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Chronic PAH exposures and associated declines in fish health indices observed for ten grouper species in the Gulf of Mexico

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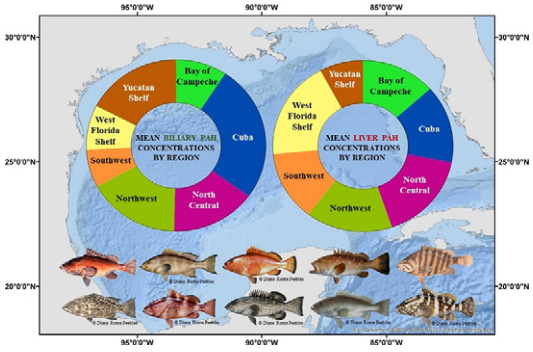
HIGHLIGHTS

- Ten grouper species from the Gulf of Mexico were analyzed for PAHs.
- Spatial, temporal and species differences were detected in PAH levels and profiles.
- Fish health metrics declined up to 65% during the first three years following DWH.
- Liver concentrations of PAHs in Yellowedge Grouper increased (>800%) over time.
- Increasing exposures and concentrations suggest grouper are chronically exposed.

GRAPHICAL ABSTRACT



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ABSTRACT

Ten grouper species grouper ($n = 584$) were collected throughout the Gulf of Mexico (GoM) from 2011 through 2017 to provide information on hepatobiliary polycyclic aromatic hydrocarbon (PAH) concentrations in the aftermath of the *Deepwater Horizon* (DWH) oil spill. Liver and bile samples were analyzed for PAHs and their metabolites using triple quadrupole mass spectrometry (GC/MS/MS) and high-performance liquid chromatography with fluorescence detection (HPLC-F), respectively. Data were compared among species and sub-regions of the GoM to understand spatiotemporal exposure dynamics in these economically and ecologically important species. Significant differences in the composition and concentrations of PAHs were detected spatially, over time and by species. The West Florida Shelf, Cuba coast and the Yucatan Shelf had a greater proportion of the pyrogenic PAHs in their livers than the other regions likely due to non-oil industry related sources (e.g., marine vessel traffic) in the regional composition profiles. Mean liver PAH concentrations were highest in the north central region of the GoM where DWH occurred. Biliary PAH concentrations and health indicator biometrics initially decrease during the first three years following the DWH oil spill but significantly increased thereafter. Increased exposures are likely explained by the resuspension of residual DWH oil as well as continued inputs from natural (e.g., seeps) sources and other anthropogenically derived sources (e.g., riverine runoff, other oil spills, and leaking oil and gas infrastructure). The increasing trend in PAH concentrations in the bile and liver of grouper species in the north central region of the GoM post-DWH suggest continued chronic exposures, however the critical stage at which permanent, irreparable damage may occur is unknown. Long-term monitoring of PAH levels and

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associated fish health biomarkers is necessary to evaluate impacts of chronic exposures, particularly in regions subject to intensive oil extraction activities.

1. Introduction

In the United States (US), the Gulf of Mexico (GoM) supports both high levels of fish catch (65% of the continental US fish supply) and marine-derived oil and gas (39% of the US supply) (Chen, 2017; Fautin et al., 2010; NMFS, 2018; NOAA, 2018). Maintaining the health and productivity of ecosystem resources while balancing potential conflicts among human extraction activities requires detailed information concerning the interactions and impacts of oil and gas development on fisheries.

The GoM ecosystem is chronically exposed to oil emanating from both natural and anthropogenic sources, including natural oil seeps, oil and gas activities, atmospheric deposition, marine vessel traffic, and land-based run-off (NRC, 2003). Chronic, small-scale oil spills and two mega-spills (Ixtoc 1 and *Deepwater Horizon*, DWH) have resulted in significant oil residues in the GoM (Lubchenco et al., 2012; Waldichuk, 1980). While infrequent, large oil spills have an important impact on the oil budget in the GoM. For example, the DWH and Ixtoc 1 spill contributed over seven and four times, respectively, of the estimated annual hydrocarbon inputs to the GoM (Murawski et al., 2014). Understanding the temporal and spatial effects of both chronic and acute oil exposures is thus an important aspect of understanding the potential impacts on fish productivity within the GoM and elsewhere where the two extractive industries interact.

Polycyclic aromatic hydrocarbons (PAHs) are a minor (0.2 to 7%), yet highly toxic component of crude oil (Council, 2003), and have been previously associated with a well-documented range of both lethal and sublethal effects in fishes and the wider marine ecosystem (Beyer et al., 2016; Collier et al., 2013; Murawski et al., 2016; Pulster et al., 2020a). Effects of PAH exposure observed in fishes following the DWH incident and subsequent exposure studies included skin lesions, cardiotoxicity, reproductive impairment and mortality (Brown-Peterson et al., 2015; Hedgpeth and Griffitt, 2016; Incardona et al., 2014; Murawski et al., 2014).

While the impacts of singular exposure events have been well documented, the effects of simultaneous repeated acute and chronic oil exposures on aquatic organisms are not well understood. However, human and animal epidemiological studies provide evidence of induced persistent cognitive deficits, gene alterations, oxidative damage, antioxidant enzyme changes, and apoptotic cell death in various organs as the result of simultaneous chronic and acute exposures to other persistent organic chemicals (Moyano et al., 2017; Nasr et al., 2016; Samsam et al., 2005). Uncertainties regarding the permanence of impairment in fish following prolonged and repeated sublethal, chronic and concurrent acute exposures to PAHs in fish underscore the need for baseline data and continued health monitoring in the GoM and elsewhere where persistent oil extraction activities occur.

Prior to DWH, baseline data on the levels of PAHs in GoM fishes were scarce, thus hindering health assessments (Kennicutt et al., 1988; Murawski and Hogarth, 2013). Limited PAH data in fish existed for the northern GoM prior to 2010. Likewise, some PAH monitoring data for crustaceans and fishes from the southern GoM were available in internal reports from the Mexican oil company (Petróleos Mexicanos, PEMEX) (Gracia, 2010). A few studies in the 1980s and 1990s analyzed PAHs in bile and muscle from mostly coastal species in the northwestern GoM (McDonald et al., 1996; NCCOS, 2019), but there are no comprehensive baselines for any fish species.

Groupers are a key group of economically and recreationally important species in the GoM, contributing to the $912 million total US landings revenue in 2016 for the GoM (NMFS, 2018). They are equally as important to commercial, recreational and subsistence fisheries in México and Cuba. The grouper fishery in México is multi-specific with Red and Black Groupers being the most important (DOF, 2014). The Yucatan Shelf area is the main fishery ground contributing 85% of the Mexican GoM landings (SAGARPA, 2013). This group of demersal species is found throughout the GoM along hard or rocky bottom structures, occupying reefs, caves and ledges at depths of 20 to c.a. 200 m (Chen, 2017; Murawski, 2018). Exposure of grouper species to PAHs induced significant hepatic morphological alterations, ethoxyresorufin *O*-deethylase (EROD) activity, cytological changes, metabolic inhibition, hepatocellular oxidative stress, possible reproductive impairment, and potential initiation of mutagenic and carcinogenic processes (Au et al., 2004; de Campos et al., 2018; Derakhshesh et al., 2019; Salvo et al., 2016).

Considering the economic importance of groupers, the goal of this study is to provide baseline concentrations for hepatobiliary PAH exposure across regions and species and to conduct time series surveillance to understand temporal changes in oil exposures for ten of the most valuable species of groupers occurring in the GoM following DWH. The ten analyzed species included were in the genera *Epinephelus*, *Hyporthodus*, and *Mycteroperca*.

2. Materials and methods

2.1. Field collections

Between 2011 and 2017, groupers specimens ($n = 584$) were collected from 104 locations during May through September using demersal longline sampling gear along transects throughout the GoM (Fig. 1). The GoM was separated into seven zones based on multivariate analysis of species composition and proximity to oil infrastructure (Murawski, 2018). The zones were designated as the north central (NC), West Florida Shelf (WFS), Cuba (CUB), Yucatan Shelf (YS), Bay of Campeche (BC), southwest (SW), and northwest (NW) regions. The demersal longline sampling design and protocols have been previously described in detail (Murawski et al., 2014; Murawski et al., 2018). Briefly, sampling efforts consisted of longline sets (5 nautical miles) deployed along transects extending from shallow (40 m) to deep continental shelf areas (300 m). At each station, leader lines with an average of 450 - #13/0 circle hooks were baited with cut Atlantic Mackerel *Scomber scombrus* and Humboldt squid wings *Dosidicus gigas*. Sensors (Star-Oddi DST Centi-TD®, Gardabaer, Iceland) were attached to the ends of the mainline during each deployment station to collect time, temperature, and depth data.

The ten grouper species included in this study were Black *Mycteroperca bonaci*, Gag *Mycteroperca microlepis*, Misty *Hyporthodus mystacinus*, Nassau *Epinephelus striatus*, Red *Epinephelus morio*, Red Hind *Epinephelus guttatus*, Snowy *Epinephelus niveatus*, Warsaw *Epinephelus nigritus*, Yellowedge *Hyporthodus flavolimbatus* and Yellowmouth *Mycteroperca interstitialis*. At retrieval, species identification and biometric data (lengths, weights, gross abnormalities) were recorded and a subsample of fish dissected and sampled for a variety of tissues, including blood, otoliths, eyes, muscle, liver, and bile. Sexes were identified macroscopically in the field. If fish were not macroscopically identifiable in the field or if the gonads were too small to identify (i.e., immature) they were labeled as "unsexed". Full gall bladders were dissected and drained into combusted amber vials. All sample matrices

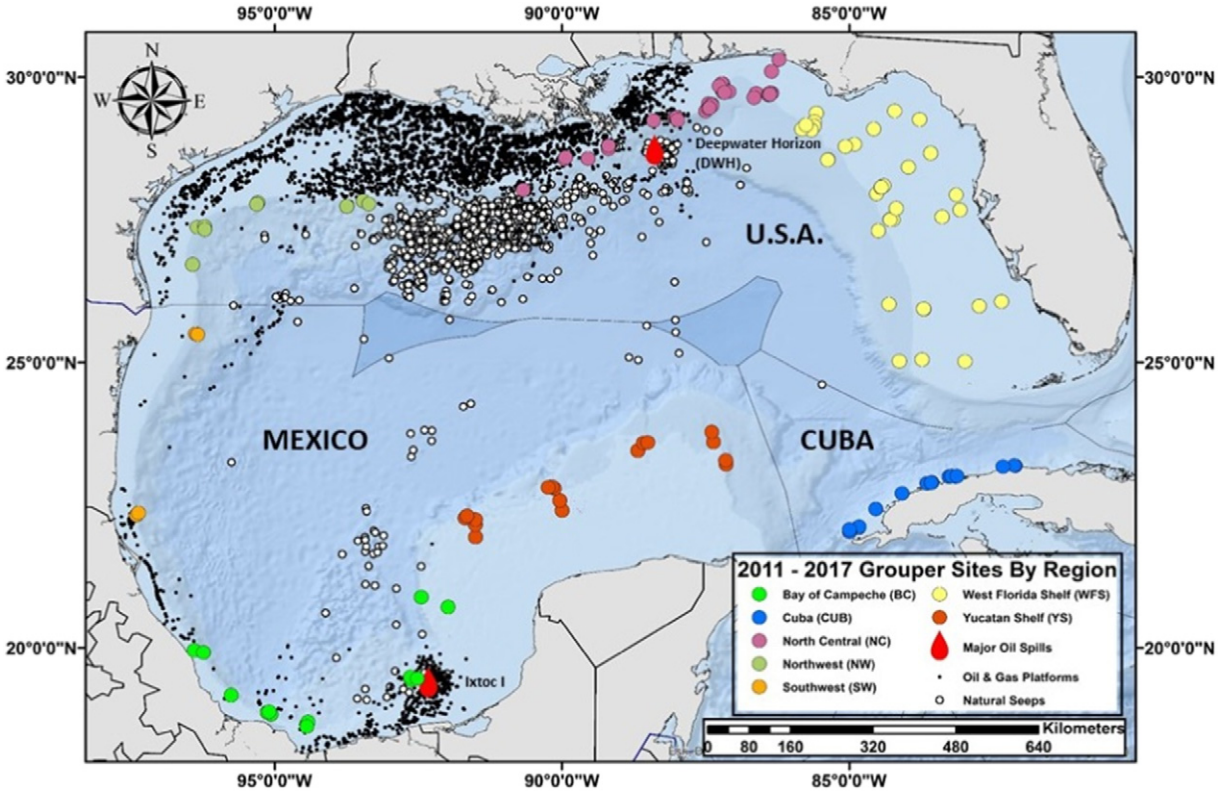

Fig. 1. The 2011–2017 site locations where ten species of grouper ($n = 584$) were collected for PAH analysis.

were frozen immediately (−20 °C) for transport to the laboratory for further analysis.

2.2. Biliary PAH analysis

Untreated bile samples (3 μL) from 337 groupers were analyzed for naphthalene, phenanthrene and benzo[*a*] pyrene metabolite equivalents using high performance liquid chromatography fluorescence detection (HPLC-F). PAH equivalents were separated using a C18 reverse-phase column (Synergi™ 4 μm Hydro-RP 80 Å, Phenomenex, Torrence, CA) set at 50 °C with a 1.0 mL min^{-1} gradient starting with 100% water with 0.5% Acetic Acid to 100% methanol (Optima LC/MS Grade). Excitation/emission wavelength pair for naphthalene (292/335 nm), phenanthrene (260/380 nm) and benzo[*a*] pyrene (380/430 nm) metabolite equivalents were used to measure fluorescence response. All peaks within the elution time window of 5 to 19 min were integrated and summed for each PAH metabolite equivalent. The fluorescence responses were converted to PAH metabolite equivalents, (e.g., fluorescent aromatic compounds, ng FAC g^{-1} bile) using naphthalene (2.5 μg mL^{-1}) phenanthrene (1 μg mL^{-1}) and benzo[*a*]pyrene (250 ng mL^{-1}) external standards via:

$$\textit{Biliary FACs} = \frac{\textit{Standard concentration}}{\textit{Mean Standard Area}} \times \frac{\textit{integrated sample area}}{\textit{bile density}} \times \frac{\textit{volume of standard injected}\ (10\,\mu L)}{\textit{volume of sample injected}\ (3\,\mu L)}$$

where bile density is 1.03 g mL^{-1} (Krahn et al., 1986).

2.3. Hepatic PAH analysis

Homogenized liver tissues ($n = 507$) were prepared for analysis using species-specific modification of the QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction and clean-up methodology (Bond Elut, Agilent Technologies, Santa Clara, CA, USA). A 2-gram aliquot of homogenized liver was spiked with a surrogate standard solution containing 19 deuterated PAHs (16 EPA PAHs plus dibenzothiophene, benzo[*e*]pyrene and perylene), vortexed for 30 s and then allowed to marinate for 10 min. Post-marination, 20 mL of acetonitrile (ACN; Optima, Fisher Chemical) and two steel homogenizing beads were added and shaken at 1000 rpm using a 1600 MiniG® automated tissue homogenizer and cell lyser (SPEX® Sample Prep, Metuchen, NJ). After 10 min of shaking, acetonitrile extracts were centrifuged at 5000 rpm for 5 min. Acetonitrile extracts (8 mL) were transferred to a Bond Elut Enhanced Matrix Removal-Lipid (EMR-Lipid) dispersive solid phase extraction tube followed by an additional shake (5 min) and centrifugation step (5 min). The extract was decanted, mixed with anhydrous magnesium sulfate (Bond Elut EMR-Lipid polish pouch) for water removal and centrifuged.

Prior to analysis, final extracts (1 mL) were spiked with an internal standard (*p*-terphenyl-d14). Extracts (2 μL) were injected in splitless mode using a two-layer sandwich with 0.2 μL of analyte protectant (20 mg mL^{-1} L-gulonolactone and 10 mg mL^{-1} D-sorbitol composite solution in ACN). Target analytes, consisting of 46 parental PAHs and their associated alkylated homologs ($TPAH_{46}$) were confirmed and quantified using gas chromatography (7890B Gas Chromatograph System, Agilent Technologies) and triple quadrupole mass spectrometry (7010 Triple Quadrupole- GC/MS/MS, Agilent Technologies) operating in multiple reaction monitoring (MRM) and full scan modes. Oven and acquisition parameters are available in the supplementary information (Table S1).

2.4. Quality assurance and quality control (QA/QC)

A QA/QC program was implemented to ensure data conformed to recommended NOAA and EPA performance guidelines (Johnson et al., 2009; Krahn et al., 1986; USEPA, 1984). The QA/QC measures for HPLC-F biliary PAH analysis included analytical standards, calibration curves, continuing calibration standards and interlaboratory calibration exercises previously described in depth (Snyder et al., 2019; Snyder

et al., 2015). Field samples were analyzed in triplicate or until QA/QC standards were met (CV < 20%) with methanol blanks between each field sample. Biliary PAHs are reported as the sum of all three PAH metabolite equivalents ($TPAH_3$) rounded to two significant figures in ng FAC g^{-1} bile.

The QA/QC measures for GC/MS/MS PAH tissue analysis followed NOAA and EPA guidelines and included analytical standards, calibration curves, method blanks, matrix spikes, standard reference material and matrix matched standards (NOAA, 2012; USEPA, 2018). Prior to sample analysis, GC/MS/MS acquisition parameters were optimized using analytical standards and British Petroleum (BP) surrogate crude oil. Solvent based (0.75 to 1000 ng mL^{-1}) and matrix matched (1 to 1000 ng mL^{-1}) calibration curves spanning three orders of magnitude verified the linearity of all parental PAHs. The method detection limit (MDL) is defined as the lowest concentration matrix match standard (MMS) analyzed (1 ng mL^{-1}) where all compounds were detected. A matrix match standard (MMS) containing all analytes (both deuterated and non-deuterated) was made and analyzed with each batch of samples to quantify surrogate recoveries and calculate relative response factors. Target analyte concentrations were quantified using the MMS relative response factors and confirmed by matching spectra, retention times and relative intensity ratios of the selected ions with matrix matched standards. *ortho*-Terphenyl (OTP 100 ng mL^{-1}) was added to each sample prior to injection to monitor instrument stability. Acceptable QA criteria were met for the surrogate standard recoveries ($TPAH_{46}$: 82 ± 12%), matrix spikes ($TPAH_{46}$: 88 ± 12%), procedural blanks ($TPAH_{46}$: 77 ± 16%), solvent blanks and internal standard monitoring.

Nonparametric Spearman's rank-order correlations were calculated using log transformed liver lipids as a predictor variable and the log transformed wet weight (w.w.) and lipid weight (l.w.) PAH concentrations as the response variables. Weak to moderate negative associations were found between wet ($\rho = -0.34$, $p < 0.0001$) and lipid ($\rho = -0.63$, $p < 0.0001$) weight concentrations with liver lipids, respectively (Fig. S1). Therefore, only wet weight concentrations are reported due to the weak to moderate relationships observed between PAHs and lipids combined with the fact that only about a third of the specimens had sufficient liver mass for both PAH and lipid analysis. Liver PAHs are reported as the sum of 46 PAH analytes ($TPAH_{46}$) rounded to three significant figures in ng g^{-1} (w.w.). The 2–3 ring and 4–6 ring PAHs and alkylated homologs were summed as the low molecular weight (LMW) and high molecular weight (HMW) PAHs, respectively.

2.5. Analysis of livers for total lipid fraction

A modified Folch method was used to extract and measure total lipids in liver tissues (Matyash et al., 2008). Briefly, 1.5 mL of methanol (Optima LC/MS Grade) and a 200 mg aliquot of homogenized liver tissue were added to a glass test tube and vortexed. Methyl tert-butyl ether (MTBE) was then added to the test tube and allowed to incubate for 1 h at room temperature on a pulsing vortex mixer. After 1 h, 1.25 mL of laboratory-grade water was added and centrifuged at 1000 rpm for 10 min. The organic phase was then transferred to a clean scintillation vial and the tissue extract tube was re-extracted using 2 mL of MTBE/methanol/water (10/3/2.5 v/v/v) and centrifuged at 1000 rpm for an additional 10 min. Organic phases were combined and allowed to evaporate to dryness. Total lipids were determined gravimetrically and reported as total liver lipids (g).

2.6. Proxy fish health indices

Hepato-somatic (HSI), gonadal-somatic (GoSI), gastrointestinal-somatic (GSI) indices were calculated via:

$$HSI, GoSI\ or\ GSI = \frac{organ\ weight\ (\mathrm{g})}{total\ weight\ (\mathrm{g})}$$

where organ weight is the weight of the liver (HSI), gonads (GoSI) or gastrointestinal tract (GSI) for each individual fish.

Fulton's condition factor (K) was calculated as:

$$K = 100 \times \frac{total\ weight\ (\mathrm{g})}{standard\ length\ (\mathrm{cm})^3}$$

where 100 is a scaling factor in order to bring K close to unity.

Length-weight relationships (LWR) were calculated as:

$$Total\ Fish\ Weight\ (\mathrm{g}) = a \times SL^b$$

where a is the power function coefficient or regression intercept, b is the regression slope and SL is the fish standard length (cm).

2.7. Statistical analyses

All statistical analysis was performed in JMP Pro 14.3 (SAS Institute, Inc.). All non-detects and levels below the method detection limit (MDL 1 ng mL^{-1}) were replaced with MDL/2 prior to statistical analysis. Nonparametric statistical analyses were performed on raw data due to the unequal variances and sample sizes among sampling regions. Hypothesis tests were used with a criterion of significant differences at $\alpha \leq 0.05$. Nonparametric Kruskal-Wallis ANOVA, post-hoc Games-Howell Honestly Significant Difference (HSD) and Wilcoxon's methods were used for multiple and pairwise comparisons on these data. Games-Howell HSD nonparametric approach for multiple comparisons was used to test the differences in means of specific variables (i.e., PAHs by year, region, species). This approach was designed for unequal variances and sample sizes and is based on Welch's correction to *df* with the *t*-test and uses the studentized range statistic (Games and Howell, 1976). Nonparametric Wilcoxon test was used for pairwise comparisons (e.g., males versus females). To evaluate differences between habitats, grouper were divided into complexes: shallow-water species (Black, Gag, Red Grouper, Red Hind, Yellowmouth) and deep-water species (Snowy, Yellowedge, Warsaw, Nassau, Misty) (Chen, 2017).

Discriminant function analysis using the linear, common covariance method was used to analyze differences in covariates (e.g., PAH composition and concentrations) by region and species. The relative abundances of the PAH composition profiles measured in livers were first normalized by region and species. The normalized relative abundance profiles were then used as the covariates to evaluate Gulf-wide regional and species differences, respectively. To eliminate the confounding factor of regional differences in sample sizes, species differences were only analyzed in the north central region. A multivariate nonparametric MANOVA Hotelling-Lawley procedure was used to test the significance of the canonical details. Principal components (PCA) on correlations and factor analysis using principal component varimax rotation was used to evaluate data structure and relationships between variable and factors contributing to the regional and species differences using Chi-square goodness-of-fit. The factors assign each variable a factor loading to determine the most representative indicator PAHs. The level of significance is normally determined by the magnitude of the sample size and the relationship between the loadings yet should be at least >0.70 (Comrey and Lee, 1992; Yong and Pearce, 2013). For increased stringency and robustness, values > 0.80 were selected as significant in this study.

3. Results

3.1. Inter-regional variations

3.1.1. Biometrics

The mean biometrics for each species analyzed by region in this study are given in Tables 1 and S2. The number of grouper species analyzed per region varied between two (NW and SW) to seven (YS). Mean

Table 1
Regional means (standard deviation) for sex ratios (F = female, M= male, U = unsexed) and biometrics for each grouper species collected in the Gulf of Mexico. Sample sizes are indicated by *n*.

Region	Species	Sex ratio (F:M:U)	*n*	Standard Length (cm)		Total weight (kg)		Liver weight (kg)		Liver lipids (g)	
Bay of Campeche (BC)			***59***	***45***	***(14)***	***2.9***	***(2.6)***	***0.03***	***(0.04)***	***0.61***	***(0.23)***
	Red	1:0:0	1	40		1.8		0.03		0.38	(0.38)
	Red Hind	5:0:0	5	**34**	(0.84)	1.3	(0.32)	0.009	(0.005)	0.71	(0.14)
	Snowy	9:0:4	13	46	(13)	2.9	(18)	0.03	(0.03)	0.76	(0.14)
	Yellowedge	31:0:4	35	48	(15)	3.4	(3.0)	0.04	(0.05)	0.60	(0.24)
	Yellowmouth	5:0:0	5	38	(4.4)	1.1	(0.17)	0.008	(0.001)	0.38	(0.13)
Cuba (CUB)			***90***	***36***	***(15)***	***1.8***	***(3.3)***	***0.01***	***(0.02)***	***0.34***	***(0.09)***
	Black	1:1:0	2	80	(29)	12	(11)	0.06	(0.02)	0.32	(0.02)
	Misty	3:0:0	3	62	(18)	6.5	(3.6)	0.04	(0.02)	0.43	(0.16)
	Nassau	6:0:0	6	53	(17)	4.5	(3.5)	0.05	(0.05)	0.34	(0.07)
	Red	4:0:1	5	49	(7.5)	3.0	(1.7)	0.03	(0.1)	0.34	(0.11)
	Red Hind	61:2:10	73	31	(3.8)	0.75	(0.29)	0.006	(0.004)	0.33	(0.09)
	Snowy	1:0:0	1	105		20		0.13	(0.13)	0.33	(0.33)
North Central (NC)			***133***	***64***	***(19)***	***7.3***	***(5.9)***	***0.07***	***(0.07)***	***0.44***	***(0.21)***
	Gag	8:0:0	8	82	(23)	11	(7.0)	0.11	(0.11)	0.43	(0.22)
	Red	24:1:3	28	52	(9.1)	3.7	(2.3)	0.04	(0.07)	0.40	(0.16)
	Snowy	11:0:0	11	72	(23)	8.5	(7.3)	0.08	(0.06)	0.38	(0.11)
	Warsaw	10:0:0	10	83	(21)	13	(7.2)	0.12	(0.07)	0.45	(0.11)
	Yellowedge	70:0:6	76	63	(17)	6.8	(5.4)	0.06	(0.05)	0.46	(0.23)
Northwest (NW)			***21***	***63***	***(20)***	***7.8***	***(8.1)***	***0.09***	***(0.17)***	***0.67***	***(0.21)***
	Yellowedge	17:1:1	19	62	(21)	7.8	(8.5)	0.09	(0.18)	0.70	(0.19)
	Warsaw	2:0:0	2	71	(9.9)	8.1	(3.3)	0.07	(0.02)	0.39	(0.02)
Southwest (SW)			***10***	***64***	***(12)***	***6.7***	***(3.4)***	***0.06***	***(0.03)***	***0.56***	***(0.17)***
	Snowy	1:0:0	1	75		11	(11)	0.07	(0.07)	0.36	(0.36)
	Yellowedge	8:0:1	9	62	(12)	6.2	(3.2)	0.05	(0.03)	0.58	(0.16)
West Florida Shelf (WFS)			***183***	***47***	***(14)***	***3.3***	***(3.5)***	***0.03***	***(0.03)***	***0.51***	***(0.19)***
	Black	1:0:0	1	34		1.01				0.76	(0.76)
	Gag	12:3:2	17	49	(21)	4.2	(5.9)	0.04	(0.05)	0.51	(0.17)
	Red	109:13:8	130	45	(13)	2.9	(2.9)	0.03	(0.03)	0.49	(0.19)
	Snowy	10:1:4	15	52	(14)	4.4	(3.5)	0.05	(0.03)	0.54	(0.22)
	Yellowedge	17:1:2	20	52	(14)	4.2	(4.3)	0.04	(0.06)	0.58	(0.22)
Yucatan Shelf (YS)			***88***	***44***	***(13)***	***2.6***	***(2.6)***	***0.03***	***(0.04)***	***0.53***	***(0.22)***
	Black	1:0:0	1	61		5.6	(5.6)	0.05	(0.05)	0.3	(0.3)
	Misty	0:0:1	1	44		2.9		0.03	(0.03)	N/A	
	Red	6:0:0	6	47	(12)	3.5	(3.5)	0.03	(0.02)	0.50	(0.21)
	Red Hind	9:1:21	31	35	(3.3)	1.2	(0.35)	0.01	(0.01)	0.40	(0.16)
	Snowy	12:0:3	15	49	(16)	3.7	(3.4)	0.05	(0.06)	0.62	(0.15)
	Yellowedge	23:0:1	24	50	(14)	3.7	(3.3)	0.05	(0.05)	0.73	(0.16)
	Yellowmouth	9:0:1	10	42	(7.2)	1.7	(0.76)	0.01	(0.005)	0.36	(0.08)

fish lengths ranged from 44 to 64 cm per region and from 31 to 83 cm per species (Table 1, Fig. S2). There were no significant relationships found using nonparametric Spearman's rank-order correlations between biometric indices (i.e., length, total weight, liver weight, gastrointestinal weight, somatic indices and condition factor) and biliary or liver PAHs for all grouper species combined, individual species or species by region.

3.1.2. Habitat complex

Mean (±SD) biliary PAH concentrations (ng FAC g^{-1} bile) in the deep-water grouper species (46,000 ± 51,000, $n = 171$) were significantly lower ($Z = 2.22$, $p = 0.0267$) than for shallow-water species (60,000 ± 67,000, $n = 166$). Similarly, mean (±SD) liver PAHs (ng g^{-1} w.w.) in the deep-water grouper species (1350 ± 2320, $n = 249$) were significantly lower ($Z = 5.78$, $p < 0.001$) than the shallow water species (1730 ± 2900, $n = 258$).

3.1.3. Regional differences

The main variables of year, region and species significantly affected biliary PAH concentrations among grouper species Gulf-wide (Table S3). We evaluated regional differences in biliary PAHs for 2015 when multiple regions were surveyed. In contrast, liver concentrations of PAHs among groupers were not significantly affected by any of the explanatory variables (Table S3); therefore, grouper species were pooled to evaluate regional differences in liver concentrations of PAHs.

There were a few significant differences between the regions for liver concentrations of PAHs among the ten grouper species combined (Table 2, Fig. 2). Liver concentrations of PAHs (ng g^{-1} w.w.) in groupers collected on the YS were significantly lower than grouper collected in the NC region ($p = 0.001$) and the WFS ($p < 0.001$). All other regions had similar levels of liver PAHs.

To eliminate year and species as confounding factors, regional differences in biliary and liver PAHs for Yellowedge and Red Grouper were evaluated for 2015 only (Fig. 3). Yellowedge Grouper were collected from three regions (BC, NC, YS) and Red Grouper were collected from four regions (BC, NC, WFS, YS) within a one month period (August 24–September 24, 2015). For both of these species, there were no significant differences in biliary PAHs across regions. In contrast, mean concentrations (±SD) of liver PAHs in Yellowedge Grouper from the NC region (4080 ± 3240, $n = 6$) during 2015 were significantly higher than those along the YS (525 ± 581, $n = 20$, $p = 0.040$) and BC (449 ± 439, $n = 12$, $p = 0.037$). Similarly, Red Grouper from the NC region (773 ± 274, $n = 9$) had significantly higher mean concentrations (± SD) of liver PAHs than those along the YS (399 ± 92.9, $n = 5$, $p = 0.009$) in 2015.

Discriminant analysis (Hotelling-Lawley $p < 0.001$) and PCA with factor analysis was able to detect differences in PAH profiles between factor 1 (χ^2 (1021) = 52,610, $p < 0.001$) and 2 (χ^2 (1014) = 42,000, $p < 0.001$) by region (Fig. 4). All regions overlapped, however there was a clear distinction between: (1) the WFS, (2) the YS, BC, and CUB; and (3) the NW and SW regions. The NC region overlapped with all regions. Significant factor 1 variables (>0.80), explaining 41% of the variation, consisted of primarily the 3–5 ring compounds including dibenzothiophene, phenanthrene, fluoranthene/pyrene, and benzo[*a*]

Table 2
Biliary (ng FAC g^{-1} bile) and liver (ng g^{-1} w.w.) concentrations of polycyclic aromatic hydrocarbons (PAH) by region and grouper species collected in the Gulf of Mexico, 2011–2017.

Collection year(s)	Region	Species	Biliary $TPAH_3$			Liver $TPAH_{46}$		
			n	Mean	(SD)	*n*	Mean	(SD)
2015–2016	Bay of Campeche (BC)		51	31,000	(32,000)	57	1390	(2970)
		Red	1	7400	.	1	384	.
		Red Hind	5	15,000	(7900)	5	945	(538)
		Snowy	9	40,000	(35,000)	12	2270	(5060)
		Yellowedge	34	32,000	(34,000)	34	961	(2100)
		Yellowmouth	2	17,000	(12,000)	5	2570	(248)
2017	Cuba (CUB)		67	86,000	(74,000)	60	1500	(2290)
		Black	2	59,000	(570)	2	5010	(2810)
		Misty	2	32,000	(8600)	3	598	(229)
		Nassau	6	140,000	(110,000)	3	1710	(75)
		Red	2	130,000	(75,000)	4	681	(308)
		Red Hind	54	82,000	(72,000)	47	1460	(2430)
		Snowy	1	52,000	.	1	1960	.
2011–2015, 2017	North Central (NC)		83	51,000	(44,000)	112	1710	(2450)
		Gag	2	29,000	(33,000)	7	1390	(676)
		Red	23	40,000	(35,000)	14	770	(341)
		Snowy	4	77,000	(50,000)	11	1370	(884)
		Warsaw	7	150,000	(34,000)	10	1970	(914)
		Yellowedge	47	41,000	(31,000)	70	1950	(3010)
2016	Northwest (NW)		21	56,000	(74,000)	21	1590	(2460)
		Warsaw	2	13,000	(1300)	2	890	(85)
		Yellowedge	19	60,000	(77,000)	19	1660	(2580)
2016	Southwest (SW)		10	24,000	(13,000)	10	1350	(1490)
		Snowy	1	28,000	.	1	482	.
		Yellowedge	9	23,000	(14,000)	9	1440	(1540)
2011, 2013–2015, 2017	West Florida Shelf (WFS)		36	28,000	(20,000)	160	1910	(3400)
		Black	0	.	.	1	1530	.
		Gag	1	21,000	.	16	2410	(3370)
		Red	33	28,000	(20,000)	109	2220	(3830)
		Snowy	1	15,000	.	14	672	(336)
		Yellowedge	1	60,000	.	20	693	(880)
2015	Yucatan Shelf (YS)		69	58,000	(73,000)	87	797	(656)
		Black	0	.	.	1	1370	.
		Misty	0	.	.	1	3500	.
		Red	5	20,000	(26,000)	5	400	(93)
		Red Hind	27	110,000	(95,000)	31	719	(284)
		Snowy	5	50,000	(23,000)	15	735	(638)
		Yellowedge	23	25,500	(29,000)	24	467	(545)
		Yellowmouth	9	20,000	(18,000)	10	1800	(268)

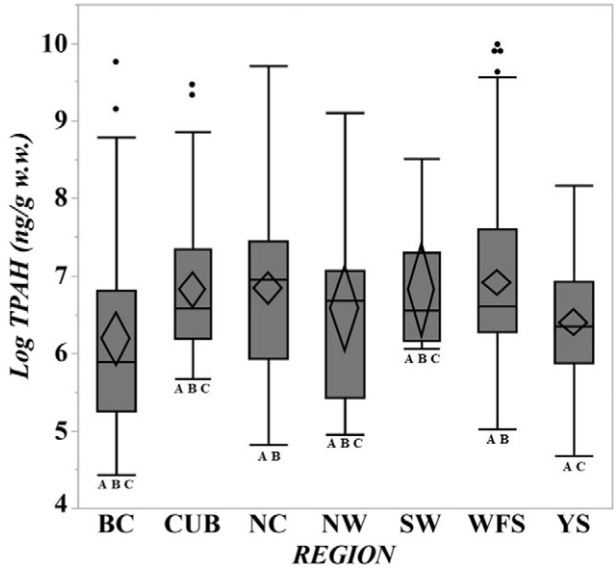

Fig. 2. Regional comparison of liver PAH concentrations in groupers collected in the Gulf of Mexico between 2011 and 2017. The acronyms for regions: BC = Bay of Campeche, CUB = Cuba, NC = north central, NW = northwest, SW = southwest, WFS = West Florida Shelf and YS = Yucatan Shelf. Regions not connected by a letter are statistically different. The line represents the median and the confidence diamond contains the mean and the upper and lower 95% confidence intervals of the mean.

anthracene/chrysene series, and benzo[*e*]pyrene and benzo[*a*]pyrene (Fig. S3). Significant factor 2 variables (>0.80) explaining an additional 19% of the variation, were primarily the result of the naphthalene homologue series and fluorene.

3.2. Intra-regional variations

Multi-year surveys conducted in the NC and WFS regions allowed temporal evaluations and species comparisons for bile and liver PAH concentrations within these regions only.

3.2.1. North central (NC) region

Species, year and sex all had significant main effects on biliary PAH concentrations in the five grouper species collected in the NC region (see Table S3 for parameter effects). Within a particular year, there was only one significant difference in mean (±SD) biliary PAHs (ng FAC g^{-1}) detected between species: in 2017, Warsaw (150,000 ± 34,000, $n = 7$) had significantly higher ($p < 0.001$) biliary PAHs than Yellowedge (61,000 ± 28,000, $n = 15$).

Biliary PAHs from all species were pooled to evaluate temporal trends in the NC region but acknowledge interpretive caution due to the species differences detected in 2017. Mean (±SD) biliary PAHs (ng FAC g^{-1}) in all grouper combined were significantly higher in 2017 (86,000 ± 48,000, $n = 24$) compared to 2012 (50,000 ± 28,000, $n = 11$, $p = 0.0262$), 2013 (19,000 ± 5800, $n = 11$, $p < 0.001$), and 2015 (20,000 ± 11,000, $n = 18$, $p < 0.001$). Mean (±SD) biliary PAHs

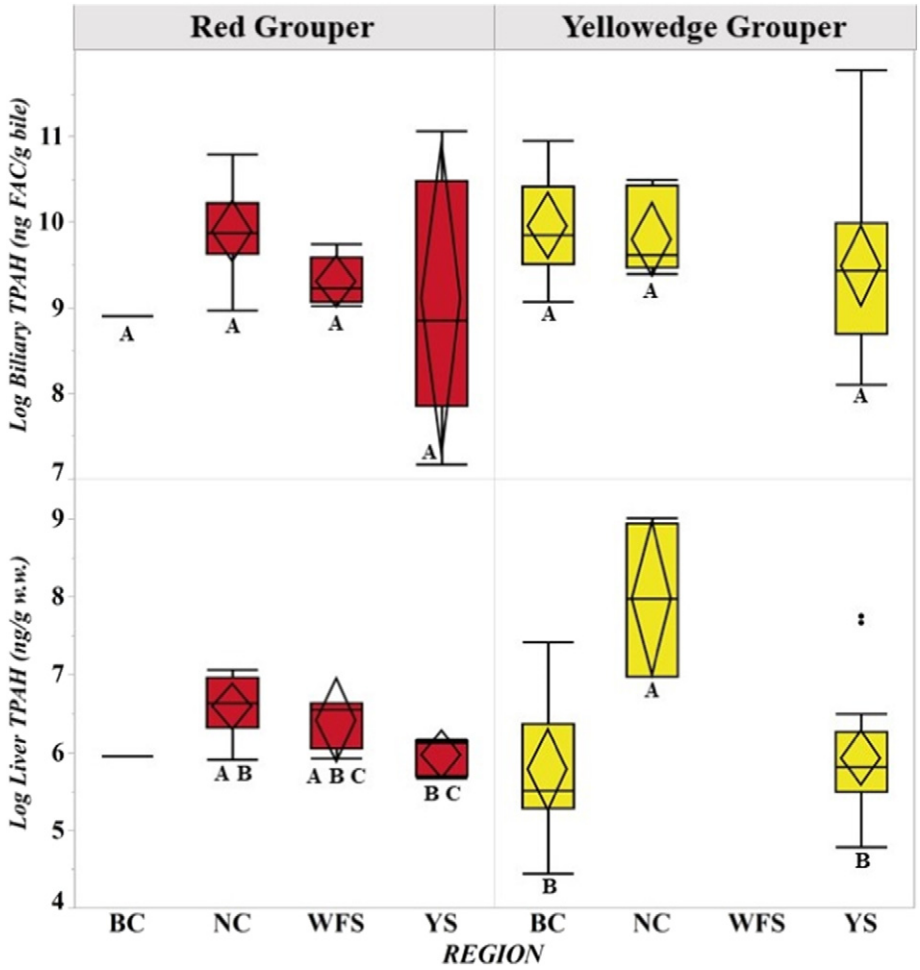

Fig. 3. Regional comparison of mean biliary (ng FAC g^{-1} bile) and liver (ng g^{-1} w.w.) PAH concentrations in 2015 for Red and Yellowedge Grouper collected in the Gulf of Mexico. The acronyms for regions: BC = Bay of Campeche, CUB = Cuba, NC = north central, NW = northwest, SW = southwest, WFS = West Florida Shelf and YS = Yucatan Shelf. Regions not connected by a letter are statistically different. The line represents the median and the confidence diamond contains the mean and the upper and lower 95% confidence intervals of the mean.

(ng FAC g^{-1}) in 2014 (51,000 ± 45,000, $n = 18$) were significantly higher than 2013 ($p = 0.0273$) and 2015 ($p = 0.0458$). Additionally, the 2012 mean (±SD) biliary PAHs (ng FAC g^{-1}) was also significantly higher compared to 2013 ($p = 0.0011$) and 2015 ($p = 0.0025$). In general, biliary PAHs in the NC region declined 85% between 2011 and 2013, followed by a 353% increase by 2017.

In order to eliminate species as a confounding factor, Yellowedge Grouper was selected for temporal analysis (Fig. 5a–b). The mean (± SD) biliary PAH concentrations (ng FAC g^{-1}) for Yellowedge collected in 2011 (130,000, $n = 1$) were nearly an order of magnitude higher than 2013 (18,000 ± 6050, $n = 10$) and 2015 (20,000 ± 11,000, $n = 7$) concentrations. The 2017 biliary PAH concentrations (61,000 ± 28,000, $n = 15$) were significantly higher than 2013 ($p < 0.001$) and 2015 (20,000 ± 11,000, $n = 7$, $p < 0.001$) concentrations for Yellowedge Grouper (Fig. 5a). Overall, biliary PAH concentrations measured in Yellowedge Grouper collected from the NC region decreased 86% between 2011 and 2013, followed by a 239% increase in 2017.

Year was the only significant factor effecting liver PAH concentrations (ng g^{-1} w.w.) in the NC region (Table S3). However, there were no significant differences in liver PAH concentrations between grouper species within a particular year in the NC region; therefore, grouper species were pooled to evaluate temporal trends in liver concentrations. Mean (±SD) liver PAHs in 2011 (582 ± 570, $n = 31$) for all grouper species combined were significantly lower than 2014 (1910 ± 1810, $n = 14$, $p = 0.0250$) and 2017 (2480 ± 3150, $n = 26$, $p = 0.0457$), demonstrating an overall average increase of 326% over time.

Even though interspecies differences were not detected in the NC region, Yellowedge Grouper were selected to evaluate separately for temporal trends in liver PAHs to make certain species is eliminated as a confounding factor. For mean (±SD) liver PAH concentrations (ng g^{-1} w.w.) in Yellowedge Grouper there were no significant differences over time, nevertheless, levels in 2011 (301 ± 193, $n = 19$) were up to 13 times lower than those measured in 2013 (3610 ± 4026, $n = 10$), 2014 (1990 ± 2150, $n = 10$), 2015 (4080 ± 3240, $n = 6$) and 2017 (2790 ± 4100, $n = 15$). In general, concentrations of PAHs in the livers of Yellowedge Grouper collected in the NC region of the GoM increased by a factor of 9 between 2011 and 2017 (Fig. 5b).

In the NC region, there were no significant differences in mean (± SD) biliary PAH concentrations (ng FAC g^{-1} bile) between habitat complex although biliary PAH concentrations in the deep-water (56,000 ± 47,000, $n = 58$) species were almost double that of the shallow-water species (39,000 ± 34,000, $n = 25$, $Z = -1.33$, $p = 0.185$). Likewise, mean (±SD) liver PAH concentrations (ng g^{-1} w.w.) were twice as high as the deep-water groupers (1880 ± 977, $n = 91$) compared to the shallow-water groupers (977 ± 550, $n = 21$, $Z = -0.563$, $p = 0.574$).

Liver PAH profile differences were also detected between species for factor 1 (χ^2 (974) = 13,720, $p < 0.001$) and 2 (χ^2 (973) = 11,170, $p < 0.001$) in the NC region using discriminant analysis (Hotelling-Lawley $p < 0.0001$) and PCA with factor analysis (Fig. 6). There was a clear distinction between Warsaw, Yellowedge and Snowy Grouper, whereas there was some overlap between the Gag and Red Grouper. Significant

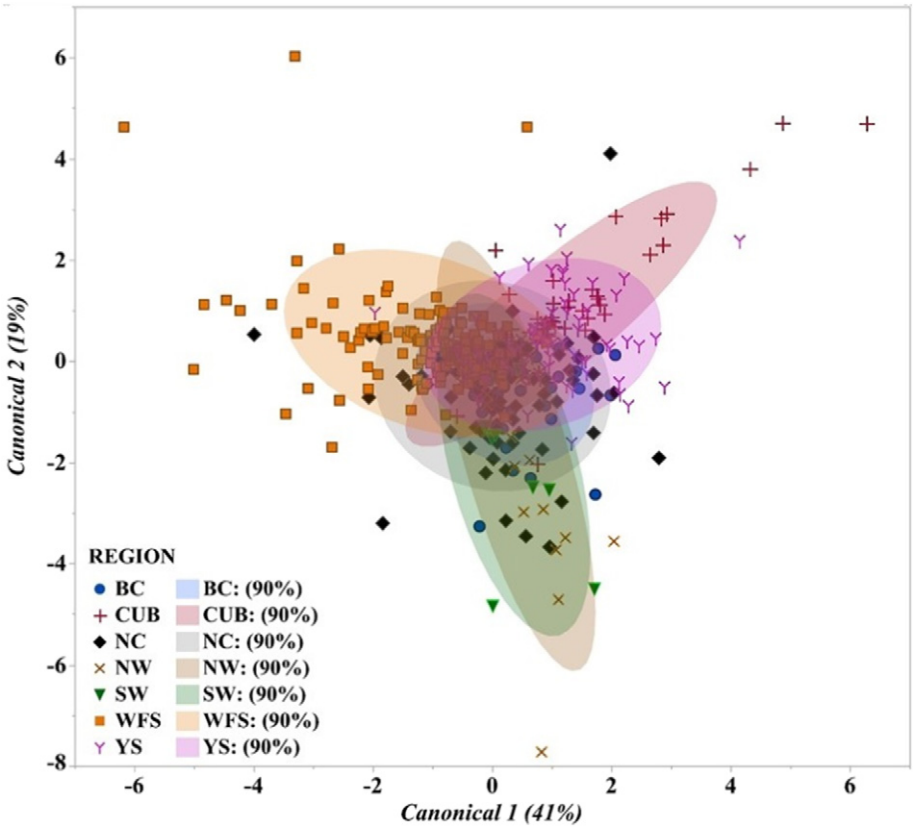

Fig. 4. Discriminant analysis plot illustrating distinct clustering of regions in the Gulf of Mexico by the regional PAH composition profiles (2011–2017). Symbols represent PAHs by regions: BC (Bay of Campeche), CUB (Cuba), NC (north central), NW (northwest), SW (southwest), WFS (West Florida Shelf) and YS (Yucatan Shelf).

factor 1 variables (>0.8), explaining 49% of the variation, consisted of primarily the 3–5 ring compounds such as the dibenzothiophene, phenanthrene, fluoranthene/pyrene, and benzo[*a*]anthracene/chrysene series, as well as benzo[*e*]pyrene and benzo[*a*]pyrene (Fig. S4). Factor 2 variables (0.6 to 0.8) explaining 12% of the data, were primarily the naphthalene homologue series, fluorene and phenanthrene.

For all years, individual years and groupers combined, there were no significant differences observed between sexes for both biliary and liver PAH concentrations in the north central region. This is most likely due to the sex skewness in the dataset and the protandrous hermaphroditism of groupers (Table 1). There were also no significant differences between sexes for individual species within a particular year. However, for all years combined, mean (±SD) biliary PAHs (ng FAC g^{-1} bile) in unsexed Red Grouper (103,000 ± 18,000, $n = 20$) were 3-fold higher than females (31,000 ± 59,000, $n = 3$, $Z = 2.33$, $p = 0.0199$).

There were significant differences among years in the standard lengths (SL), total fish, liver and gastrointestinal (G.I.) weights with all groupers combined in the NC region (Table S2, Fig. S5). All biometrics followed similar patterns over time which included an initial decline between 2011 and 2013, and a general increase in following years. Mean standard length (−10%), total weight (−35%), liver weight (−50%), gastrointestinal weight (−50%) and gonadal weight (−44%) all declined between 2011 and 2012/2013. Mean condition factors also declined by an average of 65% between 2011 and 2014 for grouper in the north central region (Fig. S6a). Additionally, mean liver lipid content declined 45% between 2011 and 2013, with an overall decline of 29% between 2011 and 2017 (Fig. S6b). There may be differences between species, however, similar trends were observed for Yellowedge Grouper biometrics in the NC region (Figs. S7 and 7a–b). Mean total weight (−30%), liver weight (−33%), gastrointestinal weight (−46%) and gonadal weight (−61%), condition factor (−24%) and lipids (−55%) all declined between 2011 and 2012/2013.

3.2.2. West Florida Shelf (WFS)

Along the WFS year was the only significant parameter effect on biliary PAHs, whereas species and sex were the significant parameter effects on liver PAH concentrations (Table S3). Sample size limitations did not warrant interspecies comparisons within a particular year. Red Groupers were the only grouper species with sufficient repeat sampling over time for biliary (2013–2015, 2017) and liver (2011, 2013, 2015, 2017) PAH analysis on the WFS (Fig. 5c–d). There were yearly fluctuations in the mean biliary PAHs in Red Grouper collected on the WFS. Mean (±SD) biliary PAHs (ng FAC g^{-1} bile) in Red Grouper increased 136% between 2013 (14,000 ± 4400, $n = 6$) and 2014 (33,000 ± 28,000, $n = 9$), followed by an average 67% decrease in 2015 (11,000 ± 3500, $n = 6$), then increasing 245% by 2017 (38,000 ± 12,000, $n = 12$). Overall, biliary PAH concentrations increased 171% between 2013 and 2017 (Fig. 5c).

Conversely, mean liver PAH concentrations in WFS Red Grouper demonstrated a consistent decline over time, although they were not significant (Fig. 5d). Mean (±SD) liver PAHs (ng g^{-1} w.w.) were 5 times higher in 2011 (2490 ± 4080, $n = 93$) than in 2017 (543 ± 179, $n = 10$). Overall, liver PAH concentrations in Red Grouper on the WFS decreased 78% between 2011 and 2017.

Biliary data was only available for female Red Grouper collected along the WFS. However, there were significant differences in mean (±SD) liver PAHs (ng g^{-1} w.w.) between sexes in Red Grouper collected on the WFS. Both male (3090 ± 2610, $n = 13$, $Z = 3.50$, $p < 0.001$) and unsexed (4110 ± 4570, $n = 8$, $Z = 3.07$, $p = 0.002$) Red Grouper had up to 2-fold higher liver PAHs than female (1910 ± 3880, $n = 88$) Red Grouper.

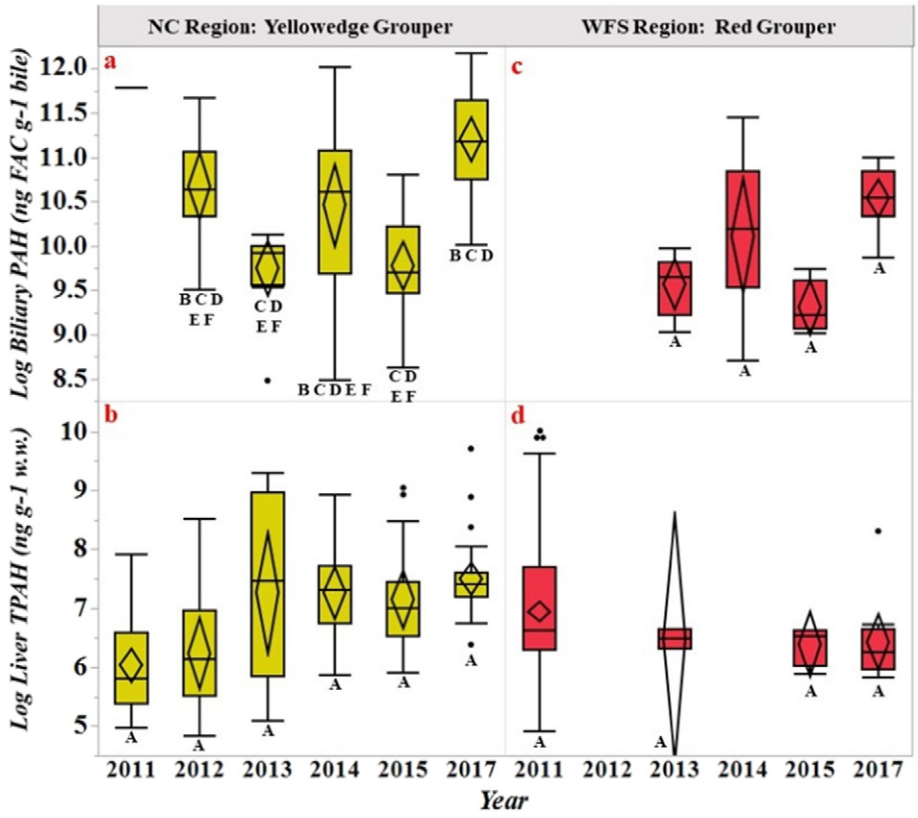

Fig. 5. Temporal trends in mean biliary (ng FAC g^{-1} bile) and liver (ng g^{-1} w.w.) PAH concentrations in Yellowedge and Red Grouper collected in the north central (NC) and West Florida (WFS) regions of the Gulf of Mexico, respectively. Years not connected by a letter are statistically different. The line represents the median and the confidence diamond contains the mean and the upper and lower 95% confidence intervals of the mean.

There were no significant differences found for most biometric variables (lengths, weights and somatic indices) for all grouper species combined collected along the WFS (Fig. S5). Condition factors and lipids were significantly higher in 2011 compared to the following years (Fig. S6c–d). For all groupers collected along the WFS between 2011 and 2017, the condition factor and lipids declined 6% and 37%, respectively. Similar trends were observed in Red Grouper biometrics from the NC region (Fig. S7). Total weight (−18%), liver weight (−10%), gastrointestinal weight (−7%) and gonadal weight (−43%), condition factor (−18%) and lipids (−48%) all declined between 2011 and 2013. For Red Grouper collected along the WFS between 2011 and 2017, the condition factor and lipids declined 8% and 35%, respectively (Fig. 7c–d).

4. Discussion

Ten grouper species were collected from seven regions of the GoM with the goal of assessing both short-term (biliary) acute and long-term (hepatic) chronic exposures to PAHs. All fish collected had detectable levels of PAHs, thus signifying the ubiquitous contamination of PAHs throughout the GoM. The temporal trends (2011–2017) observed in the NC region demonstrated an initial decrease in PAH exposures and related fish biometrics following DWH, followed by increases in exposures, biometrics and liver PAH concentrations, likely due to resuspension of sedimented PAHs in addition to chronic inputs from additional small oil spills, riverine inputs, and seeps. Similar temporal trends in PAH levels and biometrics have been observed in other demersal species in the northern GoM (Pulster et al., 2020b; Snyder et al., 2019; Struch et al., 2019). Interpretive caution should be used due to the heterogeneity in sample sizes and species by year and region, however spatiotemporal trends were similar between pooled species and individual species. Although PAH levels were not assessed in these species prior to DWH (2010), the observed decline in fish health-related biometrics is consistent with initial sublethal responses to an acute exposure (e.g., DWH spill) followed by a recovery period demonstrated by observed increases in condition factors and weights (2014–2017). Growth declines following oil exposure have been documented in laboratory and field studies for several species of fish (Brewton et al., 2013; Brown-Peterson et al., 2017; Herdter et al., 2017; Kerambrun et al., 2012; Kerambrun et al., 2011). Growth declines were not consistent across species. For example, Tilefish (*Lopholatilus chamaeleonticeps*), a major demersal fish in the GoM, did not appear to suffer growth declines post-DWH (Helmueller, 2019).

We found no statistically significant relationships between biliary or liver PAH concentrations and any pertinent biometrics (i.e., length, weight, sex, or organ weights) measured or calculated (i.e., hepatic-, gonadal- and gastro-somatic indices and condition factor). Although there were significant negative associations between lipids and PAH concentrations, differences were relatively weak to modest; explaining 34% and 63% of the wet weight and lipid weight data, respectively. Although PAHs can bioaccumulate in tissues of fish, they do not appear to biomagnify or behave as other lipophilic contaminants (i.e., PCBs) with similar partitioning coefficients (log K_{ow}). The differing behavior of PAHs compared to other lipophilic compounds has been attributed to the high metabolic capacity and low assimilation efficiencies in fish and other higher trophic level organisms (Thomann and Komlos, 1999; Wan et al., 2007).

In both the north central (NC) and West Florida Shelf (WFS) regions, all biometrics and health indices declined up to 65% during the first three years following the DWH oil spill. Condition factors increased after 2013, however liver lipids were still in decline by the final sampling event in 2017. Furthermore, standard length, total weight and organ weights all exhibited similar trends of decline until 2013. Between 2011 and 2017, the GoSI for grouper collected in both the NC and WFS region declined 30% and 64%, respectively. The higher GoSIs

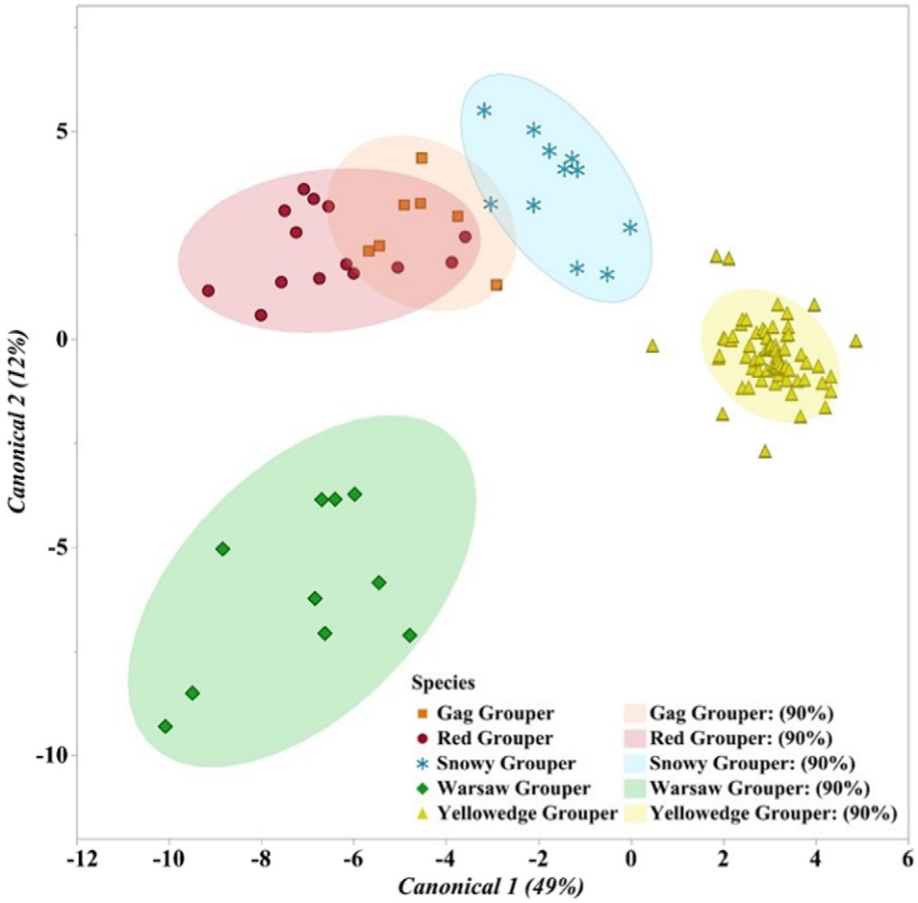

Fig. 6. Discriminant analysis plot illustrating distinct profiles in the PAH composition profiles between grouper species collected in the north central region of the Gulf of Mexico, 2011–2017.

in 2011 equates to a higher fecundity immediately following DWH. A number of studies have reported increasing environmental degradation can lead to earlier sexual maturity at smaller sizes, extended spawning, increased fecundity and high mortality (Dickerson and Vinyard, 1999; James and Bruton, 1992; Khallaf et al., 2003). The increased fecundity has been considered an adaptive response to environmental changes to ensure population viability.

Similar trends were observed in biliary PAH concentrations. Biliary PAH concentrations in Yellowedge Grouper and Red Grouper collected from the NC and WFS regions, respectively, both declined during the first three years post-DWH, followed by significant increases. Whereas liver levels of PAHs in Yellowedge Grouper from the NC region have demonstrated a consistent increase over time (+827% increase between 2011 and 2017). Conversely liver levels have declined (−78%) over time in Red Grouper from the WFS. The observed increase in liver concentrations in the NC is indicative of the chronic oil exposure in fish from this region, perhaps due to resuspension of sedimented PAHs.

Increasing levels of PAHs were observed in birds and sediments in the NC region, and have been attributed to resuspension events in the GoM (Paruk et al., 2016; Perez-Umphrey et al., 2018; Turner et al., 2014). Although there is compelling evidence for the occurrence of resuspension events (Brooks et al., 2015; Diercks et al., 2018; Hastings et al., 2016), the continued yet variable inputs of hydrocarbons into the GoM from river discharge, natural oil seeps, other annual accidental spills and chronic infrastructure leaks (i.e., Taylor Energy platform collapse) likely also contribute to the observed PAH fluctuations in bile (Sun et al., 2018). Chronically-exposed fish are known to have reduced xenobiotic metabolic capacity, slower elimination, and increased accumulation rates and thus increasing PAH levels (Varanasi et al., 1989). The increasing levels of PAHs in liver tissues observed in this study of groupers are indicative of chronically-exposed fish.

Additionally, condition factors and lipid fractions demonstrated significant declines up to six years post-DWH. Condition factors and liver lipid content are both indicators of nutritional status, energy reserves and overall fish health. These indicators can vary by season, age of fish, spawning and reproductive status, feeding quality, population differences and changes in environmental conditions (Adams, 1999; Froese, 2006; Tesch, 1968). Lipids are essential to the survival, fitness, and reproductive success of individuals (Adams, 1999). Environmental stressors, including both natural (e.g., pH and temperature) and anthropogenic (e.g., pollution) sources, can alter the quantity and quality of lipid within a fish. This may subsequently manifest in increasing disease and predation susceptibility, as well as decreasing an individual's behavioral and reproductive performance (Adams, 1999).

The length-weight relationship (LWR) in fish is another way to describe the growth allometry and body condition ("robustness", Table S4, Fig. S2). This relationship varied between grouper species, for example, in Nassau and Red Hind a positive allometric growth relationship ($b > 3$) was apparent, thus weight increases relatively rapidly as a function of length. In contrast, the other eight species exhibited a slower rate of body weight increase with length ($b < 3$), thus for these species weight increases relatively slowly as a function of length. The LWR was remarkably linear when summarizing all ten grouper species in aggregate ($n = 557$; Fig. S2). No significant differences were found between male and female standard length ($p = 0.90$, ns), weight ($p = 0.96$, ns), or condition factor ($p = 0.26$, ns). Males tended towards a positive-allometric growth relationship ($b = 3.04$) whereas females ($b = 2.82$) and unsexed ($b = 2.84$) were still lower (Table S4). The value of $b < 3$ found among the majority of the grouper species in this

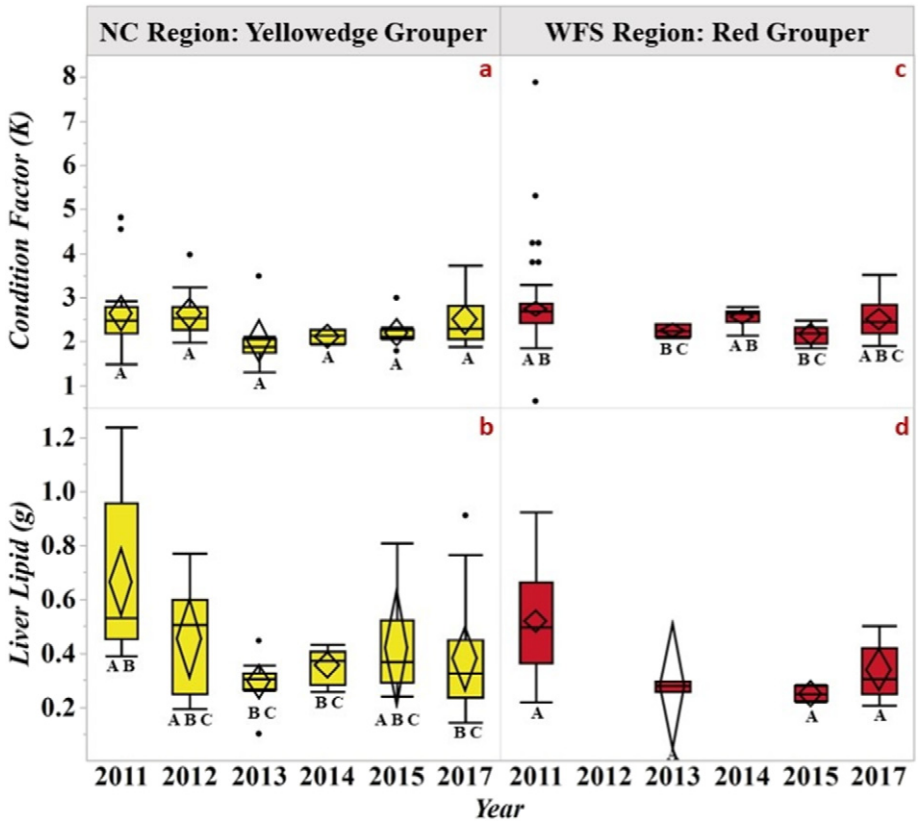

Fig. 7. Temporal trends in condition factors (K) and liver lipids (g) for Yellowedge and Red Grouper collected in the north central (NC) and West Florida Shelf (WFS) regions, respectively, in the Gulf of Mexico. Years with different letters indicate significant differences. The line represents the median and the confidence diamond contains the mean and the upper and lower 95% confidence intervals of the mean.

study was consistent with ten other grouper species collected along the southern Kenyan coast of the Indian Ocean (Ogongo et al., 2015). Conversely, 82% of the fish species analyzed by Froese ($n = 1773$) demonstrated a tendency towards positive-allometric growth ($b > 3$), though this analysis included many species other than grouper (Froese, 2006).

Mean total weights and GI weights for all grouper combined and for an individual species (i.e., Yellowedge Grouper) collected in the NC region declined 35% and 50%, respectively, between 2011 and 2012, followed by steady increases up until the last sampling event in 2017 (Table S2; Fig. S5). These trends might suggest either a lack of prey availability or a decrease in feeding intensity among grouper in the GoM for several years post-DWH. Pollution has been attributed to have negative effects on growth, age and fecundity in a number of comparative studies, which reported significant variations of LWR between polluted and non-polluted areas for a number of species (Bakhoum, 1994; Khallaf et al., 2003; Olurin and Aderibigbe, 2006). The slower growing or less robust fish ($b < 3$) combined with the decline in condition factors, weight, length and lipids observed in this study, suggests grouper are more susceptible to environmental stressors than the more robust fish ($b > 3$). Some examples of stressors include anthropogenic pollution (i.e., oil) and decreased food availability or feeding, both of which were likely encountered in the GoM in the years following the DWH oil spill. Observational studies and empirical modelling have documented declines in benthic foraminifera and impacts on reef and demersal foraging, respectively, post-DWH in the northern GoM (Ainsworth et al., 2018; Schwing et al., 2015).

Grouper sampled in this study were collected from May through September, with repeat stations in the NC region collected annually in August. Based on growth equations, all groupers collected were either sexually mature or approaching maturity. Grouper are protogynous hermaphrodites with variable spawning times throughout the year depending on species. For example, Nassau Grouper spawn in the winter (December through January), while Red Grouper spawn during the spring (February through June), Gag spawn during the summer and early fall, and Warsaw can spawn from spring through fall (April through November). There is a wide range of sizes for sex reversal in groupers (~30 to 85 cm) with an equally wide size range of fish sampled in this study (25 to 110 cm), however all were within or approaching the range for sex reversal to occur.

The sex ratio skewness of the data towards females may also explain the lack of sex-related differences observed among eight of the ten species in this study (Table 1). Male and/or unsexed Red Grouper and Red Hind did have significantly higher biliary and liver PAHs than females, potentially indicating that females may offload PAHs through maternal transfer, or that females exhibit higher metabolic activity. Maternal transfer of PAHs to embryos has been reported for some stocks of Pacific herring *Clupea pallasii* (West et al., 2014) and Northern pike *Esox lucius* (Sundberg et al., 2007). Sundberg et al. (2007) also suggested the increased maternal transfer observed in pike may be the result of decreased metabolic activity in that particular species. Decreased biotransformation activity has been demonstrated in the Dusky Grouper *Epinephelus marginatus* following PAH exposure (Salvo et al., 2016). Conversely, elevated activity was noted in the juvenile Areolated Grouper *Epinephelus areolatus* following dietary exposures to benzo[*a*]pyrene (Au et al., 2004). Additional biomarkers (e.g., DNA adducts, EROD) and analysis of gonadal tissues in Red Grouper and Red Hind would be needed to fully investigate the relationship to metabolic activity and assess the contribution of maternal transfer in this particular species group.

Polycyclic aromatic hydrocarbons (PAHs) are considered endocrine disrupting chemicals in fish. Reductions in circulating sex hormones (e.g., 17β-estradiol, 11-ketotestosterone) in plasma and a disruption of steroidogenesis have been reported in several species following exposure concentrations of crude oil and compound specific PAHs ranging

from 0.1 to 8.91 ppm; potentially resulting in sex change and gonadal maturity dysfunctions (de Campos et al., 2018; Martin-Skilton et al., 2006; Monteiro et al., 2000; Nicolas, 1999). The water concentrations of PAHs in the Gulf of Mexico following the DWH spill ranged from <0.054 (background) ppb to 154 ppm (Wade et al., 2016). Based on these laboratory studies and environmentally relevant concentrations, it is plausible that fishes in the GoM may be susceptible to endocrine disruption from PAH exposures.

Species-specific life histories, metabolic capabilities, chemical bioavailability and partitioning behavior all play a part in the species-specific differences observed in liver concentrations and profiles in this study. There were no correlations between depth (m) or temperature (°C) and either biliary or liver PAHs, however the shallow-water groupers generally had significantly higher biliary and liver concentrations of PAHs than the deep-water groupers, Gulf-wide. In contrast, there were no significant differences between the deep-water and shallow-water grouper in the NC region alone, perhaps indicating differences in the pollution load and effects of riverine runoff. Due to the proximity of anthropogenic sources, the shallower water and coastal zones within the GoM have historically tended to have higher contaminant loads and lower water quality when compared to deeper offshore waters (Kennicutt, 2017). Recent studies have however reported large-scale deposition of weathered DWH oil in both coastal and deep areas of the north central region (Adhikari et al., 2016; Romero et al., 2017), including in areas where grouper from the present study were collected.

Groupers (i.e., Yellowedge and Red) caught in the NC had significantly higher levels of liver PAHs compared to those collected in the southern GoM. Notably, all grouper analyzed in this study had detectable levels of PAHs in both bile and liver tissues, indicating that PAHs are ubiquitous in the GoM. Based on surface and subsurface circulation patterns, currents, winds and Mississippi River plumes in the GoM (Duran et al., 2018; Kourafalou and Androulidakis, 2013; Liu et al., 2011; Poje et al., 2014; Valentine et al., 2014), it is conceivable that oil from routine exploitation activities or spills have impacted all regions of the Gulf including areas where no oil infrastructure exist (i.e., WFS). There is also a network of marine transport corridors within the Gulf and through the Yucatan and Florida Straits which generate intense tanker traffic, thus increasing the risk of pollution in these areas (Botello et al., 1997).

Offshore Cuban waters do not have oil and gas infrastructure as compared to the northern and western GoM. However, north Cuba is highly influenced by oceanographic currents potentially enhancing transport and transference of pollutants. There are two Cuban regions with oil-related industrial development. The northern region includes areas with oil fields (between Havana and Varadero), an oil refinery (Havana city), and a tanker terminal (Matanzas bay). The southern region includes a large oil refinery and tanker terminal in Cienfuegos city (Echevarria-Rodriguez et al., 1991). Previous studies have reported high levels of PAHs in marine sediments in the semi-enclosed bays and adjacent areas of Havana and Cienfuegos (Martins et al., 2018; Tolosa et al., 2009).

Distinct PAH profile differences were observed among regions, with three main regional clusters: (1) the WFS, (2) the YS, BC, and CUB; and (3) the NW and SW regions. The NC region overlapped with all regions, suggesting it shares commonalities with all regions. The non-oil producing regions (WFS, YS, CUB) had a higher proportion of the pyrogenic PAHs (5 and 6 ring) suggesting these regions may be more influenced by other anthropogenic sources such as marine vessel traffic and coastal runoff rather than the direct impacts of the oil extraction activities.

Distinct interspecies PAH profiles differences were also observed within the NC region, which adds supportive evidence for species-specific metabolism. The liver PAH profiles in Warsaw Grouper were mainly comprised of the 2 and 3 ring compounds. Red Grouper liver profiles consisted of the 2 through 4 ring compounds. Composition profiles in the livers of Snowy, Gag and Yellowedge were comprised of 2 through 6 ring compounds. Comparatively, Snowy Grouper had a higher proportion of the 6 ring compounds than all other species. The Yellowedge Grouper had a higher proportion of the 3 and 4 ring PAHs compared to the other species. The differences in profiles are likely a function of species-specific metabolism rather than life histories considering there were differences among species within the same habitat complex (deep- vs shallow-water species).

Compositional profiles of PAHs in sediment, air, water and tissues are frequently used to identify potential sources (Ahmed et al., 2016; Murawski et al., 2014; Pulster et al., 2019; Wang et al., 2006). Nonetheless, profile comparisons are difficult to assess within organisms due to the complex interactions and species-specific differences between bioaccumulation (e.g., uptake, metabolism and elimination) and the physiochemical parameters effecting chemical exposure and bioavailability in the surrounding environment. The PAH profiles in groupers collected in this study were dominated by 2–3 ring low molecular weight (LMW) compounds (≥95%), which are predominantly associated with petrogenic sources (Wang et al., 2014). This dominantly LMW profile pattern has previously been described in other fish species in the GoM (Snyder et al., 2019; Snyder et al., 2015; Struch et al., 2019) as well as in other locations globally (El-Kady et al., 2018; Zhang et al., 2015).

The preferential uptake of the LMW PAHs in fish could be due to the reduced bioavailability and lipid membrane permeability of HMW compounds (partitioning coefficient). One exposure study using glass fish (*Parambassis ranga*) demonstrated that petrogenic PAHs (HMW PAHs) were less bioavailable and their bioavailability also differed depending on source (Burgess et al., 2001). Offshore water profiles in the northern GoM following the DWH event were dominated by the 2–3 ring PAHs (Payne and Driskell, 2018) with elevated subsurface (1000–1200 m) concentrations (>1 ppb) within 20 km of the wellhead (Boehm et al., 2016). It is therefore highly plausible that the PAH profiles observed in grouper are a combination of both species-specific biotransformation processes and the increased bioavailability of LMW PAHs in the surrounding environment.

5. Conclusions

The present study provides a spatiotemporal baseline for PAHs in ten economically important species of grouper collected in the GoM. Inter-regional comparisons suggest grouper collected in the NC region have some of the highest levels of PAHs and exhibited declining trends in biliary PAHs during the first three years following DWH followed by increasing levels over time. Whereas liver PAH concentrations in groupers collected in the NC region demonstrated consistent increases over time. This consistent increase in both biliary and liver PAH concentrations suggests increasing chronic environmental exposures. These increasing and chronic exposures observed in groupers and other species in the GoM signifies a more pervasive problem in this region. Chronic and repeated acute exposures to PAHs can elicit sublethal effects of varying degrees (e.g., growth declines, diet changes, habitat shifts etc.) (Pulster et al., 2020a), however the critical stage at which permanent, irreparable damage may occur is unknown. In other regions, environmental degradation and over-fishing has resulted in severe population declines of grouper, which are now considered threatened or on the verge of extinction (Ogongo et al., 2015). In order to prevent the same in the Gulf of Mexico, the data provided herein warrants continued monitoring and research on the cumulative health effects from concurrent chronic and repeated acute exposures including toxic and sublethal endpoints in these economically important species. Additionally, long-term monitoring is required to evaluate when chronic pollution overwhelms the ability for these populations to recover from intensive, acute events like DWH. Moreover, environmental regulations and industry practices should be reevaluated to reduce the amount of PAHs (and other chemicals) entering the GoM from all anthropogenic sources, including offshore oil and gas extraction and transportation activities.

Declaration of competing interest

The authors declare no conflict of interest.

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Author contributions

E.L.P. assisted in sample acquisition, completed the comprehensive data analysis and wrote the manuscript with contribution from all coauthors. B.C. and J.M. assisted in sample acquisition and processing. A.G. and M.A. assisted in sample collections and logistics in Mexico and Cuba. S.A.M. conceived and completed the study design and assisted in all sample acquisitions and study oversight.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135551.

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