

**POLYCYCLIC AROMATIC HYDROCARBONS AND
POLYCHLORINATED BIPHENYLS IN EPIBENTHIC DEEP-SEA
MEGAFUNA IN THE NORTHERN GULF OF MEXICO**

An Undergraduate Research Scholars Thesis

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ABSTRACT

Polycyclic Aromatic Hydrocarbons and Polychlorinated Biphenyls in Epibenthic Deep-Sea
Megafauna in the Northern Gulf of Mexico

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In this study, we assessed the bioaccumulation of oil-derived and man-made pollutants in deep-sea epibenthic megafauna from the northern Gulf of Mexico. Polycyclic aromatic hydrocarbons (or PAHs) were measured as representatives of oil and emission related pollution. Whereas, polychlorinated biphenyls (or PCBs) were measured as representatives of man-made industrial chemicals. Our results showed overall comparable levels for all pollutants in four separate epibenthic deep-sea megafauna, which included: Actinaria (sea anemones), Pennatulacea (sea pens), Holothuroidea (sea cucumbers), and Crinoidea (sea lilies). The analysis of PAH levels showed greater accumulation for high molecular weight compounds that are also highly lipophilic. However, the analysis of PCBs showed that the levels of two congeners (PCBs 101 and 138) were consistently higher than the rest of the PCBs measured. Our results shed light on a knowledge-gap that exists on assessing the extent to which anthropogenic pollutants may impact the deep-sea environment.

DEDICATION

Rather than dedicating this thesis to a single person, as is so frequently done, and often times for good reason, I would instead like to dedicate it to a number of different people. First, my parents, grandparents, and all other members of my family who have supported me and offered unwavering comfort and love in times of need, and in times of joy. Then, to my brother Chandler who has inspired me with his enthusiasm for this field, and looked after me during times where I felt like I had hit the end of the road. All of my friends, who's comradery has made this journey one filled with great pleasure and happiness. My advisors and professors who have mentored me and taught me important topics and lessons throughout my years here in Galveston.

Finally, to the scientific community and science itself, which will be using this knowledge and information to advance the understanding that humans have of the natural world, and use it to make the world a better place.

ACKNOWLEDGEMENTS

I would like to thank my advisors Dr. David Hala, and Dr. Gil Rowe, who have presented me with wonderful opportunities to pursue research that I was genuinely interested in, and offered tremendous amounts of support and encouragement during the process of conducting this project. I would like to acknowledge the great role that my friends and family played during this academic journey; providing support, inspiration, motivation, and comradery. I would also like to thank Dr. Clif Nunnally, and Dr. Craig McClain for allowing me to participate in the research cruise that made this thesis possible.

CHAPTER I

INTRODUCTION

It is important to understand the fate of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the marine environment given their toxicity and ability to bioconcentrate within an organism, and bioaccumulate across food webs. (Jensen, et. al, 1969; Biddinger & Gloss, 1984; Suedel, 1994; Livingstone, 1998; Ramesh, et al., 2004; El-Shahawi, et. al., 2010). Collectively, these compounds are characterized as persistent bioaccumulative, and toxic (PBT) chemicals (Gobas, et al., 2010). Ultimately, sediments and biota act as reservoirs and refuges for PAHs and PCBs. Such sequestration can ensure the long-term persistence of PAHs and PCBs, and potentially contribute to the chronic toxicity in exposed organisms (Randolph, Hardy et al. 1998; Peterson, Rice et al. 2003; Bejarano and Michel 2010). While attention is mainly focused on PAH and PCB pollution in coastal/neritic ecosystems (aBaumard, et. al., 1998; bBaumard, et. al., 1998; Soclo, et. al., 2000; Monirith, et. al., 2003; Green & Knutzen, 2003; Sanchez-Avila, et al., 2012), very little is known of their distributions in deep-ocean ecosystems.

Polycyclic aromatic hydrocarbons

The sources of PAHs in the marine environment are mainly from oil-derived hydrocarbons, which comprise a mixture of various alkyl (short or long chain), cyclic (naphthenes or cycloalkanes) and aryl (polycyclic aromatic ≥ 2 aromatic rings) hydrocarbons, some of which also contain sulfur (ethanethiol) and nitrogen atoms (carbazole) (Eneh, 2011). Of these, lower molecular weight hydrocarbons are volatile and are easily degraded, while heavier molecular weight compounds are more persistent and bioactive. For example, increasing

molecular weights (and 'ring' numbers) of polycyclic aromatic hydrocarbons positively correlate with bioaccumulation potential and toxicity (Southworth, Beauchamp et al. 1978; Black, Birge et al. 1983). The spectrum of adverse effects includes metabolic (energy loss), hemolytic (anemia), endocrine, osmoregulatory and teratogenic effects (Vandermeulen, 1987).

Polychlorinated biphenyls

While PAHs in the environment are mainly from pyrogenic (combustion of petroleum-derived hydrocarbons) and petrogenic (petroleum-derived) sources (Hylland, 2006), PCBs constitute 'legacy' persistent organic pollutants (POPs), this group now-banned substances and also includes organochlorine insecticides (such as dichlorodiphenyltrichloroethane or DDT), hexachlorobenzene (HCB), dioxins, hexabromocyclododecane (HBCD), amongst others. PCBs comprise a diverse family of compounds that possess from 1-10 chlorine atoms distributed across two fused benzene rings (Safe et al. 1985). PCBs were originally used as dielectrics and coolants in electrical devices until they were banned in the late 1970s due to environmental concerns (Boyle and Highland 1979). PCBs are still detected in the marine environment (despite the ban on their use), due to their persistence, long half-lives and low biodegradation in exposed biota and the environment (Porta & Zumeta, 2002).

Toxicology in the deep-sea environment

The deep-ocean is defined as water depths below the 200 m isobath, comprising both the pelagic and benthic realms (Gage & Tyler, 1992). At these depths, high hydrostatic pressure, lack of sunlight, low temperatures and low microbial activities can slow pollutant degradation rates, thus making the deep-sea benthos a likely long-term sink for pollutants (Ramirez-Llodra, et al., 2011). Furthermore, the hydrophobic nature of PAHs and PCBs (due to LogKow's >?) promotes their downward flux to the deep-sea benthos with particulate organic carbon from the

surface ocean (Scheringer, et. al., 2004). Such a mechanism has been shown to contribute 1.95-7.78 $\mu\text{g m}^{-1} \text{d}^{-1}$ of $\Sigma 43$ PAHs to the northern Gulf of Mexico deep sediments (Adhikari, et. al., 2015), and 14,000 mol yr^{-1} (which can be converted to $4.08 \times 10^6 \text{ g yr}^{-1}$) of PCB 52 to the northwestern Atlantic Ocean (Gustafsson, et. al., 1997).

Specific studies of deep-sea ecosystems have found significant concentrations of PBTs within some of the deepest living organisms (>10,000 m, Mariana Trench) (Jamieson, et al., 2017). Froescheis, et. al., 2000, and Looser, et. al., 2000 support the idea that the fauna, and sediments in the deep-ocean may act as a sink for semi-volatile POPs by comparing body burdens of deep-water organisms to those of their shallow water counterparts. A number of PBTs were reported by Unger, et. al., 2008, in deep-sea cephalopods taken from 1000-2000 m in the western Atlantic Ocean. Amphipods taken from the head of the Mississippi Canyon in the northwest Gulf of Mexico showed high levels of alkylated PAHs, as well as dibenzothiophenes (Soliman & Wade, 2008). A study from Suruga Bay, Japan, found no significant difference in concentrations between deep, and shallow water organisms; although none of the depths sampled exceeded 1000 m (Lee, et al., 1997). Therefore, it is evident that the deep-sea benthic environment is a likely sink for persistent pollutants. However, at present there is a lack of studies investigating the levels of such pollutants in the deep-sea benthos of the Gulf of Mexico. There is need for such investigations as the Gulf of Mexico is heavily prospected for oil and natural gas (?), and accommodates one of the busiest shipping lanes in the U.S. (the Houston Ship Channel is the second busiest shipping lane in the U.S.) (refs?).

In this study we assessed the levels of 45 PBTs (16 PAHs and 29 PCBs) in epibenthic megafauna taken from a depth of ~2000 m on the northern Gulf of Mexico (GoM) continental rise. Specifically, the organisms sampled included the family Hormathiidae (Venus flytrap

anemones: actinarians), holothurians, pennatulaceans (sea pens), and crinoid. Given the vulnerability of GoM coastal and marine ecosystems to pollution from oil-derived hydrocarbons and persistent organic pollutants, this study investigates likely impacts on the deep-sea environment.

CHAPTER II

METHODS

Sample collection

The megafauna samples were collected from May 25, 2017 to June 3, 2017 onboard the R/V Pelican. All samples were collected off the continental rise in the Northern Gulf of Mexico at depths of around 2,000 m using a vacuum suction sampler attached to the robotically operated vehicle (ROV), Global Explorer (Figure 1). Either subsamples of tissue from individual organisms, or whole-organisms were taken and immediately frozen at -20 °C until processing for PAH and PCB analysis.

Sample extraction for PAH and PCB analysis

Approximately 1 gram of tissue (or entire organism if total mass available < 1 gram) was homogenized in 3 mL of 1:1 (v/v) hexane: ethyl acetate using a Bead Mill 4 Homogenizer (Fisher Scientific) operating at a processing power of 300 g for 3-5 min. The homogenate was transferred to a 50 mL glass centrifuge tube, and spiked with 5 µL of a 100 ppm benzo[a]pyrene-d12 (Sigma-Aldrich) and PCB 65-d5 (CDN Iso-topes) as internal standards (yielding a final concentration of 2.5 ppm in final volume). The homogenates were ultrasonicated in a Branson Ultrasonics™ M2800 Ultrasonic Bath (Fisher Scientific) for 30 min. The glass tubes were then centrifuged for 10 min at 2,000 g. The resulting supernatant was removed and transferred to a pre-weighed 20 mL glass scintillation vial, and then dried under a gentle stream of nitrogen (N₂). The vial was then weighed to record the residual lipid weight. The lipid residue was then reconstituted with 1 mL of acetonitrile (ACN), and transferred into a 2 mL glass amber vial, dried under N₂, with residue reconstituted into 200 µL of ACN, and transferred to a glass insert.

All samples were then stored at -20 °C for 1 hour to precipitate lipids and other aqueous debris. Following freezing, a 50 µL of the lipid free supernatant was removed from the 200 µL glass insert and transferred to a 2 mL amber glass vial. This aliquot was dried under N₂ and finally reconstituted using 50 µL of dichloromethane (DCM). prior to gas chromatography mass spectrometry (GCMS) analysis.

GCMS analysis of PAHs and PCBs

Samples were analyzed for the presence of 45 PAHs and PCBs using GCMS. A Hewlett Packard HP-6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer was used for analysis. Sample injection was performed in splitless mode at a volume of 2 µL. Chromatographic separation was performed using a 30 m x 0.25 mm DB-5MS capillary column (J&W Scientific) with a 0.25 µm film thickness. Ultra-pure helium was used as the carrier gas with a flow rate of 1 mL min⁻¹. The GC front inlet, and the MS interface were operated at temperatures of 250 °C and 280 °C, respectively. The GC oven was initially set at 40 °C, following sample injection the oven ramped at a rate of 20 °C min⁻¹ to 180 °C, and then at 5 °C min⁻¹ until 300 °C, where it held for 10 min. The MS was run in electron impact ionization mode (EI) at an electron energy of 70 eV, and a source temperature of 230 °C. The 45 compounds were identified and measured using selected ion monitoring (SIM) mode. They were run against a 13-point linear calibration curve (R² > 0.97), constructed using serially diluted standards (2.5-10,000 ng g⁻¹) (as described in Cullen, et. al., 2019).

Data analysis

The variable n numbers for benthic megafauna sampled precluded statistical testing. Therefore, this manuscript reports on the levels of PAHs and PCBs quantified in benthic

organisms. The multivariate analysis of characteristic trends of PAHs and PCBs in organisms was investigated using principal component analysis in PAST (v2.07) (Hammer et al. 2001).

CHAPTER III

RESULTS

Body burdens

The specimens that were analyzed included 7 members of the family Hormathiidae (Actiniaria), 3 holothurians (all presumed to be from the genus *Benthothytes*), 2 *Kophobelemnon* sp. (Pennatulacea), and a crinoid. The pennatulaceans showed the highest mean concentrations of the sum of PAHs, followed by actinarians, crinoids, and holothurians, respectively. An identical trend was seen with the mean sum of PCB concentrations. The most prevalent (>400 ng g⁻¹ mean) individual compounds for the actinarians from highest to lowest mean concentrations included dibenz[a,h]anthracene, benzo[a]pyrene, PCB 101 (2,2',4,5,5'-pentachlorobiphenyl), PCB 138 (2,2',3,4,4',5'-hexachlorobiphenyl), and acenaphthene. The highest congeners (>400 ng g⁻¹ mean) found in the holothurians were dibenz[a,h]anthracene, PCB 101, PCB 138, and acenaphthene, respectively. For pennatulaceans (>400 ng g⁻¹ mean) dibenz[a,h]anthracene, PCB 138, PCB 101, benzo[a]pyrene, acenaphthene, fluoranthene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. The crinoid displayed dibenz[a,h]anthracene, PCB 101, benzo[a]pyrene, PCB 138, acenaphthene, and indeno[1,2,3-cd]pyrene as being the most abundant (>400 ng g⁻¹ mean) compounds from highest to lowest. The mean value of >400 ng g⁻¹ used in classifying a compound as being high, was employed for being ~100 ng higher than the average for all compounds (302.06 ng g⁻¹); these highest values are in the top 87.2 percentile. All values can be found in table 1.

Table 1. Mean concentrations of each compound (mean \pm standard error) for individual taxa.

Missing values are indicative of a concentration below the LOD (see methods section).

Compound	Actinaria (n = 7)	Holothuroidea (n = 3)	Pennatulacea (n = 2)	Crinoidea (n = 1)
PAHs				
Naphthalene	122.02 \pm 50.801	88.11 \pm 51.783	240.98 \pm 5.759	195.50
Acenaphthylene	123.09 \pm 51.737	77.66 \pm 60.145	248.97 \pm 7.264	203.91
Acenaphthene	558.10 \pm 285.693	400.82 \pm 345.109	1713.12 \pm 698.216	748.91
Fluorene	127.31 \pm 53.269	91.92 \pm 54.073	255.68 \pm 7.302	201.63
Phenanthrene	130.91 \pm 54.621	93.38 \pm 54.722	308.38 \pm 47.184	211.29
Anthracene	111.26 \pm 46.394	69.68 \pm 53.594	230.01 \pm 11.362	181.36
Fluoranthene	134.79 \pm 56.218	98.15 \pm 57.992	1476.78 \pm 12.951	212.93
Pyrene	119.00 \pm 49.678	86.16 \pm 50.602	237.14 \pm 5.966	198.05
Benzo[a]anthracene	92.47 \pm 43.026	16.32 \pm 16.317	322.06 \pm 5.972	257.75
Chrysene	65.61 \pm 30.458	11.58 \pm 11.580	228.61 \pm 4.308	174.36
Benzo[b]fluoranthene	103.82 \pm 48.271	132.67 \pm 77.841	362.32 \pm 5.996	289.70
Benzo[k]fluoranthene	124.51 \pm 52.105	89.70 \pm 52.420	248.86 \pm 5.900	197.27
Benzo[a]pyrene	608.99 \pm 296.890	119.60 \pm 64.059	1931.45 \pm 536.567	1150.53
Indeno[1,2,3-cd]pyrene	284.53 \pm 110.802	195.00 \pm 114.368	543.89 \pm 10.485	428.43
Dibenz[a,h]anthracene	1877.77 \pm 778.781	914.58 \pm 358.749	8169.67 \pm 45.614	5175.07
Benzo[ghi]perylene	56.18 \pm 28.680	32.54 \pm 32.542	656.87 \pm 14.009	–
PCBs				
Non-ortho				
PCB 77	56.18 \pm 30.003	10.95 \pm 10.950	216.89 \pm 4.437	164.33
PCB 81	53.29 \pm 28.528	10.39 \pm 10.388	205.58 \pm 4.154	155.75

PCB 126	84.76 ± 41.133	108.24 ± 66.704	323.56 ± 23.437	228.09
PCB 169	115.45 ± 48.077	83.76 ± 48.816	239.20 ± 1.670	186.36
Mono-ortho				
PCB 105	120.75 ± 54.907	91.94 ± 53.920	252.12 ± 5.346	201.78
PCB 114	115.48 ± 47.594	11.50 ± 11.504	227.34 ± 4.954	185.83
PCB 118	111.83 ± 46.387	80.49 ± 47.236	229.21 ± 2.335	180.71
PCB 123	119.52 ± 49.713	12.00 ± 12.004	239.53 ± 4.896	180.24
PCB 156	121.14 ± 50.475	87.84 ± 51.554	240.79 ± 4.994	194.16
PCB 167	114.28 ± 47.798	83.30 ± 48.922	228.25 ± 3.383	172.31
PCB 189	4.73 ± 4.732	–	–	–
Non-dioxin Like				
PCB 1	94.05 ± 39.297	68.06 ± 39.987	189.03 ± 4.447	149.01
PCB 18	132.85 ± 66.420	77.51 ± 45.280	226.24 ± 5.884	167.26
PCB 28	9.97 ± 6.435	–	226.54 ± 7.196	0.00
PCB 33	4.66 ± 4.662	–	207.72 ± 6.054	155.75
PCB 52	61.37 ± 43.938	–	278.38 ± 63.621	–
PCB 95	14.47 ± 6.838	–	210.11 ± 2.179	–
PCB 101	599.64 ± 294.401	530.67 ± 462.937	2160.81 ± 757.865	1182.71
PCB 149	106.47 ± 47.877	58.48 ± 58.476	221.52 ± 4.957	167.45
PCB 153	5.50 ± 5.501	–	243.06 ± 5.603	184.49
PCB 138	596.88 ± 291.794	529.36 ± 460.511	2174.79 ± 668.692	1133.35
PCB 187	11.53 ± 7.474	–	119.80 ± 119.803	178.57
PCB 183	11.44 ± 7.418	–	–	177.10
PCB 128	121.80 ± 50.913	76.13 ± 58.720	243.55 ± 3.456	185.20
PCB 177	5.32 ± 5.322	–	–	–

PCB 171	55.17 ± 49.409	60.95 ± 60.955	116.84 ± 116.838	–
PCB 157	111.52 ± 46.481	80.41 ± 46.978	223.24 ± 8.149	180.09
PCB 180	–	49.06 ± 49.059	185.59 ± 3.467	–
PCB 170	–	–	–	–

Sum normalized values display a similar profile to that of the mean concentrations (figure 1). Crinoids have the highest sum proportion of dibenz[a,h]anthracene followed by pennatulaceans, holothurians, and actinarians, respectively. Crinoids also slightly have the highest proportion of benzo[a]pyrene with pennatulaceans, and actinarians closely preceding, and holothurians being relatively lower. Acenaphthene makes up the greatest proportion of any LMW compound in all of the organisms, with the fluoranthene proportion in pennatulaceans being the only other significant LMW component. The PCB sum normalized profile shows pennatulaceans accumulating the highest proportion of both PCB 101, and 138 followed by the crinoid, holothurians, and actinarians, in order.

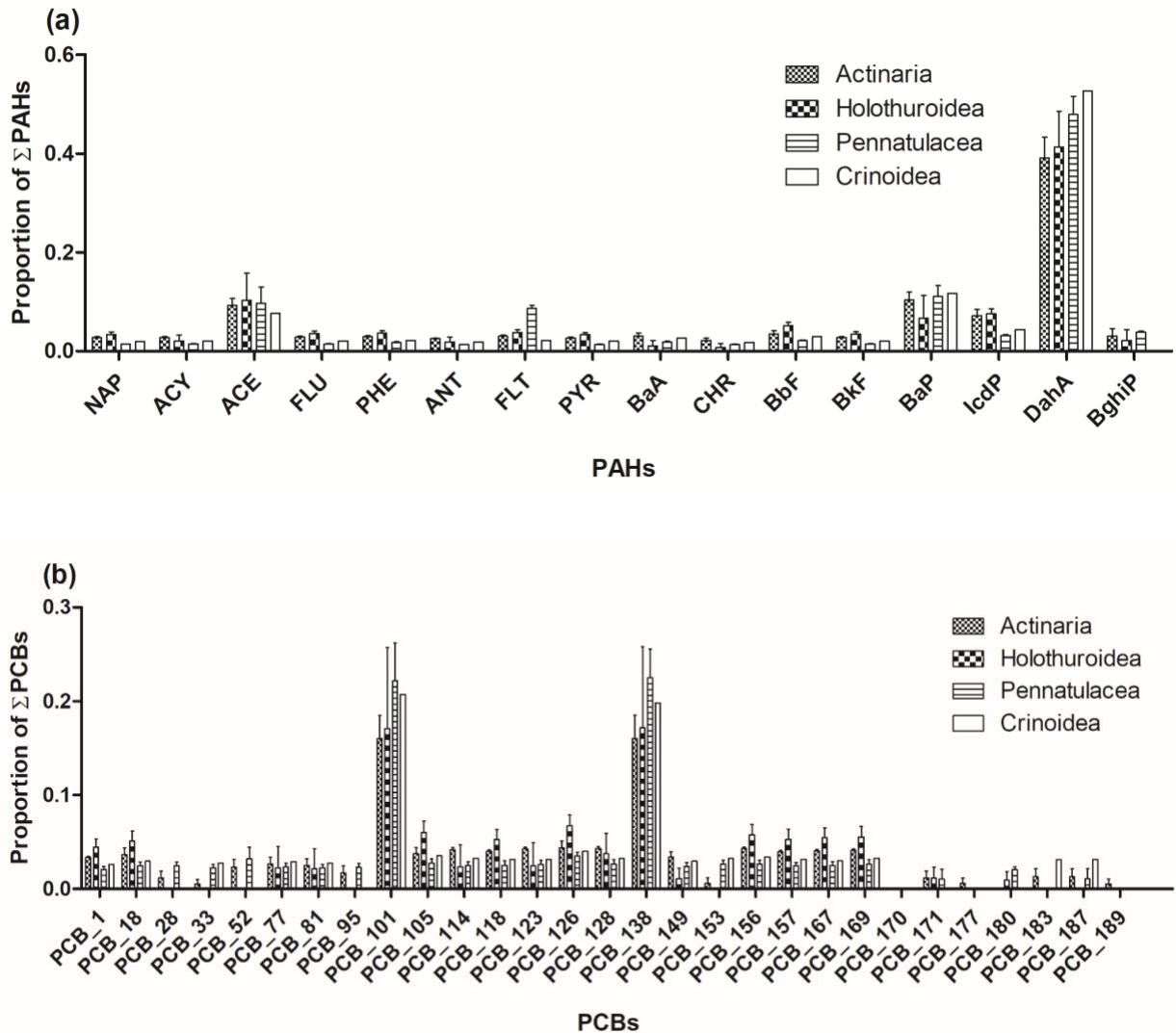


Figure 1. Mean sum normalized values for all 45 compounds (PAHs (a), PCBs (b)) across the four individual taxa. Error bars represent standard error.

Principle component analysis

Principal component analysis (PCA) of PAH and PCB body-burdens showed a majority of pollutants (78%) to cluster within a 75% data ellipse around the origin of principal components 1 (which describes 75.40% of variance) and principal component 2 (describes 13.63% of variance). Of the pollutants within the data ellipse, 88% and 66% of PAHs and PCBs

were accounted for respectively. The PAHs (ACE, BaP, IcdP and DahA) and PCBs (PCB_101 and PCB_138) found at highest levels in all four organisms span across the first principal component and with ordinations close to the origin. In contrast, the ordinations for various PCBs and a PAH (BghiP) were dispersed across the first and second principal components, indicating heterogeneous body burdens for these pollutants.

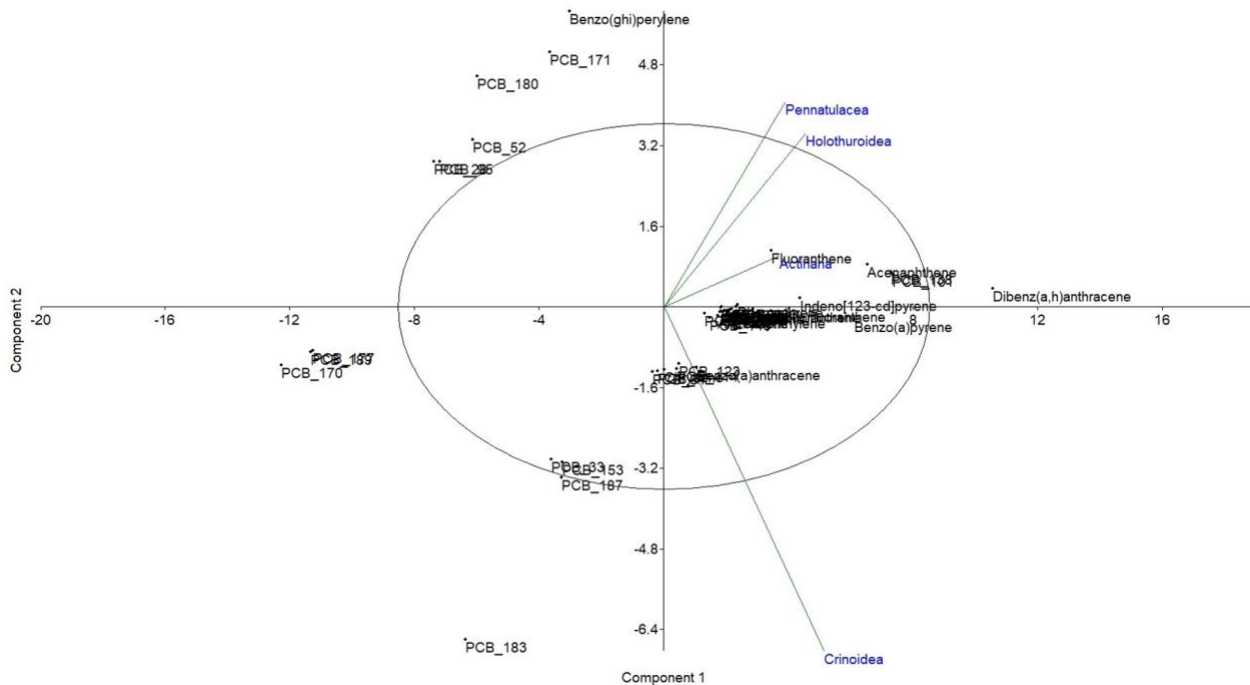


Figure 1. Principle component analysis showing ordinations for PAH and PCB body-burdens in deep sea Pennatulacea, Holothuroidea, Actinaria and Crinoidea. A 75% data ellipse around the origin is used to delineate pollutants that contribute substantially to the variance across principal components 1 and 2.

CHAPTER IV

CONCLUSION

In this study, we assessed the body-burdens of 45 PAHs and PCBs in four separate taxa (Actinaria, Pennatulacea, Holothuroidea, and Crinoidea) of epibenthic deep-sea megafauna in the northern Gulf of Mexico. We also used diagnostic ratios to determine the source of the PAHs to the deep benthos. Body burdens of PAHs and PCBs, as well as diagnostic ratios are discussed below.

Body Burdens

Body-burdens of PAHs showed a similar pattern within all four taxa, as did the sum normalized proportions. The HMW compounds, specifically dibenz[a,h]anthracene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene showed prevalence, with the exception of the LMW congener acenaphthene, also found in high concentrations. A few compounds do show a significant difference between taxa. Fluoranthene, and benzo[g,h,i]perylene showed a much higher profile in the pennatulaceans than other organisms. Concentrations across different taxa also showed a high level of similarity for the PCB profiles, with PCBs 101, and 138 being dominant (both non-dioxin-like). The pennatulaceans showed the highest overall accumulation of PBTs followed by actinarians, crinoids, and holothurians, respectively.

The prevalence of the HMW PAH congeners could be partly explained by the higher octanol/water partition coefficient (KOW) of these compounds, which leads to higher bioaccumulation factors (BAF) (Chiou, et. al., 1977; Fisk, et. al., 1998). However, the log KOW increases linearly as a function of the compound's molecular weight (Ma, et. al., 2010). If the accumulation of these compounds were only a factor of log KOW values, then it would be

expected to see a linear increase in body burdens as a function of molecular weight, which is not observed. Previous studies on bivalves (see table 2.) found pyrene and phenanthrene to be the most dominant congeners present in tissues. Bintein, et. al., 1993 suggests a parabolic relationship between log KOW and BAF, with very hydrophobic compounds having a low potential to accumulate within organisms. Contrary to the findings of this study and others, Gewurtz, et. al, 2000 found that biota-sediment accumulation factors (BSAF) in benthic invertebrates taken from Lake Erie displayed an inverse relationship to log KOW, and suggest that the larger PAHs are rapidly metabolized. PCBs show a similar linear relationship between molecular weight and log KOW (Hawker & Connell, 1988). Gewurtz, et. al, 2000, and Wu, et. al., 2008, found a parabolic relationship between log KOW of PCBs and their BAFs, similar to the previously mentioned findings for PAHs. However, many PCB congeners with higher BAFs calculated using various groups of organisms, are not seen in the profiles found in this study (Harrad & Smith, 1997; Maruya & Lee, 1998; Sobek, et. al., 2010). This disconnect may be attributed to bioavailability and overall presence of these specific compounds in the deep benthos.

Bioavailability of these compounds is dependent on a number of influences. Organic carbon content of the sediments is one of these important factors. This is because these lipophilic pollutants can bind to, and have a high affinity towards the organic portion of the sediments, and therefore the portion in the porewater is considered the bioavailable fraction (Bierman Jr, 1990). The organic carbon (OC) content in deep GoM sediments was found to range from 0.37-1.3 %, and is considered low relative to most other continental margins (Morse & Beazley, 2008). Hence, it could be said that OC concentrations alone, in deep GoM sediments have little to do with the high values of pollutants – since they are so low – which have been quantified within

these organisms. Additionally, the porewater fraction seems to be more relevant to smaller infauna which live within the sediments. What would seem more important to the large deep-sea megafauna is the bioavailability of these compounds during ingestion of the sediments and/or particulate organic matter (POM).

Dietary absorption of these organic pollutants is dependent on a number of factors such as the size of the molecule, and the log KOW. For instance, a compound with a log KOW ~ 4 is taken up by respiration at rate 20 times faster than by ingestion but, at a log KOW ~ 6 dietary uptake is 5 times faster (Mackay & Fraser, 2000). Relating this to the predominant pollutants in this study, benzo[g,h,i]perylene (log KOW 6.50), and benzo[a]pyrene (log KOW 5.97) are likely taken up through ingestion, whereas acenaphthene (log KOW 3.92) could be accumulated more readily through respiration. According to Gobas, et. al., 1988 compounds with a log KOW ~ 7 began to show absorption efficiencies of close to 0. In this study, benzo[g,h,i]perylene (log KOW 6.90), and indeno[1,2,3-cd]pyrene (log KOW 6.95) show a decrease in concentration compared to the other HMW congeners. It should be noted that acenaphthene has consistently been found in high concentrations in water relative to most PAHs, and low to absent concentrations within sediments, but still present within organisms (Wade, et. al., 1989; Witt, 1995; Zhou & Maskaoui, 2003; Kafilzadeh, et. al., 2011). This coupled with the respiratory uptake efficiency of acenaphthene, could explain its prevalence in this study, though the source is unclear.

Although most of the PAH congeners in this study which are accumulated at high concentrations do have what some literature suggests to be an optimal log KOW for bioaccumulation, no meaningful relationship was observed between the mean body-burdens for individual compounds and their log KOW. In regards to the PCBs, the two congeners found at high concentrations also have an 'optimal log KOW' but, a very weak parabolic relationship (R^2

= 0.05) was observed when plotting all of the compounds. This suggests that the accumulation of specific PBT congeners could be heavily site/source dependent, related to the bioavailability of the compound in the environment, or vary significantly across taxa based on physiological differences.

Temperature also has an effect on the uptake of organic pollutants. An increasing temperature, increases solubilities, therefore decreasing adsorption to organic matter (OM), leading to a greater portion of the compound being present in the aqueous medium (Chiou, et. al., 1997). In cold waters such as those in this study, and the entirety of the deep-ocean (~ 4 °C), this would suggest that lipophilic compounds may be bound at a higher fraction to the OM pool. Such affinity towards the sediments and POM at these lower temperatures, could pose a greater risk for benthic detritivores, and may explain some of the high concentrations. Temperature also has an effect on the reaction kinetics of enzymes which may be essential in transforming, and the excreting of these compounds from an organism.

Delivery mechanisms and sources

A The deep Gulf of Mexico has a very large number of natural gas and oil seeps that these organisms could be exposed to (Kvenvolden & Cooper, 2003; Brooks, et al., 1986; Anderson, et al., 1983; Bernard, et al., 1976). In this case, it could be expected to see a larger amount of petrogenic or LMW PAHs within the tissues of these organisms. Additionally, since this environment is so far removed from the surface ocean and the coastline, it may be reasonable to assume that these organisms are out of reach from pyrogenic PAHs that are derived from the burning of fossil fuels and other organic compounds. This however, as shown by the diagnostic ratios in table 1, is not observed. This suggests that these predominantly

anthropogenic pollutants are being delivered to the deep ocean, as well as to the organisms inhabiting it, at significant concentrations.

A possible mechanism that could transport these compounds to the deep ocean is the biological pump, and downward flux of organic material. Since these pollutants are lipophilic they should have a high affinity towards sinking POM, phytoplankton, and other biota in the water column (Jones & de Voogt, 1999.). Once delivered to the surface ocean through atmospheric deposition, they can be taken up by phytoplankton, adsorb to organic particles, or become incorporated into the food web, ultimately being transported with this system to the seafloor. This phenomenon has been reported by Fowler & Knauer, 1986; Gustafsson, et. al., 1997; and Dachs, et. al., 1999. Riverine delivery of PAHs to the GoM from the Mississippi, and Atchafalaya Rivers has also been reported, and could be major transport mechanisms as well (Adhikari, et. al., 2019). The diversity of sources for PAHs as mentioned above – natural seeps, oil spills, atmospheric deposition, riverine influx – is higher than that of PCBs. Due to the distance from possible localized contaminated sources, the main contributor of PCBs to the deep ocean are most likely atmospheric deposition and possible being bound to particles and transported by rivers.

All of the organisms analyzed in this study are detritivores, feeding on POM, also known as marine snow; the two feeding modes being suspension feeding (actinarians, pennatulaceans, and the crinoid), and deposit feeding (holothurians) (Macurda & Meyer, 1974; Roberts, et. al., 2000; Ammons & Daly, 2008; Williams, 2011). There were no meaningful differences observed between the two feeding types. Therefore, if the biological pump is a heavy contributor of these compounds to the deep-sea floor, it would be expected to impact the levels of PAHs and PCBs in these organisms significantly.

Conclusions

The lower presence of petrogenic PAHs in these organisms may be explained by a few factors. First, the proximity of these oil and gas seeps to the organisms may heavily influence the amounts of PAHs found in their tissues. They may have to be living in the near vicinity of one of these seeps in order to accumulate the lower weight compounds found in the natural seepage. Secondly, these LMW compounds do not have optimal log KOW values for accumulation within organism. So even if these organisms were exposed to high levels of LMW PAHs, their body burdens may not reflect this. Diagnostic ratios indicate a pyrogenic source however, this may be an artifact of optimal log KOW values of these compounds. A study using diagnostic ratios on OM fluxes in the Northern GoM, close to the sample sites in this study, found a heavy petrogenic source value. The POM these organisms are feeding on may be rich in LMW compounds, but may be excluded during dietary absorption. At the current time, it is unclear whether these ratios can be used on biota in the same way they are used on sediments, and particles. A new diagnostic ratio that considers in vivo processes may be needed to accurately delineate source ratios.

The only thing that can be stated for certain is that these various taxa of deep-sea megafauna are accumulating high levels of specific PAHs and PBCs in similar profiles. More experiments looking at concentrations in sediments and POM that is in proximity to sampled organisms, as well as increasing sample sizes and geographic variation are needed to better understand the sources, fate, and behavior of these compounds. Additionally, there is no manner in which to tell how these concentrations are affecting these animals physiologically. Performing enzyme assays for these groups of organisms, that could indicate physiological stress is an important next step for this research.

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