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Evaluation of muscle tissue as a non-lethal proxy for liver and brain organic contaminant loads in an elasmobranch, the Bonnethead Shark (*Sphyrna tiburo*)

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ABSTRACT

Elasmobranch ecotoxicological investigations are complicated because accessing organs that accumulate organic contaminants is usually lethal. Several metrics among liver, muscle, and brain were evaluated to determine their relative organic contaminant loads and the efficacy of using muscle as a non-lethal proxy for liver. Liver contained the highest concentrations (368–4020 ng/g wet weigth [ww]) and greatest estimated total load of contaminants. Brain had higher toxin concentrations than muscle (4.18–84.2 ng/g ww versus 0.94–4.73 ng/g ww). Liver and brain were similar to each other in terms of contaminant detection occurrence and signature overlap, whereas muscle poorly reflected those of liver and brain. However, the identity of contaminants detected in muscle constituted those that substantially contributed to summed liver and brain concentrations. Thus, studies utilizing muscle as a non-lethal liver alternative to study organic contaminant exposure in elasmobranchs should craft questions with care, considering its limited ability to serve as an accurate proxy.

1. Introduction

As a result of their highly lipophilic nature, legacy organic contaminants such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) tend to accumulate in tissues that have high lipid contents (Mackay and Fraser, 2000). For predatory animals, this tissue-specific accumulation can result in these contaminants concentrating in tissues with high fat content. For example, the highest gravimetric concentrations of organic contaminants in marine mammals are found in their blubber (Tilbury et al., 1997; Yordy et al., 2010), an insulating layer that can be composed of up to 91% lipid (Isobe et al., 2009). Since blubber can be non-lethally sampled, and because marine mammals can be predictably found as they surface to breath, the field of marine mammal toxicology has flourished. Contaminant research has been more difficult for other aquatic animals that are not easily accessible or do not have subdermal fat stores.

In particular, toxicological research on organic contaminants in elasmobranchs (sharks, skates, and rays) has lagged behind that of other aquatic taxa (Gelsleichter and Walker, 2010). Most elasmobranchs are difficult to sample; their elusive nature and their naturally lower population numbers as top predators make acquiring a large number of sex- and size-specific samples difficult. Sampling of tissues that are appropriate for organic contaminant analysis is also problematic. For example, the liver is the primary lipid storage organ in elasmobranchs, and as such tends to accumulate the highest concentrations of organic contaminants (Boldrocchi et al., 2019; Corsolini et al., 2014; Schlenk et al., 2005; Storelli and Marcotrigiano, 2001; Strid et al., 2007); however, acquisition of liver tissue is typically lethal. Given the conservation concerns surrounding many elasmobranch species, lethal sampling raises ethical questions and can constrain toxicological research.

Besides providing storage for lipids, the elasmobranch liver performs many crucial physiological functions to maintain proper homeostasis (e.g. urea production, metabolic processing; Ballantyne, 1997). Since toxins accumulate in the liver (Fisk et al., 2002; Lyons and Lowe, 2013; Weijs et al., 2015), this organ may be especially susceptible to negative impacts from contaminant exposure (Alves et al., 2016; Lyons and Wynne-Edwards, 2021; Walker, 2011), which could have downstream impacts on homeostatic regulation (Lyons and Wynne-Edwards, 2021).

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Thus, quantifying hepatic contaminant concentrations and relating them to biological outcomes is necessary for understanding how organic contaminant exposure may negatively influence elasmobranch health.

Other tissue alternatives to liver, such as muscle (Marsili et al., 2016) or skin (Fossi et al., 2017), can be used to evaluate contaminant exposures in elasmobranchs without lethal sampling. However, without an understanding of the relationship between muscle and liver concentrations, muscle biopsy contaminant data provides limited information on internal contaminant concentrations (Gelsleichter et al., 2005). Although species-specific variation exists (Cagnazzi et al., 2019; Lee et al., 2015), elasmobranch muscle is also characteristically lipid deficient (Davis et al., 2002; Schlenk et al., 2005; Storelli and Marcotrigiano, 2001). For other contaminant types (such as heavy metals), selection of an appropriate, representative tissue of internal contamination has been less confounding because strong correlations exist among different tissue types in other vertebrates (Gelsleichter et al., 2020; O'Bryhim et al., 2017; Sakai et al., 2000). However, since organic contaminants are lipophilic, analyses only using muscle could result in an underestimation of individual exposures. Previous studies have noted higher concentrations of accumulated contaminants in liver compared to muscle (Lyons and Adams, 2017; Mull et al., 2012; Storelli and Marcotrigiano, 2001), but no study has formally assessed the efficacy of using muscle tissue as a proxy for liver organic contaminant loads. Thus, while muscle represents a promising, non-lethal alternative in elasmobranch organic contaminant studies, more work is needed to verify the biological utility of this tissue.

The hypothesis that muscle tissue accurately reflects liver organic contaminant constituents, proportions, and relative concentrations was posited to evaluate the utility of muscle tissue as an alternative to invasive liver sampling for assessing organic contaminants in elasmobranchs, particularly sharks. Similar evaluations were made between liver and brain tissue to provide an alternative contrast. The Bonnethead Shark (*Sphyrna tiburo* Linnaeus, 1758) is a readily available species of low conservation concern that uses coastal environments and feeds benthically, putting it in close proximity to habitats that may be prone to accumulate contaminants (Gelsleichter et al., 2005); therefore, it is an ideal candidate for examination in this study. If muscle represents an appropriate proxy tissue, the collection of muscle biopsies would enable non-lethal sampling, which is particularly significant for vulnerable and endangered species.

2. Methods

2.1. Sample collection

Bonnethead Sharks were collected as incidental mortalities during routine fisheries-independent monitoring surveys on the Atlantic coast of Florida in 2013 and 2014. Sharks ($n = 22$) were placed on ice and transported to the lab, where sex, reproductive status, and morphometrics (total body mass, tissue mass, length) were collected before samples of liver (distal left lobe), dorsal muscle and/or brain tissues were obtained. Whole brains and subsamples of liver and muscle were frozen at -20 °C prior to extraction.

Total muscle mass was not measured for each analyzed individual, but instead low and high estimates of possible muscle mass contributions to total body mass were calculated for each fish. Sharks in this study were weighed whole or after removal of embryos (i.e. gravid females) to obtain total body mass, and a low and high estimate of muscle mass was subsequently calculated. Having both a low and a high estimate of possible muscle mass contributions enabled us to correspondingly determine a low and high estimate of muscle total contaminant load (see below). This was necessary to demonstrate the potential extent by which muscle might contribute to overall body burden. Low estimates were calculated using mean muscle mass contributions obtained from necropsy measurements of three immature Bonnethead Sharks not part of this study. Weighed specimens were denuded of muscle and reweighed to calculate muscle mass contributions to total body weight. Since it was not possible to completely denude each specimen, the proportion of muscle estimated by this technique is slightly underestimated (i.e., low estimate). The high estimate of muscle mass contribution was derived from the literature based on teleost morphometrics (Guderley et al., 1994), where muscle mass had a greater contribution to total body mass than those measured in the juvenile sharks previously described

2.2. Tissue extraction

Tissues were extracted following previously published protocols (Lyons and Lowe, 2013). Briefly, approximately one gram of liver, ten grams of muscle, and whole brains (3–10 g) were spiked with recovery surrogates (TCMX, PCB 30, 112, and 198; target recovery of 70–130%) prior to extraction on a Soxhlet apparatus. Samples were extracted with methylene chloride solvent for 12–14 h. After extraction, samples were concentrated and lipid content was determined gravimetrically by splitting the sample. Extracts were then cleaned up by elution through an Alumina-B/Silica gel first with hexane, then 30% methylene chloride (DCM) in n-hexane, followed by DCM, and concentrated. Samples were transferred to autosampler vials and spiked with internal standards (4,4′-Dibromobiphenyl and 2,2′,5,5′-Tetrabromobiphenyl) prior to injection onto an Agilent gas chromatograph (GC; 6890 N 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 inner diameter x 60 m) with 0.25 μm film thickness. The temperature profile of the GC oven was programmed from 45 °C to 125 °C at 20 °C/min, then to 295 °C at 2.5 °C/min and held for 10 min. Injector and transfer line temperatures were set at 285 °C and 300 °C, respectively. The source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. Helium was used as the carrier gas at a flow velocity of 40 cm/s. The MSD was operated in the Electron Ionization (EI) mode and scanned from 45 to 500 amu at a rate of 1.66 scans/s. Samples were screened for a total of 83 organic contaminants (Supplemental Table 1), quantified using the software in the GCMS system (Agilent Technologies), and reported on a wet weight ("ww") basis. To ensure quality control of samples, one blank, one certified standard reference material (Lake Michigan Trout tissue 1947, National Institute of Standards and Technology), two blank spikes, and a replicate tissue sample were run in tandem with study samples. Recovery surrogates were within acceptable ranges for QA/QC (104 ± 9%) samples and all tissues. However, whereas recoveries were similar between liver (111 ± 31%) and brain (95 ± 31%) they were lower for muscle (75 ± 17%). Blank spikes target compound recoveries of 95 ± 20% and replicates were within 10 ± 27% of each other. Compounds measured in tissue replicates had a relative significant difference of 9.0 ± 9.9%. The CRM had 95% of PCB and pesticide compounds that were within range of actual values (i.e. ± 35%).

2.3. Data analysis

2.3.1. Concentrations and totals

Contaminant concentrations were divided into three groups, based on their identity: PCBs, DDXs (DDT and associated metabolites), and non-DDX pesticides (herein "Pesticides"). Contaminant concentrations from individual sharks were summed to obtain group concentrations ($\sum$PCBs, $\sum$DDXs, $\sum$Pesticides) and overall organic contaminant concentrations ($\sum$OCs) for each type of tissue. Tissue total contaminant load (tOCs) was calculated as $\sum$OCs multiplied by total mass of its respective tissue type (liver, muscle, or brain). Contaminants in each sample were given a binary scored based on their presence ("1") or absence ("0") to compare how often screened contaminants were detected across tissue types. Group concentration sums, total loads, and contaminant detections were compared across tissue types using either an Analysis of Variance or a Kruskal-Wallis test, depending on

assumption violations, followed by multiple pairwise tests with Bonferroni and Šidák correction for multiple comparisons, respectively.

2.3.2. Profiles and correlations

To compare contaminant profiles across tissues, individual contaminant contributions to ∑OCs were calculated for each sample (i.e. [contaminant X]/ ∑OC), and differences in mean ranks were compared by tissue type using a Permutational Multivariate Analysis of Variance (PERMANOVA). Bray-Curtis dissimilarity was utilized as the basis for matrix calculations and the model was permuted 999 times. Only specimens with estimates of each contaminant group (i.e. ∑PCBs, ∑DDXs, ∑Pesticides) for each tissue type were included, and contaminant data were converted to proportions so that each sample contributed equally to analysis. A multivariate homogeneity of group dispersions test was additionally conducted to determine if multivariate dispersion (i.e., variance) differed significantly among tissue types (Anderson, 2006). Significant differences indicate high among-group variability that can bias *P* value interpretations and increase the chance of Type-1 error. PERMANOVA and dispersion analyses were calculated using the "adonis" and "betadisper" functions (Oksanen et al., 2019), respectively, in the *vegan* package in R (v. 4.0.0; R Core Team, 2020).

Hierarchical agglomerative cluster analysis was used to further compare contaminant signatures among sampled specimens. Calculations were conducted on raw (unstandardized) and row standardized (i.e., proportional) tissue profiles, using Ward's Minimum Variance technique (Ward, 1963), and a Bray-Curtis similarity matrix. Cluster solutions were evaluated using agglomerative coefficients (to measure the clustering structure of the dataset), cophenetic correlations (a measure of how similar two objects must be in order to be grouped into the same cluster, which was used to evaluate the goodness–of–fit between the input data and cluster output), and randomization tests (to determine significant clusters).

In addition, the multipatt function in the *indicspecies* package (De Cáceres and Legendre, 2009) was used to identify contaminants that significantly contributed to group identity both for individual tissues and pairs of tissues. Contaminant concentration correlations between tissue pairs were assessed for each individual contaminant and for each contaminant group using Spearman's correlations.

2.3.3. Lipophilicity

To investigate the influence of contaminant hydrophobic affinity on contaminant distribution in tissues, a series of analyses were performed that included each contaminant's octanol-water partition coefficient (log K_{ow}; Supplemental Table 1) as a measure of lipophilicity. Since each contaminant has different hydrophobic properties, their ability to concentrate in certain tissues will be influenced by this characteristic. To investigate the propensity of muscle and liver to accumulate contaminants with certain hydrophobic affinities, contaminants were first categorized by shark into three categories: those found in both muscle and liver, those found only in liver, and those found in only muscle. Spearman's correlation was used to determine if contaminant concentrations had a significant association with log K_{ow} by tissue type. Mean log K_{ow}'s were compared across groups using a Kruskal-Wallis test followed by a Dunn's test with Šidák correction for multiple comparisons. Finally, the probability of occurrence of an individual contaminant based on its log K_{ow} for each group of contaminants was investigated by tissue type using a series of random effects logistic regressions with total length and sex as covariates and a random intercept for individual shark ID to account for repeated measures.

3. Results

3.1. Sample distribution

Tissues were obtained from 22 sharks (47.1–117.4 cm total length [TL]) comprising three males and 19 females sampled from 2013 to 2014. One shark (BH-15) had an unreported liver mass and was omitted from total load comparisons. Muscle mass comprised 55.1–56.9% of total mass in the three fully necropsied immature sharks (54.5–57.7 cm TL, 2 males and 1 female); thus, 55% was used to calculate the low estimate of total muscle mass. A majority of females (n = 13, 68%) exhibited some degree of reproductive activity (ovulation through near-term pregnancy; Table 1); thus, the influence of maternal offloading on liver concentrations and, by extension, their contribution to total body burden cannot be discounted. Uterine eggs were estimated to contribute 2–10% of the total contaminant load in females where both liver and muscle were analyzed (data not shown).

3.2. Tissue concentrations

Contaminants were measured in samples of liver (n = 22), muscle (n = 8) and brain (n = 21), with liver tissue analyzed for every individual shark (Table 1). No significant relationships were found between ∑OCs (or tOCs) and length, mass, or lipid content within each tissue type (all $p \geq 0.10$; Supplemental Fig. 1). All tissue types displayed at least an order of magnitude difference in concentration among samples (liver: 368–4020 ng/g ww; muscle: 0.94–4.73 ng/g ww; brain: 4.18–84.2 ng/g ww). Despite the liver constituting only 2–10% (median = 3%) of total body mass, it had the highest concentration of summed contaminants (LN-transformed, ANOVA, $F_{2,48} = 350$, $p < 0.0001$) and greatest total load (Kruskal-Wallis, $\chi_2 = 41.6$, $p < 0.0001$) of all tissue types (Fig. 1A), likely due to its high lipid content (14–60%, median 45%). For sharks with paired muscle and liver samples (n = 8), liver total loads were 13–93 times higher than estimated muscle loads. Thus, despite muscle constituting 55% (low estimate) to 61% (high estimate) of total body mass, liver contributed a majority (93–99%) to total combined contaminant load (i.e. sum of muscle and liver loads). Brain tissue had significantly higher concentration of ∑OCs than muscle tissue ($p < 0.0001$; Fig. 1B). Similar to liver, brain lipid content was greater than that of muscle (max value of 1.9–6.2% and 0.008–0.097%, respectively). However, since brain mass only constituted 0.07–0.7% of total body

Table 1
Bonnethead Shark morphometrics grouped based on analyzed tissue types. The sum of all detected organic contaminants are reported on a ng/g wet weight basis for liver tissue since it was present in all sample combinations. Total length is reported in centimeters and reproductive status of individuals is indicated.

Samples	Length	Sex	Status	Liver sOCs
Muscle + brain + liver				
BH-1	114.9	F	Early-pregnancy	1361
BH-2	100.5	F	Ovulated	1034
BH-3	101.5	F	Ovulated	780
BH-7	104.7	F	Ovulated	803
BH-8	117.4	F	Pre-ovulation	1098
BH-14	111.7	F	Mid-pregnancy	1134
BH-21	93.0	M	Mature	4020
Muscle + liver				
BH-4	104.9	F	Ovulated	430
Brain + liver				
BH-6	104.7	F	Ovulated	629
BH-9	94.8	F	Mature	1630
BH-10	81.4	F	Immature	643
BH-11	95.5	F	Mature	912
BH-12	87.0	F	Immature	726
BH-13	77.0	F	Mid-pregnancy	368
BH-15	108.3	F	Late-pregnancy	965
BH-16	114.6	F	Late-pregnancy	1869
BH-17	47.1	F	Immature	1570
BH-18	91.9	M	Mature	3465
BH-19	106.2	F	Ovulated	677
BH-20	116.4	F	Ovulated	3965
BH-22	92.3	M	Mature	2081
BH-23	88.8	F	Mature	1016

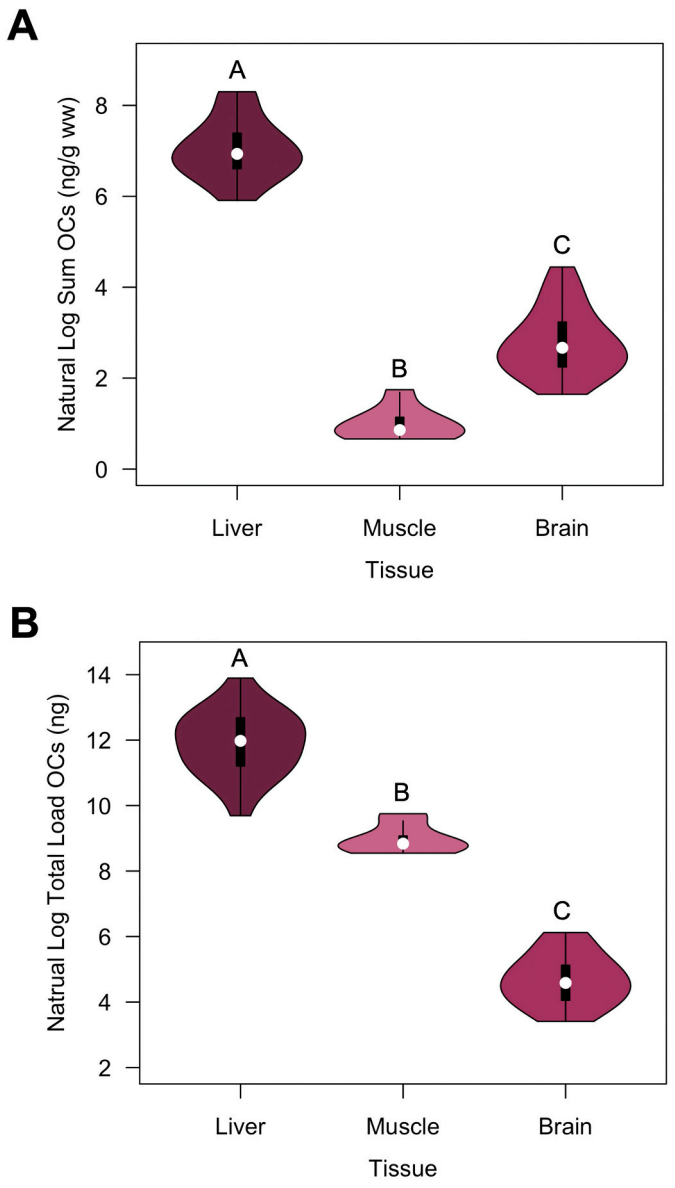

Fig. 1. Violin plots of summed organic contaminant concentrations (A) and total loads (B) by tissue type for liver ($n = 22$), muscle ($n = 8$) and brain ($n = 21$) of Bonnethead Sharks. Note values are shown on a natural log scale but analyses (Kruskal-Wallis tests) were performed on non-transformed data. Different letters denote significant differences among groups.

mass, it had a lower total contaminant load compared to either muscle or liver ($p \leq 0.0001$).

3.3. Tissue profiles

The majority of ∑OCs among tissue types was comprised of PCBs, although tissues diverged in their relative proportions of DDXs and other Pesticides (Table 2). The number of detected contaminants significantly differed among tissue types (Kruskal-Wallis $\chi_2 = 22.8$, $p < 0.0001$; Table 2). Although the number of detected contaminants was similar between liver and brain tissues ($p = 0.053$), and type 2 error due to low sample size cannot be discounted, both had significantly greater detections than muscle ($p \leq 0.0004$). In addition to differences in number of detected contaminants, tissues also significantly differed in the relative proportion of their contaminant signatures (PERMANOVA, $F_{2,18} = 22.9$, $p = 0.001$; Fig. 2), though dispersion was homogeneous among tissue types ($F_{2,18}$, $p = 0.598$). With respect to contaminants that

Table 2
Range and median (in parentheses) for the number of contaminant detections and the relative contribution of contaminant group to total contaminant concentration per tissue in Bonnethead Sharks. Subscripts indicate number of samples per tissue type.

	$Liver_{22}$	$Brain_{21}$	$Muscle_8$
PCBs			
# Detections	18–35 (28)	10–35 (20)	6–16 (7)
% Contribution	73.7–97.6 (86.9)	59.3–100 (88.9)	39.5–95.1 (47.9)
DDXs			
# Detections	1–3 (2)	0–4 (1)	0–1 (0)
% Contribution	1.6–17.6 (9.0)	0.00–19 (2.9)	0.00–7.4 (0.0)
Pesticides			
# Detections	1–8 (5)	0–8 (4)	1–4 (2)
% Contribution	0.55–10 (3.0)	0.0–31.7 (8.4)	4.9–60.4 (50.7)

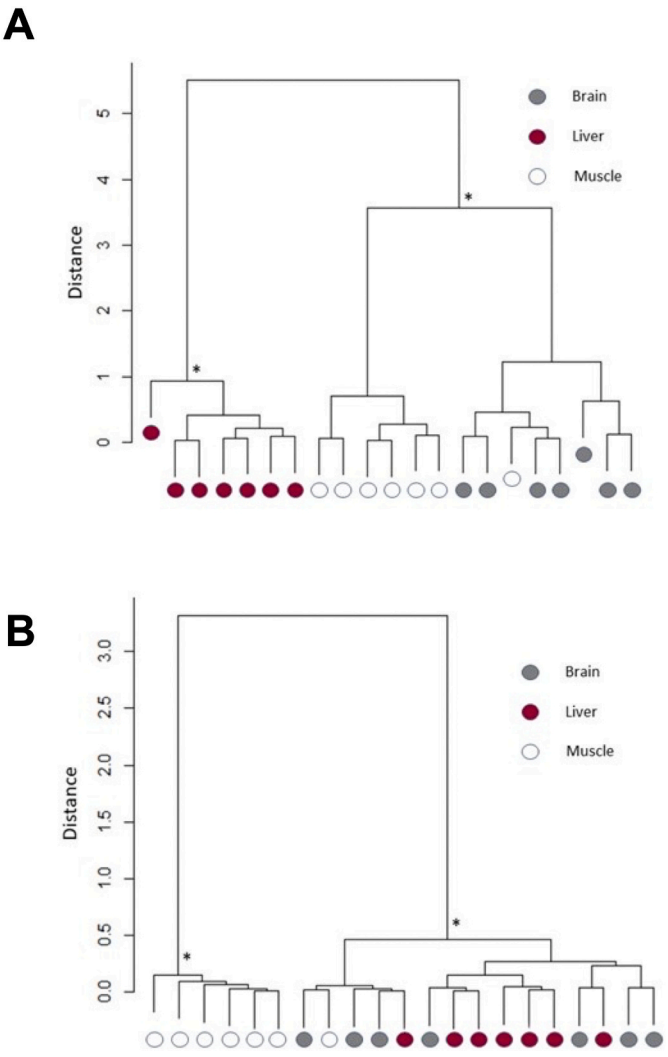

Fig. 2. Hierarchical agglomerative cluster analysis of (A) unstandardized and (B) standardized contaminant profiles among tissue types. Calculations were performed using Ward's Minimum Variance technique and a Bray- Curtis dissimilarity matrix. * = significant cluster ($p < 0.05$).

contributed to tissue group identify, at least two unique contaminants were identified for liver, muscle and brain (Supplemental Table 2). Liver and brain also further shared 13 contaminants between them, while none were uniquely shared between liver and muscle or brain and muscle.

Diagnostics of unstandardized and standardized cluster solutions confirmed that the generated dendrograms were appropriate summaries of contaminant profile data among tissue types. Agglomerative cluster coefficients of 0.973 and 0.989, respectively, indicated a high degree of clustering throughout the dissimilarity ranges. Additionally, pairwise tissue type dissimilarities and cophenetic distances associated with the cluster dendrogram were strongly correlated (unstandardized = 0.933, standardized = 0.964).

Cluster dendrograms demonstrated clear and reliable distinctions among tissue types for both unstandardized (i.e., "raw" concentrations) and standardized (i.e., proportional) data (Fig. 2). The dendrogram created using unstandardized contaminant profiles separated samples into two significant groupings, a monospecific cluster consisting of liver samples, and a cluster that contained brain and muscle tissue and further subdivided samples into generally distinct but nonsignificant groupings (Fig. 2a). When standardized data were used as a basis for clustering, most muscle samples formed a distinct, highly similar significant cluster, whereas brain and liver samples clustered together in a second distinct, significant cluster (Fig. 2b). Muscle did not group with liver samples based on either the magnitude or relative proportion of contaminant profiles (Fig. 2).

Among paired samples, muscle captured 18–42% of the number of contaminants detected in liver tissue across sharks (Fig. 3A). However, the contaminants detected in both muscle and liver constituted 46–95% of ∑OCs in the liver (Fig. 3B). Therefore, muscle tissue did not reliably reflect the composition of individual contaminants found in the liver, but it did capture the contaminants with the highest liver concentrations.

3.4. Tissue correlations

Only one contaminant (PCB206) was significantly correlated between paired liver and muscle samples ($\rho = 0.90$, $p = 0.037$, $n = 5$; Supplemental Table 3). Similarly, PCB206 was the only contaminant with a significant association between muscle and brain pairs ($\rho = 0.90$, $p = 0.037$, $n = 5$). Given the scant number of significant individual contaminant correlations between muscle and either liver or brain, it was not surprising that ∑PCB and ∑Pesticides group concentrations were also not significantly correlated between muscle and the other two tissues (muscle-brain: $p \geq 0.17$; muscle-liver $p \geq 0.27$), although ∑DDX concentrations were similar between muscle and liver tissues ($\rho = 0.68$, $p = 0.06$). In contrast to muscle, brain and liver had 14 strongly associated contaminants (Supplemental Table 3). As these shared contaminants were mostly individual PCB congeners, a significant association was found between ∑PCB concentrations in liver and brain ($\rho = 0.84$, $p < 0.0001$), whereas ∑Pesticides and ∑DDX showed no associations ($p \geq 0.08$). Thus, muscle did not correlate well with the other two tissues for any contaminant group, while ∑PCBs exhibited strong correlations between liver and brain.

3.5. Influence of lipophilicity

Within tissue type, a positive correlation was found between individual contaminant concentration and its log K_{ow} value for both liver ($\rho = 0.44$, $p < 0.0001$) and brain tissues ($\rho = 0.27$, $p < 0.0001$); however, no such significant associations were found in muscle tissue ($\rho = -0.17$, $p = 0.13$). Mean log K_{ow} in paired samples of muscle and liver also significantly differed based on whether the contaminant was present in both paired samples of liver and muscle, present in only liver, or present only in muscle (KW, $W_2 = 36.12$, $p = 0.001$). Contaminants that were present in both liver and muscle had significantly higher mean log K_{ow} (7.03 ± 0.82, $p \leq 0.0001$) than those found only liver (6.57 ± 0.85) or only in muscle (5.28 ± 1.42). For contaminants that were found only in one tissue type per shark, mean log K_{ow} was higher for those only present in liver than those only present in muscle ($p = 0.006$).

Probability of occurrence by log K_{ow} varied among the contaminant groups and by tissue type. Liver was the only tissue where all three contaminant groups demonstrated significant relationships (Fig. 4A). For all tissues, PCB presence was positively associated with log K_{ow} (all $p \leq 0.0001$), with the odds ratio highest in muscle (5.52), followed by brain (3.86), and liver (2.95; Fig. 4B). In brain tissue, PCBs had marginally significant odds of being present in female sharks (OR = 0.44, $p = 0.050$) or with increasing shark length (OR = 0.99, $p = 0.051$).

The probability of Pesticide and DDX occurrence varied with contaminant group and by tissue type. Similar to PCBs, Pesticide presence was significant and positively associated with log K_{ow} in liver (OR = 1.60, $p < 0.0001$). In contrast, no such relationship was found in muscle ($p = 0.65$). In brain tissue, Pesticide occurrence had a marginally

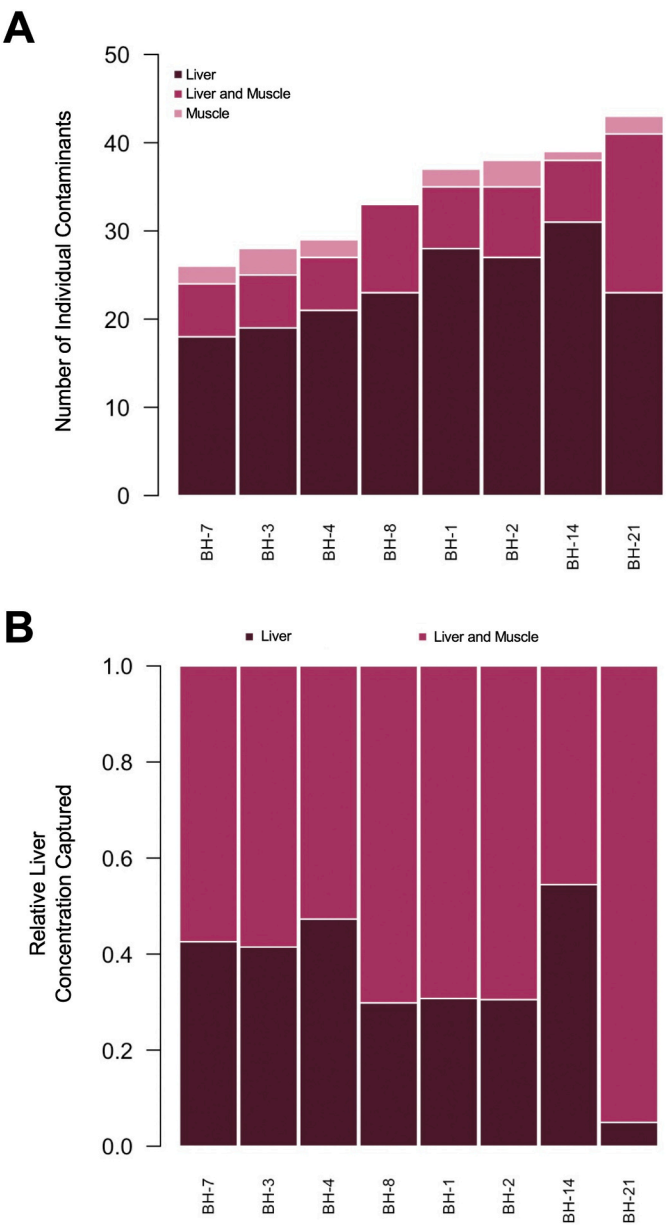

Fig. 3. For paired samples of liver and muscle, (A) number of detected contaminants were tallied based on their either their detection in liver only, in both liver and muscle, or in muscle only. (B) To access the degree to which contaminants identified in muscle reflected those contributing most to liver total contaminant concentrations, relative liver contaminant concentrations were partitioned based on contaminants that co-occurred in liver and muscle (light purple) or only liver (dark purple). For both plots, shark ID is denoted along the x-axis.

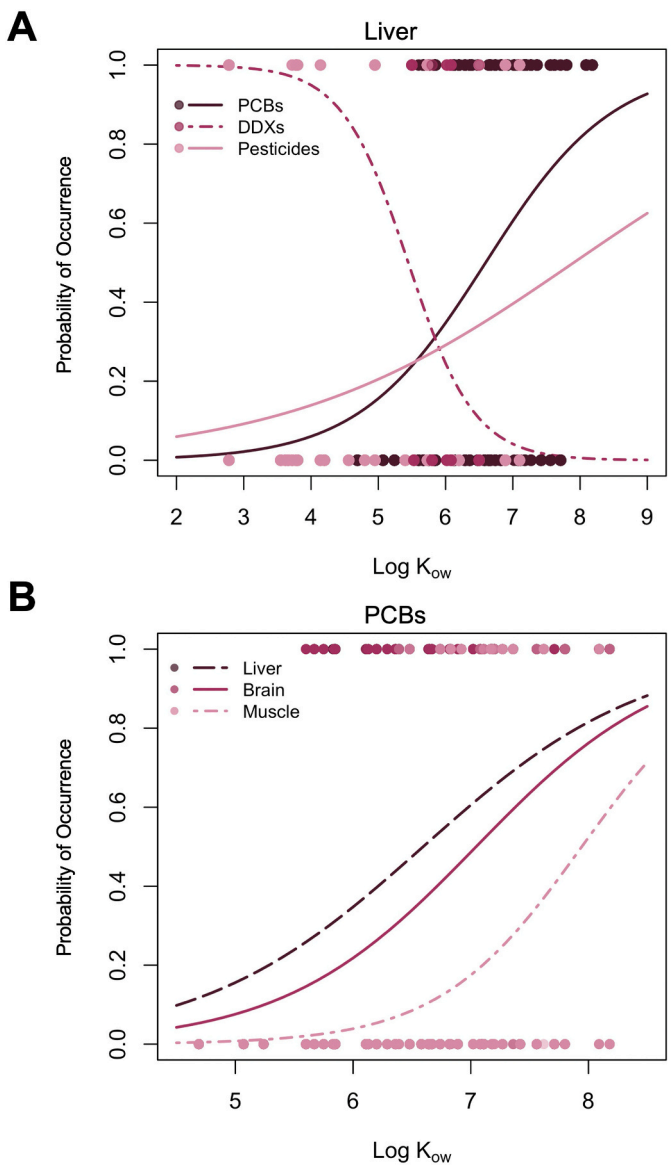

Fig. 4. Logistic regressions based on the occurrence of individual contaminants by contaminant group in the liver (A) and by tissue type for PCBs (B) against the contaminant's octanol-water partition coefficient (Log K_{ow}) for Bonnethead Sharks.

significant positive association with log K_{ow} (OR = 1.23, p = 0.051), whereas increasing shark length had a significant negative effect on occurrence of brain Pesticides (OR = 0.99, $p = 0.025$). For DDXs, a significantly negative association with log K_{ow} was found in liver (OR = 0.13, $p = 0.002$) and brain tissues (OR = 0.044, $p = 0.001$). Low log K_{ow} variance of detected contaminants in muscle prevented evaluation.

4. Discussion

Exposure and accumulation of organic contaminants remains a problem for elasmobranchs due to their predatory positions in food webs (Tiktak et al., 2020). For coastal species, exposure risk may also be elevated as a result of their close proximity to anthropogenic activities and contaminant sources (Gelsleichter and Walker, 2010; Lyons et al., 2019). However, compared to other important predators (e.g., marine mammals) with easier access to target tissues (i.e. blubber), elasmobranch research is hampered by the fact that many organic contaminants of interest accumulate in organs that are difficult to non-lethally sample, such as the liver. Although muscle has been proposed as an alternative tissue to evaluate the presence and magnitude of organic contaminants in elasmobranchs (Marsili et al., 2016), this work corroborates previous findings that muscle is an inappropriate choice as a proxy internal contaminant burden (Gelsleichter et al., 2005), at least for species with lean muscle tissue. Unlike other contaminants, such as methylmercury that distribute more widely across tissues in sharks, enabling muscle to serve as an appropriate proxy (O'Bryhim et al., 2017), organic contaminant tissue-to-tissue associations may be more nuanced due to individual contaminant pharmacokinetics and lipid distribution through the body.

The liver appears to be the organ where most organic contaminants accumulate in Bonnetheads, both in terms of contaminant concentrations and total body burden estimates. The relatively high contaminant concentration in liver tissue compared to muscle and brain is probably because the liver serves as the major lipid storage organ of elasmobranchs (Ballantyne, 1997) and has a high degree of vascularization and metabolic activity (i.e. increased potential for contaminant delivery). Likewise, lipid content composition may also be responsible for the higher concentrations of contaminants in brain tissue compared to muscle. Many elasmobranchs have very lean musculature compared to some teleosts (Økland et al., 2005), which is probably related to their inability to oxidize lipids in muscle tissue (Speers-Roesch and Treberg, 2010) leaving few physiological reasons to accumulate lipid in this tissue. However, other studies have noted substantial variation in elasmobranch lipid content from 0.18% to 40.3% (Cagnazzi et al., 2019; Storelli and Marcotrigiano, 2001). Low lipid content in Bonnethead muscle (mean 0.04 ± 0.03%) may limit the ability of muscle to accumulate lipophilic organic contaminants, despite the high proportion that it contributes to total body mass. Lean muscle may therefore be unsuitable for detecting many contaminants in certain elasmobranchs, as seen with the lower recovery rate of surrogate spikes in muscle compared to the brain or liver tissue.

Lipid composition likely also plays a role in contaminant distribution across tissues (Kammann et al., 1990; Kiceniuk et al., 1997). Lipid type was not determined in these samples but differences in occurrence of contaminant groups among tissue types were observed, suggesting that tissues have different lipid compositions that may drive tissue-specific patterns of accumulation. Indeed, among PCBs liver had the strongest "occurrences" as log K_{ow} increased, followed by brain and then muscle. Elasmobranch livers are largely composed of neutral, or storage, lipids (e.g. triacylglycerols; Ballantyne, 1997), which differ from the more polar lipids found in brain or muscle (e.g. phospholipids; Kreps et al., 1975, Økland et al., 2005). Thus, lipid content and composition probably play a role in how contaminants partition themselves among tissues (Elskus et al., 2005; Jenssen et al., 1996; Kammann et al., 1990).

Muscle was a poor proxy for liver contaminant composition in terms of contaminant identities, signatures and concentrations. The lack of significant correlations between liver and muscle for individual and group contaminant concentrations suggests that muscle should not be used as a proxy to gauge the magnitude of contamination in Bonnethead Sharks (Gelsleichter et al., 2005). This conclusion may be applicable for other elasmobranchs with lean muscle; however, greater interspecies comparisons are needed to determine how widespread this pattern may be. For those where both liver and muscle contaminant concentrations are reported along with muscle lipid content (Boldrocchi et al., 2019; Corsolini et al., 2014; Lyons and Adams, 2017; Storelli and Marcotrigiano, 2001), a negative correlation exists between muscle lipid content and the relative difference between mean muscle and liver PCB and DDX concentrations (Fig. 5). In other words, concentration differences between liver and muscle were greater in species with lean muscle. Thus, studies that use muscle as a proxy for liver contaminants in species where muscle lipid content is low may underestimate the number and relative proportions of organic contaminants as well as their potential concentrations.

Despite the reduced ability of muscle to reflect the liver in most

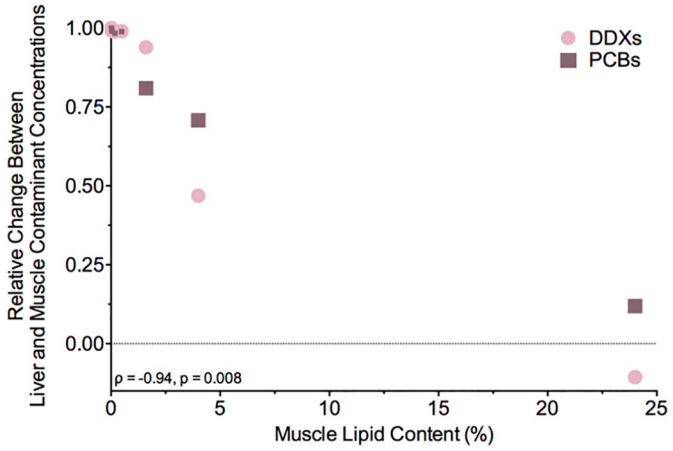

Fig. 5. The relative difference in mean PCB (purple squares) and DDX (pink circles) between liver and muscle was correlated against muscle lipid content using data from the present study and previously published works (Boldrocchi et al., 2019; Corsolini et al., 2014; Lyons and Adams, 2017; Storelli and Marcotrigiano, 2001). A significant, negative correlation was found for both contaminants.

aspects examined here, the identity of contaminants that were detected in both muscle and liver tissue appeared to comprise the primary contaminants (> 60%) of liver ∑OCs. This association suggests that though muscle may not accurately reflect the magnitude (i.e. concentration) or relative proportion (i.e. signature) of contaminants in the liver, it can capture the identity of the contaminants that are contributing most to liver OC accumulation. The positive correlation between contaminant concentration in the liver and log K_{ow}, suggests that the liver is prone to accumulating recalcitrant contaminants. Distribution of these contaminants to tissues with relatively low lipid contents (e.g., muscle) may be limited unless exposure to them is relatively high and may explain why muscle did a reasonable job of pinpointing the contaminants contributing most to liver ∑OCs; however, drawing conclusions beyond potential contaminant occurrence based on muscle contaminants should be exercised with caution.

This study implicates the brain as another possible site that undergoes significant contaminant accumulation in sharks. This is an important discovery, as accumulation of OCs in neural tissue may have implications for healthy brain functioning (Fonnum and Mariussen, 2009) and has had negative neuroendocrine impacts in teleosts (Aluru et al., 2004; Rahman et al., 2020). Summed PCB levels in Bonnethead brain tissue (mean 18.2 ± 18.4 ng/g ww) were lower than those measured in PCB-dosed Arctic Char (*Salvelinus alpinus* Linnaeus 1758, ~2000 ng/g ww)(Aluru et al., 2004) or lethally- dosed Little Brown Bats (*Myotis lucifugus* Le Conte 1831, 1300 μg/g ww)(Clark and Stafford, 1981), where negative effects were elicited from direct exposure. However, PCBs concentrations were comparable to those measured in the brain tissue of Greenland Sharks (*Somniosus microcephalus* Bloch & Schneider 1801, 24.7 ± 30.1 ng/g ww)(Corsolini et al., 2014). Further studies are needed to understand at what levels brain accumulation of contaminants elicits negative neurological impacts in elasmobranchs, an area of research that is in its infancy.

The blood-brain barrier differs structurally and physiologically between teleosts and mammals (Bernstein and Streicher, 1965). In Bonnetheads, the blood-brain barrier does not appear to be effective at excluding organic contaminant accumulation. Similar observations are noted in interspecies comparative studies where accumulation of highly chlorinated PCBs were found in fish brains but were absent in mammal brains despite similar brain lipid contents across species (Bachour et al., 1998). Interestingly, Bonnethead brain contaminant signatures were highly reflective of the liver indicating that exclusion of contaminants is likely limited. This association is in contrast to European eels (*Anguilla anguilla Linnaeus 1758)* where brain and liver signatures were distinct, and brain had higher contributions of lower chlorinated PCBs (Bonnineau et al., 2016). These findings support earlier work suggesting that species-specific factors likely play an important role in tissue accumulation (Ingebrigtsen et al., 1990).

Complicating factors in this dataset make it difficult to discount the likely influence that reproductive activity and limited sample size play in the interpretation of results. Reproductively active Bonnethead females were shedding contaminants through maternal offloading processes (data not shown). Since the liver is the main site of lipid mobilization to create egg yolk in fishes (Mommsen and Walsh, 1988), maternal offloading would negatively bias the magnitude of liver contribution to total body load and also potentially alter liver contaminant signatures through differential offloading of contaminants based on their log K_{ow} (Vanden Berghe et al., 2012). However, maternal offloading will probably not affect the detection rate substantially since only portions of total contaminant loads are transferred (Weijs et al., 2015; in this study uterine eggs constituted 2–10% of offloaded contaminants). For example, despite maternal offloading, correlations between liver and brain were still significant for many contaminants and tissue signatures shared a high degree of overlap. In contrast, the dissimilarity between liver and muscle likely reflects actual contaminant patterns, despite the range of life history stages and potential biological scenarios. Although this study was challenged by inherent problems faced from opportunistic sampling, these challenges are inescapable aspects of field sampling and tissue collection. This conclusion is not meant to discount the potential influence of uncontrolled factors (e.g. reproductive or nutritional state), but highlight the limited ability of muscle to serve as a non-lethal liver proxy since most other field studies looking to implement muscle as a liver proxy will be faced with these same challenges.

5. Conclusions

Bonnethead Sharks were used as an elasmobranch model to investigate the efficacy of using muscle tissue as a proxy for legacy organic contaminant accumulation in liver and brain tissue. Due to the low detection of legacy organochlorines, muscle did not serve as a suitable non-lethal alternative to liver or brain for most metrics examined here, which limits its utility in species with similar life-history traits or anatomy as the Bonnethead. Results indicate that lipid dynamics may play a role in contaminant distribution and should be formally examined through physiologically-based toxicokinetic modeling in elasmobranchs. Despite their propensity to accumulate contaminants and the important ecological roles they play in ecosystems, elasmobranchs are understudied with regards to toxicological modeling. The significant accumulation of organic contaminants in brain tissue implicates this organ as a potential target of organic contaminant negative impacts, which should be further investigated as the field of elasmobranch toxicology develops.

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Ethics approval

All sharks were sampled by authorized staff under official permits or scientific exemptions of U.S. state government agencies.

CRediT authorship contribution statement

Kady Lyons: Conceptualization, Investigation, Writing – original draft. **Douglas H. Adams:** Resources, Writing – review & editing,

Supervision. **Joseph J. Bizzarro:** Conceptualization, Methodology, Formal analysis, Writing – review & editing.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112327.

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