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Polychlorinated Biphenyls, Organochlorine Pesticides, and Polycyclic Aromatic Hydrocarbons in Snapper (Family Lutjanidae) from Cuba and the Wider Gulf of Mexico

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Polychlorinated Biphenyls, Organochlorine Pesticides, and Polycyclic Aromatic Hydrocarbons in Snapper (Family Lutjanidae) from Cuba and the Wider Gulf of Mexico

by

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Abstract

The snapper species assemblage is economically and environmentally important to all three countries bordering the Gulf of Mexico (GoM). Polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were investigated in seven snapper species located in the GoM, with a specific focus on the those caught along the northwestern coast of Cuba. Hepatic samples were processed using QuEChERs extraction methodology and analyzed using gas chromatography with tandem mass spectrometry (GC-MS/MS). Nearly all fish included in this study contained detectable amounts of all three groups of these contaminants, regardless of location, and across all regions of the Gulf, indicating that PCB, OCP, and PAH contamination in fisheries is ubiquitous in the Gulf of Mexico. Measured values of these three groups of persistent organic pollutants (POPs) were within the same order of magnitude as other species in the GoM and globally, with concentrations of ΣPCBs ranging from 0.80 to 427 ng g^{-1} wet weight (w.w.), ΣOCPs from 4.00 to 247 ng g^{-1} (w.w.), and ΣPAHs from 60.0 to 2,991 ng g^{-1} (w.w.). Wenchman were found to have increased hepatic PCBs compared to other snapper, and Silk Snapper had elevated hepatic PAHs. Species-specific variation in hepatic total load and composition of PAHs, OCPs, and PCBs suggest species-specific differences in xenobiotic metabolism. Contaminants are correlated with several fish biometrics and health indicators, including negative relationships between PCBs and Fulton's condition factor, and between PAHs and hepatic lipids. These correlations may indicate that snapper with elevated exposure to these contaminants are experiencing a range of sublethal effects. Regional variation observed in hepatic concentrations and compositional profiles were likely driven by local onshore contamination sources, chemical usage patterns, and riverine input. Spatial gradients observed in PCB and DDT concentrations were along the northwest coast of Cuba are likely driven by proximity to the city of Havana. The results of this study demonstrate possible connections between onshore development/industrial activity and impacts to offshore ecosystems. These data also serve to establish critical contaminant baselines for this group of

economically important GoM fishes; and will contribute to assessing the impact of any future pollution events or changes in contaminant inputs within the area. This information will also help to fill some of the gaps in our understanding of the presence and possible impacts of these organic contaminants in GoM snapper fisheries.

Introduction

Gulf of Mexico Oceanographic and Human Use Setting

The Gulf of Mexico (GoM) provides essential habitat to many economically and environmentally important fish species, and the value of maintaining healthy GoM fisheries cannot be understated. In the United States (U.S.) alone approximately 500,000-800,000 metric tons of seafood are sourced from the GoM annually (*1*), and recreational fishing is estimated to bring in an additional $11 billion in yearly revenue to the Gulf coast states (*2*). The GoM also supports a vast array of marine life, with hundreds of species of birds, marine mammals, and other marine/coastal species that rely on healthy habitats and fish populations in the GoM.

This vital ecosystem is shared and managed by three separate countries; the United States (U.S.), Mexico, and Cuba. While there has been a large body of research directed to understanding the U.S. and Mexican regions of the Gulf, there has been comparatively little research published on the offshore resources of Cuba. More information from this Cuban region of the Gulf is needed in order to construct a comprehensive understanding of both fisheries and contaminant cycling in the GoM. Understanding the entirety of the GoM large marine ecosystem (LME) is critical due to the connectivity of the ecosystems and fisheries throughout (*3, 4*). The Gulf is interconnected in part due to the loop current, which can transport fish eggs/larvae and contaminants across far distances relatively quickly. Examples of this connectivity can be seen in the genetic ties between fish populations across regions of the Gulf (*5*), and in models showing the potential transport of oil after a spill (*6*). Considering the connectivity of the ecosystems and fisheries in the Gulf, maintaining a healthy and sustainable GoM requires the U. S., Mexico, and Cuba to have well-coordinated Gulf-wide monitoring and management strategies

Contaminants in the Gulf: Background and Interactions with Fish

Fisheries of the GoM are facing a number of common challenges, including overfishing, habitat loss, and exposure to anthropogenically derived pollutants (*7*). Understanding the impacts and synergistic relationships among these pressures, and how best to mitigate them, is a key part of sustainably managing fisheries and the GoM as a whole. Part of the complexity behind understanding the role of contaminants in the Gulf comes from the multiple types and sources of inputs. The simultaneous presence of multiple chemical stressors can lead to antagonistic, additive, and/or synergistic impacts on the system (*8*), which makes it difficult to deduce the impact of a single stressor (e.g. oil). For example, possible sources for crude oil contamination in the Gulf include the offshore oil and gas industry, but also include natural seeps on the seafloor, ongoing leaks such as the Tayler Energy well, coastal refineries, and riverine inputs such as the Mississippi River. In addition, historical incidents such as the Deepwater Horizon (DWH) and Ixtoc I oil spills can leave reservoirs of contaminants which then continue to cycle through an ecosystem for decades (*9, 10*).

The ongoing challenges of poor water quality and environmental degradation in the GoM are driven by excessive nutrient inputs and anthropogenic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) (*11*). These three groups all contain compounds known to impact marine systems via various toxicological effects; and are considered contaminants of environmental concern in the GoM. These compounds are also all lipophilic organic molecules, and therefore are likely to amass in high lipid content tissues (e.g., liver, gonads, eggs) within the body (*12*). Contaminants sequestered in lipids may also be remobilized and transferred along with lipids for the development of egg and gonadal tissue during gestation or spawning, resulting in increased levels of lipophilic contaminants in eggs and larvae (*13*). These life stages are particularly vulnerable to health impacts because of their reduced ability to metabolize and excrete xenobiotics (*14*). The potential for maternal offloading allows health impacts of these contaminants to be passed on to the next generation, and can have population level effects if the contaminant loads impact survival or reproductive fitness of the cohort (*15, 16*).

Chemical and physical properties of contaminants directly impact possible routes for fish exposure and uptake, and since lipophilic compounds generally share similar properties, the possible routes for fish exposure and uptake are fairly consistent across these compounds The three main routes of chemical uptake in fish are through consumption of contaminated food, respiration (absorption across the gills), and dermal absorption (across skin) (*8, 17, 18*). The concentration of organic contaminants dissolved in water (and therefore available for absorption) depends on several physiochemical properties including solubility, water temperature, and suspended sediment concentration (*19-21*). Lipophilicity, often evaluated using the octanol/water partitioning coefficient (K_{ow}), of a compound can affect the rate of uptake from water, excretion rate, and efficiency of compound assimilation into an organism (*22*). Bioavailability is also influenced by factors such as compound size and physiochemical conditions of the environment (*18*). Rates of bioaccumulation and biomagnification of organic compounds are affected by chemical and ecological factors such as K_{ow} and seasonality (*18, 21, 23-25*). In addition, biotic factors including diet composition, individual energetic demands, developmental stage, and species-specific metabolism/biotransformation rates can all impact uptake and bioaccumulation rates for an individual organism (*22, 26, 27*).

Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic contaminants in which the molecular structures have two or more aromatic rings fused together. Sources of PAHs in the environment are largely petrogenic (e.g. crude oil) or pyrogenic (e.g. burning of hydrocarbons). Petrogenic sources include petroleum extraction, oil facility accidents, refineries, and transport, as well as naturally-occurring oil seeps on the seafloor (*28*). Pyrogenic sources include grassland and forest fires, incomplete combustion of wood or fossil fuels (e.g. coal fired power plants, car engines), and volcanism (*17*). Although some of these sources are naturally occurring, anthropogenic sources are commonly the cause of high concentrations present in an area (*29*). Alkylated PAH homologs are usually present along with

the parental molecules, and the presence and ratios of these homologs present can provide insight regarding a particular point source (e.g., petrogenic vs pyrogenic). Another smaller source of PAHs is biogenesis by plants, bacteria, and fungi, however this input is usually assumed to be too minor to influence environmental concentrations (*12, 30*). Due in part to the wide range in type and global nature of these sources, PAHs are ubiquitous across the planet. In addition, our current societal dependence on fossil fuels suggests that fossil fuel industry related sources of PAHs are unlikely to diminish within the next few decades, thus PAH levels in marine ecosystems are expected to remain relevant for decades to come (*17*). A recent survey of PAH baselines GoM fishes found that PAH exposure is pervasive throughout all regions of the Gulf, and that many species appear to be chronically affected by this exposure (*31*). The effects of PAH (and PAH metabolite) exposure on fish health include a range of both lethal and sublethal effects such as carcinogenesis/neoplasia, immunotoxicity, reduced cardiac function, genotoxicity, endocrine disruption, metabolic costs, and growth inhibition/defects (especially in larval and juvenile stages) (*8, 10, 12, 16, 32-36*).

Fish have the ability to readily metabolize PAHs into more water soluble compounds through the cytochrome P4501A enzyme system (*12, 14*). Metabolizing PAHs can help to decrease body burden, reducing the potential for adverse effects from these compounds, however some PAH metabolites can be more toxic than their parental molecule (*8, 12*). Once metabolized, the majority of PAH parental compounds and their metabolites are eliminated via excretion of urine or bile which is passed through the gastrointestinal tract. Some elimination is also possible by simple diffusion from the organism (*18, 37*). Although fish are able to metabolize and excrete PAHs with relative ease, prolonged or elevated exposure can cause PAH uptake to outpace elimination, thus leading to accumulation in lipid-rich tissues such as the liver (*10*). The functioning of this xenobiotic metabolism and elimination system causes the PAH load in hepatic tissue to reflect chronic exposure, whereas PAHs in bile are indicative of recent short-term exposure (*17*). In addition to being metabolized by fish, PAHs are also susceptible to weathering, photooxidation, and biodegradation via microorganisms (*38, 39*). Because of this PAHs are not considered to be as persistent in the environment as more recalcitrant contaminants, and the Stockholm

Convention on Persistent Organic Pollutants (POPs) does not included PAHs in the official POPs classification.

Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides (OCPs)

Polychlorinated biphenyls (PCBs) are a class of compounds that were originally manufactured for use in industrial applications such as oils, lubricants, hydraulic fluids, etc., and were even included in some pesticides and inks (*19, 40*). Production of PCBs began in the 1930s, and lasted until they were banned across most of the globe in the 1970s. Primary sources for PCBs in the environment are through historical and current leaks, spills, dispersal of products in which they are contained, and the burning of PCB containing waste (*41*). Due to their low solubility in water, the largest reservoir for PCBs in aquatic/marine systems is often the benthic sediments (*42, 43*). This group of compounds is completely anthropogenically synthesized (no naturally occurring sources are known thus far), thus high concentrations tend to occur near industrialized, developed areas (*41*). Natural conditions and biota do not significantly degrade or biotransform PCBs, so once in the environment they will continue to be cycled and accumulated for decades (*19*). PCBs are now considered to be globally ubiquitous due to this longevity and their ability to cycle through soil, water, and air (*44*).

The molecular structure of PCBs consists of a biphenyl ring with one to ten chlorine atoms attached. The number and placement of the chlorine atoms affects the physical, chemical, and toxicological properties of each PCB, with a higher degree of chlorination generally corresponding to higher viscosity, lower solubility in water, and greater toxicity (*19*). More highly chlorinated PCBs also have an increased likelihood of bioaccumulation (*21, 22*). This is due in part to the higher lipophilicity of more chlorinated PCBs (*19*), and partly because less chlorinated PCBs are more readily metabolized than their more chlorinated counterparts (*45*). Negative effects of PCBs on fish health include carcinogenesis, liver damage, and a variety of reproductive and endocrine system issues (*17, 19, 46, 47*). Effects of PCB contamination on eggs and juveniles is particularly concerning, as contaminant offloading into lipid rich

eggs has been demonstrated to lead to reduced survival of offspring and decreased reproductive success (*48*).

Pesticides is a class of compounds including herbicides, insecticides, and rodenticides. Organochlorine pesticides (OCPs) are a subclass containing carbon, hydrogen, and chlorine atoms. These compounds often are characterized by their stability and resulting persistence in the environment. This group includes compounds such as dichlorodiphenyltrichloroethane (DDT) which have been banned in the U.S., as well as compounds like lindane which are still broadly used today (*49*). It should be noted that although many of the legacy OCPs were banned in the U.S. in the 1970s, this does not always coincide with usage in Mexico and Cuba (and other tropical countries). One example is DDT, which the U.S. banned usage of in 1972, Cuba banned in 1989, and Mexico halted use in 2000, however many Caribbean countries still use DDT today to combat malaria (*50-53*). All the compounds in the OCP group are anthropogenically manufactured; and are mainly spread into the environment through agricultural use and other pest control activities. The primary source of OCPs in marine ecosystems is runoff from coastal land, as well as rivers passing through agricultural areas. Because OCPs are chemically stable in the environment, the leaching of OCPs sequestered in benthic sediments is another possible source of input to marine systems (*54*). This class of compounds is broadly classified as neurotoxins (*55*), and adverse effects of OCP exposure observed in fish include general oxidative stress, carcinogenesis, liver damage, reduced growth, and disruption of the endocrine and developmental systems (*17, 56*). Chronic exposure of humans to OCPs can negatively impact metabolism of carbohydrates, lipids, and proteins, resulting in cascading impacts through other body systems (*55*).

In contrast to PAHs, PCBs and OCPs are not easily metabolized by fish or other organisms. This causes them to be classified as POPs, meaning that they are hazardous and slow to breakdown in the natural environment (*57*). In addition to fish having a low capacity to metabolize them, the low water solubility of these compounds slows any excretion prior to metabolism. The combination of these factors causes PCBs and OCPS to buildup in adipose tissues, enabling them to both bioaccumulate within an organism over time, and biomagnify through successive trophic levels (*22, 41, 56, 58*). Rates of

bioaccumulation and biomagnification of organic compounds are affected by chemical and ecological factors such as K_{ow}, bioenergetics, dietary preferences, and seasonality (*18, 21, 23-25*). The high potential for bioaccumulation and biomagnification of these PCBs and OCPs results in these contaminant groups impacting the health of higher trophic level species even when concentrations in the abiotic environment are below usual toxicological thresholds. Lipophilic and environmentally persistent compounds, such as PCBs and OCPs, also tend to exhibit global fractionation patterns (*44*). These patterns are referred to as the global distillation effect, where optimally volatile compounds are atmospherically transported and tend to concentrate at cooler higher latitudes. In contrast, less volatile POPs are not transported as efficiently, and so concentrations found in the environment are more likely to reflect the local sources of that compound (*59*).

Cuban Fisheries and Contaminant Setting

The Cuban region of the GoM faces many of the same challenges as the rest of the Gulf, and Cuban commercial fisheries have been in steady decline for the last 50 years (*60, 61*). This is likely due to a combination of overfishing and a lack of protective regulations enforcement (*62, 63*); some problematic issues in this region include targeted fishing of spawning aggregations and the harvest of individuals prior to sexual maturity (*64*). Although the observed declines are likely a result of fishing pressures, other potential drivers may be causing or exacerbating these population declines as well (*65*).

The area offshore of Havana has been especially impacted, with untreated discharges of heavy metals, industrial effluents, sewage, and crude oil into the marine environment (*63, 66-68*). The lack of effective wastewater collection and treatment in Cuba is a concern for human health and a major source of pollution into Cuban coastal waters (*69, 70*). An estimated 13 million gallons of wastewater is produced and discharged per day in the providence of Havana (*69*), and almost all is untreated or only passed though limited pre-treatment (removal of some solids but no disinfection or nutrient removal) (*70*). This wastewater is discharged into the marine environment via submarine outfalls, which are submerged pipes releasing wastewater at around 150 m offshore and at about 10 m water depth (*70*). This wastewater

is thought to be a mix of both urban and industrial waste, and elevated heavy metal concentrations have been found in sediments near these submarine outfalls (*71*). Changes in octocoral communities have also been documented following the installation of submarine outfalls offshore of Havana (*72*).

In addition, contaminants from point sources such as a cement plant and paper mill are discharged into the Almendares River flowing through Havana, and are then carried out into the marine environment slightly to the west of Havana harbor (*63*, *73*). These contamination sources result in high levels nutrients and other organic pollutants offshore of Havana (*65*). Changes in reef community composition and damage to fish reproductive function have been observed near Havana Harbor and the Almendares River outlet, and have been attributed to these local sources of anthropogenic pollutants (*63*, *73*-*75*). Investigating and accounting for all possible factors, including organic contaminants, is an integral part of moving towards recovery and comprehensive management of these fisheries.

There is little published research investigating organic contaminants in fish from Cuban waters, however there have been some data collected from sediments and bivalves. A 2010 study along the southern coast of Cuba documented the presence of OCPs and PCBs in sediments from Cienfuegos Bay (*76*). This study found that surface sediment concentrations of PCBs and OCPs were in the range of 27 – 1,745 pg g^{-1} dry weight (d.w.), and from below the detection limit (<DL) to 9,580 pg g^{-1} (d.w.), respectively (*76*). A separate study in the Gulf of Batabanó, found individual PCBs all to be below the detection limit, and individual OCPs ranging from <DL to 0.737ng g^{-1} (d.w.) (*77*). The International Mussel Watch measured PAH, OCP and PCB concentrations in the tissues of mussels from the southern coast of Cuba in 1993 (*78*), finding that PCB concentrations in one species of oyster ranged from <DL to 2.20 ng g^{-1} (d.w.), and OCP concentrations from <DL to 2.77 ng g^{-1} (d.w.) (*79*). The inconsistency in matrix analyzed (sediment vs biological, species, tissue) and units reported (wet weight vs dry weight) obscures datasets from Cuba and the wider GoM; and complicates efforts to coalesce either a regional or a Gulf-wide understanding of these contaminants from historical datasets.

Snapper (*Lutjanidae sp.*): Comparative Life Histories in the Gulf of Mexico

As a group, snappers are particularly important to Cuban and GoM fisheries. The snapper species assemblage was chosen for analysis in this study because of their ubiquitous occurrence throughout the GoM, as well as the significant role they play both economically and ecologically in the GoM and the broader Caribbean region. The species included in this study were Wenchman (*Pristipomoides aquilonaris*, Good & Bean 1896), Silk Snapper (*Lutjanus vivanus*, Cuvier 1828), Yellowtail Snapper (*Ocyurus chrysurus*, Bloch 1791), Blackfin Snapper (*Lutjanus buccanella*, Cuvier 1828), Queen Snapper (*Etelis oculatus*, Valenciennes 1828), Black Snapper (*Apsilus dentatus*, Guichenot 1853), and Mutton Snapper (*Lutjanus analis*, Cuvier 1828). This list is a relatively broad representation of the snapper species found in Cuban waters (*80*), although a few species such as Lane Snapper and Gray Snapper were not collected but do occur frequently in this region (*81*). Some species of snapper commonly found in northern and more western GoM waters (e.g., Red Snapper) were not included because this study was designed with a focus on snappers found in the Cuban area of the Gulf (i.e. Red Snapper were not encountered in sampling off Cuba).

The seven snapper species selected for analysis occupy mainly deeper waters, with the exception of the shallow dwelling Yellowtail Snapper and Mutton Snapper. The habitat for adult snapper of these species generally consists of shelf edge rocky outcroppings and reefs, with substrates of hard rock and shell hash (*82-85*). Mutton Snapper are an exception, with habitats commonly consisting of more sandy/vegetated substrates in mangroves as well as on shallow coral reefs (*84*). Detailed life history information is not known for all of these species, however most have extended breeding periods, with peak spawning activity in either the spring, summer, or fall seasons, depending on species (*82-84, 86*). Diets of snappers generally consist of small fish and crustaceans; some species also eat polychaetes, gastropods, tunicates, and cephalopods (*82, 84*). There is limited published information available on Wenchman diet, life history, etc. (*87*), thus for the purposes of this study, it was assumed that these parameters for Wenchman are similar to other deep-water snapper species.

Thesis Objectives

The objectives of this thesis were: (1) compare organic contaminant loads across species, (2) evaluate relationships between hepatic contaminants and associated biometrics and health indices, and (3) investigate potential spatial patterns of contaminants both around the GoM and within the Cuban region of the Gulf. Despite the increasing focus on ecosystem-based management of the Gulf of Mexico (GoM), there remain many knowledge gaps, particularly in the Cuban portion of the GoM. One such gap is understanding the presence and impacts of organic contaminants. This study addresses that gap by assessing the presence and possible impacts of three types of organic contaminants in snapper populations in the GoM, with a specific focus on the northwestern coast of Cuba. The three classes of organic pollutants evaluated in this study are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs).

Methods

Field Sampling

Fish were collected in the spring and summers (May-August) of 2015, 2016, and 2017 by the Center for Integrated Modeling and Analysis of Gulf Ecosystems (C-IMAGE) consortium (*88*) using demersal longline surveys around the continental shelf of the GoM (*3*). Inshore to offshore transects (n = 44) were sampled, with each transect consisting of multiple stations across a water depth range of 20 to 200 meters. These transects were grouped into seven broad regions reflecting oceanographic features and species distributions (*3, 31, 89*) (Figure 1). Within the Cuban region nine transects were distributed from the westernmost tip of the Cuban shelf to directly offshore of the city of Havana (Figure 2). Samples collected outside of the Cuban region were mainly included for a Gulf-wide spatial context of the POPs measured.

Eight km of mainline was deployed at each station, and size 13/0 circle hooks were baited with Atlantic mackerel (*Scomber scombrus*) and Humboldt squid (*Dosidicus gigas*) wings before being attached to the mainline at regular intervals. Upon capture each fish was identified to species, and fish biometrics and general observations were recorded for all specimens caught. Target species were then dissected, and individual organs weighed and recorded to the nearest g for each fish. Liver and muscle tissue were collected and stored in amber glass jars at -20ºC until thawed for analysis. The seven snapper species included in this study were Silk Snapper (*Lutjanus vivanus*, n = 48), Queen Snapper (*Etelis oculatus*, n = 11), Blackfin Snapper (*Lutjanus buccanella*, n = 17), Yellowtail Snapper (*Ocyurus chrysurus*, n = 19), Black Snapper (*Apsilus dentatus*, n = 3), Mutton Snapper (*Lutjanus analis*, n = 3), and Wenchman (*Pristipomoides aquilonaris*, n = 89).

Analysis of Hepatic Lipid Content and Biometric Factors

Lipid content of hepatic tissue was measured using a modified version of the Folch method (*90*). Lipids were extracted from 200 mg of homogenized hepatic tissue using agitation and a solution of methyl-tert-butyl ether, methanol, and Milli-Q filtered water. The organic layer was then collected and dried to procure the weight of the lipid fraction in relation to the weight of the original tissue subsample (see (*10*) for further details). It should be noted that some hepatic samples were too small to conduct both lipid and contaminant analysis, when that was the case contaminant analysis was prioritized and lipid analysis was not completed for these samples (n = 4). Hepato-somatic index (HSI) is a ratio indicating the relative mass of the liver to the total body, and calculated using $HSI = \frac{mass\ of\ total\ body}{mass\ of\ liver}$. Fulton's condition factor (K) was calculated using $K = 100 \times \frac{body\ mass\ (g)}{standard\ length\ (cm)^3}$ (*91*).

Analysis of Hepatic Contaminants

Tissue samples were prepared for analysis using QuEChERS extraction (*92*) and clean up procedures and methodology. The QuEChERS extraction process (Bond Elut dSPE EMR-Lipid, Agilent Technologies, Santa Clara, CA, USA) was optimized for the highest possible recoveries of the three POP classes from liver tissues of each fish species (details of extraction methodology provided in Appendix B). Liver extracts were analyzed for 46 PAHs (19 parental PAHs and their associated alkylated homologs), 32 PCBs, and 32 OCPs using gas chromatography triple quadrupole mass spectrometry (GC-MS/MS, Agilent Technologies 7890 GC and 7010 MS/MS). Liver extracts (2 µL) were injected in splitless mode using a two-layer sandwich of 0.2 µL analyte protectant (composite solution of 20 mg mL^{-1} L-gulonolactone and 10mg mL^{-1} D-sorbitol in ACN), and 2µL of sample extract. Analyte separation was acheived using a 30m Rxi-5Sil fused silica capillary column (Restek, Bellefonte, PA, USA) in a 7890 Agilent gas chromatograph and confirmed using a 7010 Triple Quadrupole MS/MS (Agilent Technologies) operating in both multiple reaction monitoring (MRM) and full scan modes. Oven

parameters are summarized in Appendix B, and acquisition parameters are summarized in Tables 2 and 3 in Appendix B.

Final analyte concentrations were calculated from response factors generated using an external matrix matched standard, with five point calibration curves (1 – 200 ng mL^{-1}) run at the beginning and end of the project to assure response factors remained stable (CSV < 20) across the range of concentrations observed in samples. Method detection limits (MDL) for each analyte were set to the concentration of the lowest calibration standard in which the analyte peak signal to noise ratio > 3, and concentrations <MDL were set to ½ MDL prior to continued analysis.

Quality Assurance/Quality Control

I implemented a quality assurance and quality control program (QA/QC) to ensure data met recognized quality standards (*93*, *94*). The QA/QC program validates extraction efficiencies for each species by monitoring the recovery of appropriate deuterated standards in all sample extracts, matrix spikes, and method blanks. Prior to extraction of each species, the methodology was verified via acceptable recovery of all analytes from matrix spikes. Average recovery of analytes across matrix spikes was 94% for PCBs, 95% for OCPs, and 86% for PAHs. Solvent and method blanks were used to monitor and subtract any background contamination. Recovery of surrogate standards averaged 74% in method blanks; and averaged 81% in samples.

Data Analysis

Data are rounded to three significant digits and reported in ng g^{-1} wet weight (w.w.), with preliminary data organization conducted in Microsoft Excel. All statistical tests were conducted using MATLAB R2020a and the Fathom Toolbox for MATLAB (*95*). Species and spatial differences in contaminant concentrations were assessed using non-parametric permutation-based multivariate analysis of variance (PERMANOVA). If a grouping effect (e.g. species) was determined to be significant, then differences were further examined using pair-wise PERMANOVA. The significance level (α) was set to

0.05, and significance was assessed using Bonferroni-adjusted p-values to decrease the probability of type 1 error. Canonical analysis of principal coordinates (CAP) was used to test and visualize compositional variation between groups. Compositional analysis was conducted using relative abundances calculated using raw data and standardized by grouping (values <MDL not set to 0.5 MDL). Correlations between contaminant loads and various biometric and health indicators were assessed using Pearson's linear regressions. A ternary plot was generated using PAST software. The inputs for the ternary plot were the total PCB, OCP and PAH values for each individual fish. This software calculated and plotted each group as a fraction of the total contaminants detected.

Results

Hepatic PCBs, OCPs and PAHs

Liver tissue samples (n = 190) from seven species of snapper were analyzed for 32 PCB congeners, 32 OCPs, and 46 PAHs (19 parental PAHs and their associated homologs). For all species combined, the measured values for individual PCBs ranged from <MDL to 103 ng g^{-1} wet weight (w.w.), individual OCPs from <MDL to 238 ng g^{-1} (w.w.), and individual PAHs from <MDL to 1,870 ng g^{-1} (w.w.). The sum of the measured PCBs (ΣPCBs) ranged from 0.80 to 427 ng g^{-1} (w.w.), ΣOCPs from 4.00 to 247 ng g^{-1} (w.w.), and ΣPAHs from 60.0 to 2,990 ng g^{-1} (w.w.). Nearly all snapper analyzed had detectable levels of PCBs (95.3% of individuals), OCPs (98.4% of individuals), and PAHs (100% of individuals) in hepatic tissues (analyte detection limits provided in Appendix B).

Significant species-based differences were detected in all three POP groups (Figures 3 and 4), and were found to be nearly identical whether examining the full Gulf-wide dataset or the subsection of samples from the Cuban region. Wenchman had significantly higher hepatic ΣPCB loads compared to Silk Snapper (p = 0.002), Yellowtail Snapper (p = 0.034), and Black Snapper (p = 0.002). Wenchman also had higher hepatic ΣOCP loads compared to Silk Snapper (p = 0.002), Yellowtail Snapper (p = 0.002) and Blackfin Snapper (p = 0.046), with Queen Snapper hepatic ΣOCP higher than Yellowtail Snapper (p = 0.002) and Blackfin Snapper (p = 0.043) as well. Silk Snapper hepatic tissues had significantly higher ΣPAHs when compared to Wenchman hepatic tissues (p = 0.002).

The composition of Wenchman hepatic PCBs also varied from that of other snappers, with a higher prevalence of PCBs with a low degree of chlorination (tri and tetra chlorobiphenyls). Additionally, the most abundant congeners (PCB 153 and PCB 170) make up a lower fraction of the total PCB load in

Wenchman (Figure 4A). Regardless of species, the non-dioxin-like PCBs make up about 80% of the total hepatic PCBs measured. The most abundant congener found in these samples was PCB 153.

Disparities in hepatic OCP composition are mainly driven by DDE, a metabolite of DDT (Figure 4B). In Wenchman and Queen Snapper DDE was the most abundant OCP, while propachlor was the most abundant OCP in Yellowtail Snapper and Blackfin Snapper. Silk Snapper hepatic tissues contained a more balanced distribution, although DDE is still the most abundant OCP. Hepatic PAHs found in all species consisted mainly of low (2-3 ring) molecular weight compounds (Figure 4C). Profiles for hepatic PAHs were dominated by naphthalene, dibenzothiophene, and anthracene homologs across all species, although there was a higher relative abundance of C1 naphthalene in Yellowtail Snapper and Queen Snapper compared to the other species. Further statistical analysis (spatial patterns and correlations with biotic factors) were conducted separately for each species due to the observed differences between species. When examining relationships between the three contaminant groups (Figure 5) the only significant relationships found were positive correlations between hepatic ΣOCPs and ΣPCBs in both Wenchman and Yellowtail Snapper ($p = 0.013$ and $p = 0.004$, respectively).

Biometric and Fish Health Data

Fish biometric data and health proxy results are summarized by species in Table 1. In this data set, both hepatic ΣPCBs and ΣOCPs in Wenchman exhibited positive correlations with hepatic lipid content ($r = 0.349$ $p = 0.002$ and $r = 0.454$ $p = 0.001$, respectively), standard length ($r = 0.448$ $p = 0.001$ and $r = 0.419$ $p = 0.001$), and total body weight ($r = 0.439$ $p = 0.001$ and $r = 0.398$ $p = 0.003$). Hepatic ΣPAHs in Wenchman were not significantly correlated with hepatic lipid content, standard length, or weight. Wenchman hepatic ΣPCBs were negatively correlated with Fulton's condition factor ($r = 0.332$, $p = 0.006$; Figure 6), and ΣPAHs were negatively correlated with Wenchman HSI ($r = -0.272$, $p = 0.020$). Neither hepatic ΣPCBs or ΣOCPs were significantly correlated with Wenchman HSI, and hepatic ΣPAHs and ΣOCPs were not significantly correlated with Fulton's condition factor in Wenchman. Silk Snapper hepatic ΣPAHs exhibited positive correlations with standard length ($r = 0.606$, $p = 0.001$) and total body

weight (r = 0.519, p = 0.001), and a negative correlation with % lipid (r = -0.384, p = 0.010). Hepatic ΣOCPs and ΣPCBs were not significantly correlated with Silk Snapper hepatic lipid content, standard length, or weight. Hepatic ΣPCBs in Silk Snapper exhibited a positive correlation with HSI (r = 0.679, p = 0.004), and a negative correlation with Fulton's condition factor (r = -0.542, p = 0.008) (Figure 6). No significant correlations were found between Silk Snapper hepatic ΣOCPs and ΣPAHs and Fulton's condition factor or HSI. In both Wenchman and Yellowtail Snapper females were found to have lower hepatic ΣPAH loads compared to males (p = 0.016 and p = 0.006, respectively; Figure 7). No further significant correlations between contaminant loads and biotic factors were observed in the other species included in this study.

Gulf-wide Spatial Comparisons

Wenchman were sampled in all seven regions of the GoM (Figure 1), making this species ideal for Gulf-wide spatial comparisons. Both the Cuban (p = 0.002) and the NW (p = 0.002) regions had significantly higher ΣPCB values than the SW region. Wenchman from the NW also had significantly higher ΣOCP values than the SW (p = 0.008), although Wenchman from the Cuban region had significantly higher ΣOCP values than both the NW (p = 0.008) and the SW (p = 0.002) regions (Figure 8). The Cuban (p = 0.002), NW (p = 0.015), and SW (p = 0.015) Wenchman all had significantly higher ΣPAH values than those from the NC region, although the sample size for the NC was small (n = 3), so results related to this region are tentative.

Canonical analysis of principal coordinates on Wenchman data shows significant compositional variation between regions in all three contaminant groups (PCBs: p = 0.023, OCPs: p = 0.005, PAHs: p = 0.001) (Figures 9 and 10). Regional variation in PCB composition is driven by PCB 166, 105, 189, 118, and 169. The Cuban region is associated with PCB 44, 52, 28, and 49. PCB 37 is associated with some outliers in the YP and CAMP regions (Figure 10a). The OCP composition of the Cuban region is associated with DDT and its metabolites DDE and DDD, whereas the SW region is associated propachlor (Figure 10b). Driving compositional variability in PAHs are naphthalene, naphthalene homologs, fluorene

homologs, dibenzothiophene homologs, phenanthrene, anthracene, and phenanthrene/anthracene homologs. Compositional separation of the Cuban region is associated with fluorene, acenaphthene, and C3 dibenzothiophene, while the WFS and NC regions are associated with chrysene, benzo[a]anthracene, acenaphthylene, and C1 fluoranthene/pyrene (Figure 10c).

The other six species included in this study were only sampled in the Cuban and YP regions (with the exception of a single Silk Snapper from the WFS region). Silk Snapper from the Cuban region had higher ΣPAH levels than those from the YP region ($p = 0.001$), and Yellowtail Snapper had higher ΣPCB levels in the Cuban region compared to the YP ($p = 0.031$). Small sample sizes in the remaining species prevented any meaningful regional comparisons in hepatic POPs from being conducted.

Cuban Spatial Patterns

There were significant differences in snapper contaminant loads along the east-to-west gradient of the coastline from just east of Havana to San Antonio Bank, ~300 nautical miles to the west (Figures 11 and 12). Hepatic ΣPCBs measured in Wenchman were significantly higher to the east offshore of Havana (Transect 44) compared to the west offshore of Guanahacabibes National Park (Transect 37, $p = 0.010$), the eastern edge of the Gulf of Guanahacabibes (Transect 38, $p = 0.010$), offshore of Santa Lucia (Transect 39, $p = 0.027$), and offshore between Puerto Esperanza and Palma Rubio (Transect 40, $p = 0.010$). Hepatic ΣPCBs in Wenchman from the middle of the sampling area (Transect 40) were also higher than those from near Guanahacabibes National Park (Transect 38, $p = 0.020$). Hepatic ΣOCPs were significantly higher in Wenchman from Transect 44 compared to Transect 37 ($p = 0.040$) (Figure 11). No significant spatial trends in ΣPAHs were detected in Wenchman along the northwestern coast of Cuba. Silk Snapper from the Cuban region did not demonstrate any significant spatial gradients in hepatic ΣPCB or ΣOCP values, however Silk Snapper from offshore of Havana (Transect 44) did have lower ΣPAH values than those from offshore of Guanahacabibes National Park (Transect 37, $p = 0.003$) or the Gulf of Guanahacabibes (Transect 38, $p = 0.0390$) (Figure 12). The remaining five snapper species did not have large enough sample sizes to conduct meaningful spatial analysis within the Cuban region.

Discussion

The POP loads measured in these seven species of snapper were generally similar to those found in other Gulf reef-fish species. Hepatic ΣPAH concentrations for all snapper data combined (all species and regions) ranged from 60.0 to 2,990 ng g^{-1} w.w. These values are within the ranges of hepatic ΣPAHs measured in Gulf Red Snapper (192 to 8,530 ng g^{-1} w.w.), groupers (69.5 to 21,500 ng g^{-1} w.w.), and Golden Tilefish (116 to 8,112 ng g^{-1} w.w) collected from around the GoM (*10, 89, 96*). Another study conducted in the southern Gulf found that total PAHs in muscle tissue of Shoal Flounder ranged from <DL to 1,658 µg g^{-1} d.w. (*97*).

Hepatic ΣPCBs measured in this study ranged from <MDL to 427 ng g^{-1} w.w., and hepatic ΣOCPs from <DL to 247 ng g^{-1} w.w. Concentrations of individual OCPs in GoM mesopelagic fishes analyzed in the 1970s ranged from <DL to 120 ng g^{-1} w.w., and ΣPCBs from 2 to 926 ng g^{-1} w.w. (*98*). A study of Red Snapper fillets in the late 1970s found PCBs ranged from <DL to 464 ng g^{-1} w.w., and ΣDDT (DDT and metabolites DDD and DDE) ranged from <DL to 322 ng g^{-1} w.w. (*99*). My study found concentrations of ΣDDT to be between <DL and 240 ng g^{-1} w.w.. More recent data on shark livers from the northwest GoM found concentration of individual PCBs ranged between <DL to 950 ng g^{-1} w.w. (*100*), which is higher but still in the same order of magnitude as the <MDL to 103 ng g^{-1} w.w. range observed in this study. Muscle tissue of inshore fish from the Florida pan handle had individual parental PAH concentrations ranging from <DL to 6 ng g^{-1} w.w., total PCBs between 14.79 and 104.23 ng g^{-1} w.w., and individual OCPs between <DL and 9 ng g^{-1} w.w. (*101*). The dominance of PCB 153 in this study matches the patterns of congener abundance observed globally in most biological samples, and is due to this congeners prevalence in synthesized materials as well as its slow rate of biotransformation (*41, 102, 103*). The relative abundance of naphthalene homologs in Wenchman (Figure 4C) exhibit the classic

pattern observed in petrogenic PAHs (*104*), suggesting exposure may be driven more by petrochemical activities such as crude oil extraction and transport rather than combustion related sources.

Inter-Species Variability

Significant differences in hepatic burdens of all three contaminant groups were detected between Wenchman and Silk Snapper. Differences in PCB and OCP total burdens were also detected between Wenchman and Yellowtail Snapper, and Queen Snapper had different total hepatic OCP loads when compared to both Yellowtail Snapper and Blackfin Snapper. These results indicate there is clear variation in organic contaminant burdens between these seven species of snapper. One explanation for this observation could be that metabolism and biotransformation rates for PAHs, PCBs and OCPs vary across these species, and as such each species breaks down and excretes these compounds with varying levels of efficiency. Species-specific metabolism of xenobiotics has been observed in other marine fishes (*27*), and could result in differing levels of accumulation for individual compounds within the livers of these species (*26*). Differences in size and trophic level of the species may also be contributing to the disparities. Variability in exposure and uptake of these compounds could result from differences in diet, life history, and/or habitat (*25*). For example, elevated PAH concentrations in Tilefish from the northern GoM are hypothesized to be due in part to its burrowing lifestyle and close association with oil contaminated sediments (*105*). The high DDE concentrations observed in Wenchman and Queen Snapper may also be a result of sampling location and recent onshore use, as is discussed below.

There have not been in-depth diet or xenobiotic metabolism studies conducted on these species, therefore it is difficult to assess which factors may be accounting for the observed variation. The limited information that is available on the diets and habitats of these snapper species does not however appear to explain the observed differences, as Silk Snapper and Wenchman are known to consume small fish/crustaceans and live in the same depth range, however these two species have the most different contaminant burdens. For some species the disparities could also be explained by the small sample sizes and uneven distribution of sample locations, as all species other than Wenchman and Silk Snapper

consisted of $n > 20$ individuals from only a few fishing stations. The species-based variation found by this study is likely produced by a combination of all the above factors.

Biometric and Health Data

The positive linear relationships between PCB and OCP body burdens and biometric attributes (e.g. length, weight, and % lipid) in fishes have been well established by previous studies (*25, 99, 106*). My data further support those observations, with Wenchman exhibiting significant positive correlations between hepatic PCBs/OCPs and weight/length/hepatic lipid. The correlations with size (a proxy for age) are explained by the persistent nature of PCBs and OCPs, which leads to these compounds bioaccumulating over an individual's lifetime (*26, 54*). The lack of correlations between size/percent hepatic lipid and hepatic ΣPAHs in Wenchman is likely due to PAHs being metabolized and eliminated, and therefore not bioaccumulating in lipid stores over an individual's lifetime. Lipophilic compounds such as PCBs and OCPs are known to be stored in the lipid component of tissues (*17, 25*), therefore it is consistent that both ΣPCB and ΣOCP were found to be positively correlated with percent lipid in the hepatic tissues of Wenchman .

It is surprising that these same correlations are not observed in Silk Snapper, although the expected correlations could have been obscured by co-occurring factors such as growth dilution or recent lipid remobilization (*12, 25*). Even more unexpected are the positive correlations between length/weight and hepatic ΣPAHs in Silk Snapper. These correlations with size variables may indicate Silk Snapper are experiencing bioaccumulation of PAHs, where uptake is outpacing their ability to metabolize and excrete these compounds (*18, 25*). One alternative explanation is larger Silk Snapper may be exposed to higher PAH uptake, potentially through dietary changes as body size increases. Distribution of collection locations for Silk Snapper compared to Wenchman may explain why the correlations between PAHs and size only occur in the Silk Snapper data, as less Wenchman were collected in the western side of the sampling area where the highest levels of PAHs were found in Silk Snapper. The lack of significant biotic

and health factor correlations in the other snapper species is likely explained by the low sample sizes for those species.

A weak negative correlation between ΣPAHs and lipid content was observed in Silk Snapper hepatic tissues. This phenomenon which has been observed in other demersal fish species (*10, 96, 107*) is often attributed to high metabolic capacity and low assimilation efficiencies (*18, 108*). The hepato-somatic index (HSI) reflects the relative size of the liver compared to the whole fish. As such, correlations between contaminant concentrations and HSI suggest enlargement (positive correlation) or atrophy (negative correlation) of the liver when exposed to elevated levels of contaminants. The liver serves an important role in energy storage for fish, with higher HSI values thought to indicate increased energetic reserves and overall fitness (*109, 110*). Wenchman HSI was negatively correlated with hepatic ΣPAHs, suggesting that elevated PAH exposure may be leading to liver atrophy and depleted energy reserves in this species. In Silk Snapper, HSI is positively correlated with hepatic ΣPCBs. This correlation has also been observed in Coho Salmon (*Oncorhynchus kisutch*), where Salmon fed PCB-containing diets were found to have higher HSI values (*111*), and could indicate that elevated PCB exposure may be causing enlargement of Silk Snapper livers. Although the mechanism behind these correlations cannot be definitively determined from the data available in this study, these results indicate that exposure to elevated levels of these contaminants are affecting the organ and tissue structures of these species.

Interpretation of HSI and other biometric matrices is complicated by the likely co-occurrence of a number of other xenobiotics and effects of various anthropogenic stressors (*112*). For example, past research has shown there is fair amount of municipal sewage released offshore of Havana (*68, 75*), and elevated HSI in freshwater fish has been found to correlate with exposure to municipal sewage effluent (*113*). Because elevated levels of PCBs and municipal sewage likely co-occur in this study, this limits interpretation of the positive correlation found between ΣPCB and HSI in Silk Snapper. Additional factors which are unrelated to pollutant stressors such as sex and seasonal cycles can complicate interpretation of observed trends as well by affecting accumulation of contaminants, HSI, and condition factor (*24, 110, 112*).

Fulton's condition factor (K) is used as a general measure of fish condition, with a higher K value assumed to indicate an individual is well-fed, healthy, and therefore may have higher overall fitness (*91, 114*). The negative correlations observed between ΣPCB and K in both Wenchman and Silk Snapper implies that fish with elevated hepatic PCBs tend to have a lower body weight for their length, and therefore indicates a potential decline in fish health with increasing PCB burdens. Elevated PCB concentrations in snapper in this study may be resulting in weight loss and reduced condition by effecting the metabolism or general health of the organism, as exposure to xenobiotics has been found to increase energetic costs as well as an individual's susceptibility to parasites and diseases (*96, 115-117*). Condition factors are however also influenced by other co-occurring variables such as prey availability, sex, capture date relative to reproductive season, and age (*114, 118, 119*). The highest ΣPCB values in my study were measured in fish from near the city of Havana, where heavy fishing pressure and high levels of other anthropogenic pollutants occur (*60, 61, 65, 66, 68, 75*).

Both Wenchman and Yellowtail Snapper females had significantly lower hepatic ΣPAHs than males. No significant differences in hepatic lipid or length were observed between males and females in any of the species in this study, so it is unlikely those variables are driving the observed sex-based differences in contaminant loads. These results might suggest the occurrence of maternal offloading; where PAHs are being remobilized with lipids and transferred to eggs during female gametogenesis (*17*). The possibility of maternal offloading occurring in Wenchman and Yellowtail Snapper could result in reduced reproductive success for these species due to the negative health impacts of elevated PAH concentrations in the eggs and developing larvae (*15, 36*). Decreases in reproductive success for these fish could then result in impacts to population size for these overfished species (*61, 62, 64*). Samples for this study were collected between May and August when Wenchman and Yellowtail Snapper are known to spawn (*82, 85*), therefore it is likely that any maternal offloading occurring in the population would be reflected by the individuals sampled. It would be expected that any maternal offloading occurring in the population would be reflected across all lipophilic compounds present (as has been observed in other species (*120*)), so it is surprising that sex-based differences were not observed in PCB or OCP

concentrations. Maternal transfer of PAHs has been observed in a number of fish species including feral mesopelagic fishes from the GoM, and the effects on larval fish are known to include decreased growth and survival rates (*121-124*).

The concentrations of PCBs, OCPs and PAHs observed in these snapper populations are below the concentrations generally found to cause mortality, but within the range of concentrations known to result in sublethal effects for some fish species (*125, 126*). A recent meta-analysis of information on PCB toxicity in fish concluded that significant effects on mortality, reproduction, and growth are supported at whole body concentrations above 100 ng g^{-1} w.w. ΣPCBs (*127*). The upper range of hepatic concentrations measured in this study is over 100 ng g^{-1} w.w., although these parameters were reported for whole body concentrations while this study assessed hepatic tissue. Whole body concentrations are likely to be lower than hepatic, however this still serves as a useful threshold for evaluating potential PCB impacts. The recommended threshold for mortality impacts of DDT in fish is 600 ng g^{-1} (whole body concentration) (*128*). The DDT concentrations measured in my study are 3x lower than this recommended threshold, however this threshold does not account for any sublethal effects, which are known to occur at lower concentrations. Past studies on GoM demersal fishes have suggested that hepatic PAH concentrations within the same general range found in this study may be resulting in sublethal effects (*10, 107*). These studies have found that fish with hepatic PAH concentrations at the high end of those observed in this study exhibit signs of sublethal effects such as immunotoxicity and changes in hepatic histology (*9, 96*).

Gulf-Wide Spatial Trends

Wenchman were the only snapper species collected in all seven regions of the Gulf, therefore analysis Gulf-wide spatial trends focused mainly on this species. Hepatic PCBs in Wenchman did vary between regions, with relative abundance profiles in the northwest and Cuban regions less dominated by PCB 153, PCB 179, and PCB 170 than in the southwest region (Figure 9a). Wenchman from the Yucatán Peninsula appear to have a more even distribution of PCB congener abundance; however this may be an

artifact of the low sample size from that region (n = 4). The relative abundance profiles for hepatic OCPs in Wenchman from the Cuban and Yucatán Peninsula regions are dominated by DDE, while the northwest and southwest regions contain a greater proportion of metolachlor (dominant in the northwest region) and propachlor (dominant in the southwest region), as well as other OCPs such as dieldrin, endosulfan, and methoxychlor (Figure 9b). The regional differences observed in hepatic OCP compositions of Wenchman are likely driven by variation in onshore use of these pesticides. For example, propachlor and metolachlor are still in use as herbicides for crops such as corn which are commonly grown in the U.S., and DDT (of which DDE is a metabolite) is still used as an insecticide in Caribbean countries. Hepatic PAH compositions in Wenchman from all regions of the Gulf were dominated by low molecular weight PAHs, with the most abundant being dibenzothiophene and anthracene homologs, followed by naphthalene homologs (Figure 9c).

Concentrations of hepatic PCBs tend to vary predictably across latitudes due to the global fractionation effect (*59*), which transports PCBs across latitudes based on volatility and temperature. The relatively small latitudinal range of the GoM is however unlikely to exhibit significant PCB fractionation along this globally-scaled gradient. The regional differences in ΣPCBs (Figure 8) and PCB composition (Figures 9 and 10) in this study are therefore likely driven by more local variations in sources and/or transport of these compounds. The lower ΣPCBs observed in Wenchman from the SW region could be due to the relatively low density of industrial activity in the adjacent land mass which drains into this region of the Gulf (*129*). In contrast, the NC and NW regions have relatively higher density development onshore, and snapper collected in the NW region had relatively high hepatic ΣPCBs. Offshore transport is one of the dominant transport patterns in the NW region of the Gulf (*130*), and this may be contributing to the relatively high levels of PCBs and OCPs observed in snapper from the NW region.

Wenchman collected off Cuba had the highest levels of hepatic PCBs and OCPs compared to other GoM regions in this study. The elevated PCBs and OCPs observed in the Cuban region are likely driven in part by variation in the distances of sampling locations from land. Fishing locations were selected along transects extending from shallow (~40 m) to deep (~300 m) depths across the continental

shelf. The majority of the GoM has a very wide gradually sloping continental shelf, but the Cuban region has a very narrow and steep sloped continental shelf. This required all the fishing stations within the Cuban region to be located much closer to shore compared to those in other Gulf regions (*3*). Most anthropogenic contaminants occur in relatively higher concentrations in coastal zones, with water quality and anthropogenic influence known to improve with distance offshore (*11*). This is especially true for lipophilic contaminants such as PCBs, OCPs and PAHs which are often stored in lithogenous sediments (*42*). Although the elevated levels of PCBs and OCPs observed in snapper from the Cuban region may have been driven by distance from shore that does not diminish the importance of this observation, as it implies that Cuban fish populations may be universally exposed to contaminant levels experienced only by inshore populations elsewhere in the GoM.

Differences in historical and continued use around the GoM and wider Caribbean could also be contributing to the regional variation observed in both OCPs and PCBs. For example, the continued or recent use of DDT (*52*) likely explains the high concentrations of DDT and its metabolites DDD and DDE in the Cuban region compared to the rest of the Gulf. These high concentrations of DDT/DDD/DDE in the Cuban region in turn drive the compositional differences observed between regions, as DDT and its metabolites DDD and DDE make up the highest percentage of the Cuban regions total OCPs, whereas other regions are dominated by other OCPs such as propachlor (Figures 9 and 10). Another factor which may play a part in the high OCP and PCB levels observed in the Cuban region may be the currents in that area. Aquatic pollution could be transported into Cuban fisheries via the Yucatán Channel and Florida Straits, with contaminants discharged by Caribbean countries or other regions of the Gulf circulated along the Cuban coast.

Regional variation in onshore PCB use is convoluted to interpret. The use of PCBs was banned in the U.S. in 1979, in Mexico in 1988 (*53, 57*), and Cuban import likely decreased dramatically after the collapse of the Soviet Union in the early 1990s. Although PCBs were banned and are no longer commercially available, they may still be present in products produced prior to the ban, and minor inputs from routine leaks in transformers, landfills, etc. are expected to continue in all regions of the Gulf (*41,*

53). In addition, heavy historical use of both PCB and OCPs likely resulted in persistent environmental reservoirs which will continue to cycle for decades (*41*), further obscuring the effect of when these compounds were banned in each region.

The only significant regional differences found in ΣPAHs were comparing the NC region to other regions. These results are likely unreliable artifacts of the extremely small sample size (n = 3) from the NC region, and should be interpreted with caution. The lack of any reliable regional differences in ΣPAHs is likely explained by the high metabolic capacity of fish for these compounds, as well as having low sample sizes from areas such as the NC region. Previous studies have indicated fishes from the NC region of the Gulf may be experiencing elevated levels of chronic PAH exposure (*9*, *31*, *89*). In addition, the multitude of sources for PAHs in all regions of the GoM has likely contributed to ubiquitous distributions of these compounds across these GoM snapper fisheries (*9*).

Comparing the results of multiple studies can be challenging due to disparities in methodology and reporting (*131*), however the contaminant concentrations measured here in the GoM appear similar to those found in other regions of the world. Hepatic samples from fish in the Belgian North Sea were found to have individual PCBs from <DL to 530 ng g^{-1} w.w. DDT between 0.08 and 26 ng g^{-1} w.w., DDE from 1.7 to 270 ng g^{-1} w.w., and DDD between 0.6 and 120 ng g^{-1} w.w. (*102*). In comparison, the snapper analyzed by this study were found to have hepatic concentrations of individual PCBs within the same order of magnitude but a bit lower (from <MDL to 103 ng g^{-1} wet weight (w.w.)), DDT (between <MDL and 53.4 ng g^{-1} w.w.), DDE (from <MDL to 238 ng g^{-1} w.w.), and DDD (between <MDL and 6.0 ng g^{-1} w.w.) in this study had almost the same ranges as the Belgian samples. In 1996 whole fish from Columbia were found to contain 0.7 to 0.78 ng g^{-1} w.w. of DDT (*53*), these lower levels are likely explained by the analysis of whole body concentration compared to liver tissue concentration.

There is a significant lack of PAH data from fish populations in the wider Caribbean region (*131*), however the values observed by this study are within the same general range of hepatic PAHs observed in fish from other areas of the world. Whole fish from Nigeria were determined to have individual PAHs ranging from <DL to 29,000 ng g^{-1} d.w. (*132*). Mean concentrations of individual

parental PAHs in muscle tissue of coastal fish in Ghana ranged from <DL to 54 ng g^{-1} w.w. (*133*). Data collected on PAHs in estuarine fish livers from Europe found individual parental PAH concentrations between <DL and 166 ng g^{-1} whole weight in liver tissue (*134*). Finfish from coastal Bangladesh had individual PAHs between <LOD and 1,540 ng g^{-1} w.w. (*135*). These data sets report concentrations for individual PAHs to be in the same general range and order of magnitude and as those seen in this study (<MDL to 1,870 ng g^{-1} w.w.). The dominance of low molecular weight PAHs is a pattern consistent with other data sets of PAHs in fish. Because coastal zones generally contain higher levels of anthropogenic contaminants (*11*), the similarity observed between PAH concentrations in GoM fish and coastal fish from other areas suggests that GoM fish may be experiencing relatively high levels of PAH exposure.

Cuban Spatial Trends

Hepatic ΣPCBs concentrations in Wenchman from the Cuban region are significantly higher at the eastern locations near Havana and decline rapidly further to the west (Figure 11). The sampling area in the Cuban region extends from the heavily developed capital city of Havana in the east (Transect 44), to the rural conservation area known as Guanahacabibes National Park at the western most tip of Cuba (Transects 37 and SAB) (Figure 2). Increased PCB contamination is associated with heavy industry and urban development, so the gradient observed in Wenchman hepatic ΣPCBs is likely driven by the corresponding gradient in density of development and industrialization onshore. This explanation is further supported by the negative correlation (r = -0.688, p < 0.001) between hepatic ΣPCB loads in Wenchman and distance (km) from the mouth of Havana Harbor (Figure 12). Past studies have found similar trends, with impacts from anthropogenic contaminants decreasing further from Havana (*63, 68, 74, 75*). Concentrations of PCBs in onshore sediments have also been found to decrease with distance from Havana (*136*), demonstrating similar patterns in onshore pollution that corresponds to those found offshore. The tightly coupled onshore and offshore gradients (in development/industry and contaminant load, respectively) suggests that PCB contamination in Cuban Wenchman is driven mainly by local onshore sources. The existence of such clear spatial variation in hepatic PCBs additionally suggests that

both the fish and PCB contamination within the marine environment are staying localized and not migrating or being transported far along the Cuban coastline.

A similar pattern of elevated levels in Wenchman near Havana was observed in DDT and its metabolites (DDE and DDD). The high levels found offshore of Havana are likely driven by both recent and historical spraying of DDT in residential and commercial areas (*76, 131*). In addition to any recent onshore use of these contaminants, the sediment transported out of Havana harbor and the neighboring Almendares River is likely acting as a reservoir of historical DDT and PCBs in the marine benthos (*43, 68*). The lack of spatial patterns in other OCPs within the Cuban region could be the result of a combination of low sample sizes and conflicting point sources.

There are many potential PAH sources along the Gulf-facing coastline of Cuba, including onshore combustion (motors, power plants, etc.), oil industry (drilling, refineries, etc.) (*137*), and boat engines (local traffic and nearby international shipping channels). In addition, there are naturally occurring seeps and dense oil drilling in other areas of the Gulf (68), from which hydrocarbons could be transported to the Cuban shelf by the Gulf Stream, Florida Straits, and associated eddies. The elevated ΣPAH concentrations found in Silk Snapper from the western edge (Transects 37 and 38) of the sampling area (Figure 13) may be driven by proximity to major shipping lanes and naturally occurring offshore seeps. Shipping traffic produces PAHs via incomplete combustion of engine fuel, as well as accidental spills and leaks during petroleum transportation, and the western edge of the Cuban sampling area is in close proximity to the dense shipping traffic passing through the Yucatán Channel (*31*). Elevated PAHs in the area near the Yucatán Channel have been observed in other studies as well, with both sediment and fish datasets exhibiting elevated concentrations of PAHs on the side of the Yucatán Peninsula which is closest to the Yucatán Channel (*31, 89*). The lack of spatial trends observed in Wenchman hepatic PAHs along the Cuban coastline is likely explained by the high capacity of this species to metabolize PAHs, as any spatial variation in PAH exposure would likely not appear as accumulation in the hepatic tissue so long as the levels of exposure throughout the area are below the maximum capacity for metabolism (*17*).

An alternative explanation could be that Wenchman are more mobile than Silk Snapper, however the existence of clear spatial gradients of other contaminants in Wenchman argues against this explanation.

Conclusions

Consideration of the many anthropogenic factors affecting the GoM is an important part of sustainable ecosystem management. Many critical knowledge gaps remain in the Cuban portion of the GoM, including understanding the presence and impacts of organic contaminants. This study addresses a portion of that gap by assessing the presence of three types of problematic contaminants in important Cuban fishes. These results indicate significant differences in hepatic PCB, OCP and PAH burdens between species analyzed. This variation between species has been observed in previous studies; and is likely driven by species-specific metabolism and elimination rates. Correlations between ΣPAHs and biometric factors in Silk Snapper suggest PAH exposure rates may be outpacing PAH metabolism; and are therefore seeing increased liver concentrations. Condition factor in Wenchman and Silk Snapper is negatively correlated with hepatic ΣPCB concentrations, indicating the potential for elevated PCB concentrations to lower the overall fitness of these species. In Wenchman the negative correlation between HSI and ΣPAHs also suggest reduced energy reserves in individuals exposed to high PAH concentrations. Lower hepatic ΣPAH concentrations were observed in female Wenchman and Yellowtail Snapper compared to males. This is consistent with the hypothesis that these contaminants are being offloaded into eggs, although I did not evaluate contaminant concentrations in reproductive products. The correlations between organic contaminants and biotic/health factors observed in this study suggest that snapper populations exposed to elevated concentrations of these contaminants may thus be experiencing a range of sub-lethal negative health effects. These effects have the potential to be manifested at a population level. While the population-level implications of these sub-lethal effects can be hard to quantify, modeling of the potential impacts of elevated exposure on lifetime egg production, hatching success and larval outcomes is an obvious next step.

Spatial differences in organic contaminants are likely driven by variation in onshore point and non-point sources of pollution, riverine input, and proximity to marine vessel thoroughfares. The higher hepatic ΣPCBs and ΣOCPs found in Wenchman from the Cuban region, compared to other regions of the Gulf, may be due to the narrow continental shelf compressing Cuban snapper populations much closer to shore. The slower phasing out of PCBs and some insecticides in Cuba likely plays a role as well. Within the Cuban region PCBs and DDT/DDD/DDE in Wenchman appear to be reflecting local gradients in onshore development, with elevated levels observed offshore of Havana at the eastern edge of the sampling area. The opposite gradient is observed in Silk Snapper ΣPAHs, with high levels at the western tip of Cuba potentially driven by vessel-related pollution from the nearby Yucatán Channel and natural seeps.

Almost all fish included in this study contained detectable quantities of all three groups of these contaminants, regardless of location, and across all regions of the Gulf, indicating that PCB, OCP, and PAH contamination in fishes is ubiquitous in the GoM. As such, continued monitoring of these compounds is needed in all three countries, and the potential impacts of these contaminants should be considered when assessing the health of GoM fisheries. A Gulf-wide baseline for these contaminants is needed in order to understand their presence and fate, as well as any potential impacts they may be having on GoM biota. This data also provides critical baseline data for assessing the impact of any future pollution events or changes in contaminant inputs within this area. The information presented by this study will contribute to the larger base of knowledge on organic contaminants in the Gulf of Mexico; and will assist in future management decisions for Cuban fisheries within the study area.

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Appendix A: Tables and Figures

Table 1. Range of body measurements and hepatic POP concentrations (ng g^{-1} w.w.) observed for seven species of snapper sampled from around the Gulf of Mexico in 2015-2017.

Species	***n***	**Standard Length (cm)**	**Weight (kg)**	**Hepatic Lipid (%)**	**Σ_{32} PCBs**	**Σ_{32} OCPs**	**Σ_{46} PAHs**
Wenchman *(Pristipomoides aquilonaris)*	89	16 – 43	0.1 – 1.8	2 – 36	0.99 - 427	4.00 - 247	93.0 – 2,330
Silk Snapper *(Lutjanus vivanus)*	48	31 – 66	0.7 – 8.6	2 – 24	0.80 – 36.8	4.44 – 64.6	60.0 - 2990
Yellowtail Snapper *(Ocyurus chrysurus)*	19	23 – 41	0.3 – 1.6	2 – 17	0.80 – 37.7	5.00 – 16.4	158 – 2,140
Blackfin Snapper *(Lutjanus buccanella)*	17	24 – 52	0.4 – 5.0	5 – 20	0.84 – 30.7	4.00 – 23.0	112 – 2,350
Queen Snapper *(Etelis oculatus)*	11	31 – 55	0.6 – 3.5	4 – 17	2.00 – 41.3	8.00 - 155	409 – 2,930
Black Snapper *(Apsilus dentatus)*	3	30 – 42	0.8 – 2.2	3 – 5	1.15 – 2.10	6.82 – 13.8	523 – 1,500
Mutton Snapper *(Lutjanus analis)*	3	42 – 47	2.1 – 2.8	5 – 5	0.98 – 62.3	7.17 – 10.6	340 – 1,660

Σ_{32}PCB = sum of 32 polychlorinated biphenyls

Σ_{32}OCP = sum of 32 organochlorine pesticides

Σ_{46}PAH = sum of 46 parental polycyclic aromatic hydrocarbons and alkylated homologues.

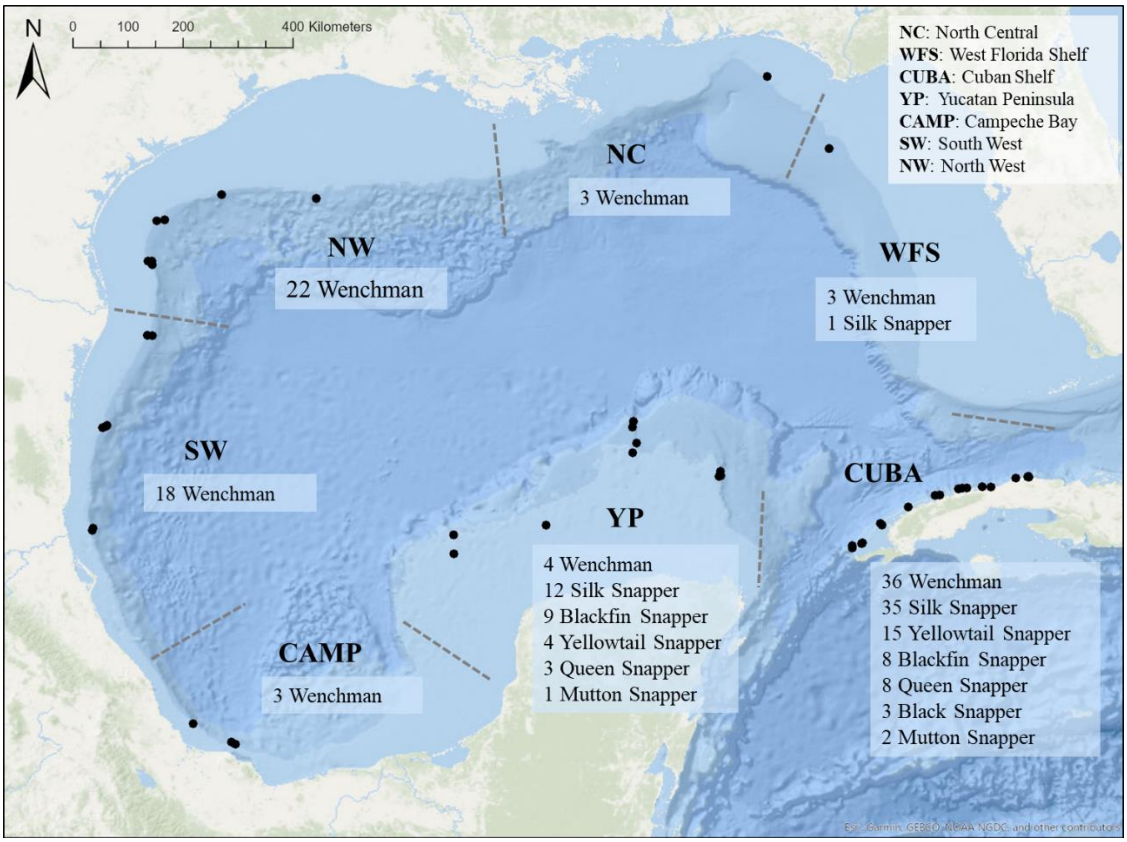

Figure 1. Station locations sampled in the Gulf of Mexico during 2015-2017 for chemical analyses. Each station is represented by a black dot; stations were grouped into regions (designated by gray dashed lines) for spatial analysis. Numbers of specimens by species and region evaluated for organic pollutants are indicated.

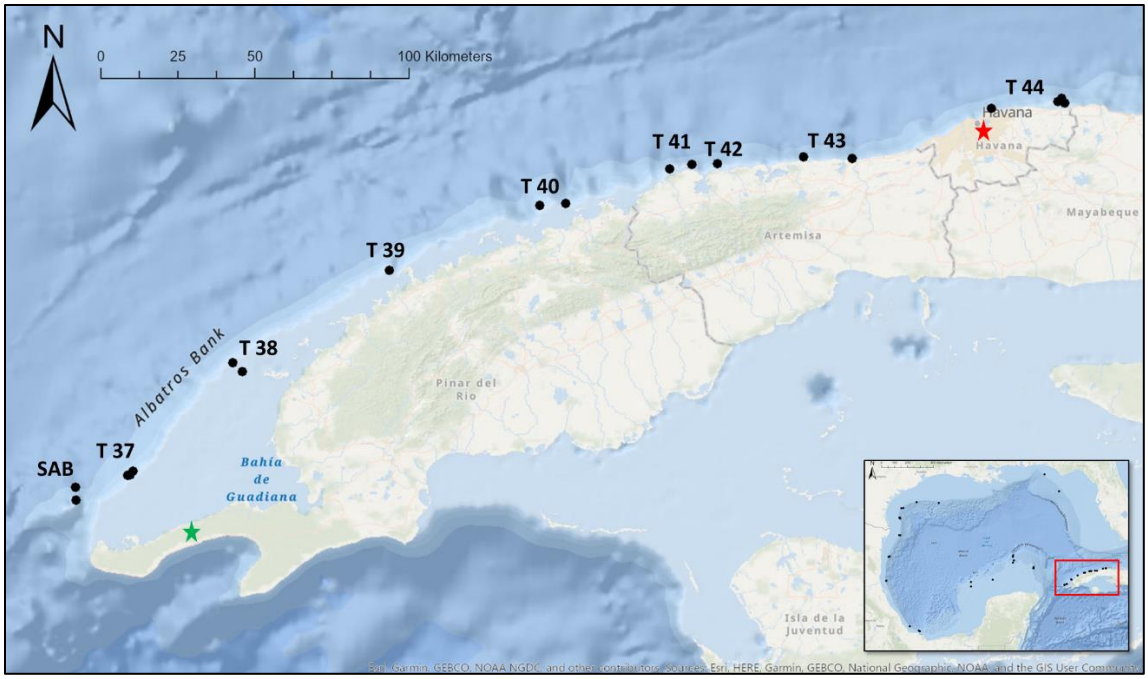

Figure 2. Sampling locations (black dots) grouped into transects (designated by letter and number) where snapper were obtained for chemical analysis off the northwest Cuban coast in 2017. Havana is designated by the red star, and Guanahacabibes national park is designated by the green star.

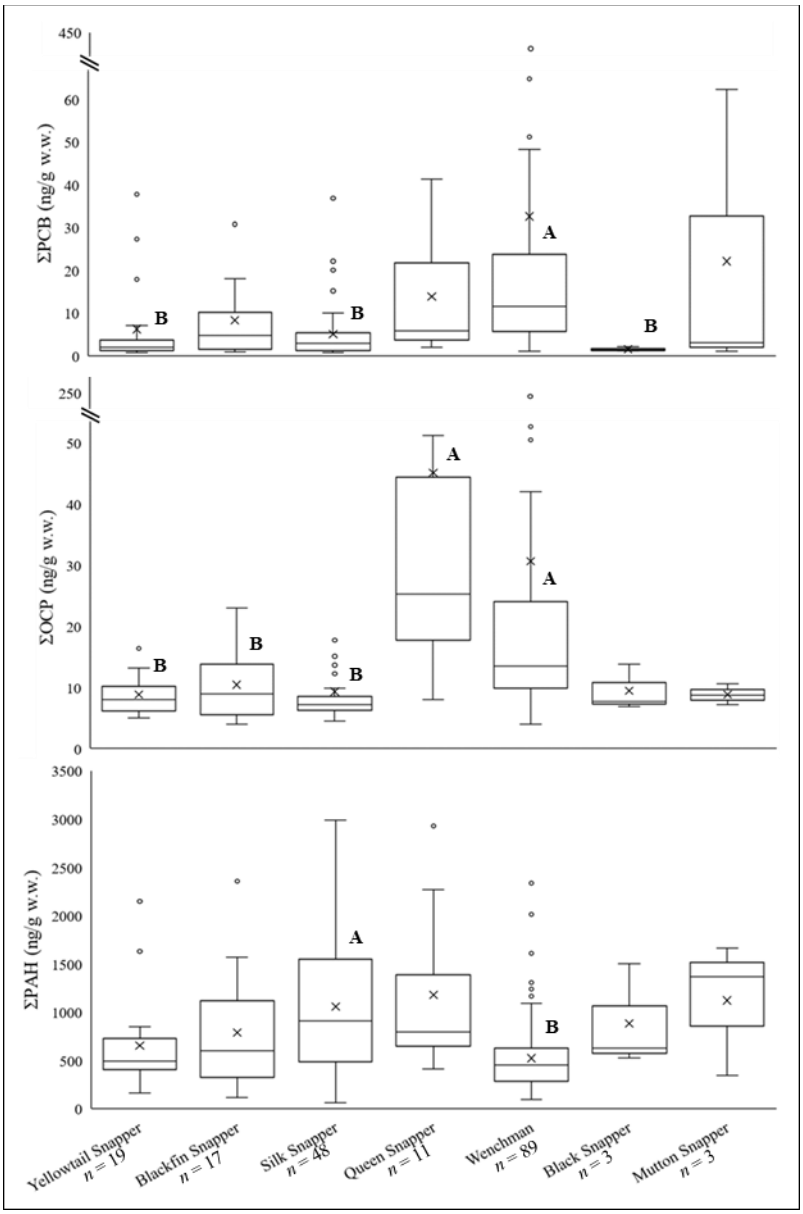

Figure 3. Gulf-wide species comparison of hepatic ΣPCBs, ΣOCPs, and ΣPAHs (ng g^{-1} w.w.) measured in seven snapper species. Significant differences between species are indicated by different letter designations. Open circles indicate outliers for each group. Some outliers fall in the y-axis break and are not shown.

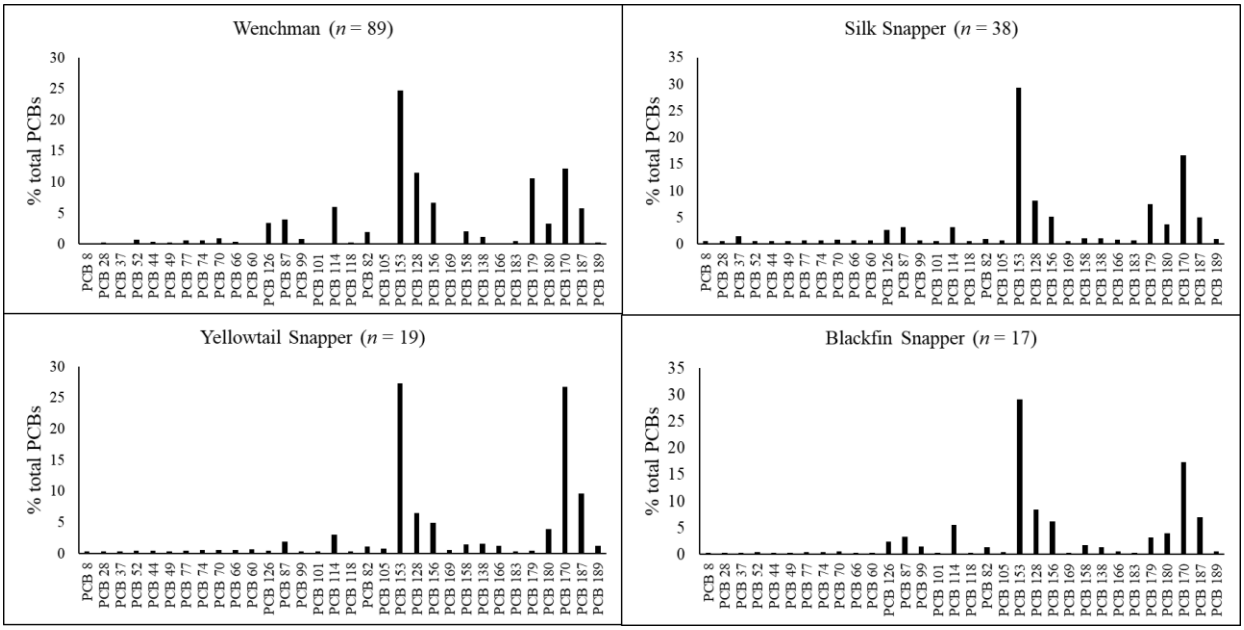

Figure 4A. Gulf-wide species comparison of the percent composition of hepatic PCBs measured in the four most abundant snapper species.

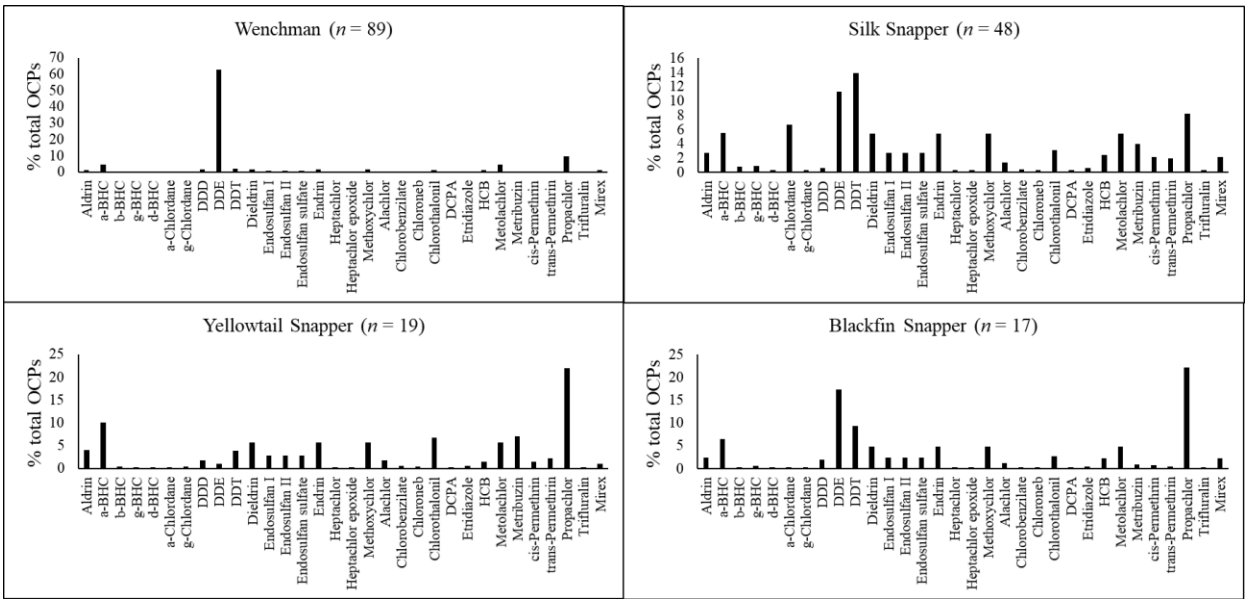

Figure 4B. Gulf-wide species comparison of the percent composition of hepatic OCPs measured in the four most abundant snapper species.

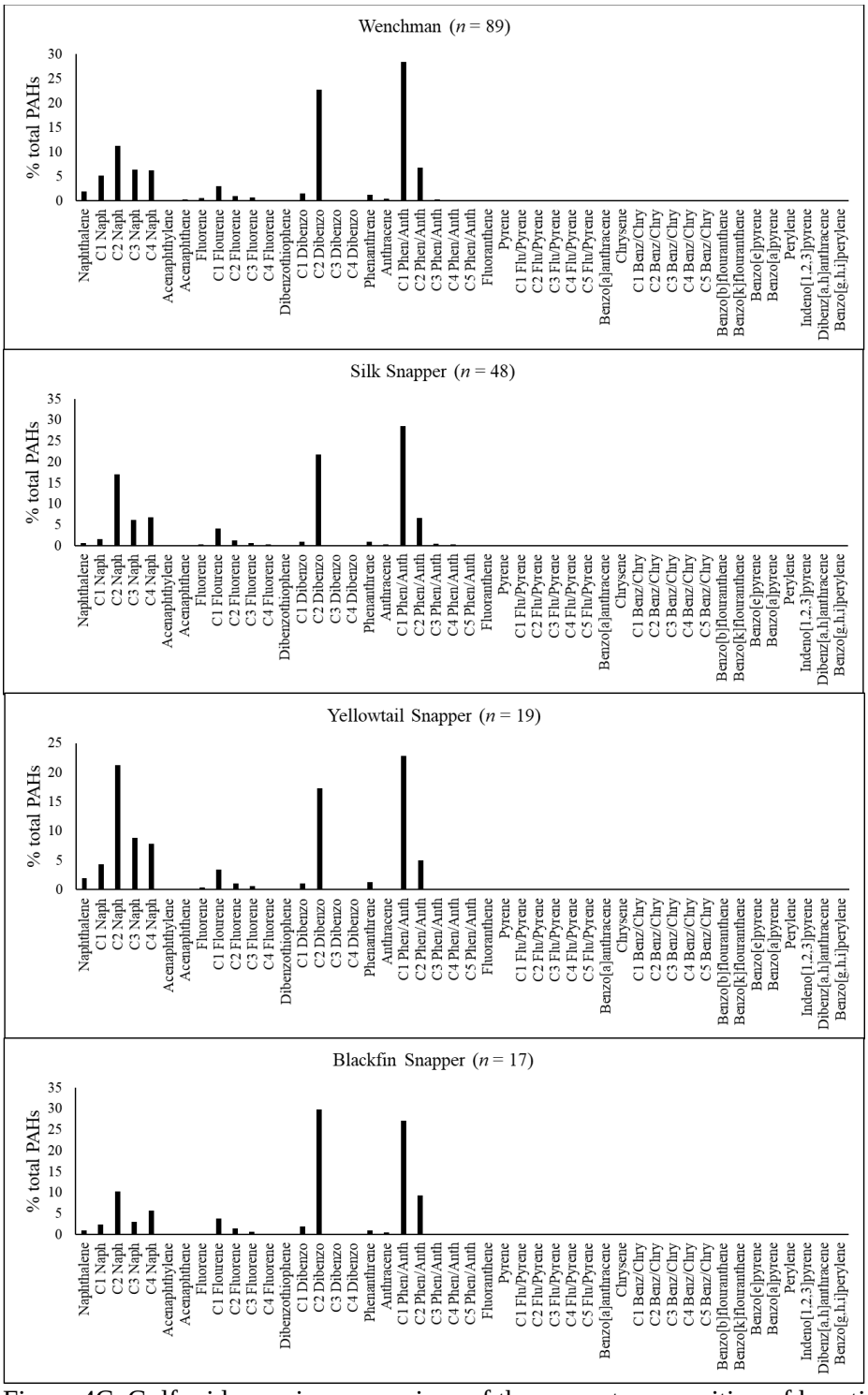

Figure 4C. Gulf-wide species comparison of the percent composition of hepatic PAHs measured in the four most abundant snapper species.

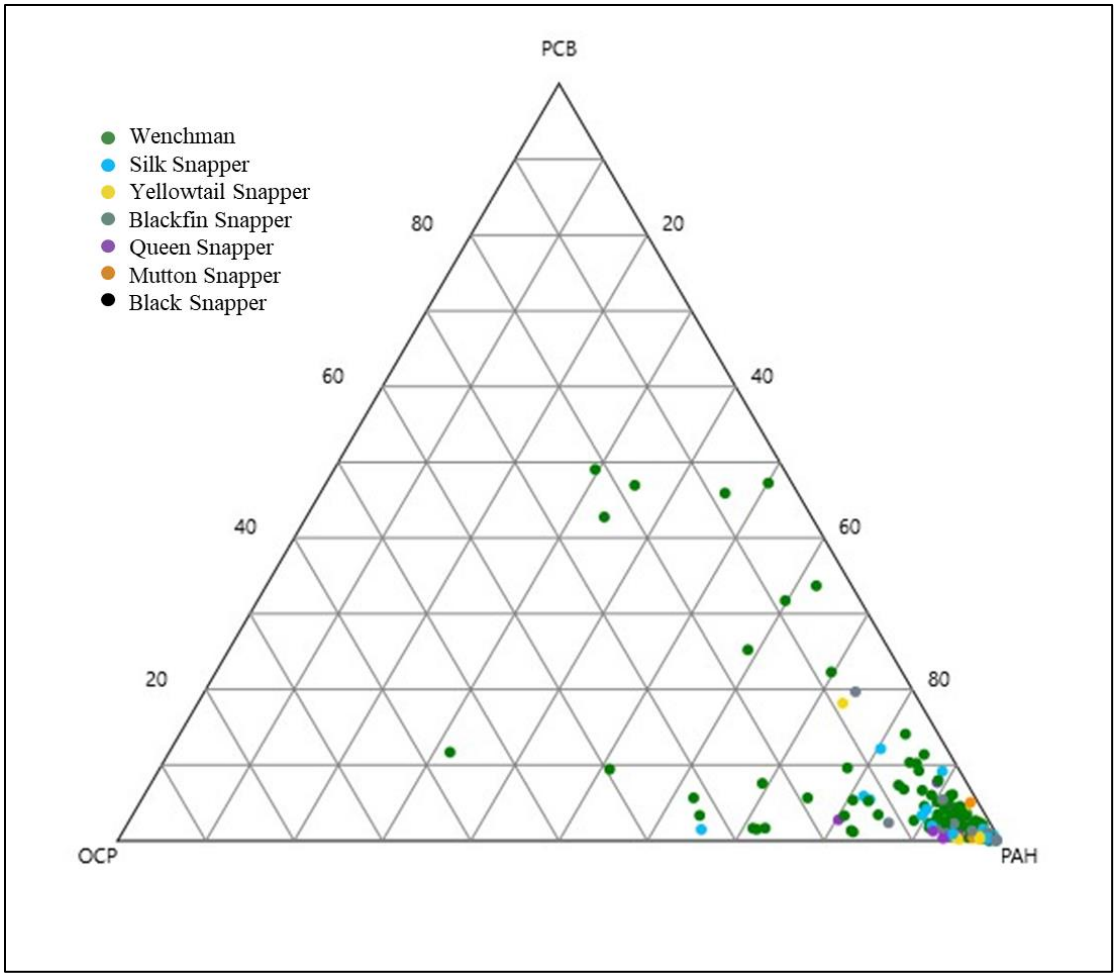

Figure 5. Ternary plot of PCBs, OCPs and PAHs in seven species of Snapper (*n* = 189) from around the GoM. Dots represent individual fish, with snapper species designated by color. The compound groups represent fractions of the total organic contaminants detected.

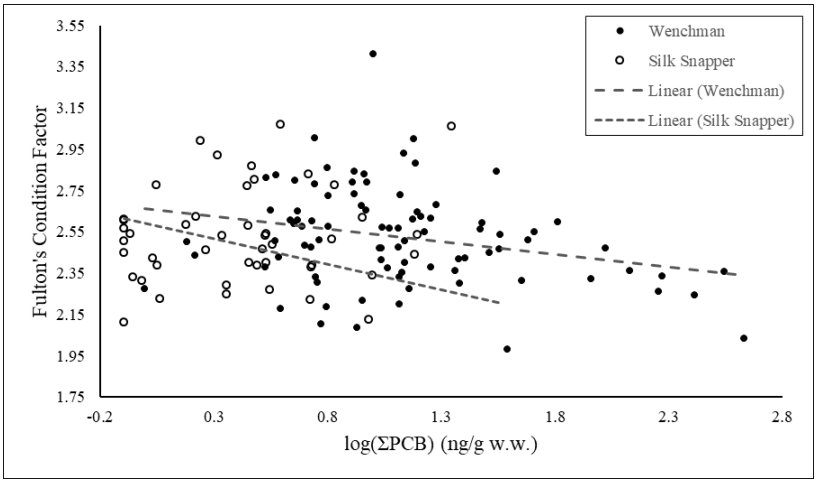

Figure 6. Log transformed Wenchman (*Pristipomoides aquilonaris*, *n* = 89) and Silk Snapper (*Lutjanus vivanus*, *n* = 48) hepatic ΣPCBs (ng g^{-1} w.w.) plotted against Fulton's condition factor. Wenchman data are indicated by solid dots; Silk Snapper by open circles. Linear correlations for each species are shown by dashed lines, with r = -0.332, *p* = 0.006 for Wenchman, and r = -0.542, *p* = 0.008 for Silk Snapper.

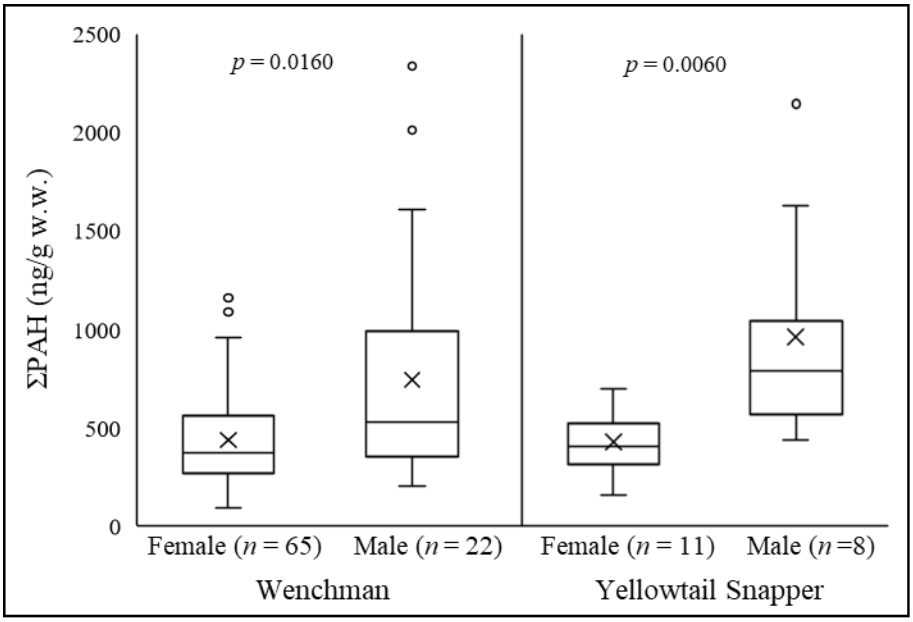

Figure 7. Comparison of hepatic ΣPAHs (ng g^{-1} w.w.) between male and female Wenchman (*Pristipomoides aquilonaris*, *n* = 87) and Yellowtail Snapper (*Ocyurus chrysurus*, *n* = 19) sampled in the Gulf of Mexico, 2015 - 2017. Open circles indicate outliers for each group.

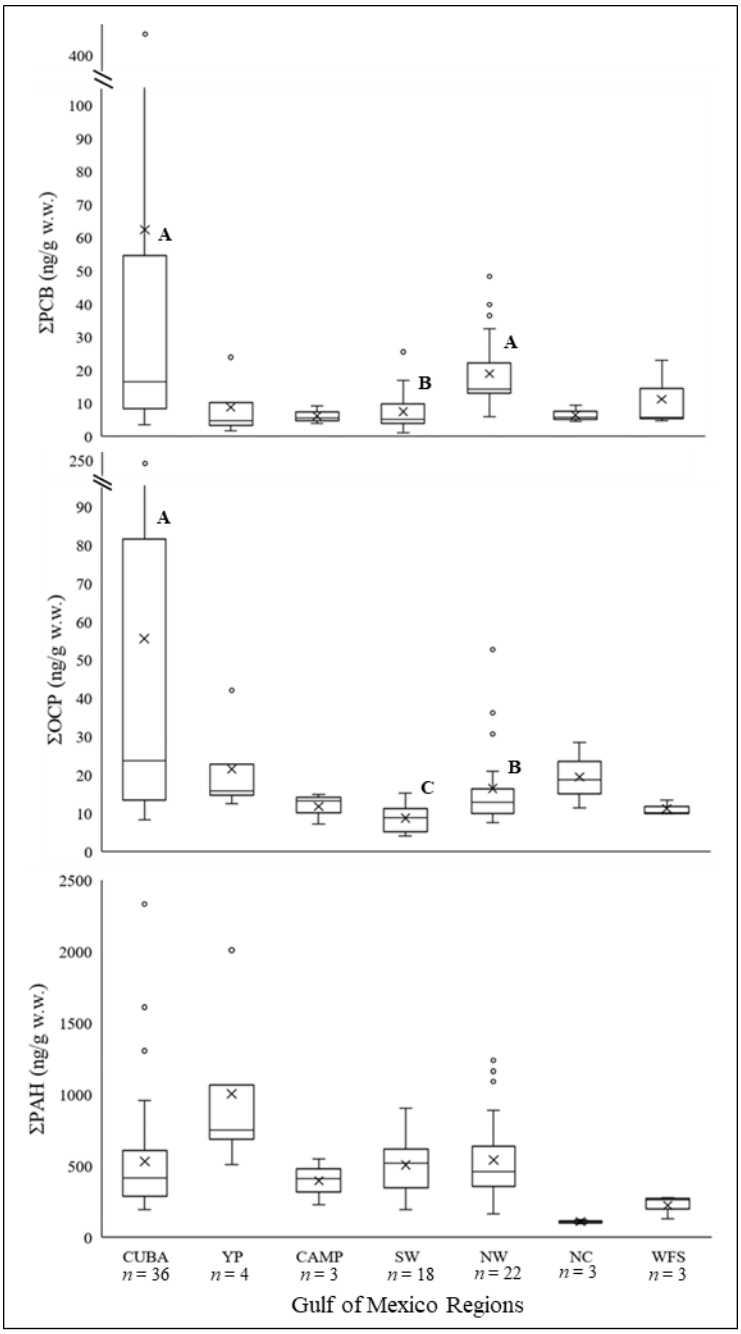

Figure 8. Gulf-wide regional comparison of hepatic ΣPCBs, ΣOCPs, and ΣPAHs (ng g^{-1} w.w.) measured in Wenchman (*Pristipomoides aquilonaris*, *n* = 89) sampled from the seven Gulf regions. Regions are designated (Figure 2) as: Cuba (CUBA), Yucatan peninsula (YP), Campeche Bay (CAMP), southwest (SW), northwest (NW), north central (NC), and west Florida shelf (WFS). Open circles indicate outliers for each group. Some outliers fall in the y-axis break and so are not shown.

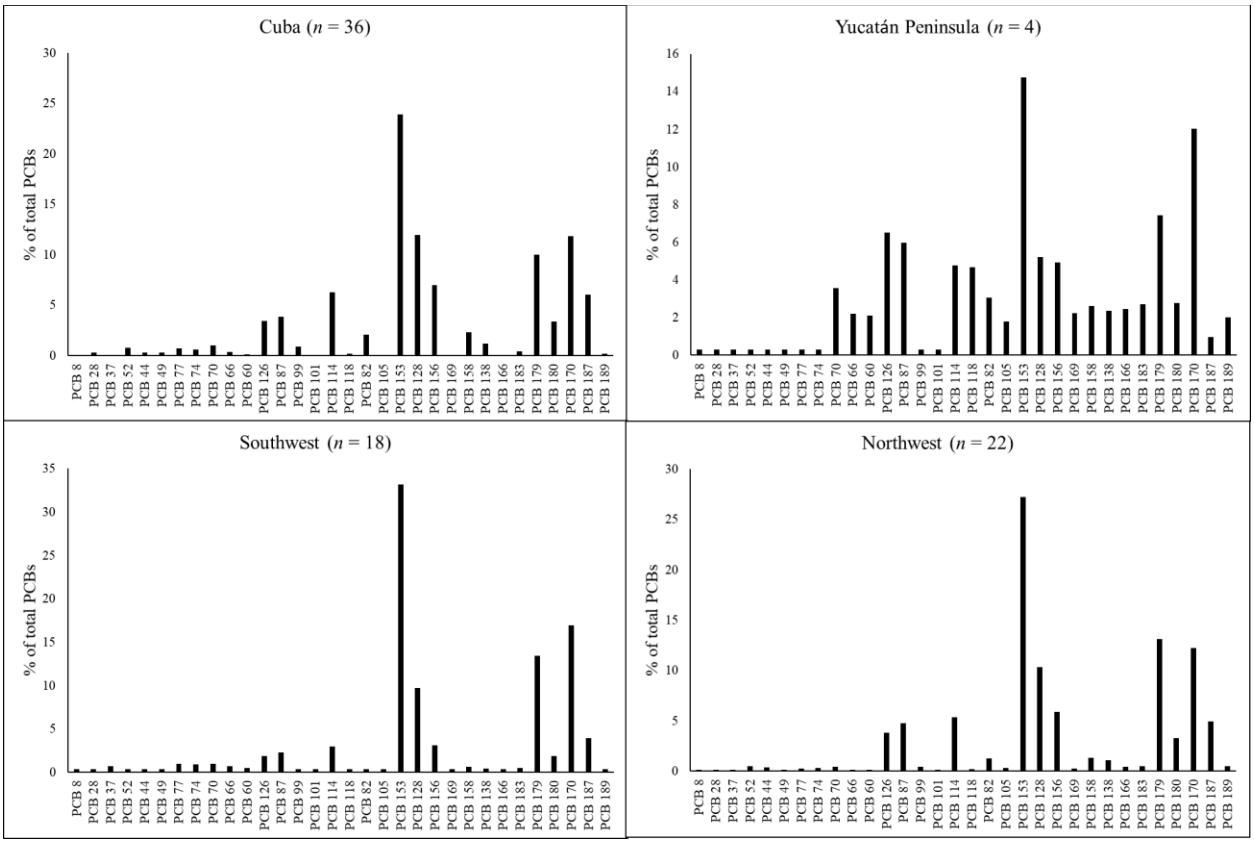

Figure 9A. Regional comparison of hepatic PCB relative abundances (percent of total) measured in Wenchman (*Pristipomoides aquilonaris, n* = 89) sampled from regions around the Gulf. Only the regions with Wenchman *n* > 3 were included.

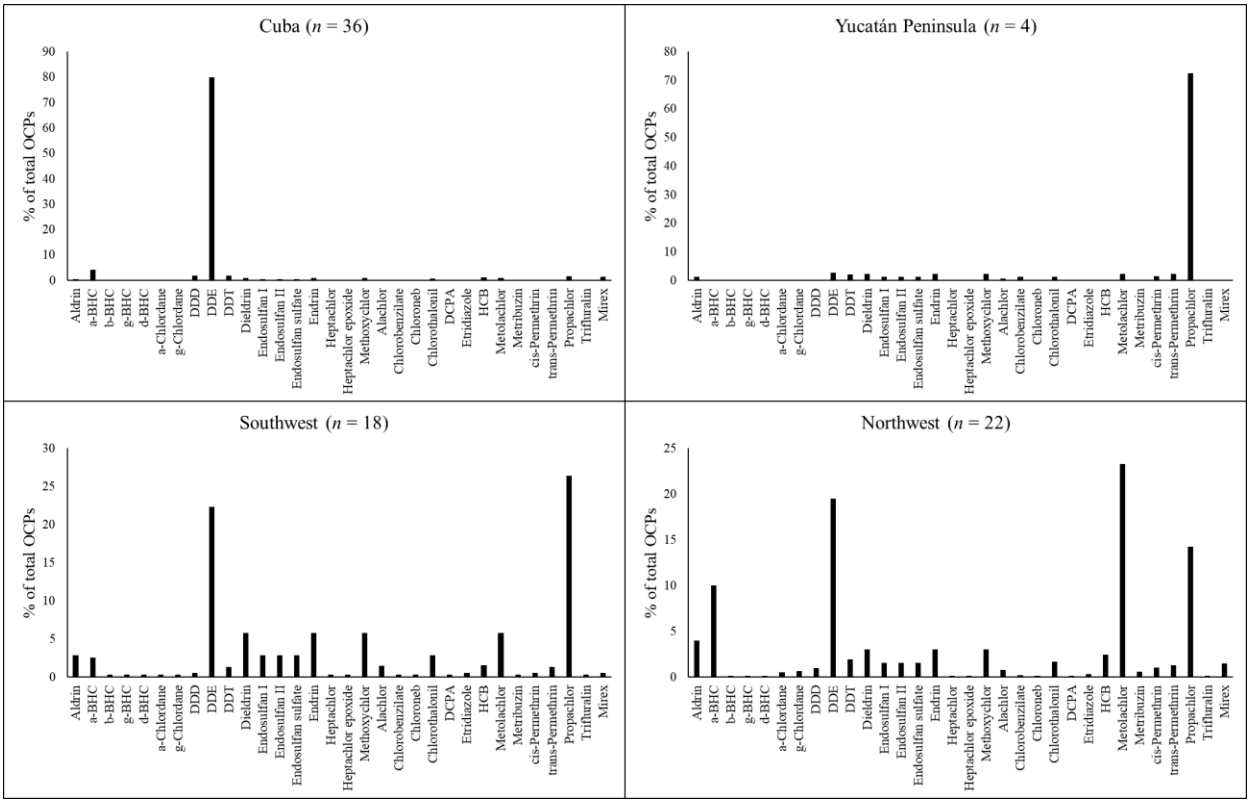

Figure 9B. Regional comparison of hepatic OCP relative abundances (percent of total) measured in Wenchman (*Pristipomoides aquilonaris*, $n = 89$) sampled from regions around the Gulf. Only the regions with Wenchman $n > 3$ were included.

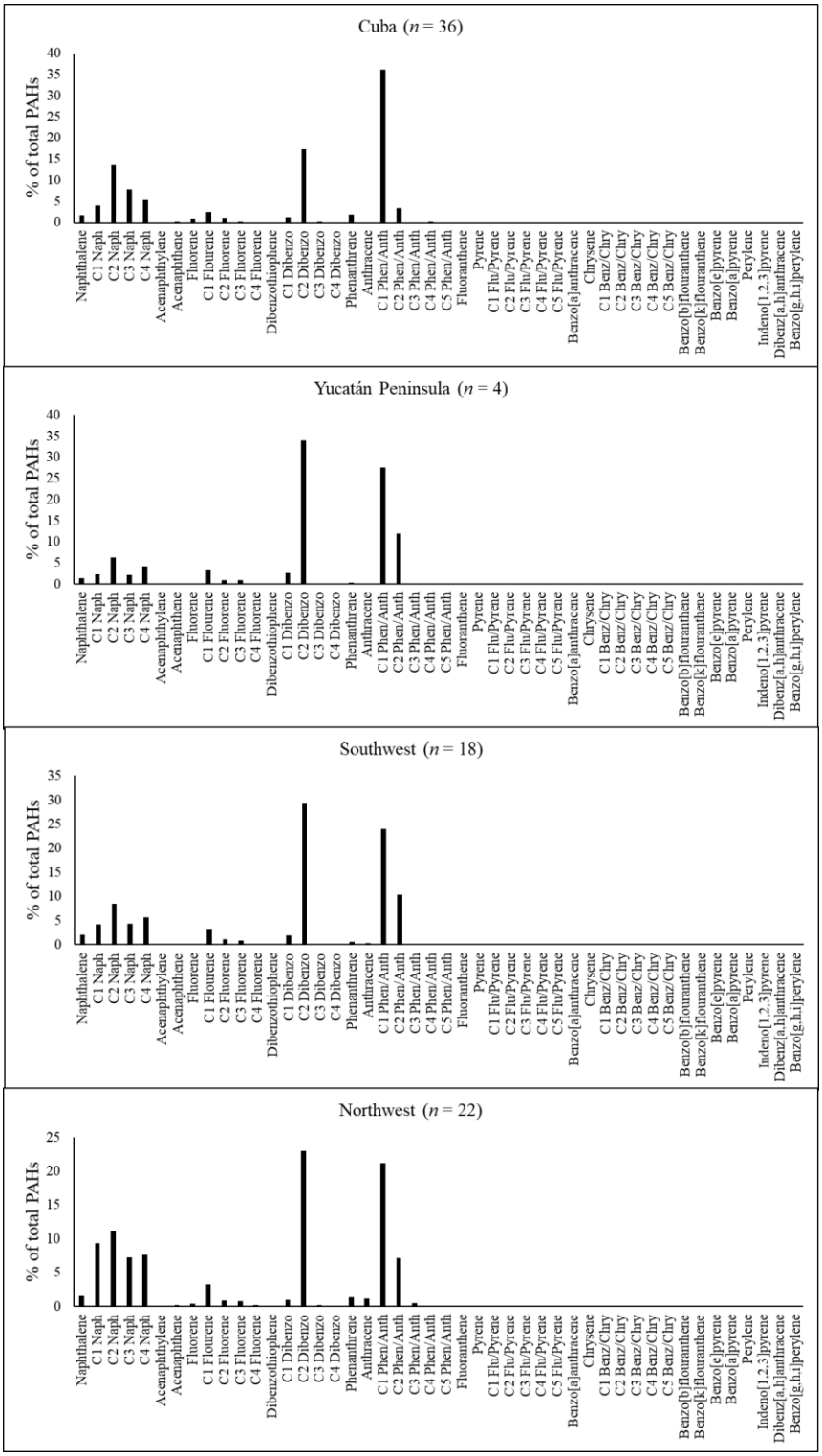

Figure 9C. Regional comparison of hepatic PAH relative abundances (percent of total) measured in Wenchman (*Pristipomoides aquilonaris, n* = 89) sampled from regions around the Gulf. Only the regions with Wenchman *n* > 3 were included.

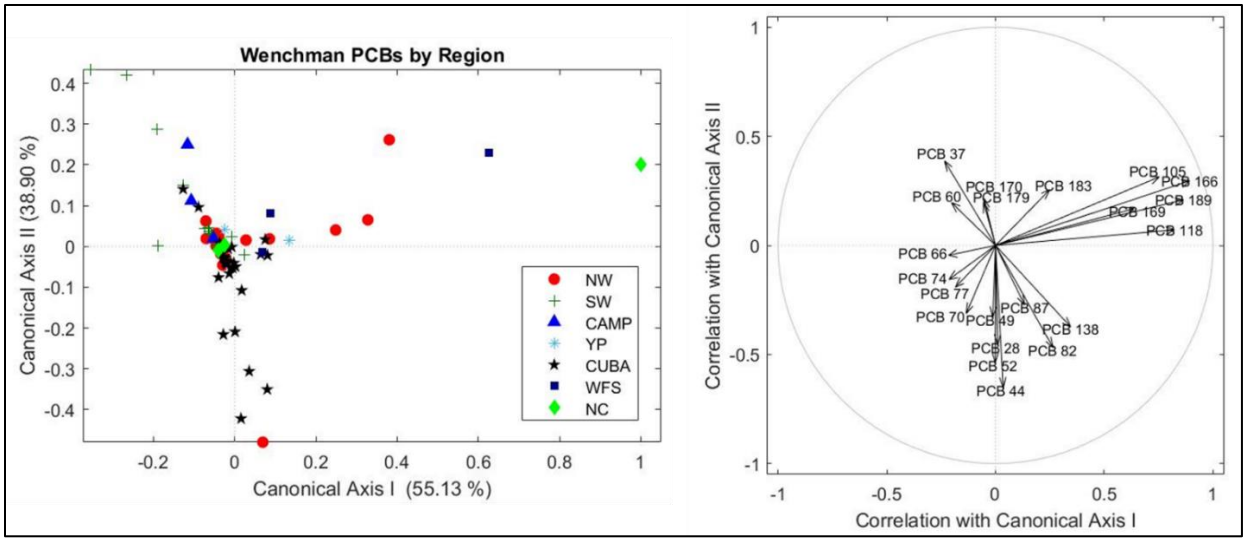

Figure 10A. Canonical analysis of principal coordinates examining regional variation in the relative abundance of hepatic PCBs measured in Wenchman (*Pristipomoides aquilonaris*, *n* = 89) sampled around the Gulf. The seven regions are defined in Figure 1, and are designated using different colors and symbols, with each marking representing one fish. The associated vectors for the relevant individual compounds are shown on the right.

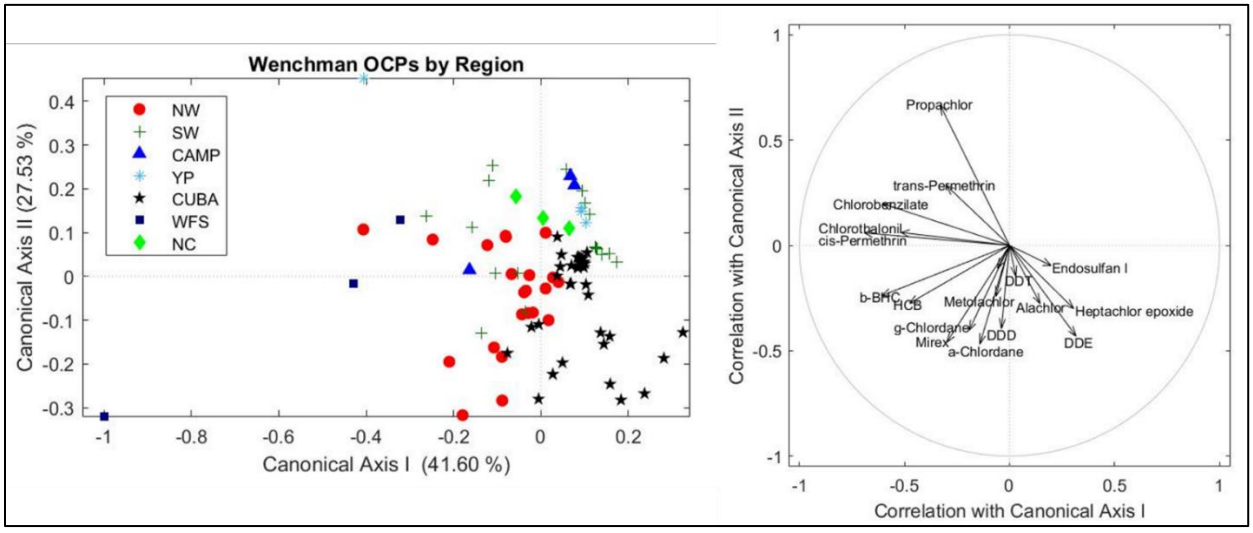

Figure 10B. Canonical analysis of principal coordinates examining regional variation in the relative abundance of hepatic OCPs measured in Wenchman (*Pristipomoides aquilonaris*, *n* = 89) sampled around the Gulf. The seven regions are defined in Figure 1, and are designated using different colors and symbols, with each marking representing one fish. The associated vectors for the relevant individual compounds are shown on the right.

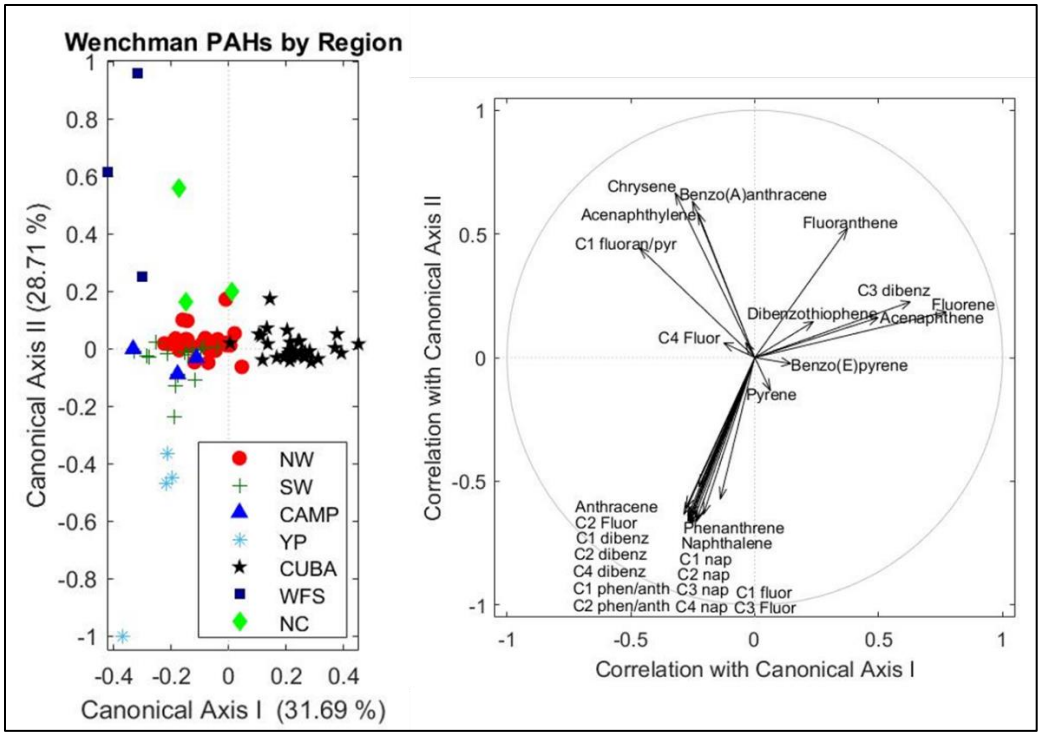

Figure 10C. Canonical analysis of principal coordinates examining regional variation in the relative abundance of hepatic PAHs measured in Wenchman (*Pristipomoides aquilonaris*, *n* = 89) sampled around the Gulf. The seven regions are defined in Figure 1, and are designated using different colors and symbols, with each marking representing one fish. The associated vectors for the relevant individual compounds are shown on the right.

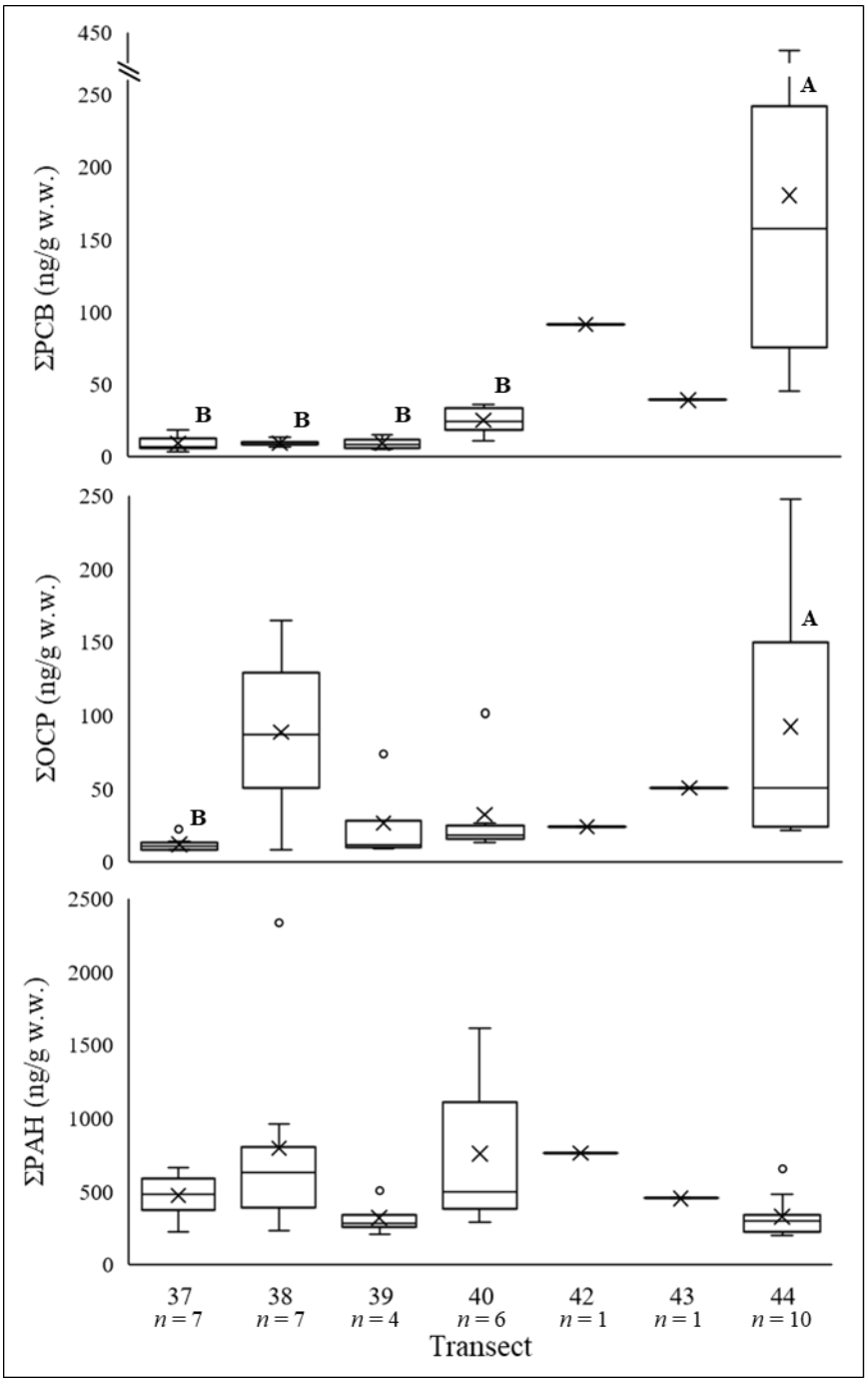

Figure 11. Spatial comparison of hepatic ΣPCBs, ΣOCPs and ΣPAHs (ng g^{-1} w.w.) measured in Wenchman (*Pristipomoides aquilonaris*, *n* = 36) sampled from transects along the north-western coast of Cuba. Transects on the x-axis are ordered from west (left) to east (right; Figure 2). Open circles indicate outliers for each group.

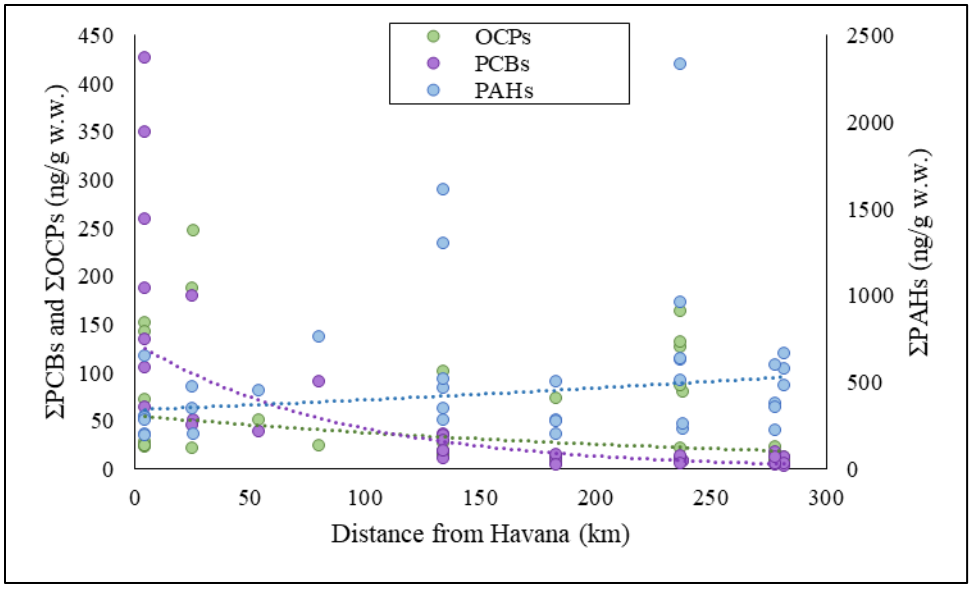

Figure 12. Hepatic ΣPCBs (purple), ΣOCPs (green), and ΣPAHs (blue) in Wenchman (*Pristipomoides aquilonaris*, $n = 36$) sampled off the northwestern coast of Cuba, plotted against distance (km) from the city of Havana. Distance was measured as a straight line between the site where the individual was caught and the mouth of Havana Harbor. Hepatic ΣPCBs are the only group significantly correlated with distance to Havana ($r = -0.689$, $p < 0.001$).

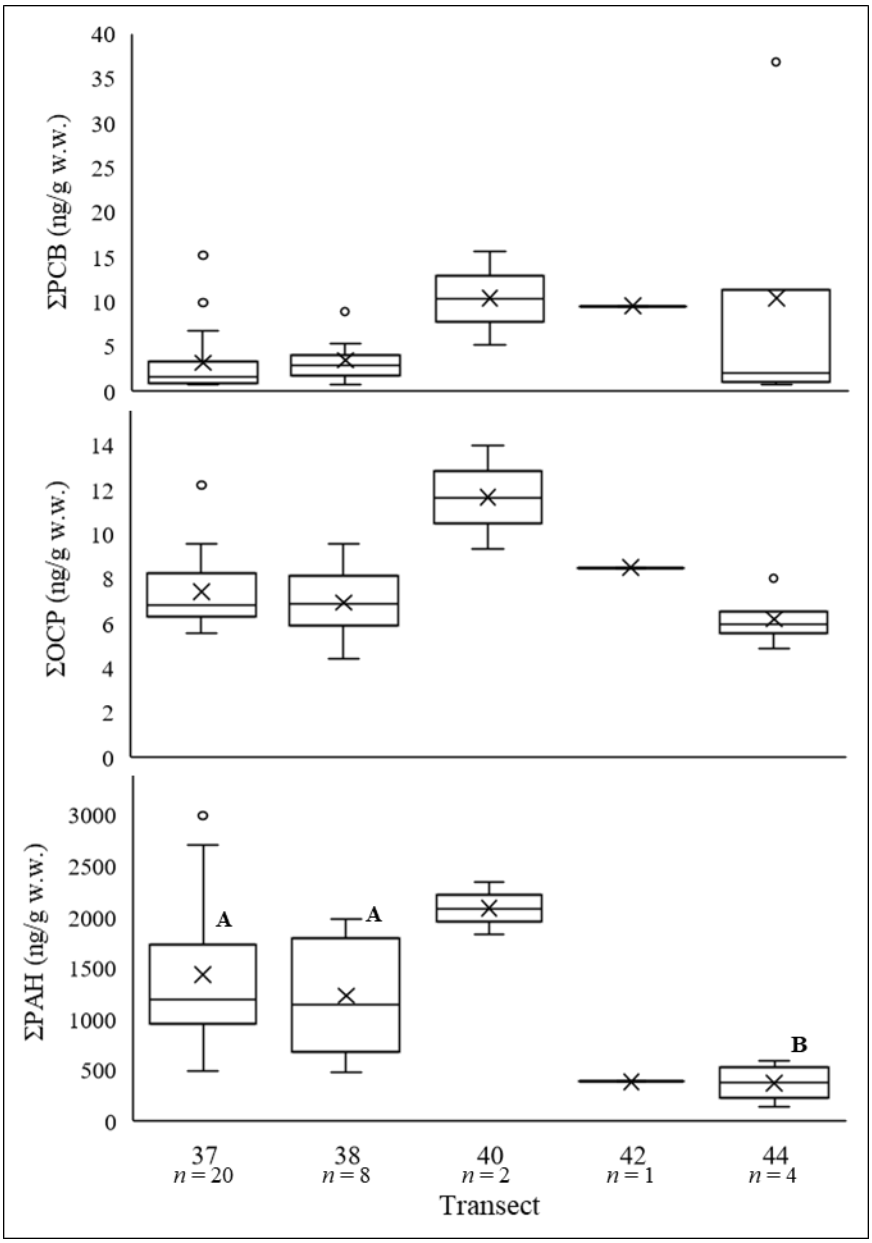

Figure 13. Spatial comparison of hepatic ΣPCBs, ΣOCPs and ΣPAHs (ng g^{-1} w.w.) measured in Silk Snapper (*Lutjanus vivanus*, *n* = 35) sampled from transects along the north-western coast of Cuba. Transects are ordered from west (left) to east (right; Figure 2). Open circles indicate outliers for each group.

Appendix B: Extraction Methodology and Acquisition Parameters

Tissue Extraction

Hepatic tissue was homogenized prior to extraction, and QuEChERS extraction (*92*) was conducted using a 2g subsample of the homogenate. Each 2g subsample was placed into a 50mL polypropylene tube and spiked with 100ng of surrogate standards (to monitor recovery of analytes), which were then vortexed into the tissue and allowed to marinate for 15 minutes. Acetonitrile (20mL) (Fisher Chemical Optima® Grade) was then added to the tube along with two steel balls, and the tube was shaken at 1,000 rpm for 15 minutes using a 1600 MiniG® GenoGrinder (SPEX® Sample Prep, Metuchen, NJ). After this mechanical extraction the tube was centrifuged for 5 minutes at 5,000rpm, and 8mL of the resulting supernatant was shaken for 5 minutes with an Agilent matrix cleaning step (Bond Elut Enhanced Matrix Removal-Lipid) to promote further clean-up of the extract. This tube was then centrifuged, and the supernatant was transferred into another 50mL tube along with an Agilent polish pouch (Bond Elut EMR-Lipid polish pouch) to remove any remaining water and water soluble interferences. After another 5 minute centrifuge, 1mL of the resulting extract was spiked with an internal standard (100ng/mL p-Terphenyl-d14) prior to instrument injection to monitor instrument stability.

GC Oven Parameters

Inlet temperature was set to 295ºC. The carrier gas (1.5 mL min^{-1}) and quench gas (2.25 mL min^{-1}) was ultra-high purity helium, and the collision gas was ultra-high purity nitrogen (1.5 mL min^{-1}). Initial oven temperature was set to 60ºC for 3 minutes, then increased to 120ºC at a rate of 12ºC min^{-1}, followed by a 8ºC min^{-1} ramp up to 300ºC, then an increase to 320ºC at 15ºC min^{-1}, and a final hold of 4 minutes at 320ºC. The transfer line was set to a temperature of 320ºC, the high efficiency source to 300ºC, and the quadrapole to 150ºC.

Table 2. Method acquisition parameters for 32 polychlorinated biphenyls, 32 organochlorine pesticides, and 46 polycyclic aromatic hydrocarbons (19 parental and associated alkylated homologs). Collision energy = CE, method detection limit = MDL.

Target Analyte	Parental ion	Quantifier Ion	CE	Qualifier Ion	CE	MDL
PCB 8 (2,4'-Dichlorobiphenyl)	221.8	152	30	187	10	50 ppt
PCB 28 (2,4,4'-Trichlorobiphenyl)	255.7	186	30	220.9	15	50 ppt
PCB 37 (3,4,4'-Trichlorobiphenyl)	255.7	186	30	220.9	15	50 ppt
PCB 44 (2,2',3,5'-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt
PCB 49 (2,2',4,5'-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt
PCB 52 (2,2',5,5'-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt

Table 2. (Continued)

Target Analyte	Parental ion	Quantifier Ion	CE	Qualifier Ion	CE	MDL
PCB 60 (2,3,4,4'-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt
PCB 66 (2,3',4,4'-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt
PCB 70 (2,3',4',5-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt
PCB 74 (2,4,4',5-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt
PCB 77 (3,3',4,4'-Tetrachlorobiphenyl)	289.7	219.9	35	254.9	15	50 ppt
PCB 83 (2,2',3,3',4-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 87 (2,2',3,4,5'-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 99 (2,2',4,4',5-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 101 (2,2',4,5,5'-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 105 (2,3,3',4,4'-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 114 (2,3,4,4',5-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 118 (2,3',4,4',5-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 126 (3,3',4,4',5-Pentachlorobiphenyl)	325.7	255.9	30	253.9	30	50 ppt
PCB 128 (2,2',3,3',4,4'-Hexachlorobiphenyl)	359.6	289.9	35	287.8	35	50 ppt
PCB 138 (2,2',3,4,4',5'-Hexachlorobiphenyl)	359.6	289.9	35	287.8	35	50 ppt
PCB 153 (2,2',4,4',5,5'-Hexachlorobiphenyl)	359.6	289.9	35	287.8	35	50 ppt
PCB 156 (2,3,3',4,4',5-Hexachlorobiphenyl)	359.6	289.9	35	287.8	35	50 ppt
PCB 158 (2,3,3',4,4',6-Hexachlorobiphenyl)	359.6	289.9	35	287.8	35	50 ppt
PCB 166 (2,3,4,4',5,6-Hexachlorobiphenyl)	359.6	289.9	35	287.8	35	50 ppt
PCB 169 (3,3',4,4',5,5'-Hexachlorobiphenyl)	359.6	289.9	35	287.8	35	50 ppt
PCB 170 (2,2',3,3',4,4',5-Heptachlorobiphenyl)	393.6	323.8	30	321.8	35	50 ppt
PCB 179 (2,2',3,3',5,6,6'-Heptachlorobiphenyl)	393.6	323.8	30	321.8	35	50 ppt
PCB 180 (2,2',3,4,4',5,5'-Heptachlorobiphenyl)	393.6	323.8	30	321.8	35	50 ppt

Table 2. (Continued)

Target Analyte	Parental ion	Quantifier Ion	CE	Qualifier Ion	CE	MDL
PCB 183 (2,2',3,4,4',5',6-Heptachlorobiphenyl)	393.6	323.8	30	321.8	35	50 ppt
PCB 187 (2,2',3,4',5,5',6-Heptachlorobiphenyl)	393.6	323.8	30	321.8	35	50 ppt
PCB 189 (2,3,3',4,4',5,5'-Heptachlorobiphenyl)	393.6	323.8	30	321.8	35	50 ppt
Aldrin	262.7	192.9	40	202.8	30	500 ppt
a-BHC	180.8	144.9	15	108.9	30	50 ppt
b-BHC	180.8	144.9	15	108.9	30	50 ppt
g-BHC	180.8	144.9	15	108.9	30	50 ppt
d-BHC	180.8	144.9	15	108.9	30	50 ppt
a-Chlordane	372.7	265.8	25	262.9	25	50 ppt
g-Chlordane	372.7	265.8	25	262.9	25	50 ppt
4,4'-DDD	234.8	165	20	199	20	50 ppt
4,4'-DDE	246	176	40	211	20	50 ppt
4,4'-DDT	234.8	165	20	199	20	50 ppt
Dieldrin	262.7	192.9	40	227.8	20	1 ppb
Endosulfan I	241	205.9	20	135.9	45	500 ppt
Endosulfan II	240.7	205.9	20	135.8	45	500 ppt
Endosulfan sulfate	271.6	236.8	15	234.8	20	500 ppt
Endrin	280.7	210.9	30	172.9	40	1 ppb
Heptachlor	271.7	236.8	15	234.8	15	50 ppt
Heptachlor epoxide (Isomer B)	352.7	262.8	15	281.9	15	50 ppt
Methoxychlor	227	141	20	212	20	1 ppb
Alachlor	187.9	160	10	132	20	250 ppt
Chlorobenzilate	250.8	139	15	111	45	50 ppt
Chloroneb	190.8	112.9	15	140.9	10	50 ppt
Chlorothalonil	263.4	167.8	30	132.9	45	500 ppt
DCPA	331.7	222.8	45	166.8	55	50 ppt
Etridiazole	210.7	139.8	20	107.9	40	100 ppt
Hexachlorobenzene (HCB)	283.7	213.8	35	178.8	50	50 ppt
Metolachlor	237.6	162.1	15	133	35	1 ppb
Metribuzin	197.8	82	15	88.9	15	50 ppt
cis-Permethrin	162.6	126.9	15	91	10	100 ppt
trans-Permethrin	162.6	126.9	15	91	10	100 ppt
Propachlor	175.9	92	20	77	35	50 ppt
Trifluralin	305.9	264	10	206	15	50 ppt
Mirex	271.6	236.8	20	234.8	20	50 ppt
Naphthalene	128	102	20	127	25	1ppb
C1N	142	141	20	115	25	1ppb
C2N	156	141	20	115	30	1ppb

Table 2. (Continued)

Target Analyte	Parental ion	Quantifier Ion	CE	Qualifier Ion	CE	MDL
C3N	170	155	20	127	30	1ppb
C4N	184	169	30	154	40	1ppb
Acenaphthylene	152	151	15	150	20	1ppb
Acenaphthene	154	153	15	152	30	1ppb
Fluorene	166	165	20	163	30	1ppb
C1F	180	165	25			1ppb
C2F	194	179	30			1ppb
C3F	208	193	40			1ppb
C4F	222	206	40	179	40	1ppb
Dibenzothiophene	184	139	30			1ppb
C1D	198	197	20	165	30	1ppb
C2D	212	197	40			1ppb
C3D	226	211	40			1ppb
C4D	240	211	40			1ppb
Phenanthrene	178	176	30	152	30	1ppb
Anthracene	178	176	30	152	30	1ppb
C1PH/A	192	191	20	189	40	1ppb
C2PH/A	206	191	20	189	40	1ppb
C3PH/A	220	205	30	189	40	1ppb
C4PH/A	234	219	50	204	20	1ppb
C5PH/A	248	247	20	246	40	1ppb
Fluoranthene	202	201	30	200	40	1ppb
Pyrene	202	201	30	200	40	1ppb
C1F/Py	216	215	30	213	40	1ppb
C2F/Py	230	229	40	215	20	1ppb
C3F/Py	244	229	40	228	40	1ppb
C4F/Py	258	243	20	228	40	1ppb
C5F/Py	272	271	10	257	40	1ppb
Benzo[A]anthracene	228	226	40	202	30	1ppb
Chrysene	228	226	40	202	30	1ppb
C1BA/C	242	241	40	239	40	1ppb
C2BA/C	256	255	20	239	40	1ppb
C3BA/C	270	255	20	239	50	1ppb
C4BA/C	284	282	50	269	50	1ppb
C5BA/C	298	296	50	283	50	1ppb
Benzo[*b*]fluoranthene	252	250.1	40	224	35	1ppb
Benzo[*k*]fluoranthene	252	250.1	40	224	35	1ppb
Benzo[*e*]pyrene	252	250.1	40	226	35	1ppb
Benzo[*a*]pyrene	252	250.1	40	224	35	1ppb
Perylene	252	250.1	40	224	35	1ppb

Table 2. (Continued)

Target Analyte	Parental ion	Quantifier Ion	CE	Qualifier Ion	CE	MDL
Indeno[*1,2,3,-cd*]pyrene	276	274	50	248.2	40	1ppb
Dibenzo[*a,h*]anthracene	278	276.1	40	252	50	1ppb
Benzo[*g,h,i*]perylene	276	274	50	248.2	50	1ppb

Table 3. Method acquisition parameters for surrogate standards and internal standards. Collision energy = CE.

Surrogate/Internal Standards	Parental ion	Quantifier Ion	CE	Qualifier Ion	CE
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
Dibenzothiophene-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
Indeno[*1,2,3,-cd*]pyrene-d12	288	284.1	50	255.9	50
Dibenzo[*a,h*]anthracene-d14	292	288.3	50	264.1	40
Benzo[*g,h,i*]perylene-d12	288	284.1	50	256.1	50
Biphenyl-d10	163.9	160.1	30	162.1	15
Carbofuran	163.9	149	15	103	20
Chlorpyrifos-d10	199.7	171.9	10	108.9	40
Parathion-d10	114.8	82.9	15	62.8	45
Tributyl phosphate	154.8	98.9	5	80.9	30
Triphenyl phosphate	324.8	169	5	76.9	30
PCNB	236.6	118.7	30	142.8	25
DBOFB	295.8	245.9	35	195.8	50
p-Terphenyl	244	237.3	50	159.3	50