Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks

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A B S T R A C T
Organic contaminant and total mercury concentrations were compared in four species of lamniform sharks over several age classes to examine bioaccumulation patterns and gain insights into trophic ecology. Contaminants found in young of the year (YOY) sharks were assumed to be derived from maternal sources and used as a proxy to investigate factors that influence maternal offloading processes. YOY white (Carcharodon carcharias) and mako (Isurus oxyrinchus) sharks had comparable and significantly higher concentrations of PCBs, DDTs, pesticides, and mercury than YOY thresher (Alopias vulpinus) or salmon (Lamna ditropis) sharks. A significant positive relationship was found between YOY contaminant loads and maternal trophic position, suggesting that trophic ecology is one factor that plays an important role in maternal offloading. Differences in organic contaminant signatures and contaminant concentration magnitudes among species corroborated what is known about species habitat use and may be used to provide insights into the feeding ecology of these animals.

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1. Introduction

Legacy organic contaminants such as DDTs and PCBs continue to pose problems for aquatic biota despite cessation of their production and use in the United States (Rapaport and Eisenreich, 1988; Turusov et al., 2002). Due to their physical properties these contaminants can persist in the environment for many decades and bioaccumulate through the food chain, reaching very high concentrations in upper trophic level marine predators, such as dolphins (Fair et al., 2010), pinnipeds (Blasius and Goodmanlowe, 2008), and sharks (Mull et al., 2012). While production and disposal of many of these compounds have been banned or heavily regulated for over 50 years, their long persistence remains an issue for maintaining and restoring healthy aquatic communities as these contaminants continue to reenter and recycle through food webs (Evans et al., 1991; Calamari et al., 2000).

Inorganic mercury, on the other hand, is continually released into the environment through both anthropogenic and natural processes such as the burning of fossil fuels or volcanic emissions, respectively (Hylander and Meili, 2003; Nriagu and Becker, 2003). The conversion of inorganic mercury into an organic form allows this heavy metal to bioaccumulate through food webs similar to organic contaminants (Mason et al., 1995). However, mercury does not predictably concentrate in specific tissue types as do organic contaminants, which accumulate in lipid storage organs such as liver and blubber (Yordy et al., 2010; Roos et al., 2010). In marine mammals, up to 95% of total organic contaminant loads may fractionate into blubber compared to blood, liver, and muscle (Schantz et al., 1993; Aguilar and Borrell, 1994; Hickie et al., 1999). In fishes, especially elasmobranchs, the liver is used as the main organ of energy storage, and the highest wet weight concentrations of organic contaminants have been found here compared to muscle.

Abbreviations: PCBs, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; 4,4’-DDE, dichlorodiphenyl dichloroethane; ANOSIM, analysis of similarity; SIMPER, similarity percentage analysis.

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term pregnancy thresher shark, Lyons and Lowe (submitted for publication) demonstrated maternal offloading to occur in elasmobranchs since in utero embryos had measurable concentrations of organic contaminants and mercury in their liver and muscle tissues as well as in their yolky stomach contents, which consisted of consumed ovulated eggs. Therefore, contaminants in newborn offspring likely reflect their mother’s accumulated contaminant burdens and the contaminant signatures (i.e., both total loads and congener profiles) measured in young animals may be used to explore adult female trophic ecology and factors that may influence maternal offloading processes in elasmobranchs.

Southern California is a known nursery area for three species of lamniform sharks (white shark Carcharodon carcharias, Weng et al., 2007a; shortfin mako Isurus oxyrinchus, Holts and Bedford, 1989; and thresher Alopias vulpinus, Cartamil et al., 2010a) and purportedly for a fourth (salmon shark Lamna ditropis; Goldman and Musick, 2006). While these species share similar physiological and reproductive characteristics (e.g., regional endothermy and oophagous reproduction; Gilmore, 1993; Carlson et al., 2004) they differ in their habitat use and trophic ecology, both among species and across age classes, which may influence the levels and types of contaminants these species accumulate. Therefore, the purpose of our study was to investigate potential factors that may affect maternal offloading processes in elasmobranch species by examining the roles that maternal trophic position, habitat use, and age at maturity have on contaminant concentrations measured in YOY sharks of four closely related species. In addition, we also aim to address how these factors may influence species bioaccumulation at different age classes.

2. Methods

2.1. Sample collection

For each species, samples of liver and white muscle tissue were collected from each individual when available. Thus, sample sizes represent the number of unique individuals examined. When possible, liver samples were taken from the tip of the left liver lobe and muscle samples from the dorsal musculature anterior to the dorsal fin. Size criteria used to define age classes are reported in Table 1 (Supplemental; Cailliet et al., 1985, Francis and Duffy, 2005, Ribot-Carballal et al., 2005; Goldman & Musick 2006; Smith et al. 2008). Sharks were considered juveniles if they were smaller than the smallest size at maturity but larger than YOY (<1 yr old) sizes, whereas sharks that measured between the smallest and largest reported size at maturity for the species were considered “near-maturity”. Individuals exceeding the largest reported size at maturity were considered adults.

2.1.1. White sharks

YOY (n = 21) and juvenile (n = 9) white shark samples were collected from incidental mortalities of sharks caught by commercial fishers collaborating with Monterey Bay Aquarium’s juvenile white shark research program in the Southern California Bight (SCB) from 2006 to 2012. Contaminant concentrations in white sharks were obtained from previously published data (Mull et al., 2012, 2013).

2.1.2. Salmon sharks

YOY and juvenile salmon sharks were sampled between 2006 and 2010 from Oregon and central California (n = 34 and n = 2, respectively). Additionally, one YOY salmon shark (80.3 cm FL) that stranded at Huntington Beach, California was donated by the California Department of Fish and Wildlife. One adult and near

(Storelli et al., 2003; Schlenk et al., 2005; Gelsleichter and Walker, 2010; Mull et al., 2012). On the other hand, higher concentrations of mercury have been documented in the muscles of some fish species rather than in tissues with highest lipid content (Mason et al., 1995; Endo et al., 2008; Suk et al., 2009), suggesting mercury partitioning is influenced by other factors besides the hydrophobic properties driving organic contaminant tissue-specific accumulation, such as mercury’s affinity for disulfide bridges.

While DDT, PCBs, and mercury can now be found throughout the world’s oceans, marine areas adjacent to heavily populated or industrialized areas tend to have significantly higher levels of these contaminants (Brown et al., 1998; Klasing et al., 2009). In particular, high levels remain near historic dumping sites or in marine areas receiving large inputs of urban or agricultural runoff (Hu et al., 2010; Webster et al., 2011). In southern and central California high levels of organic contaminants, particularly DDT, remain in sediments which lead the U.S. Environmental Protection Agency (EPA); a governmental body responsible for assessing and monitoring environmental health and remediation, to designate areas within these regions as Superfund sites (Eganhouse et al., 2000; Tomaszewski et al., 2007). For example, it was estimated that 11 tons of PCBs and 110 tons of DDT still remain in sediment on the Palos Verdes Shelf, southern California (Eganhouse et al., 2000; Lee et al., 2002). The high levels of DDT have given animals utilizing these and surrounding areas a unique “California” contaminant signature, where the relative proportion of DDTs to PCBs is higher compared to that in biota from other distant locations that are further offshore or north or south (Brown et al., 1998; Krahm et al., 2007). Conversely, animals with higher proportions of chlordane pesticides compared to PCBs are reflective of an “Alaskan” signature (Krahn et al., 2007). Therefore, relative concentrations of these contaminants can be used as an indicator of regional habitat use.

While animals may acquire these contaminants through diet, other processes such as maternal offloading may represent another important pathway of contaminant accumulation, especially during the early life stages. Maternal offloading is the process whereby females passively transfer contaminants when lipids are mobilized from fat stores for yolk or milk production (Addison and Brodie, 1987; Russell et al., 1998). However, various factors related to feeding, such as trophic position (Fair et al., 2010; Ross et al., 2000) and reproductive history (i.e. number of previous birthing events; Aguilar and Borrell, 1994; Borrell et al., 1995) have been shown to influence the amount of contaminants females may transfer to offspring in marine mammals. For instance, females tend to offload the highest amount of contaminants to offspring during their first reproductive event and fewer contaminants to subsequent litters (Borrell et al., 1995). While these processes have been well studied in marine mammals, maternal offloading is less understood in other upper trophic level marine predators, such as elasmobranchs, though it has been shown to occur (Butler and Schutzmann, 1979; Mull et al., 2013; Lyons and Lowe, submitted for publication).

Since marine mammals invest a substantial amount of resources into their offspring, females can offload a large portion of their contaminants. Therefore, the contaminant concentrations of pups and calves are typically reflective of their mothers’ accumulated loads, but will change during juvenile stages due to growth dilution and dietary accumulation (Wells et al., 1994; Ross et al., 2000; Metcalfe et al., 2004). This phenomenon may also occur for elasmobranch species, where reproductive energetic investment is also large (Carrier et al., 2004). The high levels of organic contaminants measured in young of the year (YOY) white sharks were primarily attributed to maternal offloading, as many of these young sharks could not acquire these levels through their own feeding (Mull et al., 2012, 2013). Through an opportunistic sampling of a late...
maturity female salmon sharks were obtained from the sport fishery in Prince William Sound, Alaska (collected by A. Carlisle).

2.1.3. Shortfin mako and thresher sharks

Shortfin mako (herein “mako”) and thresher shark samples were obtained from annual National Oceanic and Atmospheric Administration (NOAA) shark survey cruises and fishing tournaments conducted between 1996 and 2012 in the SCB. Mako sharks were sampled from all age classes, including YOY (n = 22), juvenile (n = 1), near-maturity females (n = 4), and adults (n = 4). Similarly, thresher shark samples were obtained from YOY (n = 19), juvenile (n = 7), and near-maturity females (n = 6). Only one adult female thresher was sampled, which happened to be pregnant when landed (Lyons and Lowe, submitted for publication). The near-term thresher embryos were included in the analysis of YOY contaminants.

2.2. Chemical analyses

Muscle samples were only analyzed for total mercury while liver was only analyzed for organic contaminants since previous studies have shown these contaminants to have higher concentrations in these respective tissues (Endo et al., 2008; Mull et al., 2012). Analysis took place at California State University Long Beach’s Institute for Integrated Research on Materials, Environment and Society and a private laboratory, PHYSIS.

2.2.1. Organic contaminants

Each liver sample was analyzed for 54 PCB congeners, DDT and its metabolites (2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDT, 2,4'-DDT), and 24 non-DDT pesticide compounds, and summed by contaminant group to obtain total concentration (abbreviations herein PCBs, DDT, pesticides) as well as total organic contaminant concentrations (i.e., ΣPCBs + ΣDDTs + Σpesticides) following Mull et al. (2012). Briefly, approximately 0.5–1 g of liver was extracted with a Soxhlet apparatus in 100% dichloromethane (DCM) solvent for 14–16 h. Before extraction, samples were spiked with four recovery surrogate analytes to measure the extraction and recovery efficiency throughout sample preparation. After extraction, lipid content was determined gravimetrically from split aliquots of the extracts after removing the solvent. Extracts were then purified by eluting them through Alumina-B/Silica gel with hexane, 30% DCM in hexane, followed by DCM. Samples were then concentrated by rotovap, spiked with internal standards, and injected using an autosampler (7683B series, Agilent Technologies, Santa Clara, California, USA) onto an Agilent gas chromatograph (GC; 6890N series) equipped with a mass selective detector (MSD; Agilent 5973 inert series). The GC column employed was a ZB-5 (Phenomenex; Torrance, California) fused silica capillary (0.25 mm ID × 60 m) with 0.25 µm film thickness. Helium was used as the carrier gas at a flow velocity of 40 cm/s. The MSD was used in the Electron Ionization (EI) mode and scanned from 45 to 500 amu at a rate of 1.66 scans/s. Data were then acquired and quantified by the software in the GCMS system. Detection limits of the system were found to be 1 ppb (ng/g) on a wet weight basis. In cases where extracts had contaminant concentrations that exceeded the linear limits of the GCMS extracts were diluted appropriately and reanalyzed.

2.2.2. Mercury analysis

Approximately 0.5 g (wet weight) of white muscle was digested in a 15 mL 9:5:1 mixture of water, trace metal grade nitric acid, and hydrochloric acid, respectively, in a MARS 5 microwave reaction system (CEM Corporation, Matthews, NC). Samples were preserved in 2% nitric acid until analysis. Samples were analyzed for total mercury (herein “mercury”) using a Hydra AF Gold + Automated Mercury Analyzer (Teledyne Leeman Labs Inc, Hudson, NH) and accompanying WinHg software using EPA mercury analysis method 245.7. Standard mercury curves ranging from 0 ppt (parts per trillion) to 10 ppb were created using 1 ppm mercury stock solution (Plasma-Pure Standard Solution, Leeman Labs, Inc) and samples were diluted to the range of the curve. Detection limits of the machine ranged from 1 to 2 parts per trillion. One ppb standards and blanks were run at the beginning and end of each batch as well as after every 14th sample to ensure accuracy and no contamination between samples in the analyzer.

2.2.3. Quality assurance quality control

With each batch of 24 samples analyzed, six were designated for data quality assurance quality control purposes and run in parallel with study samples. One blank, two sample replicates, one certified reference material (Lake Michigan Trout tissue 1947, National Institute of Standards and Technology for organics and either DOLT-3 or DORM-2, National Institute of Standards and Technology for mercury), and two blank spikes (organics only) were employed to ensure precision and accuracy in analytical process. Blank spikes were employed instead of matrix spikes since the signal of the spikes was expected to be masked by the anticipated high concentration of contaminants found in samples. No contamination was detected in blank samples and 90 ± 8% compounds measured in certified reference materials were within at least 30% of true values. The average (±SD) relative significant difference between replicates was 15 ± 4%. For organics, recovery surrogates fell within 25% or better of spiked values and 88 ± 12% of compounds measured from blank spikes samples were within 30% of spiked concentrations.

2.3. Data analysis

2.3.1. YOY species differences

Organic contaminant (OC) concentrations were compared by contaminant group (e.g., PCBs, DDTs, pesticides, mercury) and species for YOY sharks, including the embryonic thresher sharks. Due to the inability to obtain equal variances, contaminant concentrations among species were compared using Kruskal–Wallis non-parametric tests followed by Wilcoxon rank sum tests for pairwise comparisons. In addition, YOY salmon sharks were compared using Welch’s t-tests since sharks were sampled in different geographic regions (i.e. California and Oregon).

Besides differences in contaminant concentrations, we might also expect to see variation in species’ congener profiles since differences in habitat use and diet may influence what types of contaminants are accumulated (Aguilar et al., 2002). Relative proportions of organic contaminants were calculated by normalizing each detected chemical to the most abundant and recalcitrant contaminant that is accumulated (Aguilar et al., 2002). Differences in habitat use and diet may influence what types of contaminants are accumulated (Aguilar et al., 2002). Relative proportions of organic contaminants were calculated by normalizing each detected chemical to the most abundant and recalcitrant contaminant that is accumulated (Aguilar et al., 2002). Differences in habitat use and diet may influence what types of contaminants are accumulated (Aguilar et al., 2002).

2.3.2. Influence of maternal transfer

Levels measured in YOYs were assumed to be largely derived from their mothers due to their young age. Therefore, we were interested in examining the relationship between the fourth-root transformed [herein “transformed”] YOY summed OC loads with respect to maternal trophic position through a generalized linear model (GLM) to determine if mother’s trophic level may influence maternal contaminant transfer among species. YOY total OC...
concentrations were obtained by summing PCBs, DDTs, and pesticides [herein "summed OCs"] per individual. Estimated trophic levels for adult white, mako, and thresher sharks were compared from three sources utilizing different methods (e.g., Cs/K ratios, Schafer et al., 1981; stomach content and diet analysis, Cortez, 1999; stable isotope analysis, Estrada et al., 2006). Due to similarity in trophic level estimates among studies as well as consistent relative order (i.e., thresher < mako < white shark), only trophic levels obtained from Schafer et al. (1981) were used in subsequent analysis since trophic level estimates were derived from sharks sampled in southern California. While comparable trophic level estimates for adult salmon sharks were unavailable (K. Goldman, pers. comm.), we assumed that adult salmon sharks were at an intermediate trophic level between that of thresher and mako sharks based on available diet information (Nagasawa, 1998; Goldman and Musick, 2008). Salmon (Onchorhynchus spp.) is a common prey item of salmon sharks and diet analysis of salmon species (Kaeriymäki et al., 2004) suggests they feed at higher trophic levels than bait fishes, which is a common prey item of thresher sharks, but below mako shark prey, which occasionally includes tuna and marine mammals. Since adult salmon sharks feed in more pristine environments (e.g., Alaska) we were interested in the response of the relationship as adult salmon shark trophic level was varied. Therefore, we also carried out GLMs by subsequently placing adult salmon sharks at following trophic levels: low (3.9), hi-low (4.0), medium (4.10), low-hi (4.20), and high (4.30). These estimated salmon shark trophic level extremes were higher than those used for thresher sharks (3.82) and lower than that for mako and white sharks (4.40 and 5.02, respectively; Schafer et al., 1981).

Marine mammal females experience their highest contaminant loads prior to their first reproductive event and subsequently offload the greatest amount of contaminants to offspring at this time (Borga et al., 2004). Therefore, we were interested in examining the differences in OC concentrations measured among near-maturity shark species prior to reproduction as an additional factor that may influence maternal offloading. Since the age at maturity greatly varies among these four shark species (12–17 years for white, 7–14 years for mako, 5.3–7 years for thresher, and 6–9 years for salmon sharks; Snelson et al., 2008; Goldman and Musick, 2008), we hypothesized that later maturing species would have greater concentrations. Due to low sample sizes, we were only able to measure and compare summed OC levels among near-maturity thresher, salmon, and mako shark females to examine the differences in their contaminant concentrations prior to their first reproductive event. Summed transformed OC levels were used when statistical comparisons were possible.

2.3.3. Bioaccumulation of contaminants

Since samples were available from either YOY, juvenile, near-mature, and/or adult individuals for some species, we investigated how OC concentrations and DDT:PCB ratios measured in YOY sharks compared to older age classes by fork length and differences in bioaccumulation potentials among species. Transformed summed OC concentrations by age class within a species were compared using linear regression and appropriate pair-wise or multiple comparison tests depending on the transformability of the data and sample sizes.

3. Results

3.1. YOY species differences

On average (+SD), YOY white and mako sharks were found to have comparable levels of PCBs (17.4 ± 9.5 and 17.8 ± 12 µg/g, respectively, Kruskal–Wallis tests $H_3 = 55.1, p = 1.0$) and pesticide contaminants (1.6 ± 1.0 and 1.9 ± 1.6 µg/g, respectively, $H_3 = 45.7, p = 1.0$), which were significantly higher than the levels found in YOY thresher and salmon sharks ($H_3 = 55.1$ and 45.7, respectively, $p < 0.0001$; Fig. 1). Thresher and salmon shark PCB concentrations were significantly different (1.5 ± 0.7 and 0.9 ± 0.9 µg/g, respectively; $p = 0.023$), but pesticide (0.2 ± 0.1 and 0.3 ± 0.2 µg/g, respectively, $p = 1.0$) concentrations were not significantly different and had substantially less variability than mako or white sharks.

While not significantly different, white shark average (+SD) DDT concentrations were over two times higher than those of mako sharks (103.3 ± 93.1 and 40.2 ± 37.3 µg/g, respectively, $p = 0.78$) and both were significantly higher than for thresher and salmon sharks ($H_3 = 59.3, p = 0.0001$). However, thresher sharks had significantly higher concentrations of DDTs than salmon sharks (3.5 ± 1.8 and 1.3 ± 1.0 µg/g, respectively, $p = 0.00012$). When salmon sharks were compared between sampling locations (California versus Oregon) there were no significant differences between the sum of organic contaminants (Welch’s t-test, $t_{27.23} = 0.12, p = 0.90$) found in the livers. There was no correlation between liver lipid content and contaminant concentration for any of the four species (Pearson’s correlation, $p = 0.34$–0.96).

Total muscle mercury followed similar patterns as the organic contaminants with mako and white sharks having significantly higher concentrations than thresher or salmon sharks ($H_3 = 62.68, p < 0.001$). White sharks had the highest average muscle concentrations (1.21 ± 1.11 µg/g) followed by mako sharks (0.68 ± 0.43 µg/g). Salmon sharks (0.25 ± 0.13 µg/g) had significantly higher mercury concentrations than threshers (0.12 ± 0.06 µg/g, $p = 0.0002$). Approximately 30% of white and 19% of mako shark muscle samples exceeded EPA levels of consumption concern (>1.3 µg/g ww: Klaing et al., 2009), with maximum mercury concentrations of 4.66 and 1.7 µg/g for YOY white and mako sharks, respectively. In addition to higher contaminant concentrations for both organics and mercury, white and mako sharks also had substantially higher absolute and relative variability among contaminant groups compared to thresher and salmon sharks. California and Oregon salmon sharks showed no significant differences in muscle mercury concentrations ($t_{23.28} = 0.73, p = 0.47$).

Besides differences in contaminant concentrations, the species also exhibited distinct contaminant signatures. ANOSIM results indicate that relative proportions of contaminants were significantly different among species with no pair being alike (Global $R = 0.477, p < 0.001$; Fig. 2A). When comparing contaminant proportions within species, mako, thresher, and salmon shark samples were 76–78% similar to their conspecifics, while white sharks were only 63% similar to each other. However, removal of the three “outlier” white sharks by visual inspection (Fig. 2A) resulted in an increase in the similarity among white sharks to 77%, which was comparable to the other three species (Fig. 2B). Differences in the proportion of DDT and its metabolites were primarily responsible for the separation among species. In particular, the proportion of 4,4′-DDE accounted for anywhere from 38 to 74% of the differences between species in pairwise comparisons, with all other individual contaminants contributing very little to the separation (i.e., less than 10%). Salmon sharks, however, were consistently separated from the other three species by the proportions of aldrin, trans-nonachlor and dieldrin pesticides found in their tissues after 4,4′-DDE, which was not the case when white, mako, or thresher sharks were compared between each other.

Contaminant signatures related to DDT:PCB ratios also differed among species (Fig. 3). YOY white shark had the highest ratio (5.21 ± 1.2) and was significantly higher than the other three species ($H_3 = 38.11, p < 0.0001$). Thresher sharks had the next highest average DDT:PCB ratio (2.26 ± 0.58). While thresher shark ratios were not significantly different from mako shark ratios (2.14 ± 0.96,
$p = 1.0$, salmon shark ratios were significantly lower (1.52 ± 0.69, $p < 0.001$). Salmon and mako sharks had comparable ratios ($p = 0.18$). Ratios of salmon sharks sampled from central California were slightly but not significantly higher than those sampled in Oregon (1.60 ± 0.54 and 1.3 ± 0.42, respectively; $F_{26,37} = 1.64$, $p = 0.11$). However, the salmon shark from southern California had a much higher ratio compared to sharks from the more northern latitudes (4.15 and 1.45 ± 0.5, respectively).

### 3.2. Influence of maternal transfer

A significant positive relationship was found between maternal trophic position and individual YOY transformed contaminant loads for each of the three sources used to estimate maternal trophic position ($F_{50,51} = 5.7, 5.04, 4.04$, in all cases $p < 0.0001$). The relationship was maintained when salmon sharks were included in the GLM, regardless of the trophic position at which salmon sharks

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**Fig. 1.** Boxplots of contaminant concentrations of liver (organic contaminants) and muscle tissue (total mercury) among four species of YOY sharks (Log$_{10}$ scale). Whiskers and crosses represent minimum and maximum values and group means, respectively, for A) PCBs, B) DDT, C) Pesticides, and D) Mercury (Hg). Sample sizes are listed in parentheses and letters denote statistically similar groups. White and mako sharks were found to have significantly higher levels of all contaminants compared to thresher and salmon sharks. Thresher sharks had higher concentrations of PCBs and DDTs, but lower levels of mercury compared to salmon sharks.

**Fig. 2.** Spatial separation of species contaminant ratios using MDS of all samples (A) and excluding the white shark outliers (B). Species are indicated with different colors and symbols. Species were found to have significantly different contaminant signatures. White sharks within group similarity was much lower and is reflected by the wide spread of points (black diamonds) compared to the other three species (A).
were placed ($F_{92.81} = 11.7 - 7.2$, in all cases $p < 0.00001$). However, the fit of the model significantly decreased as salmon shark trophic position was increased ($F_{1.3} = 385, R^2 = 0.98$, $p < 0.001$).

Near-maturity makos had significantly higher levels of OCs than near-maturity thresher sharks ($F_{3.005} = 4.23$, $p = 0.02$; Fig. 4). The single near-maturity and adult salmon sharks had substantially lower levels than the other two species. While adult sample sizes were not adequate to perform interspecific statistical comparisons, the adult mako sharks had substantially higher OC concentrations than the adult salmon shark and pregnant thresher shark.

### 3.3. Bioaccumulation of contaminants

YOY contaminant loads for thresher sharks significantly decreased with increasing fork length ($F_{1.25} = 26.35$, $p = 0.0001$; Fig. 5B). While a positive relationship was found between transformed organic contaminant loads and increasing fork length for near-maturity thresher sharks ($F_{1.4} = 10.04$, $p = 0.034$), no significant relationships were observed for any other age class or combination of age classes regardless of whether the pregnant female was included or excluded (Table ii, supplemental). When all age classes older than YOY were examined mako sharks showed a positive relationship with contaminant concentrations as size increased ($F_{1.7} = 22.88$, $p = 0.002$; Fig. 5C). No relationship was found between OC transformed concentrations and YOY mako fork length ($p = 0.11$; Table ii, supplemental). However, removal of the largest YOY outlier from the data set resulted in a marginally significant negative relationship ($F_{1.13} = 2.81$, $p = 0.041$) for this age class.

Near-maturity and adult mako sharks had comparable levels of OCs ($t_{6} = 0.41$, $p = 0.69$) and both were significantly higher than levels measured in YOYs ($t_{22} = 3.24$, $p = 0.003$).

Unlike the mako and thresher sharks, YOY salmon and white sharks did not show a negative relationship between length and OC transformed concentration (Fig. 5A and D, respectively; Table ii, supplemental). However, when only non-YOY age classes were considered, negative relationships resulted for both salmon and white sharks ($F_{1.2} = 70.71$, $p = 0.014$ and $F_{1.2} = 6.58$, $p = 0.037$, respectively). None of the species (YOY classes only) showed significant relationships between fork length and mercury concentrations (Table ii, supplemental).

DDT:PCB ratios of both thresher and mako sharks significantly increased with fork length ($F_{1.29} = 18.91$, $p = 0.0002$ and $F_{1.25} = 44.79$, $p < 0.001$, respectively; Fig. 6) and were not significantly different between species ($F_{1.54} = 1.33$, $p = 0.25$). Near-maturity and adult mako and thresher sharks had significantly higher ratios than YOYs ($t_{21} = 7.64$, $p < 0.0001$ and $t_{6.4} = 3.85$, $p = 0.007$, respectively). Although the pregnant thresher shark had a substantially lower ratio compared to the near-maturity thresher females (2.19 versus 5.7 ± 1.56), her embryos did not have disproportionately higher ratios (2.19 versus 2.35 ± 0.07).

### 4. Discussion

The differences found in organic contaminants and mercury concentrations in YOY sharks from four closely related species provide insights into factors influencing maternal offloading in elasmobranchs. Since adults of these species vary in their habitat use and trophic level, the discrepancies in contaminant concentrations and signatures in YOYs may be used to provide insights into adult trophic ecology. The differences in the magnitude of contaminants maternally offloaded to offspring, in addition to other factors, will then likely influence contaminant bioaccumulation trajectories among these four species.

#### 4.1. Contaminant differences in YOY sharks as trophic ecology tracers

The high degree of contamination in YOY and juvenile sharks found in this study can largely be explained by maternal offloading, indicating that like marine mammals elasmobranchs have the capacity to offload contaminants to their young (Butler and Schutzmann, 1979; Mull et al., 2013). Lamnid sharks are oophagous and in utero consumption of contaminated eggs produced by their mother is the likely mechanism by which developing embryos acquire contaminants during gestation (Lyons and Lowe, submitted for publication). The concentrations measured in young white and mako sharks were comparable to the levels that have been measured in the juveniles of other upper trophic level predators such as harbor seals (Phoca vitulina; Ross et al., 2004; Drescher et al., 1977), common bottlenose dolphins (Tursiops truncatus; Kuehl and Haebler, 1995), and orcas (Orcinus orca; Ylitalo et al., 2001) for which maternal offloading has been demonstrated. Therefore, while the mechanism of transfer may differ, the results of this study demonstrate that some elasmobranch species have the potential to offload contaminants at rates comparable to those observed in marine mammals.

However, maternal transfer magnitude appears to vary among species, which was reflected in the drastically different contaminant concentrations measured among the four species. YOY white and mako sharks were found to have higher levels of all contaminant groups, including mercury, relative to salmon and thresher sharks. Since these contaminant loads are assumed to be largely reflective of contaminants accumulated by their mothers, the...
potential for offloading is likely a result of the dynamic interplay of factors such as age at maturity, foraging location, and trophic ecology (Borgå et al., 2004). Of the four species, adult white sharks feed at the highest trophic levels followed by makos (Schafer et al., 1981). Adult salmon and mako sharks are likely predominantly oceanic and thresher sharks the most coastal (Hanan et al., 1993; Goldman and Musick, 2008). While sub-adult and adult northeastern Pacific white sharks spend considerable time in oceanic habitats (Weng et al., 2007b; Domeier and Nasby-Lucas, 2008; Jorgensen et al., 2010), a substantial portion of their foraging for some individuals occurs in coastal habitats (Carlisle et al., 2012).

Besides dramatic differences in contaminant concentrations, organic contaminant profiles were also found to be significantly different among species, indicating differential adult habitat use. Since coastal California has such a strong DDT signature, the presence and proportion of this contaminant in animal tissues can therefore be used as a marker of habitat use or feeding location (Krahn et al., 2007). YOY white sharks had substantially higher DDT:PCB signatures than the other three species, which suggests a stronger coastal influence at some point in the life history of white sharks relative to the other species (Subramanian et al., 1988; Krahn et al., 2007). Juvenile white sharks tend to be associated with shelf habitats (Dewar et al., 2004; Weng et al., 2007a, 2012), and acoustically tagged sharks have been detected on and around the Palos Verdes Superfund site (C. Lowe, unpubl. data), where high levels of DDT have been measured (Eganhouse et al., 2000). However, the high DDT:PCB ratio may also be influenced by adult females feeding on coastal prey items such as California sea lions (Zalophus californianus), harbor seals (Phoca vitulina richardsi), and juvenile northern elephant seals (Mirounga angustirostris) from southern and central California (Klimley et al., 1992). Although adults may spend a considerable amount of time offshore, stable isotope data suggest that a large proportion of adult white shark diet is derived from coastal versus pelagic sources (Carlisle et al., 2012). While this signature likely represents a combination of YOY feeding and maternal offloading, since sampled YOY sharks were <1 yr old it is most likely that maternal input drives this pattern.

After white sharks, YOY thresher sharks had the next highest DDT:PCB ratio, suggesting that adult thresher prey items are less exposed to DDT than adult white shark prey. Although tagging...
data indicate that YOY and juvenile thresher sharks use coastal habitats extensively (Cartamil et al., 2010a), foraging habitat of juvenile thresher sharks is likely more pelagic (i.e., less exposed to contaminants in the sediments) than the habitat of juvenile white sharks based on diet information for these two species (Tricas and McCosker, 1984; Preti et al., 2012). While adult thresher sharks utilize more oceanic habitats than younger individuals (Cartamil et al., 2010b, 2011), this species is typically regarded as “coastal” compared to mako sharks. Therefore, the lack of difference between YOY thresher and mako shark ratios was unexpected given that adult makos tend to utilize offshore habitats (Cailliet and Bedford, 1983; Vetter et al., 2008) and suggests a greater degree of habitat overlap between these two species than once thought. However, large makos have been documented to feed on coastal marine mammals and tunas (Preti et al., 2012), which themselves may have high levels of DDT. The higher than expected levels of DDT found in YOY mako sharks may be the result of maternally offloaded contaminants acquired from prey items bearing strong coastal California signatures.

Salmon sharks had significantly lower ratios than the other three species, which is expected since adult salmon sharks utilize habitats and feed on prey farthest away from DDT hotspots in coastal Alaska and the Subarctic Gyre (Weng et al., 2008; Carlisle et al., 2011). While no differences were observed in terms of contaminant load or DDT:PCB ratio between Oregon and central California sampled YOY salmon sharks, the stranded salmon shark from Huntington Beach had a much higher DDT:PCB ratio than all other sampled salmon sharks; although it was smaller than the average size, which suggests this YOY was feeding in southern California. Since total contaminant concentrations in this individual were comparable to the low levels measured in its more northern conspecifics, the amount of contaminants acquired through its own diet may not have been substantial, but the increased proportion of DDT was likely enough to alter this ratio, given the low DDT proportions measured in other YOY salmon sharks.

Although salmon and thresher sharks had similar levels of pesticides, YOY salmon sharks had higher proportions of chlordane compounds and giving them a distinct chemical signature compared to the other three species. While banned in the U.S., these compounds are still used and produced in Asian countries and are subsequently deposited by atmospheric transport over the Arctic. This has lead to relatively higher chlordane contributions in animals that feed in polar regions, which has been described as an “Alaskan” signature (Krahn et al., 2007). While YOYs were not caught in Alaska, the chemical signatures of their tissues corroborate what is known about adult females from tagging studies that show salmon sharks occupying higher latitudes than the other three species (Weng et al., 2008; Carlisle et al., 2011).

The similarity in the relative proportion of contaminants among individuals of a species, save for white sharks, was surprisingly high. This may reflect more consistent habitat use by mako, salmon, and thresher sharks compared to white sharks. If conspecifics are consistently using similar habitats, they should acquire similar contaminant signatures. White sharks have a unique “onshore—offshore” migration pattern as adults as well as a “north—south” migration pattern between the U.S. and Mexico as juveniles (Weng et al., 2008, 2012). In addition, two different adult aggregation sites exist in the eastern Pacific (Domeier and Nasby-Lucas, 2008; Jorgensen et al., 2010), separated by nearly 10 degrees of latitude, and adults feeding in these areas likely acquire very different contaminant signatures due to differences in latitude as well as available prey items (i.e., tuna versus marine mammals). The differential habitat use and prey selection of adult white sharks may contribute to the high variability observed in YOY levels relative to the other species.

### 4.2. Influence of life history characteristics on maternal transfer potential

Since females must acquire contaminants in order to transfer them, factors influencing the ability of females to bioaccumulate contaminants could affect the amount they are able to transfer to young. Trophic position has been clearly shown to influence contaminant bioaccumulation in species (Borgå et al., 2004) and could play a role in maternal offloading processes. For example, orca calves from populations in British Columbia that prey on fish had substantially lower contaminant levels than offspring whose mothers preyed upon marine mammals (Ylitalo et al., 2001). The positive relationship we observed between YOY contaminant burdens and maternal trophic position in white, mako, and thresher sharks follows our expectation since these contaminants are magnified through the food web. Thus, mothers that feed higher on the food chain should acquire contaminants at faster rates than those that feed at lower levels and transfer higher contaminant loads to their offspring.

However, maternal trophic position is not the only factor influencing maternal offloading. Since YOY salmon shark levels were lower than expected, given that adults are presumed to feed at intermediate trophic levels compared to thresher and mako sharks, this suggests that foraging location may also play a role in maternal offloading processes. Indeed, adult salmon sharks forage in the subarctic waters of the North Pacific (Goldman and Musick, 2008; Carlisle et al., 2011), which is a relatively more pristine environment compared to the more industrialized California coastline utilized by the other species. Differential habitat use by adult salmon sharks has likely reduced their dietary contaminant input, despite their intermediate trophic position, and subsequently the amount of contaminants they may potentially offload to offspring.

In marine mammals, females have been demonstrated to attain their highest contaminant concentrations just prior to maturity (Ross et al., 2000), after which their loads decrease considerably as much of their burden is transferred to offspring (Borrell et al., 1995; Hickie et al., 1999). Species that reproduce later in life have longer opportunities to accumulate contaminants prior to reproduction and would be expected to offload higher levels of contaminants to offspring than females of other species that reproduce at younger ages. In the current study, near-maturity mako sharks, which mature relatively late in life (7–14 yrs), were found to have significantly higher levels of organic contaminants in their livers than near-maturity thresher sharks, which mature earlier (5.3–7 yrs; Snelson et al., 2008). Both near-maturity thresher and mako sharks had substantially higher loads than the single near-maturity salmon shark, despite the latter’s intermediate age at maturity (6–9 yrs; Goldman and Musick, 2008), which is likely due to the salmon shark’s lower environmental exposure. Assuming that near-maturity sharks will offload a substantial portion of these contaminants at their first reproduction similar to marine mammals, the large differential in contaminant concentrations among these near-maturity females suggests that amount of contaminants offloaded to offspring will vary among species and likely contributes to the large differences and high variability in YOY contaminant concentrations. Since white sharks mature at even later ages (12–17 yrs; Snelson et al., 2008), we might expect near-maturity female white sharks to have even higher loads than the mako sharks measured in this study. While age at maturity is likely a factor, we were unable to test the direct effect that reproductive age has on maternal offloading due to its high correlation with maternal trophic position (p < 0.001), variation in offloading dependent on females’ previous reproductive history, and differences in gestation length and number of offspring produced among species (Snelson et al., 2008; Goldman and Musick, 2008).
In addition, timing of these species reproductive cycles vary and may be another factor influencing the amount of contaminants females may transfer to offspring. Longer gestational periods would likely lead to increased transfer of contaminants since females would have more time to offload. Salmon and thresher sharks have comparably shorter gestation periods than white and mako sharks (approximately 9–12 versus 12–18 months, respectively; Snelson et al., 2008); resulting in species with the longer gestational periods also having higher YOY contaminant concentrations. However, despite the fact that length of gestation is nearly doubled in white sharks compared to salmon sharks, on average YOY white shark summed organic contaminant concentrations were approximately 50 times higher than YOY salmon shark average contaminant burdens. While length of gestation likely plays a role in the extent of maternal offloading, factors related to mothers’ ability to accumulate contaminants such as trophic level, age at maturity, and time between subsequent pregnancies are likely more important in determining how much she may offload. However, differences in any or all of these factors may account for the high variability within and among species.

4.3. Potential factors influencing patterns of bioaccumulation of contaminants

The negative relationship between YOY fork length and organic contaminant concentration in mako and thresher sharks provides support for our assumption that contaminants measured in YOY sharks would be largely the result of what they received in utero, allowing us to use YOYs to make inferences about aspects of their mother’s trophic ecology. We had assumed that sharks receive a bolus of contaminants during gestation, and we might expect to see one of two outcomes if these young sharks were obtaining contaminants through their diet at appreciable rates after parturition: 1) contaminant concentrations would remain constant as fork length increased (i.e., uptake matches growth dilution) or 2) contaminant concentrations would increase faster than growth dilution (i.e., uptake is greater than growth dilution). In the case of organic contaminants, there was a clear negative trend as size increased in threshers, suggesting that these YOY sharks have obtained a majority of their contaminants from their mothers and that their own dietary acquisition was relatively low, resulting in subsequent dilution with growth. This relationship was also observed in mako sharks, although it was weaker than that observed in thresher sharks due to the increased variability.

A negative trend in organic contaminant concentration with size was not found for YOY white or salmon sharks; however, this is likely due to several factors. First of all, the lack of neonatal or embryonic samples in our data set may be an important factor to help anchor the points needed to demonstrate a relationship and these were only available for mako and thresher sharks. In the case of white sharks, the high variance in YOY concentrations among individuals may mask any observable patterns. However, the negative relationship observed among juveniles may provide support for the hypothesis that maternally acquired contaminants are diluted with post-natal growth. While substantially larger in size, two of the juvenile white sharks had considerably lower levels than YOYs, which suggests that white sharks may not accumulate contaminants at appreciable rates through their diet at this stage. Although YOY salmon sharks had comparable contaminant levels, they did not exhibit growth dilution patterns similar to those in thresher sharks. Differential growth rates and post-partum contaminant uptake rates relative to offloaded concentrations between salmon and thresher sharks may influence the ability of YOY salmon sharks to exhibit growth dilution at this young age. However, when juvenile individuals were included a negative relationship (i.e., growth dilution) was found for salmon sharks. Unfortunately, the sample size was too small to make any definitive conclusions.

The point at which sharks begin to show patterns of organic contaminant bioaccumulation appears to vary among species and is likely influenced by species-specific ontogenetic changes in feeding ecology. The large and significant increase in contaminant concentration with fork length in mako sharks older than YOY clearly demonstrated continuing bioaccumulation of contaminants in this species. As mako sharks increase in size, they begin to incorporate a wider variety and higher trophic level (i.e., more contaminated) prey items into their diet compared to younger sharks (Preti et al., 2012). In addition, opportunistic feeding on contaminant laden marine mammals from the SCB by large mako sharks could substantially increase their dietary inputs even if marine mammals are not a regular prey item.

Thresher sharks, on the other hand, did not show any significant increases in organic contaminants with size, not including the pregnant female that had clearly lowered her burden upon transfer to embryos. Juvenile and adult threshers have been found to feed on the same types of low trophic level prey items, such as small fishes, throughout their ontogeny (Preti et al., 2012). Their relatively fast growth rate (Smith et al., 2008), low trophic position, and consistent low input of dietary contaminants throughout their ontogeny likely results in thresher sharks exhibiting low rates of organic contaminant accumulation. Thus, we would not expect to see indications of bioaccumulation in thresher sharks until individuals, in particular males that cannot offload, reach the larger age classes, which were unavailable for this study.

Unfortunately, due to low sample size of large salmon sharks and lack of samples for sub-adult and adult white sharks we were unable to draw strong conclusions about bioaccumulation patterns in these species. Despite this, the negative trend observed in contaminant concentrations of >1 year old salmon sharks possibly suggests a low bioaccumulation rate in this species. However, the only adult salmon shark sampled in this study was female and the observed negative relationship driven by her low levels may be an artifact of maternal offloading. On the other hand, we would expect to see dramatic increases in contaminant concentrations from juvenile to adult age classes in white sharks, particularly around 300 cm total length when sharks undergo a well-documented diet shift and begin foraging on marine mammals (Tricas and McCosker, 1984; McCosker, 1985). We would expect to see a substantial increase in contaminant exposure from feeding on high trophic level prey at the sub-adult and adult stages, which would greatly alter the bioaccumulation rates of white sharks through their ontogeny and this bioaccumulation rate to be substantially greater than the other three species examined. Therefore, the occurrence and timing of ontogenetic trophic shifts likely alters bioaccumulation rates within species by age classes and also exaggerates contaminant differences among species.

DDT:PCB ratios were found to significantly increase with fork length in thresher and mako sharks. Near-maturity mako sharks were found to have significantly higher DDT:PCB ratios than YOYs and this increase in proportion of DDT suggests that larger mako sharks may incorporate prey items with stronger coastal signatures. Interestingly, the pregnant female thresher had a substantially lower ratio compared to the near-maturity thresher sharks. Since the ratios of the pregnant female’s embryos were only slightly higher it is unlikely that she offloaded a disproportional amount of DDT thereby lowering this ratio. However, the narrow size range of near-maturity sharks and lack of large mature thresher sharks makes it difficult to determine if the increase in DDT:PCB ratio from
YOY to near-maturity sharks represents a true pattern. Nevertheless, the increased variability in ratios with fork length of both species may be reflective of differential patterns of habitat use with age. As sharks increase in size, they can utilize larger areas and may not be restricted to the nursery habitats used by smaller sharks. Unlike the organics, mercury concentration showed no trend with YOY size for any of the species, suggesting that mercury and organics may accumulate at differential rates. While there is direct evidence of maternal offloading of mercury in thresher sharks (Lyons and Lowe, submitted for publication), the fact that mercury concentrations did not show signs of growth dilution (i.e., decrease in concentration with size) like the organics may be indicative of differential bioaccumulation rates for these two types of contaminants. Suk et al. (2009) demonstrated mercury to accumulate proportionally with size in muscle of thresher and mako sharks from the SCB. Despite examining comparable size ranges for thresher and mako sharks, we did not find that the rate of organic contaminant bioaccumulation increased as a linear function like mercury in this study. Detectible increases in organic contaminant concentrations will likely not occur until somatic growth substantially slows, at which point concentrations of these contaminants might be expected to increase, or ontogenetic diet shifts to dramatically increase dietary input of organic contaminants.

5. Conclusions

Differences in organic contaminants and mercury concentrations among four closely related species demonstrate that multiple factors influence maternal offloading processes. Trophic position, where mothers feed geographically, and possibly age at maturity appear to be important factors that can affect the amount and types of contaminants they may transfer to their young. Therefore, the differences found in organic contaminant signatures and concentrations among YOY species can be used to provide insights into the adult feeding ecology of these animals. However, since maternal offloading occurs, management models factoring in contaminant bioaccumulation can no longer assume YOY sharks are receiving a “fresh start” at birth since sharks may be most susceptible to contaminant exposure at this age. While the physiological effects of these contaminants on elasmobranchs remain unknown, reported increases in white shark bycatch and apparent stability of thresher shark and salmon shark populations in the northeastern Pacific suggest these contaminants are not affecting these species at the population level (Goldman and Musick, 2008; Smith et al., 2008; Lowe et al., 2012). While little is known about mako shark population trends, they would likely demonstrate similar responses to exposure as the other species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2013.05.009.

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