HMW compounds compared to LMW PAHs (Baumard et al. 1998).

Spatial differences

Station trends. When species and years were combined, total biliary metabolite concentrations and total liver PAH concentrations were not directly correlated with distance from the *Deepwater Horizon* spill site ($\rho = -0.25$, $p = 0.003$ and $\rho = 0.079$, $p = 0.382$, respectively), but there were statistically significant differences between longline stations (Figure 3). Median concentrations for all sites are reported in Supplemental Data, Table S3; but stations with a sample size <5 were not included in the Kruskal-Wallis test because *p* values can be inaccurate. Biliary metabolite concentrations were expectedly higher than liver PAH concentrations in all fish sampled from the same sites. Total FAC levels at station 31-150 were significantly lower than concentrations at the following 3 north central (NC) stations: 11-150 ($p = 0.005$), 12-100 ($p = 0.03$), and 7-150 ($p < 0.001$). For reference, station 11-150 is located approximately 22 nm from the *Deepwater Horizon* spill site, whereas stations 12-100 and 7-150 are approximately 62 and 100 nm from the site, respectively. Station 31-150 is located in the southwest (SW) region, nearly 600 nm from the *Deepwater Horizon* spill site, but it is 16 nm from the *Ixtoc 1* spill site. Fish sampled at 31-150 and 30-150 (also located near the *Ixtoc 1* site) had low FAC levels, but the median total liver PAH concentrations were similar to the high levels measured in the NC region. These results suggest that hakes in the Bay of Campeche, like those in the NC Gulf of Mexico, may be exposed to chronic, low levels of recalcitrant crude oil and may bioaccumulate PAHs in their tissues over time.

Total liver PAHs were more variable by station than total biliary metabolites, and statistically similar stations were generally located within close proximity of one another. Hakes sampled at station 14-100 had significantly higher liver PAH concentrations than hakes caught at stations 5-100 ($p = 0.009$), 9-150 ($p = 0.02$), and DSH7-100 ($p = 0.007$). These results do not coincide with the positive relationship observed in 2011 to

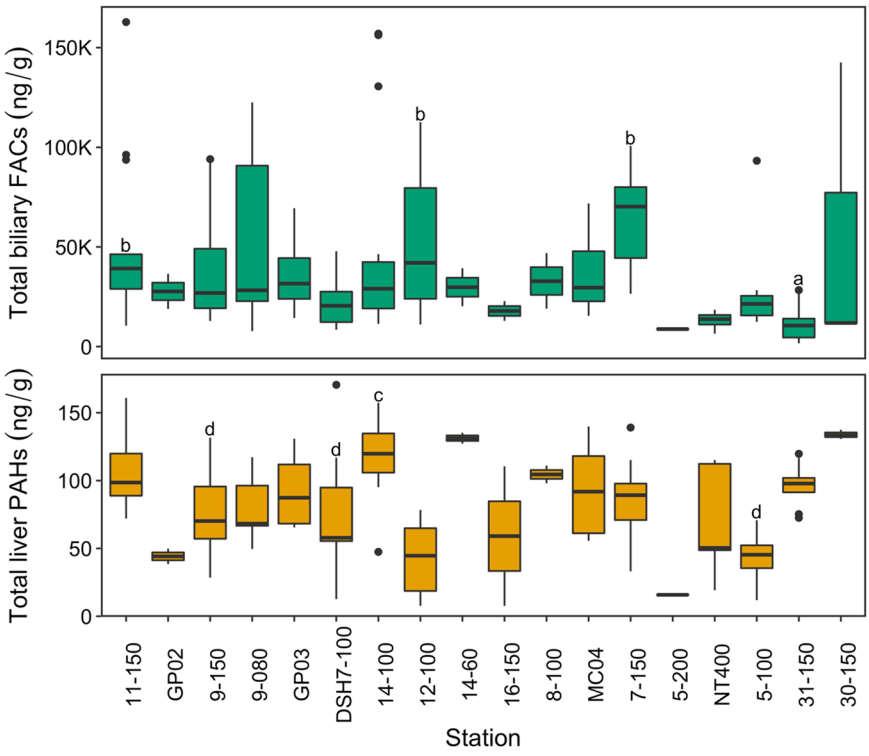

FIGURE 3: Station differences for total biliary fluorescent aromatic compounds (FACs) and total liver polycyclic aromatic hydrocarbons (PAHs) in order of increasing distance from the *Deepwater Horizon* spill site (left to right). Stations 11-150, 12-100, and 7-150 (labeled b) are significantly different ($p < 0.05$) from station 31-150 (labeled a); and stations 9-150, DSH7-100, and 5-100 (labeled d) are significantly different from station 14-100 (labeled c) for total biliary metabolites and liver PAHs, respectively.

2014 between PAH metabolite levels in hakes and distance from the *Deepwater Horizon* site (Leary 2015). However, station 14-100 is located along the DeSoto Canyon, a region heavily impacted by the *Deepwater Horizon* because of sedimentation pulses, direct oiling, and oil trajectories (Brooks et al. 2015).

Regional trends. When all species and years were combined, significant differences were found between bile samples collected from the NC and SW regions ($p < 0.001$) and the west Florida shelf (WFS) and SW regions ($p = 0.003$). Median FAC concentrations were highest in the NC Gulf of Mexico (30 000 ng/g bile), followed by the WFS (29 000 ng/g bile) and the SW (12 000 ng/g bile) regions (Figure 4). The high NPH and PHN concentrations in the NC region were unsurprising given the numerous oil platforms in this area as well as the reported presence of water-soluble aromatic compounds retained in the deep water column (Reddy et al. 2012) and offshore sediments (Romero et al. 2017; Giering et al. 2018) post–*Deepwater Horizon* spill. Although the WFS is often considered a reference area for petrogenic contamination in the NC Gulf of Mexico (Leary 2015), the subsurface transport of hydrocarbons is highly plausible and would support the similar concentrations of biliary metabolites observed in both hake species in these regions (Weisberg et al. 2016). Multiple PAH inputs, along with physical and sedimentological fluctuations caused by the Mississippi River discharge, currents, and isolated storm events, may also confound exposure variability over time and space (Kennicutt et al. 1996). Liver PAH concentrations were not significantly different between the 3 Gulf of Mexico regions, though this result was somewhat surprising given the significant differences detected between some longline stations. These findings further illustrate the ubiquitous nature of PAHs and the potential for hakes to accumulate a variety of PAHs from multiple sources because they presumably migrate throughout the Gulf of Mexico.

Temporal differences

Temporal trends were assessed for gulf and southern hakes in the NC Gulf of Mexico because this region was sampled during all 4 yr of the study. The sample size of repeated stations was too small to perform statistical analyses, but station differences were not significant within years, so total biliary FACs and liver PAHs were compared across all NC stations. No significant differences were discovered between biliary metabolites and years, though 2012 had the highest concentrations for each biliary compound. Total FAC concentrations fluctuated between years, with the highest median concentrations recorded in 2012 (42 000 ng/g) and 2015 (35 000 ng/g). Between 2012 and 2015, there was an 18% decrease in median total FACs (Figure 5A), which may be associated with decreases in environmental PAH pollution (Wang et al. 2008). It is also possible that the higher FAC concentrations in 2012 were associated with the large-scale sediment resuspension event following Hurricane Isaac. Isaac's impacts have been observed in other species and matrices (Paruk et al. 2016; Perez-Umphrey et al. 2018), and this resuspension event lasted from 28 August

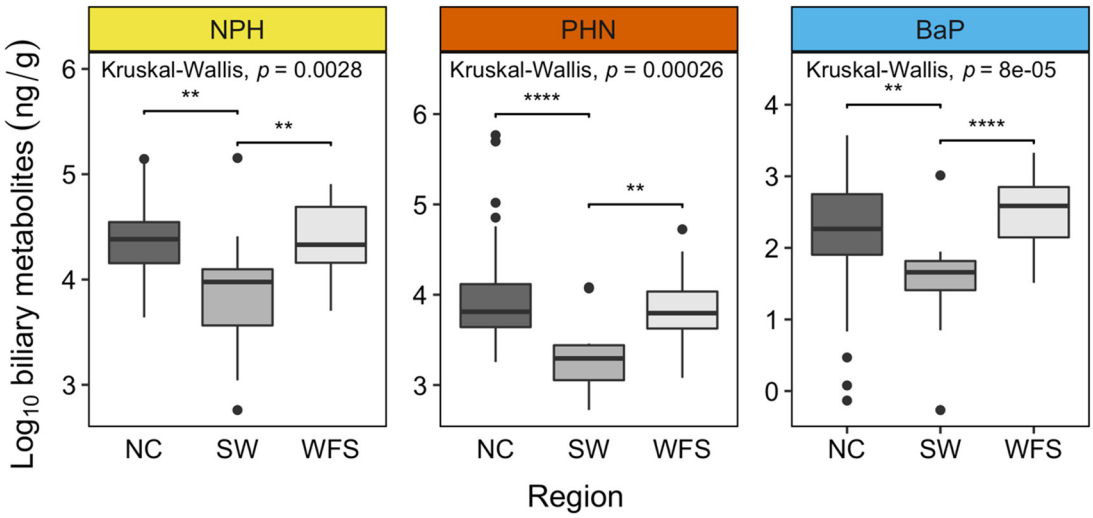

FIGURE 4: Naphthalene (NPH), phenanthrene (PHN), and benzo[*a*]pyrene (B*a*P) concentration differences in all hakes collected in the Gulf of Mexico's north central (NC), southwest (SW), and west Florida shelf (WFS) regions. Asterisks indicate statistical significance (**$p < 0.01$, ****$p < 0.0001$). B*a*P = benzo[*a*]pyrene; NC = north central; NPH = naphthalene; PHN = phenanthrene; SW = southwest; WFS = west Florida shelf.

to 2 September 2012 (Diercks et al. 2018), which overlapped with our sampling period. It is difficult to relate bile concentrations to negative health effects in fish because metabolites are excreted quickly, but these compounds can be recirculated and reabsorbed during metabolism, thereby increasing potential toxicity (Lech and Vodicnik 1985).

Total liver PAHs differed among years, with 2012 concentrations being significantly lower than those of 2013 ($p = 0.04$), 2014 ($p = 0.002$), and 2015 ($p < 0.001$). Since 2012, total liver PAHs increased 119% in hakes, with the median concentrations from 2012 to 2015 equaling 45.3, 85.4, 89.2, and 99.0 ng/g, respectively (Figure 5B). The low water temperatures in hake habitats could be contributing to the retention of dietary PAHs, as has been reported for NPH in the livers of exposed coho salmon (Collier et al. 1978) and starry flounder (Varanasi et al. 1981). Sediment cores collected in the NC Gulf of Mexico post–*Deepwater Horizon* spill have also suggested that degradation of LMW PAHs may be low in this region (Romero et al. 2015). The initial spike in liver PAHs and subsequent smaller increases over time may indicate that *Urophycis* spp. were chronically exposed to decreasing PAH concentrations but that hake metabolic rates are slower than accumulation rates in tissues (Varanasi 1989).

The HSI was also significantly different between 2012 and 2014 ($p = 0.006$), 2013 and 2014 ($p = 0.01$), and 2014 and 2015 ($p = 0.002$). In 2014, the median HSI was approximately 84%

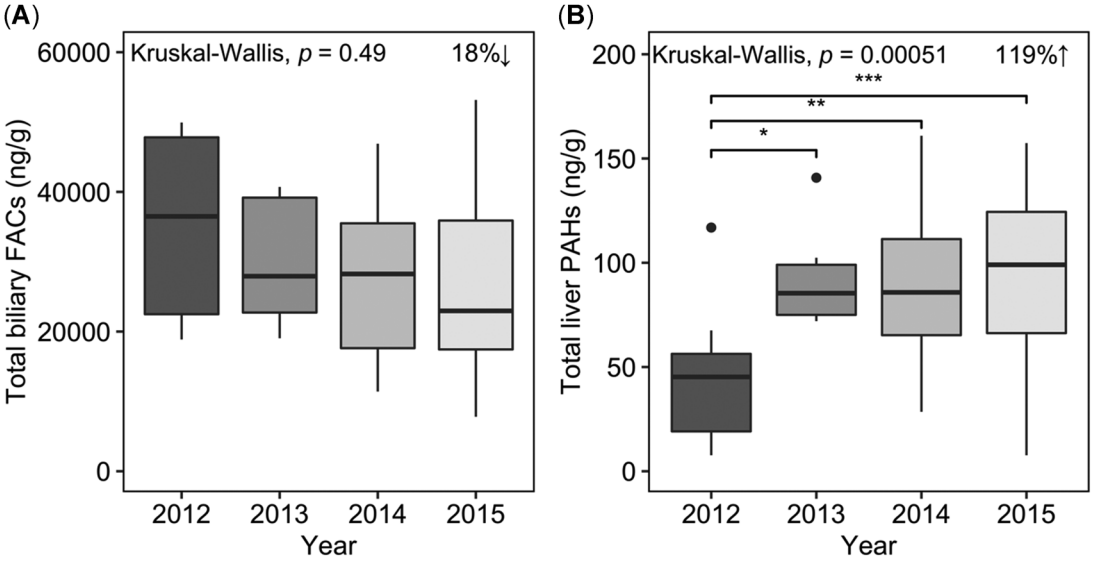

FIGURE 5: (**A, B**) Temporal differences in total biliary metabolites and total liver polycyclic aromatic hydrocarbons (PAH) for gulf and southern hakes sampled in the north central region. The percentage of decrease or increase in concentrations between 2012 and 2015 is marked with an arrow (top right), and asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Extreme outliers are not shown. FAC = fluorescent aromatic compound.

higher in southern hake and approximately 70% higher in gulf hake than the median HSI for the previous 2 yr. However, in 2015, the mean HSI for NC gulf and southern hakes decreased to 0.033 and 0.029, respectively, nearing the 2012 and 2013 values. The HSI is used as an indirect index for fish health and energy reserves, and it is often correlated with the degree of environmental pollution (Pointet and Milliet 2000). It is also influenced by female reproductive status, among other seasonal and annual variables, so we investigated the potential for sex as a confounding factor in our analyses. Hakes with the highest HSI values had the highest gonad weights (indicative of sexual maturity), but this subset included females, males, and an unidentified individual. Furthermore, all of the fish with high HSI values were sampled in 2014, but HSI and gonad weight were not linearly related.

To our knowledge, the present study is the first to report significant ongoing exposure to and metabolism of PAHs in gulf hake and southern hake in the Gulf of Mexico. Spatiotemporal analysis results suggest that acute PAH exposures have decreased from 2012 to 2015, as indicated by a downward trend in biliary metabolite levels. In contrast, liver PAH concentrations are increasing in hakes, which is indicative of chronic PAH exposures in these fishes. These findings further our understanding of PAH bioavailability, metabolism, and partitioning in hake tissues and will facilitate comparisons with other demersal species and environmental data. The present study also adds to the body of knowledge needed to strengthen our understanding of the fate and effects of oil exposures and health impacts in the Gulf of Mexico. Ultimately, the present study will contribute to the development of an ecosystem health model for Gulf of Mexico benthos and will serve as a baseline of PAH exposure for *Urophycis* spp. that will be critical for mitigating future environmental impacts and informing management decisions that improve damage-assessment policy.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4596.

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Data Accessibility—Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (DOI: 10.7266/N7X34W1J; 10.7266/n7-tv8b-7h23).

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