Spring 3-26-2015

Polycyclic Aromatic Hydrocarbon determination in liver of Menhaden exposed to the Deep Water Horizon Oil Spill

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Polycyclic Aromatic Hydrocarbon determination in liver of Menhaden exposed to the Deep Water Horizon Oil Spill and urban and non-urban sources of PAHs

By

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Submitted in partial fulfillment of the requirements for the
degree of Master of Science in Biology from the
Department of Biological Sciences of Seton Hall University
Spring 2015

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Abstract

The April 2010 BP® oil spill has caused great concern for ecological impacts in the Gulf of Mexico. Crude oil contains polycyclic aromatic hydrocarbon (PAHs) and oil spills increase the exposure of aquatic organisms to this class of chemicals. The overall detrimental health effects associated with PAH exposure depend on the exposure period, the concentration of PAHs, and the toxicity of the PAHs in the mixture. *Brevoortia tyrannus* and *Brevoortia patronus* are filter feeding fish commonly known as menhaden and account for over 40% of the commercial industry fisheries catch. As primary consumers, they are a major link in the food chain. In this study, liver metabolites of PAHs were measured in menhaden using fixed emission fluorescence spectroscopy (FEFS). Liver PAH analyses were performed on fish samples from North Atlantic to Gulf coasts with the anticipation of comparing a BP® oil signature with ones from urban environments. Adult menhaden were collected from Delaware Bay, NJ (September 2010-September 2011), James River, Virginia (November 2010), Grand Isle, Louisiana (September 2010- September 2011), and Vermillion Bay, Louisiana (June 2011-October 2011). The findings of this study show that a high Naphthol-like/Hydroxypyrene-like ratio is indicative of petroleum exposure. The Barataria Bay (BB), LA 2010 and 2011 samples, Vermillion Bay, LA 2011 samples, and Delaware Bay, NJ 2010 and 2011 data all showed evidence of PAH exposure. The difference of HNP-like and HPY-like PAHs at BBLA from 2010 to 2011 indicated that the site was affected. This indicated continuing exposure to

DWH crude oil one year after the event. In addition, there was seasonal correlation for HNP-like vs HPY-like PAHs across the different sample sites. The accumulation of PAHs was site and/or season related and not due to the age of the fish. *B. tyrannus* and *B. patronus* species were found to have similar types and concentrations of PAHs despite sample location and can serve as a useful tool to monitor PAH exposure over time. Results suggested that menhaden could be a useful biomonitoring organism regardless of species.

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Acknowledgements

I would like to express my sincere thanks, respect, and gratitude to my advisor, Dr. Carolyn Bentivegna for her guidance, support, and encouragement for my Masters study and research. She has provided me with endless patience, opportunity, and knowledge. She is an excellent coach and mentor.

In addition, I would like to take the time to thank the rest of my thesis committee Dr. Tin-Chun Chu and Dr. Marian Glenn. Thank you for your insights, questions, feedback, and encouragement.

I would like to send a special thanks to my fellow lab mates and co-collaborators at Seton Hall University: Lauren Ridely, Edwin Pena, Angelo Monero, and Megan Durham.

Thank you to the following supporters and sponsors of this research effort: Louisiana Fish and Wildlife, Dr. Portier and Gregory Olsen of Louisianan State University, Dr. Keith R. Cooper and Sean Bugel of Rutgers State University, John Tiedermann of Monmouth University, Florida Keys Community College, and the New Jersey Department of Environmental Protection.

Last but not least, I would like to thank my Mom and Neil. Thank you for never giving up on me.

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Introduction

In April 2010, an explosion on a BP® deep water horizon oil rig in the Gulf of Mexico resulted in release of over 4.9 million barrels of crude oil into the Gulf over the course of xx days, before sealing the three leaks. President Barack Obama referred to the spill as “"the worst environmental disaster America has ever faced". The leaks that were discovered where closed on July 15, 2010 at which point recovery efforts for the removal of contaminants were well underway (Office of the Press Secretary, 2010).

Crude oil varies in carbon content and composition. The crude oil released into the Gulf of Mexico in 2010 was considered to be ‘light and sweet’, meaning that it would require less refining for the modification of hydrocarbons and sulfur. This particular type of oil is usually processed into gas, heating oil, kerosene, and fuels. Polycyclic aromatic hydrocarbons, PAHs, are a class of chemicals found within this type of oil (Gulf of Mexico Research Institute, 2011).

PAHs are a class of over 100 chemicals that are found naturally in organic materials such as coal, oil, natural gas, wood, garbage, and tobacco. This class of chemicals contains two or more aromatics rings sharing carbon atoms. “Small” PAHs are those that contain up to three aromatic rings and those containing more than three rings are referred to as “large”. Major characteristics of these compounds include high melting point, high boiling points, low water solubility, low vapor pressure, light sensitivity, heat resistance, corrosion resistance, and they are very lipophilic and can therefore be stored in the fatty areas of animals. These compounds can enter the food web and remain there for an indefinite

period of time unless they are metabolized and eliminated (Center for Disease Control, 2013).

Two general types of PAHs are the focus of this study: naphthalene-like and pyrene-like (Figure 1a and 1b). Both have been included in the Agency of Toxic Substances and Disease Registry, a US Department of Health and Human Resources, as 2 of the 17 PAHs that are considered to the be the most harmful to humans. However, the overall health effects of PAHs depends on the extent of exposure, the concentration of PAH to which the organism is exposed, the toxicity of the PAH, and the degree of PAH metabolism. Some PAH metabolites are naphthol and hydroxypyrene. These metabolites are generated by the cytochrome P4501A, a common enzyme in many organisms for detoxifying PAHs and or bio activating them (Global Marine Oil Pollution Information Gateway, 2010). Naphthol is a "small" PAH consisting of two six membered rings sharing one edge with one hydroxyl group. Hydroxypyrene is a "large" PAH consisting of four six membered rings and a hydroxyl group.

Due to their carcinogenic and mutagenic properties, crude oil toxicity is a major focus of research studies. Crude oil components can be found in a water-soluble fraction, dispersed oil droplets, particle bound, or as emulsions within the sea. Marine organisms and ecosystems are readily exposed to these compounds. Crude oil will typically contain about 0.2% to 7% PAHs (Global Marine Oil Pollution Information Gateway, 2010). The uptake of these PAHs in microorganisms can have a wide variety of impacts. The impacts can affect a

number of physiological processes including biodegradation, bioaccumulation, and biotransformation (Gomez et al, 2010).

Kutzen (1995) reported two main mechanisms that cause damage due to PAH exposure in marine organisms: reaction with lipids in different cell components/membranes (narcosis) and the reaction with macromolecules including nucleic acids and various proteins (adduct formation). PAHs have detrimental effects on the immune systems of vertebrates, including reduced macrophage activity, a lower than normal number of lymph nodes, a lower lymphocyte response, metabolic disturbances that lead to improper hormone synthesis and metabolism (Kutzen, 1995).

The physiological reaction that disrupts the proper functioning of cell organelles and cellular membranes has many downstream consequences. Many of the enzyme systems that are associated with any of the above mentioned components are severely impacted. One example is tissue damage caused by improper lysosome system function due to autolysis or cellular self - destruction. Skin lesions, liver lesions, and cataracts were also seen in fish species (Kutzen, 1995).

The carcinogenic effects of PAHs are an area of great focus. Different PAH compounds have different roles in tumor development. Fish are also believed to be more susceptible to carcinogenic toxicities due to their degradation and metabolite detoxification pathways as well as their capacity for repairing modified DNA. This has led to reported chromosomal damage (Kutzen, 1995).

Temperature and age of the fish also had effects on carcinogenesis caused by PAH exposure.

In addition to the above described effects of PAH exposure, reproductive issues in fish have also been reported. This includes delayed/decreased hatching, larval malformations, yoke sac damage, reduced larval growth, and disruption in egg/larval development (Kutzen, 1995).

The specific chemical and physical properties of crude oil will determine the effects on the marine ecosystem. The lightest classes (Class A and Class B) of oil will rapidly spread on solids and water surfaces and can be toxic to fish, humans, and other organisms. These types of oils do no typically adhere to solid surfaces but penetrate porous materials including sand and other sediment. The heavier classes (Class C and D) will attach to solid materials but do not penetrate porous materials. These types of oils are dense and therefore sink. They are considered less toxic than Class A and Class B, but they can still affect marine ecosystems. Specifically, these oils can be transported by ocean currents and contaminate other sites (Gomez et al, 2010). The crude oil at the DeepWater Horizon oil spill is characterized as a 'lighter' class.

Mitra et al. reported the presence of PAHs from DWH oil in phytoplankton in the Gulf of Mexico (2012). PAHs were recovered from Mesozooplankton, size range 0.2-20 mm, collected in August and September 2010 from the northern Gulf of Mexico indicating that the DWH oil spill contributed to the contamination in the Gulf of Mexico. Furthermore, the study showed that the PAHs were found two trophic levels above prokaryotic

hydrocarbon consumers in the food web documenting the movement of PAHs to higher tropic levels. Menhaden are a filter feeding fish that would be affected by this bioaccumulation effect.

Bioaccumulation of PAHs by fish in the Gulf of Mexico is of major concern as this specific site has a huge role in the commercial fishing industry. Both bait fish and sport fish inhabit these waters. Menhaden are a common bait fish species known as 'bunker' or 'pogy' and they are considered to be one of the most important fish of the sea (Franklin, 1999). In 1996, about 36% of U.S. Atlantic coast commercial fisheries catch were menhaden (*Chesbay.org*, 2013). Menhaden are a major link in the food chain (US Department of Fish and Wildlife, 1983). These fish are not limited to the sport fishing industry but are also used in purse-seine fish industry and fertilizer industries (Ahrenholz, 1991).

The purse seine fish industry is a reduction process that turns menhaden into valuable consumer products such as fish meal, fish oil and soluble fish products. Fish meal is commonly used in livestock feed as it is over 60% protein. Fish oil is used in cooking oils and margarine after undergoing further refinement. In 1989, the United States Food and Drug Administration (FDA) reported that hydrogenated menhaden oil is safe for human consumption and in 1997 menhaden oil became an omega-3 nutrient source with a large market demand. Menhaden are also used as chum or as live bait for sport fishing (*Chesbay.org*, 2013).

Menhaden are a toothless filter feeding species feeding on zooplankton, phytoplankton, and diatoms making these fish a major link in the food chain

between primary producers and secondary consumers. Adult menhaden filter approximately 6 to 7 gallons of water per minute. They are an extremely oily fish where oil can be roughly 4% of their total body weight (Ahrenholz, 1991). Menhaden were chosen as the model to study crude oil contaminants based upon the concept that "like dissolves like". Oil contaminants might accumulate in oily fish and the body burdens would be indicative of their recent and long term exposure. Specifically, the liver tissue would be analyzed. In addition, the filter feeding lifestyle of menhaden could lead to increased exposure including contamination of gills, as plankton is filtered from the water by gill rackers, and of the diet as the plankton would move into the GI tract tissues and be distributed to the liver by the circulatory system.

There are two main groups of menhaden: large scaled menhaden and small scaled menhaden. The large scale menhaden species are the sister species *Brevoortia patronus*, Gulf menhaden, and *Brevoortia tyrannus*, Atlantic menhaden. The small scale menhaden species are the sister species *Brevoortia smithi*, yellow fin menhaden and *Brevoortia gunteri* (Ahrenholz, 1991).

Atlantic Menhaden, *Brevoortia tyrannus*, have a large geographic range from Nova Scotia to eastern Florida (Figure 1A). This menhaden has a very large scale-less head, small ventral fins and a prominent forked tail. The color of this species ranges from a dark green or blue green spine with silver sides. A dark humeral spot is located on the side posterior to the gill opening with small irregular spots running caudal along the body. They travel in schools and can reside in both freshwater and salt water (Ahrenholz, 1991).

Gulf menhaden, *Brevoortia patronus*, range from Florida to Mexico with abundance off the shores of Louisiana and Mississippi (Figure 2A). They are a food source for many sport and commercial fish species including bluefish, bass, sharks, and more. They also compose a large portion of the diet for shorebirds and pelicans. The back of the fish is a bluish grey and the sides of the body are silver. A major characterization is the dark humeral spot on the body side with other smaller spots running parallel with the spine down the body. The spawning season of this species occurs October to April with spawning occurring offshore in the ocean (Ahrenholz, 1991). Embryos move with currents into estuaries where they spend their first year. Young of the year return to the ocean in the fall.

Yellow fin menhaden, *Brevoortia smithi*, have a large geographic range and are found from the central Atlantic coast to the southern coast of the Gulf of Mexico. *Brevoortia gunteri* are the sister species of yellow fin menhaden. They tend to be localized to the western Gulf of Mexico with overlap in the habitat range of *Brevoortia patronus*. Both of these small scaled menhaden lack lateral spots and tend to have firmer flesh (Ahrenholz, 1991). *Brevoortia smithi and Brevortia gunteri* were not used in this study.

PAH determination in fish has been documented and investigated by using fluorescence spectrophotometry. Jonsson et al. (2010) reported that PAH determination in fish can be accomplished using fluorescence. There are two major events involved with measuring fluorescence of PAHs- excitation and emission. Excitation occurs when photons are absorbed by a molecule as a result of exposure to particular wavelengths of light. The structure of the PAH

determines which wavelength(s) it will absorb. The PAH becomes "excited" when it absorbs particular wavelengths of light in the ultra violet and or visible light range, from 200 nm to 900nm. Emission is the amount of photons that are emitted as the molecule returns to its ground state. The emission is at a lower energy level and seen as light of a particular wavelength. The amount of light to which molecule is exposed must be enough to cause the molecule transition from its low energy state to its high energy state. Therefore, it must be the equivalent to the difference between the two states within the molecule (Fluorescent Microsphere Resource Center, 1999).

This difference between the two states is specific to each molecule so that each PAH will have unique wavelengths at which it has maximum excitation and emission. Both excitation and emission properties can be used to establish 'spectra'. Excitation spectra can be generated by scanning the excitation wavelengths while measuring the intensity of emission. Likewise, the emission spectra can be generated by fixing the excitation wavelength and scanning the emission wavelength (Fluorescent Microsphere Resource Center, 1999).

Fixed-wavelength fluorescence has been a successful method in measuring concentrations of various PAH metabolites. The fixed wavelength approach has been developed and supported by many research groups including Lin et al. (1996), Aas et al. (2000), Yang et al. (2003), and Beyer et al. (2010). Lin et al. did a comparative analysis study of fixed wavelength fluorescence to high-performance liquid chromatography with fluorescence detection (HPLC-F) as an estimation of PAHs in fish bile. The findings indicated that fixed wavelength

emission could be used as a successful measure for PAHs in fish species. Yang et. al (2003) published their findings on the measurements of PAH metabolites from the bile of the brown bullheads fish species collected from the Ottawa River. It provided evidence that fixed emission wavelength analysis was an effective method to determine fish exposure to PAHs. Beyer et al (2010) reported the findings of various analytical methods for detection of PAH metabolites in bile including fixed fluorescence detection. Fixed emission fluorescence is considered to be a tool for easy, rapid, and quantitative assessment of PAH metabolites in fish.

Preliminary studies for this research determined two settings in order two analyze small and large PAHs separately. The small two and three ringed, naphthol-like PAHs have a fixed emission wavelength of 350nm and were scanned for excitation wavelengths from 250nm to 320nm. The second setting was used for detecting four to six ringed PAHs such as hydroxypyrene. The samples had a fixed emission of 450nm and were scanned for excitation wavelengths from 250nm to 420nm. Spectra generated by small or large PAHs will have optimal excitation wavelengths that produced one or more major peaks. Values of fluorescence at these peaks can be used for quantification as well as molecular profiling of naphthol-like vs hydroxypyrene-like PAHs.

The purpose of this study was to determine the presence of PAHs in menhaden species from different estuaries including the DWH site. Their body burden was measured using fixed emission fluorescence spectroscopy (FEFS) to detect and quantify the presence of PAHs using spectra generated for naphthol-

like and hydroxyprene-like compounds and standard curves. Spectra generated information on abundance and types of PAHs. Therefore, the higher the relative fluorescence unit (RFU) value, at the major excitation peak, the higher the PAH concentration. Adult menhaden were collected from Delaware Bay, NJ; James River, VA; Barataria Bay and Vermillion Bay, LA in 2010 and/or 2011. The Delaware Bay region of NJ was selected for collection of *Brevoortia tyrannus* and was expected to contain high levels of PAHs due to urbanization. However, this area has not had a major oil spill. Barataria Bay, LA was selected for the collection of *Brevoortia patronus* and to assess the impact of the DWH oil spill as the estuary in this bay was heavily oiled. Vermillion Bay, LA was selected for collection of *Brevoortia patronus* as well. This bay is located west of Barataria Bay and received less oiling. The James River, VA was selected as a point of reference for *Brevoortia tyrannus* and *Brevoortia smithi* migration zone.

The objectives of this investigation were to determine if FEFS could be used to establish standards for naphthol-like and hydroxypyrene- like PAHs; to investigate whether concentrations of naphthol-like and hydroxypyrene-like PAHs could be analyzed in liver samples of *Brevoortia tyrannus* and *Brevoortia patronus*; and to determine if *Brevoortia tyrannus* and *Brevoortia patronus* from sample dates ranging from Fall 2010 to Fall 2011 contained similar types and concentrations of PAHs, thereby establishing seasonal variation patterns and monitoring levels of PAHs in menhaden exposed to the DWH oil and control sites over that time period.

The hypotheses were that menhaden would be exposed to and accumulate PAHs from their diet; and therefore, hydroxypyrene- and naphthollike PAHs would be found within the liver of menhaden. Also, *Brevoortia patronus* from the Gulf of Mexico would exhibit a greater concentration of PAHs in liver due to recent crude oil exposure. Seasonal variation would be significant due to changes in phytoplankton availability and different sampling sites will have distinct PAH profiles due to exposure to urban versus oil spill PAHs.

Methods

A. Fish Origin, Preparation, and Catalogue

Brevoortia tyrannus and *Brevoortia patronus* fish were collected from one of the following locations: Delaware Bay Region/East Coast, NJ; James River, VA; Barataria Bay, LA; and Vermillion Bay, LA. The collections were performed either by this research group or by state Fish and Wildlife organizations.

Fish weight, length, species, and location were recorded for identification purposes with photographs. The photographs were used for species identification. Atlantic *Brevoortia tyrannus* were collected on 9/8/2010, 9/21/2010, 7/11/2011, 7/24/2011, 7/30/2013, 7/5/2011, 8/31/2013, and 10/8/2013. The fish were labeled according to the commercial fishing vessel from which they were obtained and/or location and included Mount Vernon (MVNJ 2010), Enterprise (EPNJ 2010), Sea Huntress (SHNJ 2010), Evening Star (ESNJ 2011), Eva Marie (EMNJ 2011), and James River, VA (MSVA 2010). The *Brevoortia patronus* collected from Barataria Bay, LA were collected on the following dates 10/30/2010, 7/28/2011, 8/24/2011, 9/13/2011, and 10/11/2011. The *Brevoortia patronus* collected from Vermillion Bay, LA were collected on 7/6/2011, 8/23/2011, and 9/12/2011.

B. Liver PAH Determination

Fish were dissected and liver was removed. Remaining parts of the fish were transferred to another research group for further analysis. Approximately, 0.1g of liver was weighed and added to homogenizer with 1mL of 75%EtOH. Liver was homogenized, vortexed, and centrifuged for 20 minutes at 13,000RPM. Supernatant was transferred and pellet was saved for further analysis.

Supernatant, 100µl, was added to 75%EtOH for a final volume of 1mL. Replicates and duplicates of fish samples were made approximately every one out of every three fish when enough liver was available. In addition, one out of every three samples was used as duplications for the percent recovery analysis of naphthol and hydroxypyrene. Spikes were made by combining100µl of original supernatant with 100µl of PAH (2500ng/mL) and brought up to a final volume of 1mL in 75%EtOH. Samples were frozen until analyzed with the SpectraMax® M5/M5 Microplate Reader.

Liver dry weight was used to calculate ng of PAH/mg of liver by weighing original pellet after centrifugation. Samples were left to air dry overnight. The 1.5mL tube weight, wet pellet weight, and tube with dry pellet weight were recorded to calculate dry pellet weight (tube with dry pellet – tube weight).

C. Sample Analysis with SpectraMax® M5/M5 Microplate Reader

The samples were analyzed using the SpectraMax® M5/M5 Microplate Reader for FEFS. Samples were loaded into a quartz cuvette. Samples were analyzed under two specific settings. One setting was for the determination of two/three ring PAHs such as naphthol and phenthrol. Specifically, the emission wavelength was fixed at 350nm and samples were scanned with excitation wavelengths from 250nm to 320nm. The second setting was for the determination of four or five ringed PAHs such as hydroxypyrene. The emission at 450nm was measured as samples were scanned for Ex wavelengths from 250nm to 420nm. 75%EtOH was used as background reference/blank.

D. Data Analysis and Statistics

Data sets generated from SpectraMax® M5 software were imported to an Excel document. Data presented as spectra were generated by subtracting the 75% EtOH background from each wavelength of the liver sample. Axes were relative fluorescence intensity (RFU) by excitation wavelength (nm) for either Em350 or Em450. This represented naphthol and hydroxypyrene-like PAHs, respectively. It was observed that there were multiple spectrum types, i.e. different major excitation peaks, for each fixed emission. Therefore, data were presented for each spectrum type. For naphthol-like PAHs, the liver samples were run with a fixed Em350 and were scanned for excitation. An excitation peak was observed at 280nm. This spectrum was designated type 1. Another spectrum type was also observed in which the major excitation peak was at 290nm and was designated as type 2. For hydroxypyrene-like PAHs, emission was fixed at Em450. Spectra designated as type 1 HPY-like had two peaks present: one at Ex270 and one at Ex350. Fluorescence intensity was measured at 350nm and not 270nm. To be considered type 2, spectra had to be observed with one excitation peak at 350nm. Data were also presented as concentrations of naphthol or hydroxypyrene-like PAHs using specific wavelengths of Ex280/Em350 and Ex350/Em450, respectively.

These data were generated by 1) subtracting background, 2) adjusting RFU for percent recovery, 3) converting RFU to ng/ml using a standard curve, 4) multiplying by 10 to adjust for the dilution of homogenate, 5) dividing by pellet dry weight (ng/g) and 6) dividing by 1000 to achieve final units of ng PAH/mg

pellet. Some pellet dry weight values were clearly erroneous or inaccurate. Therefore, any data that had a pellet weight not within two significant figures of the average was omitted and not used for analysis. The standard curve equation for naphthol- like PAHs was y= 3.6542x with an average percent recovery of 25% (spiked homogenate 1250ng/mL). Please refer to figure 7 in Results. The standard curve equation for hydroxypyrene like PAHs was y= 2.7137x with an average percent recovery of 35% (spiked homogenate 1250ng/mL). Please refer to figure 8 in Results.

Statistics were performed using IBM SPSS Statistics Data® software with a one way parametric ANOVA Tukey test and post-hoc test where values with $p>0.05$ were not significantly different.

Results

Fish Identification and preparation

Fish weight and length, species, and location were recorded. Fish were photographed for identification purposes. The locations of the fish were designated by region/state and ship from which they were obtained. Fish were classified by using the information in Table 1 from *Ahrenholz*, 1991. A fish catalog was created to maintain records. Fish were identified by comparison of external features with the illustrations in Ahrenholz, 1991.

Figure 1a shows a schematic representation of *B. tyrannus*; 1b is a photograph taken by this researcher; and figure 1c shows the migratory range of *B. tyrannus*. This menhaden hasa large scale-less head with small ventral fins, a prominent forked tail, and a dark humeral spot located on the side posterior to the gill opening with small irregular spots running caudal along the body. The color of this species ranges from a dark green or blue green spine with silver sides.

Figure 2a provides a schematic representation *B. patronus*; 2b provides a photograph taken by this researcher; and figure 2c provides the migratory range of *B. patronus*. This menhaden has a bluish grey color and the sides of the body are silver and there is a dark humeral spot on the body side with other smaller spots running parallel with the spine down the body.

The fish were frozen upon capture and thawed just prior to dissection. The liver was taken for FEFS analysis. The remaining organs were separated out and stored for later use. The heads were removed and separated for future analysis.

The fish were skinned and the filets were stored for further investigation by this research group.

Table 1 Distinguishing and comparative characteristics of North American Coastal Menhadens: Chart used to classify fish samples used for this study. Taken from Ahrenholz: "Population Biology and Life History of the North American Menhadens, *Brevoortia* spp." N.p., 1991. Web. 27 Mar. 2013.

1A

2A

1B

2B

1C

2C

Figure 1 and 2 Menhaden Species: Figure 1A: A schematic representation of *B. tyrannus* provided by Britannica(http://www.britannica.com/animal/Atlantic-menhaden); Figure 1B: A photograph of *B. tyrannus* taken by this researcher prior to dissection. Figure 1C: Computer generated distribution maps for *Brevoortia tyrannus* (Atlantic menhaden), with modeled year 2100 native range map based on IPCC A2 emissions scenario. www.aquamaps.org, version of Aug. 2013. Web Accessed Aug. 2013 (http://fishbase.org/Summary/SpeciesSummary.php?ID=1592&AT=menhaden) ; Figure 2A: A schematic representation of *B. patronus* provided by UVM.edu (http://www.uvm.edu/~jbartlet/nr260/animal%20life/animallife.html) 2012; Figure 2B: A photograph of *B. patronus* taken by this researcher prior to dissection. Figure 2C: Computer generated distribution maps for *Brevoortia patronus* (Gulf menhaden), with modeled year 2100 native range map based on IPCC A2 emissions scenario. www.aquamaps.org, version of Aug. 2013. Web.Accessed Aug 2013 (http://fishbase.org/Summary/SpeciesSummary.php?ID=1589&AT=menhaden).

Fish Origination, Sample Site, Date of Catch, and total fish samples

The fish samples used in this study were obtained by this research group or provided by local fish and wildlife agencies. *B. tyrannus* and *B. patronus,* fish were collected from one of the following locations: Delaware Bay Region/East Coast, NJ; James River, VR; Barataria Bay, LA; and Vermillion Bay, LA. Table 2 provides the date of capture, the location of capture, and total fish obtained. *B. smithi* were not utilized in this study. Figure 3 and Figure 4 shows the location of sample sites.

Table 2. Fish Sample Sites, Date of Capture, and Total Sample Size

Sample Site	Naming Convention	Date of Capture	Sample Size
Delaware Bay Region, NJ: Mount Vernon Ship	**MVNJ1**	9/7/10	11
Delaware Bay Region, NJ: Mount Vernon Ship	**MVNJ2**	9/23/10	8
Delaware Bay Region, NJ: Enterprise Ship	**EPNJ**	9/21/10	9
Delaware Bay Region, NJ: Sea Huntress Ship	**SHNJ**	10/25/10	19
James River, VA	**JRVA**	10/1/2010	15
Barataria Bay, LA	**BBLA1**	10/30/10	15
Barataria Bay, LA	**BBLA2**	7/28/11	15
Barataria Bay, LA	**BBLA3**	8/24/11	21
Barataria Bay, LA	**BBLA4**	9/13/11	16
Barataria Bay, LA	**BBLA5**	10/11/11	19
Vermillion Bay, LA	**VBLA1**	7/6/11	40
Vermillion Bay, LA	**VBLA2**	8/23/11	20
Vermillion Bay, LA	**VBLA3**	9/12/11	20
Delaware Bay Region, NJ: Eva Marie Ship	**EMNJ1**	7/5/11	5
Delaware Bay Region, NJ: Eva Marie Ship	**EMNJ2**	8/31/11	10
Delaware Bay Region, NJ: Eva Marie Ship	**EMNJ3**	10/8/11	10
Delaware Bay Region, NJ: Evening Star NJ	**ESNJ1**	6/24/11	13

Delaware Bay Region, NJ: Evening Star NJ	**ESNJ2**	6/30/11	10
Delaware Bay Region, NJ: Evening Star NJ	**ESNJ3**	8/31/11	9
Delaware Bay Region, NJ: Evening Star NJ	**ESNJ4**	10/8/11	9

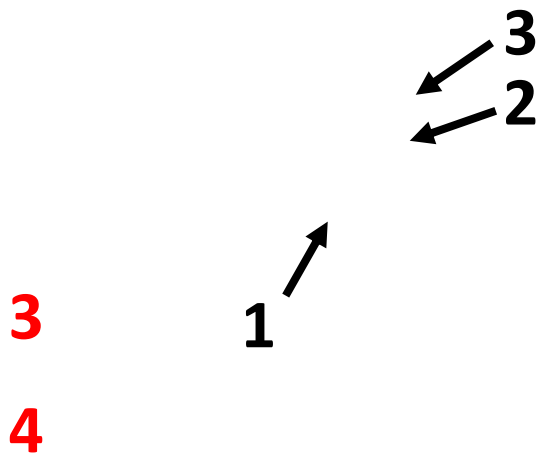

Figure 3. Map provided by freeworldmaps.net (http://www.freeworldmaps.net/printable/us/us-blank-map-physical.jpg); Modified by this researcher. Depicts all sample sites used for this study. **1**. Vermillion bay and Barataria bay, LA Regions; **2**. James River, VA; **3**. Delaware Bay Region, NJ.

Figure 4. Magnified View of LA Sampling Sites: Grande Isle, LA: heavy DWH oil exposure. Vermillion bay, LA: no apparent exposure to DWH oil. *Created by Greg Olsen, LSU and modified by this researcher.

Fixed Emission Fluorescence Spectrophotometry (FEFS) Settings

The samples were run using a SpectraMax® M5/M5 Microplate Reader with appropriate fluorescence settings and samples in a quartz cuvette. Samples were analyzed under two specific settings: a setting for the determination of two/three PAHs such as naphthol (Figure 5A). Specifically, the samples had a fixed emission wavelength (aka Em) of 350nm and were scanned for excitation wavelengths (aka Ex) from 250nm to 320nm. The second setting was for the determination of four to five ring PAHs such as hydroxypyrene (Figure 5B). Samples had a fixed Em of 450nm and were scanned for Ex wavelengths from 250nm to 420nm. 75%EtOH was used as background reference/blank. Figure 5a and 5b provide chemical structures for naphthol and hydroxypyrene PAHs and the schematic flowchart for fluorescence detection using the Spectra Max® M5/M5.

5A **5B**

5C

Figure 5 PAH Type and Process Overview: A: Naphthol Structure; **B:** Hydroxypyrene Structure; **C:** A schematic representation of scanning fluorescence spectrophotometry (SFS) taken from Fluorescence Microsphere Resource Center database, 1999.

Standard Curves and Percent Recovery

Standard curves were created by Edwin Pena of Seton Hall University for naphhol-like and Hydroxypyrene-like PAHs in order to establish background information for this study. Figure 6A and 6B provides the characterization of standard PAHs using FEFS in order to establish expected excitation peaks. Figure 6a characterizes naphthol (HNP), Fluorine (FL), Anthracene (AN), hydroxpyrene (HPY), and Benzo a Pyrene (BaP), all with a final concentration of 1250ng/mL, with an emission wavelengths held constant at 350nm. HNP had a single excitation wavelength peak at 270/280nm with 5005RFUs. FL had an excitation wavelength peak at 270nm and a smaller peak at 310nm with 16396RFUs. AN had an excitation wavelength peak at 290nm and a smaller excitation peak at 260nm with smaller RFU values of 197 and 253, respectively, as compared to HNP and FL. HPY had an excitation wavelength peak at 340nm and a smaller excitation peak at 270nm with smaller RFU values of 85 and 65, respectively. BaP had an excitation wavelength peak at 340nm and a smaller excitation peak at 270nm with smaller RFU values of 85 and 39, respectively. Therefore, 2-3 ring PAHs (NL and FL) had a greater fluorescence when detected at Em350 compared to 4-5 ring PAHs (PY and BaP). Compounds having a spectrum with an excitation peak pattern similar to that of NL (single excitation peak at 270/280nm) are referred to as 'naphthol-like' PAHs.

Figure 6b characterizes FN, AN, HPY, and BaP, all with a final concentration of 1250ng/mL and a fixed emission held constant at 450nm. Excitation wavelengths were scanned from 250 to 420. FN had an excitation wavelength peak at 280nm and smaller excitation peak at 350nm with RFU values of 6477 and 2439. AN had an excitation wavelength peak at 250nm and smaller excitation peak at 350nm with RFU values of

4025 and 1232. BaP had an excitation wavelength peak at 260nm, 280nm, 360nm, and 380nm with RFU vales of 7493, 8455, 5142, and 4672. This was the only compound to have greater than two prominent peaks. HPY had an excitation wavelength at 270nm and 340nm with an RFU value of 4329 and 4044. Compounds having a spectrum with an excitation peak pattern similar to that of pyrene (second excitation peak at 340-360nm) are referred to as 'hydroxypyrene-like' PAHs.

The standard curves for naphthol (HNP) and hydroxypyrene (HPY) were generated in order to convert RFUs into PAH concentrations in samples. The results are expressed in term of ng/mL of PAH vs total RFU. To construct the naphthol-like standard curve, the RFUs are those from the 270nm excitation wavelength using a fixed Em of 350nm. 75% Ethanol was used as a blank as well as the extraction solvent. Figure 7 is the standard curve for Naphthol-like PAHs. To construct the hydroxypyrene standard curve, the RFUs are those from the 340nm excitation wavelength using a fixed Em of 450nm. Figure 8 is the standard curve for hydroxypyrene-like PAHs. Detection limits were determined to be 5ng/mL.

6A

6B

Figure 6A&6B Fluorescent Spectra of PAHs: Fluorescent spectra of PAHs standards with emission wavelengths held constant at 350 (A) or 450 (B) nm. Excitation wavelengths were scanned from 250 to 340 or from 250 to 420. PAHs were naphthol (HNP), Fluorine (FL), Fluoranthene (FN), Anthracene (AN), hydroxpyrene (HPY), and Benzo a Pyrene (BaP) with a final concentration of 1250ng/mL. ***Above data and graphs generated by Edwin Pena, Seton Hall University.

Figure 7: Standard curve for Naphthol-like PAHs used through the entirety of this study. This Figure was constructed by Edwin Pena of Seton Hall University.

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Figure 8: Standard Curve for Hydroxypyrene-like PAHs used through the entirety of this study. This Figure was constructed by Edwin Pena of Seton Hall University.

Percent recovery was established for both naphthol-like and hydroxypyrene-like PAHs. The percent recovery was expressed as two values, as the 'recovery' from a spiked supernatant sample or as the 'recovery' from a spiked homogenate sample.

For naphthol-like percent recovery from the spiked supernatant method, fish from the Delaware Bay region Eva Marie ship, caught on July 5, 2011, were used for the calculation (Fish samples 005, 061, 070). To generate homogenate, 0.1g of liver was homogenized in 1mL of 75% EtOH. The homogenate was vortexed and centrifuged for 20 minutes at 13,000 RPM. Supernatant was transferred and pellet was saved for further analysis. 100ul of supernatant was added to 75% EtOH for a volume of 1ml (non-spiked samples) or 0.950mL (spiked samples). Samples included 1) two extracts with no spike, 2) duplicates of the same extracts with 50µl spike of naphthol added to the homogenate (final concentration: 1250ng/ml in 1mL EtOH), and a 1250ng/ml concentration of naphthol in 75% EtOH.

Results showed that the spiked samples with 50µl of naphthol had a higher RFU values (RFU values) as compared to the non-spiked samples (RFU values). The control, 1250ng/mL of naphthol in 75% EtOH had a nearly identical RFU value as the spiked samples, 061 and 070. This indicated a loss of naphthol as the combination of naphthol from the supernatant plus naphthol from the spike should have been higher than the spike alone. All three of these samples had an excitation peak at 270nm. The spiked fish sample 005 had the highest RFU value of 4435 as compared to the other samples with a single excitation peak at 280nm. The non-spiked fish sample of 005 had 2957 RFUs. There was a consistent outcome of the spiked supernatant samples having a higher RFU value as compared to the non-spiked samples. The average percent recovery for the method was 49%. Please refer to Figure 9 for these results.

For naphthol-like percent recovery from the spiked homogenate method, fish from the Delaware Bay region Eva Marie ship, caught on July 5, 2011, were used for the calculation (Fish samples 033 and 040). Homogenates were generated as described above and the spikes were added directly to the homogenate instead of its supernatant. Samples included 1) two homogenate samples with no spike, 2) two duplicates of the same samples with 50µl spike of naphthol (final concentration: 1250 ng/ml) added to the homogenate, and 3) a 1250ng/ml concentration of naphthol in 75% EtOH.

Results showed that the spiked homogenate samples with 50µl of naphthol had higher RFU values as compared to the non-spiked samples as expected. RFU values for non-spiked and spiked were 863 and 1833, respectively. The control of 1250ng/mL concentration of naphthol in 75% EtOH had the highest RFU value recovered in the sample population. Its RFU value was 3794. All of these samples had a nearly identical excitation peak pattern with a peak at 270nm. There was a consistent outcome of the spiked supernatant samples having a higher RFU value as compared to the non-spiked samples. The average percent recovery was 25%. Please refer to Figure 10 for these results.

For Hydroxypyrene-like percent recovery only homogenates were spiked (Fish 031, 033, and 040). The procedures and spike concentrations were the same as for naphthol (Figure 10). The average percent recovery for was 35%. Please refer to Figure 11 for these results. The spiked homogentate samples with a 50µl of hydroxypyrene had higher RFU values (3766, 1698, and 1833) as compared to the non-spiked samples (14, 19, and 23) as expected. The control of 1250ng/mL

concentration of naphthol in 75% EtOH had the highest RFU value (3010) recovered in the sample population. All of these samples had a nearly identical excitation peak pattern with a peak at 270/280nm and a peak at 340/350nm. There was a consistent outcome of the spiked supernatant samples having a higher RFU value as compared to the non-spiked samples.

Tables 3A and 3B provide the date for the percent recovery calculations. Table 3A combines the percent recovery results for naphthol (HNP) from Figure 9 and Figure 10. The average percent recovery for the results of spiked supernatant (EMNJ 005, 061, and 070) was 45% as compared to the average percent recovery for spiked homogenates (EMNJ 031, 033, and 040) which was 25%. Table 3B provides the percent recovery for Figure 11 with the EMNJ031, 033, and 040 fish. The average percent recovery for this method for hydroxypyrene (HPY) was 35%. Recovery of PAHs from homogenates was not as high as those from supernatants. However, those from homogenates best represented extraction of PAHs from tissues and were therefore used to calculate PAH concentrations in liver tissue when using standard curves (see Methods).

Figure 9: Percent recovery for naphthol with spiked supernatant (1250ng/mL). EMNJ fish samples were used. This Figure was constructed by Edwin Pena of Seton Hall University.

Figure 10: Percent recovery for naphthol with spiked homogenate (1250ng/mL). EMNJ fish samples were used. This Figure was constructed by Edwin Pena of Seton Hall University.

Figure 11: Percent recovery for hydroxypyrene with spiked homogenate (1250ng/mL). EMNJ fish samples were used. This Figure was constructed by Edwin Pena of Seton Hall University.

Table 3A Percent Recovery for Spiked Supernatent for HNP: Percent recovery of samples spiked with 1250ng/mL of naphthol (HNP). HNP-like PAHs were detected by fluorescence (RFU). Samples were either spike supernatents (EMNJ005, 061, and 070). The control was HNP alone.

Sample Number:	Non Spiked Value (RFU):	Spiked with 1250ng/ml of HNP	Percent Recovery (%):
EMNJ061	**1106**	**2875**	**49**
EMNJ070	**2748**	**3380**	**37**
EMNJ005	**2957**	**4435**	**36**
HNP	**3794**		**Average: 49%**

Table 3B Percent Recovery for Spiked Supernatent for HPY: Percent recovery of samples spiked with 1250ng/mL of HP-like PAHs were detected by fluorescence (RFU Fish (031, 033 and 040) obtained from the ship Eva Marie (EMNJ) were used for this analysis. There was an average of 35% recovery for this method.

Sample Number:	Non Spiked Value (RFU):	Spiked with 1250ng/ml of HP	Percent Recovery (%):
EMNJ031	**14**	**3766**	**32**
EMNJ033	**19**	**1698**	**56**
EMNJ040	**23**	**1833**	**39**
Control	**3010**		**Average: 35**

Naphthol-like PAH Determination in 2010 Liver Samples

Liver PAH was analyzed for 2010 samples. This included two catch dates from the Delaware Bay region, NJ Mount Vernon ship (MVNJ), one catch date from the Delaware Bay region Enterprise ship (EPNJ), one catch date from the Delaware Bay region Sea Huntress ship (SHNJ), one catch date from James River, VA (JRVA), and one catch from Barataria Bay, LA (BBLA).

The MVNJ samples were caught on 9/7/10 and on 9/23/10 (n=19). The samples were run with a fixed Em350 and were scanned for excitation. An excitation peak excitation was observed at 280nm. This spectrum was designated type 1 (n = 10). Another spectra type was also observed in which the major excitation peak was at 290nm. This spectrum was designated as type 2 (n=9). The reason for the different spectra types was unknown. Figure 12a provides the type 1 spectra for MVNJ. At Em280, the lowest value observed was 227 RFUs and the highest value observed was 1038 RFUs. Figure 12b provides the type 2 spectra for MVNJ. At Em290, the lowest value observed was 304 RFUs and the highest value observed was 1406 RFUs. All of the fish were identified as *B. tyrannus*.

The Enterprise Ship (EPNJ) that collected from the Delaware Bay Region on 9/21/10 provided 9 fish samples (n=9). The samples were run with a fixed Em350 and were scanned for excitation. There were two different spectra types as presented for MVNJ. Type 1 spectra had an excitation peak at 280nm (n = 8) and type 2 spectra had an excitation peak at 290nm (n = 1). Figure 13a provides the type 1 spectra for EPNJ. At Em280, the lowest value observed was 712 RFUs

and the highest value observed was 1500 RFUs. Figure 13b provides the type 2 spectra for EPNJ. At Em290, the value observed was 903 RFUs. All of the fish were identified as *B. tyrannus*.

The Sea Huntress ship (SHNJ) that collected from the Delaware Bay Region on 10/25/10 provided 19 samples (n = 19). The samples were run with a fixed Em350 and were scanned for excitation. There were two different spectra types. Type 1 spectra had an excitation peak at 280nm (n = 9) and type 2 spectra had an excitation peak at 290nm (n = 9). Figure 14a provides the type 1 spectra for SHNJ. At Em280, the lowest value observed was 745 RFUs and the highest value observed was 1241 RFUs. Figure 14b provides the type 2 spectra for SHNJ. At Em290, the lowest value observed was 304 RFUs and the highest value observed was 949 RFUs. All of the fish were identified as *B. tyrannus*.

The James River, VA (JRVA) samples were collected on 10/1/10 and provided 15 fish samples (n = 15). The samples were run with a fixed Em350 and were scanned for excitation. There were two different spectra types present in JRVA. Type 1 spectra had an excitation peak at 280nm (n = 10) and type 2 spectra had an excitation peak at 290nm (n = 5). Figure 15a provides the type 1 spectra for JRVA. At Em280, the lowest value observed was 575 RFUs and the highest value observed was 1424 RFUs. Figure 15b provides the type 2 spectra for JRVA. At Em290, the lowest value observed was 1368 RFUs and the highest value observed was 1945 RFUs. Fish samples JRVA002, JRVA003, JRVA004, JRVA007, JRVA014, and JRVA017 were identified as *B. patronus* and were all type 2 spectra. All of the remaining samples were identified as *B. tyrannus*.

The Barataria Bay, LA (BBLA) samples were collected on 10/30/10 and provided 10 fish samples (n = 10). The samples were run with a fixed 350em and were scanned for excitation. There was only one spectra type present. Type 1 spectra had an excitation peak at 280nm (n = 10). Figure 16 provides the type 1 spectra for BBLA. At Em280, the lowest value observed was 118 RFUs and the highest value observed was 781 RFUs. All of the species were identified as *B. patronus*.

Figure 12A: Mount Vernon, NJ type 1 spectra for HNP- like PAH determination in *B. tyrannus* fish in 2010. There is a 280nm excitation peak.

Figure 12B: Mount Vernon, NJ type 2 spectra for HNP- like PAH determination in *B. tyrannus* fish in 2010. There is a 290nm excitation peak.

Figure 13A: Enterprise ship, NJ type 1 spectra for HNP- like PAH determination in *B. tyrannus* fish in 2010. There is a 280nm excitation peak.

Figure 13B: Enterprise ship, NJ type 2 spectra for HNP- like PAH determination in *B. tyrannus* fish in 2010. The type 2 spectra is shown here with the EtOH baseline values. There is a 290nm excitation peak.

Figure 14A: Sea Huntress ship, NJ type 1 spectra for HNP-like PAH determination in *B. tyrannus* fish in 2010. There is a 280nm excitation peak.

Figure 14B: Sea Huntress ship, NJ type 2 spectra for HNP-like PAH determination in *B. tyrannus* fish in 2010. There is a 290nm excitation peak with higher RFU values.

Figure 15A: James River, VA type 1 spectra for HNP- like PAH determination in *B. tyrannus* fish in 2010. There is a 280nm excitation peak.

Figure 15B: James River, VA type 2 spectra for HNP- like PAH determination in *B. patronus* fish in 2010. There is a 280nm and 290nm excitation peak.

Figure 16: Barataria Bay, LA type 1 spectra for HNP- like PAH determination in *B. patronus* fish in 2010. There is a 280nm excitation peak.

Each catch site from 2010 was organized by spectra type. The type 1 (Ex280) and type 2 (Ex290) spectra were compared separately. Figure 17 provides the comparison of type 1 Naphthol-like spectra for all 2010 sample sites from fish liver for both *B. patronus* and *B. tyrannus* combined. The values shown are the average RFUs with standard error bars for the Ex280 for all fish samples from James River, VA (JRVA), Barataria Bay, LA (BBLA), Mount Vernon, NJ (MVNJ1 and MVNJ2) and Delaware Bay, NJ (SHNJ and EPNJ). The Barataria Bay, LA samples had the lowest RFU average with an Ex280 peak at 475 RFUs. The James River, VA had the highest RFU average with an Ex280 peak at 1100 RFUs.

Figure 18 provides the comparison of type 2 Naphthol-like spectra for all 2010 sample sites from fish liver for both *B. patronus* and *B. tyrannus* combined. The values shown are average RFUs with standard error bars for the Ex280/Ex290 for all fish samples from James River, VA (JRVA), Mount Vernon, NJ (MVNJ1 and MVNJ2) and Delaware Bay, NJ (SHNJ and EPNJ). The Barataria Bay, LA samples did not exhibit a type 2 spectra type and are therefore not shown here. The MVNJ1 had the lowest RFU average with an Ex290 peak at 222 RFUs. The James River, VA had the highest RFU average with an Ex290 peak at 1455 RFUs.

Comparisons of spectra type, sampling locations, and fish species found the following. Each of the catch sites had both type 1 and type 2 spectra naphthol- like PAHs with the exception of Barataria Bay, LA which only had type 1. In addition, type 1 and type 2 spectra were found in both types of species, *B.*

patronus and B. tyrannus, with similar major peaks of Ex280 or Ex290, despite the sample location. However, *B. patronus* from the James River, VA sample had only a type 2 spectra for naphthol-like PAHs.

Figure 17: Type 1 HNP-like PAH Spectra for all sample sites in 2010. Standard error bars are shown to demonstrate the differences between sites. MVNJ1 refers to the catch date of 9/7/10 and MVNJ2 refers to the catch date of 9/23/10. EPNJ2 refers to the catch date 0f 9/21/10.

Figure 18: Type 2 HNP-like PAH Spectra for all sample sites in 2010. Standard error bars are shown to demonstrate the differences between sites. MVNJ1 refers to the catch date of 9/7/10 and MVNJ2 refers to the catch date of 9/23/10. EPNJ2 refers to the catch date 0f 9/21/10. BBLA is not present as there was no type 2 spectrum observed in samples.

In order to investigate species related effects, *B. tyrannus* species and *B. patronus* from all sample sites in 2010 were compared. Figure 19 shows the type 1 naphthol-like spectra data for all *B. tyrannus* species across all sample sites. The Barataria Bay, LA samples are not included here since *B. tyrannus* is not present in this region. The Mount Vernon, NJ samples from 9/7/10 (MVNJ1) had the lowest RFU average with an Ex280 peak at 418 RFUs. The James River, VA had the highest RFU average with an Ex280 peak at 1100 RFUs. Fluorescence intensities (RFU) for type 1 spectra were overall very similar to each other regardless of capture site, with the single exception of MVNJ, which was lower.

Figure 20 shows the type 2 Naphthol-like spectra data for all *B.tyrannus* species across all sample sites. The Barataria Bay, LA samples are not included here since *B. tyrannus* is not present in this region. Also, there were no type 2 spectra from James River, VA for the *B. tyrannus* fish. As it did for the type 1 spectrum, the Mount Vernon, NJ samples from 9/7/10 (MVNJ1) had the lowest RFU average with an Ex280 peak at 418 RFUs. However, the Mount Vernon, NJ samples from 9/23/10 (MVNJ2) had the highest RFU average with an Ex280 peak at 1200RFUs. Type 2 spectra had higher average of RFU values compared to type 1 spectra. The fluorescence intensity of type 2 was similar between locations with the exception of MVNJ1; although, it was more viable among individuals of a particular catch compared to type 1.

The *B. patronus* species for each spectra type was also compared. Figure 21 shows both type 1 naphthol-like spectra and type 2 naphthol-like spectra for all *B. patronus* species found in James River, VA and Barataria Bay, LA. *B.*

patronus was only present in these two sample locations. James River, VA had higher type 1 spectra and type 2 spectra average RFU values as compared to Barataria Bay, LA. The James River, VA type 2 spectra had the highest average RFU compared to *B. patronus* and *B. tyrannus* from all other sites with an Ex290 of 1458 RFUs.

Figure 19: Type 1 HNP-like PAH Spectra for all *B. tyrannus* fish in sample sites in 2010. Standard Errors bars are shown to demonstrate the differences between sites. MVNJ1 refers to the catch date of 9/7/10 and MVNJ2 refers to the catch date of 9/23/10. EPNJ refers to the catch date 0f 9/21/10.

Figure 20: Type 2 HNP-like PAH Spectra for all *B. tyrannus* fish in sample sites from 2010. Standard Errors bars are shown to demonstrate the differences between sites. MVNJ1 refers to the catch date of 9/7/10 and MVNJ2 refers to the catch date of 9/23/10. EPNJ refers to the catch date 0f 9/21/10.

Figure 21: Both Type 1 and Type 2 HNP-like PAH Spectra for all *B. patronus* fish in sample sites from 2010. Standard Errors bars are shown to demonstrate the differences between sites. JRVA1 refers to type 1 naphthol-like spectra and JRVA2 refers to type 2 naphthol-like spectra. BBLA provides type 1 and type 2 spectra for Barataria Bay, Louisiana.

Hydroxypyrene-like PAH Determination in 2010 Liver Samples

Hydroxypyrene-like (HPY) PAHs were also investigated. The same liver samples analyzed for the HNP-like PAHS were also analyzed for the HPY-like PAHs, including MVNJ, EPNJ, SHNJ, JRVA, and BBLA. The samples were run with a fixed 450nm emission wavelength and scanned for excitation wavelengths from 250nm-440nm. Fluorescence from this longer wavelength represented 4-5 ring PAHs. Two types of spectra were observed called type 1 and type 2 HPY-like spectra. In order for a spectrum to be considered as type 1, two peaks had to be present; one at Ex270 and one at Ex350. Fluorescence intensity was measured at 350nm and not 270nm. To be considered type 2, spectra had only one observed excitation peak at 350nm. It was found that each HPY-like spectrum corresponded with one of the two naphthol-like spectra. Meaning, a sample that expressed a type 1 spectrum for HNP usually expressed a type 1 spectrum for HPY. Results for individual fish collections were as follows.

MVNJ1 samples were caught on 9/7/10 and MVNJ2 samples were caught on 9/23/10 (n=19 combined dates) from the Delaware River region of NJ. Ten fish had type 1 spectra and nine had type 2 spectra. Figure 22a provides the type 1 spectra for MVNJ1 and MVNJ2. At Ex350, the lowest value observed was 73 RFUs and the highest value observed was 427 RFUs. Figure 22b provides the type 2 spectra for MVNJ1 and MVNJ2. At Ex350, the lowest value observed was 361 RFUs and the highest value observed was 562 RFUs. Type 2 spectra typically had higher RFU values than type 1 spectra. All of the MVNJ fish were identified as *B. tyrannus*.

The Enterprise Ship (EPNJ) that collected from the Delaware Bay Region on 9/21/10 provided nine fish samples. Eight fish had type1 spectra and one fish had a type 2 spectra. Figure 23a provides the type 1 spectra for EPNJ. At Ex350, the lowest value observed was 47 RFUs and the highest value observed was 110 RFUs. Figure 23b provides the type 2 spectra for EPNJ. At Ex350, the peak value observed was 410 RFUs. All of the EPNJ fish were identified as *B. tyrannus*.

The Sea Huntress ship (SHNJ) collected menhaden from the Delaware Bay Region on 10/25/10 (n=18). Nine fish had type 1 spectra and nine had type 2 spectra. Figure 24a provides the type 1 spectra for SHNJ. At Ex350, the lowest value observed was 73 RFUs and the highest value observed was 125 RFUs. Figure 24b provides the type 2 spectra for SHNJ. At Ex350, the lowest value observed was 188 RFUs and the highest value observed was 441 RFUs. All of the SHNJ fish were identified as *B. tyrannus*.

The James River, VA (JRVA) samples were collected on 10/30/2013 and provided 15 fish samples (n = 15). There were ten type 1 spectra and five type 2 spectra. Figure 25a provides the type 1 spectra for JRVA. At Ex350, the lowest value observed was 54 RFUs and the highest value observed was 132 RFUs. Figure 25b provides the type 2 spectra for JRVA. At Ex350, the lowest value observed was 413 RFUs and the highest value observed was 689 RFUs. Fish samples JRVA002, JRVA003, JRVA004, JRVA007, JRVA014, and JRVA017 were identified as *B. patronus* which all had type 2 spectra. All of the remaining samples were identified as *B. tyrannus*.

The Barataria Bay, LA (BBLA) samples were collected on 10/30/10 and provided 10 fish samples (n = 10). Only type 1 spectra were observed. Figure 26 provides the type 1 spectra for BBLA. At Ex350, the lowest value observed was 14 RFUs and the highest value observed was 15 RFUs. All of the fish were identified as *B. patronus*.

Figure 22a: Mount Vernon, NJ fish samples from 9/7/10 and 9/23/10. This is type 1 HPY-like PAH determination in *B. tyrannus* fish. There is an excitation peak at 270nm and 350nm

Figure 22b: Mount Vernon, NJ fish samples from 9/7/10 and 9/23/10. This is type 2 HPY-like PAH determination in *B. tyrannus* fish. There is one excitation peak at 350nm.

Figure 23a: Enterprise Ship samples from the Delaware Bay Region, NJ. This is type 1 HPY-like PAH determination in *B. tyrannus* fish. There is an excitation peak at 280nm and 350nm.

Figure 23b: Enterprise Ship sample from the Delaware Bay Region, NJ. This is type 2 HPY- like PAH determination in *B. tyrannus* fish. There is an excitation peak at 350nm. The red dotted line represents ETOH control.

Figure 24a: Sea Huntress Ship samples from the Delaware Bay Region, NJ. This is type 1 HPY-like PAH determination in *B. tyrannus* fish. An excitation peak is seen at 280nm and 350 nm.

Figure 24b: Sea Huntress Ship samples from the Delaware Bay Region, NJ. This is type 2 HPY-like PAH determination in *B. tyrannus* fish. An excitation peak is seen at 350 nm.

Figure 25a: James River, VA fish samples. This is type 1 spectra for HPY- like PAH determination in *B. tyrannus* fish. There is a 270nm and 350nm excitation peak.

Figure 25b: James River, VA fish samples. This is type 2 spectra for HPY- like PAH determination in *B. patronus* fish. There is a 350nm excitation peak.

Figure 26: Barataria Bay, Louisiana fish samples. This is type 1 spectra for HPY- like PAH determination in *B. patronus* fish. There is a 270nm and 350nm excitation peak.

Each catch site from 2010 was compared by spectrum type. Figure 27 provides the comparision of type 1 HPY-like spectra for all 2010 sample sites from fish liver for both *B. patronus* and *B. tyrannus* combined on one graph. The values shown are the average RFUs with standard error bars for the fixed Em450 scans for fish samples from James River, VA (JRVA), Barataria Bay, LA (BBLA), Mount Vernon, NJ (MVNJ1 and MVNJ2) and Delaware Bay, NJ (EPNJ and SHNJ). The Barataria Bay, LA samples had the lowest RFU average with an Ex350 peak at 19 RFUs. Similarly, the Barataria Bay, LA samples also had the lowest average of RFU value for type HNP-like PAHs in 2010 samples. The Mount Vernon, NJ from 9/7/2010 had the highest RFU average with an Ex350 peak at 129 RFUs. These results indicated that there were differences between locations for PAH-like compounds using type 1 spectra.

Figure 28 provides the comparision of type 2 HPY-like spectra of fish liver from all 2010 sample sites of both *B. patronus* and *B. tyrannus* combined on

one graph. The values shown are the average RFUs with standard error bars for the fixed Em450 of all fish samples from James River, VA (JRVA), Delaware Bay, NJ (EPNJ and SHNJ), and Mount Vernon, NJ (MVNJ1 and MVNJ2). The Barataria Bay, LA (BBLA) samples did not exhibit a type 2 spectra type. Type 2 spectra demonstrated one major peak at 350nm unlike type 1 which had a peak at 270nm and 350nm. The Delaware Bay Region, NJ (SHNJ) had the lowest RFU average with an Ex350 peak at 276. The James River, VA samples had the highest RFU average with an Ex350 peak at 549 RFUs. Therefore, these results indicated that there were differences between locations for PAH-like compounds using type 2 spectra.

Figure 27: Average type 1 HPY- like PAH spectra for all sample sites in 2010. Standard errors bars are shown to demonstrate the differences between sites. MVNJ1 refers to the catch date of 9/7/10 and MVNJ2 refers to the catch date of 9/23/10.

Figure 28: Average type 2 HPY like PAH spectra for all sample sites in 2010. Standard errors bars are shown to demonstrate the differences between sites. EPNJ refers to the catch date of 9/21/10.

In order to investigate species-related effects, *B. tyrannus* and *B. patronus*, from all sample sites in 2010 were compared on separate graphs. Figure 29 shows type 1 HPY-like spectra data for *B. tyrannus* species. Barataria Bay, LA was not included since *B. tyrannus* was not found in this region. The Delaware Bay, NJ samples (EPNJ) had the lowest RFU average with an Ex350 peak at 83 RFUs. The Mount Vernon, NJ from 9/23/10 had the highest RFU average with an Ex350 peak at 129 RFUs. Overall, *B. tyrannus* with type 1 spectra appeared similar between collections in NJ and between collections in NJ and Barataria Bay, LA.

Figure 30 shows the type 2 HPY- like spectra for *B. tyrannus* fish. The Barataria Bay, LA samples are not included here since *B. tyrannus* was not present in this region. The James River, VA samples are not included here as there were no type 2 HPY-like spectra for the *B. tyrannus* collected. The Delaware Bay Region, NJ (SHNJ) had the lowest RFU average with an Ex350 peak at 276 RFUs. The Mount Vernon, NJ samples from 9/23/10 (MVNJ2) had the highest RFU average with an Ex350 peak at 487 RFUs. The MVNJ2 also had the highest RFU values for type 1 HNP-like spectra in *B. tyrannus.* Type 2 spectra had higher average RFU values compared to type 1 spectra. However, type 2 spectra of *B. tyrannus* did not appear to distinguish between NJ collections nor between NJ and Barataria Bay, LA collection sites.

The *B. patronus* fish for each spectra type were also compared. Figure 31 presents both type 1 HPY- like spectra and type 2 HPY-like spectra for all *B. patronus* fish found in James River, VA and Barataria Bay, LA. *B. patronus* was

only present in these two sample locations. James River, VA had higher average RFU values for type 1 and type 2 spectra compared to Barataria Bay, LA. The James River, VA type 2 spectra had the highest average with an Ex350 of 549 RFUs. This was the same result as for HNP-like PAHs in 2010 *B. patronus* fish. However, type 1 spectra for *B. patronus* did not appear to distinguish between VA and LA menhaden.

Figure 29: Average type 1 HPY-like PAH spectra for all *B. tyrannus* fish in sample sites from 2010. Standard errors bars are shown to demonstrate the differences between sites. MVNJ1 refers to the catch date of 9/7/10 and MVNJ2 refers to the catch date of 9/23/10. EPNJ refers to the catch date 0f 9/21/10.

Figure 30: Average type 2 HPY- like PAH spectra for all *B. tyrannus* fish in sample sites from 2010. Standard errors bars are shown to demonstrate the differences between sites. MVNJ1 refers to the catch date of 9/7/10 and MVNJ2 refers to the catch date of 9/23/10. EPNJ refers to the catch date 0f 9/21/10.

Figure 31: Average type 1 and type 2 HPY-like PAH spectra for all *B. patronus* fish in sample sites from 2010. Standard errors bars are shown to demonstrate the differences between sites. JRVA1 refers to type 1 and JRVA2 refers to type 2 HPY-like spectra. BBLA only had type 1 HPY-like spectrum.

Comparison of Naphthol like and Hydroxypyrene like PAH in 2010 Liver Samples

Statistical analyses were performed in order to determine if there were any signficant differences between sample sites in 2010 (Table 4). The sample sites were each analyzed in terms of both spectra type and species of menhaden. Units were in ng PAH/mg tissue dry weight.

The type 1 spectra values were compared for both species in all sample sites. Concentrations of HNP-like PAHs were calculated using the major peak at excitation wavelength of 280nm. Concentrations of HYP-like PAHs were calculated using the major peak at excitation wavelength of 350nm. Results for HNP-like PAHs using type 1 spectra showed that there were no significant differences between JRVA, SHNJ, or EPNJ for *B. tyrannus*. However, MVNJ1 *B. tyrannus* was significantly lower than JRVA, SHNJ, and EPNJ samples. HNP-like PAHs in *B. patronus* of BBLA were similar to those of *B. tyrannus* of MVNJ1 and also signficantly lower than the other sites. *B. tyrannus* of MVNJ2 and *B. patronus* of JRVA could not be compared to other sites due to their limited sample size of type 1 spectra. For the type 1 spectra of HPY- like PAHs, there were no significant differences between *B. tyrannus* of JRVA, SHNJ, EPNJ, and MVNJ1. The HPY-like PAHs were signifantly lower in *B. patronus* of BBLA. Overall, BBLA from fall 2010 had lower HNP and HPY-like PAHs based on type 1 spectra than Delaware Bay, NJ fish, which was unexpected given the recent oil spill in the Gulf of Mexico.

Ratios of the HNP-like and HPY-like PAHs for type 1 spectra were analyzed (Table 4). High ratios are associated with petrogenic PAH exposure

(petroleum source) and low ratios with pyrogenic PAH exposure (urbanzied areas). Ratios showed no significant differences between JRVA *B. tyrannus*, BBLA *B. patronus*, SHNJ *B. tyrannus*, and EPNJ *B. tyrannus*. The HNP/HPY ratio of MVNJ1 *B. tyrannus* was significantly lower than those of JRVA *B. tyrannus*, BBLA *B. patronus*, and EPNJ *B. tyrannus*. It was not signifcantly different than SHNJ *B. tyrannus*. MVNJ2 *B. tyrannus* and JRVA *B. patronus* were not included in comparisions due their sample size of one. Overall, this data from type 1 spectra did not indicate that menhaden from BBLA had been exposed to a different source of PAHs than the other sites despite the recent oil spill.

The same statistical analyses were performed for type 2 HNP- like and HPY-like PAHs of menhaden species analyzed in 2010 (Table 5). Type 2 spectra of HNP-like PAHs had their major peak at Ex290/Em350, while type 2 spectra of HPY-lk PAHs had their major peak at Ex350/Em450. Results using type II spectra for HNP-like PAHs showed that there were no significant differences between JRVA *B. patronnus* , SHNJ *B. tyrannus*, or MVNJ2 *B. tyrannus*. However, MVNJ1 *B. tyrannus* was significantly lower than those three sites. This finding for HNP-like type 2 was consistent with the results for HNP-like type 1. For type 2 HPY- like PAHs, there were no significant differences between JRVA *B. tyrannus*, MVNJ1 *B. tyrannus* and MVNJ2 *B. tyrannus*. The HPY- like PAHs were signifantly lower in SHNJ *B. tyrannus* as compared to JRVA *B. patronus* and MVNJ2 *B. tyrannus*. SHNJ *B. tyrannus* was not signifcantly different from MVNJ1 *B. tyrannus*. The lower HPY-like PAHs measured using type II spectra in SHNJ *B. tyrannus* were not consistent with the type 1 result, which was similar

to the other sites. EPNJ *B. tyrannus* was not included in the statistical analysis due to its sample size of one. BBLA *B. patronus* spectra were not included as it had no type 2 spectra for either HNP or HPY-like PAHs.

The ratios of HNP- like and HPY-like PAHs for type 2 spectra were analyzed (Table 5). The only signficant difference between sites was SHNJ *B. tyrannus,* which was higher than MVNJ1and MVNJ1 *B. tyrannus*. MVNJ1 *B. tyrannus* had a low ratio for type 2 spectra as it did for type 1. This indicated consistent support for pyrogenic PAH exposure at this site. Again, EPNJ *B. tyrannus* was not included due to its sample size of 1. BBLA *B. patronus* was not included as it had no type 2 spectra for either PAH. Ratios calculated using type II spectra were lower than those calculated using type I spectra. HPY-like PAH concentrations appeared to account for the differences as concentrations of HNP-like PAHs were similar for type 1 and type 2 spectra.

Overall, the data did indicate the following. Menhaden from BBLA had exposure to a different source of PAHs. *B. patronus* species from BBLA did not exhibt HNP-like and HPY-like type 2 spectra and was statistically different for type 1 HPY-like PAHs from all other catch sites. The *B. tyrannus* species exhibited both type 1 and type 2 spectras for both HNP-like and HPY-like spectra unlike *B. patronus* indicating a more diverse PAH exposure type for these species and/or greater accumulation of PAHs by *B. patronus*. The only signficant difference between sites was SHNJ *B. tyrannus,* which was higher than MVNJ1and MVNJ1 *B. tyrannus.* MVNJ1 *B. tyrannus* had a low ratio for type 1 and type 2 spectra indicating pyrogenic exposure for fish in this collection.

Table 4: Type 1 Spectra for Naphthol-like and Hydroxypyrene-like PAHs and Their Ratios, in *B. tyrannus* and *B. patronus*. N = sample size. HNP-Like = Naphthol-like PAHs (ng/mg). HPY-like = Hydroxypyrene-like PAHs (ng/ml). If letters are the same, then the values were not significantly different from site to site.

Location	Collection Date	Species	Spectra	n	HNP-like	HPY-like	Ratio HNP/HPY
JRVA	10/31/2010	*B. tyrannus*	I	9	703±265[a]	57±20[a]	15±11[a]
		B. patronus	I	1	563	51	11
BBLA	10/30/2010	*B. tyrannus*	I	0			
		B. patronus	I	10	303±144[b]	19±11[b]	15±5[a]
SHNJ	10/25/2010	*B. tyrannus*	I	9	603±110[a]	59±13[a]	10±1[ab]
		B. patronus	I	0			
EPNJ	9/21/2010	*B. tyrannus*	I	9	717±130[a]	55±20[a]	14±4[a]
		B. patronus	I	0			
MVNJ1	9/7/2010	*B. tyrannus*	I	9	267±105[b]	79±43[a]	4±3[b]
		B. patronus	I	0			
MVNJ2	9/23/2010	*B. tyrannus*	I	1	664	66	10
		B. patronus	I	0			

Table 5: Type 2 Spectra for Naphthol-like and Hydroxypyrene-like PAHs and their Ratios, in *B. tyrannus* and *B. patronus*. N = sample size. HNP-Like = Naphthol-like PAHs (ng/mg). HPY-like = Hydroxypyrene-like PAHs (ng/ml). If letters are the same, then the values were not significantly different from site to site.

Location	Collection Date	Species	Spectra	n	HNP-like	HPY-like	Ratio HNP/HPY
JRVA	10/31/2010	*B. tyrannus*	II	0			
		B. patronus	II	5	932±307[a]	338±60[a]	3±1[ab]
BBLA	10/30/2010	*B. tyrannus*	II	0			
		B. patronus	II	0			
SHNJ	10/25/2010	*B. tyrannus*	II	9	719±209[a]	170±57[b]	4±1[a]
		B. patronus	II	0			
EPNJ	9/21/2010	*B. tyrannus*	II	1	578	252	2
		B. patronus	II	0			
MVNJ1	9/7/2010	*B. tyrannus*	II	2	198±5[b]	256±9[ab]	0.77±0.01[b]
		B. patronus	II	0			
MVNJ2	9/23/2010	*B. tyrannus*	II	7	767±130[a]	299±48[a]	2.65±0.72[b]
		B. patronus	II	0			

Naphthol-like PAH Determination in 2011 Liver Samples

Liver PAHs were analyzed for 2011 samples listed in Table 2. They included four catch dates from Barataria Bay, LA (BBLA2, BBLA3, BBLA4, and BBLA5), two catch dates from Vermilion Bay, LA (VBLA1 and VBLA2), and seven catch dates from Delaware Bay, NJ (EMNJ1, EMNJ2, EMNJ3, ESNJ1, ESNJ2, ESNJ3, and ESNJ4).

Samples from the Eva Marie ship from the Delaware Bay region, NJ were caught on 7/5/11 (EMNJ1), 8/31/11 (EMNJ2), and 10/8/11(EMNJ3) and provided 25 fish. The samples were run with a fixed Em350 and were scanned for excitation. An excitation peak was observed at 280nm for type 1 spectra (n =25). Figure 32 provides the type 1 spectra for EMNJ. This data also exhibited a 270nm peak in some samples. However, it is still classified as type 1 spectra due to the major peak at 280nm. At Em280, the lowest value observed was 821 RFUs and the highest value observed was 4589 RFUs. There was only one type of spectrum observed in this data. All of the species were identified as *B. tyrannus*.

Samples from the Evening Star ship from the Delaware Bay Region, NJ were caught on 6/20/11(ESNJ1), 6/24/11(ESNJ2), 8/31/11(ESNJ3), and 10/8/11(ESNJ4) and provided 48 fish samples. The samples were run with a fixed Em350 and were scanned for excitation. An excitation peak was observed at 280nm for type 1 spectra (n =28). An excitation peak was also observed at 290nm for type 2 spectra (n =13). Figure 33a provides the type 1 spectra for all ESNJ catch dates. At Em280, the lowest value observed was 577 RFUs and the highest value observed was 2232 RFUs. Figure 33b provides the type 2 spectra

for all ESNJ catch dates. At Em290, the lowest value observed was 1679 RFUs and the highest value observed was 4589 RFUs. All of the species were identified as *B. tyrannus*.

Samples from Vermillion Bay, LA were collected on 8/23/11 (VBLA1) and 9/12/11 (VBLA2) and provided 37 fish. The samples were run with a fixed Em350 and were scanned for excitation. An excitation peak was observed at 280nm for type 1 spectra (n = 37). Figure 34 provides the type 1 spectra for VBLA samples. At Em280, the lowest value observed was 602 RFUs and the highest value observed was 2568 RFUs. There were no other spectrums observed in these samples. All of the species were identified as *B. patronus*.

Samples from Barataria Bay, LA were collected on 7/28/11 (BBLA2), 8/24/11(BBLA3), 9/13/11(BBLA4), and 10/11/11(BBLA5) provided 68 fish. The samples were run with a fixed Em350 and were scanned for excitation. An excitation peak was observed at 280nm for type 1 spectra (n = 59). An excitation peak was observed at 280nm and 290nm for type 2 spectra (n= 9). Figure 35a provides the type 1 spectra for BBLA. At Em280, the lowest value observed was 708 RFUs and the highest value observed was 3169 RFUs. Figure 35b provides the type 2 spectra for BBLA. At Em280, the lowest value observed was 613 RFUs and the highest value observed was 1175 RFUs. Again, a second excitation peak at 290nm was present in all type 2 spectra. All of the species were identified as *B. patronus*.

Each catch site from 2011 was organized by spectra type. The type 1 (Ex280) and type 2 (Ex280/290) spectra were compared separately. Figure 36

provides the comparison of type 1 HNP-like spectra for all 2011 sample sites from fish liver for *B. patronus* and *B. tyrannus* combined. The values shown are the average RFUs with standard error bars for the Ex280 for fish samples from Barataria Bay, LA (BBLA2, BBLA3, BBLA4, and BBLA5), and Delaware Bay, NJ (EMNJ1, EMNJ2, EMNJ3, ESNJ1, ESNJ2, ESNJ3 and ESNJ4). The Delaware Bay ESNJ samples had the lowest RFU average with an Ex280 peak of 1250 RFUs. The Delaware Bay, NJ (EMNJ) had the highest RFU average with an Ex280 peak at 1707 RFUs.

Figure 37 provides the comparison of type 2 HNP- like spectra for all 2011 sample sites from fish liver for *B. patronus* and *B. tyrannus* combined. The values shown are the average RFUs with standard error bars for the Ex280/Ex290 for all fish samples from Barataria Bay, LA (BBLA2, BBLA3, BBLA4, and BBLA5), and Delaware Bay, NJ (ESNJ1, ESNJ2, ESNJ3 and ESNJ4). The Vermillion Bay, LA samples (VBLA1 and VBLA2) and Delaware Bay, NJ (EMNJ1, EMNJ2, and EMNJ3) did not exhibit a type 2 spectra type and are therefore not shown here. BBLA had the lowest RFU average with an Ex280 peak at 820 with another Ex290 peak at 829 RFUs. The Delaware Bay, NJ (ESNJ1, ESNJ2 and ESNJ3) had the highest RFU average with an Ex280 peak at 1279 RFUs with another Ex290 peak at 1224 RFUs.

Each of the catch sites had both type 1 and type 2 HNP-like present with the exception of Delaware Bay Region, NJ (EMNJ1, EMNJ2, and EMNJ3) and Vermilion Bay, LA (VBLA1 and VBLA2) which only had type 1 HNP-like spectra. However, type 1 and type 2 HNP-like spectra were present in both

species; *B. patronus* and *B. tyrannus*, with similar profiles of Ex280 or Ex280/Ex290, despite the different sample locations. For the 2011 data, separate species analysis was not done as each sample location only had one species type present. There was no overlap of speciation in the same sample site as seen in James River, VA 2010 data.

Figure 32: Eva Marie Ship Vessel from the Delaware Bay Region, NJ (EMNJ1, EMNJ2, EMNJ3). EMNJ1 catch date from 7/5/11, EMNJ2 catch date from 8/31/11, and EMNJ3 catch date from 10/8/11. This is type 1 spectra for HNP-like PAH determination in *B. tyrannus* species. There is a 280nm excitation peak.

Figure 33A: Evening Star Ship from the Delaware Bay Region, NJ (ESNJ1, ESNJ2, ESNJ3, and ESNJ4). ESNJ1 catch date from 6/24/11, ESNJ2 catch date from 6/30/11, ESNJ3 catch date from 8/31/11, and ESNJ4 catch date from 10/8/11. Type 1 spectra for HNP- like PAH determination in *B. tyrannus* species. There is a 280nm excitation peak.

Figure 33B: Evening Star Ship from the Delaware Bay Region, NJ (ESNJ1, ESNJ2, ESNJ3, and ESNJ4). ESNJ1 catch date from 6/24/11, ESNJ2 catch date from 6/30/11, ESNJ3 catch date from 8/31/11, and ESNJ4 catch date from 10/8/11. Type 1 spectra for HNP-like PAH determination in *B. tyrannus* species. There is both a 280nm and 290nm excitation peak.

Figure 34: Vermillion Bay, LA caught on 8/23/11(VBLA1) and 9/12/11(VBLA2). Type 1 spectra for HNP- like PAH determination in *B. patronus* species. There is a 280nm excitation peak.

Figure 35A: Barataria Bay, LA caught on 7/28/11 (BBLA2), 8/24/11(BBLA3), 9/13/11(BBLA4), and 10/11/11(BBLA5). Type 1 spectrum for HNP-like PAH determination in *B. patronus* species. There is a 280nm excitation peak.

Figure 35B: Barataria Bay, LA caught on 7/28/11 (BBLA2), 8/24/11(BBLA3), 9/13/11(BBLA4), and 10/11/11(BBLA5). Type 2 spectra for HNP-like PAH determination in *B. patronus* species. There is a 280nm and 290nm excitation peak.

Figure 36: Type 1 HNP like PAH spectra for sample sites in 2011. Standard errors bars are shown to demonstrate the differences between sites. The graphed values represent averages for all of the sample dates within each site. BBLA refers to the average of BBLA2, BBLA3, BBLA4, and BBLA5 type 1 HNP-like spectra. VBLA refers to the average of VBLA1 and VBLA2. EMNJ refers to the average of EMNJ1, EMNJ2, and EMNJ3. ESNJ refers to the average of ESNJ1, ESNJ2, ESNJ3, and ESNJ4. There is an Ex280 peak.

Figure 37: Type 2 HNP- like PAH Spectra for sample sites in 2011. Standard errors bars are shown to demonstrate the differences between sites. The graphed values represent averages for all of the sample dates within each site. BBLA refers to the average of BBLA2, BBLA3, BBLA4, and BBLA5 type 2 HNP-like spectra. ESNJ refers to the average of ESNJ1, ESNJ2, ESNJ3, and ESNJ4. EMNJ and VBLA are not present as there were no type 2 spectra observed in samples. There is an Ex280 and Ex290 peak.

Hydroxypyrene-like PAH Determination in 2011 Liver Samples

Hydroxypyrene-like (HPY) PAHs were also investigated. The same liver samples analyzed for HNP-like PAHs were also analyzed for HPY-like PAHs including EMNJ, BBLA, VBLA, ad ESNJ. The samples were run with a fixed 450nm emission wavelength and scanned for excitation wavelengths. Two different types of spectra were identified among the 2011 sample sites as with the 2010 sample site, type 1 and type 2. In order for a spectrum to be considered as type 1, two peaks had to be present; one at Ex270/280 and one at Ex330-Ex350. Fluorescence intensity was measured at 350nm and not 270/280nm. To be considered type 2, spectra had only one observed excitation peak at 350/360nm.

Delaware Bay, NJ samples were caught on 7/5/11 (EMNJ1), 8/31/11 (EMNJ2), and 10/8/11(EMNJ3) and provided twenty five fish samples. Type 1 spectra were observed in all fish samples. Figure 38 provides the type 1 HPY-like spectra for EMNJ samples. At Ex350, the lowest value observed was 9 RFUs and the highest value observed was 120 RFUs. All of the fish were identified as *B. tyrannus*.

Delaware Bay, NJ samples were caught on 6/24/11 (ESNJ1), 6/30/11 (ESNJ2), 8/31/11 (ESNJ3), and 10/8/11 (ESNJ4) and provided forty one fish samples. Two different HPY-like spectra types were observed; type 1 and type 2. Figure 39a provides the type 1 spectra for ESNJ (n=21). At Ex.350, the lowest value observed was 9 RFUs and the highest value observed was 105 RFUs. Figure 39b provides the type 2 spectra for ESNJ (n=20). At Ex350, the lowest

value observed was 156 RFUs and the highest value observed was 300 RFUs. All of the fish were identified as *B. tyrannus*.

The Vermilion Bay, LA samples were caught on 7/6/11 (VBLA1), 8/23/11 (VBLA2), and 9/12/11 (VBLA3) and provided eighty fish samples. Two different HPY-like spectra types were observed; type 1 and type 2. Figure 40a provides the type 1 spectra for VBLA (n=55). At Ex350, the lowest value observed was 13 RFUs and the highest value observed was 224 RFUs. Figure 40b provides the type 2 spectra for VBLA (n=25). At Ex350, the lowest value observed was 21 RFUs and the highest value observed was 98 RFUs. All of the fish were identified as *B. patronus*.

The Barataria Bay, LA samples were caught on 7/28/11 (BBLA2), 8/24/11 (BBLA3), 9/13/11 (BBLA4), and 10/11/11 (BBLA5) and provided seventy one samples. Two different HPY-like spectra types were observed, type 1 and type 2. Figure 41a provides the type 1 spectra for BBLA (n=60). At Ex350, the lowest value observed was 15 RFUs and the highest value observed was 175 RFUs. Figure 41b provides the type 2 spectra for BBLA (n=11). At Ex350, the lowest value observed was 130 RFUs and the highest value observed was 414 RFUs. All of the fish were identified as *B. patronus*

All catch sites for 2011 expressed both type 1 and type 2 HPY-like PAHs with the exception of EMNJ which only had type 1. *B. tyrannus* and *B. patronus* expressed both types of spectra.

Figure 38: Type 1 spectra of Eva Marie ship samples from Delaware Bay Region, NJ. This is type 1 spectrum for HPY- like PAH determination in *B. tyrannus* species. There is a 280nm and 330/350nm excitation peak.

Figure 39A: Type 1 spectra of Evening Star samples from Delaware Bay Region, NJ. This is type 1 spectrum for HPY-like PAH determination in *B.tyrannus* species. There is a 280nm and 330/350nm excitation peak.

Figure 39B: Type 2 spectra of Evening Star samples from Delaware Bay Region, NJ. This is the type 2 spectra for HPY-like PAH determination in *B. tyrannus* species. This has a 290nm and 360nm excitation peak.

Figure 40A: Type 1 spectra of samples from Vermillion bay, LA caught on 7/6/11 (VBLA1), 8/23/11 (VBLA2) and 9/12/11 (VBLA3). This is the type 1 spectra for HPY-hydroxypyrene like PAH determination in *B. patronus* species. There is a 280nm and 350/360nm excitation peak.

Figure 40B: Type 2 spectra of samples from Vermillion Bay, LA caught on 7/6/11 (VBLA1), 8/23/11 (VBLA2) and 9/12/11 (VBLA3). This is type the 2 spectra for HPY- like PAH determination in *B. patronus* species. There is a 290nm and 360nm excitation peak.

Figure 41A: Type 1 spectra of samples from Barataria Bay, LA caught on 7/28/11(BBLA2), 8/24/11(BBLA3), 9/13/11(BBLA4), and 10/11/11(BBLA4). This is the type 1 spectra for HPY- like PAH determination in *B. patronus* species. There is a 280nm and 330/350nm excitation peak.

Figure 41B: Type 2 spectra of samples from Barataria Bay, LA caught on 7/28/11(BBLA2), 8/24/11(BBLA3), 9/13/11(BBLA4), and 10/11/11(BBLA4). This is the type 2 spectra for HPY- like PAH determination in *B.patronus* species. There is a 290nm and 360nm excitation peak.

Each catch site from 2011 was compared by spectrum type. Figure 42 provides the comparision of type 1 HPY-like spectra for all 2011 sample sites from fish liver for *B. patronus,* and *B. tyrannus* combined on one graph. There is a 280nm and 330/350nm excitation peak for 2011 type 1 HPY-like spectra. The values shown are the average RFUs with standard error bars for the fixed Em450 scans for fish samples from the Delaware Bay region, NJ (EMNJ and ESNJ), Barataria Bay, LA (BBLA), and Vermilion Bay, LA (VBLA). The ESNJ samples had the lowest RFU average with an Ex350 peak at 36 RFUs. In 2010 data, the ESNJ samples also had the lowest average of RFU value for type 1 HPY-like PAHs. The BBLA samples had the highest RFU average with an Ex350 peak at 158 RFUs. These results indicated that catch sites from Barataria Bay, LA had on average higher or more chronic exposure to PAHs.

Figure 43 provides the comparision of type 2 HPY-like spectra of fish liver from all 2011 sample sites for *B. patronus* and *B. tyrannus* combined on one graph. The Delaware Bay, NJ EMNJ samples did not exhibit type 2 HPY-like PAHs in 2011. The values shown are the average RFUs with standard error bars for the fixed Em450 of all fish samples from the Delaware Bay region, NJ (ESNJ), Barataria Bay, LA (BBLA), and Vermilion Bay, LA (VBLA). The type 2 HPY-like spectra demonstrated an Ex290nm and Ex360nm peak. VBLA had the lowest RFU average with an Ex350 peak at 133. The ESNJ samples had the highest RFU average with an Ex350 peak at 273 RFUs. In 2010, BBLA only had type 1 HPY-like spectra; therefore, the presence of type 2 HPY-like spectra indicated a profile shift in 2011.

Figure 42: Average type 1 HPY- like PAH spectra for all sample sites in 2011. Standard errors bars are shown to demonstrate the differences between sites.

Figure 43: Average type 2 HPY-like PAH spectra for sample sites in 2011. EMNJ had no type 2 HPY-like spectra for 2011. Standard errors bars are shown to demonstrate the differences between sites.

Comparison of Seasonal Naphthol-like and Hydroxypyrene-like PAHs in 2011 Liver Samples

Statistical analyses were performed in order to determine if there were any signficant differences between samples dates in 2011. The dates of collections were analyzed for a paticular site to determine if there were seasonal effects on PAH concentrations. Analyses were done on Ex280/Em350 for HNP-like PAHs and Ex 350/Em450 for HPY-like PAHs wihout distinguishing between different spectra types. This was done as not all spectra types were present for each catch sites/dates. Data were converted to ng/mg of PAHs using standard curves for HNP and HPY.

Barataria Bay, LA had four catch dates in 2011 including 7/28/2011 (BBLA2), 8/24/2011 (BBLA3), 9/13/2011 (BBLA4), and 10/11/2011 (BBLA5) (Table 6). All fish were identified as *B. patronus*. For the HNP- like PAHs, there were no significant differences between catch dates of 7/28/2011 and 8/24/2011. However, there was a significant difference between catch date 7/28/2011 and catch dates of 9/13/2011 and 10/11/2011. There were no significant differences between 8/24/2011, 9/13/2011,10/11/2011. These results indicated that early summer samples contained more HNP-like PAHs than late summer/early fall samples. For HPY-like PAHs, there were no significant differences between catch dates of 8/24/2011, 9/13/2011,10/11/2011. However, there were significant differences between catch date 7/28/2011 and all other catch dates. Catch date 7/28/2011 was significantly higher than all other catch dates. These results indicated that early summer samples contained more HPY-like PAHs than late

summer/early fall samples. There were significant differences between BBLA ratios of HNP-like PAHs and HPY-like PAHs. The catch date of 7/28/2011 was significantly higher than all other catch dates, which were themselves not different from one another. Results shows that the 7/28/2011 catch had a higher HPY-like concentration, approximately 6X higher, which probably accounted for the lower ratio.

Vermillion Bay, LA had three catch dates in 2011 including 7/6/2011 (VBLA1), 8/23/2011 (VBLA2), and 9/12/2011 (BBLA3) (Table 7). All fish were identified as *B. patronus*. For the HNP-like PAHs, there were no significant differences between the 7/6/2011, 8/23/2011, and 9/12/2011 catch dates. For the HPY- like PAHs, there were no significant differences between catch dates 8/23/2011, and 9/12/2011. However, catch date 7/6/2011 was significantly higher than the other catch dates. It had a HPY–like concentration approximately 2-3X higher than the other dates. These results also indicated that early summer samples contained more HPY-like PAHs than late summer/early fall samples. There were no significant differences for the HNP-like and HPY-like ratios among catch dates.

Eva Marie, NJ had three catch dates in 2011 including: 7/5/2011 (EMNJ1), 8/31/2011 (EMNJ2), and 10/8/2011 (EMNJ3) (Table 8). All fish were identified as *B. tyrannus*. For the HNP- like and HPY-like PAHs, there were no significant differences between 8/31/2011 and 10/8/2011 catch dates. However, the 7/5/2011 catch date was significantly higher than both 8/31/2011 and 10/8/2011 catch dates for HNP-like and HPY like PAHs. Results showed that for

the 7/5/2011 HNP-like concentrations were approximately 2X higher and HPY-like concentrations were 3-4X higher than the other catch dates. There were no significant differneces between the HNP- like and HPY- like ratios between catch dates. These results also indicated that early summer samples contained more HPY-like PAHs than late summer/early fall.

Evening Star, NJ had four catch dates in 2011 including 6/24/2011 (ESNJ1), 6/30/2011 (ESNJ2), 8/31/2011 (ESNJ3), and 10/8/2011 (ESNJ4) (Table 9). All fish samples were identifed as *B. tyrannus*. For the HNP- like PAHs, there were no significant differences between 6/24/2011, 6/30/2011, and 10/8/2011 catch dates. However, catch date 8/31/2011 was significantly higher than the 6/24/2011 and 6/30/2011 catch dates. The results showed that the HNP-like concentration was approximately 1.5-2X higher. The 8/31/2011 and 10/8/2011 catch dates were not significantly different from each other. The results showed that for the HPY-like PAH concentrations, the 6/24/2011 and 6/30/2011 catch dates were significantly higher than both 8/31/2011 and 10/8/2011 catch dates, approximately 5-7X higher. For HNP-like and HPY like ratios, the results showed that both the 8/31/2011 and 10/8/2011 catch dates were significantly higher than other catch dates, approximately 6-7X higer. These results also indicated that early summer samples contained more HPY-like summer/early PAHs than late fall samples.

Overall, comparison of catch dates showed that fish collected in fall (September and October) contained more HNP-like PAHs than early summer samples (June and July). Conversely, fish samples from an early summer catch

date had higher average HPY-like PAH concentrations than fish from fall samples dates. The results demonstrated seasonal variability for HNP-like PAHs vs HPY-like PAHs regardless of species and regardless if the were collected on the Atlantic coast or Gulf of Mexico.

Table 6: Barataria Bay, LA spectra for Naphthol like (HNP) and Hydroxypyrene like (HPY) PAHs and their Ratios for menhaden from different sample dates in 2011. *B. tyrannus* and *B. patronus* are fish type. N = sample size. HNP-Like = naphthol like PAHs (ng/mg). HPY-like = hydroxypyrene like PAHs (ng/mg). If letters are the same, then the values are not significantly different from site to site. For HPY-like PAHs, BBLA2 was significantly higher than BBLA3 and BBLA4. For HNP-like PAHs, BBLA2 was significantly lower than BBLA4 and BBLA5. For the HNP/HPY ratio, BBLA2 was significantly different from all other BBLA sites.

Location	Collection Date	Species	n	HNP-like	HPY-like	Ratio HNP/HPY
BBLA2	7/28/2011	*B. tyrannus*	0			
		B. patronus	15	580±327[a]	140±68[a]	5±3[a]
BBLA3	8/24/2011	*B. tyrannus*	0			
		B. patronus	21	839±343[ab]	22±15[b]	51±25[b]
BBLA4	9/13/2011	*B. tyrannus*	0			
		B. patronus	17	989±318[b]	22±15[b]	57±23[b]
BBLA5	10/11/2011	*B. tyrannus*	0			
		B. patronus	19	897±357[b]	24±9[b]	42±20[b]

Table 7: Vermilion Bay, LA spectra for Naphthol like (HNP) and Hydroxypyrene like (HPY) PAHs and their Ratios for menhaden from different sample dates in 2011. *B. tyrannus* and *B. patronus* are fish type. N = sample size. HNP-Like = naphthol like PAHs (ng/ml). HPY-like = hydroxypyrene like PAHs (ng/mg). If letters are the same, then the values are not significantly different from site to site. There were no significant differences between sites for HNP- like PAHs and HNP/HPY like ratios. There was a significant difference between VBLA1 and other catch dates for HPY-like PAHs.

Location	Collection Date	Species	n	HNP-like	HPY-like	Ratio HNP/HPY
VBLA1	7/6/2011	*B. tyrannus*	0			
		B. patronus	33	993±438 [a]	86±67[a]	19±13[a]
VBLA2	8/23/2011	*B. tyrannus*	0			
		B. patronus	19	802±372 [a]	31±29[b]	34±18[a]
VBLA3	9/12/2011	*B. tyrannus*	0			
		B. patronus	19	1030±494[a]	40±25[ab]	34±22[a]

Table 8: Eva Marie, NJ spectra for Naphthol like (HNP) and Hydroxypyrene like (HPY) PAHs and their Ratio for menhaden from different sample dates in 2011. *B. tyrannus* and *B. patronus*, are fish type. N = sample size. HNP-Like = naphthol like PAHs (ng/mg). HPY-like = hydroxypyrene like PAHs (ng/mg). If letters are the same, then the values are not significantly different from site to site. There were no significant differences between sites for HNP/HPY like ratios. There was a significant difference between EMNJ1 and all other catch dates for HNP-like and HPY-like PAHs/

Location	Collection Date	Species	n	HNP-like	HPY-like	Ratio HNP/HPY
EMNJ1	7/5/2011	*B. tyrannus*	5	3892±1275[a]	95±51[a]	46±14[a]
		B. patronus	0			
EMNJ2	8/31/2011	*B. tyrannus*	10	1407±60[b]	30±18[b]	61±22[a]
		B. patronus	0			
EMNJ3	10/8/2011	*B. tyrannus*	10	1208±591[b]	20±14[b]	76±43[a]
		B. patronus	0			

Table 9: Evening Star, NJ spectra for Naphthol like (HNP) and Hydroxypyrene like (HPY)PAHs and their Ratios for menhaden from different sample dates in 2011. *B. tyrannus* and *B. patronus* are species type. N = sample size. HNP-Like = naphthol like PAHs (ng/mg). HPY-like = hydroxypyrene like PAHs (ng/mg). If letters are the same, then the values are not significantly different from site to site. There were no significant differences between ESNJ 1, ESNJ2, and ESNJ4 for HNP- like PAHS. There were no significant differences between ESNJ 1 and ESNJ2 for HPY-like PAHs and HNP/HPY like ratios. There were no significant differences between ESNJ3 and ESNJ4 for HNP- like, HPY-like PAHs and HNP/HPY like ratios. There were significant differences between ESNJ1and ESNJ2 and between ESNJ3 and ESNJ4 for HNP- like, HPY-like PAHs and HNP/HPY like ratios.

Location	Collection Date	Species	n	HNP-like	HPY-like	Ratio HNP/HPY
ESNJ1	6/24/2011	*B. tyrannus*	14	434±228[a]	59±29[a]	10±8[a]
		B. patronus	0			
ESNJ2	6/30/2011	*B. tyrannus*	11	623±256[a]	95±59[a]	9±6[a]
		B. patronus	0			
ESNJ3	8/31/2011	*B. tyrannus*	10	1103±402[b]	16±8[b]	76±36[b]
		B. patronus	0			
ESNJ4	10/8/2011	*B. tyrannus*	10	712±469[ab]	13±10[b]	60±24[b]
		B. patronus	0			

Comparison of Collection Sites for Naphthol-like and Hydroxypyrene-like PAHs in 2011 Liver Samples

Statistical analyses were performed in order to determine if there were any significant differences between fish collection sites for a particular season including early summer (June –July), late summer (August), and fall (September-October) in 2011. Analyses were done on Ex280/Em 350 for HNP- like PAHs and Ex350/Em450 for HPY-like PAHs withough distinguishing between the different spectra types. Data were converted to ng/mg liver PAHs using standard curves for HNP and HPY.

BBLA2, VBLA1, EMNJ1, and ESNJ2 all had sample catch dates during June or July 2011 (Table 10). BBLA1 was caught on 7/28/2011 with all of the fish identified as *B. patronus*. VBLA1 was caught on 7/6/2011 with all of the fish identifed as *B. patronus*. EMNJ1 was caught on 7/5/2011 with all of the fish identified as *B. tyrannus*. ESNJ2 was caught on 6/30/2011 with all of the fish identified as *B. tyrannus* species. Although this sample date was not in the calendar month of July, it was grouped in this analysis.

For the HNP-like PAHs, EMNJ1 had the highest concentrations, 3892 ±1275 ng/mg, which were significantly higher than all other sites. BBLA2 had the overall lowest conentraitons, 560±327 ng/mg. VBLA1 was statistically higher than BBLA2 and EMNJ1 but not ESNJ2. Concentrations in BBLA2 and ESNJ2 were statistically similar. Therefore, there were significant differences in HNP-like PAHs between sites in summer 2011with EMNJ1 having the highest concentrations.

For the HPY-like PAHs, BBLA2, EMNJ1, and ESNJ2 were not significantly different. BBLA2 had the highest concentrations of HPY-like PAHs 140±68 ng/mg. VBLA1 had the overall lowest concetrations of HPY-like PAHs, 87±67 ng/mg, and was statistically lower from BBLA2 but not different from EMNJ1 and ESNJ2. EMNJ1 and ESNJ2 were not significantly different from each other. EMNJ1 was statistically similar to all other catch dates for HPY-like PAHs in early summer. Therefore, menhaden from BBLA had the most HPY-like PAH contamination in summer 2011.

For the ratios of HNP- like to HPY- like PAHs, BBLA2 had the lowest ratio among the sites for June/July collections, 5±3 ng/mg. However, it was not significantly different than ESNJ2. EMNJ1 was significantly higher than all catch dates, 47±14 ng/mg. VBLA1 was significantly higher than BBLA2. The low ratio of BBLA2 compared to VBLA1 indicated pyrogenic not petrogenic exposure to PAHs at BBLA, which was surprising given that the oil spill came ashore in Barataria Bay. This suggested that something else was affecting the ratio in June/July catches. To summarize, there were significant differences for the HNP-like PAHs, HPY-like PAHs, and ratios of HNP-like to HPY-like PAHs for the early summer catch dates showing differences between collection sites.

Collection sites were also compared for fish captured in August 2011 (Table 11). BBLA3 was caught on 8/24/2011 with all of the fish identified as *B. patronus*. VBLA2 was caught on 8/23/2011 with all of the fish identified as *B.patronus*. EMNJ2 was caught on 8/31/2011 with all of the fish identified as *B.*

tyrannus. ESNJ3 was caught on 8/31/2011 with all of the fish identified as *B. tyrannus*.

For the HNP- like PAHs, BBLA3 , VBLA2, and ESNJ3 were not significantly different. ESNJ3 had the lowest concentrations of HNP-like PAHs, 712±469 ng/mg. EMNJ2 was significantly higher than all other catch dates with concentrations of 1408±602 ng/mg. This was approximately 1.5-2X higher than the other catch dates for late summer. For the HPY- like PAHs, BBLA3, VBLA2, EMNJ2, and ESNJ3 were not significantly different. The lowest concentration was 16±7a for ESNJ3 and the highest was 31±28 ng/mg for VBLA2.

The ratios of HNP-like to HPY-like PAHs for BBLA3, EMNJ2, and ESNJ3 were not significantly different. VBLA2 was significantly lower than EMNJ2 and ESNJ3 but similar to BBLA2. The lowest ratio was 33±17 ng/mg for VBLA3 and the highest ratio was 76±36 ng/mg for ESNJ2. To summarize, EMNJ2 had a signifcantly higher concentration of HNP-like PAHs than other sites for late summer catch date. There was no significant differences for the HPY-like PAHs concentrations. The higher ratios of HNP-like to HPY-like PAHs in NJ fish compared to LA fish were attributed to higher HNP concentrations, which suggested petrogenic exposure in NJ fish in late summer catches.

Collection sites were compared for fish captured in October 2011 (Table 12). BBLA5, EMNJ3, and ESNJ4 all had sample catch dates during October 2011. BBLA5 was caught on 10/11/2011 with all of the fish identified as *B.*

patronus. EMNJ3 was caught on 10/8/2011 with all of the fish identified as *B. tyrannus*. ESNJ4 was caught on 10/8/2011 with all of the fish classified as *B. tyrannus*.

For the HNP- like PAHs, BBLA5 was not signficantly different from EMNJ3 and ESNJ4, which were significantly different from each other. The lowest concentration was 712±469 for ESNJ4 and the highest concentration was 1208±590 for EMNJ3. For the HPY- like PAHs, BBLA5, EMNJ3, and ESNJ4 were not significantly different. The lowest concentration was 13±10 for ESNJ4 and the highest concentration was 23±9 for BBLA5. The ratios of HNP-like to HPY-like PAHs for BBLA5 was not signficantly different from EMNJ3 and ESNJ4, which were signficantly different from each other. The lowest ratio was 42±20 for BBLA5 and the highest was 75±42 for EMNJ3.

Overall, when comparing sampling locations, HNP-like PAHs, HPY-like PAHs, and their ratios in liver had the highest occurrence of significant differences between sites for July. HNP-like PAHs were lowest in BBLA2 and highest in EMNJ. HPY-like PAHs were lowest in VBLA1 and highest in BBLA2. EMNJ and ESNJ were significantly different from each other for HNP-like PAHs on all catch dates. This indicated that Atlantic coast fish had different levels of exposure through out the year. HPY-like PAHs were similar between sites in late summer and fall. This differed from early summer catch dates when HPY-like PAHs were significantly higher at some locations. This indicated a seasonal effect on HPY-like PAHs accumulation. Ratios of PAHs were generally

higher in NJ fish than LA fish, which suggested petrogenic PAH exposure along the Atlantic compared to the Gulf of Mexico coast and/or species differences.

Table 10: July Naphthol like (HNP) and Hydroxypyrene like (HPY) PAHs and their ratios for menhaden species from sample dates in 2011. *B. tyrannus* and *B. patronus* are the species present at a particular site. N = sample size. HNP-Like = naphthol like PAHs (ng/ml). HPY-like = hydroxypyrene like PAHs (ng/ml). If letters are the same, then the values are not significantly different from site to site. There was a significant difference in EMNJ1 from all catch sites for HNP- like. There was a significant difference in VBLA1 from BBLA3 for HPY-like. There were no significant differences between VBLA2 and ESNJ2 for the ratio of HNP-like to HPY-like.

Location	Collection Date	Species	n	HNP-like	HPY-like	Ratio HNP/HPY
BBLA2	7/28/2011	*B. tyrannus*	0			
		B. patronus	15	560±327[a]	140±68[a]	5±3[a]
VBLA1	7/6/2011	*B. tyrannus*	0			
		B. patronus	33	993±438[b]	87±67[b]	19±13[b]
EMNJ1	7/5/2011	*B. tyrannus*	4	3892±1275[c]	95±50[ab]	47±14[c]
		B. patronus	0			
ESNJ2	6/24/2011	*B. tyrannus*	5	623±256[ab]	99±59[ab]	9±6[ab]
		B. patronus	0			

Table 11: August Naphthol like (HNP) and Hydroxypyrene like (HPY) PAHs and their ratios for Menhaden species from sample dates in 2011. *B. tyrannus* and *B. patronus* are the species present at a particular site. N = sample size. HNP-Like = naphthol like PAHs (ng/mg). HPY-like = hydroxypyrene like PAHs (ng/mg). If letters are the same, then the values are not significantly different from site to site. There were no significant differences between sites for HPY-like PAHs. EMNJ2 was significantly higher than all catch dates for HNP-like PAHs. VBLA2 was significantly different from EMNJ2 and ESNJ3 for HNP-like to HPY-like ratios but not from BBLA3.

Location	Collection Date	Species	n	HNP-like	HPY-like	Ratio HNP/HPY
BBLA3	8/24/2011	*B. tyrannus*	0			
		B. patronus	21	839±343[a]	22±15[a]	51±26[ab]
VBLA2	8/23/2011	*B. tyrannus*	0			
		B. patronus	19	802±372[a]	31±28[a]	33±17[b]
EMNJ2	8/31/2011	*B. tyrannus*	10	1408±602[b]	29±17[a]	65±21[a]
		B. patronus	0			
ESNJ3	8/31/2011	*B. tyrannus*	10	712±469[a]	16±7[a]	76±36[a]
		B. patronus	0			

Table 12: October Naphthol like (HNP) and Hydroxypyrene like (HPY) PAHs and their ratios for menhaden species from sample dates in 2011. *B. tyrannus* and *B. patronus* are species present at a particular site. N = sample size. HNP-Like = naphthol like PAHs (ng/mg). HPY-like = hydroxypyrene like PAHs (ng/mg). If letters are the same, then the values are not significantly different from site to site. There were no significant differences between sites for the HPY-like PAHs. EMNJ3 was significantly higher than ESNJ4 for HNP-like PAHs but not for BBLA5. BBLA5 was significantly lower than EMNJ3 HNP-like to HPY-like ratios but not from ESNJ4.

Location	Collection Date	Species	n	HNP-like	HPY-like	Ratio HNP/HPY
BBLA5	10/11/2011	*B. tyrannus*	0			
		B. patronus	19	896±357^{ab}	23±9^{a}	42±20^{a}
EMNJ3	10/8/2011	*B. tyrannus*	10	1208±590^{a}	19±15^{a}	75±42^{b}
		B. patronus	0			
ESNJ4	10/8/2011	*B. tyrannus*	10	712±469^{b}	13±10^{a}	60±24^{ab}
		B. patronus	0			

Analysis of 2010 and 2011 of Naphthol-like and Hydroxypyrene-like PAHs for B. patronus species

The Barataria Bay, LA was the only location where *B. patronus* was captured in consecutive years, 2010 and 2011. Again, Barataria Bay, LA was the site of the DWH oil spill. Barataria Bay, LA had BBLA1 samples captured on 10/30/10 and BBLA5 captured on 10/11/11.

Both 2010 and 2011 catch dates had HNP-like type 1 spectra (Figure 44). There was an increase in HNP-like PAHs in 2011, one year after the spill. HNP-like PAHs concentrations in liver increased significantly from 283 ±152 ng/mg to 857± 357 ng/mg in 2010 and 2011, respectively. BBLA1 and BBLA5 had type 1 spectra of HPY- like PAHs for both 2010 and 2011 (Figure 45). HPY-like PAHs in liver were not significantly different, 19±11 ng/mg and 24±9 in 2010 and 2011, respectively. The ratios of HNP-like to HPY-like PAHS in BBLA1 and BBLA 5 (Figure 46) were statistically different, 13±6 ng/mg and 42±20 ng/mg in 2010 and 2011, respectively. Overall, there was a significant difference from 2010 to 2011 for HNP-like PAHs and ratios. Levels of HPY-like PAHs increased but were not significantly higher. The higher levels of HNP-like PAHs compared to HPY-like PAHs suggested the possibility of continuing petroleum exposure.

Figure 44: Type 1 Naphthol-like PAHs from Barataria Bay, LA from 2010 to 2011; HNP- like PAH concentrations comparison of 2010 (BBLA1) to 2011 (BBLA5) from Barataria Bay, LA expressed in ng/mg of liver. There was a significant increase of PAH concentrations (ng/mg liver) from 283 ±152 ng/mg to 857± 357 ng/mg in 2010 to 2011. Oil from the DWH spill came ashore at Barataria Bay, LA.

Figure 45: Type 1 Hydroxypyrene-like PAHs from Barataria Bay, LA from 2010 to 2011: HPY-like PAH concentrations comparison of 2010 (BBLA1) and 2011(BBLA5) from Barataria Bay, LA expressed in ng/mg of liver. There was no significant increase of HPY-like PAH concentrations (ng/mg liver) from 19±11 ng/mg to 24±9 in 2010 and 2011. Oil from the DWH spill came ashore at Barataria Bay, LA.

Figure 46: Ratios of Naphthol-like to Hydroxypyrene-like PAHs in Barataria bay, LA from 2010 to 2011 Ratios of HNP-like to HPY-like PAHs concentrations comparison from 2010 (BBLA1) to 2011(BBLA5) from Barataria Bay, LA expressed in ng/mg of liver. There is significant increase of the ratios of HNP-like to HPY like PAHs concentrations (ng/mgliver) 13±6 ng/mg to 42±20 ng/mg in 2010 and 2011. Oil from the DWH spill came ashore at Barataria Bay, LA.

Correlation of PAH concentration (ng/mg) and fish weight (g)

In order to determine if there was a relationship between PAH concentration (ng/mg) and total fish weight (g), a Pearson two tailed correlation was performed. For 2010 sample sites including Barataria Bay, LA (BBLA1), Mount Vernon, NJ (MVNJ1 and MVNJ2), Delaware Bay Region, NJ (EPNJ and SHNJ), and James River, VA (JRVA) there was no significant correlation between the HNP- like PAH concentrations and total fish weight, $p>0.05$. There was a very small negative correlation of 0.044. Similarly, there was no significant relationship between the HPY-like PAH concentrations and total fish weight, $p>0.05$. There was a very small positive correlation of 0.045. These results indicated that the size of the fish was unrelated to PAH-like compounds in menhaden liver.

Table 13: Pearson Correlation for 2010 Sample Sites Naphthol-like and Hydroxypyrene-like PAH concentrations (ng/mL) and Fish Weight (grams):
B. tyrannus and *B. patronus* were used for analyses. N = sample size. HNP-Like = naphthol like PAHs (ng/ml). HPY-like = hydroxypyrene like PAHs (ng/ml), HNP-like to HPY- like ratios.

		Fish Weight
HPYCONCN	Pearson Correlation	.045
	Sig. (2-tailed)	.710
	N	72
HNPCONCN	Pearson Correlation	-.044
	Sig. (2-tailed)	.713
	N	72

Discussion

The Deep Water Horizon (DWH) spill released more crude oil than ever before recorded (The Toxicology Society, 2011). It is estimated that over 4.9 million barrels of crude oil containing a complex mixture of thousands of naturally occurring chemicals including polycyclic aromatic hydrocarbons (PAHs) were released into the Gulf of Mexico. In addition to the largest amount of crude oil released in history, as compared to earlier spills like the Exon Valdez spill in 1989, this spill also occurredmuch deeper, approximately 1,544 meters below sea level. The Exon Valdez spill occurred at only 50 meters below sea level (NOAA, 2014). The impact of the PAH exposure to marine life is an area of major concern due to the possibility of bioaccumulation and contamination of the marine foodweb. The temperature difference affected the rate of bacterial metabolism in removing the oil.

Several key findings resulted from this study including a method forfixed emission fluorescence spectroscopy (FEFS) to establish standards for naphthol-like (HNP) and hydroxypyrene- like (HPY) PAHs; to establish that concentrations of HNP-like and HPY-like PAHs could be analyzed in liver samples of *B. tyrannus* and *B. patronus,* and the determination that *B. tyrannus* and *B. patronus* PAH levels and ratios vary seasonally, from sample dates ranging from Fall 2010 to Fall 2011.

The null hypothesis of this study is that HNP-like and HPY-like PAHs in liver of menhaden would not differ due to species of *Brevoortia*, season of catch date, location of catch or fish size. It was expected that due to recent crude oil

exposure *B. patronus* from the Gulf of Mexico would exhibit a greater concentration of both HNP-like and HPY-like PAHs in liver. Seasonal variation would also be significant due to changes in phytoplankton availability, and different sampling sites would have distinct PAHs profiles due to exposure to urban versus crude oil spill sources of PAHs

Kreitsburg *et al. (2010)* reported that fish have a variety of responses to PAHs and that metabolism of PAHs typically occurs in the liver. Based on his work and that of other researchers, the liver was selected and utilized to determine the PAH exposure of menhaden in this study. They recovered over 15 different types of PAHs (as a result of an oil spill in the Baltic Sea) in the liver, and they found that liver was more effective for the recovery for the 4-5 ringed PAHs compared to other organs.

Kreitsburg *et al.* also reported that all PAHs are soluble in water and tend to remain relatively near their point of origination. Rivers, estuaries, and coastal waters tend to be where PAHs are introduced to the aquatic environments. PAHs derived from urban sources are largely due to burning of fossil fuels and called pyrogenic PAHs. bodies of water located near urban areas have a high risk of pyrogenic PAH contamination. Pyrogenic PAHs are comprised of a larger proportion of high molecular weight PAHs than low molecular weight PAHs. This is the opposite of PAHs in crude oil and petroleum in which low molecular weight PAHs dominate (*Kreitsburg et. al*, 2010).

The sample sites selected for this study included two bodies of water along the Atlantic coast near urban centers- James River, VA and Delaware Bay, NJ. It

included two sites along the Gulf coast-Barataria Bay, LA, which was impacted by the DWH oil spill, and Vermillion Bay, LA, which was less impacted and contained the same menhaden species as Barataria Bay. Neither of these sites was near urban centers, so sources of PAHs would primarily have been due to petroleum from natural seeps and/or crude oil spills.

To document the impact on Barataria Bay, LA due to the DWH spill, Schwacke *et al.* (2013*)* reported the findings of health assessments of dolphins in Barataria Bay. The study reported that those dolphins found at Barataria Bay were 5 times more likely to have moderate to severe lung disease than wild dolphins from previous studies. Over 48% of the dolphins were given a 'guarded or worse' prognosis and 17% were considered 'poor or grave', which indicated that they were not expected to survive.

In our study, FEFS was successfully used to profile PAHs within the livers of menhaden collected from the Atlantic and Gulf coast locations. Multiple PAH standards were tested in order to establish their fluorescence patterns. Preliminary work established Em350 and Em450 as wavelengths that multiple 2-3 and 4-5 ring PAHs could be detected, respectively (data not shown). Figure 6a and 6b provided excitation spectra patterns of the 2-3 ringed PAHs Naphthanol (HNP), Fluorene (FL), Anthracene (AN), and the 4-5 ringed PAHs Hydroxypyrene (HPY), and Benzo(a)pyrene (BaP). The spectra showed that fluorescence intensity of HNP-like PAHs was highest using Em350, while those for HPY-like PAHs were highest using Em450. For example, HNP and HPY had fluorescence intensities of approximately 1000 RFUs and 75RFUS at Ex280/Em350,

respectively. The wavelength profiles that had an excitation peak at 270/280nm were reported as 'naphthol-like' (HNP-like) PAHs. The wavelength profiles that had an excitation at 340/350nm were reported as 'hydroxypyrene-like' (HPY-like) PAHs.

Bayer *et al* reported that fluorescence spectrophotometry is a useful and efficient means of reporting PAH metabolites in fish liver (2010). This process is effective as most PAHs are strong fluorophores whereas other compounds within the liver (bile) do not exhibit fluorescence. In addition, Bayer *et al* also reported that PAHs will exhibit different excitation and emission characteristics depending on their molecular structures. The smaller ringed PAHs, like HNP, require a greater excitation energy meaning excitation light at shorter wavelengths. This excitation/emission wavelength pairs are referred to as EEWP and these pairings indicate a specific type of PAH.

It is important to also clarify the distinction between a 'pure' sample as compared to a real world environmental sample. The fluorescence for a 'pure' sample, or those that only contain one specific PAH type and a non-fluorescence solvent, is that the emission spectrum will remain the same regardless of the excitation light. However, for those mixtures that contain multiple PAHs as well as other potential fluorophores, changing the excitation wavelength may change which PAHs are being excited and therefore which spectrum of light is emitted (Aldstadt *et al*, 2002).

Fluorescence detection of PAHs has been utilized in field studies as a screening and monitoring tool to distinguish contaminated locations versus less or

uncontaminated areas. The fluorescent assay utilized by several researchers (Bayer *et al*, 2010; Lin *et al*, 1996; Aas *et al*, 2000b) biomonitored exposure to PAHs in bile. This study utilized the fish liver instead of bile. Menhaden had an unusual anatomy associated with its planktivorous lifestyle, for example, a very long intestine and loose-corded liver. This made it difficult to find a distinct gall bladder; therefore, liver was used for PAH analyses. The other researchers dissolved their bile into 48% EtOH. Preliminary data in this studied indicated that 75% EtOH was better at extracting low as well as high molecular weight PAHs (data not shown).

Fluorescence detection of PAHs in field studies has been utilized and reported in a variety of investigations including Beyer *et al*, 1996, 1998; Cheevaporn and Beamish, 2007; Cormier *et al*, 2000; Gagnon and Holdway, 2002; Gorbi *et al*, 2005; Hanson *et al*, 2006, 2009; Haugland et al, 2005; Krca *et al*, 2007; Neves *et al*, 2007; Ribeiro *et al*, 2005; Vuorinen *et al*, 2006; Wang *et al*, 2008; Webb *et al*, 2005; Yang *et al*, 2003; and Aas and Klungsøyr, 1998, as well as in laboratory exposure studies including Beyer *et al*, 1997; Boleas *et al*, 1998; Camus *et al*, 1998; Goanvec *et al*, 2008; Gravato and Santos, 2003; Sandvik *et al*, 1997; and Aas *et al*, 2000a. This list of investigations was reported in the analytical review paper by *Tuvikene (*2010) and demonstrates how common it is to biomonitor PAHs using fluorescence detection.

The ability of FEFS to distinguish between specific PAHs was limited by the overlapping excitation wavelengths seen with standards (Figure 6a and 6b). However, results for fish livers did find multiple excitation patterns associated

with HNP-like and HPY-like PAHs. Therefore, HNP-like and HPY-like spectra were further subdivided into 'types', e.g type 1 vs. type 2, depending on the excitation wavelength profile exhibited by the sample. These slight variations in fluorescent compounds may have been due to PAHs metabolites within the liver (Bayer *et al*, 2010) or other fluorescent compounds found in organisms such as proteins. For HNP-like PAHs, a type 1 spectrum had a major peak at Ex280 while a type 2 spectrum had a major peak at Ex290. For HPY- like PAHs, a type 1 spectrum had two excitation peaks: one at Ex270/280 and one at Ex330-Ex350. Fluorescence intensity was measured at Ex350 and not Ex270/280. This allowed the type 1 spectrum to be compared to the type 2 spectrum. To be considered type 2, the spectrum had only one observed excitation peak at 350/360nm.

A variety of endogenous fluorophores may have been present in the samples and that may have impacted the results. This includes amino acids, enzymes, coenzymes, structural proteins, and vitamins. Ramanajum included the findings of excitation and emission maxima of endogenous fluorophores in his study on *Fluorescence Spectroscopy of Neoplastic and Non-Neoplastic Tissues* (2010). For example: tryptophan, an amino acid found within the liver, has an EEWP of Ex280/Em350; NADH has an EEWP of Ex290 and 350/Em440; vitamin D has a EEWP of Ex390/Em480; vitamin A has a EEWP of Ex327/Em510; collagen has a EEWP of Ex325/360Em400/405; and vitamin E has a EEWP Ex290/Em350 (Ramanajum, 2010 and Roberage *et al*, 2014). The ability to record the endogenous fluorophores is influenced by the biochemical environment as the fluorescence spectrum is highly sensitive to this. The spectra

of fluorophores can be affected by the concentration of metabolites and pH levels. Many fluorophores also respond to environmental changes, like oxygen levels (So *et al*, 2002). HNP-like and HPY-like PAH fluorescence was recorded *in lieu* of the fluorophores because PAHs have higher water solubility content and can remain at a higher level of magnitude then other compounds (SCG Industries, 2013).

For 2010 and 2011 PAH sample analysis, FEFS was utilized as a successful monitoring tool. The 'types' of PAHs observed in 2010 and 2011 for HNP-like and HPY-like were recorded and analyzed. In addition, the results provided evidence that the HNP-like spectrum type correlated with the HPY-like spectrum type regardless of species type and sample location. Meaning, that if a sample expressed type 1 HNP-like PAH spectrum then it would also express a type 1 HPY-like PAH spectrum.

In 2010, all catch dates exhibited both type 1 HNP-like and HPY-like PAHs in spite of sample location and species analyzed (Figures 12A, 13A, 14A,15A, 16, 22a, 23a, 24a, 25a, and 26). Statistical analysis for type 1 HNP-like 2010 catch dates indicated that BBLA and MVNJ1 were significantly lower than all catch dates and type 1 HPY-like analysis indicated that BBLA was significantly lower than all other catch sites in 2010 (Table 4). The 2010 type 1 HNP-like and HPY-like disprove the null hypothesis that *B. patronus* fish from the Gulf of Mexico would exhibit a greater concentration of HNP-like and HPY-like PAHs. In fact, the EPNJ *B. tyrannus* catch date from 9/11/10 had the highest

type 1 HNP-like PAHs and MVNJ1 *B. tyrannus* catch date from 9/7/10 had the highest type 1 HPY-like PAHs (Table 4).

The 2010 data also disproves the null hypothesis where there would be no differences in HNP-like and HPY-like PAHs in liver of menhaden due to location of catch. *B. patronus* from BBLA only exhibited type 1 of HNP-like and HPY-like spectra whereas all other 2010 catch sites demonstrated both type 1 and type 2 HNP-like and HPY-like spectra (Figures 12A, 12B,13A, 13B, 14A,14B, 15A, 15B, 22A, 22B, 23A, 23B, 24A, 24B, 25A, and 25B) which indicated a more diverse PAH exposure type. This may have been due to the different migratory patterns of the fish analyzed. Atlantic menhaden have a greater migratory range and all catch dates from 2010 were caught offshore. The gulf menhaden were recovered from the bay area where salinity would have varied. The northeast samples had a higher degree of PAH exposure. Seasonal variation was not analyzed in 2010 as there were no multiple catch dates from the same location.

In 2011, all catch dates exhibited both type 1 HNP-like and HPY-like PAHs despite sample location and species analyzed (Figures 32, 33A, 35, and 36A). For 2011, all catch dates exhibited both type 2 HNP-like and HPY-like PAHs with the exception of VBLA (no type 2 HNP-like) and EMNJ (no type 2 HPY-like) (Figures 40B, 42B, and 43B). From 2010 to 2011, BBLA had a shift in the HPY-like PAH profile observed. The 2011 type 1 HNP-like and HPY-like disprove the null hypothesis that *B. patronus* fish from the Gulf of Mexico would exhibit a greater concentration of HNP-like and HPY-like PAHs. In fact, the EMNJ *B. tyrannus* samples had the highest type 1 HNP-like PAHs (Figure 36).

The ESNJ *B. tyrannus* samples had the highest type 2 HNP-like and HPY-like PAHs (Figure 37 and Figure 43) whereas BBLA had the highest type 1 HPY-like PAHs (Figure 42). These results indicated that for 2011 HNP-like and HPY-like data that catch dates from the northeast had a higher or more chronic exposure to PAHs as seen in 2010 data. In 2011, separate species analysis was not performed as there was no overlap in speciation in the same sample site as seen in 2010.

It was expected that different sampling sites would have distinct PAH profiles due to exposure of PAH sources in highly urbanized areas versus the DWH oil spill. The data presented shows that the same HNP-like or HPY-like PAH type can be found in both species and at various locations. It did not suggest that those fish that were exposed to the DWH oil spill were more likely to exhibit a more diverse profile 'type' or a greater level of contamination despite recent exposure. In fact, those samples that have migratory ranges in highly urbanized environments, exhibited a greater amount of 'types' for either HNP-like or HPY – like PAHs. The data suggested that the significance of the spectrum type is linked to concentration of PAH which can be impacted by those areas that have suffered long term PAH exposure like the northeast/Delaware region.

Barataria Bay, LA was the location sampled in consecutive years. There as a significant increase of in HNP-like PAHs from 2010 to 2011 (Figure 46). HPY-like PAHs were not significantly different from 2010 and 2011 (Figure 47). The higher levels of HNP-like PAHs compared to HPY-like PAH suggest continuing petroleum exposure.

The 2010 and 2011 results indicated that levels of PAHs were likely linked to the PAH 'source' and level of exposure. Yan *et al.* reported concentrations of PAHs observed in the northeast Atlantic region over time. Concentrations of PAHs as well as their source type, aka petrogenic vs. pyrogenic, fluctuated over decades beginning in the 1940s. Petrogenic sources of PAHs were observed from 1950's to the 1970's. Following that time, pyrogenic sources were recovered and observed as the dominant PAH source type in that region (Yan *et al*, 2006). Exposure to pyrogenic sources PAHs may have explained the higher levels of HNP-like and HPY-like PAHs from northeast sample sites as compared to the Gulf locations.

The species of *Brevoortia* did not have a measureable effect on the levels of PAHs accumulated in 2010 and 2011. The FEFS has proved to be valid for assessing PAH exposure in multiple species. Lin *et al.* (1996) reported that fluorescence spectroscopy could be used for PAH exposure in three different species of fish including brown bullhead, white sucker, and common carp. Both type 1 and type 2 HNP-like and HPY-like PAHs were recorded in both species of *Brevoortia*. The differences in liver concentrations were better explained by PAH source than sample location. The *B. tyrannus* species from the northeast sample locations had the highest level of both HNP-like and HPY- like PAHs supporting a higher degree of PAH contamination along the Atlantic coast despite the DWH oil spill in the Gulf. This may have been due to the long term urbanization in that area.

Results for HNP-like and HPY-like PAH spectra observed may be due to a variety of factors. Firstly, the emission/excitation settings utilized for this study are slightly different than those reported in the literature for fluorescence spectroscopy. A PAH type can be characterized based on the wavelength pairs observed. Typically, strong fluorescence at Ex290/Em335 is observed in fish exposed to petrogenic contamination like the DWH oil spill. Strong fluorescence at Ex340/Em380 and Ex380/430nm is observed in fish exposed to combustion PAH sources due to the presence of larger ringed PAHs (Aas *et al*, 1998; Bayer *et al*, 2000; Lin *et al*, 1996). For this study, the samples were analyzed using slightly different settings like EM450 and EM350 while scanning for excitation.

PAH mixtures have a high degree of diversity. These variations can cause dispersed results. It is recommended needs a reference to utilize a secondary method to validate results such as High Pressure Liquid Chromatography (HPLC) or Gas Chromatography Mass Spectroscopy (GCMS). Beyer *et al* and other researchers reported in their summary of analytical methods used to evaluate PAH concentrations in fish liver, that it is challenging to analyze samples with 'optimal accuracy and precision' (2010).

The reported varying levels of PAHs within different sample dates and sample locations may also have been due to salinity levels. As reported by Ramachandran *et al* (2006), fish exposed to PAHs will accumulate more in areas of low salinity as compared to those of full salinity. They found greater PAH toxicity following oil spills in areas of lower salinity like estuaries and coastal waters. Salinity was not measured when collecting samples for this study and

therefore cannot be confirmed. However, *B. patronus* was collected from an estuarine environment and *B. tyrannus* from an ocean environment. The results reported here found similar or higher levels of PAHs in *B. tyrannus* versus *B. patronus*, which does not support the increased bioaccumulation of PAHs from lower salinity environments found by Ramachandran *et al*.

Seasonal variation was analyzed in 2011 data. Levels of PAHs were compared by date from one site or by site from one date. The comparison of catch dates within the same site showed that fish collected in fall (September and October) contained more HNP-like PAHs than early summer samples (June and July). Fish samples from early summer catch dates had a higher average of HPY-like PAH concentrations than fish from fall samples (Table 6, 7, 8, and 9). The results support the hypothesis of this study as the results demonstrated seasonal variability for HNP-like and HPY-like as PAHs. This may have been due to food source availability over time.

Comparing sites from a particular season found the following. There were no statistical differences in July between gulf menhaden from BBLA and Atlantic menhaden; however, there were statistical differences between BBLA and VBLA for HNP-like and HPY-like despite location proximity (Table 10). For August and October analysis, there were no statistical differences between any locations for HPY-like PAHs (Table 11 and Table 12). For HNP-like PAHs, there were no significant differences between gulf menhaden and Atlantic menhaden. EMNJ did have statistically higher levels of HNP-like PAHs than ESNJ and BBLA (Table 12). These findings disproved the null hypothesis that there would be no

significant differences between catch sites for a given date. Results showed that gulf menhaden had higher levels of both HNP and HPY-like PAHs at BBLA than VBLA in 2011. Other catch dates showed no differences between Gulf sites or between Gulf sites and Atlantic coast menhaden. This finding supported crude oil exposure at BBLA when compared to VBLA and the important influence of season on PAH detection.

The results reported for the ratios of HNP-like/HPY-like for 2010 and 2011 provide further evidence of exposure to different sources of PAH, petrogenic versus pyrogenic. For 2010 data of type 1 HNP-like/HPY-like, BBLA was significantly higher than MVNJ1 but similar to the other NJ collection and JRVA collection (Table 4). This showed no consistent differences in type 1 spectra ratios between Atlantic coast and Gulf coast collections in 2010. For the type 2 HNP-like/HPY-like ratio analyses, MVNJ1 and MVNJ2 were significantly lower than JRVA and SHNJ despite geographic proximity (Table 5). This showed possible differences in types of exposure along the Atlantic coast.

BBLA was not analyzed as there were no type 2 spectra recorded in 2010. These results do not support exposure of BBLA menhaden to petrogenic PAHs in 2010. However, the collections were in the fall when differences between sites were not found in PAH levels. Therefore, the lack of differences between ratios could have been due to a seasonal effect in PAH bioaccumulation.

The 2011 HNP-like/HPY-like ratios were compared between seasons for a particular site and between sites for a particular season. BBLA was the only site to have any significant differences (Tables 6, 7, and 8). The ratio was lower for

the July catch compared to those from August, September and October. A lower ratio indicated pyrogenic not petrogenic PAHs. In addition, EMNJ3 had the highest ratio values recorded in October despite BBLA's recent exposure to the DWH oil spill (Table 8). BBLA had the lowest ratio of HNP-like to HPY-like PAHs in July 2011 when compared to other sites (Table 10). For August 2011, BBLA was statistically similar to VBLA, but was statistically lower than both NJ catch sites (Table 11). For October 2011, BBLA was statistically similar to ESNJ but statistically lower than EMNJ (Table 12). These results support the null hypothesis that Gulf menhaden would not be significantly exposed to petrogenic PAHs as evidenced by high a ratio HNP/HPY-like PAHs. The high exposure to HPY as opposed to HNP controlled the ratio.

seasonal variability in PAH exposure may have been due to the phytoplankton availability in various sampling locations. As menhaden are a filter feeding fish, the phytoplankton food source may have been contaminated with PAHs as a result of petrogenic and pyrogenic sources. Mitra *et al* reported PAH contamination of zooplankton following the DWH spill (2012). They reported a potential for DWH oil derived PAHs to bioaccumulate to higher trophic levels within an aquatic ecosystem. The present study also investigated the correlation of fish weight and PAH uptake. The results indicated that there was no correlation for either HNP-like or HPY-like PAHs (Table 13). Therefore it was likely the accumulation of PAHs was site and/or season related and not due to the age of the fish.

In summary, fixed emission fluorescence spectroscopy has proven to be a useful tool for determining the PAH levels in liver samples. The findings of this study indicated that a high HNP-like/HPY-like ratio was not related to petroleum exposure at the DWH site. Changes in HPY-like PAHs rather than HNP-like PAHs influenced the ratio. All samples sites in 2010 and 2011 showed evidence of PAH exposure even though the types of spectra could not distinguish between sources of exposure. *B. tyrannus* and *B. patronus* species were found to have similar types and concentrations of PAHs despite sample location. Therefore, results suggested that menhaden could be a useful biomonitoring organism regardless of species. The higher levels of HNP-like and HPY-like PAHs at BBLA compared to VBLA in 2011 indicated that BBLA was affected. This showed continuing exposure to DWH crude oil one year after the event. In addition, there was seasonal connection for HNP-like and HPY-like PAHs across the different sample sites. Menhaden from early summer were found to have higher HPY-like PAH concentrations and menhaden from fall were likely to have higher HNP-like PAH concentrations regardless of geographic location. Seasonal variability of PAH levels was established, and species type and sample location did not affect the findings. This indicates the need for future research on the importance of phytoplankton blooms on PAH bioaccumulation.

Suggested additional research includes studying PAH levels in menhaden populations over time to determine the potential long term effects of the DWH oil spill as well as studies of chronic PAH exposure due to urbanization. For example, the only samples of menhaden to be captured from the same site in

consecutive years were Barataria Bay, LA *B. patronus* samples. In 2010 and 2011, the results showed type 1 HNP-like spectra concentrations significantly increased from 2010 to 2011 (Figure 46). Type 1 HPY-like PAH spectra were also reported in 2010 and 2011 and there was no significant differences observed from year to year (Figure 47). Monitoring of this trend over time would provide an effective means to determine the potential long term effects of the DWH oil spill on the Gulf environment, in comparison to the effects of urbanization.

Lastly, it is suggested that the potential bioaccumulation of PAHs be studied using menhaden and other species associated with the human palate, like bluefish. This would provide additional support to the importance of the continued effort to monitor areas affected by petrogenic and pyrogenic PAH sources.

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