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AGE-RELATED POLYCHLORINATED BIPHENYL DYNAMICS IN IMMATURE BULL SHARKS (*CARCHARHINUS LEUCAS*)

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Abstract: Polychlorinated biphenyls (PCBs) were quantified in liver tissues of bull sharks (*Carcharhinus leucas*) ranging in age from <4 wk to >3 yr. Summed values of PCBs (ΣPCBs) ranged from 310 ng/g to 22 070 ng/g (lipid wt) across age classes with ΣPCB concentrations for the youngest sharks in the present study (<4 wk; 5230 ± 2170 ng/g lipid wt) determined to not significantly differ from those quantified in >3-yr-old sharks, highlighting the extent of exposure of this young life stage to this class of persistent organic pollutants (POPs). Age normalization of PCB congener concentrations to those measured for the youngest sharks demonstrated a significant hydrophobicity (log octanol/water partition coefficient [K_{OW}]) effect that was indicative of maternal offloading of highly hydrophobic ($\log K_{OW} \geq 6.5$) congeners to the youngest individuals. A distinct shift in the PCB congener profiles was also observed as these young sharks grew in size. This shift was consistent with a transition from the maternally offloaded signal to the initiation of exogenous feeding and the contributions of mechanisms including growth dilution and whole-body elimination. These results add to the growing pool of literature documenting substantially high concentrations of POPs in juvenile sharks that are most likely attributable to maternal offloading. Collectively, such results underscore the potential vulnerability of young sharks to POP exposure and pose additional concerns for shark-conservation efforts. *Environ Toxicol Chem* 2014;33:35–43. © 2013 SETAC

Keywords: Hydrophobicity Maternal transfer Shark liver Umbilical scar stage Young-of-year

INTRODUCTION

As apex predators in aquatic food webs, most sharks are at a heightened risk of exposure to persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), through food-web biomagnification [1]. Biological characteristics, including the highly lipid-rich livers of sharks that can contain up to 80% lipid and can constitute up to 20% of the body mass, can provide substantial capacity for POP bioaccumulation in these fishes [2,3]. In addition, life-history characteristics including slow growth, late maturation, and low reproductive rates are predicted to contribute to the high levels of POP bioaccumulation reported for a range of shark species [4–7]. For example, ΣPCB congener concentrations of 3040 ng/g and 10 000 ng/g (lipid wt) have been reported for liver tissues collected from blue (*Prionace glauca*) and Greenland (*Somniosus microcephalus*) sharks, respectively [6,8]. Moreover, Serrano et al. [2] reported average ΣPCB congener concentrations ranging from 45 ng/g to 5410 ng/g (lipid wt) in liver tissues from 8 deep-water shark species. Such ranges in PCB bioaccumulation represent a potential concern for shark species where maternal energy investment in embryo development occurs in both egg- and live-bearing species via yolk and/or placental nourishment and the potential exists for the transfer of the maternal PCB burden.

Recent evidence indicates that female carcharhinid sharks mobilize a significant proportion of their liver lipids to developing embryos during gestation via placental transfer [3]. For the dusky shark (*Carcharhinus obscurus*), such maternal provisioning can result in neonates with sufficiently enlarged livers that can represent 20% of the body mass [3]. This maternal provisioning is hypothesized to provide newborn sharks with energy reserves following parturition to sustain them as they develop foraging skills and begin independent feeding [3,9,10]. Gelsleichter et al. [11] suggested that the high concentrations of ΣPCBs observed in young-of-year blacktip (*Carcharhinus limbatus*) sharks were representative of maternal transfer processes. Mechanisms such as placental transfer have been widely demonstrated in marine mammals to result in developing fetuses receiving substantial POP burdens [12–15]. As well maternal offloading of POPs has been observed in egg-laying reptiles and teleost fishes [16–18]. When considering this mechanism, questions remain regarding the extent of potential POP transfer to young sharks and how these burdens change with growth. This is especially relevant given the capacity for sharks to bioaccumulate substantial concentrations of a range of POPs that have the potential for heightened toxicological risk. For example, Storelli et al. [7] reported sum toxic equivalents of 150 pg/g (lipid wt) for dioxin-like PCBs, polychlorinated dibenzo-*p*-dioxin, and polychlorinated dibenzofuran compounds quantified in blue shark livers. In addition, toxic equivalents of 110 pg/g and 170 pg/g (lipid wt) have been determined for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, respectively, in Greenland shark livers [8]. These results highlight the potential for exposure of highly sensitive developing embryos to a range of endocrine-disrupting compounds during gestation [15]. From this perspective, the potential toxicological consequences associated with maternally provisioned resources represent an additional concern for the already threatened conservation status of numerous shark species [19,20].

Ecological tracers including the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and dietary fatty acids have demonstrated significant relationships between maternal foraging activities and resource allocation to neonatal bull

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sharks [10,21]. In the present study, liver tissues from immature bull sharks (*Carcharhinus leucas*) were collected for PCB analysis to investigate the extent of PCB contamination in this viviparous species and to compare congener profiles across age classes of individuals ranging in age from <4 wk to >3 yr. It is hypothesized that neonatal bull sharks will have ΣPCB burdens that are more similar to those of juvenile bull sharks owing to maternal energy investment in this species. In addition, we hypothesize a shift in PCB congener profile from the youngest to the juvenile age classes that reflects the transition from maternal provisions to exogenous feeding and dietary exposure. It is anticipated that the present study will provide novel information regarding PCB dynamics in young sharks and further our understanding of maternal influences on growing sharks.

MATERIALS AND METHODS

Sample collection

Thirty-nine bull sharks including neonate, young-of-year, and juvenile age classes were collected from nursery habitats of the Caloosahatchee (26°30′N, 81°54′W) and Myakka (82°12′W, 26°57′N) Rivers of Florida, USA. Sharks were caught using shallow water (<10 m) bottom-set longlines (length 400–800 m) set for periods from 0.5 h to 2.0 h between May and October of 2006 to 2008 in cooperation with the Florida Fish and Wildlife Conservation Commission. All sharks were killed in the field directly following capture in accordance with the University of Windsor's Animal Use and Care Guidelines (AAUP 07-13). Each shark was measured for precaudal, total, and fork length (cm), sexed, and assessed for relative age by umbilical scar stage following a qualitative 5-point umbilical scar stage scale outlined by Bass et al. [22] and Duncan and Holland [9]. Scar stages include open wound with umbilical remains attached (umbilical scar stage 1), open wound without remains (umbilical scar stage 2), wound partially open (umbilical scar stage 3), wound completely closed (umbilical scar stage 4), and faint scar present (umbilical scar stage 5). Available information indicates a period between 4 wk and 6 wk for complete healing of the umbilical scar (i.e., umbilical scar stage 5) and approximately 1 yr for complete disappearance of the scar [9,22]. Age estimates of sharks lacking an umbilical scar were determined based on fork length estimates following Branstetter and Stiles [23] and Neer et al. [24]. Body mass (kilograms) estimates were derived from Cliff and Dudley [25]. Liver biopsies (~5 g) were collected from each individual and stored frozen (−20 °C) in cryogenic Teflon vials until analysis.

Sample analysis

The PCB extractions were completed using between 0.5 g and 1 g of liver tissue following the sodium sulfate cold column extraction procedures described in Lazar et al. [26] and Daley et al. [27]. Sample extraction efficiencies were determined using either 1,3,5-tribromobenzene or a combination of PCB34 and brominated diphenyl ether 71 recovery standards. Sample lipid contents were determined gravimetrically using 1 mL of sample extract and a microbalance [28]. The remaining extract was concentrated to 2 mL under vacuum with sample cleanup performed by Florisil chromatography as described by Lazar et al. [26], followed by collection of the first (50 mL hexane; ACP) and second (50 mL; hexane/dichloromethane 85/15 v/v; Fisher Scientific) fractions. After Florisil chromatography, extracts were concentrated to 1 mL under vacuum and transferred to 1.8 mL gas chromatography vials. Samples were analyzed for individual PCB congeners by gas chromatography-electron capture detection. For each batch of 6 samples, an in-house reference homogenate tissue, method blank, and external PCB recovery standard (Quebec Ministry of Environment Congener Mix; AccuStandard) were analyzed. A total of 33 PCB congeners were scanned for during analysis: PCBs 18/17, 31/28, 33, 52, 49, 44, 70, 95, 101, 99, 87, 110, 151/82, 149, 118, 153, 105/132, 138, 158, 187, 183, 128, 177, 156/171, 180, 191, 170, 201, 195/208, 194, 205, 206, and 209. All congeners were detected with sufficient concentration to be included in the data analysis (detection limits 12–41 pg/g wet wt). Recoveries of individual PCB congeners in the homogenate reference tissue extracted with each batch of samples were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts from the Great Lakes Institute for Environmental Research's accredited organic analytical laboratory (Canadian Association for Laboratory Accreditation and ISO17025 certified). Recovery efficiencies for 1,3,5-tribromobenzene, PCB34, and brominated diphenyl ether 71 standards were 86.0 ± 4.2%, 79.8 ± 12.3%, and 80.7 ± 3.1%, respectively, and sample PCB concentrations were not recovery-corrected.

Statistical analyses

Sex, season, year, and river did not significantly influence percentage of lipid content and ΣPCB congener concentration values of bull shark liver tissues (Supplemental Data, Table S1). Multiple regression analysis demonstrated that there was no effect of season, year, and/or their interactions with age class for ΣPCB values ($F_{19,19} = 1.148$, $p = 0.384$). The data were therefore grouped per age class for all following analyses. Analysis of variance followed by a Tukey's honestly significant difference post hoc test was used to test for differences in lipid contents, ΣPCB congener concentrations (lipid wt), and percentage of total PCB and lipid-normalized PCB congener ratios for select congeners (PCB153 vs 18/17, 31/28, 52, 99, 180, and 206) among bull shark age classes. These congener ratios were chosen to compare potential age-related changes in individual congener bioaccumulation across a range of hydrophobicities. To further examine potential changes in PCB congener profiles with shark age, individual PCB congener concentrations were normalized to the average concentration determined for the youngest shark age class (umbilical scar stage 2) collected in the present study using lipid-corrected PCB congener concentrations determined for each shark liver sample. This approach was designed to normalize the differences in baseline environmental concentrations for each congener and to allow for comparison of bioaccumulation patterns among congeners following Burtnyk et al. [29]. The umbilical scar stage 2–normalized PCB congener concentrations were then plotted against congener octanol/water partition coefficients ($\log K_{OW}$) obtained from Hawker and Connell [30]. The PCB congener partition ratios were estimated by dividing the average PCB congener concentrations (lipid wt) of umbilical scar stage 2 sharks by the average congener concentrations (lipid wt) of the oldest female sharks (>3 yr old, $n = 4$) [14,15]. The PCB data were $\log_{10}$-transformed, and all analyses were performed in R 2.13.0 [31] with a criterion for significance of $p < 0.05$ for all statistical tests.

RESULTS

Bull shark liver lipid content ranged from 37.4% to 70.8% across age class (Figure 1A). Liver lipid content was significantly correlated with fork length (−0.7 fork length + 109.8, $r^2 = 0.443$, $p < 0.001$) and demonstrated a significant decline with increasing age class ($F_{6,32} = 7.06$, $p < 0.0001$;

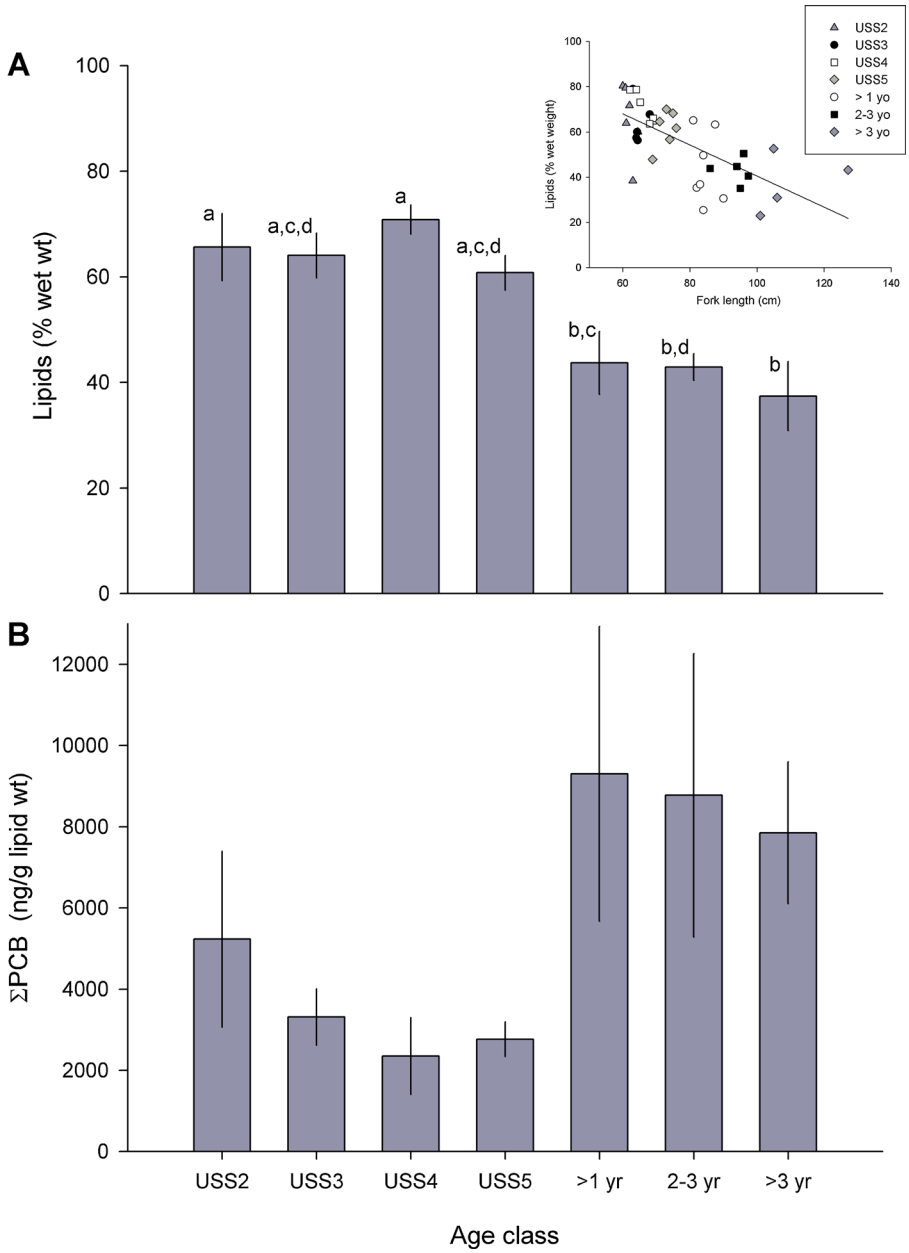

Figure 1. (**A**) Mean (± standard error) liver lipid contents (% wet wt) and (**B**) summed values of polychlorinated biphenyl (ΣPCB) concentrations (ng/g lipid wt) for liver tissues sampled from immature age classes of bull sharks collected from southwestern Florida. Age classes are defined as follows: umbilical scar stage 2, open wound without remains; umbilical scar stage 3, wound partially open; umbilical scar stage 4, wound completely closed; umbilical scar stage 5, faint scar present; >1 yr, no umbilical scar, 80 cm to 90 cm fork length; 2 yr to 3 yr, 95 cm to 100 cm fork length; and >3 yr, >100 cm fork length. Inset panel represents relationship between liver lipid content and shark fork length (cm). Letters represent significant results of Tukey's honestly significant difference post hoc comparison. Where letters are absent, no significant differences were observed. USS = umbilical scar stage.

Figure 1A). Significant differences in lipid content were not observed between umbilical scar stage 2 and umbilical scar stage 5 age classes or between the 3 oldest age classes (Figure 1A). However, liver lipid content was significantly higher in umbilical scar stage 2 through umbilical scar stage 5 compared with >3-yr-olds (Figure 1A).

Mean ΣPCB concentrations ranged from 1660 ng/g to 3540 ng/g (wet wt) among the shark age classes with the highest mean ΣPCB concentration determined for livers from 2-yr-old to 3-yr-old sharks (Table 1). The predominant congeners were PCBs 99, 118, 138, 153, 180, and 187, constituting approximately 77% of ΣPCBs (Supplemental Data, Table S2). There were no significant differences ($F_{6,32} = 1.905$, $p = 0.110$) in ΣPCB concentrations (lipid wt) among age classes (Figure 1B). However, ΣPCB concentrations (lipid wt) decreased consistently and by approximately 47% across umbilical scar stage 2 through umbilical scar stage 5 age classes and increased by approximately 3-fold between umbilical scar stage 5 and 1-yr-olds (Figure 1B). At approximately 1 yr, ΣPCB concentrations in liver tissues averaged (± standard error) 9300 ± 3630 ng/g lipid weight but did not increase significantly across the 3 oldest age classes (Figure 1B).

Among PCB congeners 18/17, 31/28, 52, 99, 153, 180, and 206, PCB153 was consistently the dominant congener in bull shark PCB profiles, averaging 27.4 ± 2.4% of ΣPCBs for all age classes included in the present study (Supplemental Data, Figure S1). For PCBs 18/17, 31/28, 52, and 99, their proportional contributions to ΣPCBs declined with increasing

Table 1. Fork length, estimated mass, and summed values of polychlorinated biphenyl(ΣPCB) congener concentrations detected in liver tissues of bull (*Carcharhinus leucas*) sharks from immature age classes sampled from southwestern Florida

Age class	*n*	Length (cm)	Mass (kg)[a]	ΣPCB(ng/g wet wt, mean ± SE)[b]
Umbilical scar stage 2	6	61.9 ± 0.7	4.5 ± 0.2	3350 ± 1550
Umbilical scar stage 3	5	64.7 ± 0.9	4.6 ± 0.4	2230 ± 562
Umbilical scar stage 4	6	66.2 ± 1.2	5.2 ± 0.2	1650 ± 640
Umbilical scar stage 5	6	73.0 ± 1.1	5.6 ± 0.4	1640 ± 370
>1yr	7	84.5 ± 1.2	11.0 ± 1.0	3110 ± 1050
2–3yr	5	93.7 ± 2.0	14.4 ± 1.3	3540 ± 990
>3yr	4	109.8 ± 5.9	19.3 ± 0.9	2860 ± 590

[a]Body mass estimates were derived from Cliff and Dudley [25].
[b]Values represent the sum total of 33 congeners. See Supplemental Data, Table S2, for mean ± standard deviation values of each congener.
SE = standard error.

shark age. Concentrations of these 4 congeners also represented <10% of ΣPCBs for each age class, with PCBs 18/17 and 52 averaging <1% of total PCB concentrations quantified in bull shark livers. In contrast, proportional contributions of PCBs 153, 180, and 206 to bull shark ΣPCBs increased with shark age. For example, PCB180 concentrations for umbilical scar stage 2 sharks averaged 7.5 ± 0.7% of ΣPCBs and increased to an average of 10.5 ± 1.1% for >3-yr-old sharks.

Concentration ratios for the highly recalcitrant PCB153 ($\log K_{OW} = 6.92$) relative to less hydrophobic PCB congeners including 18/17, 31/28, 52, and 99 ($\log K_{OW} = 5.25$, 5.67, 5.84, and 6.39, respectively) were generally highest for umbilical scar stage 2 sharks relative to other scar stages (Figure 2). Umbilical scar stage 3 shark congener ratios were significantly lower than those of 2-yr-old to 3-yr-old and >3-yr-old sharks for PCB153:31/28 ($F_{6,32} = 2.665$, $p = 0.033$) and PCB153:99 ($F_{6,32} = 2.823$, $p = 0.025$). The PCB153:52 congener ratios for umbilical scar stage 3 sharks were also significantly lower than those of 2-yr-olds ($p = 0.009$); however, among all other age classes, no significant differences in PCB153:52 ratio values were evident ($F_{6,32} = 1.992$, $p = 0.097$). For PCB153:18/17, umbilical scar stage 2 sharks had the highest average ratio, which was approximately 1.6-fold higher than that determined for >3-yr-old sharks (Figure 2A; $F_{6,31} = 1.176$, $p = 0.344$). For PCB153:31/28, ratios determined for umbilical scar stage 2 sharks were 2.0-fold to 3.6-fold higher than those calculated for umbilical scar stage 3 through umbilical scar stage 5 sharks and 1.3 times that determined for >1-yr-old sharks (Figure 2B). This pattern was also observed for PCB153:52 and PCB153:99 ratios, whereby the values determined for umbilical scar stage 2 sharks were substantially higher than those for umbilical scar stage 3 through umbilical scar stage 5 sharks and most similar to those determined for the oldest 3 age classes (Figure 2C and D). Relative to more hydrophobic congeners including PCB180 ($\log K_{OW} = 7.36$) and 206 ($\log K_{OW} = 8.09$), PCB153 ratios exhibited a generally negative relationship with increasing shark age (Figure 2E and F). Significant differences were evident among shark age classes for PCB153:180 values, with umbilical scar stage 3 shark ratios determined to be significantly higher relative to >3-yr-old fish ($F_{6,32} = 2.707$, $p = 0.031$). For PCB153:206 values, no significant differences were determined among shark age classes ($F_{6,32} = 0.810$, $p = 0.570$).

Umbilical scar stage 2–normalized PCB congener profiles quantified in each age class demonstrated a significant hydrophobicity ($\log K_{OW}$) effect where the relationships between umbilical scar stage 2–normalized PCB concentrations and $\log K_{OW}$ transitioned from negative to positive with increasing shark age (see Figure 3 for regression statistics). Specifically, this normalization demonstrated the predominance of highly hydrophobic ($\log K_{OW} \geq 6.5$) congeners in the livers of umbilical scar stage 2 relative to umbilical scar stage 3 sharks (Figure 3A). Concentration ratios for these highly hydrophobic congeners in umbilical scar stage 3 sharks were <1 and averaged 0.67% relative to umbilical scar stage 2 sharks. This hydrophobicity effect was not evident for umbilical scar stage 2 normalized PCB congener concentrations for umbilical scar stage 4 and umbilical scar stage 5 age classes (Figure 3B and C) as the umbilical scar stage 2–normalized congener concentrations for these age classes were predominantly <1. The majority of PCB congeners quantified for umbilical scar stage 2–normalized concentrations of >1-yr-old sharks exceeded 1, indicating a higher degree of PCB bioaccumulation in livers of older sharks relative to umbilical scar stage 2 through umbilical scar stage 5 sharks (Figure 3D). Significant hydrophobicity effects were observed for umbilical scar stage 2–normalized concentrations for the 2-yr-old to 3-yr-old and >3-yr-old sharks (Figure 3E and F). The PCB congener concentrations for these age classes averaged approximately 1.7 times greater than those quantified in umbilical scar stage 2 sharks.

The relationship between PCB congener partition ratios (lipid wt) and $\log K_{OW}$ of umbilical scar stage 2 versus >3-yr-old sharks was best described by a second-order polynomial and demonstrated a significant hydrophobicity effect (Figure 4). Log-transformed partition ratios ranged from −0.4 to 0.4 and averaged −0.07 ± 0.19 standard deviation, with a total of 11 congeners having positive partition ratios (>0). All partition ratios estimated for PCB congeners with $\log K_{OW} \geq 7.36$ were negative (<0).

DISCUSSION

The present study is among the first to quantify PCB congener concentrations among multiple age classes of immature sharks. The youngest age class of bull shark included in the present study exhibited ΣPCB congener concentrations that were comparable to those of older immature individuals and congener profiles that were dominated by increasingly hydrophobic ($\log K_{OW} > 6.5$) compounds relative to successively older immature sharks. The ΣPCB concentrations (wet wt) determined for young-of-year bull sharks in the present study were similar to those reported for similarly aged blacktip (2930 ± 580 ng/g wet wt) and sandbar (*Carcharhinus plumbeus*, 2050 ± 470 ng/g wet wt) sharks [11]. Carcharhinid sharks mobilize substantial liver lipid reserves during gestation [3], and the patterns and magnitude of PCB contamination quantified for neonatal bull sharks in the present study provide strong evidence for the transfer of PCBs to bull shark pups during such maternal resource provisioning.

Stable isotope analyses have demonstrated that bull sharks rely on maternally provided resources for their first year of growth, with gradual loss of the maternal isotope signal owing to development of foraging skills and assimilation of external

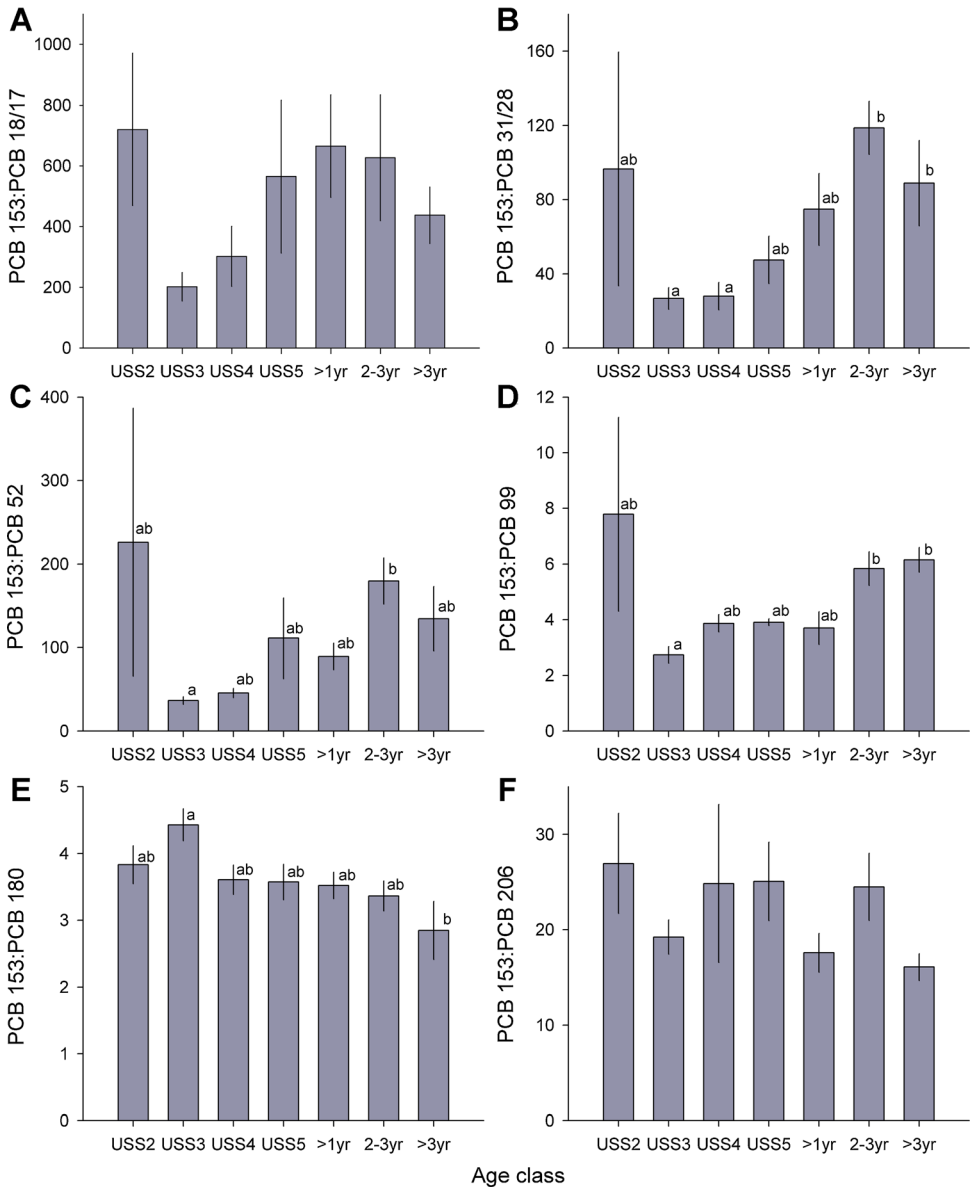

Figure 2. Comparison of polychlorinated biphenyl (PCB)153 concentrations relative to (**A**) PCB18/17, (**B**) PCB31/28, (**C**) PCB52, (**D**) PCB99, (**E**) PCB180, and (**F**) PCB206 concentrations (ng/g lipid wt) across immature age classes of bull sharks. Age classes are defined as follows: umbilical scar stage 2, open wound without remains; umbilical scar stage 3, wound partially open; umbilical scar stage 4, wound completely closed; umbilical scar stage 5, faint scar present; >1 yr, no umbilical scar, 80 cm to 90 cm fork length; 2 yr to 3 yr, 95 cm to 100 cm fork length; and >3 yr, >100 cm fork length. Bars in each panel represent mean concentration ratio (± standard error), and letters represent significant results of Tukey's honestly significant difference post hoc comparison. Where letters are absent, no significant differences were observed. USS = umbilical scar stage.

dietary carbon [10,21]. The changes observed in the umbilical scar stage 2–normalized PCB congener profiles are consistent with such observations regarding the reliance of this species on maternally provided resources during early growth and development. Specifically, age normalization of the umbilical scar stage 3 age class PCB concentrations demonstrated higher proportions of increasingly hydrophobic ($\log K_{OW}$ >6.5) congeners in the youngest shark age class. The general decline in ΣPCB congener concentrations between umbilical scar stage 2 and umbilical scar stage 5 sharks can likely be attributed to a combination of mechanisms. These include redistribution of the hepatic PCB burden, growth dilution, and biotransformation and whole-body elimination [32]. Importantly, PCB congener elimination rates are generally negatively correlated with log K_{OW} and body size [16,33,34]. Such elimination kinetics dictates more rapid loss of the less hydrophobic congeners for smaller, younger sharks and aid in describing the observed changes in age-normalized PCB congener profiles across umbilical scar stage 3 through umbilical scar stage 5 age classes.

By the first full year of growth, bull sharks are predicted to have increased in mass by approximately 2.5-fold relative to umbilical scar stage 2 individuals [25]. Such increases in body mass are predicted to be accompanied by reduced capacity for the elimination of increasingly hydrophobic ($\log K_{OW}$ >6.5) pollutants [34]. In addition, the transition from reliance on maternally provided resources to exogenous feeding during the first year of growth will result in growing exposure to such increasingly hydrophobic congeners via dietary consumption. For example, Cliff and Dudley [25] indicated that bull shark diets exhibit size selectivity, suggesting that larger, older sharks feed on larger prey items that generally occupy higher trophic positions in the food web. For fish species, body size is also

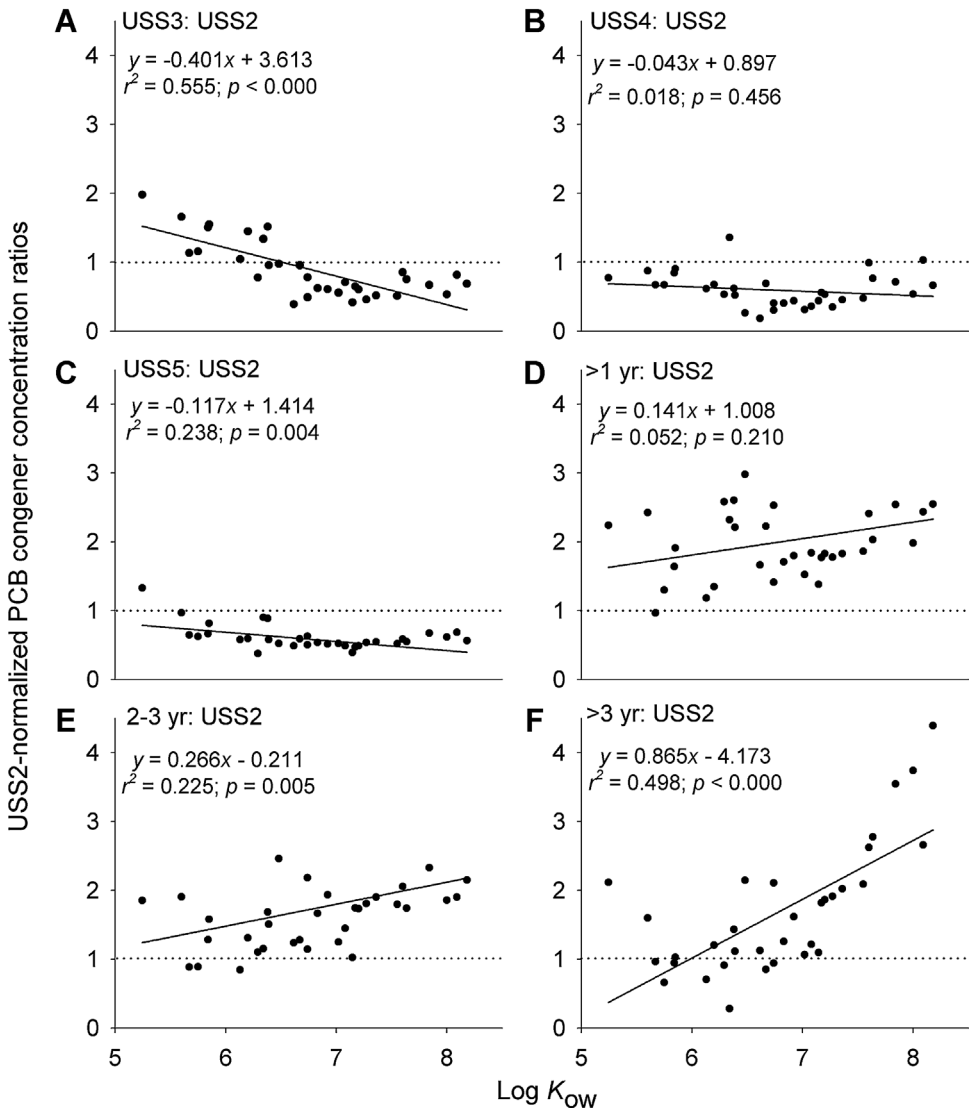

Figure 3. Relationship between umbilical scar stage 2–normalized polychlorinated biphenyl (PCB) congener concentrations and log octanol/water partition coefficient (K_{OW}) for (**A**) umbilical scar stage 3, (**B**) umbilical scar stage 4, (**C**) umbilical scar stage 5, (**D**) >1 yr old, (**E**) 2 yr old to 3 yr old, and (**F**) >3 yr old age classes of bull sharks. Each point represents a different PCB congener. USS = umbilical scar stage.

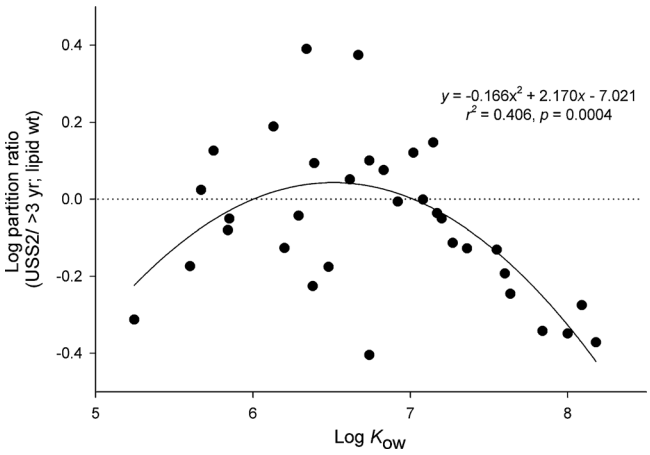

Figure 4. Average partition ratios for polychlorinated biphenyl congeners (ng/g lipid wt) plotted against log octanol/water partition coefficient (K_{OW}) for bull sharks. Partition ratios were calculated as the congener concentration quantified in umbilical scar stage 2 individuals relative to the average value quantified for >3-yr-old female bull sharks and $\log_{10}$-transformed. USS = umbilical scar stage.

typically a good predictor of the extent of PCB bioaccumulation [35], with the degree of chlorination of the PCB burden also increasing with body size or trophic level [36]. Such alterations in elimination kinetics and foraging ecology support the resultant changes observed in the relationships between age-normalized PCB concentrations and log K_{OW} for ≥1-yr-old individuals and aid in understanding the changes in PCB bioaccumulation patterns observed across these age classes of immature bull sharks.

Comparison of PCB153 concentrations with those of PCBs 18/17, 28/31, 52, 99, 180, and 206 across the bull shark age classes included in the present study provides strong evidence for this transition and of the significance of maternal offloading for this species. For example, PCB153:PCB99 values were most similar between umbilical scar stage 2 and >3-yr-old sharks. In addition, ΣPCB concentrations measured in bull sharks did not exceed or equal those measured in umbilical scar stage 2 individuals until sharks were ≥1 yr old. Growth dilution is most important for high log K_{OW} compounds [32], and growth rates for larger (>120 cm) immature and adult bull sharks represent a

time frame of approximately 32 yr for this species to reach 90% of the maximum asymptotic size [37]. The diets of older bull sharks have also been demonstrated to transition from being dominated by smaller, primarily teleost prey to increasing proportions of larger prey species such as reptiles, elasmobranchs, and small mammals [25]. Such a change in dietary composition to larger prey species is likely to result in a dietary PCB profile that is increasingly dominated by more highly hydrophobic congeners owing to slower congener elimination rates and reduced growth dilution in larger prey [33,35,38]. From these perspectives, the similarity of PCB153 congener ratios determined for the umbilical scar stage 2 sharks with those for sharks >1 yr old demonstrates that bull shark neonates likely receive the majority of their PCB burden via maternal offloading.

Partition ratios describing the extent and patterns of PCB transfer between mother–fetus pairs for marine mammals have indicated a distinct hydrophobicity pattern in that increasingly hydrophobic PCBs ($\log K_{OW} > 6.5$) are offloaded to developing fetuses to a reduced extent relative to less hydrophobic pollutants [14,15]. Although liver tissues were not collected from mature female bull sharks for the present study, the partition ratios estimated using PCB congener concentrations from the oldest female sharks demonstrated a similar hydrophobicity pattern to that observed by Desforges et al. [15]. For increasingly hydrophobic congeners having $\log K_{OW} > 6.5$, partition ratio estimates in the present study averaged 0.9, which contrasts with an average of >1.0 for less hydrophobic congeners. While this difference is limited in magnitude, it confirms prior conclusions regarding the greater potential for the offloading of less hydrophobic congeners to developing young [14,15].

The reduced extent of maternal offloading for highly hydrophobic PCBs has been attributed to their increasing hydrophobicity and the lower capacity of polar lipids in blood for these congeners relative to nonpolar lipids that dominate storage fats such as blubber [15,39,40]. Although less hydrophobic congeners are predicted to be more readily offloaded, maternal PCB profiles would be dominated by more hydrophobic congeners owing to maturation times of up to 20 yr for female bull sharks [23,37,41]. Consequently, PCB congeners transferred to developing sharks would reflect this maternal profile. Partition ratios estimated for some of the least hydrophobic congeners quantified in umbilical scar stage 2 sharks were lower than would be predicted based on the relationship described between partition ratios and $\log K_{OW}$ for beluga whale mother–calf pairs [15]. Partition ratio estimates in the present study were not based on matched mother–pup pairs or calculated using PCB concentrations of reproductively mature female sharks. These together likely contributed to the error of these estimates for a number of congeners. The umbilical scar stage 2 sharks also represent a time frame during which immature sharks have been removed from maternal placental nourishment [9,22]. Consequently, there is potential for redistribution of maternally offloaded PCBs from the liver in addition to growth dilution, biotransformation, and whole-body elimination during this early life-history period, especially for more readily eliminated congeners having $\log K_{OW} \leq 6.5$ [33].

Female bull sharks are estimated to reach reproductive maturity between 15 yr and 20 yr of age at >200 cm total length [23,37]. This contrasts with faster-growing species such as the blue shark, for which maturity occurs at approximately 5 yr of age [42]. Thus, the potential exists for female bull sharks to bioaccumulate substantial PCB burdens prior to their first reproductive event [41]. For marine mammals, the greatest extent of PCB offloading to developing fetuses occurs during the initial pregnancy event [12,14,43]. During subsequent reproductive events, a reduced extent of offloading occurs owing to the reduced time frame for feeding and PCB bioaccumulation between future reproductive events [12,14,43]. Therefore, developing embryos could be subject to a high degree of variability with respect to PCB exposure depending on maternal age at 1st reproduction in addition to the time between reproductive events. Mull et al. [44] observed high variability for ΣPCB concentrations in liver tissues from young-of-year great white sharks (*Carcharodon carcharias*), similar to findings reported in the present study. Bull shark litters also typically range in number from 6 pups to 14 pups [25], which contrasts with species such as the blue shark, for which litters have been reported to include up to 75 individuals [45], suggesting that the potential distribution of the maternally offloaded PCB burden may also vary depending on litter size. These life-history characteristics are likely important parameters contributing to the variability observed in ΣPCB concentrations quantified for umbilical scar stage 2 sharks in the present study and would be anticipated to contribute to differences in the magnitude of maternal PCB offloading among elasmobranch species.

Growth rates for carcharhinid species such as the dusky shark (*Carcharhinus obscurus*) have been demonstrated to result in increases in average mass up to approximately 400% from umbilical scar stage 2 through umbilical scar stage 5 ages [3]. However, during such rapid growth, hepatosomatic indices for these age classes also decline from approximately 15% to 6%, representing an approximate 30% loss of liver mass [3]. Such reductions in mass for this lipid-rich tissue represent an extensive loss in storage capacity for POPs such as PCBs [46]. When lipids are mobilized as an energy reserve, the potential exists for the mobilization of PCBs from the lipid reserves into the bloodstream and redistribution into other somatic tissues [26,47,48]. In the present study, PCB concentrations were quantified in bull shark liver tissues only. The reductions in ΣPCB concentrations observed in the present study for umbilical scar stage 3 through umbilical scar stage 5 relative to umbilical scar stage 2 sharks are hypothesized to primarily represent the redistribution of the liver PCB burden into other somatic tissues as these individuals grew in size. In addition, when combined with the potential for such high growth dilution and changes in trophic position for young sharks, these are likely key parameters contributing to the observed changes in the umbilical scar stage 2–normalized PCB congener profiles for the umbilical scar stage 3 through umbilical scar stage 5 age classes.

Limited information currently exists regarding the extent of maternal offloading of POPs that occurs across the diverse range of reproductive strategies exhibited by elasmobranchs. Maternal offloading of contaminants is not limited to placental species as this mechanism has been observed in egg-laying fishes and reptiles [16–18]. Gelsleichter et al. [11] and Mull et al. [44] did not observe changes in ΣPCB congener concentrations with age or size in juvenile blacktip and white sharks, likely a consequence of grouping individuals into broader size and age classes and/or not sampling a size range of young-of-year individuals. However, the results of the present study agree well with those of Gelsleichter et al. [11] and Mull et al. [44], who documented ΣPCB concentrations of similar magnitude to older individuals and consistent with maternal offloading patterns. Combined, these studies also indicate that the extent of such maternal offloading for shark species is sufficient to generate PCB concentrations in neonates that are of consistent magnitude with those associated with

increased toxicological risk. For example, Strid et al. [8] quantified ΣPCB concentrations ranging from 990 ng/g to 10 000 ng/g (lipid wt) in Greenland shark livers, which are similar to the findings of the present study for umbilical scar stage 2 sharks. Critically, such high PCB burdens in shark livers are often accompanied by elevated concentrations of highly toxic compounds including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and tetrachlorodibenzofuran as well as coplanar and mono-ortho–substituted PCB congeners [7,8]. Although such compounds were not quantified in the present study, it is likely that concentrations of these highly toxic pollutants are also offloaded during maternal resource provisioning in shark species. For example, ΣPCB concentrations averaging approximately 17 500 ng/g ± 12 750 ng/g (lipid wt) have been reported for young-of-year white shark liver tissue [44]. This result is roughly double the highest result reported for Greenland shark livers with demonstrated toxic equivalent potential [8,44]. Given the limited understanding of toxic equivalents in sharks and the high concentrations of POPs being reported in young individuals of variety of shark species, future analyses should focus on determining toxic equivalent values whereby toxicity can be observed.

The youngest life stages of animal species are typically the most developmentally sensitive with respect to pollutant exposure. The results of the present study underscore the potential vulnerability of young sharks with respect to possible toxicity effects associated with POP exposure. Many elasmobranch species, including high–trophic level sharks are threatened worldwide [20]. Additional research emphasizing species-specific pollutant offloading patterns and associated toxicity, in addition to age- and growth-related changes in POP dynamics, will prove valuable for understanding the risks to global shark populations that are associated with POP bioaccumulation and exposure.

SUPPLEMENTAL DATA

Tables S1–S2.
Figure S1. (256 KB DOC).

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