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Integration of multi-tissue PAH and PCB burdens with biomarker activity in three coastal shark species from the northwestern Gulf of Mexico

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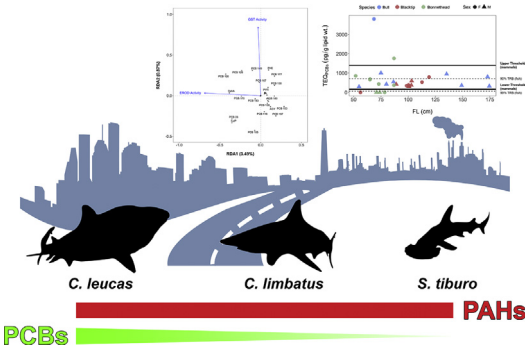
HIGHLIGHTS

- PAH/PCB burdens and biomarker activity were quantified in three shark species.
- Potential toxicity was evaluated by measuring TEQs.
- Sharks accumulated higher than expected PAH burdens.
- Multivariate analyses found correlations among some congeners and biomarkers.
- Comparison with toxicity benchmarks suggests potential physiological impacts.

GRAPHICAL ABSTRACT



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ABSTRACT

Tissue-based burdens of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were integrated with ethoxyresorufin-*O*-deethylase (EROD) and glutathione *S*-transferase (GST) enzyme activity in bull (*Carcharhinus leucas*), blacktip (*Carcharhinus limbatus*), and bonnethead (*Sphyrna tiburo*) sharks from Galveston Bay, TX. The potential toxicity of these burdens was evaluated by calculation of toxic equivalents (TEQs). Concentrations of total PAHs (∑PAHs) were significantly greater in blacktip and bonnethead sharks than bull sharks in liver, but did not exhibit differences in muscle among species. Hepatic concentrations of ∑PAHs in these sharks (range of means: 1560–2200 ng/g wet wt.) were greater than concentrations previously reported in oysters from Galveston Bay (range of means: 134–333 ng/g dry wt.), which suggests that trophic dilution of PAHs may not be reflected in sharks. Total PCBs (∑PCBs) were significantly greatest in bull sharks and lowest in bonnetheads, while blacktips were intermediate to these species. EROD activity was greater in bonnetheads than the other species, whereas GST activity was significantly higher in blacktips and bonnetheads than in bull sharks. Integration of hepatic burdens with biomarker activity via constrained multivariate analysis found correlations for only a small number of individual PAH/PCB congeners. Hepatic TEQ measurements suggest potential physiological effects of these burdens compared to established TEQ thresholds for other taxa, although the likelihood of similar effects in sharks requires further study and the inclusion of toxic endpoints. Our findings indicate that sharks may be prone to the accumulation of PAHs and PCBs, which may result in negative health outcomes for these cartilaginous fishes.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are among the most ubiquitous environmental contaminants in estuarine and coastal ecosystems that are commonly introduced through anthropogenic activities (Islam and Tanaka, 2004; Hylland, 2006). Some of these pollutants can accumulate in exposed organisms due to long half-lives and high lipophilicity (Fisk et al., 2001; Van der Oost et al., 2003; Borgå et al., 2004). Consequently, these persistent contaminants can be transferred from the base of the food web to higher trophic levels, resulting in biomagnification of potentially toxic burdens of contaminants (Fisk et al., 2001; Borgå et al., 2004). Although the production of PCBs was banned in many countries by the late 1970s, this legacy contaminant still persists in aquatic systems worldwide. In contrast, PAHs are currently continually released into coastal ecosystems from a combination of pyrogenic (combustion-derived) and petrogenic (petroleum-derived) sources (Hylland, 2006).

There are marked differences in the accumulation potential of PAHs and PCBs by many marine organisms. While PCBs undergo bioaccumulation (over ontogeny) and biomagnification (across trophic levels), PAHs may exhibit trophic dilution (Wan et al., 2007; Gilbert et al., 2015; Romero-Romero et al., 2017; Sun et al., 2018). These differences are proposed to be a result of intrinsic variability in organismal metabolic capabilities (Baumard et al., 1998; Livingstone, 1998). PAHs (and PCBs to a lesser extent) are metabolized by phase I cytochrome P450 enzymes (e.g. CYP1A) subsequent to activation of the aryl hydrocarbon receptor (AhR) in exposed vertebrate organisms (Meador et al., 1995; Nilsen et al., 1998; Billiard et al., 2002). Phase I metabolites can be further biotransformed by phase II conjugation reactions (with glucuronic acid, sulfate, or glutathione groups), increasing their polarity prior to final elimination in urine or feces. Both phase I and II enzymes can be induced by some of these contaminants and often show elevated activities in exposed organisms (Livingstone, 1998; Van der Oost et al., 2003; Hylland, 2006).

Since sharks often occupy high trophic positions, they are especially vulnerable to the bioaccumulation and biomagnification potential of PAHs and PCBs. Their large, lipid-rich livers provide a significant compartment (up to 20% of body mass) for the accumulation of these lipophilic pollutants (Hussey et al., 2010; Corsolini et al., 2014). Many large-bodied sharks are long-lived, slow to mature, and have low fecundity (Cortés, 2000) and these traits contribute to increased individual- and population-level exposure risks relative to other sympatric fish species (Gelsleichter and Walker, 2010). Although only a limited number of studies have evaluated the effects of PAHs and PCBs on the health of sharks and their relatives (Gelsleichter et al., 2006; Marsili et al., 2016; Alves et al., 2016; Sawyna et al., 2017), PCB bioaccumulation in marine mammals (such as pinnipeds and cetaceans) is associated with poor health outcomes such as immunosuppression, endocrine disruption, and reproductive impairment (Nomiyama et al., 2014; Desforges et al., 2016; Jepson et al., 2016). Additionally, the uptake of PAHs in fishes and mammals can cause genotoxicity (and associated carcinogenesis), as well as endocrine and metabolic disruption (Hawkins et al., 2002; Lemiere et al., 2005; Schwacke et al., 2014).

While considerable attention has focused on the quantification of tissue-based PCB burdens in sharks (Serrano et al., 2000; Storelli et al., 2005; Corsolini et al., 2014; Olin et al., 2014; Beaudry et al., 2015; Gilbert et al., 2015), to our knowledge only Al-Hassan et al. (2000) and Marsili et al. (2016) have measured PAH burdens in elasmobranchs. The northern Gulf of Mexico (GoM) region is highly impacted by offshore-drilling for fossil fuels, which also includes a high-intensity shipping and transportation corridor (Steichen et al., 2012). In particular, Galveston Bay, Texas, USA is one of the most impacted bodies of water in this region due to its proximity to the numerous industrial facilities in Houston (Santschi et al., 2001; Wade et al., 2014) and whose associated waterways host the largest petrochemical complex in the country (and second largest in the world; Port of Houston Authority, 2018). The frequent dredging of the Houston Ship Channel likely contributes to the resuspension and increased availability of PAHs and PCBs that have been bound to sediment particles and buried (Bocchetti et al., 2008). Additionally, there are nine US Environmental Protection Agency (USEPA) Superfund sites that border Galveston Bay (USEPA, 2017), demonstrating legacy pollution. Therefore, there is a need to assess the extent of PAH and PCB exposure in resident organisms, especially those occupying high trophic positions.

Exposure to PAHs and PCBs was evaluated in three species of sharks common in the GoM, bull (*Carcharhinus leucas*), blacktip (*Carcharhinus limbatus*), and bonnethead sharks (*Sphyrna tiburo*). These species were selected due to their differences in life history characteristics (Cortés, 2000) and ecological niche (Snelson et al., 1984; Cortés, 1999; Plumlee and Wells, 2016), which likely influence their exposure to these pollutants. With respect to trophic ecology, bull and blacktip sharks are tertiary consumers while bonnethead sharks are secondary consumers (Cortés, 1999). Additionally, these sharks are likely prone to the exposure of high levels of PAHs and PCBs since these contaminants can enter estuarine and coastal habitats from point and non-point sources (Kennish, 2002; Gelsleichter et al., 2008; Gelsleichter and Walker, 2010). This is of great concern since many coastal sharks use these habitats as nursery grounds (Castro, 1993; Heupel et al., 2010). Furthermore, chronic exposure to these pollutants in sensitive juvenile fishes can result in sublethal effects that affect physiology, growth and development, fitness, and survival (Varanasi et al., 1987; Meador et al., 2002; Incardona et al., 2004; Meador et al., 2006). Therefore, PAH/PCB burdens and biomarker activity were investigated over multiple size classes of sharks from Galveston Bay, TX.

The present study measures and characterizes patterns of tissue-based burdens of PAHs and PCBs in each of the three species of sharks. Biochemical responses to PAH/PCB burdens were assessed by quantifying phase I (ethoxyresorufin-*O*-deethlyase; EROD) and phase II (glutathione *S*-transferase; GST) enzyme activity assays in hepatic tissue. Last, toxic equivalents (TEQs) were used to assess the likelihood of toxicity due to both PAHs and PCBs. The direct integration of tissue-based burdens with enzymatic biomarker activities provides a comprehensive approach to evaluating pollution-induced stress.

2. Materials and methods

2.1. Sample collection

Bull ($N = 9$), blacktip ($N = 24$), and bonnethead sharks ($N = 21$) were opportunistically sampled from fishing charters or from routine long-line surveys conducted by the Texas Parks and Wildlife Department in Galveston, Texas, USA from April to October in 2015 and 2016. Of the 54 sharks sampled, 48 were used for quantifying EROD activity (bull: $n = 3$; blacktip: $n = 24$; bonnethead: $n = 21$) and 41 of these for quantifying GST activity (bull: $n = 3$; blacktip: $n = 20$; bonnethead: $n = 18$). PAH/PCB tissue-based burdens were quantified in 29 sharks (bull: $n = 9$; blacktip: $n = 10$; bonnethead: $n = 10$), of which only 3 bull, 10 blacktip, and 10 bonnethead sharks also had biomarkers measured. Sample sizes for each of these measurements varied since some samples were not immediately stored at −80 °C or tissue mass was limited (Table 1). Sex was determined for each shark and total (TL, cm), fork (FL, cm) and pre-caudal length (PCL) morphometrics were collected (Table 1). Size classes for each species were distinguished based upon previous studies from Texas or from a nearby location at a similar latitude, which has been shown to affect growth rates in bonnethead sharks (Branstetter, 1987; Branstetter and Stiles, 1987; Lombardi-Carlson et al., 2003). Four size classes were delineated in bull sharks, which included young-of-the-year (YoY; TL < 90.0 cm), juvenile (90.0 < TL < 160.0 cm), sub-adult (160.0 < TL < 210.0 cm), and adult (TL > 210.0 cm) groups (Branstetter and Stiles, 1987); YoY individuals are sharks within the first year of life. Additionally, four size classes were delineated in blacktip sharks, which included YoY (TL

Table 1
Sample sizes (N), sex ratios, and mean (±SD) body length measurements (min–max) for three species of sharks.

Species	N	Sex ratio F/M/NI	TL (cm)	FL (cm)	PCL (cm)
Bull (*C. leucas*)	9	1/8/0	137.1 ± 56.4 (69.9–215.0)	110.8 ± 47.0 (54.9–174.5)	100.6 ± 42.9 (49.8–159.0)
Blacktip (*C. limbatus*)	24	11/13/0	130.1 ± 19.9 (72.5–167.9)	102.9 ± 15.9 (56.2–134.8)	93.2 ± 14.7 (50.8–122.5)
Bonnethead (*S. tiburo*)	21	14/5/2	92.5 ± 14.0 (67.7–124.5)	73.4 ± 12.7 (51.8–106)	67.5 ± 11.5 (48.2–93.2)

TL = total length; FL = fork length; PCL = pre-caudal length; F = female; M = male; NI = not identified.

< 83.0 cm), juvenile (83.0 < TL < 111.5 cm), sub-adult (111.5 < TL < 140.0 cm), and adult (TL > 140.0 cm) classes (Branstetter, 1987). Only three size classes were delineated for bonnetheads (YoY, juvenile, adult) since this species reaches maturity much faster (3–4 years) than bull (14–18 years) and blacktip sharks (4–8 years) (Branstetter, 1987; Branstetter and Stiles, 1987; Lombardi-Carlson et al., 2003). Due to a latitudinal gradient in growth rate, age-growth curves of bonnethead sharks from northwest Florida were used to develop size classes for individuals sampled from Galveston, TX since these locations share a similar latitude (Lombardi-Carlson et al., 2003). These size classes were delineated as YoY (TL < 70.0 cm), juvenile (70.0 < TL < 88.5 cm), and adult (TL > 88.5 cm). Liver samples (~5 g) were collected from the lower left lobe and muscle samples (~5 g) were taken from the epaxial region near the anterior dorsal fin. Samples were transported on ice for up to 30 min before storage at −80 °C for further analysis.

2.2. Sample extraction and clean-up

A ~1 g sub-sample was excised in duplicate from each tissue (i.e. muscle or liver). Each sub-sample was homogenized in a 7-mL polypropylene tube containing ceramic beads (Fisher Scientific) and filled with 3 mL of 1:1 (v/v) hexane:ethyl acetate. Tubes were placed in a Fisherbrand™ Bead Mill 4 Homogenizer (Fisher Scientific) and homogenized at a processing power of 150 *g* for 2 min. Homogenate was transferred to an acid -washed 50-mL glass tube. A 5 μL aliquot of 100 ppm benzo[*a*]pyrene-d_{12} (Sigma-Aldrich) and 100 ppm PCB 65-d_5 (CDN Isotopes) was spiked to each sample as internal standards (2.5 ppm at final volume). Recovery efficiency for spiked PAH/PCB analytes (as standard addition) was also assessed (Pethybridge et al., 2010a; Corsolini and Sara, 2017). The mixture used for standard addition comprised two PAHs (Acenaphthene, Benzo[*a*]pyrene) and two PCBs (PCBs 101, 138) at a 2.5 ppm final concentration (along with internal standards benzo[*a*]pyrene-d_{12} and PCB 65-d_5). The standard addition samples were compared with matching 'control' samples (spiked only with internal standards). After spiking tissue homogenates, glass tubes were placed in a Branson Ultrasonics™ M2800 Ultrasonic Bath (Fisher Scientific) for 30 min to further extract PAHs and PCBs into 1:1 (v/v) hexane: ethyl acetate solvent. Phase separation of the solvent from the tissue matrix was assisted by centrifugation at 2000*g* for 10 min. The supernatant was pipetted into pre-weighed 20-mL glass vial and dried under N_2 gas for 30 min. Following extraction, lipid content of each sample was determined gravimetrically. The remaining residue was rinsed with 1 mL of acetonitrile (ACN) and pipetted into a 2-mL amber vial. All samples were then dried in a Savant™ SPD121P SpeedVac™ Concentrator (Thermo Scientific) and reconstituted into 200 μL ACN before transferring to a glass insert. Following Hong et al. (2004), sample freezing was conducted at −20 °C for 1 h to precipitate lipids out of solution. Afterwards, a clean 50 μL sub-aliquot was removed, dried (SpeedVac™) and reconstituted into 50 μL dichloromethane (DCM) prior to gas chromatography and mass spectrometry (GC–MS) analysis.

2.3. Sample analysis

Concentrations of the USEPA's 16 priority PAHs and 29 individual PCB congeners were quantified in shark liver and muscle tissues. All PAHs with two or three rings (naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene) were classified as low molecular weight (LMW) PAHs, while congeners with four to six rings (fluoranthene, chrysene, pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene, indeno[1,2,3-*cd*]pyrene) were classified as high molecular weight (HMW) PAHs. Of the 29 PCB congeners, 12 were dioxin-like (DL-PCBs): PCBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. Analytical grade standards were obtained from the following sources: acenaphthene (ACE), acenaphthylene (ACY), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbF), benzo[*g*,*h*,*i*]perylene (BghiP), fluoranthene (FLT), pyrene (PYR), and PCBs 1, 18, 52, 101, 138, and 180 from Sigma-Aldrich; anthracene (ANT), chrysene (CHR), benzo[*a*]anthracene (BaA), benzo[*k*]fluoranthene (BkF), dibenz[*a*,*h*]anthracene (DahA), fluorene (FLU), indeno[1,2,3-*cd*]pyrene (IcdP), phenanthrene (PHE), and naphthalene (NAP) from Supelco; PCBs 28, 33, 77, 81, 95, 105, 114, 118, 123, 126, 128, 149, 153, 156, 157, 167, 169, 170, 171, 177, 183, 187, and 189 from Ultra Scientific. All PCBs are identified according to the IUPAC numbering system.

Samples were analyzed for the 45 individual PAH/PCB congeners by GC–MS. This analysis was conducted on a Hewlett Packard HP-6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer. Samples were injected in splitless mode (2 μL) equipped with a DB-5MS (J&W Scientific) capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness). Helium was the carrier gas at a flow rate of 1.0 mL/min. Temperatures at the front inlet and the MS interface were set at 250 and 280 °C, respectively. Following injection of the sample, the GC oven was programmed at 40 °C and held for 1 min, then ramped up to 180 °C at 20 °C/min, and finally ramped up to 300 °C at 5 °C/min and then held for 10 min. The MS was operated in electron impact (EI) mode at an electron energy of 70 eV while the MS source temperature was maintained at 230 °C. Selected ion monitoring (SIM) mode was used for identification and quantification of all 45 analytes. Quantification of all PAH and PCB congeners were performed against a linear 13-point calibration curve ($R^2 > 0.97$) using serially diluted standards that were prepared in DCM (2.5 to 10,000 ng/mL).

Sample quality assurance and quality control measures were conducted by running a solvent blank and a mixed standard after every eight samples analyzed. The limit of detection (LOD) was quantified by the signal-to-noise ratio of 5:1 for the lowest detectable calibration point (Supplemental Data, Table S1). Blanks showed no signs of external contamination above the LOD and accuracy of the mixed standards fell within ±30% for all 45 analytes, with the exception of benzo[*b*]fluoranthene (67.1 ± 5.85%). Mean (±SD) intra-day variability (via coefficient of variation) of mixed standards was 7.85 ± 1.99%, while inter-day variability was 9.36 ± 5.73%. Recovery rates for the four analytes used in the standard addition spike were as follows: acenaphthene = 95.0 ± 22.6%, benzo(a)pyrene = 100 ± 2.61%, PCB 101 = 70.7 ± 11.7%, PCB 138 = 91.9 ± 22.0%. All samples were analyzed in duplicate, for which mean (± SD) variability between these samples was 4.06 ± 2.10% for all analytes. Sample concentrations were not corrected for recovery.

2.4. EROD and GST quantification

For quantification of enzyme activity, 200 mg liver samples were homogenized in a 1:5 (w/v) of 0.1 M monobasic sodium phosphate buffer

(pH 7.4), containing 0.15 M potassium chloride, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol (DTT), and 10% (v/v) glycerol (Nilsen et al., 1998). Homogenates were centrifuged for 20 min at 12,000*g* and 4 °C, after which the supernatant (postmitochondrial fraction) was obtained for subsequent enzyme assays. Protein content was quantified by Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as a standard. Absorbance was measured at 595 nm on a Cytation™ 5 Multi-Mode microplate reader (BioTek Instruments).

EROD activity was quantified by a modification of the fluorimetric method conducted by Burke and Mayer (1974). Hepatic S9 fractions were incubated for 20 min at 25 °C, in a 200 μL final volume containing 180 μL of S9 (1 mg/mL final concentration), 10 μL NADPH as cofactor (2 mM final concentration), and 10 μL 7-ethoxyresorufin as substrate (2 μM final concentration). Reactions were stopped by adding 200 μL of ice-cold methanol. Samples were centrifuged at 2000*g* for 5 min and fluorescence was quantified in a 100 μL aliquot of supernatant at 535 nm/590 nm excitation/emission wavelengths. All reactions were run in duplicate and in parallel with controls, including no NADPH control (i.e. reaction mix comprising all components except NADPH) and negative control (7-ethoxyresorufin and buffer only). EROD activity was expressed as pmol/min/mg protein.

GST activity was determined by modification of the procedure described by Habig et al. (1974). Reactions took place in a 200 μL final volume containing 170 μL of S9 (1 mg/mL final concentration), 20 μL reduced glutathione as cofactor (2 mM final concentration), and 10 μL 1-chloro-2,4-dinitrobenzene as substrate (CDNB; 1 mM final concentration). Change in absorbance was quantified once per minute for 10 min at an absorbance of 340 nm at 25 °C. All reactions were run in duplicate and in parallel with controls. GST activity was quantified using a molar extinction coefficient of 9.6 mM^{-1} cm^{-1} (Habig et al., 1974) and expressed as nmol/min/mg protein.

2.5. TEQs – potential toxicity of PAH and PCB burdens

To evaluate the potential toxicity of PAH and PCB burdens, toxic equivalents (TEQs) were calculated in both liver and muscle samples. The TEQ concept assumes a common toxic mechanism of action, in which the congeners included in the calculation activate the aryl hydrocarbon receptor (AhR) in a similar fashion to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Giesy and Kannan, 1998). It is also assumed that the effects among dioxin-like congeners are additive within a mixture of contaminants and are the critical effects on an organism (Giesy and Kannan, 1998). TEQs were calculated for both the non-*ortho* and mono-*ortho* substituted DL-PCBs (TEQ_{PCBs}) based upon toxic equivalent factors (TEFs) for fish as proposed by Van den Berg et al. (1998). Additionally, TEQs were calculated for PAHs (TEQ_{PAHs}) using relative potency factors for fishes (fish potency factors; FPFs) based upon AhR binding and CYP1A induction relative to 2,3,7,8-TCDD (Barron et al., 2004). These FPFs were available primarily for four-, five-, and six-ring PAHs that would activate a transduction pathway similar to that of TCDD. Fish-specific TEFs and relative potencies are used due to the toxicokinetic differences in these congeners among taxonomic groups (Hahn and Stegeman, 1992; Van den Berg et al., 1998; Zhou et al., 2010). Since six of the ten PAHs with FPFs are considered to be probable human carcinogens (benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, and indeno[1,2,3-*cd*]pyrene), negative health outcomes to the local population may result if these compounds are found in high concentrations (NTP, 2016).

2.6. Statistical analyses

Sex, size, and species comparisons were conducted for variables such as lipid content, concentrations of ∑PAHs and ∑PCBs, biomarker activity, and TEQ values in liver and muscle tissues. Since lipid content has previously been reported to differ between sexes, by reproductive status, and among species, it was necessary to determine whether this variable could confound comparisons of tissue-based burdens (Rossouw, 1987; Lucifora et al., 2002; Pethybridge et al., 2010b, 2011; Lyons and Lowe, 2013). Within the subset of individuals analyzed for tissue-based burdens, only two bull sharks were mature, while one blacktip and five bonnethead sharks were mature. Of these adults, one individual of each species was captured during its mating season. Intraspecific analyses were conducted to evaluate sex differences and bioaccumulation of PAH/PCB burdens over ontogeny, in addition to interspecific comparisons of burdens among species; comparisons by sex were not conducted on bull sharks due to the presence of only a single female. All PAHs and PCBs were analyzed on a ng/g wet weight (ww) basis unless otherwise noted. If data exhibited normality and homoscedasticity, Welch's *t*-test was conducted for pairwise comparisons and an ANOVA on a weighted generalized least squares (GLS) model was performed for more than two groups to explicitly account for unequal sample sizes. Non-parametric data were analyzed using Mann-Whitney *U* for pairwise comparisons or were log_{10}-transformed to meet parametric assumptions before conducting an ANOVA on a weighted GLS for more than two groups. Significant results for ANOVAs were followed by post-hoc general linear hypothesis tests using Tukey contrasts and Westfall-adjusted *p*-values. Bioaccumulation of PAHs and PCBs over ontogeny were analyzed by linear or quadratic regression depending on the fit with the data. Since these contaminants are expected to accumulate with increasing body size, ANCOVAs were conducted to account for size (FL) when testing for differences in tissue-based burdens between sexes of each species. Data that did not meet assumptions of normality and homoscedasticity were log_{10}-transformed and analyzed via a weighted GLS model for the ANCOVA. If the interaction of FL and sex was not significant in the ANCOVA, the model was reduced to only the main effects. This approach was also applied to interspecific comparisons of tissue-based burdens and biomarker activity, where FL was also treated as the covariate. Pearson correlations were implemented for parametric data while Spearman correlations were conducted for non-parametric data when assessing the relationship between contaminant classes and biomarker activity, as well as burdens between tissues. Sex and FL were accounted for in species comparisons of TEQ_{PCBs} and TEQ_{PAHs} by conducting ANCOVAs following log_{10}-transformation of the response variable. If interaction terms were significant, no post-hoc testing was conducted. All statistical analyses were conducted within the R statistical program (ver. 3.3.3; R Core Team, 2017) and significance was set at $\alpha = 0.05$ for all tests.

Comparisons of liver and muscle congener profiles among the three species were conducted using one-way PERMANOVAs on Bray-Curtis dissimilarity matrices with the vegan package (ver. 2.4–4; Oksanen et al., 2017) in R. If significant differences were detected, pairwise comparisons with Bonferroni-adjusted *p*-values were calculated. Additionally, a SIMPER analysis was conducted using a Bray-Curtis dissimilarity matrix to determine the congeners contributing to the greatest differences between species. All concentrations that were below the LOD (< LOD) were treated as zero and included in subsequent multivariate analyses. If concentrations of a particular congener were zero in all samples of a given tissue (liver or muscle), these congeners were removed from all further multivariate analyses in that tissue. This included the removal of BbF from the analysis of individual congeners in liver tissue, in addition to the removal of BaA, BbF, BghiP, PCB 28, PCB 33, PCB 53, PCB 77, PCB 81, PCB 95, PCB 101, PCB 114, PCB 171, PCB 177, and PCB 189 from analysis of muscle tissue.

Multivariate ordination methods were performed to explore relationships among species via congener profiles, and to explicitly test for correlations with biomarker activity using the vegan package in R. Principal component analysis (PCA) was conducted to visually evaluate

relationships of individual sharks using congener profiles, which helped to reduce the effect of contaminants with disproportionately high concentrations. This was followed by a partial redundancy analysis (pRDA) to determine which congeners were correlated with EROD and GST activity while the effect of species differences was held constant. An adjusted R^2 (bimultivariate redundancy statistic; R^2_{adj}) and a permutation test (using 999 permutations) of marginal effects (Type III sum of squares) were calculated for the pRDAs to determine if there were significant relationships of congener profiles with biomarker activity. Angles between the positions of individual congeners and enzymatic biomarkers (with respect to the origin) were reflective of correlations between these variables.

3. Results

3.1. Comparisons of lipid content and PAH/PCB burdens

Lipid content did not exhibit a significant relationship with body size (FL) in liver or muscle tissue for any species ($p > 0.05$). Of the two species that could be evaluated for sex differences (blacktips and bonnetheads), only the lipid content of the liver in bonnethead sharks exhibited a significant difference in which males were greater than females ($df = 6.809$, $t = 2.855$, $p = 0.025$). No significant interspecific differences were found for lipid content in the liver ($F_{2,26} = 1.309$, $p = 0.287$) or muscle ($F_{2,26} = 1.217$, $p = 0.312$; Table 2). Since lipid content

Table 2
Percent lipid content and individual congener concentrations of PAHs and PCBs (mean ± SE; ng/g ww).

	Bull (*C. leucas*)		Blacktip (*C. limbatus*)		Bonnethead (*S. tiburo*)	
	Liver (n = 9)	Muscle (*n* = 9)	Liver (*n* = 10)	Muscle (n = 10)	Liver (n = 10)	Muscle (n = 10)
% Lipid	63.0 ± 8.16	0.911 ± 0.178	62.8 ± 3.21	1.02 ± 0.227	50.6 ± 6.95	1.11 ± 0.0924
PAHs						
NAP	61.8 ± 5.90	38.3 ± 0.765	73.9 ± 5.70	37.4 ± 0.176	77.0 ± 7.87	37.7 ± 0.153
ACY	52.4 ± 2.78	8.36 ± 5.53	47.8 ± 1.97	38.9 ± 0.398	85.0 ± 25.0	36.6 ± 4.35
ACE	85.6 ± 11.2	61.6 ± 3.79	83.0 ± 4.17	58.8 ± 0.413	82.8 ± 3.21	59.2 ± 0.364
FLU	57.4 ± 4.62	40.3 ± 0.950	44.7 ± 1.50	38.8 ± 0.149	39.5 ± 4.54	39.1 ± 0.119
PHE	83.5 ± 7.22	41.6 ± 1.02	68.3 ± 9.99	39.9 ± 0.151	58.9 ± 3.59	40.2 ± 0.168
ANT	48.9 ± 2.81	23.5 ± 5.93	47.0 ± 1.80	10.3 ± 5.23	42.9 ± 1.35	10.3 ± 5.23
FLT	60.7 ± 3.89	43.4 ± 1.44	46.6 ± 1.04	41.2 ± 0.151	46.8 ± 1.46	41.6 ± 0.139
PYR	48.9 ± 10.4	38.1 ± 1.11	7.80 ± 5.20	36.4 ± 0.131	30.7 ± 5.13	36.7 ± 0.120
BaA	23.6 ± 9.31	<LOD	5.73 ± 5.73	<LOD	<LOD	<LOD
CHR	8.65 ± 5.73	4.42 ± 4.42	<LOD	<LOD	<LOD	<LOD
BbF	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BkF	39.7 ± 7.74	41.3 ± 1.66	46.1 ± 1.96	38.5 ± 0.164	41.8 ± 5.13	39.0 ± 0.217
BaP	5.16 ± 5.16	45.6 ± 1.63	<LOD	43.1 ± 0.151	<LOD	43.3 ± 0.134
DahA	361 ± 81.4	548 ± 99.5	514 ± 84.5	393 ± 53.6	488 ± 80.0	357 ± 40.7
BghiP	5.06 ± 5.06	<LOD	<LOD	<LOD	<LOD	<LOD
IcdP	618 ± 181	392 ± 57.8	1140 ± 104	370 ± 23.1	1210 ± 181	343 ± 29.3
PCBs						
Non-*ortho*						
77	37.4 ± 7.76	<LOD	3.71 ± 3.71	<LOD	3.71 ± 3.71	<LOD
81	8.06 ± 5.33	<LOD	<LOD	<LOD	<LOD	<LOD
126	80.2 ± 13.3	4.77 ± 4.77	48.5 ± 6.31	<LOD	27.7 ± 7.59	<LOD
169	38.1 ± 7.87	29.6 ± 5.64	<LOD	11.1 ± 5.65	<LOD	7.19 ± 4.80
Mono-*ortho*						
105	78.5 ± 7.59	4.41 ± 4.41	51.5 ± 1.94	<LOD	30.0 ± 6.60	<LOD
114	36.2 ± 4.64	<LOD	<LOD	<LOD	<LOD	<LOD
118	205 ± 31.1	36.7 ± 0.777	82.4 ± 8.75	36.1 ± 0.375	46.1 ± 3.01	33.5 ± 3.73
123	52.2 ± 2.32	17.1 ± 6.80	8.31 ± 5.54	25.9 ± 5.66	3.85 ± 3.85	26.3 ± 5.75
156	59.6 ± 4.31	4.20 ± 4.20	31.4 ± 6.95	<LOD	8.42 ± 5.67	<LOD
157	40.6 ± 1.37	32.2 ± 4.12	7.50 ± 5.00	35.1 ± 0.175	3.80 ± 3.80	35.3 ± 0.169
167	55.5 ± 5.00	13.6 ± 6.88	31.5 ± 9.66	3.59 ± 3.59	12.9 ± 6.65	<LOD
189	22.2 ± 7.06	<LOD	<LOD	<LOD	3.41 ± 3.41	<LOD
NDL						
1	18.5 ± 5.86	3.58 ± 3.58	3.04 ± 3.04	<LOD	2.89 ± 2.89	2.88 ± 2.88
18	12.4 ± 6.23	<LOD	<LOD	3.65 ± 3.65	<LOD	<LOD
28	45.5 ± 2.22	<LOD	34.7 ± 3.90	<LOD	22.6 ± 6.17	<LOD
33	20.4 ± 6.48	<LOD	<LOD	<LOD	3.22 ± 3.22	<LOD
52	56.1 ± 9.11	<LOD	<LOD	<LOD	<LOD	<LOD
95	52.2 ± 9.81	<LOD	<LOD	<LOD	<LOD	<LOD
101	81.7 ± 10.9	<LOD	<LOD	<LOD	<LOD	<LOD
128	102 ± 23.8	21.7 ± 8.72	73.2 ± 22.4	3.96 ± 3.96	31.0 ± 6.84	3.95 ± 3.95
138	471 ± 104	25.8 ± 6.50	136 ± 26.3	24.8 ± 5.42	55.5 ± 5.08	3.49 ± 3.49
149	92.5 ± 18.2	<LOD	<LOD	6.95 ± 4.64	11.6 ± 5.89	<LOD
153	950 ± 226	38.0 ± 5.09	268 ± 65.6	35.3 ± 3.95	93.2 ± 15.8	7.66 ± 5.11
170	161 ± 31.6	12.7 ± 6.34	64.8 ± 12.3	7.07 ± 4.71	17.3 ± 7.25	<LOD
171	55.4 ± 8.37	<LOD	9.40 ± 6.31	<LOD	3.63 ± 3.63	<LOD
177	82.3 ± 11.2	<LOD	13.3 ± 6.81	<LOD	7.64 ± 5.09	<LOD
180	282 ± 63.0	17.8 ± 5.66	103 ± 25.4	17.7 ± 4.83	45.2 ± 6.87	<LOD
183	119 ± 22.0	4.14 ± 4.14	44.6 ± 5.74	<LOD	11.7 ± 5.96	<LOD
187	286 ± 61.0	17.5 ± 6.93	92.0 ± 15.4	7.40 ± 4.94	52.8 ± 4.17	<LOD
∑PAHs	1560 ± 197	1330 ± 164	2120 ± 106	1150 ± 75.7	2200 ± 219	1080 ± 44.2
∑PCBs	3600 ± 594	284 ± 52.7	1110 ± 203	219 ± 24.1	498 ± 80.1	120 ± 11.6

PAHs = polycyclic aromatic hydrocarbons; NAP = naphthalene; ACY = acenaphthylene; ACE = acenaphthene; FLU = fluorene; PHE = phenanthrene; ANT = anthracene; FLT = fluoranthene; PYR = pyrene; BaA = benzo[*a*]anthracene; CHR = chrysene; BbF = benzo[*b*]fluoranthene; BkF = benzo[*k*]fluoranthene; BaP = benzo[*a*]pyrene; DahA = dibenz[*a*,*h*]anthracene; BghiP = benzo[*g*,*h*,*i*]perylene; IcdP = indeno[1,2,3-*cd*]pyrene; PCBs = polychlorinated biphenyls; NDL = non-dioxin-like.

was essentially equivalent within and among species for each tissue, all tissue concentrations were reported and analyzed on a ng/g ww basis.

PAH burdens did not significantly differ by sex in blacktip liver ($F_{1,7} = 0.211$, $p = 0.660$) or muscle tissue ($F_{1,7} = 5.486$, $p = 0.052$). Additionally, no sex differences were found for PCB burdens in either liver ($F_{1,7} = 0.417$, $p = 0.539$) or muscle ($F_{1,7} = 0.494$, $p = 0.505$) in blacktips. Likewise, no significant differences between sexes were detected for ∑PAHs in the liver ($F_{1,6} = 0.001$, $p = 0.975$) and muscle ($F_{1,6} = 0.087$, $p = 0.778$) of bonnetheads, as well as ∑PCBs in the muscle ($F_{1,6} = 0.146$, $p = 0.716$). Although ∑PCBs did significantly differ between sexes in the liver of bonnetheads ($F_{1,6} = 13.372$, $p = 0.011$), only two males were evaluated. Therefore, both sexes were treated equivalently since PCB burdens in the males (273 and 347 ng/g ww) were close to that of a female at a similar size (385 ng/g ww). The interaction term was not significant in any of the ANCOVAs ($p > 0.05$) and was removed prior to final analysis of the results. Comparisons between sex were not analyzed for bull sharks since only a single female had PAH/PCB burdens quantified.

Interspecific comparisons of PAH and PCB burdens found that relationships among species differed by tissue. Significant differences in hepatic ∑PAHs ($F_{2,24} = 6.793$, $p = 0.005$) were detected among species, for which bull sharks had significantly lower concentrations of ∑PAHs relative to blacktips ($p = 0.007$) and bonnetheads ($p = 0.006$; Fig. 1A). Significant differences in hepatic ∑PCBs ($F_{2,24} = 25.423$, $p < 0.0001$) were also detected among species, but all species significantly differed in concentrations of ∑PCBs from each other ($p < 0.05$). PCB burdens were greatest in bull sharks, followed by blacktip and bonnethead sharks (Fig. 1A). While no interspecific differences were found for ∑PAHs in the muscle ($F_{2,24} = 0.508$, $p = 0.608$), significant differences were detected for ∑PCBs ($F_{2,24} = 4.432$, $p = 0.023$; Fig. 1B). A post-hoc test determined that bonnetheads had significantly lower burdens of PCBs than bull ($p = 0.023$) or blacktip sharks ($p = 0.025$) in muscle tissue. The interaction of FL and species was not significant in any of the ANCOVAs ($p > 0.05$). Lipid normalized concentrations of individual PAH and PCB congeners by species and tissue can be found in the Supplemental Data (Table S2). Correlations conducted separately on PAH and PCB burdens between liver and muscle tissues were only found to be significant in the case of ∑PCBs for bull ($r = 0.721$, $p = 0.028$) and blacktip sharks ($r = 0.770$, $p = 0.014$). Only weak and non-significant correlations were found for all other relationships (ranges: $r = -0.115$–0.343, $p = 0.332$–0.810).

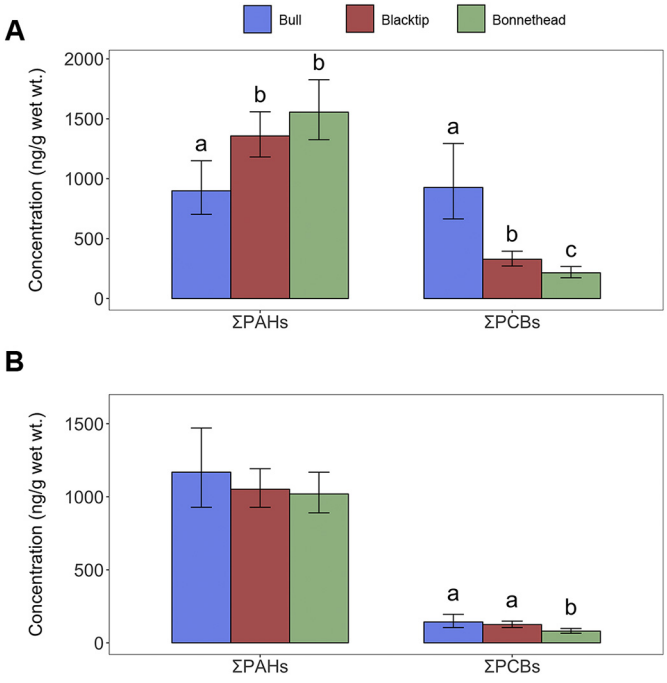

Fig. 1. Log_{10}-transformed PAH/PCB burdens were compared among species for each tissue within an ANCOVA framework that included FL as a covariate. Back-transformed adjusted means (±SE) from the ANCOVA are presented for both liver **(A)** and muscle **(B)**. Significant differences in PAH and PCB burdens were found in the liver (**A**), but only for PCBs in the muscle (**B**). Lowercase letters denote significant differences ($p < 0.05$).

3.2. Bioaccumulation of PAHs and PCBs

The linear regressions of PAH and PCB burdens with FL showed significant bioaccumulation of PCBs in the liver of bull sharks ($F_{1,7} = 6.205$, $p = 0.042$), but no significant relationship in muscle nor for either PAH regression ($p > 0.05$; Supplemental Data, Fig. S1). Unlike bull sharks, blacktips accumulated PCBs in both liver ($F_{2,7} = 30.840$, $p = 0.0003$) and muscle ($F_{1,8} = 9.333$, $p = 0.016$), but similarly did not accumulate PAHs ($p > 0.05$) with increasing length (Supplemental Data, Fig. S1). However, the regression of hepatic PCB burdens over increasing FL in blacktip sharks was best explained by a second-order polynomial that increased the R^2 from 0.49 to 0.87 compared to a simple linear regression. No significant bioaccumulation of PAHs or PCBs was found in the liver or muscle for bonnethead sharks ($p > 0.05$; Supplemental Data, Fig. S1).

Of the PAHs quantified, species means of indeno[1,2,3-*cd*]pyrene and dibenz[*a*,*h*]anthracene accounted for 57 to 77% of ∑PAHs in liver tissue (Fig. 2A), which was similar to proportions of these congeners in muscle (64–69% of ∑PAHs; Fig. 2B). Furthermore, species means of PCB 153 and PCB 138 comprised 32 to 36% of ∑PCBs in liver (Fig. 2A), whereas PCB 118 and PCB 157 were in greatest proportions (29–63% of ∑PCBs) in muscle (Fig. 2B). Overall congener profiles significantly differed among species in both liver (pseudo-$F_{2,26} = 5.532$, $p = 0.001$) and muscle (pseudo-$F_{2,26} = 4.277$, $p = 0.001$) samples. In the liver, the congener profile of bull sharks differed from both blacktips ($p = 0.003$) and bonnetheads ($p = 0.003$) following a Bonferroni-adjusted pairwise test. In muscle, however, only bull and bonnethead sharks had significantly different congener profiles ($p = 0.009$). Indeno[1,2,3-*cd*]pyrene, dibenz[*a*,*h*]anthracene, PCB 153, and PCB 128 were consistently responsible for the greatest separation among species (mean ± SE: 37.4 ± 1.43%) in the liver of the total 45 PAH and PCB congeners. The SIMPER analysis of hepatic congener profiles determined that blacktip and bonnethead sharks exhibited the least dissimilarity (~23%) on an individual congener basis, while the pairwise comparisons of bull – blacktip (~31%) and bull – bonnethead (~36%) displayed greater differences in their congener profiles. PCB 123 was responsible for the greatest separation among all comparisons in the muscle (mean ± SE: 12.8 ± 1.18%), but subsequent influential congeners varied with each pairwise comparison; many were DL-PCBs, however. Comparisons of overall dissimilarity in the muscle were lower for bull – blacktip and blacktip – bonnethead (~30%) than the comparison of bull – bonnethead (~38%). These relationships among species appear consistent with those established using ∑PAHs/∑PCBs in associated tissues.

To visualize species relationships in multivariate space, PCA ordinations were plotted for each species using congener profiles that were also scaled to unit variance. Ordinations of the liver congener profiles displayed a separation among species along the PC1 axis, where bull sharks occupied space along the positive PC1 axis and the other two species were found on the negative side of this axis (Fig. 3A). Blacktip and bonnethead sharks were primarily separated along the PC2 axis. While no size patterns were apparent in bonnetheads, relationships were present for the other species. The single YoY blacktip shark was on the opposite side of the PC2 axis from the larger conspecifics. In bull sharks, the largest conspecifics and a single YoY individual were found near the origin, another YoY individual was found on the opposite side of the PC2 axis, and the remaining three juveniles were clustered to the far side of the positive PC1 axis. Clustering of bull sharks was primarily driven by greater proportions of LMW PAHs compared to the other

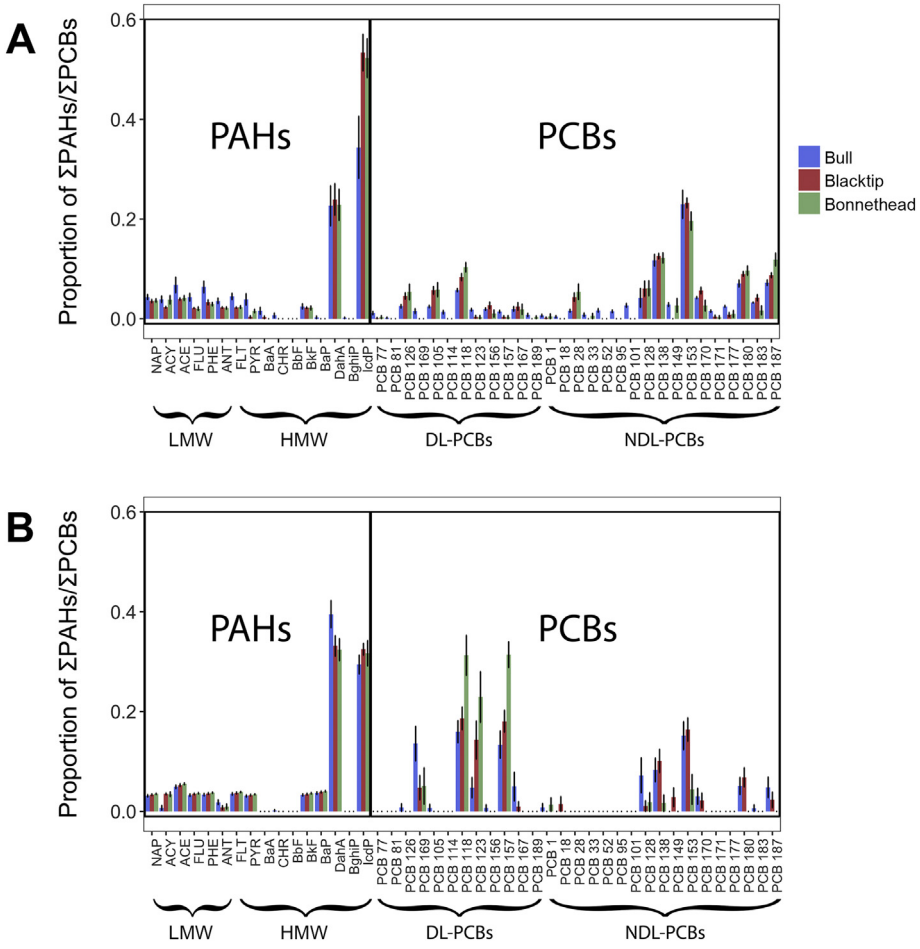

Fig. 2. The individual congener profiles were compared among species, normalized to ΣPAHs and ΣPCBs (mean ± SE). PAHs are denoted as either low (LMW) and high molecular weight (HMW) congeners while PCBs are grouped as either dioxin-like (DL-PCBs) or non-dioxin-like PCBs (NDL-PCBs). In the liver (**A**), PAHs were dominated by the heavy congeners indeno[1,2,3-*cd*]pyrene and dibenz[*a*,*h*]anthracene while hexa- and heptachlorinated congeners were the prevalent PCBs for all species. The profile in the muscle (**B**) was slightly different, with a change in the most abundant PCB congeners and substantially fewer PAH and PCB congeners detected. Significant species differences were detected for congener profiles in both liver and muscle ($p < 0.05$).

species, while most blacktips were influenced by HMW PAHs and bonnetheads were associated with greater proportions of recalcitrant PCB congeners. Although the sample size of bull sharks ($n = 3$) precluded the analysis of a relationship between biomarker activity and LMW/HMW PAHs, relationships were examined in bonnetheads. Proportions of LMW and HMW PAHs did not exhibit a significant relationship with $\log_{10}$-transformed EROD activity ($F_{1,8} = 4.853$, $p = 0.059$). High overlap was found within the multivariate ordination of muscle congener profiles, where blacktip sharks were located between bull and bonnethead sharks (Fig. 3B). Despite the level of overlap, conspecifics were relatively dispersed compared to interspecific overlap. However, bonnetheads were primarily concentrated on the positive side of the PC1 axis and negative side of the PC2 axis. Clustering of bonnethead sharks in the muscle PCA was primarily affected by greater proportions of a few mono-*ortho* DL-PCBs and indeno[1,2,3-*cd*]pyrene. While there was no clear pattern for the congeners driving the distribution of bull and blacktip sharks, these species were broadly impacted by HMW PAHs and the remaining PCB congeners. Additionally, there did not appear to be any patterns due to size within or among species.

3.3. Biomarker activity

EROD and GST activities did not significantly differ by sex or with FL in blacktip or bonnethead sharks (ANCOVA: $p > 0.05$) and could not be evaluated in bull sharks. Differences in $\log_{10}$-transformed EROD activity were found among species ($F_{2,45} = 5.027$, $p = 0.011$); mean activity was greater in bonnethead sharks (4.11 pmol/min/mg protein) than blacktip (2.19 pmol/min/mg protein; $p = 0.005$) and bull sharks (2.02 pmol/min/mg protein; $p = 0.034$) (Fig. 4A). Significant differences in $\log_{10}$-transformed GST activity were also detected among species ($F_{2,38} = 14.394$, $p < 0.0001$); mean activity in bull sharks (121 nmol/min/mg protein) was significantly lower than blacktip (200 nmol/min/mg protein; $p < 0.0001$) and bonnethead sharks (197 nmol/min/mg protein; $p < 0.0001$) (Fig. 4B). No significant correlations of EROD or GST activity with hepatic burdens of $\sum$PAHs, $\sum$PCBs, DL-PCBs, or non-dioxin-like PCBs (NDL-PCBs) were found for bull or bonnethead sharks ($p > 0.05$). Significant correlations were detected in the liver of blacktips for concentrations of $\sum$PCBs ($\rho = 0.69$, $p = 0.035$) and DL-PCBs ($\rho = 0.79$, $p = 0.0098$) with GST activity. However, correlations between GST activity and other classes of contaminants ($\sum$PAHs and NDL-PCBs) were not significant ($p > 0.05$). No significant correlations were measured with respect to EROD in blacktip sharks ($p > 0.05$).

3.4. Integration of tissue burdens and biomarker activity

The pRDA explained little variance ($R^2_{adj} = -0.064$) of the congener profiles with EROD ($p = 0.551$) and GST ($p = 0.983$) following permutation tests, but tentatively displayed some relationships of individual congeners with specific biomarkers (Fig. 5). An assessment of the angles between explanatory and response variables of the pRDA ordination in the liver showed a positive correlation between EROD activity and

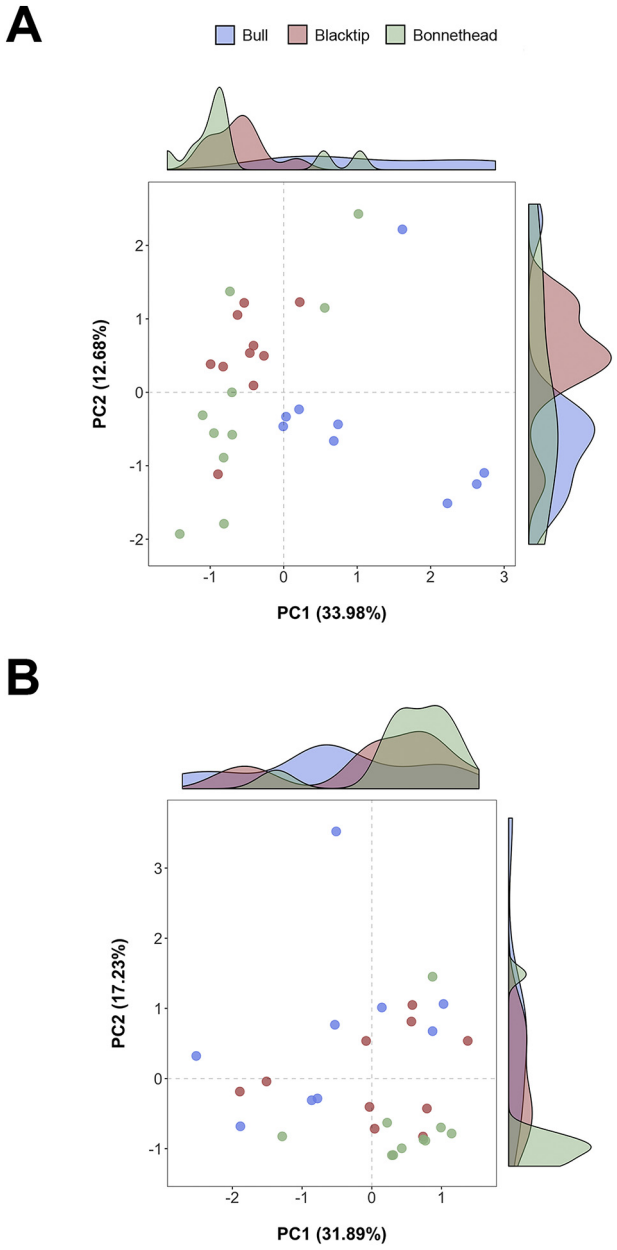

Fig. 3. Congener profiles were used to determine relationships among species by PCA in liver (**A**) and muscle (**B**). Marginal density plots are included on the PC1 and PC2 axes to visualize the distribution of individuals within each species. Hepatic congener profiles showed a separation of bull sharks from blacktips and bonnetheads along the PC1 axis, while the latter species primarily separated along the PC2 axis (**A**). Species were not clearly distinguished by muscle congener profiles, for which overlap was present across both axes (**B**).

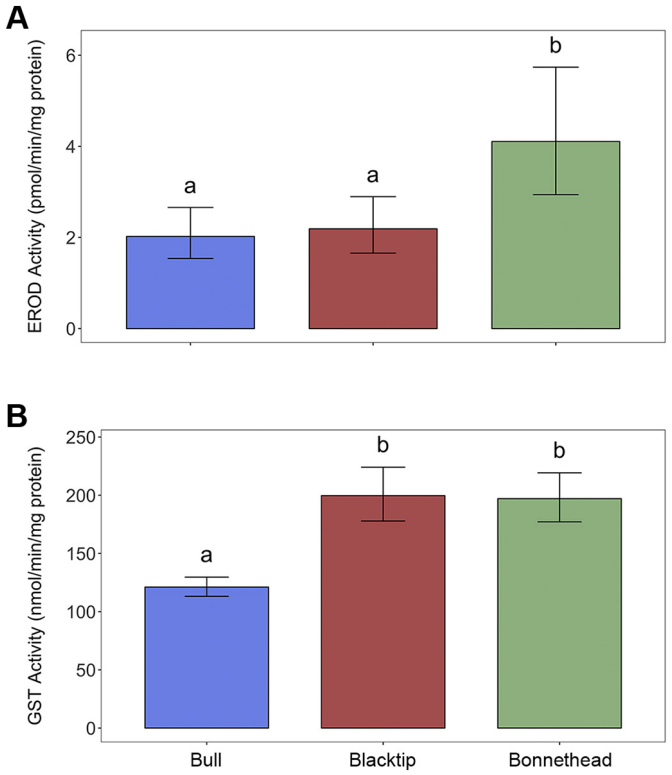

Fig. 4. Log_{10}-transformed biomarker data were analyzed by a weighted GLS ANOVA to determine relationships among species with unequal sample sizes. Although transformed data were analyzed for omnibus and pairwise comparisons, back-transformed mean ± SE are displayed for interpretability. EROD activity in bonnethead sharks was significantly greater than bulls ($p = 0.034$) or blacktips ($p = 0.005$), for which greater variance was also observed in bonnetheads (**A**). GST activity was significantly greater in blacktip ($p < 0.0001$) and bonnethead sharks ($p < 0.0001$) than bulls (**B**). Lowercase letters denote significant differences ($p < 0.05$).

proportions of congeners dibenz[*a*,*h*]anthracene and PCB 170, as well as between GST activity and PCB 167. Additionally, PCBs 126, 128, and 149 were found between the two constraints (EROD and GST activity), which suggests positive correlations with both biomarkers. Alternatively, PCB 105 was found to be strongly opposite of the GST activity vector and therefore displayed a negative correlation with this biomarker. A similar relationship was observed between PCB 153 and EROD activity, which suggests a negative relationship as well.

3.5. Potential toxicity of tissue burdens (TEQs)

Comparisons of TEQ_{PCBs} in the liver among species was confounded by interactions of species with sex ($p = 0.0003$) as well as FL ($p = 0.0037$), which prevented direct comparisons. Liver TEQ_{PCBs} in bull sharks appeared greater than in the other species with no discernible differences over FL (Supplemental Data, Fig. S2), while the two male bonnetheads had lower TEQ_{PCBs} than most females. Although there did not appear to be any sex differences in blacktip sharks, liver TEQ_{PCBs} appeared to increase with increasing FL. Similarly, interspecific comparisons of TEQ_{PCBs} in muscle tissue were confounded by an interaction between species and sex ($p < 0.0001$). The only apparent sex difference occurred within blacktip sharks, for which most males had greater muscle TEQ_{PCBs} than females (Supplemental Data, Fig. S2). When comparing between tissues, the range of mean TEQ_{PCBs} by species in the liver (140–414 pg/g ww) was much higher than in the muscle (0.840–25.9 pg/g ww) across all species (Table 3); TEQs calculated on a lipid weight basis are reported in the Supplemental Data (Table S3). Of all the DL-PCBs, PCB 126 represented the greatest contributor to TEQ_{PCBs} calculations (median 98.6%) across all species in the liver. In the muscle, more mono-*ortho* and other non-*ortho* DL-PCBs contributed to the TEQ_{PCBs} calculation besides PCB 126. For comparison with established threshold levels of physiological effects from the literature, hepatic TEQ_{PCBs} were also calculated on a pg/g lipid weight (lw) basis. Although these interspecific comparisons were also confounded by interactions with sex ($p = 0.0005$) and FL ($p = 0.0066$), the mean for each species (range: 445–1550 pg/g lw TEQ_{PCBs}) was well above the lower threshold for physiological effects in multiple taxonomic groups. Kannan et al. (2000) suggested that the lower threshold for physiological effects induced by PCB burdens in pinnipeds (*Phoca vitulina*), cetaceans (*Tursiops truncatus*, *Delphinapterus leucas*), and mustelids (*Lutra lutra*, *Mustela vison*) is 160 pg/g lw TEQ_{PCBs}. Furthermore, the 99% tissue-residue benchmark (TRB) for early life stage fish is suggested to be 57pg/g lw TEQ_{PCBs} (Steevens et al., 2005) (Fig. 6). Outliers from

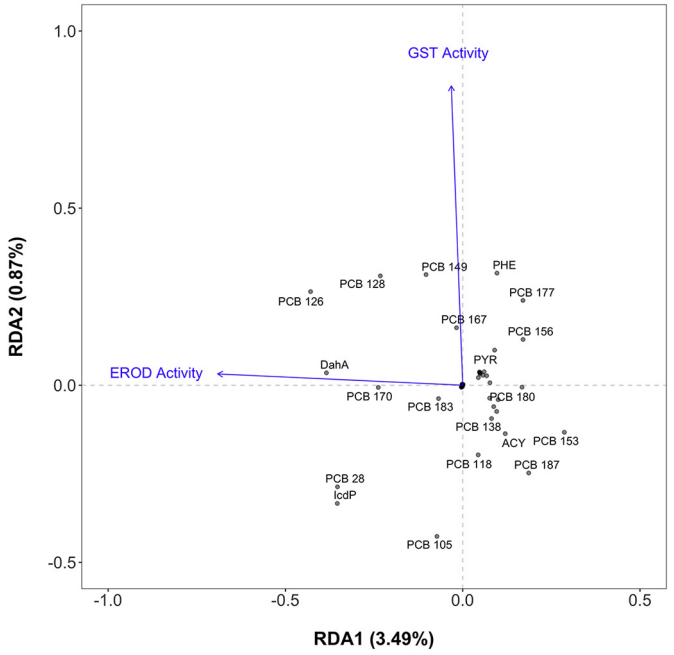

Fig. 5. The pRDA did not appear to be strongly informed by biomarker activity in the liver, but some correlations with individual congeners were present. Hepatic proportions of dibenz[*a*,*h*]anthracene and PCB 170 appeared to show positive correlations with EROD activity, albeit not very strong. Meanwhile, PCB 126, PCB 128, and PCB 149 displayed varying levels of positive correlation with EROD and GST, falling between the vectors for both constraints. Congeners that were tightly clustered, particularly around the origin, were not labeled to improve interpretation of the ordination. Additionally, these unlabeled congeners are not well-explained by either biomarker due to their presence close to the origin.

individual bull (3810 pg/g lw) and bonnethead sharks (1760 pg/g lw) were much higher than the upper limits set by both Kannan et al. (2000) and Steevens et al. (2005).

By comparison, TEQ_{PAHs} were much greater than TEQ_{PCBs} despite having fewer compounds contributing to its calculation. In the liver, mean TEQ_{PAHs} for the three species ranged from 1320 to 2460 pg/g ww (Table 3) and significant differences were detected among species ($F_{2,24} = 11.464$, $p = 0.0003$). Similar to the analyses using $\sum$PAHs and congener profiles, TEQ_{PAHs} were lower in bull sharks than blacktips ($p < 0.001$) and bonneteads ($p < 0.001$; Supplemental Data, Fig. S2). Proportions of indeno[1,2,3-*cd*]pyrene in the liver accounted for nearly the entire amount of TEQ_{PAHs} for each species (range of means: 85–91%). Although no differences were found among species for muscle TEQ_{PAHs} ($F_{2,25} = 0.623$, $p = 0.5446$; Supplemental Data, Fig. S2), mean values for each species (803–950 pg/g ww) were still higher than in either tissue for TEQ_{PCBs}. The greatest contributor to TEQ_{PAHs} in the muscle was also indeno[1,2,3-*cd*]pyrene for all species (range of means: 77–81%), followed by dibenz[*a*,*h*]anthracene (range of means: 12–16%). The only significant correlation found between TEQs and biomarker activity was a negative relationship ($\rho = -0.68$, $p = 0.035$) between hepatic TEQ_{PCBs} and EROD activity in blacktip sharks.

4. Discussion

4.1. Comparisons of PAH and PCB burdens in Galveston Bay sharks

Interspecific relationships defined by $\sum$PAHs and $\sum$PCBs varied by tissue, for which blacktip and bonnethead sharks were most similar in their hepatic burdens of both contaminants (Fig. 1A), but blacktips and bulls were more similar in their muscle PCB burdens (Fig. 1B). Since diet and life history characteristics are known to affect differences

Table 3
Mean concentrations of DL-PCBs and PAHs (ng/g ww) and associated TEQs (pg/g ww) in the liver and muscle.

		Bull (*C. leucas*)				Blacktip (*C. limbatus*)				Bonnethead (*S. tiburo*)			
		Liver		Muscle		Liver		Muscle		Liver		Muscle	
Non-*ortho*	TEF[a]	Conc.	TEQ	Conc.	TEQ	Conc.	TEQ	Conc.	TEQ	Conc.	TEQ	Conc.	TEQ
77	0.0001	37.4	3.74	<LOD	0.00	3.71	0.370	<LOD	0.00	3.71	0.370	<LOD	0.00
81	0.0005	8.06	4.03	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00
126	0.005	80.2	401	4.77	23.9	48.6	243	<LOD	0.00	27.7	139	<LOD	0.00
169	0.00005	38.1	1.91	29.6	1.48	<LOD	0.00	11.1	0.555	<LOD	0.00	7.19	0.360
Total		164	411	34.4	25.4	52.3	243	11.1	0.555	31.4	139	7.19	0.360
Mono-*ortho*													
105	0.000005	78.5	0.393	4.41	0.0221	51.5	0.258	<LOD	0.00	30.0	0.150	<LOD	0.00
114	0.000005	36.2	0.181	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00
118	0.000005	205	1.03	36.7	0.184	82.4	0.412	36.1	0.181	46.1	0.231	33.5	0.168
123	0.000005	52.2	0.261	17.2	0.0860	8.31	0.0416	25.9	0.130	3.85	0.0193	26.3	0.132
156	0.000005	60.0	0.300	4.20	0.0210	31.4	0.157	<LOD	0.00	8.42	0.0421	<LOD	0.00
157	0.000005	40.6	0.203	32.2	0.161	7.50	0.0375	35.1	0.176	3.80	0.0190	35.3	0.177
167	0.000005	55.5	0.278	13.6	0.0680	31.5	0.158	3.59	0.0180	12.9	0.0645	<LOD	0.00
189	0.000005	22.2	0.111	<LOD	0.00	<LOD	0.00	<LOD	0.00	3.41	0.0171	<LOD	0.00
Total		550	2.76	108	0.542	213	1.06	101	0.505	108	0.543	95.1	0.477
Total PCBs		714	414	142	25.9	265	244	112	1.06	139	140	102	0.837
PAHs	FPF[b]												
FLT	0.000000002	60.7	1.21×10^{-4}	43.4	8.68×10^{-5}	46.6	9.32×10^{-5}	41.2	8.24×10^{-5}	46.8	9.36×10^{-5}	41.6	8.32×10^{-5}
PYR	0.000000385	48.9	0.0188	38.1	0.0147	7.80	3.00×10^{-3}	36.4	0.0140	30.7	0.0118	36.7	0.0141
BaA	0.0002	23.6	4.72	<LOD	0.00	5.73	1.15	<LOD	0.00	<LOD	0.00	<LOD	0.00
CHR	0.0000659	8.65	0.570	4.42	0.291	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00
BbF	0.000166	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00
BkF	0.00128	39.7	50.8	41.4	53.0	46.1	59.0	38.5	49.3	41.8	53.5	39.0	49.9
BaP	0.00024375	5.16	1.26	45.6	11.1	<LOD	0.00	43.1	10.5	<LOD	0.00	43.4	10.6
DahA	0.000272	361	98.2	548	149	514	140	393	107	488	133	357	97.1
BghiP	0.0000102	5.06	0.0516	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00	<LOD	0.00
IcdP	0.00188	618	1160	392	737	1140	2140	370	696	1210	2270	343	645
Total PAHs		1170	1320	1110	950	1760	2340	922	863	1820	2460	861	803

TEF = toxic equivalency factor; Conc. = concentration; TEQ = toxic equivalent; <LOD = below the limit of detection; FPF = fish potency factor; refer to Table 2 for other abbreviations.
[a] Van den Berg et al. (1998).
[b] Barron et al. (2004).

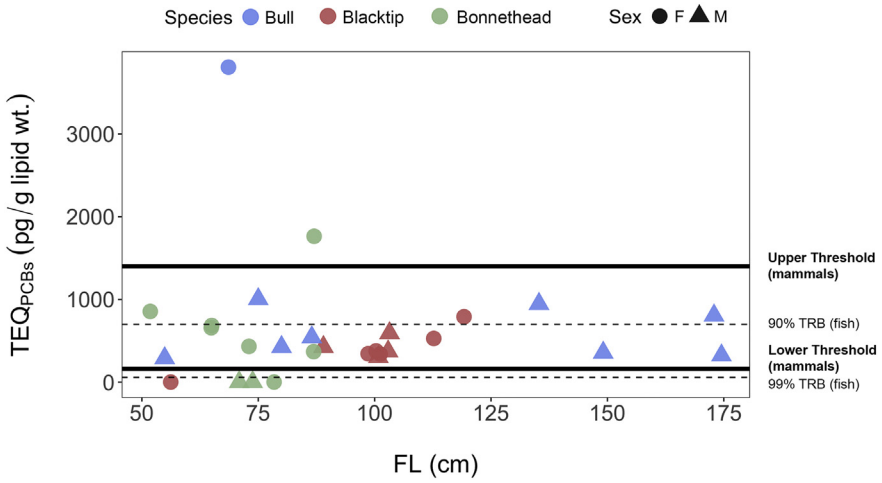

Fig. 6. A comparison with established thresholds in aquatic mammals (pinnipeds, cetaceans, mustelids; Kannan et al., 2000) and tissue residue-based toxicity benchmarks (TRBs) for early life stage fishes (Steevens et al., 2005) shows that the TEQ_{PCBs} in the liver of these sharks may result in possible physiological impacts. Since significant interactions of species with sex and body size (FL) were detected, direct species comparisons could not be made. FL is shown on the x-axis and sex is denoted by shape for interpretation within and among species in comparison to each other and the established thresholds. With the exception of four individuals, all sharks had liver TEQ_{PCBs} above the lower residue effect concentrations. Two high outliers (bull: 3807.24 pg/g lw; bonnethead 1762.80 pg/g lw) had measurements above the upper threshold established for aquatic mammals.

in the accumulation of PAHs and PCBs (Baumard et al., 1998; Fisk et al., 2001; Van der Oost et al., 2003; Borgå et al., 2004; Gelsleichter and Walker, 2010), the similarity of blacktip sharks with bonnetheads is unexpected given how closely related the former is to bull sharks phylogenetically, as well as by trophic position and life history. Crustaceans comprise the majority of the diet in bonnetheads (Bethea et al., 2007; Plumlee and Wells, 2016), while blacktips primarily consumes teleost fishes (Barry et al., 2008; Plumlee and Wells, 2016) and bull sharks consume crustaceans, teleosts, marine mammals, and other elasmobranchs at varying ontogenetic stages (Snelson et al., 1984; Cliff and Dudley, 1991). Niche partitioning may provide an explanation for the apparent similarity of blacktip and bonnethead sharks in and around Galveston Bay, in which blacktips and bonnetheads overlap by geographic location and bull sharks occupy different sections of this coastal system. Furthermore, ontogenetic shifts in diet and habitat can impact exposure to PAHs and PCBs by changes in prey size, composition, and contamination status of foraging locale (Cliff and Dudley, 1991; Bethea et al., 2007; Barry et al., 2008; Plumlee and Wells, 2016). It is important to note that not all shark size classes were evenly sampled from each species, which might obscure some relationships.

Since sharks rely upon hepatic lipid stores for energy, burdens of organic contaminants can become concentrated during periods of stress-induced lipid mobilization (Kelly et al., 2011; Belicka et al., 2012; Daley et al., 2014; Olin et al., 2014). Liver burdens are considered to be indicative of acute exposure because of the highly dynamic nature of this tissue compared to muscle (Albaigés et al., 1987). However, muscle burdens are useful to assess chronic exposure because of the slower turnover rate of this tissue and lower likelihood of concentrating these contaminants due to lipid mobilization (Albaigés et al., 1987; Daley et al., 2014; Beaudry et al., 2015). Since muscle concentrations of ∑PAHs did not differ among species and ∑PCBs were more similar between bull and blacktip sharks than with bonnetheads, these results indicate that all species are exposed to similar chronic concentrations of PAHs, but PCBs accumulate at greater concentrations in the higher trophic position carcharhinids (bulls and blacktips). However, it appears that blacktip and bonnethead sharks are more similar in terms of recent exposure to PAHs and PCBs than bull sharks based on hepatic burdens. Additionally, significant positive correlations of ∑PCBs between tissues in bull and blacktip sharks suggest that PCB burdens in muscle can serve as a non-lethal measure of liver burdens in these species. Future studies should determine whether this relationship is only reliable in high trophic level organisms.

4.2. Bioaccumulation of PAHs and PCBs

PAHs did not significantly bioaccumulate over increasing FL in any species, but mean concentrations were consistently higher than expected in both liver (1560–2200 ng/g ww) and muscle (1080–1330 ng/g ww). To date, only two other studies have quantified PAH burdens in shark tissue (Al-Hassan et al., 2000; Marsili et al., 2016). Mean concentrations of ∑PAHs measured by Al-Hassan et al. (2000) varied from 130 to 33,460 ng/g ww in the liver and <LOD to 34,840 ng/g ww in muscle of sharks sampled from the Arabian Gulf after the first Gulf war. Additionally, concentrations of ∑PAHs measured in white sharks (*Carcharodon carcharias*) by Marsili et al. (2016) ranged from 2769.20–7278.40 ng/g dry weight (426.46–1120.87 ng/g ww; mean of 84.6% water content) in muscle tissue. Samples in the present study fall within the middle of these ranges quantified by previous studies. High concentrations of PAHs in muscle and liver demonstrate chronic exposure to these contaminants and an inability to effectively metabolize and eliminate them faster than they are absorbed. This can be partially attributed to low CYP1A activity (as measured by EROD) of elasmobranchs compared to teleosts (min – max: ~0.5–5 and 1.1–205.9 pmol/min/mg protein, respectively; Gorbi et al., 2004; Solé et al., 2009, 2010) and marine mammals (min–max: 199–2167; Van den Berg et al., 1998; Tanabe, 2002; Letcher et al., 2014). However, it is likely that this does not fully account for the concentrations found in both tissues in sharks from the present study. These findings suggest that trophic dilution of PAHs may not occur with respect to elasmobranchs since concentrations measured in oysters from 2010 (range of means: 134–333 ng/g dry wt.; Apeti et al., 2013) in Galveston Bay (not including the Houston ship channel) are lower than the sharks from the present study.

PCBs bioaccumulated in the liver of bull sharks as well as the liver and muscle of blacktips, which was similar to data reported by Gilbert et al. (2015). Previous studies of bull sharks (Olin et al., 2014) and blacktips (Gelsleichter et al., 2007) may not have detected PCB bioaccumulation with increasing body size since their datasets had a limited ontogenetic range of samples. Mean hepatic ∑PCB concentrations in these prior studies were lower for bulls (2654 ng/g ww), but higher for blacktips (2930 ng/g ww). Compared to the muscle ∑PCB concentrations in bull sharks collected from two different time periods (1993–1994: 6440 ng/g lw; 2002–2004: 71200 ng/g lw; Johnson-Restrepo et al., 2005) in Florida, the mean ∑PCBs in the present study (35,400 ng/g lw) was similar. Although bonnethead sharks did not bioaccumulate PCBs, mean hepatic ∑PCBs were very similar to burdens

measured in conspecifics from a historically polluted site in Georgia (520 ng/g ww; Gelsleichter et al., 2008). Since many invertebrates lack comprehensive PAH/PCB biotransformation capabilities (Livingstone, 1998; Hylland, 2006), the consumption of crabs and other benthic invertebrates may explain the exposure of bonnetheads to such high concentrations of these contaminants. Thus, it appears that the low trophic position of this species does not buffer it from the accumulation of high concentrations of PCBs in contaminated habitats.

4.3. Congener profiles of PAH/PCB burdens

Results analyzed on an individual congener basis corroborated the relationships found by comparing ∑PAHs and ∑PCBs, with the exception that the congener profile of blacktip sharks did not significantly differ from bull or bonnethead sharks in muscle tissue. PCB 123 was the primary driver of separation for this different pattern in muscle tissue, for which blacktips had greater proportions of this congener than bulls, but less than what was measured in bonnetheads. It is currently unclear why these species have accumulated different proportions of this particular PCB congener. Additionally, bull and bonnethead sharks were consistently found to be the most different based on congener profiles from both tissues. Differences between these species were primarily influenced by greater proportions of indeno[1,2,3-*cd*]pyrene in bonnetheads for liver, but by greater proportions of PCB 123 in bonnetheads for muscle. Congener profiles in the muscle, reflective of chronic exposure, may indicate that blacktip sharks share sources of PAHs with the other two species, but sources of PCB exposure primarily with bull sharks.

With respect to contributions of all congeners, PAHs were dominated by indeno[1,2,3-*cd*]pyrene and dibenz[*a*,*h*]anthracene among all species for both tissues. LMW PAHs are often indicative of petrogenic sources, whereas a greater proportion of HMW PAHs reflect pyrogenic sources. It is difficult to assess the history of PAH exposure from tissue concentrations since this organic contaminant is readily metabolized by most vertebrates and ratios can be altered during trophic transfer (Varanasi et al., 1987; Meador et al., 1995; Baumard et al., 1998). Galveston Bay is primarily impacted by pyrogenic sources as a result of incomplete combustion (Brooks et al., 1992), which would explain the large proportions of HMW PAHs indeno[1,2,3-*cd*]pyrene and dibenz[*a*,*h*]anthracene. Dominant PCB congeners differed by tissue for all species: two highly recalcitrant congeners (PCB 153 and PCB 138) were measured in the greatest proportions for all species in the liver, but mono-*ortho* substituted DL-PCBs 118 and 157 were the primary contributors in the muscle (with the exception of PCB 153 instead of PCB 157 in bull sharks). The differences between tissues may result from the redistribution of congeners among these compartments, especially during lipid mobilization (Van den Berg et al., 1998; Daley et al., 2014). Additionally, DL-PCBs may be accumulating in greater proportions in muscle since this tissue has negligible CYP1A1 activity when compared to the liver (Wilson et al., 2010; Nielsen et al., 2017). Comparing PCB congener profiles between tissues, there is a greater proportion of DL-PCBs in the muscle of all species compared to the liver (Fig. 2). This is particularly true of bonnethead sharks, whose muscle burden of DL-PCBs (91% of ∑PCBs) was nearly twice that of its hepatic burden (46% of ∑PCBs). So while the muscle may serve as a compartment reflective of chronic exposure to ∑PCBs, the congener profiles are likely distorted as a result of multiple physiological processes.

Patterns of species distributions in the PCA biplot of hepatic congener profiles appear to be reflective of differences in biotransformation capability and exposure. Clustering of bonnethead sharks was positively associated with greater proportions of recalcitrant PCB congeners, which differed from the positive correlations of HMW PAHs with blacktips and LMW PAHs in bull sharks. Although bonnetheads had greater EROD activity than the other two species, which would presumably indicate greater metabolism of PAHs by CYP1A, the relationship between EROD and proportions of LMW/HMW PAHs was not significant. Further characterization of PAH and PCB biotransformation capability in these species is needed to determine the possible factors driving these differences. Additionally, blacktip sharks had greater proportions of HMW PAHs than the other species, which is reflective of its high exposure and lesser ability to biotransform these burdens (lower EROD activity than bonnetheads). The association of bull sharks with LMW PAHs is likely an effect of having lower proportions of HMW PAHs than the other species, as well as lower proportions of the recalcitrant PCBs due to more PCB congeners being detectable in this species. The outlier YoY bull shark had much lower hepatic lipid content (16.6%) compared to older conspecifics (mean of 68.8% for others). This is likely a result of lipid mobilization as the YoY shark depletes its intrinsic energy store in the liver, which may cause redistribution and concentration of the quantified PAH and PCB congeners. Therefore, the relationships of bull and bonnethead sharks regarding the loadings of the liver PCA values appear to be a product of differences in biotransformation capability and exposure to these pollutants.

Patterns of species clusters and PC loading values were less discernible in the PCA of muscle congener profiles. Due to similar proportions of many congeners, bull and blacktip sharks exhibited high overlap. Most bonnetheads were clustered due to high proportions of the mono-*ortho* substituted DL-PCBs 118, 123, and 157. Since concentrations of PCBs 118, 123, and 157 in the muscle were comparable across all species (Table 2), these species clusters reflect the accumulation of a greater number of PCB congeners in bull and blacktip sharks compared to bonnetheads (Fig. 2B).

4.4. Biomarker activity and integration with tissue-based burdens

Although comparisons of exposure can be drawn from the quantification of tissue-based burdens, additional measurements are necessary to determine if any physiological effects are elicited since these are often species-specific. EROD is a commonly measured phase I biomarker that is reflective of CYP1A1 induction, often by dioxin-like compounds and other planar aromatic hydrocarbons. The comparison of EROD activity among the sharks from the present study indicates higher and more variable activity in bonnethead sharks than in bulls or blacktips. Since this biomarker can also be affected by extrinsic and intrinsic factors, variance may be partially attributable to variables besides known AhR ligands (Whyte et al., 2000). Additionally, none of the contaminant classes (∑PAHs, ∑PCBs, DL-PCBs, NDL-PCBs) were found to significantly correlate with EROD in any species. Therefore, further investigation that includes the measurement of fluorescent aromatic hydrocarbons (FACs) and hydroxylated PCBs in the bile may be necessary to detect a significant relationship with exposure.

GST is another commonly measured biomarker (phase II) that conjugates electrophilic compounds for elimination from the body, often after CYP1A oxidation of a xenobiotic (Van der Oost et al., 2003). Greater GST activity was measured in the two species with higher hepatic burdens of ∑PAHs (blacktip and bonnethead sharks), although no significant correlations were measured between these variables. Significant positive correlations of GST activity with hepatic ∑PCBs and DL-PCBs were found for blacktips, but no other significant correlations were detected. Without knowing the baseline activity rates of EROD and GST for each species, it is difficult to assess whether these values represent significant induction of biotransformation pathways. Therefore, future studies should quantify activity rates for these enzymes in both sexes of each species over multiple seasons to determine a robust baseline to compare against.

Despite the increased attention sharks and other elasmobranchs have recently received in the field of environmental toxicology, the integration of tissue-based burdens with biomarkers and toxic-endpoints has been very limited (Lyons et al., 2014; Alves et al., 2016). The direct approach of integrating burdens of individual congeners with biomarker activity provides a better understanding of metabolic differences regarding bioaccumulation as opposed to the approach of

summing all analyzed congeners. This sum total approach is also limited in explaining the actions of complex mixtures, especially with regard to toxic effects. In the present study, relationships between congeners and biomarker activity in the pRDA were not very clear, as indicated by the permutation test and R^2_{adj}. Congeners known to induce CYP1A (dibenz[*a*,*h*]anthracene, indeno[1,2,3-*cd*]pyrene, PCB 126) and those that are not typically associated with the activation of this transduction pathway (PCBs 28, 128, 170) both showed positive relationships with EROD activity. These unexpected relationships may be a result of using proportions rather than raw or transformed concentrations for each PAH/PCB congener. Conversely, PCB 153 appears to display a negative relationship with EROD activity. When at high concentrations, this recalcitrant congener has demonstrated a negative effect on EROD activity in previous studies as a result of competitive inhibition (Suh et al., 2003; Chen and Bunce, 2004). If this congener were to inhibit the AhR complex, agonists of this receptor (e.g. PAHs, DL-PCBs, PCDDs, PCDFs) may accumulate at greater than expected concentrations (Suh et al., 2003). Since bull sharks accumulate particularly high concentrations of PCB 153, likely as a result of its high trophic position, this species may experience further accumulation of PAHs and DL-PCBs if CYP1A activity is substantially impacted. Only two congeners showed noticeable positive (PCB 167) and negative (PCB 105) correlations with GST activity. Since relationships of individual PAH and PCB congeners with GST have not been explored as thoroughly as its CYP450 counterparts, it is currently unclear what mechanisms may be driving these patterns.

4.5. Potential toxicity of PAH and PCB burdens

Although many studies have quantified tissue-based burdens of organic contaminants in sharks, few have assessed the potential toxicity of these accumulated contaminants (Serrano et al., 2000; Storelli et al., 2005; Corsolini et al., 2014). Mean TEQ_{PCBs} values were greatest in bull sharks and lowest in bonnetheads, while blacktips were intermediate. An unexpected negative correlation of hepatic TEQ_{PCBs} with EROD activity was detected in blacktip sharks, for which possible causes remain unclear. Since few data are available that compare toxic endpoints with TEQ_{PCBs} in elasmobranchs, comparisons were made against thresholds set for a sensitive group of fish (early life stage) and aquatic mammals. It is not currently known whether sharks and other elasmobranchs elicit physiological responses at similar levels as these other groups, but if they do, then these species could be prone to negative health outcomes such as decreased vitamin A concentrations, thyroid hormone deficiency, and immunosuppression (Kannan et al., 2000). As a caveat, however, TEFs for DL-PCBs in fish were derived from studies that used early life stage mortality as the toxic endpoint (Van den Berg et al., 1998). Therefore, these TEFs may not be directly applicable to older age classes. Additionally, TEQs cannot explain the toxic effects resulting from high concentrations of NDL-PCBs. Although di-*ortho* through tetra-*ortho* substituted PCBs do not induce CYP1A1 activity via AhR signal transduction, these congeners may still cause toxic effects such as carcinogenicity, neurotoxicity, and endocrine disruption (Safe, 1994; Giesy and Kannan, 1998). Therefore, further work is warranted for characterizing the toxic endpoints of NDL-PCBs that accumulate at high concentrations in elasmobranchs, such as PCB 153.

The toxicity of PAH burdens is often overlooked in place of quantifying their metabolites since they are considered to be readily biotransformed in vertebrates (Van den Berg et al., 1998). However, it appears that PAHs are not readily metabolized by sharks measured in the present study. Due to the lack of studies that correlate TEQ_{PAHs} (relative to TCDD) with toxic effects in fish, it is not currently feasible to assess the potential toxicity of these measurements. Additionally, the FPFs used to calculate TEQ_{PAHs} were based upon studies measuring CYP1A induction and AhR affinity as endpoints rather than toxicity, for which FPFs would be expected to be lower. It is also important to mention that the FPF for the dominating PAH congener indeno[1,2,3-*cd*]pyrene was derived from eight studies, of which only one was conducted on fish (Barron et al., 2004). Therefore, it is difficult to assess the potential toxicity of these PAH burdens without the inclusion of biomarkers of effect and other toxic endpoints. For comparative purposes, the TEQ_{PAHs} were greater in blacktip and bonnethead sharks than in bulls, but only for measurements in the liver. However, mean values of TEQ_{PAHs} could not be directly compared against TEQ_{PCBs} since the former are based upon biomarkers of exposure, which would result in higher TEQ_{PAHs} than if based upon toxic endpoints. Although it is currently difficult to discern the toxic effects of the TEQ_{PAHs} measured in the present study, the use of TEQ_{PCBs} alone may underestimate the potential toxicity exhibited by the accumulated PAH/PCB burdens in these sharks.

While most of the TEQs measured in the present study did not significantly correlate with EROD activity, it is possible that these levels represent a sequestered depot of pollutants stored in lipid-rich tissues (e.g. liver; McElroy et al., 2011). Although CYP1A is an inducible enzyme system that is sensitive to certain classes of organic contaminants, such as PAHs and DL-PCBs (Bucheli and Fent, 1995), it is possible that the tissue-based burdens of these contaminants are not readily available to this system. We cannot definitively comment on this as we are unable to contrast EROD activities with a reference dataset. It is possible that the EROD activities measured in the present study are not entirely reflective of the burdens measured for PAHs and DL-PCBs. However, the TEQ levels reported in the present study are within range of those shown to induce physiological effects in other animal systems. Therefore, we hypothesize that there are likely to be physiological effects in these sharks from the northwestern GoM. The use of a broader suite of biomarkers and long-term sampling efforts are likely to delineate these effects.

5. Conclusions

Tissue-based burdens of PAHs were found at higher than expected concentrations in all species, suggesting chronic exposure to this contaminant within the Galveston Bay region. By comparison, there appeared to be a general increase in tissue-based PCB burden with estimated trophic level (bull > blacktip ≥ bonnethead) and FL. Species-specific differences in EROD and GST activity were also detected, for which the major proponents of these relationships remain unclear. The integration of biomarker activity with congener profiles suggested that only a small subset of individual PAH and PCB congeners displayed noticeable positive or negative correlations with EROD and GST. This result underlines the importance of measuring individual congener burdens during risk assessment since only certain PAHs and PCBs are key drivers of biochemical responses (CYP1A, GST) to exposure. Furthermore, effects of dioxin-like compounds (used for calculating TEQs) are assumed to be the critical effects on an organism. The TEQs of accumulated DL-PCB burdens indicate risk of physiological effects in these sharks when compared to established thresholds in mammals and fish. While TEQ_{PAHs} were much greater than TEQ_{PCBs} for all species, it is difficult to determine the extent of toxicity from this measurement since the relative potency factors for PAHs were based upon CYP1A induction and AhR affinity rather than toxic endpoints. Future work should integrate feeding ecology, additional biomarkers, and toxic endpoints to discern sources of exposure and determine which congeners may be responsible for negative health effects.

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Appendix A. Supplementary data

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