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Hepatobiliary Polycyclic Aromatic Hydrocarbons in Pelagic Fishes of the Gulf of Mexico

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by

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of the requirements for the degree of
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Abstract

Fisheries populations and their ecosystems are negatively impacted by both chronic and acute inputs of pollutants, including oil spills such as the *Deepwater Horizon* platform blowout in 2010 in the Gulf of Mexico (GoM). After *Deepwater Horizon*, toxicological studies of demersal fishes of the northern GoM were undertaken to characterize impacts and to establish baseline contaminant levels in the aftermath of the spill. In this study, I quantify polycyclic aromatic hydrocarbon (PAH) concentrations in eight pelagic fish species to demonstrate oil exposure differences between species, region and time. Analysis of biliary PAH metabolite equivalents using high performance liquid chromatography was used to estimate short-term and acute exposure in pelagic fishes (n = 102). Hepatic PAH concentrations of 19 parental PAHs and their alkylated homologs in pelagic liver samples (n = 142) were quantified using QuEChERS extractions and GC/MS/MS to quantify long-term and chronic exposure. The hepatic $\sum_{46}$PAH (sum of 46 parental and homolog PAH compounds) concentration ranges quantified for all species and regions (21.2 to 468 ng g^{-1} wet weight) are within the range found previously in the GoM region for demersal and reef fishes. Biliary PAH equivalent $\sum_{3}$FAC concentrations (fluorescent aromatic compounds of three PAH indicators) ranged from 4,400 to 1,400,000 ng FAC g^{-1} bile, which were also within the same range as demersal and reef species in this region. However, mean biliary PAH concentrations measured in Yellowfin Tuna (855 µg FAC g^{-1}) from the north central GoM were more than three times higher than Golden Tilefish (263 µg FAC g^{-1}) and 20 times higher than Yellowedge Grouper (*Hyporthodus flavolimbatus,* 41 µg FAC g^{-1})

collected in the same region. Significant negative relationships between PAH concentrations, biometrics (e.g. fish length), and putative indices of fish health including Fulton's condition factor and the hepatosomatic index were identified for multiple species. Intraspecies and regional differences were also identified for Greater Amberjack, *Seriola dumerili,* and Yellowfin Tuna, *Thunnus albacares,* suggesting variation in PAH sources across region. Produced waters from oil and gas platform discharges among other sources including riverine input, atmospheric deposition, burning of fossil fuels, resuspension of contaminated sediments, and chronically leaking wells are likely contributing to regional differences within species. Furthermore, the high levels of PAH exposure in pelagic species compared to demersal and reef species, indicated by biliary fluorescent aromatic compounds (FACs), warrants further investigation to identify the prominent source of surface water PAHs and the specific physiological species differences that govern the observed high rates of exposure and metabolic clearance for species such as the Yellowfin Tuna.

Introduction

The Gulf of Mexico (GoM) encompasses an array of diverse ecosystems that support sectors of the marine economy including the oil and gas industry, and commercial, and recreational fisheries. United States crude oil production in the GoM averaged 1.88 million barrels per day, accounting for about 15% of the nation's average 12.23 million barrels per day in 2019 [1]. Additionally, the Gulf Coast is home to 45% of United States crude oil refining capacity [2]. In 2018, commercial fisheries landing revenues (first-sale) alone totaled $896 million dollars in the GoM, 16% of the total commercial fisheries landings for the United States [3]. There were also 56.3 million recreational fisheries trips in the GoM, making the region the largest contributor of any region in the US total recreational fisheries trips [3].

Of course, the oil and gas industry and fisheries in the GoM interact, and multiple studies have documented oil contamination in the region's fish populations as a result of both large-scale oil spills and chronic inputs from various sources [4-6]. The impact of crude oil on fisheries must be quantified to accurately assess the health of marine resources and ecosystems in the Gulf as a whole. Multiple oil spills in recent history, including the *Deepwater Horizon* blowout that spilled 4.9 million barrels of crude oil between April 20th and July 15th in 2010, further emphasizes the need to understand of the short- and long-term consequences that petroleum-derived compounds have on GoM ecosystems and the highly valuable marine resources therein [7].

Polycyclic aromatic hydrocarbon (PAH) sources, transport, and fate

The most toxic components of crude oil, polycyclic aromatic hydrocarbons (PAHs), are a class of compounds characterized by two or more fused benzene rings [8]. These compounds are ubiquitous in the aquatic environment, and many are known carcinogens [9]. Polycyclic aromatic hydrocarbons are often present in the marine environment as complex mixtures of compounds with different physical and chemical properties such as molecular weight and their octanol partitioning coefficients, K_{ow}, which describe the degree of lipophilicity of compounds. The physical and chemical properties of the target PAHs evaluated in this study are presented in Table 1. These different properties impact the dispersion and fate of the compound in an ecosystem and how they are taken up by organisms. As these compounds are often separated by their molecular weights, PAHs can be divided into two classes: low molecular weight (LMW) PAHs, two to three benzene rings, and high molecular weight (HMW) PAHs, four or more benzene rings [10].

When discussing xenobiotic contaminants, it is important to understand what impacts a compound's bioavailability as well as its potential for bioconcentration, bioaccumulation and biomagnification. Bioavailability of a PAH depends on many variables pertaining to the source, the environment (water, sediments) and the compound itself [11]. It is generally understood that large, hydrophobic compounds are more readily absorbed onto particulate organic matter in the water column, thus decreasing the bioavailability of larger compounds in the water column [12]. Additionally, large hydrophobic compounds may be hindered in their ability to penetrate a lipid membrane due to physical characteristics including size and shape of the compound [11]. The degree of bioaccumulation and susceptibility to biomagnification is impacted by the compound's log K_{ow}. It is generally understood that compounds with log K_{ow} values greater than five are more susceptible to bioaccumulation and more likely to biomagnify than compounds with lo

K_{ow} values less than five [13]. However, species-specific differences will also likely introduce variation in bioaccumulation and bioconcentration of specific PAHs [11].

Table 1. *Target PAH parent compounds and associated properties (compound class, molecular weight, and log octanol-water partition coefficient). Compound classes are separated into low molecular weight (LMW) and high molecular weight (HMW).*

PAH Compound	Compound Class	Molecular Weight	Log K_{ow}
Naphthalene	LMW	128	3.3
Acenaphthylene	LMW	152	3.9
Acenaphthene	LMW	154	3.9
Fluorene	LMW	166	4.2
Dibenzothiophene	LMW	184	4.4
Phenanthrene	LMW	178	4.5
Anthracene	LMW	178	4.4
Fluoranthene	HMW	202	5.1
Pyrene	HMW	202	4.9
Benzo[*a*]anthracene	HMW	228	5.8
Chrysene	HMW	228	5.8
Benzo[*b*]fluoranthene	HMW	252	5.8
Benzo[*k*]fluoranthene	HMW	252	6.1
Benzo[*e*]pyrene	HMW	252	6.4
Benzo[*a*]pyrene	HMW	252	6.1
Perylene	HMW	252	6.3
Indeno[*1,2,3,-cd*]pyrene	HMW	276	6.7
Dibenzo[*a,h*]anthracene	HMW	278	6.7
Benzo[*g,h,i*]perylene	HMW	276	6.6

These various PAHs accumulate in the marine environment through both natural and anthropogenic routes [8]. There are four general categories of PAH origins: biogenic (produced by living organisms), diagenic (produced in sediments), petrogenic (derived from oil) and pyrogenic (produced during combustion) [14]. The primary sources to the marine environment are petrogenic (e.g., surface and deep sources of petroleum) and pyrogenic (e.g., atmospheric deposition and coastal runoff of combustion products from fossil fuels) sources [14]. Surface

releases of oil originate from boats and ships, tanker accidents, oil and gas platform discharges, and pipeline leaks. Subsurface sources include submarine groundwater discharge, natural oil seeps, leaking infrastructure (eg., Taylor MC20), and deep platform blowouts, such as the 2010 *Deepwater Horizon* (DWH) oil spill, which discharged at a depth of 1,500 m below the surface for three months. The Louisiana Sweet Crude (LSC) oil from DWH was comprised of 3.9% PAHs by weight [15]. Even prior to the DWH blowout, elevated petrogenic PAHs were found in marine organisms associated with natural oil seepage events in the GoM [16]. The GoM tends to exhibit elevated PAH contaminant loads due its proximity to natural seeps, oil and gas platforms (Figure 1), riverine input, and terrestrial runoff, among other sources [17].

An additional source of PAH input to the marine environment is produced waters, which is the largest stream of waste from oil and gas production from active onshore and offshore wells [18-20]. Produced waters are composed of formation water (water trapped with oil or gas in a geologic reservoir), and injection water (water that is injected into the well during oil and gas production to improve recovery of the natural products and sometimes safety) [18, 20]. These waste waters contain elevated concentrations of metals, radioisotopes, and organic chemicals including PAHs [18]. Although the total oil present in produced waters is regulated not to exceed an average concentration of 29 ppm of "oil and grease", concerns have been raised about the impacts of long-term chronic exposure in marine organisms because of the shear volumes of the amount of produced water discharged [21].

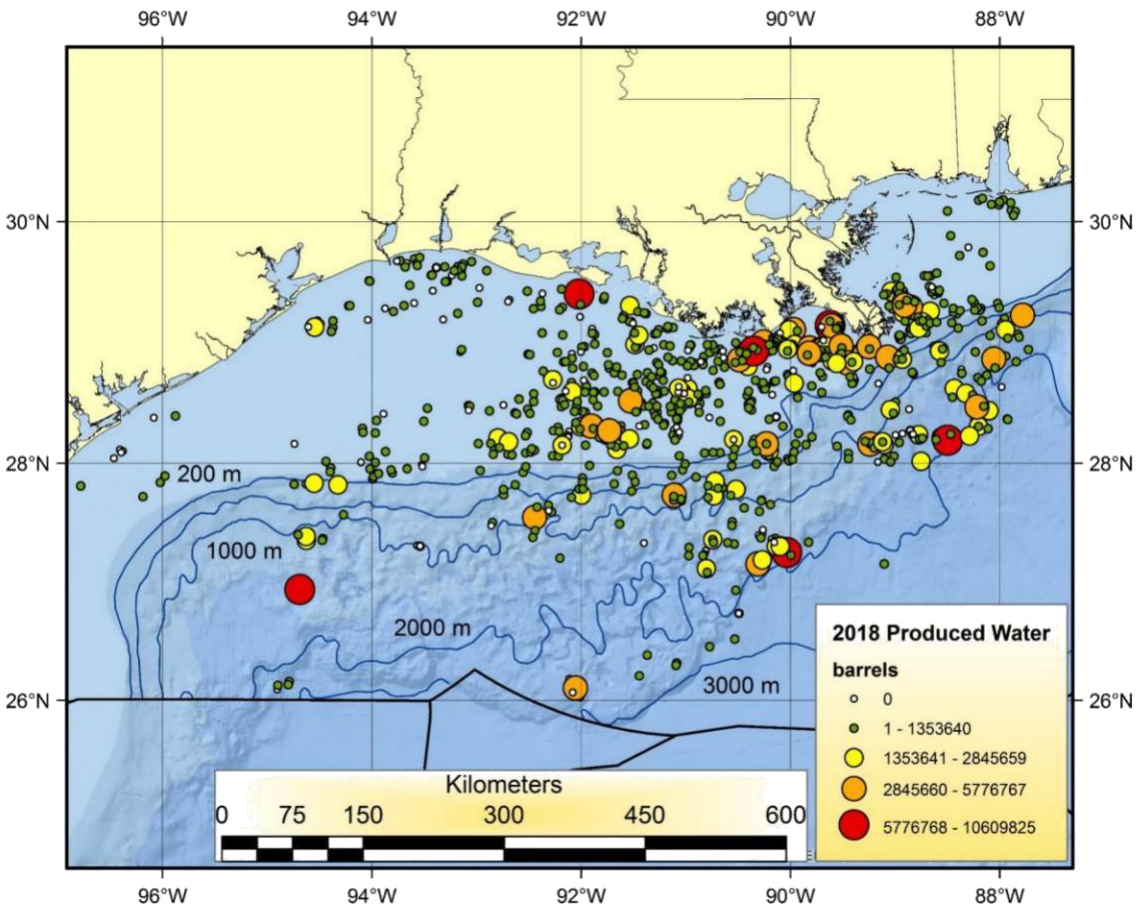


Figure 1. *Measurements of produced waters discharged by 960 oil and gas platforms in 2018 (map: Murawski, unpublished).*

Polycyclic Aromatic Hydrocarbons (PAHs) in fishes

After the DWH accident, oil was deposited onto sediments by way of subsurface intrusions ("plumes") and through the deposition of oiled marine snow in the Northern GoM [22-24]. Once released, PAHs can be absorbed by plankton and be incorporated into the food web. Fishes absorb PAHs through skin contact, across their gills, or through ingestion of contaminated sediments or food [26, 27]. The levels of xenobiotic (foreign to the body of a fish) contaminants in organisms are determined by the balance of uptake and elimination of those contaminants [10].

Fishes with relatively high gill ventilation rates, large gill surface area and countercurrent blood flow may have correspondingly high levels of uptake and accumulation of xenobiotic contaminants [28]. As PAHs are readily absorbed by a fish through one or multiple of the routes described above, the PAH concentrations in tissues may become elevated as compared to the surrounding environment as lipophilic PAHs are absorbed into lipid-rich tissues [29]. Conversely, there are six potential routes of elimination through the fish body: respiration, dermal diffusion, fecal egestion, metabolic transformation, reproductive losses, and growth dilution [27]. Environmental factors including temperature, pH, oxygen content, and salinity as well as physiological factors such as reproductive state, age, sex, and stress level will impact rates of uptake and elimination of xenobiotics in fish [10]. Physical and chemical properties such as lipophilicity of specific compounds, including PAHs, will also impact bioavailability and accumulation rates (Table 1) [10, 27, 30, 31].

Metabolic transformation through enzymatic pathways alters the structure of the parent PAHs resulting in more polar compounds which are consequently more easily excreted [29, 32]. Metabolism converts parent PAHs into more excretable PAH metabolites, through metabolization pathways of PAHs utilizing the cytochrome P450 induction pathway [33]. However, metabolization also makes PAH metabolites more reactive and electrophilic which can increase the toxicity of these compounds as compared to the original PAH compounds. Retention and distribution of PAH metabolites in fish are impacted by environmental temperature [34]. Metabolites and parent PAHs also undergo enterohepatic recirculation (reabsorption by the intestines and transport to the liver), which can increase retention time, absorption potential, and toxicity [35]. Impacts have been noted in fish when metabolites bind to proteins, DNA, and RNA [33].

Sub-lethal impacts associated with PAH contamination have been observed in contaminated embryos, juveniles and adults [36-38]. Liver lesions and neoplasia in PAH contaminated fish have been observed in the wild and in laboratory-based studies [39-42]. Other studies have shown a reduction in reproductive potential in the form of reduced gametogenesis, gonad size, and egg production as consequences of PAH contamination[33, 43, 44]. Documented sublethal effects also include cataract formation, decreased growth, lowered condition factor, and cardiac disfunction, reduced survival to maturity, DNA damage, and immunosuppression [4, 37, 45, 46].

After the 2010 DWH blowout and the resulting spill, demersal and reef fishes were the primary group of fishes studied as 4-31% of crude oil was estimated to be sequestered in deep ocean sediments [22, 47]. Researchers found ubiquitous PAH contamination in benthic and demersal fish species including groupers, hakes (*Urophycis* spp.), Tilefish, *Lopholatilus chamaeleonticeps*, Red Snapper, *Lutjanus campechanus*, and King Snake Eel, *Ophichthus rex*, among other species [4, 5, 48]. Significant differences in biliary PAH equivalent concentrations were found among species with differing life histories and physiology [48, 49]. Increasing hepatic $\sum_{46}$PAH concentrations (summed concentrations of 46 different PAHs) in grouper species indicated chronic exposure in the north central region of the GoM, and suggested various sources in addition to the 2010 DWH spill result in chronic PAH contamination in this region [4, 49].

Initial post-DWH studies focused primarily on demersal species due to their coupling with sediments and their economic importance. However, researchers have found that pelagic fishes in the GoM also exhibited increases in tissue PAH concentrations and corresponding sublethal impacts [36, 50]. In particular, concentrations of PAHs in the muscle tissues of

mesopelagic fishes increased seven to ten-fold following DWH [51]. Pelagic fish embryos were also likely exposed to oil that traveled to upper surface waters. This is especially relevant for the species known to be spawning at the time, including Yellowfin Tuna, *Thunnus albacares*, Dolphinfish, *Coryphaena hippurus*, and Greater Amberjack, *Seriola dumerili* [36, 50, 52]. Pelagic fish embryos exposed to petrogenic PAH mixtures have developed cardiac dysfunction, reduction in size of cranial features, and spinal curvature [36, 50].

Pelagic Fishes

Pelagic fishes including billfishes (family *Istiophoridae*) and tunas (family *Scombridae*) are ecologically as well as economically important. In 2018, commercial landings of Yellowfin Tuna from the west coast of Florida and Louisiana alone were valued at $3.5 million (first sale ex-vessel) [53]. Ecologically, these large pelagic fishes play important roles as top predators in marine food webs [54]. Chronic or acute pollution events resulting in lethal or sublethal health effects to these large pelagic fishes could lead to cascading effects on GoM pelagic food webs [55]. Monitoring the health of these species is thus an important aspect of the health of the GoM large marine ecosystem (LME).

Eight pelagic fish species with varying physiology and life histories were evaluated in this study. These species utilize various water column zones, habitats, and have variation in their diets. These factors may result in variable exposures to PAHs through diet, diffusion, respiration and the proximity to sources. Other important factors affecting uptake of PAHs by these species include species-specific metabolism, spawning location and timing, and the propensity of maternal offloading which may decrease PAH concentrations in females spawning at the time of collection.

Various pelagic organisms exhibit zonation in the water column, namely: the epipelagic (0 to 200 meters), mesopelagic (200 to 1000 meters), bathypelagic (1000 to 4000 meters), and abyssopelagic (4000 to 6000 meters). Species included in this study occupy three of four of these zones. The primary epipelagic species include Yellowfin Tuna, Dolphinfish, Great Barracuda, *Sphyraena barracuda*, and Almaco Jack, *Seriola rivoliana* [56]. Greater Amberjack utilize both the epipelagic and mesopelagic zones. Escolar, *Lepidocybium flavobrunneum*, is principally epipelagic. Swordfish, *Xiphias gladius,* and Longnose Lancetfish, *Alepisaurus ferox,* diel vertical migrators utilizing zones from the epipelagic zone to the bathypelagic [57, 58].

Yellowfin Tuna, Swordfish, and Dolphinfish are highly migratory species that travel long distances and often cross international boundaries. Highly migratory species including scombrids and billfishes have adopted high ventilation rates and large gill surface area to body ratios in order to improve gas exchange and meet demands associated with continuous swimming [59, 60]. These physiological factors may increase the volume of water passing over their gills and increase exposure to PAHs. The five species in this study that are not highly migratory utilize various habitats. Great Barracuda, Almaco Jack, and Greater Amberjack are all reef-associated, but Almaco Jacks are primarily associated with reef slopes and drop-offs. Escolar, which is known to vertically migrate, and Longnose Lancetfish, which known as an opportunistic feeder, are non-reef-associated mesopelagic species [61, 62]. Generally, non-continuous swimmers will have lower gill surface area, gill filament lengths, and observe lower swimming speeds [60]. This will likely result in decreased volumes of water passing over their gills and decreased uptake across the gills.

Pelagic fish species often occupy high trophic levels ranging from 3.5 to 5 (Table 2). Species in this study typically occupy trophic levels 3.5 to 4.6, and consume primarily fishes, crustaceans, and cephalopods. A more detailed account of the diets of these species is presented in Table 2. Persistent organic pollutants (POPs) which are not easily metabolized (e.g. organochlorine pesticides and polychlorinated biphenyls) can bioaccumulate and biomagnify across trophic levels. Contrarily, PAHs are readily metabolized by fishes, thus trophic dilution is likely to occur and trophic magnification has not been observed in previous studies [10, 13]. Bioaccumulation of PAHs may still result if the rate of exposure and uptake exceeds the rate of metabolism and elimination or if the mechanisms of PAH metabolism are undermined by pollutant exposure [10].

Table 2. *Trophic ranges and typical diets of pelagic fish species within this study.*

Species	Trophic Range	Diet	References
Yellowfin Tuna	3.7 – 4.5	Fishes, crustaceans, cephalopods	[63-66]
Swordfish	4.5 – 4.6	Fishes, crustaceans, cephalopods	[64, 67]
Dolphinfish	4.4 – 4.5	Fishes, zooplankton, squids and crustaceans	[65, 68]
Longnose Lancetfish	3.4 – 4.5	Fishes, cephalopods, tunicates, and crustaceans	[64, 67, 68]
Escolar	4.3	Fishes, crustaceans, cephalopods	[69]
Barracuda	3.6 – 4.5	Fishes, cephalopods, shrimps	[70, 71]
Almaco Jack	4.3 - 4.5	Fishes and invertebrates	[56, 70, 72]
Greater Amberjack	3.7 – 4.6	Fishes and invertebrates	[67, 70]

Study Objectives

The objectives of this study are (1) to quantify PAH biliary metabolite equivalents in eight pelagic species sampled in the GoM, (2) to quantify hepatic concentrations of PAHs in the same species, (3) to relate levels of contamination in individual fish to indices of their overall health,

including Fulton's condition factor (K) and hepatosomatic indices (HSI), (4) to evaluate differences in PAH concentrations between species, (5) to identify regional and temporal differences by evaluating compositional profiles in hepatic tissues, and (6) to assess relationships between PAH concentrations and biometric factors (e.g. length, weight, sex).

Quantifying PAH concentrations across trophic levels of the marine ecosystem is an important part of understanding how contaminant concentrations vary within an ecosystem and how they may affect ecosystem function. PAH concentrations have previously been quantified in sediments and demersal as well as mesopelagic fish tissues from the GoM, however limited data exist for PAHs in pelagic species. Quantification of PAH concentrations in pelagic fish species will add to the current understanding of how PAHs are transferred through the ecosystem and across trophic levels (e.g. 3.5 – 5) in pelagic predators.

Primary null hypotheses in this project are:

1. There are no significant differences in hepatic PAH concentrations across eight pelagic species.
2. There are no significant differences in biliary PAH equivalent concentrations across eight pelagic species.
3. There are no significant relationships between PAH concentrations and associated biometrics and health indices.
4. There are no significant differences between PAH concentrations across regions and years within each species.

Methods

Field Sample Collection

The Gulf of Mexico Research Initiative (GoMRI) through its Center for Integrated Modeling and Analysis of Gulf Ecosystems (C-IMAGE) sponsored and conducted Gulf-wide demersal longline surveys between 2011 and 2017 followed by a pelagic longline sampling expedition in August of 2018 and a pelagic hook and line fishing expedition in February 2020 [73]. Demersal longline surveys were completed at stations between 40 m and ~300 m using a five nautical mile mainline with attached leader lines and #13/0 circle hooks [73]. The 2018 pelagic longline cruise consisted of eight stations with paired day and night sets (16 total sets). Ten nautical miles of mainline were deployed within the upper 200 m (epipelagic) using 150-foot leader lines with baited #16/0 hooks at each station. All hooks were baited by alternating between Atlantic Mackerel (*Scomber scombrus*) and squid (*Illex sp.)*. Upon retrieval of each set, the time, depth, temperature, species identification of each specimen and associated biometrics (standard length, fork length, total length, total weight, organ weights, sex and fish health observations) were recorded (Table 3). The 2020 pelagic hook and line cruise consisted of two sampling stations associated with offshore oil platforms and sampling consisted of trolling at the surface with artificial lures as well as jigging near the platforms. All sampling locations at which fish used in this study were caught are plotted in Figure 2.

Target species included in this study were collected at 42 fishing sites between 2016 and 2020 (Table 3, Figure 2). Samples were immediately weighed and frozen at -20°C onboard

and subsequently transferred to a laboratory freezer at the end of the research cruise. A total of 159 pelagic fishes were collected for PAH analysis from the demersal (n = 84) and pelagic (n = 75) surveys.

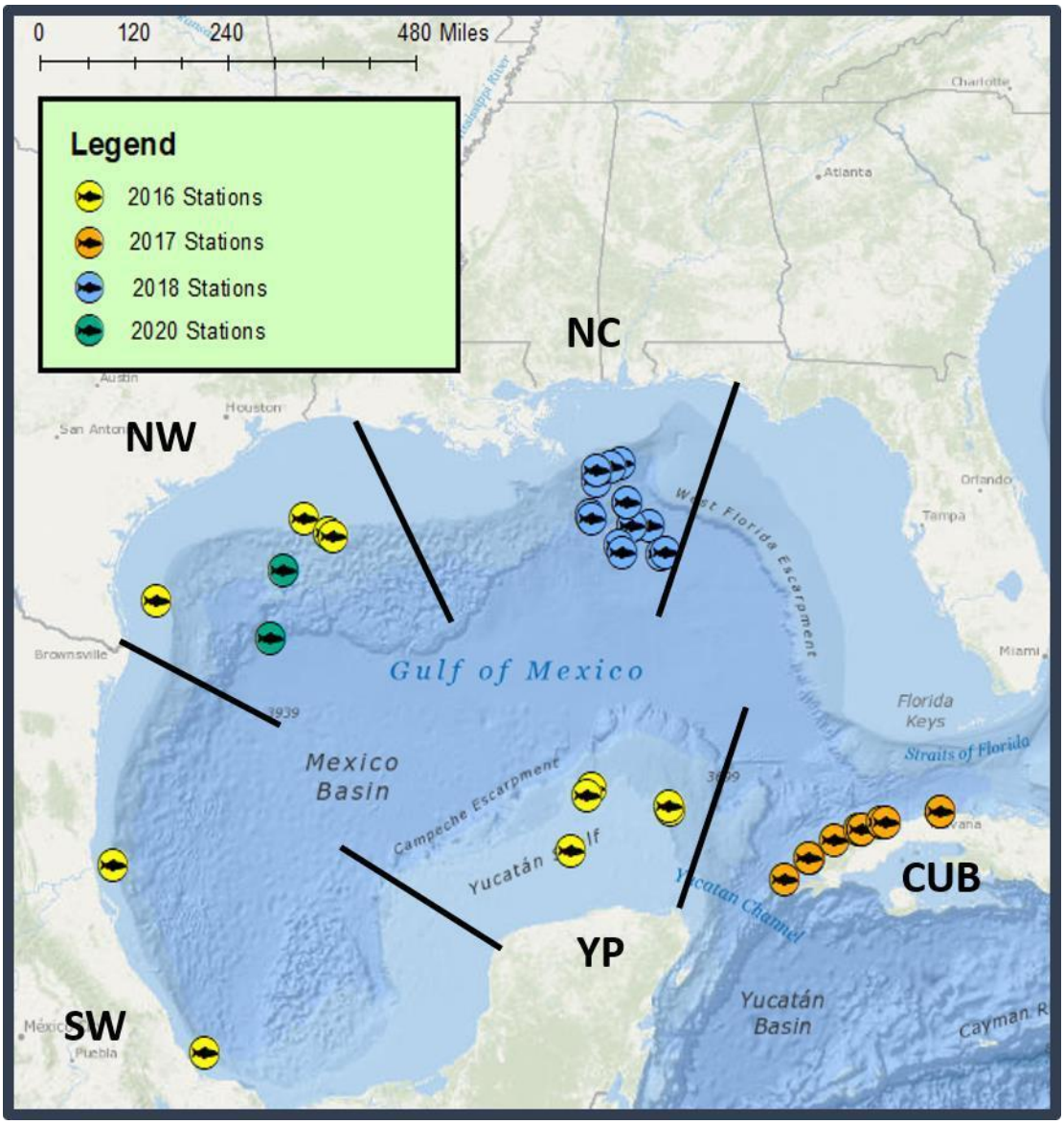


Figure 2*. Station locations of the target pelagic species used in this study caught in 2016, 2017, 2018, and 2020.*

Table 3. *Sample sizes (n), mean and standard deviations (SD) for biometric measurements by region and species. Regional means for all species combined are bolded. Sex ratio indicates the number of females (F), males (M), and those in which sex could not be determined (U).*

Region	Species	Sex ratio	n	Standard Length (cm)		Total Weight (kg)		Liver Weight (kg)		*n* lipid samples	% Liver Lipid Content	
		F:M:U		Mean	(SD)	Mean	(SD)	Mean	(SD)		Mean	(SD)
Cuba	Almaco Jack	4:1:1	6	59.0	(17.2)	4.57	(3.24)	0.04	(0.03)	5	37	(15)
	Barracuda	1:16	17	74.2	(12.6)	3.08	(1.55)	0.03	(0.04)	16	30	(10)
	Region (species combined)	**5:17:1**	**23**	**70.2**	**(15.1)**	**3.46**	**(2.14)**	**0.03**	**(0.04)**	**12**	**32**	**(12)**
North Central	Dolphinfish	2:1:2	5	73.6	(17.3)	5.38	(4.01)	0.04	(0.01)	4	43	(15)
	Escolar	7:0:0	7	71.7	(12.4)	6.63	(3.61)	0.05	(0.02)	7	68	(28)
	Longnose Lancetfish	2:0:8	10	73.2	(21.2)	1.16	(1.08)	0.003	(0.002)	1	60	
	Swordfish	2:6:0	8	103.7	(13.3)	15.37	(5.71)	0.25	(0.31)	8	49	(19)
	Yellowfin Tuna	2:5:0	7	138.4	(12.6)	54.77	(12.74)	0.26	(0.03)	7	50	(13)
	Region (species combined)	**15:12:10**	**37**	**91.6**	**(30.4)**	**16.27**	**(21.09)**	**0.13**	**(0.18)**	**27**	**53**	**(21)**
Northwest	Greater Amberjack	12:6:3	21	80.8	(21.0)	10.48	(8.53)	0.08	(0.10)	17	36	(12)
	Yellowfin Tuna	14:23:1	38	85.0	(11.8)	13.78	(6.35)	0.09	(0.03)	32	34	(24)
	Region (species combined)	**26:29:4**	**59**	**83.5**	**(15.6)**	**12.61**	**(7.31)**	**0.08**	**(0.06)**	**49**	**35**	**(20)**
Southwest	Greater Amberjack	3:0:1	4	44.8	(9.8)	1.96	(0.84)	0.02	(0.01)	4	32	(16)
	Region (species combined)	**3:0:1**	**4**	**44.8**	**(9.8)**	**1.96**	**(0.84)**	**0.02**	**(0.01)**	**4**	**32**	**(16)**
Yucatan Shelf	Almaco Jack	11:14:2	27	50.5	(10.6)	3.23	(2.03)	0.02	(0.02)	26	29	(8)
	Greater Amberjack	8:1:0	9	99.6	(15.7)	15.61	(5.34)	0.13	(0.07)	6	45	(21)
	Region (species combined)	**19:15:2**	**36**	**62.8**	**(24.6)**	**6.32**	**(6.26)**	**0.05**	**(0.06)**	**32**	**32**	**(13)**

Biliary PAH Analysis

Analysis of biliary PAH equivalents is used to estimate short-term and acute exposure. Biliary PAH equivalents of three parental PAHs, naphthalene, phenanthrene, and benzo[*a*]pyrene (Figure 3) ranging from low to high molecular weights were quantified. High performance liquid

chromatography with a fluorescence detector (HPLC-F, Hitachi, Tokyo, Japan) has been used widely to study exposure of fish and other marine organisms to PAHs and estimate metabolite equivalents [74]. This study used HPLC-F to conduct semi-quantitative analysis of PAH metabolite equivalent concentrations in untreated bile from the eight target pelagic fish species. Bile samples (n = 102) were injected (3 µL) onto a C-18 reverse phase column (Synergi™ 4 µm Hydro-RP 80 Å, LC Column 150 x 4.6 mm, Phenomenex, Torrance, CA) and eluted with a gradient from 95% water 5% acetic acid to 100% methanol with a flow of 1.0 mL minute^{-1}.

Continuing calibration standards were analyzed every 10-12 field samples. Field samples were analyzed in triplicate, with 100% methanol blanks between each sample. Quality assurance/quality control measures included maintaining a CV of less than 20% for each sample triplicate and the use of analytical standards. The area of fluorescence response across the region of interest (5-19 min) for each sample chromatogram were manually integrated and converted to PAH equivalents, referred to as fluorescent aromatic compounds (FACs), using the following equation with a bile density of 1.03 g mL^{-1} [75]:

$$\frac{\text{standard concentration}}{\text{standard mean area}} \times \frac{\text{integrated sample area}(6 - 19\text{min})}{\text{density of bile}} \times \frac{\text{uL of standard injected}}{\text{uL of sample injected}}$$

Biliary PAH equivalents are reported to two significant figures in ng FAC g^{-1} bile.

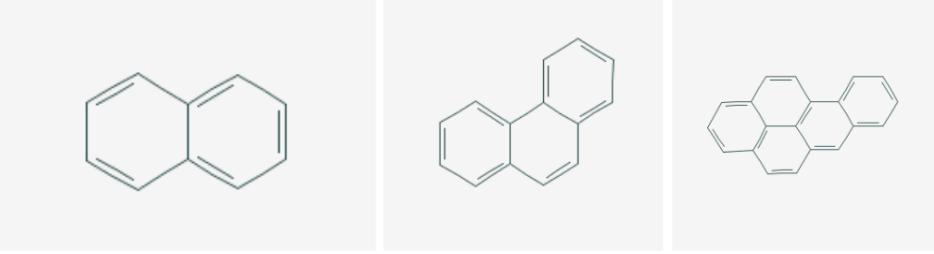


Figure 3. *Chemical structures of parent PAHs analyzed via HPLC-F. Naphthalene (left), phenanthrene (middle), and benzo[*a*]pyrene (right).*

Hepatic PAH Analysis

Analysis of liver PAH concentrations is utilized to understand long-term and chronic exposures. Liver tissues (n = 142) were thawed and homogenized prior to using a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction method first utilized in pesticide analysis of fruits and vegetables [76]. This methodology has since been used to extract fish tissues for POP analysis [77, 78]. For each sample, a two-gram subsample of homogenized liver was spiked with a deuterated PAH standard mix containing 19 PAHs. The tissue was then vortexed for thirty seconds and left to marinate for a previously optimized species-specific amount of time (10– 20 min). After the addition of two steel homogenizing spheres and 20 mL acetonitrile (ACN) each sample was shaken using a 1600 MiniG homogenizer (SPEX® Sample Prep, Metuchen, NJ) and subsequently centrifuged at 5000 RPM for five minutes. An 8 mL aliquot of supernatant was transferred to a Bond Elut Enhanced Matrix Removal-Lipid (EMR-Lipid, Agilent Technologies, Santa Clara, CA) dispersive solid phase extraction tube and shaken (five minutes) and centrifuged (five minutes) once more. Once decanted, a Bond Elut EMR-Lipid polish pouch was added to each extract followed by manual vortexing (30 seconds) and centrifugation (5 minutes).

Prior to injection, each extract was spiked with an internal standard (*p*-terphenyl-d14). A 2 µL aliquot of extract was injected between a sandwich of 0.2 µL analyte protectant (solution of 20 mg mL^{-1} L-gulonolactone and 10mg mL^{-1} D-sorbitol in ACN) into an inlet operating in splitless mode with a temperature set at 295°C. A 7890B Gas Chromatograph System (Agilent Technologies, Santa Clara, CA) outfitted with a 30m Rxi-5Sil fused silica capillary column (Restek, Bellefonte, PA, USA) was utilized to separate analytes. The temperature parameters of the oven were as follows: 60°C for three minutes followed by an increase to 120°C at a rate of

12°C min^{-1}. A subsequent ramp to 300°C using a rate of 8°C min^{-1} and an increase to 320°C at 15°C min^{-1} where temperature was held for four minutes. A triple quadrupole mass spectrometry (7010 Triple Quadrupole – GC/MS/MS, Agilent Technologies) operating in multiple reaction monitoring (MRM) mode and full scan mode was used to identify and quantify 46 PAHs including 19 parental PAHs and their associated alkylated homologs. Acquisition parameters for target analytes and surrogate standards are summarized in Tables 4 and 5. Ultra-high purity helium was used as the carrier gas and quench gas at rates of 1.5 mL min^{-1} and 2.25 mL min^{-1}, respectively. The transfer line, source, and quadrupole were set to 320°C, 300°C, and 150°C, respectively.

Final analyte concentrations were calculated using response factors calculated from matrix-matched standards specific to each species and each day of extractions. A five-point matrix matched calibration curve was run prior to this project to assure linearity of analytes within a concentration range of 1 to 1000 ng mL^{-1}. The hepatic concentrations for 19 PAHs and their associated alkylated homologs are reported to three significant figures and summed concentrations are presented as $\sum_{46}$PAHs in ng g^{-1} wet weight (w.w.). All analytes identified at less than the method detection limit (MDL = 1 ng/g w.w.) were replaced with ½ MDL (0.5 ng/g w.w.) prior to statistical analysis.

Quality assurance and quality control (QA/QC) measures were developed based on NOAA's Analytical Quality Assurance Plan for the Mississippi Canyon 252 Natural Resource Damage Assessment and EPA Method 8270E [79, 80]. Measures of QA/QC included method blanks, matrix spikes, analytical standards, and matrix-matched standards. Method blanks and solvent blanks were utilized to monitor background contamination

while matrix spikes were utilized to validate the extraction method. Each analyzed sample, method blank, and matrix spike was monitored to maintain recoveries of analytes were within accepted protocol ranges. Average recoveries across compounds were 70% in samples, 65% in method blanks, and 72% in matrix spikes.

Table 4. *Method acquisition parameters for target PAH analytes.*

Target Analyte	Parental Ion	Quantifying Ion	Collision Energy	Qualifying Ion	Collision Energy
Naphthalene	128	102	20	127	25
C1N	142	141	20	115	25
C2N	156	141	20	115	30
C3N	170	155	20	127	30
C4N	184	169	30	154	40
Acenaphthylene	152	151	15	150	20
Acenaphthene	154	153	15	152	30
Fluorene	166	165	20	163	30
C1F	180	165	25		
C2F	194	179	30		
C3F	208	193	40		
C4F	222	206	40	179	40
Dibenzothiophene	184	139	30		
C1D	198	197	20	165	30
C2D	212	197	40		
C3D	226	211	40		
C4D	240	211	40		
Phenanthrene	178	176	30	152	30
Anthracene	178	176	30	152	30
C1PH/A	192	191	20	189	40
C2PH/A	206	191	20	189	40
C3PH/A	220	205	30	189	40
C4PH/A	234	219	50	204	20
C5PH/A	248	247	20	246	40
Fluoranthene	202	201	30	200	40
Pyrene	202	201	30	200	40
C1F/PY	216	215	30	213	40
C2F/PY	230	229	40	215	20

Table 4 (Continued).

Target Analyte	Parental Ion	Quantifying Ion	Collision Energy	Qualifying Ion	Collision Energy
C3F/PY	244	229	40	228	40
C4F/Py	258	243	20	228	40
C5F/Py	272	271	10	257	40
Benzo[*a*]anthracene	228	226	40	202	30
Chrysene	228	226	40	202	30
C1BA/C	242	241	40	239	40
C2BA/C	256	255	20	239	40
C3BA/C	270	255	20	239	50
C4BA/C	284	282	50	269	50
C5BA/C	298	296	50	283	50
Benzo[*b*]fluoranthene	252	250.1	40	224	35
Benzo[*k*]fluoranthene	252	250.1	40	224	35
Benzo[*e*]pyrene	252	250.1	40	226	35
Benzo[*a*]pyrene	252	250.1	40	224	35
Perylene	252	250.1	40	224	35

Table 5. *Method acquisition parameters for internal and surrogate standards.*

Standard	Parental Ion	Quantifying Ion	Collision Energy	Qualifying Ion	Collision Energy
p-Terphenyl	244	237.3	50	159.3	50
Naphthalene-d8	136	108	25	134	10
Acenaphthylene-d8	160	158	30	132	30
Acenaphthene-d10	164	162.1	20	134	40
Fluorene-d10	176	174	40	146	30
Dibenzothiphene-d8	192	160.1	40	146	40
Phenanthrene-d10	188	184.1	10	158	30
Anthracene-d10	188	184.1	10	158	30
Fluoranthene-d10	212	208.1	50	184.1	40
Pyrene-d10	212	208.1	50	184.1	20
Benzo[*a*]anthracene-d12	240	236.1	50	212.1	40
Chrysene-d12	240	236.1	50	212.1	40
Benzo[*b*]fluoranthene-d12	264	260.1	50	236	40
Benzo[*k*]fluoranthene-d12	264	260.1	50	236	40
Benzo[*e*]pyrene-d12	264	260.1	50	236	40
Benzo[*a*]pyrene-d12	264	260.1	50	236	40
Perylene-d12	264	260.1	50	236	40
Ideno[*1,2,3,-cd*]pyrene-d12	288	284.1	50	255.9	50

Table 5 (Continued).

Standard	Parental Ion	Quantifying Ion	Collision Energy	Qualifying Ion	Collision Energy
Dibenzo[*a,h*]anthracene-d14	292	160.1	50	264.1	40
Benzo[*g,h,i*]perylene-d12	288	237.3	50	256.1	50

Total Hepatic Lipid Analysis

Total liver lipid content in hepatic tissue was extracted utilizing a modified Folch method explained in detail in Pulster et al., 2020 [4]. Each 200 mg liver aliquot underwent an initial extraction step using methyl tert-butyl ether (MTBE) followed by a re-extraction step using a mixture of MTBE/methanol/water (10:3:2.5, v/v/v). After each step, the organic phase was collected in an amber vial and allowed to dry. Upon attaining target dryness, total lipid for each sample was weighed and reported as percentage of liver weight.

Statistical Analysis

Prior to statistical analysis assessing species differences and biometric correlations, all hepatic PAH levels below the method detection limit (MDL) were replaced with 1/2 MDL. All statistical analyses were performed using R and RStudio [81]. Group differences in hepatic and biliary PAH concentrations (sex, species, region) were assessed using non-parametric permutation-based analyses of variance (np-MANOVA) (Vegan package, R) as the data are not normally distributed and as to not reduce statistical power associated with transformation. If the resulting p-value was smaller than the set significance level ($\alpha = 0.05$), the null hypothesis was rejected. A pairwise np-MANOVA was then utilized as a *post hoc* test to determine which pairs of groups were significantly different. To reduce type I error, the Bonferroni-adjusted p-value was used at the test statistic.

Biometric analyses were used to assess relationships between PAH concentrations and factors including length, weight, sex, and condition for each species. Biometric analyses were completed after logarithmic transformation of the raw PAH concentrations to meet the assumption of normality within chosen Pearson's correlation test. Correlations between log10-transformed PAH data and biometric data were assessed using Pearson's linear correlation. Canonical analysis of principle coordinates (CAP) tests were used to assess multivariate differences between groups by evaluating compositional profiles using the relative abundances of PAHs. Relative abundances were calculated utilizing the raw hepatic PAH data without replacement of <MDLs and normalized by the maximum PAH concentration by each region.

Results

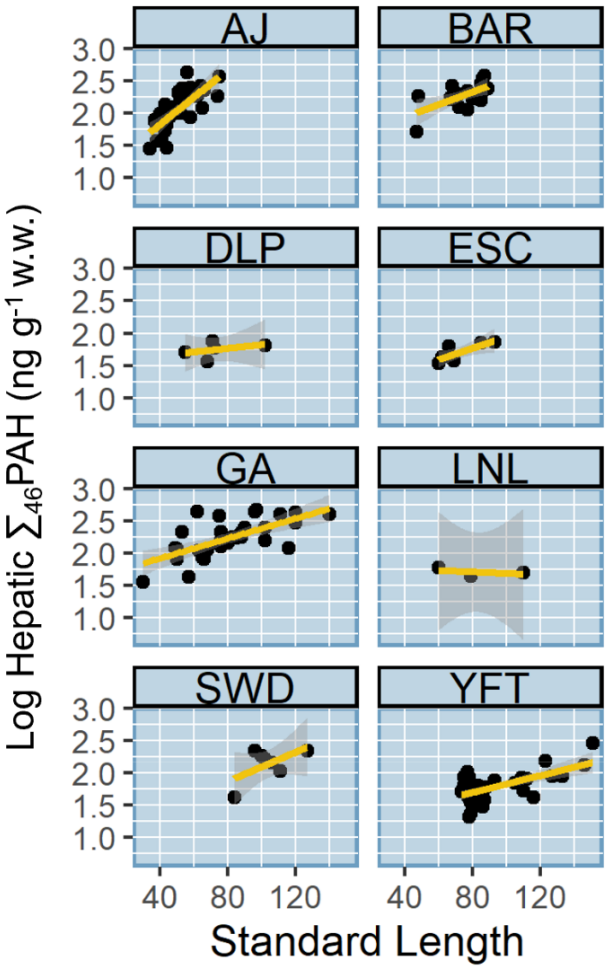
Biometric Correlations

Correlation analysis was utilized to first test the null hypothesis that there was no correlation between hepatic lipid content and hepatic $\sum_{46}$PAHs concentrations (r = -0.145, p = 0.10), therefore PAH concentrations were not lipid normalized. The p-value was greater than the alpha level, indicating that we cannot reject the null hypothesis of no correlation between percent lipid and $\sum_{46}$PAHs concentration. Additionally, no significant differences in hepatic lipid content were found when separated by species (r^2 = 0.0074, p = 0.292), regions (r^2 = 0.00036, p = 0.828), or years (r^2 = 0.00036, p = 0.825). When separated by species, no significant differences between lipid content were identified between regions, years, or sexes. No significant correlations were identified between length and lipid content for any species.

Correlation analysis was utilized to test the null hypothesis that there is no relationship between fish standard length and log transformed hepatic $\sum_{46}$PAHs (Figure 4). For five out of eight pelagic species, there was a significant positive correlation between standard length and the log transformed hepatic $\sum_{46}$PAH, thus rejecting the null hypothesis of no correlation between length and log transformed hepatic $\sum_{46}$PAH concentrations. The species with significant relationships between length and log transformed hepatic $\sum_{46}$PAH are Almaco Jack (r = 0.739, p < 0.001), Great Barracuda (r = 0.614, p = 0.009), Escolar (r = 0.800, p = 0.031), Greater Amberjack (r = 0.662, p < 0.001), and Yellowfin Tuna (r = 0.419, p = 0.005). Significant correlations between length and log transformed biliary naphthalene (r = 0.546, p = 0.0351), length and log transformed biliary benzo[*a*]pyrene (r = 0.552, p = 0.0330), as well as length and

log transformed biliary ∑3FAC concentrations were observed in Yellowfin Tuna (r = 0.55, *p* = 0.034) (Figures 5a-c).

Figure 4. *Correlation analysis of standard length versus log transformed total PAHs for all species. AJ: Almaco Jack, BAR: Barracuda, DLP: Dolphinfish, ESC: Escolar, GA: Greater*



Amberjack, LNL: Longnose Lancetfish, SWD: Swordfish, YFT: Yellowfin Tuna. The yellow line represents the linear regression and gray shading represents the confidence interval. Significant correlations were found in AJ (r = 0.739, p < 0.001), BAR (r = 0.614, p = 0.009), ESC (r = 0.800, p = 0.031), GA (r = 0.662, p < 0.001), and YFT (r = 0.419, p = 0.005).

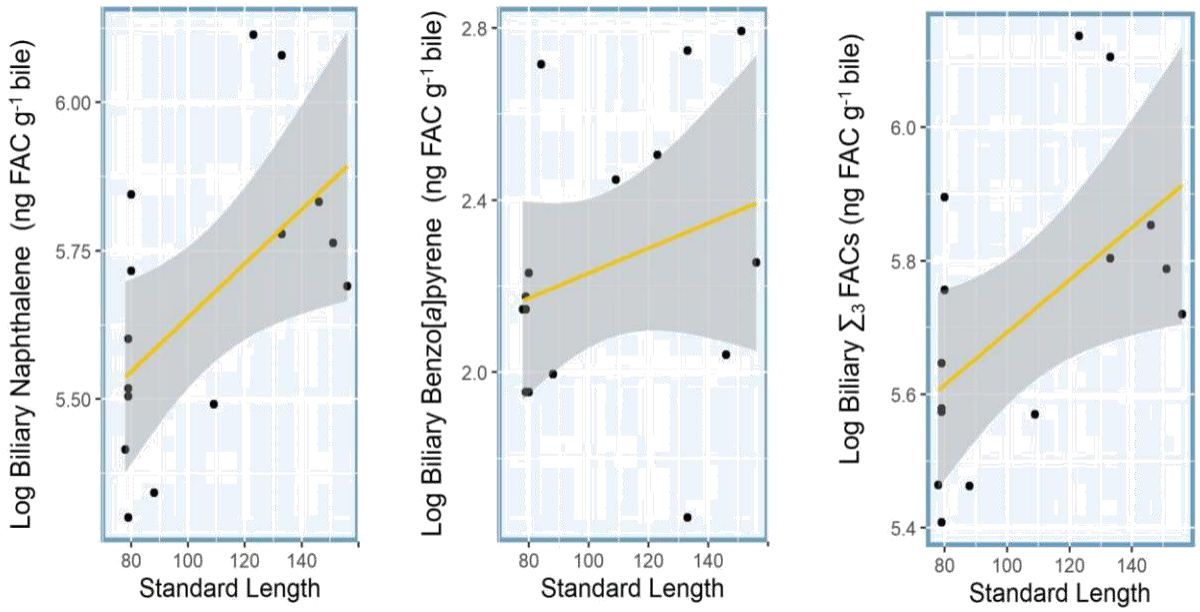


Figure 5a-c. *Correlations between log transformed biliary naphthalene and standard length (SL) (left) (r = 0.55, p = 0.04), log transformed biliary benzo[*a*]pyrene and length (middle) (r = 0.55, p = 0.03), and log transformed biliary ∑3FAC concentrations (right) (r = 0.55, p = 0.03) for Yellowfin Tuna. The yellow line represents the linear regression and gray shading represents the confidence interval.*

Fulton's condition factor (K) is often used as an index of fish health, as it incorporates both body length and weight in the calculation. A relatively higher condition factor is attributed to a "fatter" fish and a relatively lower condition factor is attributed to a "skinnier" fish for a given length [82]. The null hypothesis tested here was that there is no significant relationship between Fulton's condition factor and log transformed hepatic $\sum_{46}$PAHs. A correlation test using Pearson's adjusted p-values indicated only one species had a significant relationship between Fulton's condition factor and log transformed hepatic $\sum_{46}$PAHs: Greater Amberjack exhibited a significant, negative correlation (r = -0.462, p = 0.008) between the two factors, indicating a relationship between a higher condition factor and lower log transformed hepatic $\sum_{46}$PAH (Figure 6).

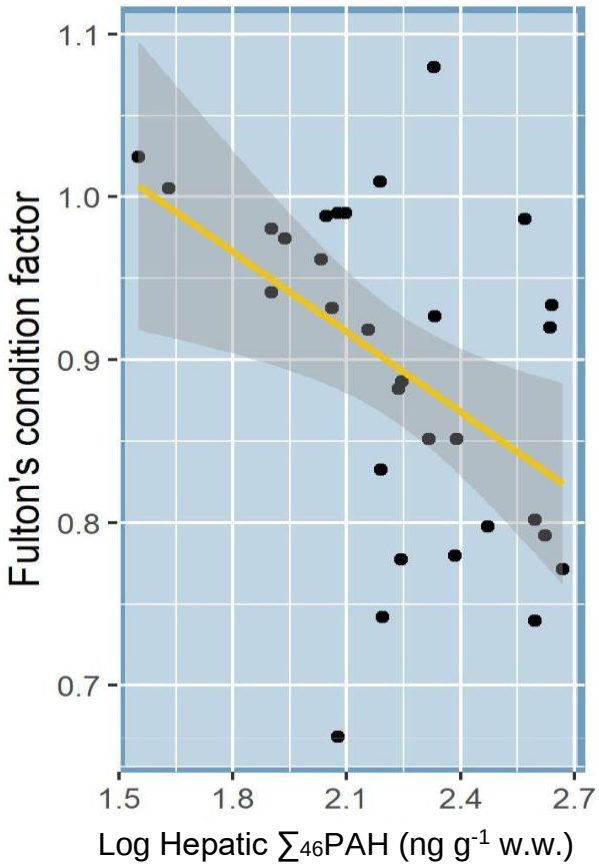


Figure 6. *Correlation between log transformed total hepatic $\sum_{46}$PAHs and Fulton's condition factor for Greater Amberjack (r = -0.46, p = 0.01). The yellow line represents the linear regression and gray shading represents the confidence interval.*

As the liver plays a large part in contaminant metabolism, the hepatosomatic index (HSI) is commonly used as an indicator of contaminant impacts on species [83, 84]. The HSI was calculated as liver weight divided by the total body weight. After confirming that HSI was not significantly different between sexes for any species, sexes were combined and relationships between HSI and both biliary PAHs and hepatic PAHs were assessed for each species. Only one species had a significant relationship between log transformed hepatic $\sum_{46}$PAH and HSI: a negative correlation in Greater Amberjack (r = -0.51, p = 0.010) (Figure 7). Only Almaco Jack exhibited a significant negative correlation between HSI and log transformed biliary

benzo[*a*]pyrene (r = 0.417, p = 0.031) (Figure 8).

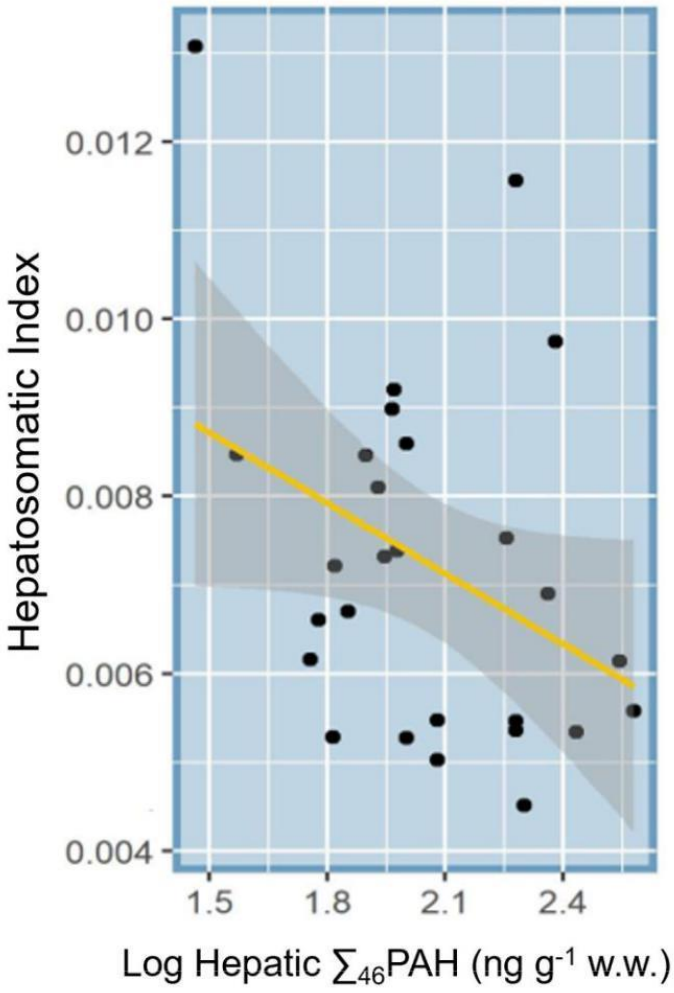


Figure 7. *Correlation between log transformed total hepatic* $\sum_{46}$*PAH and hepatosomatic index for Greater Amberjack (r = -0.51, p = 0.01). Yellow line shows linear regression and gray shading shows the confidence interval.*

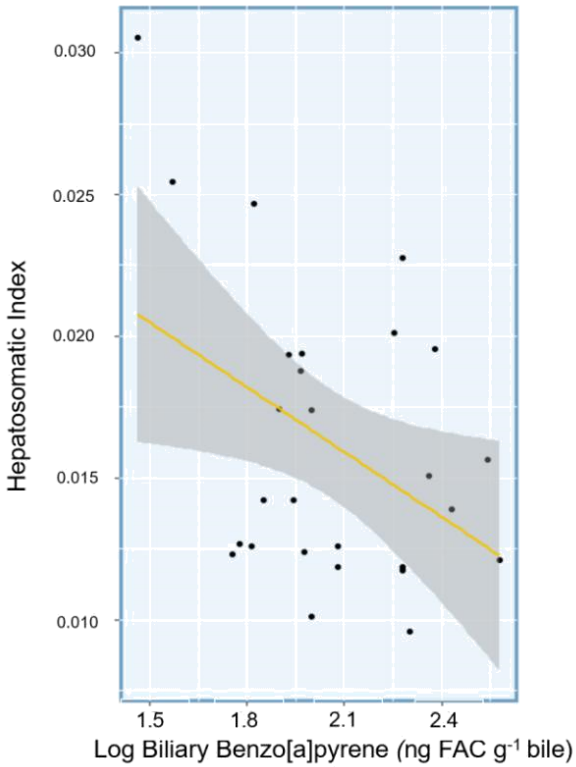


Figure 8. *Correlation between log transformed biliary benzo[*a*]pyrene FAC concentrations and hepatosomatic index for Almaco Jack (r = -0.417, p = 0.031). Yellow line shows linear regression and gray shading shows the confidence interval.*

Total biliary $\sum_3$FAC concentrations were significantly different between sexes for two species, Swordfish and Greater Amberjack (Figure 9). In Swordfish, females had significantly higher $\sum_3$FAC concentrations than males ($r^2 = 0.36$, $p = 0.008$) and, alternatively, males had significantly higher $\sum_3$FAC concentrations than females in Greater Amberjack ($r^2 = 0.32$, $p = 0.034$). When separated by parental PAH compound, males exhibited higher concentrations of biliary naphthalene than females in Greater Amberjack ($r^2 = 0.33$, $p = 0.042$) (Figure 10) and males exhibited higher concentrations of biliary benzo[*a*] pyrene than females in Almaco Jack ($r^2 = 0.68$, $p = 0.049$) (Figure 11). Conversely, in Swordfish, females exhibited significantly higher naphthalene ($r^2 = 0.67$, $p = 0.046$) and phenanthrene ($r^2 = 0.82$, $p = 0.042$) concentrations

than males (Figure 12).

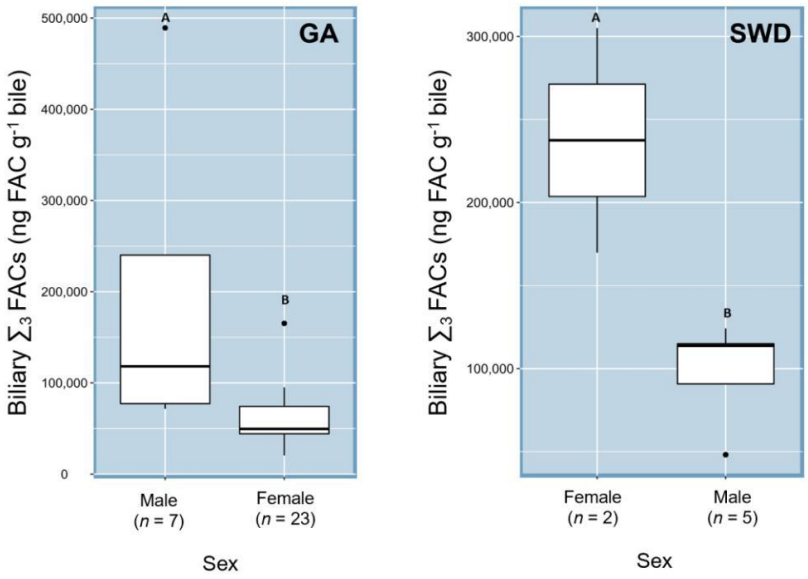


Figure 9. *Measured biliary ∑3FAC concentrations by sex in Greater Amberjack (r^2 = 0.32, p = 0.034) and Swordfish (r^2 = 0.36, p = 0.008) for all regions combined. Significantly different groups are indicated by letters and outliers are represented by circles.*

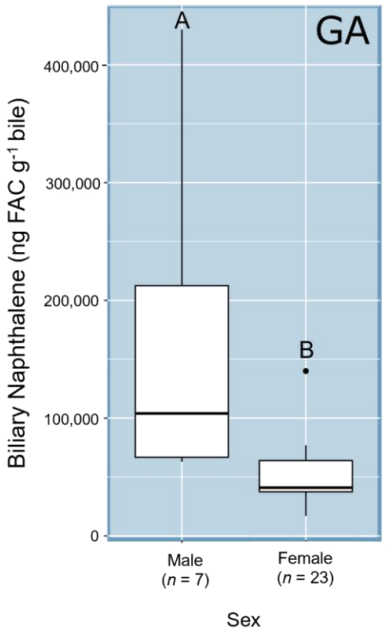


Figure 10. *Measured concentrations of biliary naphthalene equivalents by sex in Greater Amberjack (r^2 = 0.33, p = 0.042). Significantly different groups are indicated by letters and outliers are represented by circles.*

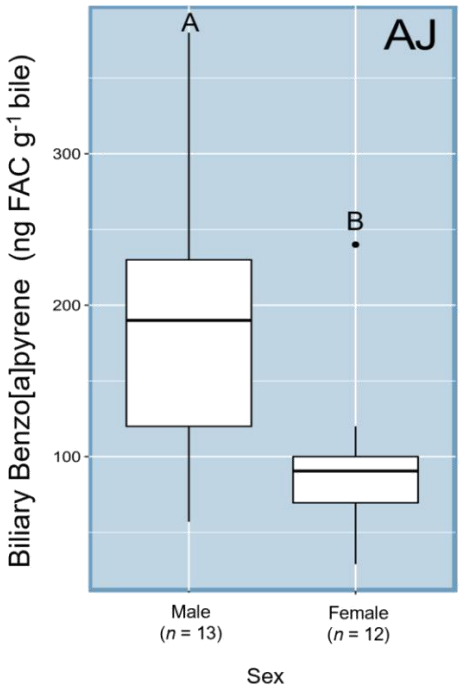


Figure 11. *Measured concentrations of biliary benzo[a]pyrene by sex in Almaco Jack (r^2 = 0.68, p = 0.049). Significantly different groups are indicated by letters and outliers are represented by circles.*

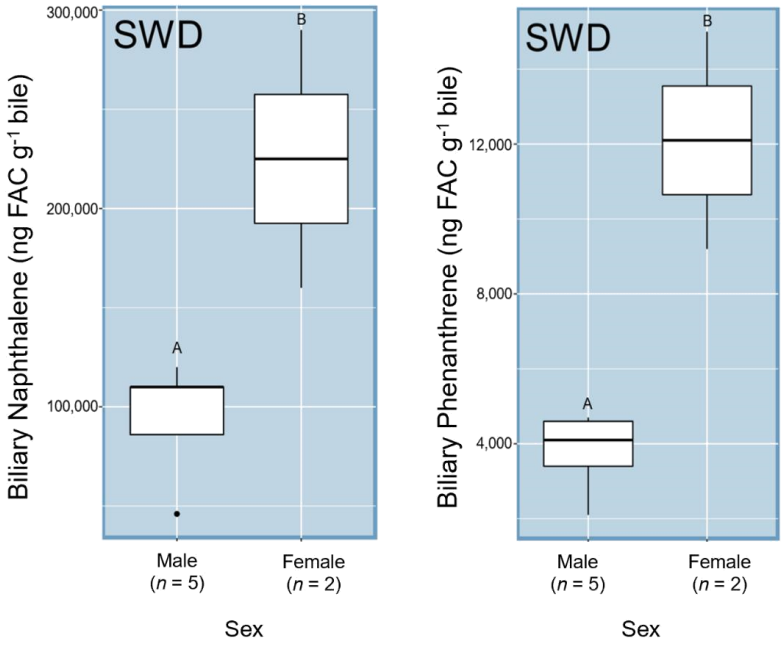


Figure 12a and b. *Concentrations of (a) biliary naphthalene and (b) phenanthrene in Swordfish, grouped by sex. In both, males had significantly lower naphthalene (r^2 = 0.67, p = 0.046) and phenanthrene (r^2 =0.82, p = 0.042) concentrations. Significantly different groups are indicated by letters and outliers are represented by circles.*

Hepatobiliary PAH Concentrations

Mean and standard deviation for biliary PAH equivalents and total hepatic PAHs are presented in Table 6, separated by species and region.

Table 6. *Mean and standard deviation (SD) of concentrations of hepatic polycyclic aromatic hydrocarbons (PAHs) and biliary fluorescent aromatic compounds (FACs) for all species, separated by regions. Biliary and hepatic sample sizes (n) are also presented. Regional means calculated from the combination of all species within that region are presented in bold.*

Region	*Species*	*Biliary Samples (n)*	*Biliary ∑3FACs (ng FAC g-1 bile)*		*Biliary NAP (ng FAC g-1 bile)*		*Biliary PHN (ng FAC g-1 bile)*		*Biliary BaP (ng FAC g-1 bile)*		*Hepatic Samples (n)*	*Hepatic ∑46PAH (ng g-1 w.w.)*	
			Mean	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**	**Mean**	**(SD)**		**Mean**	**(SD)**
Cuba	Almaco Jack	4	35,000	20,000	29,000	19,000	5,800	3,200	71	28	6	92.1	84.4
	Barracuda	15	56,000	30,000	49,000	24,000	7,300	4,600	140	96	17	205	81.8
	Region Mean (species combined)	**19**	**51,000**	**29,000**	**45,000**	**24,000**	**6,900**	**4,300**	**120**	**89**	**23**	**175**	**95.2**
North Central	Dolphinfish	3	44,000	5,700	29,000	16,000	6,600	4,700	840	610	5	57.5	14.9
	Escolar	6	150,000	130,000	140,000	120,000	6,500	6,400	110	51	7	51.7	16.3
	Longnose Lancetfish	9	110,000	63,000	100,000	55,000	11,000	24,000	55	29	3	50.8	7.67
	Swordfish	7	140,000	82,000	130,000	78,000	6,200	4,500	240	200	8	140	70.6
	Yellowfin Tuna	6	860,000	370,000	810,000	350,000	47,000	19,000	310	240	7	108	101
	Region Mean (species combined)	**31**	**270,000**	**340,000**	**245,000**	**320,000**	**16,000**	**22,000**	**230**	**300**	**30**	**89.2**	**69.7**
Northwest	Greater Amberjack	13	58,000	34,000	50,000	31,000	8,200	3,300	120	37	21	176	134
	Yellowfin Tuna	9	420,000	170,000	360,000	160,000	52,000	19,000	180	130	38	56.1	19.7
	Region Mean (species combined)	**22**	**210,000**	**210,000**	**180,000**	**190,000**	**27,000**	**2,500**	**150**	**94**	**59**	**98.7**	**98.7**
Southwest	Greater Amberjack	3	68,000	18,000	59,000	16,000	9,400	2,400	80	13	4	87.3	38.8
	Region Mean (species combined)	**3**	**68,000**	**18,000**	**59,000**	**16,000**	**9,400**	**2,400**	**80**	**13**	**4**	**87.3**	**38.8**
Yucatán Shelf	Almaco Jack	23	52,000	21,000	44,000	18,000	7,800	3,700	150	95	27	133	111
	Greater Amberjack	6	160,000	170,000	130,000	150,000	22,000	19,000	150	97	9	273	134
	Region Mean (species combined)	**29**	**73,000**	**85,000**	**63,000**	**75,000**	**11,000**	**11,000**	**150**	**96**	**36**	**168**	**130**

∑3FACs = summation of biliary naphthalene (NAP), phenanthrene (PHN) and benzo[a]pyrene (BaP); ∑46PAH: summation of 46 PAHs; w.w. = wet weight

Hepatobiliary PAH concentrations

Polycyclic aromatic hydrocarbons were found in all of the liver tissues analyzed, with mean hepatic Σ_{46}PAH concentrations ranging from 21.2 to 468 ng g^{-1} (w.w.) and individual PAH compound concentrations ranging from below the method detection limit (< MDL) to 214 ng g^{-1} (w.w.). Hepatic Σ_{46}PAH concentrations differed among species (for all sexes and regions combined) with Yellowfin Tuna exhibiting the third lowest mean concentration (Figure 12). The Yellowfin Tuna mean hepatic Σ_{46}PAH concentrations were significantly lower than Almaco Jack ($r^2 = 0.24$, $p = 0.028$), Great Barracuda ($r^2 = 0.52$, $p = 0.52$), and Greater Amberjack ($r^2 = 0.55$, $p = 0.028$) (Figure 13). Additionally, concentrations in Great Barracuda were significantly higher than Escolar ($r^2 = 0.028$, $p = 0.39$) (Figure 13).

Biliary PAH concentrations for 104 samples were quantified for the equivalents of three PAHs: naphthalene, phenanthrene, and benzo[*a*]pyrene. For all species, sexes, and regions combined, the $\sum_3$FAC concentrations range from 4,400 to 1,400,000 ng FAC g^{-1} bile. Concentrations for individual PAH equivalents ranged from 0.93 to 1,300,000 ng FAC g^{-1} bile. With all sexes, regions, and years combined, $\sum_3$FAC concentration distributions by species are as follows: Almaco Jack (50,000 ± 22,000 ng FAC g^{-1} bile), Barracuda (56,000 ± 30,000 ng FAC g^{-1} bile), Dolphinfish (44,000 ± 5,700 ng FAC g^{-1} bile), Escolar (148,000 ± 130,000 ng FAC g^{-1} bile), Greater Amberjack (86,000 ± 97,000 ng FAC g^{-1} bile), Longnose Lancetfish (112,000 ± 63,000 ng FAC g^{-1} bile), Swordfish (138,000 ± 82,000 ng FAC g^{-1} bile), and Yellowfin Tuna (593,000 ± 337,000 ng FAC g^{-1} bile).

Significant differences in biliary $\sum_3$FAC concentrations occurred by species ($r^2 = 0.35$, $p = 0.001$) (Figure 14). Yellowfin Tuna $\sum_3$FAC concentrations were significantly greater than

those of Almaco Jack (r^2 = 0.64, p = 0.028), Great Barracuda (r^2 = 0.58, p = 0.001), Greater Amberjack (r^2 = 0.56, p = 0.028), and Longnose Lancetfish (r^2 = 0.45, p = 0.028). Additionally, Almaco Jack $\sum_3$FAC concentrations were significantly lower than those of Swordfish (r^2 = 0.45, p = 0.028) and Longnose Lancetfish (r^2 = 0.37, p = 0.028) (Figure 14). Log transformed hepatic $\sum_{46}$PAHs were plotted against log transformed $\sum_3$FAC concentrations and no significant correlations were identified across pelagic species (Figure 15).

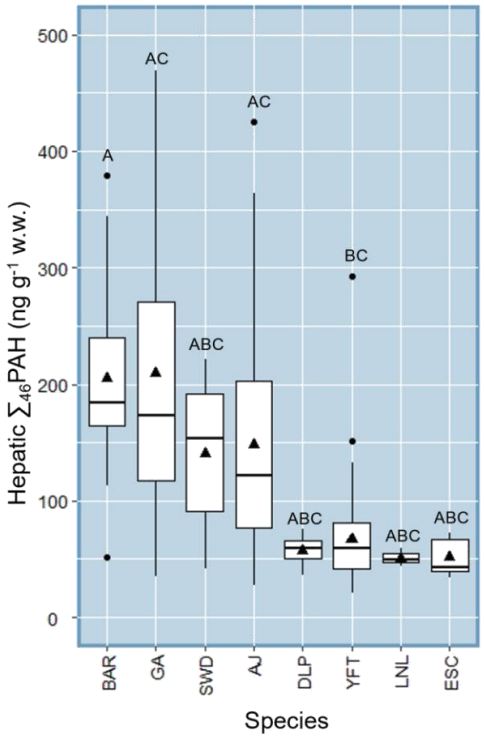


Figure 13. *Hepatic $\sum_{46}$PAHs concentrations determined for eight pelagic fish species from the Gulf of Mexico. Significantly different groups are distinguished by letter groupings. AJ: Almaco Jack, BAR: Barracuda, DLP: Dolphinfish, ESC: Escolar, GA: Greater Amberjack, LNL: Longnose Lancetfish, SWD: Swordfish, YFT: Yellowfin Tuna. Outliers are represented by circles and group means are represented by triangles. Species on the x-axis are arranged in decreasing hepatic $\sum_{46}$PAH concentration order.*

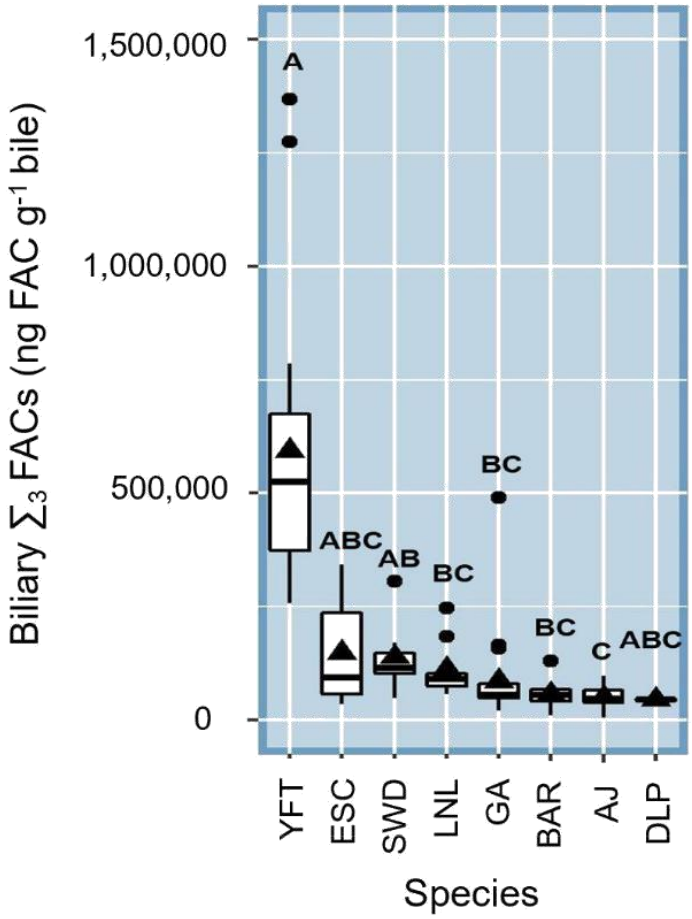


Figure 14. *Biliary∑3 FAC concentrations across eight pelagic species. Triangles are indicative of means and circles are indicative of outliers. Significant group differences are indicated by letter groupings. AJ: Almaco Jack, BAR: Barracuda, DLP: Dolphinfish, ESC: Escolar, GA: Greater Amberjack, LNL: Longnose Lancetfish, SWD: Swordfish, YFT: Yellowfin Tuna.*

Significant species differences were also found in both the biliary naphthalene concentrations ($r^2 = 0.34$, $p = 0.001$) (Figure 16a) and for phenanthrene ($r^2 = 0.31$, $p = 0.001$) (Figure 16b), however no significant differences were found in biliary benzo[*a*]pyrene ($r^2 = 0.0047$, $p = 0.49$) concentrations between species (Figure 16c). Pairwise np-MANOVAs yielded significant differences in biliary naphthalene concentrations between Yellowfin Tuna and Greater Amberjack ($r^2 = 0.54$, $p = 0.028$), Great Barracuda ($r^2 = 0.54$, $p = 0.028$), and Almaco Jack ($r^2 = 0.61$, $p = 0.028$). Biliary naphthalene concentrations were also significantly different between Swordfish and Almaco Jack ($r^2 = 0.50$, $p = 0.028$). Longnose Lancetfish concentrations were

significantly different from Barracuda (r^2 = 0.31, p = 0.028) and Almaco Jack (r^2 = 0.41, p = 0.028). Significant pairwise differences were found in biliary phenanthrene concentrations between Yellowfin Tuna and Almaco Jack (r^2 = 0.77, *p* = 0.028), Great Barracuda (r^2 = 0.70, *p* = 0.028), Escolar (r^2 = 0.61, *p* = 0.028), Greater Amberjack (r^2 = 0.63, *p* = 0.028), Longnose Lancetfish (r^2 = 0.47, *p* = 0.028), and Swordfish (r^2 = 0.64, *p* = 0.028). No significant differences were found between biliary equivalent concentrations measured in Dolphinfish and the other species likely due to small sample sizes.

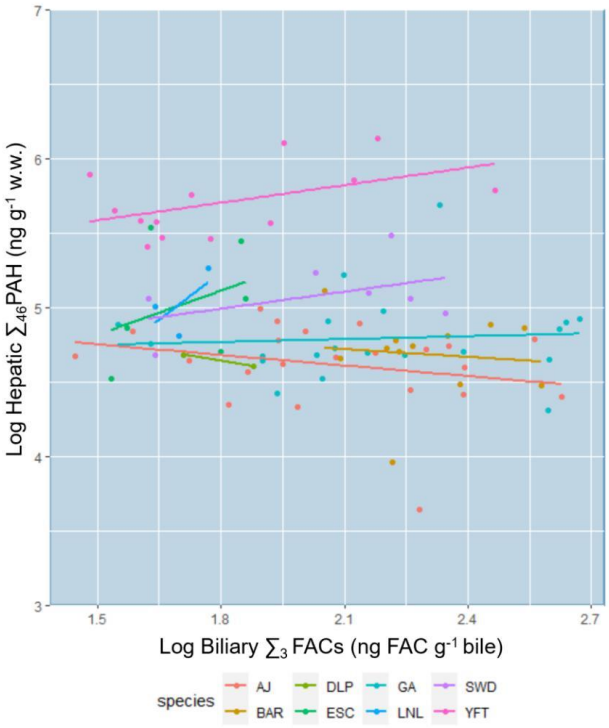


Figure 15. *Correlations between log transformed biliary ∑3FAC concentrations and log transformed hepatic ∑46PAHs for eight species of pelagic species. No significant correlations were identified.*

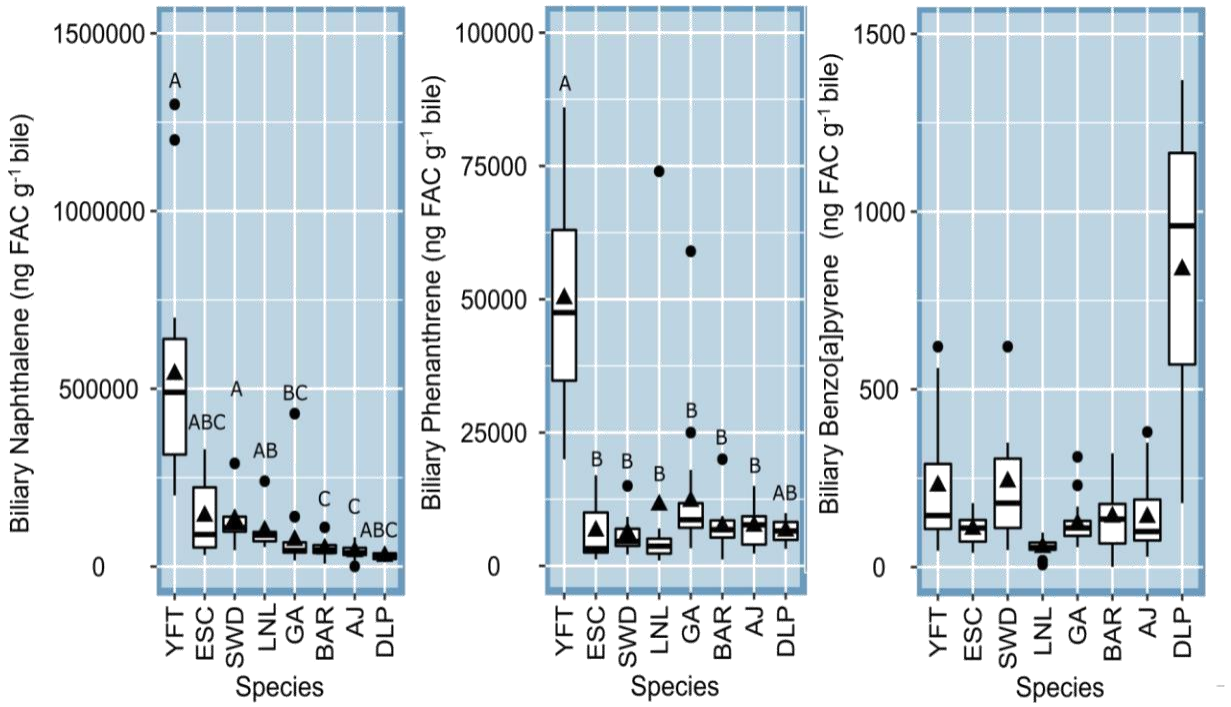


Figure 16a-c. *Biliary naphthalene (a), phenanthrene (b), and benzo[*a*]pyrene (c) equivalent concentrations (ng FAC* g^{-1} *bile) across species. Triangles are indicative of means and circles are indicative of outliers. Significant group differences are indicated by letter groupings. AJ: Almaco Jack, BAR: Barracuda, DLP: Dolphinfish, ESC: Escolar, GA: Greater Amberjack, LNL: Longnose Lancetfish, SWD: Swordfish, YFT: Yellowfin Tuna.*

Regional and Temporal Differences

Yellowfin Tuna were collected in 2018 and 2020 in the north central and north west regions, respectively. For biliary $\sum_3$FAC concentrations, biliary naphthalene and hepatic $\sum_{46}$PAHs, 2018 concentrations from the north central region were significantly higher than 2020 from the north west region, however while differences between these two groups were significant ($\sum_3$FACs: r^2= 0.44, p = 0.008, naphthalene: r^2 = 0.47, p = 0.006 and hepatic $\sum_{46}$PAHs, r^2= 0.17, p = 0.008) (Figures 17 and 18), these two factors are confounded and therefore non-separable. Greater Amberjack (GA) were collected in three regions in 2016: the

Yucatán Peninsula (YP), the south west region (SW), and the north west region (NW). As Greater Amberjack sexes had significant different biliary $\sum_3$FAC concentrations, all males were removed prior to regional assessment. Female Greater Amberjack (GA) caught in the NW region had biliary $\sum_3$FAC concentrations ($r^2 = 0.37$, $p = 0.015$) significantly lower than female Greater Amberjack from the Yucatán Peninsula region (Figure 19). No other regional or temporal differences were significant between the species analyzed in this study.

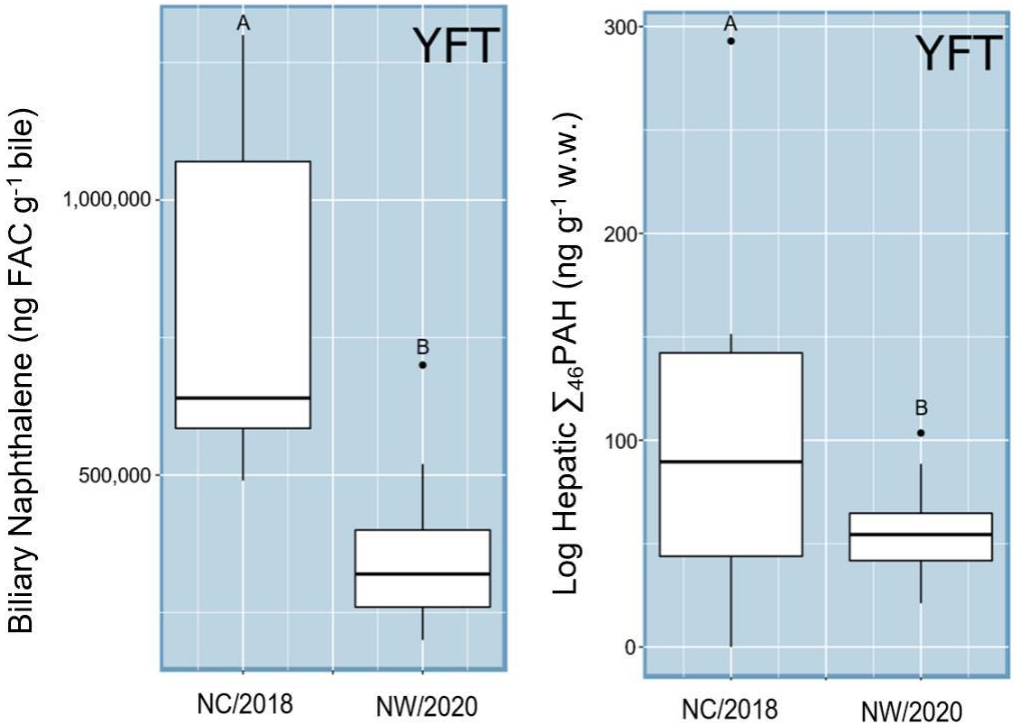


Figure 17. *Distribution of biliary naphthalene and hepatic Σ_{46}PAHs in Yellowfin Tuna caught in 2018 (NC) and 2020 (NW). In both, 2018 concentrations were significantly different from 2020 (NAP: $r^2 = 0.47$, $p = 0.006$) (Σ_{46}PAHs: $r^2 = 0.17$, $p = 0.008$) Significantly different groups are indicated by letter groupings and outliers are represented by circles.*

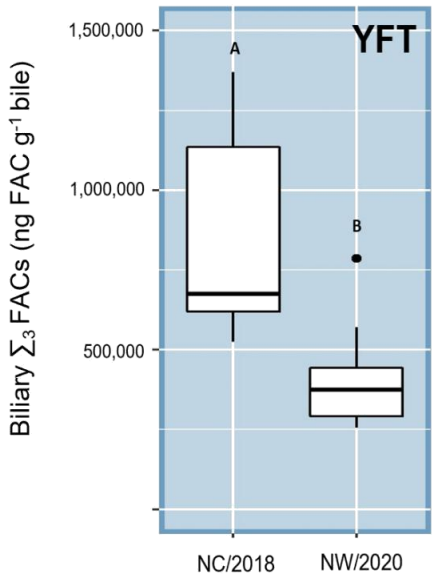


Figure 18. *Distribution of biliary Σ3FAC concentrations in Yellowfin Tuna in 2018 and 2020 (r^2 = 0.43, p = 0.008). Significantly different groups are indicated by letter groupings and outliers are represented by circles.*

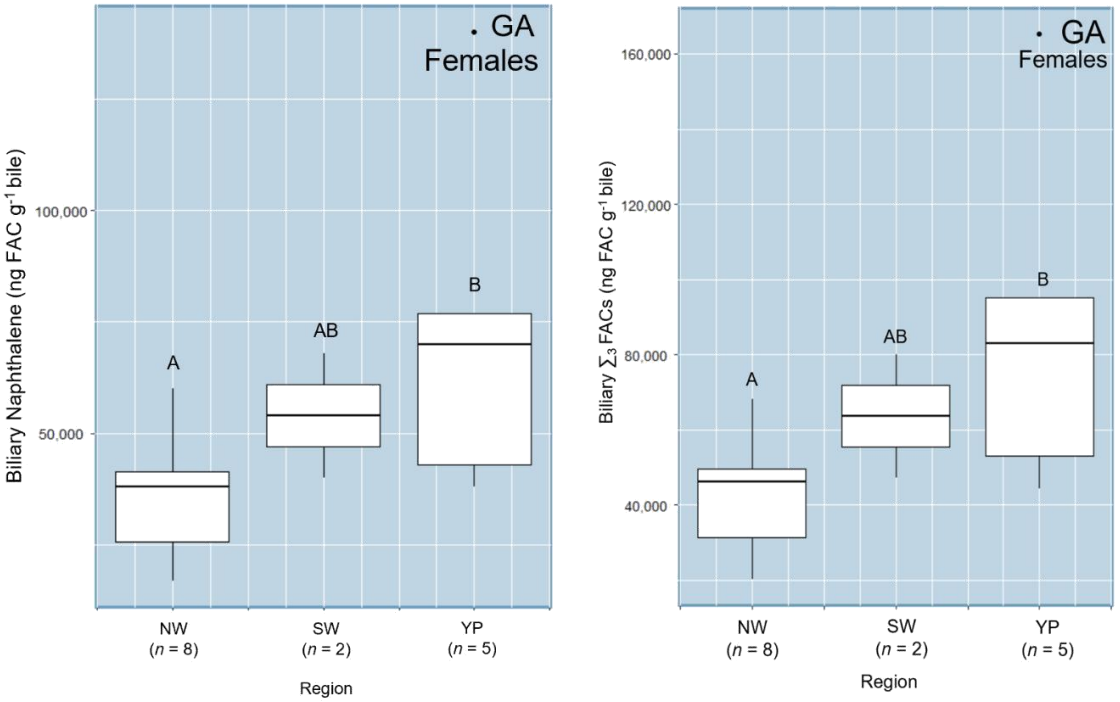


Figure 19. *Distribution of biliary naphthalene and biliary ∑3FAC concentrations where female Greater Amberjack (GA) were caught in 2016. Concentrations in fish from the Yucatán Peninsula (YP) region were significantly higher than concentrations in fish from the north west (NW) region in biliary ∑3FAC concentrations (r^2 = 0.37, p = 0.015). Significantly different groups are indicated by letter groupings and outliers are represented by circle.*

Canonical analysis of principal components (CAP) was utilized to assess group separation of relative abundance of PAH compounds and to determine what compounds are driving separations. The Greater Amberjack collected in the Yucatán Peninsula and north west regions were significantly different based on the relative abundances of PAHs ($p = 0.001$) (Figure 20). The primary compounds driving separation were determined as C1 and C2 phenanthrene/anthracene as well as C1 and C2 fluorene. A bar plot of average relative concentrations of PAH compounds across three regions where Greater Amberjack were collected indicated that the dominant PAHs are homologs of naphthalene, dibenzothiophene, and phenanthrene/anthracene (Figure 21). The fish collected in the Yucatán Peninsula region presented nominally higher C1 Phenanthrene/Anthracene concentrations relative to the south west and north west regions.

Although CAP was utilized to assess group separation in Yellowfin Tuna, the groups were not found to be significantly different between regions/years. However, variation in predominant PAHs is apparent in the relative abundance plots of Yellowfin Tuna collected in 2018 in the north central region and in 2020 in the north west region (Figure 22). The average relative abundance plots show that Yellowfin Tuna collected in 2018 in the north central region present a higher relative abundance of dibenzothiophene homologs than Yellowfin Tuna collected in 2020. Additionally, the Yellowfin Tuna collected in 2020 in the north west region present higher relative abundances of parental naphthalene than those collected in the north central region in 2018.

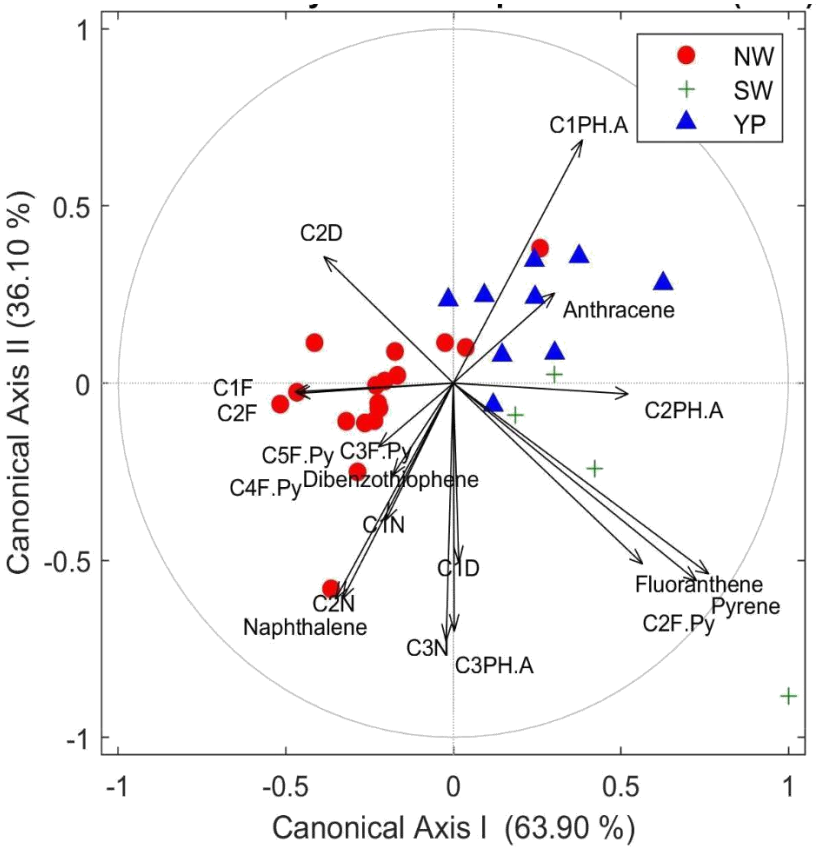


Figure 20. *Multivariate analysis (Canonical Analysis of Principal Coordinates – CAP) of relative abundances of PAHs in Greater Amberjack collected in 2016. NW: north west, SW: south west, YP: Yucatán Peninsula. Significant differences were found between the NW and YP regions (p = 0.001).*

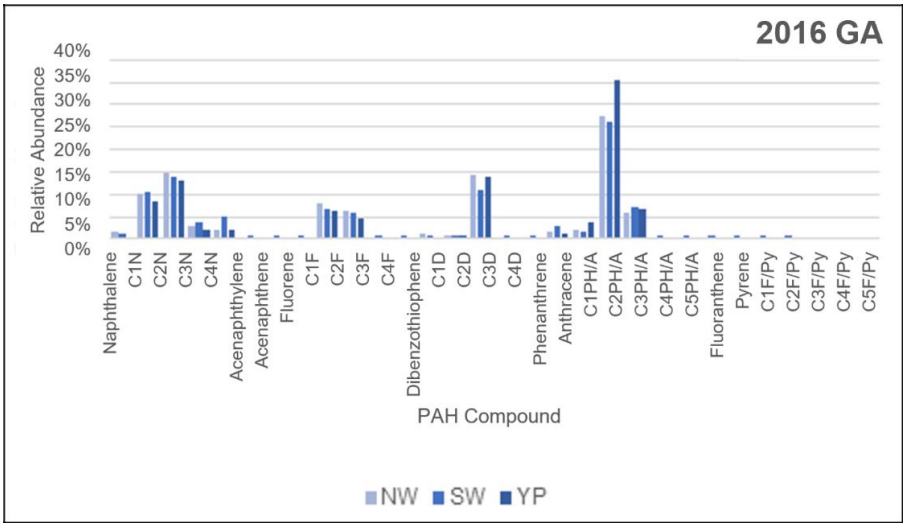


Figure 21. *Relative concentrations of PAHs in Greater Amberjack collected across three regions (NW, SW, YP).*

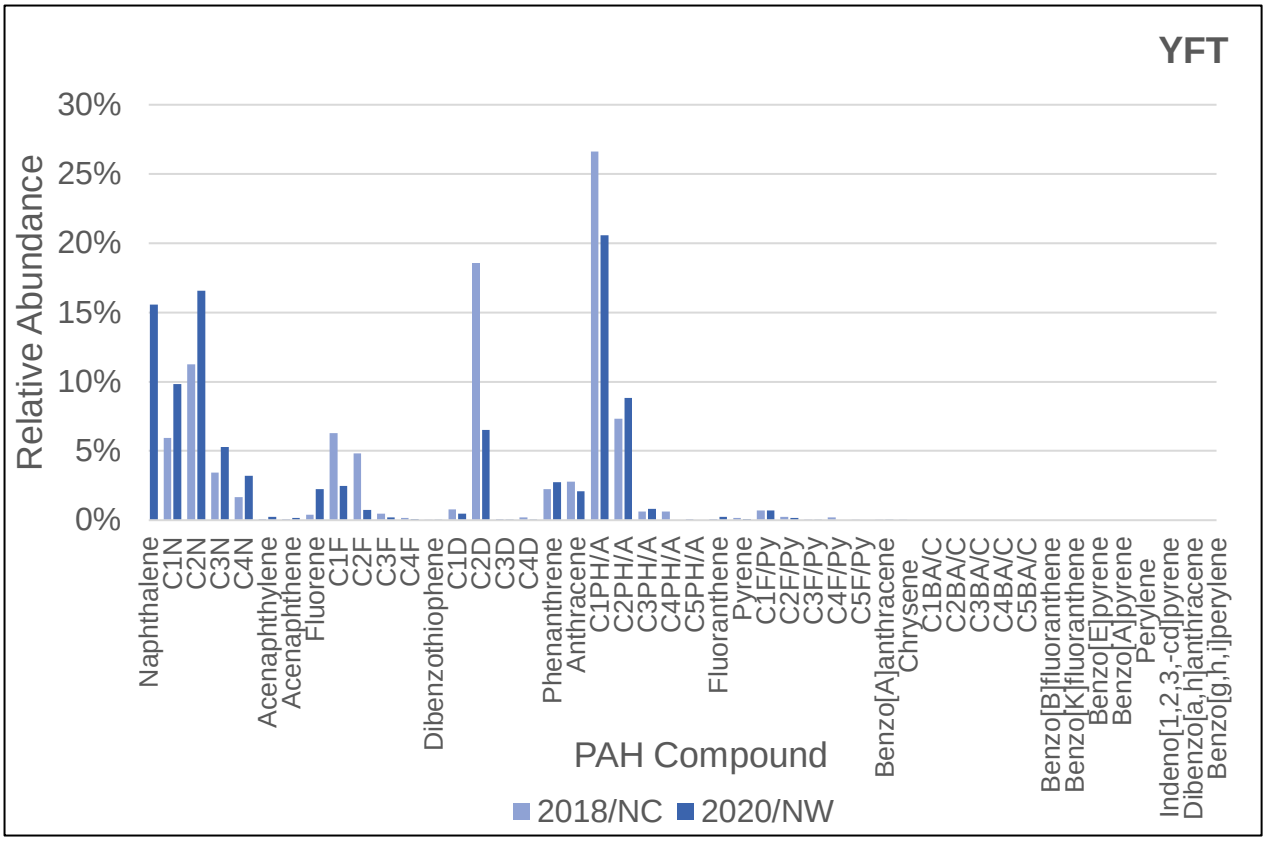


Figure 22. *Relative concentrations of PAHs in Yellowfin Tuna collected in 2018 in the north central region and in 2020 in the north west region.*

Discussion

Hepatobiliary PAHs - Interspecies Variability

Biliary PAH metabolite concentrations are often used to assess recent exposure of fish to PAH sources [74, 85, 86]. Biliary PAH concentrations within this dataset highlighted recent exposure differences between highly migratory species (Yellowfin Tuna and Swordfish) and the other species within this study. With all sexes and regions combined, biliary naphthalene concentrations were significantly higher in Yellowfin Tuna than Almaco Jack, Great Barracuda, and Greater Amberjack. Additionally, biliary naphthalene concentrations in Swordfish were significantly higher than those of Almaco Jack. Biliary phenanthrene concentrations were significantly higher in Yellowfin Tuna when compared to Almaco Jack, Great Barracuda, Escolar, and Longnose Lancetfish. Biliary $\sum_3$FAC concentrations were significantly higher in Yellowfin Tuna than Almaco Jack, Great Barracuda, Greater Amberjack, and Longnose Lancetfish. Additionally, Almaco Jack biliary $\sum_3$FAC concentrations were significantly lower than Swordfish and Longnose Lancetfish.

Potential factors contributing to interspecies variation include variation in diet, physiological differences, and migratory behavior. Swordfish and Yellowfin Tuna found in the Gulf of Mexico are highly migratory species ranging throughout the North Atlantic Ocean [87, 88]. The physiology of the highly migratory species may also play a role in their relatively high biliary FAC concentrations. Swordfish and Yellowfin Tuna can reach burst speeds of up to 0.39 m s^{-1} and 20.7 m s^{-1}, respectively [89, 90]. As these species travel long distances at high speeds, they likely encounter larger volumes of water compared to species with more limited migrations.

To meet increased metabolic demands, both Yellowfin Tuna and Swordfish, among other scombrids and billfishes including dolphinfish, have disproportionately higher gill surface area to bodyweight ratios than other marine teleosts allowing for increased gill ventilation [60, 91]. Unfortunately, gill surface area calculations were not listed for Almaco Jack, Escolar, Greater Amberjack, Great Barracuda or not Longnose Lancetfish. Additionally, these migratory species may travel closer to sources of PAH pollution as their ranges include coastal habitats and the upper pelagic zones [87, 88]. Multiple species within this study are known to undergo diel vertical migrations driven by predator-prey interactions in the pelagic food web [62, 87, 92]. This vertical migration could increase their potential exposure to contaminated waters and contaminated food items [51].

All liver samples exhibited some level of PAH contamination, suggesting that all pelagic fish sampled experienced at least some long-term chronic exposure [93]. However, the highly migratory (Yellowfin Tuna and Swordfish) species did not have higher hepatic $\sum_{46}$PAH concentrations when compared to the non-highly migratory species. This result confirms that highly migratory species are not accumulating higher levels of PAHs, when compared to non-migratory species even though they may be exposed to higher levels in the short term, as indicated by biliary PAH concentrations. Fish are capable of rapid PAH biotransformation, resulting in low levels of PAHs in tissues including liver tissues when compared to concentrations in bile [74]. Consequently, liver tissues are not often indicative of recent exposure but of long-term accumulation. These contrasting results suggest that although the highly migratory species could be exposed to higher concentrations of PAHs, they are not accumulating PAHs more than non-highly migratory species within this study.

Regional Comparisons

Hepatic $\sum_{46}$PAH concentrations found across all regions and pelagic species (125 ± 111 ng g^{-1} w.w) in this study, were within the same range as demersal and reef species assessed in the GoM between 2011 and 2017. Species previously studied for hepatic $\sum_{46}$PAH concentrations in throughout the GoM include Golden Tilefish, *Lopholatilus chamaeleonticeps* (888 ± 677 ng g^{-1} w.w.), Red Snapper, *Lutjanus campechanus* (1,580 ± 1,340 ng g^{-1} w.w.), and groupers, *Serranidae* (1,604 ± 2,760 ng g^{-1} w.w.) [4, 6, 94]. Specifically in the northern GoM, Gulf Hake, *Urophycis cirrata* (89 ± 56 ng g^{-1} w.w.), and Southern Hake, *Urophycis floridana* (87 ± 38 ng g^{-1} w.w.), and Red Snapper collected in the north west, *Lutjanus campechanus* (612 ± 36.9 ng g^{-1} w.w.) were assessed between 2012 and 2017 [5, 95]. Mean concentrations of hepatic PAHs in the pelagic species in this study collected Gulf were similar to those measured in Hake species but were up to 13 times lower than those measured in Golden Tilefish, Red Snapper and Grouper species. Contributing factors to variation in long-term accumulation between demersal and pelagic fishes could be variation in contact with PAH reservoirs such as sediments, whether primary sources of exposure are of chronic or acute nature, as well as species-specific metabolism of PAHs [48, 96].

Previously, researchers found Golden Tilefish from the northern GoM to have the highest biliary PAH concentrations measured, likely related to their sediment burrowing lifestyle [48]. The $\sum_{3}$FAC concentrations associated with Golden Tilefish collected in the north central region averaged 263 µg FAC g^{-1} with a standard deviation of 125 µg FAC g^{-1} [97]. Comparatively, Yellowfin Tuna collected in the North Central region had $\sum_{3}$FAC concentrations (855 µg FAC g^{-1}) three times higher. This suggests that biliary concentrations can be significantly higher in pelagic fish species than in the most contaminated demersal species, regardless of habitat type or relation to sediments [48, 49]. As Yellowfin Tuna are not closely related to sediments, these high biliary PAH concentrations were unexpected. Potential factors that may contribute to the

observed high exposure in Yellowfin Tuna may be related to aspects of their physiology (gill uptake rate, gill surface area) or due to increased interaction with pelagic sources of PAH pollution as a result of long distances traveled (e.g., produced waters discharges from oil and gas platforms, riverine input, atmospheric deposition of combustion products) [60, 98, 99]. Produced waters could be an important potential source of PAH exposure to pelagic fishes that interact with surface waters. Although oil and gas platforms treat produced waters to separate some oil from the water, PAHs are still discharged with produced waters at typical concentrations ranging from 80 to 3,000 µg/L [100].

Relationships with Biometric Data

Significant, positive correlations were observed between log transformed hepatic $\sum_{46}$PAH concentrations and standard lengths in five species: Almaco Jack, Great Barracuda, Escolar, Greater Amberjack, and Yellowfin Tuna. As length is often utilized as a proxy for size and age, positive correlations with $\sum_{46}$PAH concentrations were unexpected because of a fish's ability to metabolize PAHs and lower bioaccumulation potential. Increases in hepatic $\sum_{46}$PAH concentrations only result when a fish's exposure to contamination increases to a degree where it cannot metabolize PAHs fast enough to prevent bioaccumulation. Increased exposure could be a result of ontogenetic dietary shifts or habitat preference changes [101, 102]. Relationships between gill surface area, skin surface area or swimming speed may also be contributing to increased exposure to PAH contaminated waters.

Yellowfin Tuna exhibited significant relationships between standard length and biliary $\sum_{3}$FAC concentrations and between length and biliary benzo[*a*]pyrene, suggesting elevated contemporary exposure in larger Yellowfin Tuna. This positive relationship between length and PAH exposure could be a result of larger Yellowfin Tuna having increased gill or skin surface

areas, allowing for proportionally increased uptake. Past studies have identified an ontogenetic shift, occurring at around 40 to 50 cm fork length (estimated 10 to 12 months) at which Yellowfin Tuna increase their foraging range dramatically. This allows larger Yellowfin Tuna to prey on a broader range of organisms and is a potential route for increased exposure to PAHs [102]. As the Yellowfin Tuna collected in this study had fork lengths between 73.5 cm and 165 cm, they would likely be past this period of ontogenetic shift. Other studies focusing on GoM fish species (Golden Tilefish, Hakes) have identified significant negative relationships between biliary benzo[*a*]pyrene and fish length [5, 48]. Negative relationships between length and biliary PAHs in fish have previously been attributed to ontogenetic shifts in diet as species shift towards preying on fish capable of PAH metabolism and away from benthic invertebrates incapable of metabolism [101]. No other species evaluated herein has a similar significant relationship between length and biliary PAHs, as such warrants further investigation into the species-specific metabolic differences.

Fulton's condition factor (*K*), a function of weight to length of a fish, is often used to interpret the health (robustness) of a fish and is used to measure an individual fish's energy reserves [103]. A lower condition factor indicates a lower weight per length and potentially lower overall fitness [104]. An individual's condition factor may also vary with season, spawning status and temperature, prey availability, sex, and age [82, 105]. Researchers have previously observed exposure to pollutants (and PAHs) correlating to declining condition factors [4, 106-108].

Greater Amberjack was the only species that had a significant, negative correlation between Fulton's condition factor and hepatic $\sum_{46}$PAH concentrations. The negative correlation observed here potentially indicates that Greater Amberjack with higher hepatic $\sum_{46}$PAH

concentrations, and potentially higher chronic exposure to PAHs, experienced decreased energy status and lower overall fitness. The strength of the relationship ($r = -0.462$, $p = 0.008$) between condition factor and hepatic PAH concentrations indicates weak significance and is likely affected by other simultaneous factors including exposure to other pollutants (PCBs, heavy metals) and dissolved oxygen levels [106, 107], among others. No other species presented significant relationships between Fulton's condition factor and PAH concentrations.

The hepatosomatic index is often used to interpret the status of energy reserves in fishes. A higher value for the hepatosomatic index (HSI) indicates a larger liver/bodyweight whereas a lower value for the HSI indicates a smaller liver/bodyweight. The HSI has been used as an indicator for contamination in the past as it is sensitive to contamination but is also reflective of the nutritional and spawning status of a fish [103, 109]. Previous studies have identified declines in HSI in polluted versus non-polluted waters in African Sharptooth Catfish (*Clarias gariepinus*), Grey Mullet (*Mugil cephalus*), and Grass Goby (*Zosterisessor ophiocephalus*) [110, 111]. Greater Amberjack observed a negative correlation between HSI and hepatic $\sum_{46}$PAH concentrations, therefore, as hepatic $\sum_{46}$PAH concentrations increased, the liver to bodyweight ratio decreased. Almaco Jack presented a relationship between increasing biliary benzo[*a*]pyrene and decreasing HSI, also suggesting that the liver may be undergoing atrophy, indicating that the fish is experiencing a loss of energy storage and fitness when exposed to benzo[*a*]pyrene. An alternative interpretation of this result may suggest that fish with larger liver to body mass ratios are increasingly capable of metabolism of PAHs [112].

Three species presented significant differences in biliary FAC concentrations between sexes. No significant differences were observed between sexes for lipid concentrations, condition factors, length, or weight within these species, so it is unlikely that these factors are driving the

sex-based differences of these species. In previous studies, increased biliary PAH metabolite concentrations and cytochrome P450 activity have been noted in male fish, likely related to activity and sex hormone differences [5, 33]. In this study, males exhibited higher biliary benzo[*a*]pyrene concentrations in Almaco Jack and higher biliary $\sum_3$FAC concentrations in Greater Amberjack. Alternatively, female Swordfish exhibited higher biliary $\sum_3$FAC concentrations than their male counterparts. Potential contributors to differential biliary $\sum_3$FAC concentrations in males and females may be related to diet or energy expenditure differences between the two groups. However, these results should be interpreted with caution as only two female Swordfish and five male Swordfish were analyzed.

Regional Variation

Although there were significant differences between Yellowfin Tuna with respect to region, year is a confounding variable. Yellowfin Tuna caught in the north central region in 2018 (n = 6) had significantly higher biliary $\sum_3$FAC concentrations (855,000 ± 367,000 ng FAC g^{-1} bile) than those caught in the north west region in 2020 (n = 9; 418,000 ± 167,000 ng FAC g^{-1} bile), potentially indicating higher short-term exposure in the north central Yellowfin Tuna caught in 2018. Additionally, the Yellowfin Tuna caught in 2020 in the northwest region were significantly smaller than the 2018 specimens from the north central region. As there was a significant, positive correlation identified between biliary FAC concentrations and fish length, size is also a confounding variable in regional analyses of these data. As such, the comparison of regional/temporal groups of Yellowfin Tuna should be interpreted with caution.

Some factors leading to exposure differences between these two groups may be variation in diet or environmental PAH concentrations within the water taken up across the gills. Environmental PAH concentrations are likely higher in the north central region due to a

high density of active oil rig platforms discharging produced waters, riverine input from the Mississippi River, resuspension of *Deepwater Horizon* contaminated sediments, and actively leaking wells (i.e. Taylor Energy platform MC20) [49, 113, 114].

It is well understood that PAH profiles dominated by low molecular weight compounds (2 to 3 rings) versus higher molecular weight (4 to 6 rings) compounds are indicative of petrogenic sources as opposed to pyrogenic sources [115]. All samples within this study indicate a primarily petrogenic source based on the predominance of low molecular weight PAHs rather than high molecular weight PAHs. Furthermore, compositional differences between samples can indicate variation in the sources of contamination. Crude oil is typically predominated by two and three ring PAHs, primarily naphthalene, fluorene, and phenanthrene in both parental and alkylated forms [116]. Four to six ring PAHs do appear in crude oil in much smaller concentrations aside from chrysene, which was listed as having a mean concentration of 30.36 mg L^{-1} in 48 crude oils [116]. Comparatively, benzo[*a*]pyrene had a mean concentration of 1.5 mg L^{-1}oil and naphthalene had a concentration of 427 mg L^{-1} oil [116]. In DWH crude oil samples, parental naphthalene, phenanthrene, and fluorene as well as their alkylated homologs were predominant [15]. Discharged produced waters from both gas and oil platforms primarily have relatively high concentrations of low molecular weight PAHs including parental naphthalene and phenanthrene as well as their alkylated homologs [100, 116]. Dibenzothiophenes were found in high concentrations in produced waters from Norwegian North Sea platforms (0.0086 mg L^{-1}) [117]. High molecular weight PAHs are typically only present, if at all, in trace amounts due to their low solubility in water [118].

The average relative abundances of hepatic PAHs of the fish from the north central region indicate that the most abundant compounds are C1 phenanthrene/anthracene (24%), C2 dibenzothiophene (21%), and C2 naphthalene (12%) in the liver 2018 Yellowfin Tuna.

Alternatively, the major compounds in the 2020 Yellowfin Tuna livers from the north west region were abundant in C1 phenanthrene/anthracene (28%), C2 naphthalene (16%), and naphthalene (13%). There were only two samples in which only one high molecular weight PAH, C1 Fluoranthene/Pyrene, was present at greater concentrations than the MDL, indicating clear dominance of low molecular weight PAHs over high molecular weight PAHs throughout the Yellowfin Tuna studied herein.

As the north central samples present a considerable relative abundance of dibenzothiophene along with naphthalene, this indicates that produced waters may be a contributing source here. The samples from the north west region were not predominated by dibenzothiophene, indicating that samples from this region may be less impacted by sources known to present high concentrations of dibenzothiophene as a route of exposure although the weathering of crude oil and differential metabolism of compounds doubtlessly plays a role in what compounds are found. The samples presented higher parental naphthalene relative abundances in the north west region than the north central region, potentially suggesting the influence of pyrogenic sources or, alternatively, reduced weathering in the north west region. The naphthalene homolog relative abundances from the north central region present a clear bell-shaped curve, indicating the influence of a petrogenic origin [119].

After removal of male Greater Amberjack due to the significant difference between sexes, female Greater Amberjack collected in the Yucatán Peninsula region in 2016 (n = 5) were found to have significantly higher biliary naphthalene and biliary $\sum_3$FAC concentrations than Greater Amberjack collected in the north west Region in 2016 (n = 8). This suggests greater short-term exposure in female Greater Amberjack from the Yucatán Peninsula in 2016. High PAH concentrations have been observed in both sediments and fish from the Yucatán Peninsula and

researchers have identified heavy marine vessel traffic through this region as a potential contributor to PAH pollution in the Yucatán Peninsula region [49, 120].

The major contributors to average relative abundance of hepatic PAHs for all three regions were C2 naphthalene (NW: 15%, SW: 15% YP: 13%), C2 dibenzothiophene (NW: 16%, SW: 15%, YP: 11%), and C1 phenanthrene/anthracene (NW: 28%, SW: 26%, YP: 35%). Additionally, the distribution of relative abundance of hepatic PAHs were significantly different between these two groups, with C1 phenanthrene/anthracene, C2 naphthalene, and naphthalene driving separation between these groups. There were seven samples in which only one high molecular weight PAH, C1 Fluoranthene/Pyrene, was present at greater concentrations than the MDL, indicating clear dominance of low molecular weight PAHs over high molecular weight PAHs throughout Greater Amberjack within this study. All three regions in which Greater Amberjack were collected presented bell-shaped naphthalene distributions in their relative abundances which suggests a petrogenic origin.

Alternatively, combustion-derived products from marine vessels are a source of PAHs to marine sediments and waters. To distinguish further between combustion and petroleum-derived sources in samples, one can utilize ratios of parental PAHs including the ratio of anthracene to the sum of anthracene and phenanthrene. A ratio of less than 0.10 is indicative of petrogenic sources and a ratio greater than 0.10 is indicative of combustion sources [121]. All Greater Amberjack throughout all regions collected in this study had ratios of greater than 0.10 (0.32 – 0.95), suggesting primarily combustion sources of anthracene and phenanthrene although biotransformation can further complicate the ratios observed in fish. This indicates that marine vessel traffic in the Yucatán Peninsula region as well as other sources of combustion around the GoM may be contributing to PAH pollution in these pelagic fishes while the shape of

naphthalene homologs indicates influence of petrogenic sources, suggesting that both types of sources are contributing to PAH contamination in Greater Amberjack.

Conclusions

Polycyclic aromatic hydrocarbons are known to be the most toxic component of crude oil to a wide variety of marine wildlife [122]. Previous studies have observed negative impacts of PAH exposure on the health and physiology of embryonic, juvenile, and adult pelagic fishes, likely impacting the evolutionary fitness of the fish [36, 123]. One study noted impacts on swimming performance, optimal swim speed, maximum metabolic rate, and aerobic scope of adult Dolphinfish when exposed to PAH concentration levels below what pelagic fish experienced during the *Deepwater Horizon* spill [38]. The goal of this study was to quantify hepatic and biliary PAH concentrations to establish baselines for these species in the GoM and to further our collective understanding of how PAH distributions differ across water column zones and life histories. Liver (n = 142) and bile (n = 102) samples were analyzed for eight pelagic fish species caught between 2016 and 2020.

Significant interspecies differences in biliary PAH concentrations were found between Yellowfin Tuna and Swordfish (highly migratory species) and other, non-highly migratory species, highlighting increased exposure potentially related to diet, exposure to surface waters, diel migrations, or filtering of high volumes of water. Differences found between migratory and non-migratory species were not mirrored in hepatic PAH concentrations as Swordfish and Yellowfin Tuna were not significantly greater than any other species, indicating that although highly migratory species may be exposed to increased PAH levels in the short term, efficient

metabolism has prevented significantly higher hepatic accumulation when compared to non-migratory species. The findings in this study of hepatobiliary PAH concentrations in pelagic fishes could suggest significant separations of short-term exposure based on life history characteristics including vertical and horizontal migration habits as well as water column zone occupied. Significant negative relationships between health indices and PAH concentrations in multiple species (Greater Amberjack, Almaco Jack) indicated decreasing energy reserves with increasing hepatic and or biliary PAH concentrations. Significant positive relationships between length and hepatobiliary PAH concentrations suggests increasing exposure with increasing surface area, however ontogenetic shifts in diet could also be contributing to this relationship.

This study also identified hepatic PAH compositional differences between samples collected in regions with dense oil and platform activity and those with less dense oil and platform activity. The predominance of dibenzothiophene in north central fish suggests discharged produced waters as a potential source of exposure for Yellowfin Tuna, an epipelagic species. As a chronic and ongoing potential source of exposure to economically and ecologically important apex predators, the impacts of produced waters on epipelagic fishes should be further analyzed. Unexpected high biliary levels identified in Yellowfin Tuna indicate a need for further monitoring of PAH concentrations in epipelagic fish species.

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