INTRODUCTION

The Deepwater Horizon blowout began on 20 April 2010, lasted for 87 d, and ultimately released 4 million barrels of oil into the Gulf of Mexico, becoming the largest accidental marine oil spill in global history (United States of America v. BP Exploration & Production 2015). Early in the response efforts, scientists realized that there was a critical lack of pre-spill data on oil contamination levels in Gulf of Mexico fishes and how concentrations varied over space and time. The lack of comprehensive pre-spill data is not unique to Deepwater Horizon, because similar circumstances occurred in the case of the 1989 Exxon Valdezo oil spill in Alaska’s Prince William Sound and the 1979 Ixtoc I blowout in the southern Gulf of Mexico (Pulster et al. 2020).

Due to the 4-dimensional and highly dynamic distribution of the oil, the effects of Deepwater Horizon spanned almost all ecosozones of the Gulf of Mexico, from coastal to oceanic, including both pelagic and benthic (Beyer et al. 2016). Petroleum hydrocarbons at the ocean surface and mid-water depths weather rapidly, but once sequestered in deep sea sediments, oil residues may persist for decades (Mackay and McAuliffe 1989). An estimated 21 ± 10% of the total Deepwater Horizon oil not recovered (3.19 million barrels) was deposited on the seafloor (Romero et al. 2017). Multiple mechanisms, including a sedimentation pulse of oiled marine snow, sinking of in situ burn resides, and impingement of deep hydrocarbon plumes, were responsible for oil sequestration. Sedimented oil residue is bioavailable to demersal fish through inhalation of resuspended and redissolved hydrocarbons, dermal uptake, consumption of contaminated benthic prey, and direct ingestion of contaminated sediment (Meador et al. 1995). Sedimented oil has been linked with negative impacts on Gulf of Mexico benthic fauna following Deepwater Horizon, including 80 to 93% declines in foraminifera density, changes in meio- and macrofauna diversity and abundance, oil-damaged corals, dietary and trophic shifts in red snapper (Lutjanus campechanus), and elevated frequency of external skin lesions in demersal fishes (Beyer et al. 2016).
Polycyclic aromatic hydrocarbons (PAHs) are the most toxic component of crude oil, although they made up only approximately 3% of the total hydrocarbons released from Deepwater Horizon by weight (Reddy et al. 2012). Negative effects of PAHs on fish health include reproductive impairment, cardiotoxicity, developmental defects, reduced growth, decreased condition factor, immunotoxicity, and hepatic lesions (Collier et al. 2014). Polycyclic aromatic hydrocarbons are efficiently metabolized in the teleost liver by a series of oxidation and conjugation reactions, which increase the solubility of parent PAHs for easy elimination via the bile and gastrointestinal tract (Meador et al. 1995). Due to this efficient bio-transformation, PAHs rarely accumulate to high levels in fish tissue, making tissue concentrations a poor indicator of previous or ongoing PAH exposure. A more suitable biomarker of exposure to PAHs is measurement of biliary PAH metabolites (Beyer et al. 2010). Concentrations of PAHs in tissue reflect any bioaccumulation that may occur over and above metabolism and excretion.

An initial study of PAH exposure in Gulf of Mexico demersal fishes following Deepwater Horizon found tilefish (Lopholatilus chamaeleonticeps) to have significantly higher concentrations of low molecular weight, often considered petrogenic, biliary PAH metabolites compared with 2 other demersal fishes, king snake eel (Ophichthus rex) and red snapper (Snyder et al. 2015). When contrasted with biliary PAH metabolite levels from published studies using similar quantification methods globally, tilefish ranked as the third highest fish species for low molecular weight PAH exposure. That study also documented a decline in low molecular weight PAH exposure for king snake eel (2012–2013) and red snapper (2011–2013), but no change over time (2012–2013) for tilefish, with levels of low molecular weight biliary PAH metabolites remaining consistently high. Tilefish habitat, physiology, and diet most likely accounted for their high levels of petrogenic PAH exposure (Snyder et al. 2015).

Tilefish are highly susceptible to exposure to sedimented contaminants because of their burrow-forming lifestyle. Remotely operated vehicle observations have recorded their primary habitat as large (meters × meters), funnel-shaped vertical burrows constructed in silt-clay sediments, which an individual tilefish inhabits throughout its lifetime (Able et al. 1982; Grimes et al. 1986; Jones et al. 1989). Fine grained silt-clay sediments tend to retain hydrophobic contaminants, such as PAHs, due to their high surface area and generally high organic matter content. In a depositional environment, burrows rapidly accumulate sediment, and considerable burrow maintenance and enlargement via frequent oral excavation has been observed (Grimes et al. 1986). Secondary burrowing by other species in the community, such as crustaceans and small fishes, also helps shape and maintain the burrows. Tilefish have been observed preying on these associated species, which are likely to be highly contaminated due to their benthic nature. Strong association with sediments via burrow maintenance and diet high in benthic prey is hypothesized to be why tilefish have some of the highest levels of PAH exposure ever measured, as well as the highest skin lesion frequency, following Deepwater Horizon (Murawski et al. 2014; Snyder et al. 2015).

As a follow-up to the findings of Snyder et al. (2015) on tilefish, a lengthier time series of PAH exposure and hepatic accumulation, as well as biometric data in the northern Gulf of Mexico, was completed during 2012 to 2017. We monitored 9 sampling locations in the northern Gulf of Mexico around the DeSoto Canyon, aiming to track a “return to baseline” following Deepwater Horizon, and provide data on the variability of that baseline over a 6-yr period. Tilefish are fished commercially and recreationally in the Gulf of Mexico, and their life history characteristics (long-lived, slow-growing, late-maturing, complex reproductive strategy, nonmigratory) make them especially sensitive to the negative individual and population-level impacts of chronic contaminant exposure.

MATERIALS AND METHODS

Field sampling

Fisheries-independent demersal longline surveys were conducted at repeat stations in the northern Gulf of Mexico during 2012 to 2015 and 2017. Tilefish were caught consistently at 9 stations in the northern Gulf of Mexico, which ranged from west of the Mississippi River, around the DeSoto Canyon, to the northern West Florida Shelf (Figure 1). Sampling occurred in the months of July (2017) and August (2012, 2013, 2014, 2015) and apart from the R/V Weatherbird II. At each station, an average of 474 size 130 circle hooks, baited with cut Atlantic mackerel (Scomber scombus) or various squid (mainly Humboldt squid [Dosidicus gigas] wings), were attached to 2.4-m 136-kg-test leaders and a 3.2-mm galvanized steel (2012) or 544-kg-test monofilament (2013–2017) main line for an average soak time of 2 h. Temperature/depth/time recorders (Star:Oddi CDST Centi-TD) were attached to the main line of each longline, set to record bottom temperature, depth, and fish depth. More detailed methods for longlining and sampling have been previously described in Murawski et al. (2014), Snyder et al. (2015), and Murawski et al. (2018).

Tilefish were caught at depths of 147 to 438 m, with a mean depth of 265 m. Once landed, fish were sampled immediately or placed on ice prior to processing. Standard and total lengths, total body weight, sex, and organ weights (liver, gastrointestinal, and gonad) were determined. Sexes were identified visually as male, female, or unknown. If present in sufficient volume, bile was collected by draining the contents of the gall bladder into an 8-mL combusted amber vial. Livers were collected in either a combusted glass jar or combusted aluminum foil and inserted into Whirl-Paks™ if jars were not available. Samples were frozen immediately and stored at −20 °C until analysis.

Chemicals and reagents

Analytical standards were purchased from Absolute Standards. Surrogate MC252 crude oil was provided by British Petroleum (BP). All solvents were Fisher Chemical Optima® grade,
except for high-performance liquid chromatography (HPLC)-grade methyl tert-butyl ether (MTBE). All glassware was washed with Alconox®, combusted for a minimum of 4 h, and rinsed with acetone and hexane prior to use.

**Analysis of biliary PAH metabolites using HPLC-with fluorescence detection**

Two separate laboratories, Mote Marine Laboratory and the University of South Florida, analyzed the bile samples; however, the analyst remained the same. At each facility, an interlaboratory comparison was performed in conjunction with the National Oceanic and Atmospheric Administration (NOAA) Northwest Fisheries Science Center (NWFSC), Seattle, WA, USA, whose quality-assurance plan regularly monitors accuracy through a fish bile control sample from Atlantic Salmon (*Salmo salar*) exposed to 25 µg/mL of Monterey Crude oil for 48 h. As described in Snyder et al. (2015), the interlaboratory comparison used bile samples from 3 fish species and measured biliary PAH metabolite equivalents of naphthalene, phenanthrene, and benzo[a]pyrene (BaP) over a wide range of concentrations. The comparison agreed, with a coefficient of variation of <15% for biliary PAH metabolite equivalents between all 3 analytical facilities for each of the 3 quality-control samples.

A bile screening method based on semiquantitative HPLC with fluorescence detection (HPLC-F), developed by the NWFSC, was used to analyze all bile samples (Krahn et al. 1984; Krahn et al. 1986; Snyder et al. 2015). In summary, 3 µL of untreated bile was injected directly onto the HPLC-F system (Mote Marine Laboratory: Agilent Technologies, 1100 Series; University of South Florida: Hitachi High-Technologies, Elite LaChrom L-2000 Series). Throughout the present study, the same model of C-18 reverse-phase column (Phenomenex Synergi™ 4 µm Hydro-RP 80 Å) was used. With the column oven held at 50 °C, fluorescent aromatic compounds (FACs) were eluted at a flow rate of 1 mL/min using a linear gradient from 100% solvent A (water containing 5 µL/L acetic acid) to 100% solvent B (methanol). Chromatograms were recorded at representative wavelength pairs of 292/335 nm for 2- to 3-ring FACs (e.g. naphthalene metabolite equivalents) and 380/430 nm for 4- to 5-ring FACs (e.g. BaP metabolite equivalents). All peaks within a time window of 6 to 19 min on the chromatogram were integrated and summed, and FACs were calculated for each wavelength pair using an external standard of the representative parent PAH (naphthalene or BaP) to quantify FACs from fluorescence response. Biliary PAH data are reported to 2 significant figures as µg FACs/g of bile.

Quality assurance was initially evaluated through the interlaboratory comparison, and continuously monitored via methanol blanks between samples, sample analysis in duplicate (2012–2013) or triplicate (2014–2017), and continuing calibration of PAH standards (naphthalene, 2.5 µg/mL; BaP 250 ng/mL) with each batch of 12 field samples.

**Analysis of livers for PAHs and alkylated homologs using gas chromatography–tandem mass spectrometry**

Liver tissue was extracted using a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method (Lucas and Zhao 2015). The entire tissue sample was homogenized,
and a 2-g aliquot was spiked with a surrogate standard solution containing the deuterated form of each of the 19 parent PAH analytes (Supplemental Data, Table S2; 16 US Environmental Protection Agency PAHs plus dibenzothiophene, benzo[e] pyrene, and perylene). After a 10-min marination, acetonitrile was added, and samples were shaken with 2 clean steel beads using a 1600 MiniG® automated tissue homogenizer and cell lyser (SPEX SamplePrep). Acetonitrile extracts were transferred to a Bond Elut Enhanced Matrix Removal-Lipid (EMR-Lipid) dispersive solid-phase extraction tube (Agilent Technologies) and shaken in the MiniG. The extract was decanted and mixed with Bond Elut EMR-Lipid Polish Pouch (Agilent Technologies), containing anhydrous magnesium sulfate for water removal, and agitated. Final extracts were spiked with a postextraction standard of p-terphenyl-d14 and brought to a volume of 1 mL. The QuEChERS extraction method was optimized for this species and matrix by varying duration of marination and extraction in the MiniG for maximum recovery of matrix spikes.

Extracts were injected in splitless mode as a 2-layer sandwich composed of 2 µL of sample extract and 0.2 µL of analyte protectant (20 mg/mL L-gulonolactone and 10 mg/mL D-sorbitol composite solution in acetonitrile). Analytes were separated by gas chromatography (Agilent Technologies, 7890B) on a 30-m Rxi-5Sil fused silica capillary column (Restek) and analyzed by a triple quadrupole mass spectrometer (Agilent Technologies, 7010) operating in multiple reaction–monitoring and full-scan modes. Operating and acquisition parameters for gas chromatography–tandem mass spectrometry analysis can be found in the Supplemental Data (Tables S1 and S2).

A matrix-matched standard containing all analytes (both deuterated and nondeuterated forms) was made and analyzed with each batch of samples to quantify surrogate recoveries as well as relative response factors for each analyte within each sample. Target analyte concentrations were quantified using the relative response factors from the matrix-matched standard, and identities were confirmed by matching spectra, retention times, and relative intensity ratios of the selected ions with matrix-matched standard. Liver PAH concentrations are reported to 3 significant figures as ng/g wet weight. Target analytes (n = 46) include 19 parent PAHs and selected alkylated homologs (Supplemental Data, Table S2). The sum total of the analytes is reported as TP AH46. The sum of low molecular weight PAHs comprised 2- to 3-ring PAHs and alkylated homologs. The sum of high-molecular-weight PAHs comprised 4- to 6-ring PAHs and alkylated homologs. Commercial standards in both solvent and matrix, as well as BP surrogate crude oil, were used to optimize instrument parameters initially and as needed throughout the present study.

Quality-assurance measures followed NOAA’s MC252 Analytical Quality Assurance Plan’s method performance criteria (National Oceanic and Atmospheric Administration 2012). Prior to sample analysis, linearity of all analytes within an appropriate concentration range (1 to 1000 ng/mL) was verified via 7-point matrix-matched and solvent calibration curves. In addition, a standard reference material (NIST SRM 1974c, organics in mussel tissue) was analyzed at the beginning of the project to verify accurate quantification of all analytes. A series of matrix spikes using the appropriate species and tissue was performed to optimize extraction methods and assess precision. Procedural blanks were extracted and analyzed with every batch, and acetonitrile solvent blanks were analyzed in between samples to monitor for background contamination. The postextraction standard in each sample was monitored for changes in instrument stability. Recoveries of surrogate standards in sample extracts (low molecular weight: 82 ± 12%; high-molecular-weight: 78 ± 15%), SRM (82 ± 9%), and matrix spikes (84 ± 5%) as well as, procedural blanks, solvent blanks, and post-extraction standard monitoring all met acceptable quality assurance criteria established in National Oceanic and Atmospheric Administration (2012).

**Analysis of livers for total lipid**

Total lipid in liver tissue was extracted using a modified Folch method (Matyash et al. 2008). A 200 mg aliquot of homogenized liver was extracted in a 2-series extraction, first using MTBE, and second using a mixture of MTBE/methanol/water (10/3/2.5 v/v/v). Extracts were evaporated to dryness and total lipid was determined gravimetrically and reported as percentage of liver lipid.

**Statistical analyses**

All statistics were performed in MATLAB R2017a using the Fathom Toolbox for Matlab (Jones 2017). All hypothesis tests used permutation-based p-values (1000 iterations) assessed at α = 0.05. Pearson’s correlations or regressions were used to determine the concordance in variation between continuous data. A modified permutational multivariate analysis of variance (PERMANOVA) was used to test the difference in mean between groups, allowing for accuracy of p-values when between-group dispersions were heterogenous (Anderson et al. 2017). If modified PERMANOVAs were significant, they were followed by a pair-wise modified PERMANOVA. Analysis of covariance (ANCOVA) was run to test the difference in the mean of specific variables that were found to vary by another continuous variable, such as year. A chi-square test of independence was used to test the difference in sex ratio by year.

Fulton’s condition factor (K) was calculated as

\[
\frac{\text{total body weight}}{\text{total length}} \times 100, \text{where weight is expressed in kg and length in cm. Hepatosomatic index (HSI) was calculated as} \\
\frac{\text{total length}}{\text{total body weight (kg)}} \times 100. \text{Change percentage was calculated between the first and last year sampled for each variable in the time series.}
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**RESULTS**

**Biometric and liver lipid data**

A total of 286 tilefish were sampled at 9 repeat stations via 38 longline sets during 2012 to 2015 and 2017 (Table 1). The sex ratio of sampled fish was dominated by females and did not
significant changes over time (Table 1, $\chi^2 = 28.9$, $p = 0.162$). There was no significant difference in mean total length (TL) of tilefish over time for all stations combined ($F = 1.61$, $p = 0.164$), nor at any individual station except for 7-150 (Table 1, $F = 4.83$, $p = 0.006$). There was a significant decrease in mean total body weight of tilefish over time for all stations combined (Table 1, $F = 2.87$, $p = 0.023$), and for 2 out of 9 individual stations (Table 1): 7-150 ($F = 4.99$, $p = 0.004$) and 8-100 ($F = 3.14$, $p = 0.032$). At station 7-150, the decrease in total body weight was significantly correlated with the decrease in total length over time ($r = 0.935$, $p = 0.001$).

A significant 22% decrease in the mean of Fulton’s condition factor ($K$) occurred over the present study period for all stations combined (Figure 2, $F = 29.6$, $p = 0.001$) and for 6 out of 9 individual stations (Figure 3): 7-150 ($17\%$, $F = 3.45$, $p = 0.018$), 8-100 ($28\%$, $F = 13.1$, $p = 0.001$), 9-150 ($21\%$, $F = 8.77$, $p = 0.001$), 14-60 ($27\%$, $F = 12.9$, $p = 0.001$), 11-150 ($10\%$, $F = 3.34$, $p = 0.039$), and MC04 ($26\%$, $F = 28.9$, $p = 0.001$). Mean $K$ for all stations combined was 1.26 ± 0.165 in 2012 and 0.986 ± 0.089 in 2017.

Mean $K$ varied significantly by sex ($F = 5.57$, $p = 0.007$), with males having higher $K$ than females ($t = 2.78$, $p = 0.012$), and unknowns ($t = 3.25$, $p = 0.003$). There was no significant difference in mean $K$ between females and unknowns ($F = 1.35$, $p = 0.197$). Mean $K$ was 1.17 ± 0.163 for males, 1.10 ± 0.175 for females, and 1.06 ± 0.173 for unknowns. Because mean $K$ was found to vary over time, an ANCOVA was also used to evaluate differences in mean $K$ by sex, with year as a covariate. Both sex and year effects on $K$ were significant, with year having a larger impact, as seen in the F-ratio, which is approximately 22 × higher ($F_{\text{sex} \times \text{year}} = 0.615$, $p_{\text{sex} \times \text{year}} = 0.526$; $F_{\text{sex}} = 4.75$, $p_{\text{sex}} = 0.010$; $F_{\text{year}} = 104$, $p_{\text{year}} = 0.001$). For males, mean...
K varied significantly between years (Figure 2, F = 10.7, p = 0.001), with the year 2012 removed because n = 1 for males in 2012. Male K increased from 2013 to 2014, followed by a significant decrease in 2015, then followed by a significant increase in 2017. For females, K decreased significantly, 22% from 2012 to 2017 (Figure 2, F = 23.8, p = 0.001), from a mean of 1.27 ± 0.181 in 2012 to 0.981 ± 0.076 in 2017. Because the sex ratio of sampled fish was dominated by females, the overall trend in K is mirrored by the trend in females alone. There was no significant change in mean K over time for tilefish of unknown sex (Figure 2, F = 2.11, p = 0.121); however, the dominant trend was a decrease over time, which was not detected by statistical testing due to low sample size.

There was a significant change in mean HSI over time for all stations combined (F = 17.8, p = 0.001), and at 6 out of 9 individual stations: 7-150 (%) = 23.2, p = 0.001, 8-100 (%) = 14.3, p = 0.001, 9-150 (%) = 12.6, p = 0.001, 14-60 (%) = 43.2, p = 0.001, and 11-150 (%) = 2.97, p = 0.036. For all stations combined, the mean percentage of liver lipid was 12.8 ± 4.9% in 2012 and 6.0 ± 2.9% in 2017. There was no significant difference in mean percentage of liver lipid by sex with year as a covariate (FSex×Year = 1.31, pSex×Year = 0.334; FSex = 1.79, pSex = 0.182; FYear = 24.3, pYear = 0.001). Percentage of liver lipid was significantly correlated with K (r = 0.2967, p = 0.001).

There was a significant 53% decrease in mean percentage of liver lipid over time for all stations combined (Figure 4, F = 17.4, p = 0.001), and at 5 out of 9 individual stations: 7-150 (%) = 166, F = 23.2, p = 0.001, 8-100 (%) = 165, F = 14.3, p = 0.001, 9-150 (%) = 156, F = 12.6, p = 0.001, 14-60 (%) = 115, F = 43.2, p = 0.001, and 11-150 (%) = 12, F = 2.97, p = 0.036. For all stations combined, the mean percentage of liver lipid was 12.8 ± 4.9% in 2012 and 6.0 ± 2.9% in 2017. There was no significant difference in mean percentage of liver lipid by sex with year as a covariate (FSex×Year = 1.31, pSex×Year = 0.334; FSex = 1.79, pSex = 0.182; FYear = 24.3, pYear = 0.001). Percentage of liver lipid was significantly correlated with K (r = 0.2967, p = 0.001).

Relationship between total lipid and PAHs in liver tissue

There was no relationship between the percentage of liver lipid and liver TPAH46 concentration (R² = 0.009, p = 0.266). Regressions were insignificant for all stations combined and for individual stations. Without a direct relationship between the percentage of liver lipid and liver TPAH46 concentration, TPAH46 concentrations were not lipid-normalized for data analysis (Hebert and Keenleyside 1995).
PAHs and alkylated homologs in liver tissue

A total of 230 tilefish livers were analyzed for PAHs and alkylated homologs (Table 1). For all stations combined, there was no significant change in mean liver TPAH46 concentration over the present study period (Figure 5, \( F = 0.351, p = 0.876 \)). Mean concentration for all stations and all years combined was 956 ± 773 ng/g wet weight, ranging from 288 to 8110 ng/g wet weight. For individual stations, only 1 station (14-60) had a significant change in liver TPAH46 concentration over the study period (\( F = 4.60, p = 0.004 \)), which was a significant increase from 2012 to 2013, and a decline until 2017.

The composition of the 46 PAHs and alkylated homologs measured in liver tissue did not change over the study period (\( F = 1.04, p = 0.396 \)). Liver PAH profiles were consistently dominated by low molecular weight PAHs and homologs, which

FIGURE 3: Fulton’s condition factor (K) over time for tilefish sampled 2012 to 2015, and 2017 at individual stations (station number designated on plot) in the northern Gulf of Mexico. Sample size (n) noted by year. Letters (ABC) denote significantly different years. Solid line = median; dotted line = mean.

FIGURE 4: Percentage of liver lipid over time for tilefish sampled 2012 to 2015, and 2017 in the northern Gulf of Mexico. Data are combined for all stations. Sample size (n) noted by year. Letters (ABC) denote significantly different years. Solid line = median; dotted line = mean.

FIGURE 5: Total liver polycyclic aromatic hydrocarbon (PAH) concentration (TPAH46) for tilefish sampled 2012 to 2015, and 2017 in the northern Gulf of Mexico. Data are combined for all stations. Sample size (n) noted by year. Solid line = median; dotted line = mean.
constituted >99% of the TPAH$_{46}$ concentration. The mean sum of low molecular weight PAHs in liver tissue was 953 ± 773 ng/g wet weight, whereas the mean sum of high-molecular-weight PAHs in liver tissue was 4.49 ± 8.24 ng/g wet weight. All high-molecular-weight PAHs identified were less than the method detection limit (MDL; 1 ng/g). Analytes identified at <MDL were reported at one-half MDL. Therefore, the total high-molecular-weight PAH concentration in liver tissue was solely composed of analytes identified at <MDL, and quantities should thus be interpreted with caution.

There was a significant difference in mean liver TPAH$_{46}$ concentration by sex ($F = 3.46$, $p = 0.044$), with individuals of unknown sex having significantly lower concentrations compared with males ($t = 2.38$, $p = 0.023$) and females ($t = 2.71$, $p = 0.007$). Mean liver TPAH$_{46}$ concentrations were not different between males and females ($t = 0.017$, $p = 0.992$). The mean liver TPAH$_{46}$ concentration was 739 ± 417 ng/g wet weight for unknown, 998 ± 534 ng/g wet weight for male, and 1000 ± 884 ng/g wet weight for female tilefish. Liver TPAH$_{46}$ concentrations did not vary significantly with total length ($r = 0.100$, $p = 0.064$). However, mean total length did vary by sex ($F = 31.8$, $p = 0.001$), with males being significantly larger than females ($t = 6.29$, $p = 0.001$), and both males ($t = 7.75$, $p = 0.001$) and females ($t = 3.35$, $p = 0.001$) being significantly larger than unknowns. Mean total length was 56.9 cm for unknowns, 63.5 cm for females, and 77.0 cm for males. An ANCOVA with total length as the covariate could not assess the relationship between sex and mean liver TPAH$_{46}$ concentration, because the relationship between TPAH$_{46}$ and total length was not homogenous by year ($F_{Sex×TL} = 3.47$, $\rho_{Sex×TL} = 0.005$).

**Biliary PAH metabolites**

A total of 256 tilefish bile samples were analyzed for PAH metabolites (Table 1). There was a significant increase in mean total biliary PAH metabolite equivalents (naphthalene + BaP) over time for all stations combined (Figure 6, $t_{1178} = 21.8$, $p = 0.001$), and at 6 out of 9 individual stations (Figure 7): 7-150 (1166%, $F = 2.92$, $p = 0.043$), 8-100 (1153%, $F = 4.33$, $p = 0.009$), 9-150 (1155%, $F = 3.75$, $p = 0.009$), 11-150 (1235%, $F = 5.45$, $p = 0.018$), 14-60 (1317%, $F = 7.65$, $p = 0.001$), and 14-100 (1811%, $F = 7.34$, $p = 0.001$). The 3 stations (9-80, MC04, and GP03) that did not have a significant increase in total biliary PAH metabolite equivalents each had 1 yr where $n = 1$ for bile samples (Table 1); therefore, the modified PERMANOVA would not assess significance, although the dominant trend was an increase in concentration over time (215, 8.38, 72.5% increases respectively). For all stations combined, mean total biliary PAH metabolite equivalents increased by a factor of 2.8, from 230 ± 68 µg FACs/g in 2012 to 640 ± 410 µg FACs/g in 2017 (Figure 6).

Concentrations of naphthalene metabolite equivalents were consistently 3 orders of magnitude higher than concentrations of BaP metabolite equivalents, meaning that total biliary PAH metabolite equivalents were dominated by naphthalene metabolite equivalents. Therefore, trends in biliary naphthalene metabolite equivalents mirrored those of total biliary PAH metabolite equivalents. There was no difference in mean biliary BaP metabolite equivalents over time for all stations combined ($F = 1.46$, $p = 0.207$). There was no significant difference in mean total biliary PAH metabolite equivalents by sex, with year as a covariate ($F_{Sex×Year} = 1.87$, $P_{Sex×Year} = 0.168$; $F_{Sex} = 0.347$, $P_{Sex} = 0.706$; $F_{Year} = 71.1$, $P_{Year} = 0.001$).

**Relationship between PAH exposure and fish condition factor**

There was a significant negative correlation between K and total biliary PAH metabolite equivalents for all stations, all sexes, and all years combined (Figure 8, $r = −0.150$, $p = 0.021$), and at 2 out of 9 individual stations: 8-100 ($r = −0.330$, $p = 0.033$), and 14-60 ($r = −0.273$, $p = 0.050$). The significant negative correlation between K and total biliary PAH metabolite equivalents occurred for females ($r = −0.166$, $p = 0.014$) but not males. The correlation between K and total biliary PAH metabolite equivalents was significant, but positive for males ($r = 0.311$, $p = 0.029$). The correlation was insignificant for tilefish of unknown sex ($r = −0.195$, $p = 0.115$). There were no significant correlations between liver TPAH$_{46}$ concentrations and K for all data combined ($r = −0.032$, $p = 0.329$), males ($r = 0.060$, $p = 0.344$), females ($r = −0.054$, $p = 0.258$), or unknowns ($r = −0.1323$, $p = 0.202$).

**DISCUSSION**

Tilefish were selected as a target species for the present extended time series study due to their high levels of biliary PAH metabolites, a biomarker of exposure to PAHs, compared with other demersal fish species in the Gulf of Mexico, and their proximity to the Deepwater Horizon. Snyder et al. (2015) found tilefish sampled in 2012 and 2013 had higher mean concentrations of biliary naphthalene metabolite equivalents compared...
with king snake eel and red snapper sampled in the same region, assorted demersal fish sampled offshore of Texas in the early 1990s, and Atlantic croaker (*Micropogonias undulatus*) sampled in Louisiana waters pre- and post-hurricane Katrina. Since that study was published (Snyder et al. 2015), we sampled an additional 186 tilefish and extended the time series by 3 yr (2014, 2015 and 2017). Since 2012, there has been a significant 178% increase in mean total biliary PAH metabolite equivalents (dominated by naphthalene metabolite equivalents) from $230 \pm 68 \mu g F A C$s/g to $460 \pm 410 \mu g F A C$s/g in 2017.

Tilefish sampled in 2017 now have the highest levels of biliary naphthalene metabolite equivalents measured in comparable studies (Snyder et al. 2015). Contrasted against additional biliary PAH metabolite data from 12 Gulf of Mexico demersal fishes sampled in the same region in the same time window (2012–2015), tilefish have higher mean total biliary PAH metabolite equivalents compared with all species, which ranged from approximately 10 to 180 $\mu g F A C$s/g bile (Pulster et al. 2020). Other demersal fishes sampled from the northern Gulf of Mexico post Deepwater Horizon, Gulf hake (*Urophycis cirrata*), snowy grouper (*Epinephelus niveatus*), yellowedge grouper (*Hyporthodus flavolimbatus*), red snapper, and red grouper (*Epinephelus morio*), also had increased total biliary PAH metabolites over time (Pulster et al. 2020).

The exceptionally high exposure of tilefish to low molecular weight PAHs may be attributed to their burrow-forming lifestyle and limited movement. Tilefish excavate large vertical funnel-shaped burrows in silt-clay sediments, which they use for protection over their lifetime. Direct observations of tilefish have noted frequent maintenance of burrows, with their mouths and bodies, to keep the burrows from filling in (Grimes et al. 1986). The hypothesis for explaining high PAH exposure in tilefish is that maintaining their burrows exposes them to sedimented pollution at significantly higher levels than other demersal fishes. The tilefish diet consists primarily of benthic organisms living among the burrows, which also perform secondary burrowing. Benthic invertebrate prey, especially infaunal or burrowing organisms, are prone to high tissue pollutant accumulation, and are likely another source of high exposure for tilefish.

Following Deepwater Horizon, numerous studies described marine oil snow sedimentation and flocculent accumulation as a mechanism for transferring oil and its residues from the sea.
surface and the water column to the seafloor (Beyer et al. 2016). Over time, deposited oil residues may become resuspended by physical oceanographic processes (e.g. bottom currents, internal waves, and storms) and redistributed to new locations via transport of the benthic nepheloid layer downslope to be redeposited onto the sediments at other locations (Ziervogel et al. 2016; Diercks et al. 2018). This resuspension and secondary deposition is likely a mechanism of impact on benthic communities that were marginally affected by the initial marine oil snow sedimentation and flocculent accumulation or Deepwater Horizon event, possibly explaining why PAH exposure is increasing over time for tilefish and other Gulf of Mexico demersal fishes. Resuspension events will result in the renewed bioavailability of oil residues that were previously sequestered in sediments, unavailable to both tilefish and their prey.

Other time-series studies following Deepwater Horizon note varied patterns of PAH exposure over time in which resuspension events are implicated. Cytochrome P4501A expression in seaside sparrows (Ammodramus maritimus) decreased post Deepwater Horizon, but then abruptly increased in 2013 at sites that had, and had not been, directly oiled by Deepwater Horizon (Perez-Umphrey et al. 2018). The authors concluded that weather, storms, and hurricanes, such as 2012’s Isaac, influenced spatial and temporal exposure to oil in their post Deepwater Horizon time series by resuspending and redistributing contaminated sediments. Gulf menhaden (Brevoortia patronus) exhibited increases in BaP concentrations from 2012 to 2013, which Olson, Meyer, and Portier (2016) concluded were not due to a new source, but to the resuspension of Deepwater Horizon oil. In addition, blood PAH concentrations in common loons (Gavia immer) increased significantly post Deepwater Horizon to a maximum in 2013, which was also hypothesized to be related to sediment resuspension following Isaac (Paruk et al. 2016).

In contrast to biliary PAH metabolites, mean concentrations of liver TPAH46 in tilefish did not vary significantly over the present study period. Liver TPAH46 concentration did not vary with total length or percentage of liver lipid, but did vary by fish sex. Tilefish gonads were difficult to sex macroscopically in the field, in part due to their protogynous hermaphrodite reproductive strategy, and also due to sampling of the fish postspawning season; therefore, sex data should be interpreted cautiously. Tilefish of unknown sex, which were also significantly smaller in total length, had lower liver TPAH46 concentrations compared with those identified as males or females. Although the relationship between tilefish total length and liver TPAH46 was not significant, it is possible that the smaller tilefish, which are more commonly identified as unknown sex, consume different prey items, possibly leading to differences in exposure. Limited information on tilefish food habits indicate that juveniles consume more echinoderms and mollusks compared with larger individuals (Freeman and Turner 1977). Burrowing behavior of smaller tilefish may also be different, because juveniles have been observed in simple vertical shafts instead of the larger funnel-shaped burrows (Able et al. 1982).

Compared with other demersal fishes sampled in the same northern Gulf of Mexico region and time period, tilefish have comparable concentrations of liver TPAH46 (288–8110 ng/g wet wt). Liver TPAH46 concentrations ranged from 7.7 to 407 ng/g wet weight for hakes (Urophycis sp.) and 67.6 to 17 300 ng/g wet weight for groupers (Epinephelus sp.; Pulster et al. 2020). Although the data were highly variable year-to-year, these other demersal fishes exhibited a general increasing trend in liver TPAH46 concentrations over time, in contrast to stable concentrations as exhibited in tilefish.

The significant increase in biliary PAH metabolite equivalents over time, concurrent with no change in TPAH46 concentrations in liver tissue, suggests that exposure of tilefish to PAHs is increasing; however, metabolism continues to efficiently eliminate the compounds, thus limiting accumulation in liver tissue. After exposure, liver enzymes (e.g. cytochrome P450) metabolize PAHs to more water-soluble metabolites for elimination via the bile. If the level of exposure to PAHs overwhelms enzymatic capacity to metabolize and eliminate, the compounds will accumulate in the liver and other lipid-rich extrahepatic tissues (Meador et al. 1995). The lack of a clear increasing trend in TPAH46 concentration in liver tissue implies that tilefish have heretofore been able to efficiently metabolize and eliminate PAHs. However, although tilefish appear to be keeping up with this metabolic demand, the energetic cost to the individual may result in other negative health impacts, perhaps as manifested in decreased condition factor or other health indices (Figures 2–4).

Fulton’s condition factor (K) is a length-weight based metric commonly used as a proxy for fish health at individual and population levels, with higher values (heavier fish at a specific length) signifying more robust fish (Blackwell et al. 2000). Higher K values thus imply favorable environmental conditions, including suitable water quality, habitat, and prey availability. Condition factor can be used as a measure of a fish’s energy reserves, and a basic biomarker of effect of contaminants (Chellappa et al. 1995; Lambart and Dutil 1997; van der Oost, Beyer, and Vermeulen 2003).

Fish condition factor may vary by sex due to differences in sex-specific physiology and energy allocation (Blackwell et al. 2000). For tilefish, K varied by sex, with males having significantly higher mean K values compared with females and to fish of unknown sex. This result is logical in postspawning fishes, because females will lose greater mass from spawning due to both higher biomass of eggs compared with sperm and the substantially higher energetic burden of reproduction on females compared with males (Blackwell et al. 2000; Hayward and Gillooly 2011). The energetic cost of gamete biomass production is estimated to be approximately 3.5 orders of magnitude higher in females as compared to males (300 vs 0.1% of energy for basal metabolism used for gamete biomass production; Hayward and Gillooly 2011).

For all individuals combined, there was a statistically significant 22% decrease in K and a 38% decrease in total body weight in Gulf of Mexico tilefish between 2012 and 2017 (Figure 2, Table 1). With no significant change in total length over time, the decrease in K is due to a decrease in fish total weight at a given length. A log-transformed regression of total length versus total weight by year calculated that an
average-sized tilefish caught in our survey (total length = 65 cm) weighed 21% less in 2017 (2.709 kg) than in 2012 (3.427 kg). Condition factor varied significantly over time for both male and female tilefish (Figure 2). The mean K for males exhibited an initial increase 2013 to 2014, decreased from 2014 to 2015, and rebounded to 2013 values in 2017. In contrast, female tilefish exhibited a strong decline (22%) in K over the entire present study period. Variation in K over time may be related to reproductive state, season, water temperature, nutritional status/prey availability, and/or poor environmental conditions such as exposure to contaminants (Blackwell et al. 2000; Ratz and Lloret 2003).

Although reproductive state in these fish was not specifically assessed, all fish were sampled in late July to August. The spawning season for tilefish in this region is generally January to June, based on histological determination of spawning-capable females; therefore, all fish caught in the present study are likely to be in a postspawning state (Lombardi-Carlson 2012). Female fish typically exhibit a drop in condition factor immediately postspawning, with condition factor increasing steadily thereafter (Blackwell et al. 2000). Thus, condition factors should be recovering by our sampling dates in late July to August. Because our sampling periods were in a tight window, it is unlikely that the significant decrease in K over the present study period was due to sampling the spawning cycle at different times.

Average bottom temperature at each station, as recorded by sensors on each longline set, was not associated with mean K ($R^2 = 0.032, p = 0.306$). Bottom temperature at occupied stations remained relatively stable over the time series, with a mean temperature of 12.4 ± 1.86 °C.

Nutritional status of the sampled fish was not evaluated, and is unknown. However, the percentage of liver lipid (measured in the present study) is generally indicative of nutrition status and energy reserves. Percentage of liver lipid and K both declined significantly from 2012 to 2017 (53 and 22%, respectively). Condition factor and percentage of liver lipid were significantly correlated ($r = 0.297, p = 0.001$). The significant and concordant decreases over time in K and the percentage of liver lipid indicates a simultaneous decrease in overall condition factor and energy reserves of these Gulf of Mexico tilefish.

The 22% declines in K were significantly negatively correlated with total biliary PAH metabolite concentrations for all individuals combined, and for females (Figure 8). The increasing and chronic exposure to PAHs and the energetic burden of their biotransformation are likely related to the significant decrease in condition factor of tilefish, specifically adult females. Numerous studies reviewed in Collier et al. (2014) found reduced K (9–21%) in fishes exposed to PAHs and other environmental contaminants in both field and laboratory-based studies. Conversely, a variety of other studies reviewed in van der Oost, Beyer, and Vermeulen (2003) found no significant change in K with exposure to contaminants; however, the majority of these studies were short-duration exposure studies, with either single injections or short exposure periods (days) compared with our study, which included over 6 yr of adult fish chronically exposed to PAHs. For adult fish, it is expected that a significant change in K would occur over a time period longer than the standard time of laboratory-exposure experiments.

Multiple studies have shown a significant energetic cost of biotransformation of xenobiotics, although the physiological link between xenobiotic exposure and K is currently unknown. Exposure to and detoxification of PAHs is associated with increased metabolic demand in fish, with studies concluding that the increased demand was due to the associated significant energetic costs of xenobiotic metabolism (Bains and Kennedy 2004; Alves dos Santos et al. 2006; Klinger et al. 2015). Bains and Kennedy (2004) note that the energetic cost of PAH metabolism may be even higher than that of other xenobiotics, because most PAHs undergo both phase I and phase II metabolism, which require separate enzyme molecules and reactions. Quantitative research on the cost of xenobiotic metabolism has more frequently been performed with terrestrial organisms and plant secondary metabolites, although studies often fail to differentiate the direct cost of xenobiotic detoxification from the cost of toxicological impact of the xenobiotic, such as feeding inhibition or reduced digestive efficiency, which together limit total energy assimilation. The energetic costs to ruffed grouse (Bonasa umbellus) of detoxifying plant secondary metabolites in quaking aspen tree buds was found to be 10 to 14% of metabolizable energy intake, which was enough to alter foraging behavior (Guglielmo et al. 1996).

Previous studies and our data indicate that xenobiotic metabolism, particularly of PAHs, significantly increases energetic costs to fishes, often resulting in decreased body condition and energy reserves. Energy that would otherwise be used for fitness (growth and reproduction) is likely being diverted to enzyme induction and function, and to repair cellular damage caused by xenobiotics. Studies have shown that this reallocation of energy occurs within a hierarchy, where the costs of physiological maintenance are paid first, followed by those of growth and reproduction (Beyers et al. 1999).

It is likely that the declines in both condition factor and stored lipids occurring for Gulf of Mexico tilefish chronically exposed to PAHs may impact reproductive capacity. Stored energy is required for quality gamete production. Female fish with lower condition factors generally have lower fecundity, lower egg quality, atresia of oocytes, and lower larval quality and survivorship; they may also mature at a later age and possibly even skip spawning (Hislop et al. 1978; Demartini 1991; Solemdal et al. 1993; Koslow et al. 1995; Chambers and Waatwood 1996; Kjesbu et al. 1998; Marteinsdottir and Steinarsson 1998; Rideout et al. 2000; Morgan 2004). Reduced condition factor of tilefish may also have implications at the population level. Fish stocks in poorer condition generally have slower growth and lower recruitment with concomitant lower production, and natural mortality may be higher (Marshall and Frank 1999; Dutil and Lambert 2000; Ratz and Lloret 2003). An analysis of ten Atlantic cod (Gadus morhua) stocks in the north Atlantic revealed that individuals in poorer condition had lower mean weight at age and lower recruitment potential at low spawning stock biomass, and were less likely to be able to...
sustain long-term fishery exploitation (Ratz and Lloret 2002). The weight of an average-length tilefish from our survey decreased 21% from 2012 (3.427 kg) to 2017 (2.709 kg). This translates into lower interannual production of the mass of the population and lower overall egg production.

CONCLUSION

Our 6-yr of study of Gulf of Mexico tilefish post Deepwater Horizon indicated chronic and relatively high PAH exposure and associated negative health effects that may have serious consequences for long-term population viability. Exposure to low molecular weight (often deemed “petrogenic”) PAHs increased 2.8-fold from 2012 to 2017. Fulton’s condition factor (K) for tilefish, predominately adult females, declined by 22%. The decline in condition was correlated with the 178% increase in PAH exposure and the 53% decrease in the percentage of liver lipid over the present study period. Tilefish appear to be efficiently metabolizing and eliminating PAHs due to lack of increase in hepatic PAH concentrations; however, the energetic cost of chronic PAH metabolism may be related to the decrease in condition factor and liver lipid reserves. Chronic pollution, particularly exposure to PAHs, is having ongoing effects on health indices in tilefish, and ultimately effects on fecundity, stock productivity, and fitness may occur. This time series confirms the need for continued monitoring of the health of Gulf of Mexico organisms chronically exposed to contaminants. Additional analyses on temporal and spatial variability in species-specific physiology (e.g., xenobiotic metabolism capability), nutritional status, and other baseline health indices will aid in interpretation of these studies and future evaluations of Gulf of Mexico fish health as it relates to chronic pollution.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4583.

Acknowledgment—The present study was supported by a grant from The Gulf of Mexico Research Initiative through its Center for Integrated Modeling and Analysis of the Gulf Ecosystem (C-IMAGE I No. SA 12-10; C-IMAGE II No. SA 15-16; C-IMAGE III No. SA 18-16). We would like to express our appreciation to Agilent Technologies (LSAC and Research Support Programs) for providing instrumentation for the present study. Sampling was performed in accordance with Protocol ISO0000515 approved by the Institutional Animal Care and Use Committee at the University of South Florida. We also thank the captain and crew of the RV Weatherbird II, the field team (E. Herdter, K. Deak, S. O’Leary, S. Gilbert, A. Wallace, J. Ortega Ortiz, D. Portnoy), M. Campbell and L. Brandenburg for laboratory assistance, and G. Ylitalo and B. Anulacion for analytical guidance.

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/n7-g27a-x012; doi 10.7266/N7X3W1J).

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