Final Report

South Carolina State Wildlife Grant

Project Title

Concentrations of organic contaminants in Carolina and scalloped hammerhead sharks. Implications for success and survival in nursery habitats

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FINAL REPORT
South Carolina State Wildlife Grant SC-T-F18AF00964
South Carolina Department of Natural Resources
October 1, 2018 – September 30, 2021

Project Title: Concentrations of organic contaminants in Carolina (*Sphyrna gilberti*) and Scalloped *(Sphyrna lewini*) Hammerheads: Implications for success and survival in nursery habitats

Executive Summary: The main objectives of the study were to quantify a suite of legacy organic contaminant concentrations (83 compounds) in hepatic tissue of young-of-year (YOY) sharks of two hammerhead species, Scalloped Hammerhead (*Sphyrna lewini*) and Carolina Hammerhead (*Sphryna gilberti*). YOYs were sampled from across three states in the Atlantic Southeast, with most samples coming from South Carolina (n = 104), followed by Florida (n = 29) and Georgia (n = 13). As these are cryptic species, samples were genetically assigned post-hoc as either *S. lewini* (n = 56), *S. gilberti* (n = 74), or hybrid (n = 11), and contaminant concentrations and signatures were compared between species (and hybrids when analyses allowed). Samples that could not be genetically confirmed were removed from analyses (n = 5). Organic contaminants were grouped by class (i.e. polychlorinated biphenyls [PCBs], dichlorodiphenyltrichloroethane [DDT] and its metabolites [DDX], and non-DDT pesticides) and the sum of total organic contaminant (Sum OCs) concentrations were quantified for each sample. While overall Sum OCs did not differ between species, significant differences were found for DDXs and PCBs, with *S. gilberti* tending to have higher DDX concentrations and *S. lewini* having higher PCB concentrations. A Random Forest Analysis conducted only on *S. lewini* and *S. gilberti* was able to correctly identify species 85% of the time. Interestingly, when hybrid samples were included, the ability of the model to correctly predict *S. lewini* assignment increased and hybrids were overwhelmingly (81%) assigned as *S. gilberti*, which likely reflects maternal species identity. In general, Sum OCs were highest in the smallest (i.e. youngest) sharks with concentrations decreasing with length for both species, suggesting that growth dilution may account for the decrease in mean concentrations. To test if growth dilution could account for this decrease with length, we created two growth dilution models based on previously published allometry relationships for these species. In general, a larger proportion of *S. gilberti* than *S. lewini* appeared to have contaminant concentrations that exceeded their ability to undergo growth dilution as established by our models. This may possibly be attributed to *S. lewini* having an earlier purported parturition timeframe (i.e. more "growing days") than *S. gilberti*. When these individuals were removed from the data set, the inverse relationship between fork length and Sum OCs was abolished for *S. gilberti* only, possibly suggesting a decrease in fitness and removal from the population. The results of our study suggest that maternal offloading significantly shapes YOY contaminant signatures and may have implications for survival and fitness during the first months of life, the latter of which is yet to be empirically tested.

Objective 1: Determine maternal offloading potential of contaminants in near-term pregnant sharks to quantify the degree of contaminant transfer and exposure to embryonic sharks.

Accomplishments: A total of 12 days of directed fishing (one funded by this grant) were conducted for pregnant female Scalloped *(Sphyrna lewini*) and Carolina (*Sphyrna gilberti*) Hammerheads (hereafter, hammerheads), however despite these extensive efforts, none were captured. Efforts resulted in the capture of 34 mature male hammerheads (31 *S. lewini* and 3 *S. gilberti*), and 1 immature female hammerhead (*S. lewini*). We also reached out to multiple fishery independent surveys (NMFS Narragansett, and NMFS Pascagoula Longline Surveys), as well as the NMFS Bottom Longline

Observer Program), who agreed to collect samples for mature females if encountered. Unfortunately, none were encountered despite extensive coverage.

Significant deviations: Despite these extensive efforts, we were unsuccessful at collecting two pregnant hammerheads to directly quantify maternal offloading. Looking through historical databases, encounters and captures of mature female hammerheads are exceedingly rare, despite their known occurrence nearshore during parturition. Evidence from encounters suggests female hammerheads may not feed nearshore during parturition, as the couple mature females collected off of the Bulls Bay nursery (early 1980's) were incidentally captured using gillnets. A collaborating commercial fisherman based in North Carolina (Manteo, NC) occasionally captures mature females; however, all are post-partum by the time he encounters them (mid-June). Despite the lack of direct evidence of maternal transfer in *S. gilberti,* there is ample evidence that contaminants are offloaded in other Sphyrnid species such as *S. lewini* (Lyons and Adams 2015), *Sphyrna mokarran* (Lyons unpublished data), and *Sphyrna tiburo* (Weijs et al. 2015); therefore, we believe the following analyses are valid in assuming maternal transfer occurs at a comparable rate between these two species.

Objective 2: Compare concentrations in embryonic sharks to those measured in neonates to determine how these concentrations may change during early life with growth dilution.

Accomplishments: Over the course of this study, a suite of organic contaminants (OCs) were measured in 146 sharks (See Lyons and Adams 2015 for details on contaminant analytical procedures). Contaminants include a range of polychlorinated biphenyls (PCBs; 53 congeners), dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDXs), and a number of chlorinated pesticides (Table 1). Contaminant concentrations were summed to obtain group totals for each of the main categories of contaminant type (i.e. total PCBs ["tPCB"], total DDXs ["tDDX"], total non-DDT Pesticides ["tPesticides"]) as well as the total concentration of all contaminants summed (i.e. Sum OCs). These were compared between species and by size/age class. To explore differences in contaminant signatures between the two species (as well as the two species and the Hybrids) a ratio for each contaminant for each shark was calculated by dividing individual concentrations against the concentration of PCB153 (Wolkers et al. 2004). This contaminant was selected to create proportions against, as it is reliably measured in every sample. PCB153 was removed from analysis and shark contaminant ratios were evaluated using a Random Forest Analysis (Breiman 2001). Finally, we examined the potential for growth dilution and apparent loss of variability in contaminant concentrations across sample size bins (see objective 6).

Sharks were collected by multiple fisheries surveys conducted from 2014–2018: the South Carolina Department of Natural Resources Turtle Trawl, Cooperative Atlantic States Shark Pupping and Nursery Survey (COASTSPAN) participants, Kennedy Space Center Ecological Monitoring Program (KSCEMP), Southeast Area Monitoring and Assessment Program (SEAMAP), and the Florida Fish and Wildlife Research Institute's Fisheries-Independent Monitoring (FIM) program. The majority of samples were collected from Bulls Bay, South Carolina (33.0109 N, 79.4879 W), followed by Cape Canaveral, Florida (28.4195 N, 80.5635 W). Mortalities encountered during sampling operations were placed on ice and brought back to the lab where morphometric information (fork length, total body mass, liver mass) were collected. Sharks were assigned a scar stage based on Lyons et al. (2020) and determined to be Young-of-Year (YOY) or one-year-olds. In addition, sharks were assigned to one of nine 50mm size bins based on their fork length: 250-300 mm, 301-350 mm, 351-400 mm, 401-450 mm, 451-500 mm, 501-550 mm, 551 – 600 mm, 601-650 mm, or 651-700 mm. Since *S. lewini* and *S. gilberti* are a cryptic species pair, fin clips were taken from every animal and preserved in a dimethyl sulfoxide

solution for subsequent species genetic identification following the protocols of Barker et al. (2019). Individuals were then assigned a species identification (*S. lewini* or *S. gilberti*) and analyses were conducted by species. In some instances, animal IDs were ambiguous as they were either identified as hybrids (n = 11) or they were unable to be assigned a proper species ID for some other reason (e.g. degraded DNA, missing fin clip, etc.). These latter individuals (n = 5) were not included in data analyses. The majority of the samples of this project were YOYs (*S. gilberti*: 99%, *S. lewini*: 96%, Hybrids: 100%, Missing ID: 100%), with most of the samples represented in 2014 and 2018 (Table 2). In addition, most samples (104/146, ~71%) were collected from South Carolina, followed by Florida (29/146, ~20%), and Georgia (13/146, 9%).

Significant deviations: Because we were unsuccessful at capturing two near-term pregnant female hammerheads, we were unable to compare embryonic measurements to those measured in free-swimming YOYs. However, as noted in the significant deviations for objective 1, we believe that the concentrations measured in free swimming neonates are indicative of those found in near-term embryo's (Mull et al. 2013; Lyons et al. 2013), and it is possible that concentrations in some embryos may lead to individuals born with reduced fitness causing increased susceptibility to mortality.

Objective 3: Compare concentrations in YOY *S. gilberti* and *S. lewini* to determine relative vulnerability as well as maternal contaminant signatures.

Accomplishments: Contaminant concentrations were compared between species, excluding individuals with Missing IDs (Table 3). Because of the low sample size of hybrids, they were not formally included in the analysis but are included visually at times. Total concentrations of contaminants (i.e. "Sum OCs") was not statistically different between *S. gilberti* and *S. lewini* ($p = 0.31$), or when only individuals with open or healing scars (scar ranks 0-2) were compared ($p = 0.07$). However, there were differences by contaminant groups (Table 3). Primarily, tDDX concentrations were significantly higher in *S. gilberti* than *S. lewini* samples ($W = 1190$, $p < 0.0001$), while other contaminant groups were similar (tPCBs: $p = 0.12$; tPesticides: $p = 0.96$). In addition, there were significant differences between species in the distribution of contaminant concentrations. As expected for tDDX, *S. gilberti* had distributions that were shifted to the right compared to *S. lewini* (K-S test: $D = 0.33$, $p = 0.0011$; Figure 1); however, for tPCBs, *S. lewini* was marginally shifted to the right compared to *S. gilberti* (Figure 1; $D = 0.24$, p-value = 0.044). Contaminant distributions of tPesticides and total Sum OCs did not differ between the two species.

A Random Forest Analysis was employed to determine if species could be distinguished based on their contaminant signatures (i.e. relative proportions of contaminants). In the first analysis, Hybrids were removed and the remaining dataset (excluding animals with Missing IDs) was divided 70:30, with the model created from 70% of the data (training) and the remaining 30% (testing) were used to evaluate the accuracy of the model. Among the training dataset, the model had an out-of-bag error rate of ~15% to correctly classify sharks based on their contaminant signatures (Figure 2). Between the two species, *S. lewini* had a higher rate of misclassification (~26%) compared to *S. gilberti* (~8%). A confusion matrix was used to evaluate the accuracy of the model itself on the testing data set, which was 98% accurate. Variables in the model (i.e. individual contaminants) were assessed for their importance (Table 4). Among the top 10 most important contaminants were 4,4'-DDT and its metabolites (i.e. 4,4'-DDE and 4,4'-DDD), which corroborated previous evidence of higher levels that would result in different signatures. In the second analysis, all data (excluding Missing IDs) were included to investigate how hybrids would be classified by the model based on their contaminant signatures. When hybrids were included, the model was able to better correctly classify *S. lewini* (9% error rate); however, it failed to correctly classify any of the Hybrids. Rather, of the 11 Hybrids, nine were classified as *S. gilberti* and

two as *S. lewini*. This inability of the model to "correctly" classify Hybrids is likely due to influence of maternal offloading depending on which species was the mother in the hybrid cross. This data suggests that most of the Hybrids had *S. gilberti* mothers, as reflected by their contaminants signatures, which supports similar findings in genetic results where females of the rarer species (in this case *S. gilberti*) are more likely to breed with males of the more prevalent species (*S. lewini*)(Barker et al. 2019).

Significant deviations: Not applicable. Goals of this objective were met.

Objective 4: Determine contaminant concentrations in YOY sharks from a range of sizes and stages of umbilical scar healing as a proxy for time-since-birth to quantify the variability in levels and how these levels change with growth.

Accomplishments: To determine how contaminant concentrations changed with neonatal aging, two proxies were used: size bin and scar ranking. Because of variability in litter size (Lyons and Adams 2015), neonatal animals could be of different sizes but have had the same time at liberty (i.e. same scar rank). Therefore, Sum OCs were compared by species against both indices. With regards to concentrations by size, both species exhibited a downward trajectory (Table 5; Figure 3). In general, the highest concentrations were found in the smallest sharks (size bins 1 – 2, 251 – 350 mm) and the lowest concentrations in the oldest YOYs (size bins 5-6, 451– 550 mm FL) and one-year olds (Bin 8– 9, 601 – 700 mm FL) for both species. While there were fewer data points for Hybrids, they showed a similar decreasing trend in mean contaminant concentration with increasing size.

Mean Sum OC concentration by scar rank was more complicated between the species (Table 6). For *S. lewini*, concentrations decreased with increasing scar rank (i.e. with time at liberty); however, sample size was skewed towards the older YOY ranks with only a few samples in each of the younger ranks of 0-2. For *S. gilberti*, mean contaminant concentration against scar rank demonstrated a "U-shape", with rank 0 and 4 having lower mean concentrations than ranks 1-3, which were more similar to each other. Like *S. lewini*, samples were skewed towards the older YOY scar rankings and the greatest variability in group means was at scar rank 3, which may be an artifact of this being a difficult group to stage umbilical healing rank. Hybrids were the most difficult because sample distribution was highly skewed towards scar rank 3 and 4 (89% of samples).

Significant deviations: Not applicable. Goals of this objective were met.

Objective 5: Determine contaminant concentration trajectories (increasing, steady-state, or decreasing) over the first five months of life.

Accomplishments: Contaminant trajectories were examined for both species using two indices: Day-of-Year (DOY) and fork length at the time of capture. In both cases, log-transformed data was used to meet assumptions of linear model tests (i.e. normality of residuals). For both *S. lewini* and *S. gilberti*, contaminant concentrations significantly decreased with increasing fork length (*S. lewini*: $F_{1,54}$: 24.16, $p < 0.0001$, $R^2 = 0.30$; *S. gilberti*: $F_{1,72}$: 19.76, $p < 0.0001$, $R^2 = 0.20$) with no effect of species (ANCOVA, $p = 0.57$). Hybrids showed no significant change in concentration with fork length ($p = 0.16$); however, there were fewer samples compared to the other two species (Figure 4). Contaminant concentrations with DOY was not as consistent between the species as was fork length. Although both species showed similar decreasing trends (*S. lewini*: $F_{1,54}$: 34.14, $p < 0.0001$; *S. gilberti*: $F_{1,72}$: 11.95, $p = 0.0009$), there was a significant effect of species (ANCOVA, $p = 0.002$). In addition, DOY explained a greater proportion of the variance than fork length ($R^2 = 0.38$) for *S. lewini*, whereas the opposite trend was found for *S. gilberti* ($R^2 = 0.13$).

Significant deviations: Not applicable. Goals of this objective were met.

Objective 6: Quantify the variability of contaminant concentrations in sharks both over time and among umbilical scar cohorts (i.e. sharks born around the same time) to determine if we can detect the loss of sharks with the highest concentrations, which may serve as a lethality indicator

Accomplishments: While the highest concentrations tended to be found in the smallest and oftentimes youngest individuals, we wanted to determine if the overall decreasing trends in contaminant concentration with length and time were likely due to growth dilution or possibly representing a loss of the most contaminated individuals from the data set. While contaminant concentrations are predicted to decrease with growth dilution (Lyons et al. 2019), we would anticipate variability to not change within a given cohort. In contrast, a change in group variability may indicate the loss of the most contaminated individuals, either through natural selection or capture bias. When comparing changes in standard deviation among size bins where sample size was sufficient, both species demonstrated a weakly, insignificant relationship with group variability (i.e. standard deviation) decreasing with increasing size (*S. lewini*: $F_{1,3} = 9.51$, $p = 0.054$, $R^2 = 0.68$; *S. gilberti*: $F_{1,4} = 6.49$, $p = 0.063$, $R^2 = 0.52$)(Figure 5).

To determine the possibility that growth dilution could explain decreasing trends in mean contaminant concentrations with size and time, we developed two models to predict whether sharks would be physically able (in the best circumstances) to undergo growth dilution in their first summer after birth. Mean of the largest and oldest YOYs for each species (i.e. Bin 6) was designated as the "dilution bench mark to reach" and was used in the subsequent calculations. To determine the liver size each individual shark would need to achieve, we used the following formula:

$$C_1 * V_1 = C_2 * V_2$$

where, C_1 is the concentration (ng/g ww) quantified in each shark for their liver mass (V_1) at that time, and C_2 represents the "dilution benchmark to reach" (i.e. 1,587 ng/g ww for *S. lewini* or 1,156 ng/g ww for *S. gilberti*), and V_2 represents the liver mass the animal would need to achieve by end of the summer. Next, the following morphometric relationships were needed to calculate the length that the shark would need to grow to achieve V_2:

$$M = aL^b$$

where M represents liver mass, L represents fork length and a and b are previously determined coefficients (see Table 7). Solving for L enabled us to calculate the amount of growth (in mm) each shark would need to dilute their starting concentrations to our set bench mark. We then calculated the maximum potential growth in a season by subtracting the length of the smallest YOY (*S. lewini* = 307 mm FL; *S. gilberti* = 270 mm FL) from the largest YOY (*S. lewini* = 533 mm FL; *S. gilberti* = 518 mm FL) in our data set by species. This maximum potential growth was used as our cut off to determine if the growth needed to dilute was physically achievable for each species by size bin and scar stage.

We took our predictions a step further by attempting to account for temporal differences in sampling since sharks caught later in the year would have less time to grow for the season than sharks caught earlier in the summer. Thus, we divided the maximum potential growth by the difference in time between when the smallest and largest YOY for each species were captured in our data set to estimate a "growth rate per day" (i.e. *S lewini* = 1.6259 mm FL/day; *S. gilberti* = 2.1565 mm FL/day). We then determined the number of "growing days available" for each shark as the difference between October 31st and the date of capture. The number of "growing days available" was multiplied against "growth rate per day" to determine the potential "growth possible" a shark could achieve for the remainder of the season. "Growth possible" was compared against "growth needed" (see above) and a 1:1 relationship was used to determine if growth dilution was possible for each shark.

These models represent conservative predictions to quantify if growth dilution is possible. First, these models assume there is no additional contaminant input during their first growing season (i.e. no new inputs via feeding); therefore, contaminant numbers represent an underestimation of exposure since young hammerheads are assumed to be voracious predators (Galloway et al. *in prep*). This model also assumes that there is no change in trajectory between known relationships of fork length and liver mass for YOYs, which is likely not always the case in the field as some sharks will be less successful at feeding than others, which would affect their body condition and potential growth. Finally, we included two extra weeks of "growing buffer" by setting October 31st as the "end date" for a YOY's growing season, considering that the largest YOYs captured in the data set occurred on October 14th for *S. lewini* and Oct 17th for *S gilberti*.

When considering absolute growth needed (i.e. not accounting for sampling date), 11% of *S. lewini* (6/55) and 33% *S. gilberti* (24/73) would not have been physically able to grow large enough to account for the mean decrease in contaminant concentrations needed by the end of their first summer season (Figure 6). For *S. gilberti*, a majority of the individuals where growth dilution was not predicted were also the smallest animals (i.e. size bins 1-2); however for every size bin there was at least one individual that appeared to not be capable of growth dilution according to our model. In contrast, individual *S. lewini* where growth dilution was not predicted to be possible was restricted to the smaller size bins (Bin 2 & 3). Likewise for scar stage, *S. gilberti* had a greater proportion of individuals who were predicted to not be able to meet our contaminant benchmark compared to *S. lewini* (Figure 7). Finally, when accounting for "growing days available", a similar proportion of 34% *S. gilberti* (25/73) would not have met our benchmark, whereas only 5% (3/66) of *S. lewini* would not (Figure 8). The decrease in proportion for *S. lewini* is likely attributable to the extra growth buffer we included in the model. Nevertheless, growth dilution as the reason to account for contaminant decreases with length seems less likely for *S. gilberti* than *S. lewini*. The greater proportion of *S. gilberti* individuals seemingly incapable of growth dilution could be related to the later purported parturition time of this species compared to *S. lewini*, which would leave fewer potential "growing days" for *S. gilberti*. When the relationship between fork length and Sum OC concentration for each species was re-examined after removing individuals that could not undergo growth dilution, the relationship was weaker for *S. lewini* ($F_{1,47} = 13.38$, $p = 0.0006$, $R^2 = 21$) and became insignificant for *S. gilberti* ($p = 0.13$). For *S. gilberti* in particular, this suggests that growth dilution was not the reason for the initially observed decrease with fork length, but decrease in YOY concentrations with size may be occurring for other reasons, such as removal from the population, potentially due to decreased fitness.

Significant deviations: Not applicable. Goals of this objective were met.

Literature Cited:

Barker, A.M., Adams, D.H., Driggers III, W.B., Frazier, B.S. and Portnoy, D.S., 2019. Hybridization between sympatric hammerhead sharks in the western North Atlantic Ocean. *Biology letters*, *15*(4), p.20190004.

Breiman, L. 2001. Random Forests, Machine Learning 45(1), 5-32.

Lyons, K., Carlisle, A., Preti, A., Mull, C., Blasius, M., O'Sullivan, J., Winkler, C. and Lowe, C.G., 2013. Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks. *Marine Environmental Research*, *90*, pp.27-38.

Lyons, K. and Adams, D.H., 2015. Maternal offloading of organochlorine contaminants in the yolk-sac placental scalloped hammerhead shark (*Sphyrna lewini*). *Ecotoxicology*, *24*(3), pp.553-562.

Lyons, K., Kacev, D., Preti, A., Gillett, D., Dewar, H. and Kohin, S., 2019. Species-specific characteristics influence contaminant accumulation trajectories and signatures across ontogeny in three pelagic shark species. *Environmental science & technology*, *53*(12), pp.6997-7006.

Lyons, K., Galloway, A.S., Adams, D.H., Reyier, E.A., Barker, A.M., Portnoy, D.S. and Frazier, B.S., 2020. Maternal provisioning gives young-of-the-year Hammerheads a head start in early life. *Marine Biology*, *167*(11), pp.1-13.

Mull, C.G., Lyons, K., Blasius, M.E., Winkler, C., O'Sullivan, J.B. and Lowe, C.G., 2013. Evidence of maternal offloading of organic contaminants in white sharks (Carcharodon carcharias). *PloS one*, *8*(4), p.e62886.

Weijs, L., Briels, N., Adams, D.H., Lepoint, G., Das, K., Blust, R. and Covaci, A., 2015. Maternal transfer of organohalogenated compounds in sharks and stingrays. *Marine pollution bulletin*, *92*(1-2), pp.59-68.

Wolkers, H., Lydersen, C. and Kovacs, K.M., 2004. Accumulation and lactational transfer of PCBs and pesticides in harbor seals (*Phoca vitulina*) from Svalbard, Norway. *Science of the Total Environment*, *319*(1-3), pp.137-146.

Federal Cost: $ 30,741

Recommendations: Close the grant.

Tables:

Table 1. List of polychlorinated biphenyls (PCB) and pesticide compounds screened for by gas chromatography/mass spectrometry.

PCB's	Pesticides
PCB003	BHC-alpha
PCB008	Hexachlorobenzene
PCB018	BHC-beta
PCB031	BHC-gamma
PCB028	BHC-delta
PCB033	Heptachlor
PCB052	Aldrin
PCB049	Heptachlor epoxide
PCB044	Oxychlordane
PCB037	4,4'-DDMU
PCB074	Chlordane-gamma

PCB070	2,4'-DDE
PCB066	Endosulfan I
PCB095	Chlordane-alpha
PCB056 & 060	Trans-Nonachlor
PCB101	4,4'-DDE
PCB099	Dieldrin
PCB119	2,4'-DDD
PCB097	Perthane
PCB087	Endrin
PCB081	Endosulfan II
PCB110	4,4'-DDD
PCB077	2,4'-DDT
PCB151	Cis-Nonachlor
PCB149	Endrin aldehyde
PCB123	Endosulfan sulfate
PCB118	4,4'-DDT
PCB114	Endrin ketone
PCB153	Methoxychlor
PCB168 & 132	Mirex
PCB105	
PCB141	
PCB138	
PCB158	
PCB126	
PCB187	
PCB183	
PCB128	
PCB167	
PCB174	
PCB177	
PCB156	
PCB199 & 200	
PCB157	
PCB180	
PCB169	

PCB170
PCB201
PCB189
PCB195
PCB194
PCB206
PCB209

Table 2. Sample distribution across years of the study for Young-of-Years / One-year old Carolina (*Sphyrna gilberti*), Scalloped *(Sphyrna lewini*), Hybrid and unidentified hammerheads ("Missing ID") by species assignment.

Year	*S. lewini*	*S. gilberti*	Hybrids	Missing ID
2014	17	23		4
2015	2			
2016	6 / 1	7		
2017	19	9	2	
2018	10 / 1	34 /1	9	1

Table 3. Morphometric information, capture location, and total contaminant concentration range and median (in parentheses) for total polychlorinated biphenyls (tPCB), non-DDT pesticides (tPEST) and dichlorodiphenyltrichloroethane and its metabolites (tDDX) for samples in the study by species for Carolina (*Sphyrna gilberti*), Scalloped *(Sphyrna lewini*), Hybrid and unidentified hammerheads. Sample sizes by species are in parentheses and their distribution by state sampled as a subscript (SC = South Carolina, GA = Georgia, FL = Florida).

Species	States sampled	Fork Length (mm)	tPCB	tPEST	tDDX	Sum
S. lewini (n = 56)	SC_{31}, GA_5, FL_{20}	307-664 (373.5)	574-10286 (2085)	116-7734 (899)	70-6220 (668)	809 – 17677 (3098)
S. gilberti (n = 74)	SC_{59}, GA_8, FL_7	270-605 (365)	385 – 9323 (1631)	151 – 15482 (1618)	27-13743 (1382)	632 – 22348 (3149)
Hybrid (n = 11)	SC_{11}	319-493 (400)	492-2188 (825)	230 – 3394 (834)	174 – 2795 (666)	722 – 5582 (1542)
Missing ID (n = 5)	SC_3, FL_2	306-500 (325)	538 – 14317 (3262)	361 – 20754 (1922)	332 – 17698 (1730)	899 – 35071 (5185)

Table 4. Gini values ranked in order of most important for signature differentiation between Carolina (*Sphyrna gilberti*) and Scalloped *(Sphyrna lewini*) Hammerheads

Contaminant	Mean Decrease Gini Value

PCB128	2.78426224
PCB206	2.57501507
PCB209	2.43960783
PCB138	2.3530131
4,4’-DDT	2.21030419
4,4’-DDE	2.18044323
PCB187	1.51783575
4,4’-DDD	1.33617256
PCB156	1.12538592
PCB099	1.09786392
Mirex	1.09153387
PCB158	1.08409979
PCB074	0.82672052
PCB066	0.75293568
PCB119	0.65781895
2,4’-DDT	0.64623727
PCB194	0.64323332
Trans-nonachlor	0.61953113
PCB180	0.603927
PCB118	0.58737107
PCB183	0.56279405
PCB199 & 200	0.55277126
PCB201	0.53816637
PCB170	0.49303009
Heptachlor	0.46145242
PCB008	0.44310021
PCB070	0.43224943
Alpha-chlordane	0.42792976
PCB105	0.40037188
PCB157	0.38410677
PCB031	0.36693013
PCB028	0.36465753
PCB123	0.35480004
Cis-Nonachlor	0.31218643
PCB167	0.30984787
PCB126	0.28805314
PCB087	0.27079542
PCB077	0.25100123
PCB114	0.23926962
BHC beta	0.23820328
PCB056 & 60	0.21906129

PCB101	0.21368503
4,4’-DDMU	0.21146483
PCB177	0.21094884
PCB044	0.20833716
2,4’-DDE	0.20390682
BHC gamma	0.19712595
BHC alpha	0.19660759
PCB049	0.18797371
PCB003	0.18680828
Chlordane gamma	0.18325949
PCB081	0.17173923
PCB149	0.16404028
2,4’-DDD	0.16347527
Methoxychlor	0.15344549
PCB195	0.15177698
Endosulfan sulfate	0.14979246
PCB110	0.14205535
PCB097	0.14119221
PCB052	0.13918272
PCB189	0.1345545
Endrin	0.13286881
BHC delta	0.12905359
Aldrin	0.12474379
PCB168 & 132	0.12140908
PCB033	0.11296959
Oxychlordane	0.10882913
Perthane	0.10598708
PCB037	0.08813874
PCB018	0.08783346
Heptachlor epoxide	0.08276545
Dieldrin	0.07709243
PCB169	0.07549439
Endrin ketone	0.06827271
Hexachlorobenzene	0.05961309
PCB095	0.05509643
Endosulfan II	0.05028412
PCB151	0.04967045
PCB174	0.04742158
Endrin aldehyde	0.04109656
Endosulfan I	0.02099881
PCB141	0.01722731

Table 5. Range and median for Sum OCs (ng/g ww) by species for Carolina (*Sphyrna gilberti*), Scalloped *(Sphyrna lewini*), and Hybrid hammerheads in 50 mm fork length increments (i.e. size bins). Sample size for each grouping is reported in the parentheses.

Fork Length Bin	Bin #	*S. lewini*	*S. gilberti*	Hybrid
251 – 300 mm	1	-	3132-12411, 7660 (4)	
301 – 350 mm	2	1234-16364, 4796 (18)	1019 – 22348, 5914 (23)	1523 – 5582, 3552 (2)
351 – 400 mm	3	1419-17677, 3531 (19)	632 – 11892, 3597 (25)	1493 – 2242, 1692 (3)
401 – 450 mm	4	850-6011, 2153 (14)	948-9365, 2515 (16)	723 – 3082, 1087(4)
451 – 500 mm	5	898 – 2180, 1539 (2)	843 – 8596, 1470 (3)	1542 (1)
501 – 550 mm	6	809 – 2365, 1587 (2)	1044 – 1268, 1156 (2)	
551 – 600 mm	7	-		
601 – 650 mm	8	-	809 (1)	
651 – 700 mm	9	872 (1)		

Table 6. Range and median for Sum OCs (ng/g ww) by scar rank and species assignment for Carolina (*Sphyrna gilberti*), Scalloped *(Sphyrna lewini*), and Hybrid hammerheads. Sample size for each grouping is reported in the parentheses.

Scar Number	*S. lewini*	*S. gilberti*	Hybrid
0	16364 (1)	2607-6914, 3132 (3)	
1	4107-6807, 5842(3)	4677 – 9590, 7716 (4)	
2	3531 - 4827(2)	7010(1)	723 (1)
3	899 – 17677, 3102 (35)	1252-22348, 6777(16)	1493 – 3082, 1916 (4)
4	809 – 4778, 2180(15)	632 – 13029, 2925(50)	964 – 5582, 1617 (6)

Table 7. Parameters for the relationship between liver mass and fork length of late-stage neonate for Carolina (*Sphyrna gilberti*) and Scalloped *(Sphyrna lewini)* Hammerheads for the equation $M = aL^b$, as previously reported in Lyons et al. 2020.

Species	a	b
S. lewini	1.010648×10^{-8}	3.569
S. gilberti	1.095217×10^{-8}	3.567

Figures:

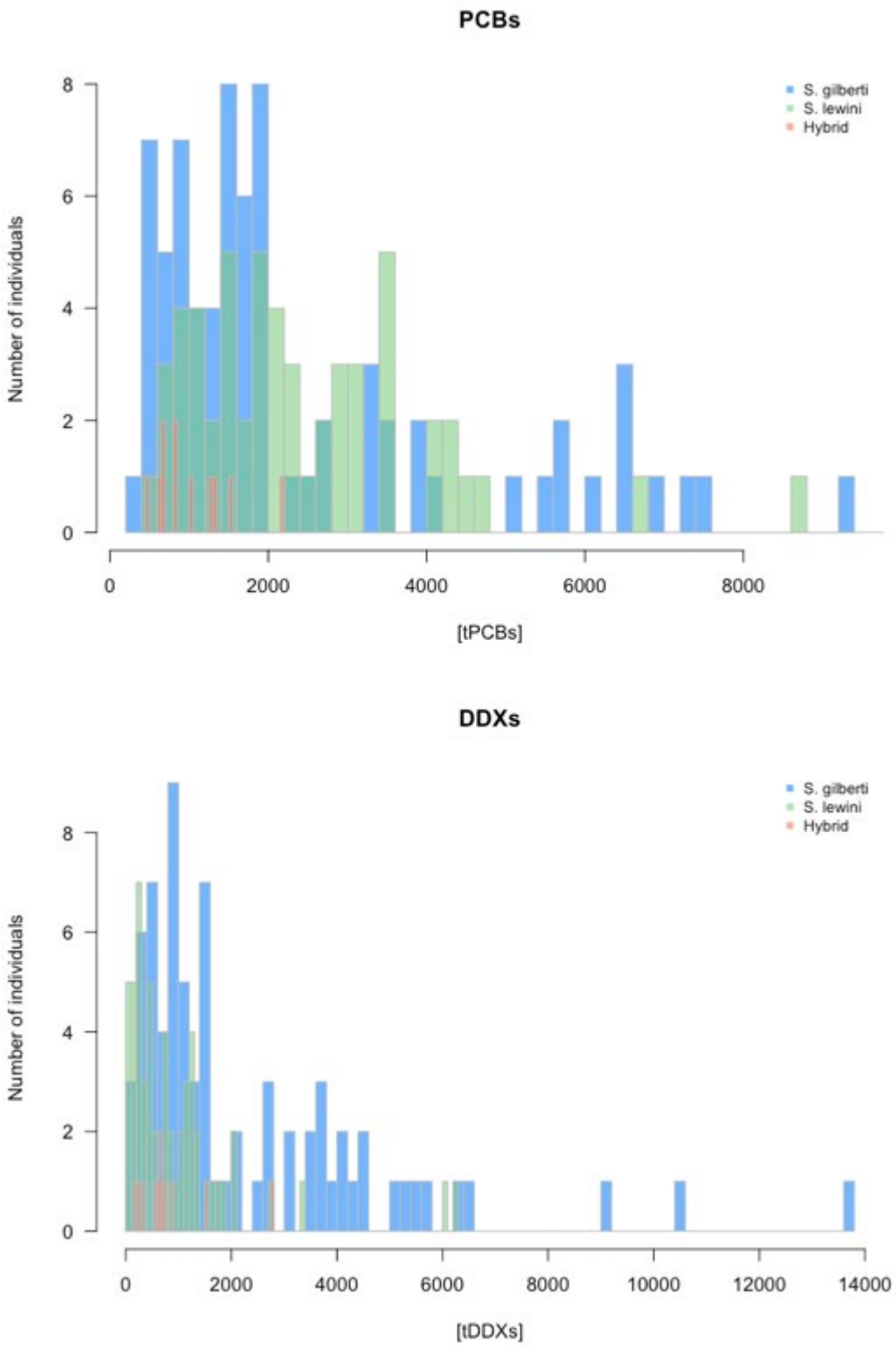

Figure 1: Histogram distribution of individuals for liver total PCB contaminants (top) and total DDX contaminants (bottom) for Scalloped (*Sphryna lewini*, green), Carolina (*Sphryna gilberti*, blue) and Hybrid (pink) Hammerheads. Distributions are significantly different between *S. lewini* and *S. gilberti*.

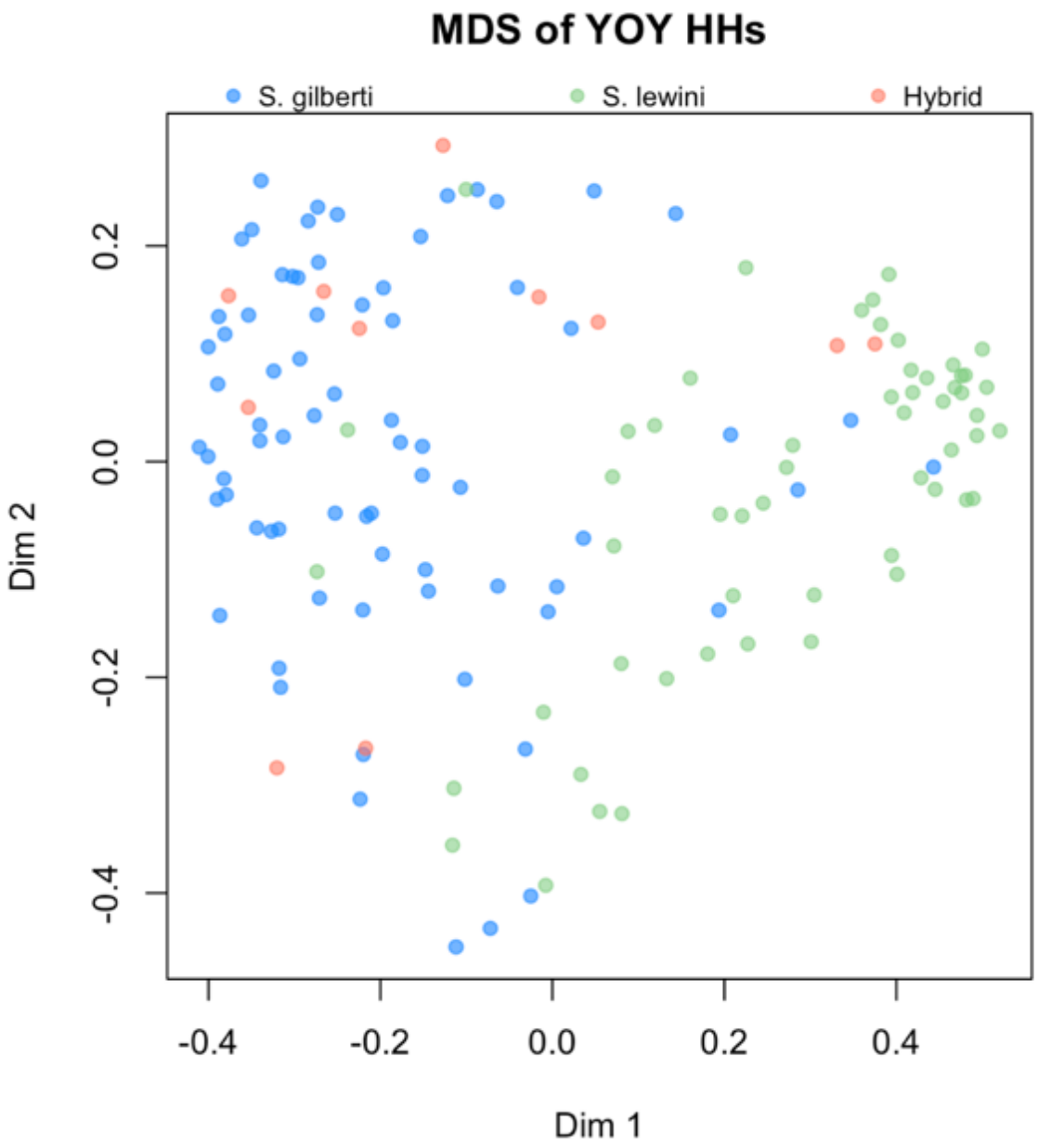

Figure 2. Multidimensional scaling plot of contaminant ratios for Scalloped (*Sphryna lewini*, green), Carolina (*Sphryna gilberti*, blue) and Hybrid (pink) Hammerheads.

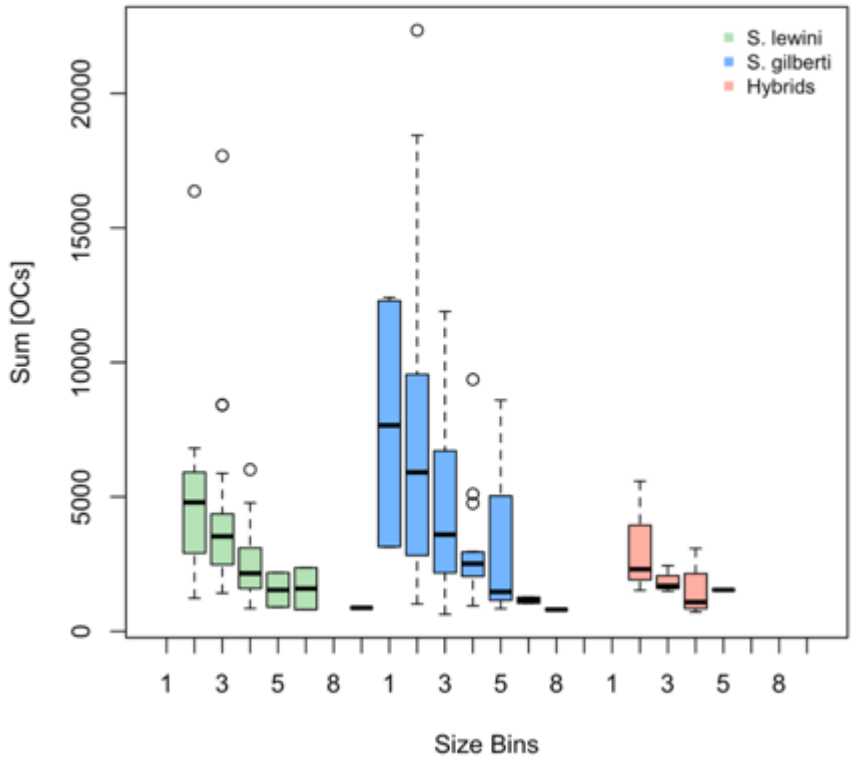

Figure 3. Sum of total organic contaminant concentrations across 50 mm fork length size bins for Scalloped (*Sphryna lewini*, green), Carolina (*Sphryna gilberti*, blue) and Hybrid (pink) Hammerheads. Whiskers represent ±1.5 times of the interquartile range and empty circles represent outliers. Fork lengths (mm) for size bins are as follows: 1: 251-300, 2: 301 – 350, 3: 351 – 400, 4: 401 – 450, 5: 451 – 500, 6: 501 – 550, 7: 551 – 600, 8: 601 – 650, 9: 651 – 700.

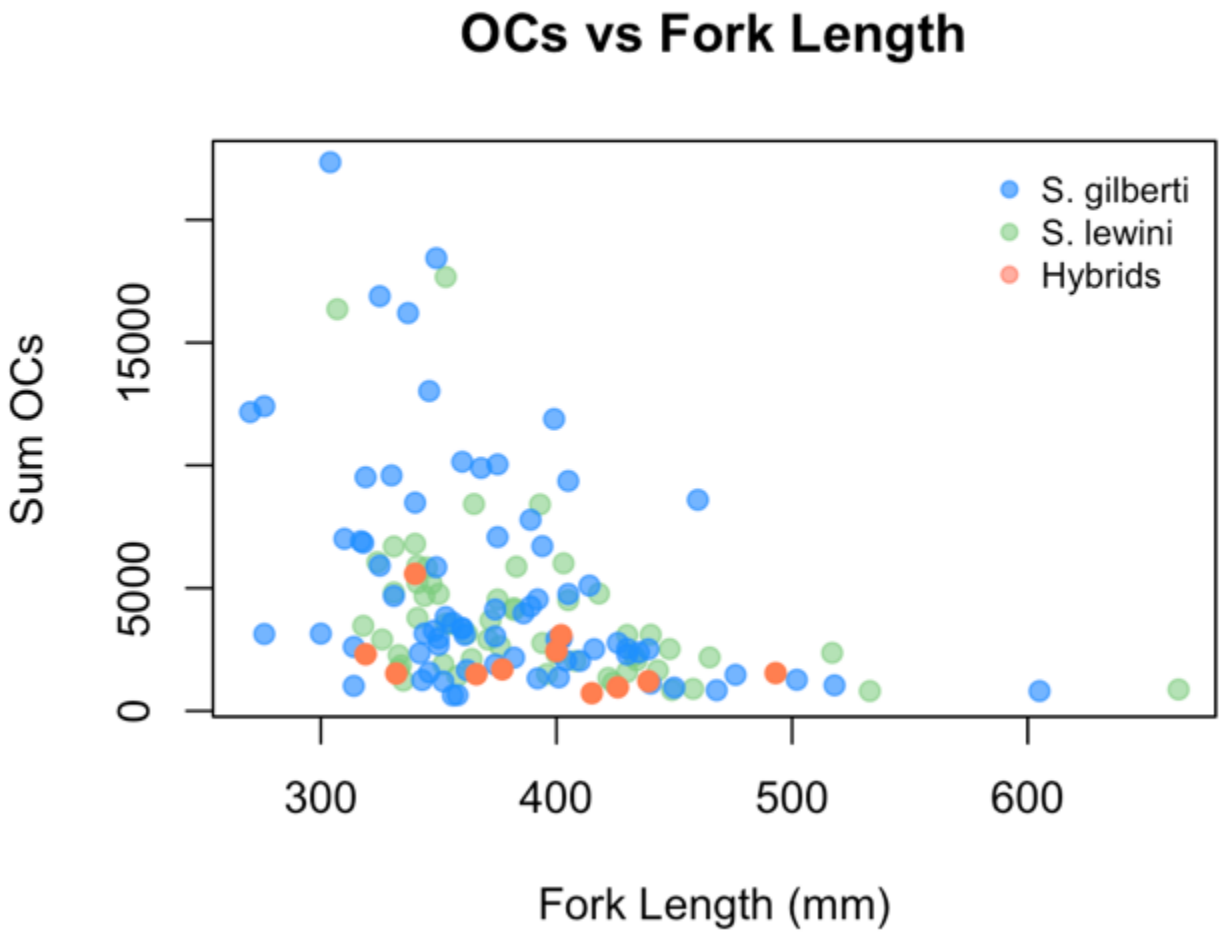

Figure 4. Liver total contaminant concentrations ("Sum OCs" in ng/g ww) by shark fork length for Scalloped (*Sphryna lewini*, green), Carolina (*Sphryna gilberti*, blue) and Hybrids (pink) Hammerheads. *S. lewini* and *S. gilberti* demonstrated a significant negative relationship (regression lines not shown here).

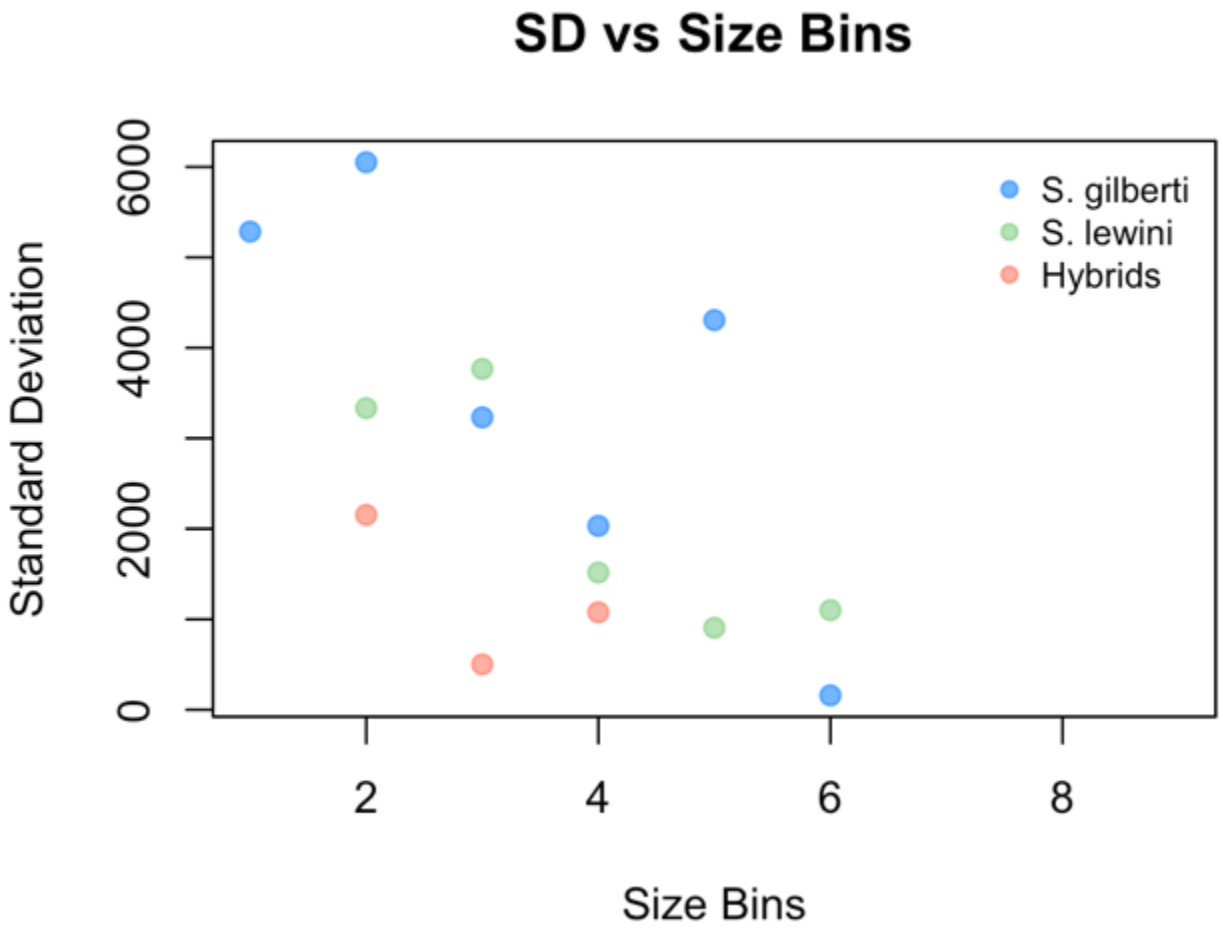

Figure 5. Within group standard deviation of sum OCs by fork length size bins for Scalloped (*Sphryna lewini*, green), Carolina (*Sphryna gilberti*, blue) and Hybrids (pink) Hammerheads. A weakly significant decrease with increasing size bin was found for *S. lewini* and *S. gilberti*. Fork lengths (mm) for size bins are as follows: 1: 251-300, 2: 301 – 350, 3: 351 – 400, 4: 401 – 450, 5: 451 – 500, 6: 501 – 550, 7: 551 – 600, 8: 601 – 650, 9: 651 – 700.

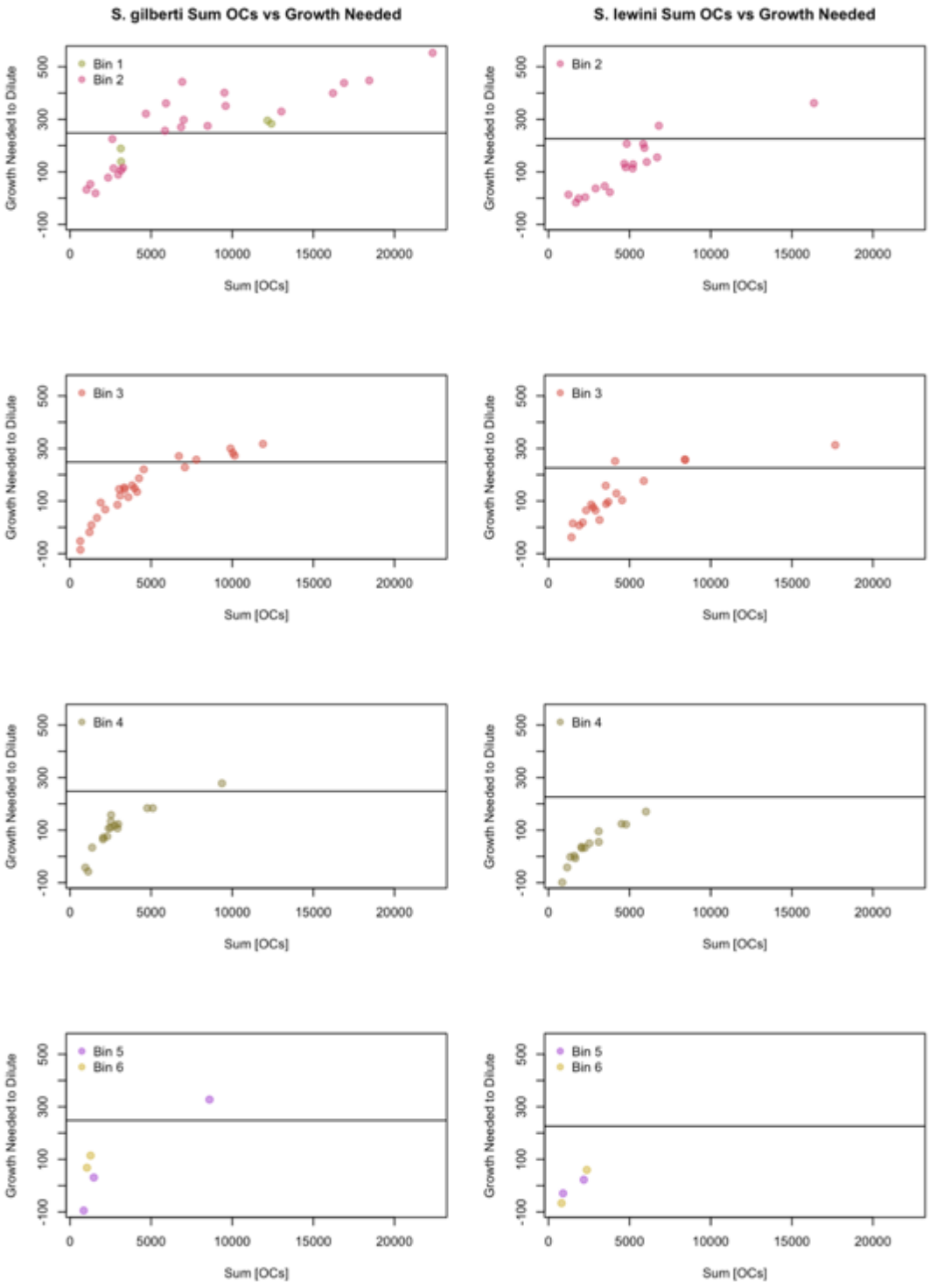

Figure 6. Quantified liver sum of organic contaminants measured at the time of capture against predicted growth needed in order to meet our set "dilution bench mark" (solid horizontal line) for each species; thus, points falling above the line represent animals with concentrations too high to achieve growth dilution according to our model. Individuals are grouped by bin size for Carolina (*Sphryna gilberti*, left) and Scalloped (*Sphryna lewini,* right) Hammerheads. Fork lengths (mm) for size bins are as follows: 1: 251-300, 2: 301 – 350, 3: 351 – 400, 4: 401 – 450, 5: 451 – 500, 6: 501 – 550, 7: 551 – 600, 8: 601 – 650, 9: 651 – 700.

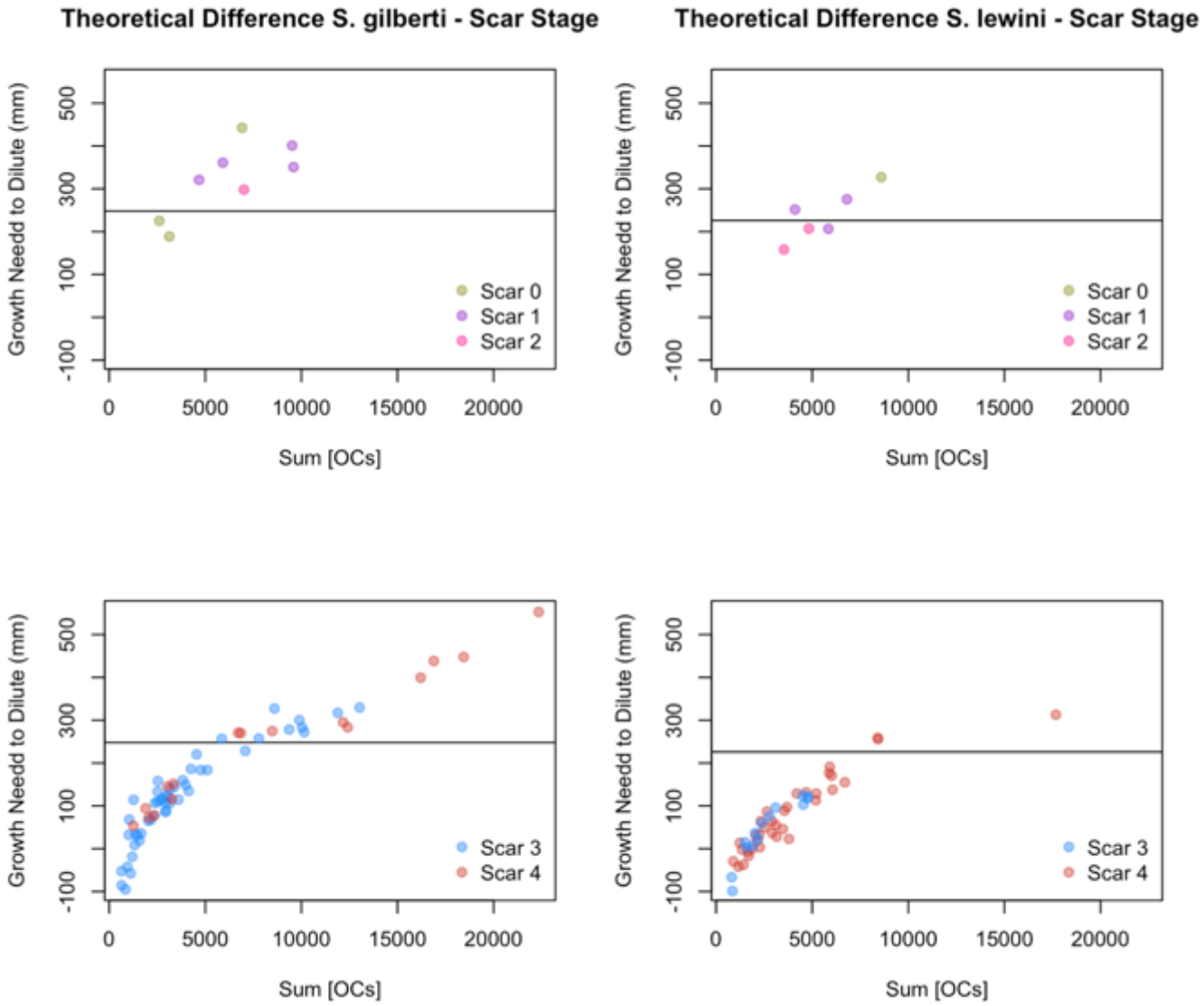

Figure 7. Quantified liver sum of organic contaminants measured at the time of capture against predicted growth needed in order to meet our set "dilution benchmark" (solid horizontal line) for each species. Individuals are grouped by scar rank for Carolina (*Sphryna gilberti*, left) and Scalloped (*Sphryna lewini,* right) Hammerheads with scar ranks of 0 representing newborn individuals, and 4 representing older (~1 month old) individuals.

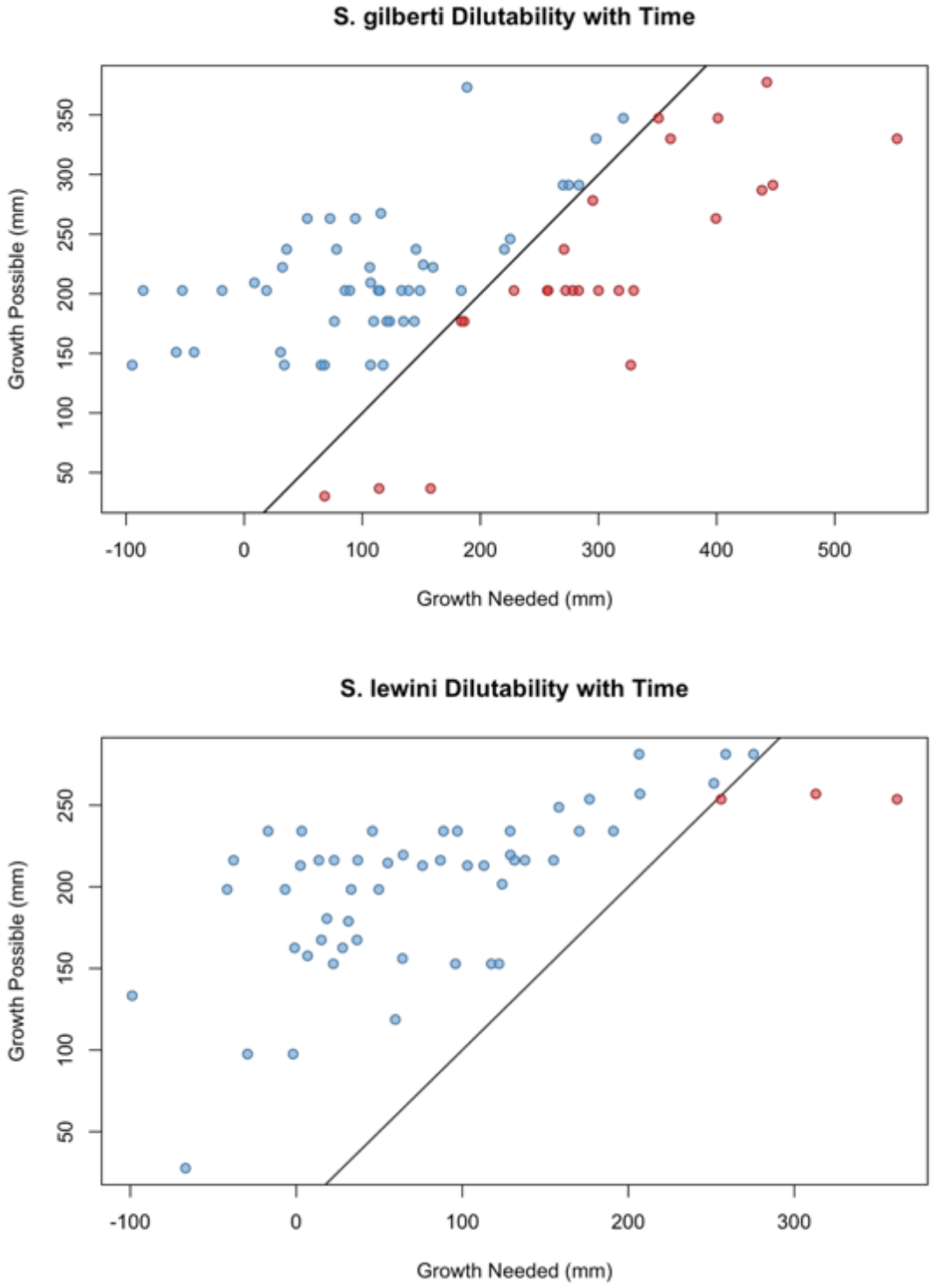

Figure 8. Growth needed to reach the dilution benchmark against the amount of growth estimated to be possible from time of capture to October 31st. Solid black line represents 1:1 ratio of growth needed to growth possible. Points falling to the right of the line (red) represent animals where growth dilution is not predicted to be possible. Points falling to the left of the line represent animals where growth dilution is estimated to be possible for Carolina (*Sphryna gilberti*, top) and Scalloped (*Sphryna lewini,* bottom) Hammerheads.