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# Temporal and Spatial Assessment of PAHs in Water, Sediment, and Oysters as a Result of the Deepwater Horizon Oil Spill

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# Temporal and Spatial Assessment of PAHs in Water, Sediment, and Oysters as a Result of the Deepwater Horizon Oil Spill

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# ABSTRACT

On April 20, 2010, BP's Deepwater Horizon oil rig exploded leaking over 200 million gallons of crude oil into the Gulf of Mexico for 84 days. Exposure to oil-associated polycyclic aromatic hydrocarbons (PAHs) in the water and sediment could severely impact the aquatic organisms inhabiting the Gulf of Mexico (i.e. developmental defects, reproductive effects, death, etc.). Therefore, water, sediment and oyster, *Crassostrea virginica*, samples were collected approximately bimonthly between May 26 and November 30, 2010 from multiple sites along the Gulf Coast, namely, two sites in Mobile Bay (Denton and Sand Reefs at 1 or 0.1 m above the bay floor), one site near Orange Beach, AL (Perdido), and one near Pointe aux Pines, MS. Water, sediment, and oysters were extracted for quantitation of 24 PAHs by gas chromatography mass spectrometry (GC/MS). The concentration range for total PAHs (tPAH) in water was non-detectable to 1100 ng/L and non-detectable to 7450 ng/g for sediment. The highest water tPAH concentrations were observed on 6/28/10 for Sand (1 m), 7/21/10 for Denton (0.1 m), 9/9/10 for Perdido, and 9/22/10 for Pointe aux Pines. The highest sediment concentrations were observed on 9/20/10 for Denton Reef, 7/7/10 for Sand Reef, 7/28/10 for Perdido, and 9/22/10 for Pointe aux Pines. Twenty other sites along the northern Gulf coast collected less frequently also indicated relatively low (<20 ng/L) tPAH water concentrations.

*Fundulus heteroclitus* embryos were exposed to water collected from three of the sites from 4.5 hours post-fertilization (hpf) to 10 days post-fertilization (dpf). Embryos were assessed on 5 and 10 dpf for lethality and cardiac toxicities (including blood clot, edema and tube heart), and cytochrome P450 enzyme induction was measured by an *in ovo* ethoxyresorufin-*O*-deethylase assay. *F. heteroclitus* embryos were not significantly affected by the water collected from these sites. There was less than 4% and 2% incidence of edema and blood clot, respectively, and there were no significant differences in deformity index or lethality.

None of the 73 sediment and 70 water samples collected between May 26, 2010 to February 9, 2011 was definitively impacted by the Deepwater Horizon Oil Spill at the times collected based on the tPAHs measured. However, samples with relatively higher percent compositions of naphthalenes and/or phenanthrene occurred in the Sand and Denton 6/28/10 water samples and in the 7/12/10 Gulf Island National Seashore sediment sample. Other sediment samples with tPAH concentrations greater than 1300 ng/g dry weight had diverse mixtures of the individual PAH compounds suggesting multiple potential sources of contamination. Also, none of the sediment samples at the times collected had tPAH concentrations which exceeded NOAA regulatory SQuiRT guidelines. Therefore, none of the PAH concentrations measured were expected to cause acute toxicity to aquatic life, a result supported by the *F. heteroclitus* bioassay.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| µg/g | microgram per gram |
| µg/L | microgram per liter |
| AhR | Aryl hydrocarbon Receptor |
| AL | Alabama |
| ANOVA | Analysis of Variance |
| ARNT | Aryl hydrocarbon Receptor Nuclear Translocator |
| ASW | Artificial Seawater |
| BaP | Benzo(a)pyrene |
| BBB | Biloxi Back Bay |
| BG | Biloxi Gulf |
| BP | British Petroleum |
| BTEX | Benzene, Toluene, Ethyl-benzene, Xylene |
| C | Carbon |
| C:N | Carbon to Nitrogen ratio |
| CWEG | Clean Water Effluent Guidelines |
| CYP1A | Cytochrome P450-1A |
| DB | Denton Bottom (0.1 m) |
| DISL | Dauphin Island Sea Lab |
| DMBA | 12-dimethylbenz(a)anthracene |

| | |
|---|---|
| DO | Dissolved Oxygen |
| DOSS | Dioctyl Sodium Sulfosuccinate |
| DT | Denton Top (1 m) |
| DWH | Deepwater Horizon |
| EMAP | Environmental Monitoring and Assessment Program |
| EPCRA | Emergency Planning & Community Right-to-know Act |
| ERL | Effects Range Low |
| ERM | Effects Range Median |
| EROD | Ethyoxyresorufin-*O*-deethylase |
| FDA | Food and Drug Administration |
| FH | Fairhope |
| FID | Flame Ionization Detector |
| FL | Florida |
| GBBC | Grand Bay Bayou Combust |
| GBBH | Grand Bay Bayou Heron |
| GC | Gas Chromatography |
| GINS | Gulf Island National Seashore |
| GPC | Gulfport Courthouse |
| GPG | Gulfport Gulf |
| HAH | Halogenated Aryl Hydrocarbons |

| | |
|---|---|
| He | Helium |
| HPAH | High molecular weight Polycyclic Aromatic Hydrocarbon |
| IARC | International Agency for Research on Cancer |
| IL | Interleukin |
| LCO | Louisiana Crude Oil |
| LOC | Levels of Concern |
| LPAH | Low molecular weight Polycyclic Aromatic Hydrocarbon |
| m | Meter |
| MB | Mobile Bay |
| MDS | Mean Deformity Score |
| MS | Mass Spectrometry |
| MS | Mississippi |
| MSDS | Material Safety Data Sheet |
| N | Nitrogen |
| NCA | National Coastal Assessment |
| NCP | National Contingency Plan |
| nd | Non-detectable |
| Ne | Neon |
| ng/g | nanogram per gram |
| ng/L | nanogram per liter |

| | |
|---|---|
| NIEHS | National Institute of Environmental Health and Safety |
| NOAA | National Oceanographic and Atmospheric Administration |
| NRDA | Natural Resources Damage Assessment |
| NS&T | National Status and Trends |
| OPA | Oil Pollution Act of 1990 |
| OPEC | Organization of the Petroleum Exporting Companies |
| OSHA | Occupational Safety and Health Administration |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PAP | Pointe aux Pines, AL |
| PCB | Polychlorinated biphenyl |
| PDB | PeeDee belemnite |
| PEL | Probable Effects Level |
| Per | Perdido |
| PHG | Phytohemagglutinin |
| POM | Particulate Organic Matter |
| PR | Pascagoula River |
| PRP | Potentially Responsible Parties |
| QuEChERS | Quick Easy Cheap Effective Rugged Safe |
| RCRA | Resource Conservation and Recovery |
| SB | Sand Bottom (0.1m) |

| | |
|---|---|
| SIM | Selected ion mode |
| SQuiRTs | Screening Quick Reference Tables |
| ST | Sand Top (1 m) |
| TCDD | 2,3,7,8-tetrachlorodibenzodioxin |
| TX | Texas |
| UM | University of Mississippi |
| USEPA | United States Environmental Protection Agency |
| VOO | Vessels of Opportunity |
| WAF | Water Accommodated Fraction |
| WSF | Water Soluble Fraction |
| XRE | Xenobiotic Responsive Element |

# CHAPTER 1 INTRODUCTION

## 1.1 GULF OF MEXICO

The Gulf of Mexico is a complex water system located along the southeastern border of North America. The Gulf is bordered by Florida, Alabama, Mississippi, Louisiana, and Texas in the United States; five Mexican states of Tamaulipas, Veracruz, Tobasco, Compeche, and Yucatan; and by Cuba. Its surface area is approximately 1.5 million square kilometers, or $5.8 \times 10^5$ square miles. The United States Gulf coastline is approximately 2700 kilometers, or 1630 miles long (Binninger 2011). The volume of water in the Gulf is estimated to be 643 quadrillion gallons (Binninger 2011). In addition to deep water habitats, coastal waters consist of estuaries, wetlands, seagrass meadows, coral reefs, mangrove or kelp forests, and upwelling areas (Summers *et al.* 2005). These coastal habitats provide spawning grounds, nurseries, shelter, and food for wildlife (Summers *et al.* 2005). The coast is a highly productive ecosystem with over 15,000 species living in the Gulf waters (Kennedy *et al.* 2011). For example of the diversity of species in the Gulf of Mexico, near shore marine organisms that spawn in coastal waters consist of killifish, vermilion snapper, Gulf sturgeon, smalltooth sawfish, Nassau grouper, snowy egret, roseate spoonbills, Brown pelicans, Louisiana Shrimp, blue crab, seagrasses, corals, sponges, oysters, and multiple dolphin species (NOAA 2008). Many endangered species are home on the Gulf Coast, nine of which are marine organisms (NOAA 2008). This includes the smalltooth sawfish, the Perdido Key beach mouse, the manatee, red-cockaded woodpecker, humpback

whale, blue whale, finback whale, sperm whale, and the hawksbill sea turtle (NOAA 2008; Summers *et al.* 2005). The West Indian Manatee prefer areas of the gulf where water is 6-7 feet deep, densely vegetated, and they spawn in warmer waters (Oceanic Research Group 2007). Also in the Gulf Coast, shorebirds, colonial nesting birds, and migratory waterfowl are dependent upon its wetlands (Summers *et al.* 2005). It produces "more food per acre than the most productive Midwestern farmland" (Summers *et al.* 2005). In 2000, more than $900 million worth of commercial fish and shellfish were caught, and shrimp from the Gulf of Mexico consisted of 80% of the total U.S. shrimp harvest (Summers *et al.* 2005). However, total commercial fishing revenue has been decreasing to a total of approximately $629 million in 2009 (NOAA 2009).

As well as the critical environmental resources represented by the Gulf of Mexico ecosystem, there are many human uses. People depend on the Gulf for transportation and shipping routes, fish and shellfish harvesting, recreation, and petroleum production. Related to these advantages and industries, the population along the Gulf Coast has increased more than 100 percent from 1960 to 2000 and is predicted to have a total of 27 million people by 2015 (Summers *et al.* 2005).

The U.S. Navy uses waterways and ports as a means of transportation, as do commercial boats for shipments and cruises (Summers *et al.* 2005). Some of the important ports in the eastern Gulf of Mexico are South Louisiana, LA; New Orleans, LA; Pascagoula, MS; Mobile, AL; Panama City, FL; Pensacola, FL; and Tampa, FL (Kennedy *et al.* 2011; NCB 2012). The important ports in the western Gulf of Mexico are Corpus Christi, Houston, and Galveston, TX (Kennedy *et al.* 2011; NCB 2012). South Louisiana, LA has been the number one ranked port in the United States for over a decade, moving approximately 213 million short tons of cargo in

2009 (Kennedy *et al.* 2011). The major commodities going through South Louisiana port are food, petroleum products, and crude petroleum (Kennedy *et al.* 2011). Houston is ranked second for most cargo with 211 million short tons of cargo in 2009, including crude petroleum, petroleum products, and chemicals (Kennedy *et al.* 2011). Other ports in the top ten are Corpus Christi, TX; New Orleans, LA; Beaumont, TX; and Texas City, TX.

The Gulf shores fisheries are extremely valuable, yielding more finfish, shrimp, and shellfish per year than the United States East Coast fisheries combined (Binninger 2011). In 2010, commercial harvests approximated $630 million in revenue from fish and shellfish (Binninger 2011). Also in 2010, 82 and 59% of the United States' shrimp and oysters, respectively, were caught along the Gulf coast (Binninger 2011). From 2007 to 2009, commercial fishing ports averaged 978, 106, 104, 55, and 22 million average pounds per year of menhaden, brown shrimp, white shrimp, blue crab, and Eastern oyster, respectively (Kennedy *et al.* 2011). Commonly caught recreational fish include the spotted seatrout, red drum, sheepshead, red snapper, king mackerel, and black drum at 14.5, 11.9, 4.4, 3.6, 3.3, and 2.9 million pounds, respectively, in 2009 (Kennedy *et al.* 2011).

Coastal areas provide recreational value. American beaches are top vacation spots, with 180 million people visiting every year for sport fishing, boating, diving, and just relaxing along the seaside (Cunningham and Walker 1996). The Gulf of Mexico's shores and beaches maintain a $20 billion tourism industry (Binninger 2011). Employment from tourism and recreation along the Gulf Coast supports over 648,000 people through eating and drinking establishments to boat dealers to water tours to sporting goods dealers (Kennedy *et al.* 2011).

The Gulf Coast region provides for the United States approximately one fourth of its natural gas and one eighth of its oil from the Gulf (Minerals Management Service (MMS) 2002). The oil industry also adds almost 55,000 offshore jobs for U.S. workers, as of 2002 (Minerals Management Service (MMS) 2002). There are approximately 3700 active oil and gas platforms in the Gulf of Mexico with enough pipeline to wrap around the Earth's equator when placed end to end (Kennedy *et al.* 2011). Oil fields from the Gulf of Mexico in 2009 produced 1.6 million barrels of crude oil and 2.6 trillion cubic feet of natural gas a day (USEIA 2009). The wells are divided into three categories by depth: shallow water (0-999 ft), deepwater (1,000-4,999 ft), and ultra-deepwater (5,000+ ft) (USEIA 2009). In 2009, shallow water, deepwater, and ultra-deepwater in the federal waters of the Gulf of Mexico had 1334, 503, and 565 production wells, respectively.

The impacts of the human activities on the Gulf of Mexico natural environment is an ongoing controversy made more poignant after the Deepwater Horizon oil spill occurred in 2010. Many federal and state agencies have ongoing monitoring programs to assess human impacts including those associated with more solid waste production, increased nutrient runoff and resulting hypoxic conditions, decreased wildlife habitats and overfishing, and more energy production.

## 1.2 WHAT IS OIL

Oil is considered any substance that is liquid at room temperature and does not mix with water but with other oils and organic solvents. General oil classes are vegetable oil, essential plant oil, petrochemical oil (petroleum), and synthetic oils, with petrochemical being of most importance. Petroleum is a naturally occurring, flammable liquid comprised of hydrocarbons and

other liquid organic compounds, found below the Earth's surface in geological formations, with the main components being carbon and hydrogen. Specific hydrocarbons commonly found in oils are shown in Figure 2 and include paraffin, cycloalkanes, aromatics, naphthenoaromatics, and resin. Component ratios within a petroleum mixture vary depending on subsurface temperatures and pressures based on their location around the globe (Fingas 2010; Hyne 2001). Oils are classified according to their geographic location, American Petroleum (API) gravity, and sulfur content (Fingas 2010). The seven oil classifications are West Texas Intermediate, a high-quality, sweet, light oil found in Texas; Brent Blend, oil originating from the North Sea; Dubai-Oman, sour crude oil shipping to Asia; Tapis, a reference for light crude oil in the Far East; Minas, a reference for heavy crude oil in the Far East; OPEC Reference Basket, a mixture of oil from OPEC (Organization of the Petroleum Exporting Companies) countries; and Midway Sunset, reference of heavy oil for pricing in California (International Marine Consultancy 2011). These oil classifications are also known as benchmark oils because they are used to reference oil types for buyers and sellers (Hyne 2003).

Oil from the Macondo 252 Well from the Deepwater Horizon incident was classified as light sweet crude (OSATF 2010). This means that the oil contains large amounts of chemicals needed for the production of gasoline, kerosene, and high-quality crude oil. The "sweet" indicates the low amount of sulfur in the oil.

### 1.2.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

PAHs are organic compounds consisting of two or more aromatic rings with varying toxic properties, from acute to chronic, such as mortality, wasting syndrome, hepatotoxicity, immunotoxicity, and reproductive impairment (Khim *et al.* 1999). These properties cause 16

PAHs to appear on USEPA's 126 Priority Pollutants list (Environmental Protection Agency 2012). The 16 are denoted in Figure 1 with asterisks. PAHs can be natural or man-made. PAHs are created when combustion of fossil fuels (oil, coal, gas) during power or heat generation is incomplete, from vehicle emissions, cigarette smoke, and various cooking methods, such as barbeque (Harvey 1991). PAHs can occur naturally from volcanic activities and forest fires, but man-made sources are of greater concern for air pollution.

Petrogenic PAHs are produced in organic-rich source rocks, exposed to high temperatures for long periods of time (Page *et al.* 2002). Petroleum (petrogenic) PAHs are mostly introduced into the environment via oil spills, but also through natural seeps or natural gas. PAHs are the most stable component in petroleum and most important to oil spill assessments (Wang and Fingas 1995). The major hydrocarbon classes are shown in Figure 2. Petroleum PAHs are usually characterized by their alkylated or substituted PAH components, which are more stable than their parent compounds (unsubstituted PAHs), consist of different distribution profiles per different oils, and, due to weathering processes, more truly reveal the composition of the PAHs in the oil (Meador *et al.* 1995; Wang and Fingas 1995).

### 1.2.2 WHAT ARE DISPERSANTS

One remediation method for the Deepwater Horizon Oil Spill was the use of dispersants; the first attempted use in deepwater (Kujawnski *et al.* 2011). Little is known about deepwater application of dispersants (Gohlke *et al.* 2011). Dispersants are a blend of surfactants and hydrocarbon-based solvents meant to relieve the interfacial tension between oil and water to allow the oil to dissolve into the water column which facilitates microbial breakdown (Kujawnski *et al.* 2011; Schmidt 2010). Approximately 2.1 million gallons of dispersant were

distributed between the surface (~1.4 million gallons) and wellhead (~0.77 million gallons) during the Deepwater Horizon Oil Spill. Corexit 9527 (manufactured by Nalco Energy Services; Sugarland, Texas) was the dispersant used for surface applications, applied by plane or small boats; and Corexit 9500A was the dispersant used for wellhead, through a jet placed in the oil flow, and for surface application.

The main component of these Corexit mixtures is the anionic surfactant dioctyl sodium sulfosuccinate (DOSS, Figure 3), which in some studies was used as a dispersant marker to trace the fate and transport of dispersants (Kujawnski *et al.* 2011). Other components of Corexit are propylene glycol, serving as a stabilizer, and 2-butoxyethanol (2-BE), found in Corexit 9527, or petroleum distillates, found in Corexit 9500A (Schmidt 2010). The material safety data sheet (MSDS) for Corexit 9527 suggested that 2-BE could cause hemolysis or kidney or liver damage with excessive exposure in animal studies. Also, Corexit 9527 could cause skin and gastrointestinal irritation. The MSDS claims Corexit 9527 was of moderate risk to human health while Corexit 9500A was only of slight risk to human health. The only compositional difference between Corexit 9527 and 9500A was the use of petroleum distillates as a substitute for 2-BE. The petroleum distillates are mostly hydrocarbons. It was thought that with the large input of hydrocarbons from the oil spill, the hydrocarbon input from the dispersant would be insignificant (Schmidt 2010).

Dispersants have been commercially available for oil responses since the mid-1960s and always come with an environmental trade off (Schmidt 2010). During a surface oil spill, oil floats on top of the water causing birds and marine mammals to suffer as well as ruining coastal resources. When oil becomes dispersed, the oil is transported throughout the water column where

it is more bioavailable to marine organisms. The oil droplets can be mistaken for food, causing missing links in the food web due to organisms such as plankton or rotifers disappearing due to oil poisoning (Schmidt 2010). There is still much debate about the impacts of dispersants and whether dispersants are safe to use. Hemmer *et al* (2011) investigated the acute toxicity and LC50 of eight dispersants listed on the USEPA National Contingency Plan (NCP) Product Schedule (Hemmer *et al.* 2011). Two aquatic species native to the Gulf of Mexico, the mysid shrimp and the inland silverside, an aquatic invertebrate and small estuarine fish, respectively, were chosen. Hemmer *et al* (2011) found Corexit 9500A to be slightly toxic to the mysid shrimp but practically nontoxic to the inland silverside due to the LC50 values of 42 and 130 ppm, respectively. Judson *et al* (2010) performed *in vitro* cytotoxicity and endocrine screening assays with human cell lines. Cytotoxicity was observed at concentrations above 10 ppm, and no biologically significant estrogenic or androgenic activity was seen by any of the eight dispersants (Judson *et al.* 2010). These two experiments show relatively low dispersant toxicity but were not designed to test for potential chronic impacts of dispersant exposure.

### 1.3 PREVIOUS GULF OIL SPILLS

There have been approximately 36 significant oil spills greater than 260,000 gallons worldwide since 1967 (Anonymous. 2012b). Table 1 highlights some of the spills which have occurred in the Gulf of Mexico. The first well known spill was in the summer of 1979. The Ixtoc I drilling rig, off the coast of Mexico, had a blowout causing approximately $140 \times 10^6$ gallons of oil to leak into the Gulf of Mexico (Boehm *et al.* 1983). Then in November 1979, an oil tanker *Burmah Agate* had a wreck with a freight ship off the Texas shore, spilling approximately 2 million gallons of oil. These two spills initiated the Natural Resources Damage Assessment

(NRDA) protocol that specifically examines the amount of damage to the offshore benthic environments. This was also the first time that gas chromatography coupled with mass spectrometry and stable isotopes for fingerprinting weathered oil were used (Boehm *et al.* 1983).

Natural Resources Damage Assessments (NRDA) include a three-phase legal process to determine the necessary type and amount of restoration to restore natural resources for the public that were harmed as a result of a hazardous substance release, such as an oil spill. Natural resources included in the assessment are land, air, water, fish, wildlife, biota, groundwater, drinking water supplies, and habitats. The three processes are pre-assessment, restoration planning, and restoration implementation (State of Florida 2012). Pre-assessment involves determining through scientific papers and research, whether damage has actually occurred (Office of Response and Restoration 2012). Once it has been determined that damage has occurred, then restoration planning, which involved developing a plan to speed up the recovery of the damaged resources, took place. Once a plan was developed, the third phase of NRDA involved enacting the plan and monitoring its progress. Another goal of NRDA was to determine potentially responsible parties (PRP). With respect to oil spills, NRDA worked through the Oil Pollution Act of 1990 (OPA). OPA forces PRP, such as oil companies, to pay for their damages by footing the bill for remediation processes. The NRDA process for the Deepwater Horizon incident was still ongoing in 2012.

In the summer of 1990, the tanker *Mega Borg* released approximately 3.9 million gallons of crude oil into the Gulf coast off the shore of Texas, and resulted in tar balls on the coast of Louisiana (Payne *et al.* 1993; Swannell *et al.* 1996). This spill was seen as an opportunity to determine the effectiveness of dispersants (Corexit 9527) in the field. Half of the oil spill was

treated with dispersant, and the other half was not (Swannell *et al.* 1996). Visually, this caused the oil to go from a dark brown color and sheen to an inconsistent mixture of brown and yellow oil and sheen almost instantly. Sixteen hours after the dispersant was applied; aerial photography did not indicate the presence of oil. Water samples taken within eight hours of dispersant application showed no signs of acute toxicity to marine organisms (Swannell *et al.* 1996). Within eight hours of treatment, there was no oil visual in the treated half of the spill, compared to the control which appeared unchanged. There were difficulties with uniform dispersant application, representative sampling due to lack of uniformity, and ambiguity of the fate of oil. Although many more oil spills have occurred in the Gulf, these three specifically caused new bioremediation and monitoring methods to be created (Swannell *et al.* 1996).

An additional regulatory and environmental complication when considering petroleum impact in the Gulf of Mexico was the significant natural release of oil and natural gas from seeps in the sea floor (Fingas 2010). The accumulations of underground oil and natural gas caused these seeps to continuously leak. Some estimates ranged from five to one million barrels (210 to 42 x $10^6$ gallons) of oil naturally seeping into the Gulf of Mexico each year from its 600 plus natural seeps (National Research Council 2003); though this number was difficult to measure and is, therefore, controversial.

## 1.4 DEEPWATER HORIZON OIL SPILL

### 1.4.1 WHAT HAPPENED

On April 20, 2010, an explosion occurred on British Petroleum's (BP) Deepwater Horizon oil rig causing 11 deaths and more injuries (Anonymous. 2012a). A current theory for

the cause was that not enough cement was used to create a reliable seal on the wellhead (Berman 2010). The blowout caused oil to leak into the Gulf of Mexico for 84 days, until the well was plugged on July 15, 2010, after allowing approximately 4.9 million barrels, (800 million liters or approximately 211 million gallons) of oil into the Gulf at an approximate rate of 50,000 barrels per day (Barron 2011; Engle 2011; Hsieh 2011; McNutt *et al.* 2011; Tao *et al.* 2011). Approximately 300,000 tons of natural gas, consisting of methane, ethane, and propane, were also leaked into the Gulf as a result of the spill (Mendes 2011). The spill was the deepest, occurring at 1500 m below sea surface, and largest offshore spill in the United States history (Camilli *et al.* 2010; Lu *et al.* 2012). The spill site was located at 28.25N, 88.81W in Mississippi Canyon Block 252 in the Gulf of Mexico approximately 50 miles from the Louisiana coast (Berman 2010).

Seafood safety from the Gulf of Mexico was an important and controversial issue during the oil spill. Gulf waters were closed to commercial and recreational fishing beginning on May 2, 2010 to prevent contaminated seafood from entering the market, closing approximately 24% of the US economic zone and 10% of the total surface area of the Gulf of Mexico (McCrea-Strub and Pauly 2011). Waters were closed if oil was visible on the water's surface, including a precautionary zone around the closed area (Ylitalo *et al.* 2012). The reopening of commercial and recreational fisheries began July 22, 2010, seven days after the capping of the Deepwater Horizon well, according to the Deepwater Horizon seafood protocol (Gohlke *et al.* 2011; Ylitalo *et al.* 2012). With the exception of a few state waters of Louisiana, all federal and almost all state waters were reopened as of November 9, 2011 (Gohlke *et al.* 2011; Ylitalo *et al.* 2012).

According to NOAA oil location maps, found at http://docs.lib.noaa.gov/noaa_documents/DWH_IR/maps/oil_approx_locations/, the oil's first land fall was May 1, 2010 on the Louisiana coast. Also from the previous website, NOAA has created maps of oiled shorelines including the degree to which they were oiled. According to these maps, by late October there was no observable oil, except for some coastal areas of Louisiana, where light to moderate oiling was still occurring, but tar balls were still accumulating on the coast.

It was estimated that approximately 46,000 personnel participated in response to the spill, including the BP staff, BP contractors, volunteers, federal and state and local government workers (Kitt and Kiefer 2010). As part of the response, volunteers were able to participate through a program known as "Vessels of Opportunity" (VOO) (restore the gulf 2010). This program was created to allow local boat operators to aid with response activities while being compensated. This increased the response force in the Gulf and allowed locals who might have been put out of business due to the oil spill to make some money.

### 1.4.2 FATE OF DEEPWATER HORIZON PETROLEUM

After an oil leak, petroleum PAHs underwent weathering, evaporation, biodegradation, and chemical processes that breakdown the components of the oil. Evaporation of components with a boiling point of less than 250°C occured during the first 24-48 hours after the oil was spilled. This caused n-alkanes of less than C14 and aromatic hydrocarbons of similar molecular weight to be reduced up to 40% (Harayama *et al.* 1999; OSATF 2010).

Methane, the major component of natural gas, was the most plentiful hydrocarbon discharged during the Deepwater Horizon Oil Spill, which allowed for methane fate and

transport to be specifically examined. Accumulation of oceanic methane influences climate change due to its ability to absorb infrared radiation, and ocean chemistry as it becomes oxidized (Kessler *et al.* 2011). The high concentrations of methane caused a methanotrophic bacterial bloom which metabolized the majority of the methane, preventing methane escape into the atmosphere. The BTEX compounds (benzene, toluene, ethyl-benzene, xylene) were the most abundant hydrocarbons larger than C5 found in the oil plume (Reddy *et al.* 2011).

Due to a lack of information regarding soluble hydrocarbon degradation via indigenous microbes, it has been difficult in the past to predict activities, such as metabolic potential and physiological mechanisms, of *in situ* hydrocarbon-degrading microbes (Kostka *et al.* 2011). This spill was an opportunity to investigate these microorganisms. Lu et al. (2012) observed increased expression of metabolic genes related to petroleum hydrocarbon degradation along with other microbial functional genes related to carbon cycling during the oil plume leak, suggesting that microbial communities have a significant impact in biodegradation of oil spills in the deep sea. Kostka et al. (2011) confirmed there was a selective response of bacterial communities in sediments as a response to oil presence from the Deepwater Horizon Oil Spill due to a shift in the bacterial community structure. The current method of biostimulation for biodegradation of crude oil contaminated soils and water depends on oxygen supply, enhancing aerobic processes (Boopathy *et al.* 2012). With this spill occurring at such great depths and oxygen supply being extremely limited, cutting-edge experiments with microbial mechanisms occurred. Boopathy et al (2012) observed microorganism mechanisms of degrading petroleum hydrocarbons, considering the electron acceptor (anaerobic) conditions. It was concluded that mixed electron acceptor conditions were optimal for petroleum hydrocarbon degradation, with sulfate-reducing

and nitrate-reducing conditions allowing for significant degradation to occur. Another study observed effects of natural gas and temperature on microbial community structure (Redmond and Valentine 2011); finding that natural gas and temperature, at the plume's depth, were favorable to hydrocarbon-degrading microbes. This was further confirmed by the lack of microbes once the well was capped in July due to the lack of food for microbes. Camilli et al (2010) suggested that it takes months for microorganisms to naturally break down the oil plume and that the low oxygen levels that occur, would threaten Gulf fisheries. This was due to the low rates of microbial respiration. Microbial degradation was a key remediation process to cleaning up the oil spill, but specific degradation pathways were still being studied.

Light molecular weight PAHs (LPAHs) more readily undergo microbial degradation and heavy molecular weight PAHs (HPAHs) undergo photooxidation and sedimentation (Meador *et al.* 1995). These weathering processes are affected by redox states, temperature, nutrient content, sediment structure, and biological activity in the sediment. Due to these many types of processes that PAH mixtures, such as petroleum, may encounter, it is difficult to link an oil sample back to its original source. By using ratios of specific compounds and looking at HPAHs, which are more stable than LPAHs, the length of weathering and sample source can be estimated.

Because of our understanding of the differential fate of different petroleum constituents once released into the environment, fingerprinting methods were used to determine the source of the petrogenic PAHs and the amount of weathering that has occurred. Hansen (2007) developed a flow chart for fingerprinting oil samples. First, the spilled oil is visually compared to suspected source samples, and then oil petroleum hydrocarbons progress through extractions and clean-up procedures as preparation for analytical characterization (Hansen *et al.* 2007). Then samples

undergo gas chromatography (GC) coupled with flame ionization detection (FID) screening to determine the dominating hydrocarbon class, the extent of weathering, and distinctive features or contaminants in the oil sample. Aliphatic hydrocarbons are usually the dominating hydrocarbon class in oil samples. Also, diagnostic ratios, indicating the extent of weathering the spilled oil has undergone, can be calculated from GC/FID chromatograms by comparing n-alkanes to isoprenoids, for example the C17/pristine ratio. Hansen (2007) evaluates the degree of weathering per sample by overlaying GC/FID chromatograms of a spill sample compared to a potential source sample or integrating a homologous series, i.e. n-alkanes. These comparisons are normalized and compared in bar chart form. Source matching can be ruled out at this step due to obvious differences in hydrocarbon distribution, unresolved complex mixture distribution, or the acyclic isoprenoid ratios not caused by weathering are significantly higher than the analytical variance.

The next step is to conduct GC/MS fingerprinting to characterize and evaluate composition and distribution of alkylated PAHs and oil biomarkers if differences are thought to be caused by weathering or samples appear to be similar (Hansen *et al.* 2007). Spill oil samples are compared with suspected source sample fingerprints from the GC/MS. The patterns of biomarkers and PAHs are examined, and if found to be different due to weathering, diagnostic ratios are used to determine if spill sample is a weathered version of its parent. There are ten commonly used diagnostic ratios for alkylated PAHs, as shown in Table 2, due to their ability to show degradation and differences between different oil sources. Alkylated PAHs are evaluated comparing peak areas of two diagnostic compounds. For example, the peak area of 2-methylphenanthrene is compared to the peak area of 1-methylphenanthrene, this number

becomes a diagnostic ratio. However, it is preferred to use peak height integration when assessing oil biomarkers because the diagnostic ratios are based on peaks that are ill resolved with noisy baselines (Hansen *et al.* 2007).

Hansen (2007) suggests two ways to calculate diagnostic ratios; A/B or A/(A+B), where A and B are the areas or peak height of two distinct peaks. A/B statistically lowers the analytical variance, while A/(A+B) always gives values between 0 and 1, resulting in lower relative standard deviations (RSD). Due to A/(A+B) RSD depending on the resulting ratio and the need for adjusting the confidence interval, it is more difficult to compare with others so A/B is most commonly used.

A fingerprinting example is described by Sun and coworkers (Sun *et al.* 2009), where samples were taken from suspected sites to determine which site had leaked oil into the China Bohai Sea. Chemical composition was analytically determined from the spill site and 15 suspected spill sources. Cluster analysis was used to determine sample similarities based on relative peak area of biomarker compounds, such as hopane and terpane. Overton et al (1981) used ratios between alkyl phenanthrenes and alkyl dibenzothiophenes to determine weathered oil contamination in sediment one year after an oil spill and fire occurred in West Hackberry, Louisiana. Elevated levels of fluoranthene and pyrene were used to identify contamination of pyrogenic sources due to the fire (Overton *et al.* 1981). If the ratio of fluoranthene/pyrene is greater than 1 a pyrogenic source is indicated, while a ratio less than one indicates a petrogenic source (Iqbal *et al.* 2008). Lemkau (2010) investigated source and short-term fate of the M/V *Cosco Busan* spill in San Francisco, California. To determine which tanker was responsible for leaking in the San Francisco Bay, Lemkau et al (2010) used a modified version of the Nordtest,

the name of the fingerprinting process described above developed by Hansen and coworkers (2007). Lemkau and coworkers (2010) found that due to changes in the n-C18/phytane and benz(a)anthracene/chrysene ratios, the route of weathering was biodegradation and photodegradation, respectively (Lemkau *et al.* 2010).

As suggested above, alkylated PAHs can be used to determine degree of weathering and the source of the contamination, weathering ratios and source ratios, respectively (Douglas *et al.* 1996). The most common alkylated ratios used are the 2-methylphenanthrene/1-methylphenanthrene and the 4-methyldibenzothiophene/1-methyldibenzothiophene (Hansen *et al.* 2007). Methylphenanthrene/ phenanthrene (its parent compound) ratio is indicative of whether the contamination is of petrogenic or pyrogenic origination (Kim *et al.* 2008). If the ratio is >2, the source is considered petrogenic, while a ratio of < 2 is considered pyrogenic. The C2-dibenzothiophene/C2-phenanthrene ratio and the C3-dibenzothiophene/C3-phenanthrene ratio are considered source ratios (Iqbal *et al.* 2008). Dibenzothiophenes are a common constituent among oils and therefore allow differentiation between different oil sources. C3-naphthalene/C2-phenanthrene ratios are indicative of the degree of weathering during the early stages of weathering due to the high sensitivity towards weathering processes (Douglas *et al.* 1996). In contrast, C3-dibenzothiphene/C3-chrysene ratios are indicative of the extent of weathering at late stages, due to the low sensitivity towards weathering processes. Retene/C4-phenanthrene ratios are commonly used to distinguish between plant and other similar oils (Daling and Faksness 2002). Retene is an aromatic compound derived from plant resin and is extremely resistant to weathering, allowing this ratio to be useful (Table 2).

Another use of alkylated-PAH ratios are double-ratio plots, line graphs that account for weathering by comparing ratios of alkylated-PAHs. The amount of weathering increases as the ratio plots move towards zero (Michel and Hayes 1999). Because alkylated-PAHs are more stable than their parent compound, their ratios remain stable throughout the weathering process, allowing weathered oil to be sourced. This is considered to be a better method than analyzing for oil biomarkers, such as hopane and sterane, used during exploratory drilling procedures because analyzing for PAHs and alkylated PAHs are a conventional procedure and they are found in high concentrations in many different petroleum products (Brown and Boehm 1993; Iqbal *et al.* 2008). The double ratio plot of C2-dibenzothiophenes/C2-phenanthrenes versus C3-dibenzothiophenes/C3-phenanthrenes is a valuable tool in distinguishing between crude oils and petroleum products, and determining sources, because the two alkyl-PAH ratios degrade at approximately the same rate (Douglas *et al.* 1996). Double ratio plots of C2-chrysenes/C2-dibenzothiophene versus 3C-chrysene/C3-dibenzothiophenes are valuable indicators of oil weathering and biodegradation because alkyl-chrysenes are more resistant to biodegradation. Double-ratio plots also allow the ability to plot source ratios versus weathering ratios, which allows for differentiating between multiple sources as well as differences in degradation of multiple samples from the same source. For example, on a double-ratio plot of C3-dibenzothiophene/C3-chrysene versus C3-dibenzothiphene/C3-phenanthrene, if the ratio of C3-dibenzothiphene/C3-phenanthrene is constant, indicating the same source, the change in C3-dibenzothiphene/C3-chrysene shows the extent of weathering that has occurred between the samples. Iqbal et al (2008) used the double-ratio plot C2-dibenzothiphene/C2-phenanthrene

versus C3-dibenzothiphene/C3-phenanthrene to distinguish between natural oil seeps in the Gulf of Mexico and oil contamination from South Louisiana crude oil in sediment samples.

In this study, the following ratios will be used to determine a source of the PAH mixture, ratios in parenthesis indicate petrogenic origin: fluoranthene/pyrene (<0.4), benz(a)anthracene/ chrysene (<0.2), anthracene/phenanthrene (<0.1) (Wang *et al.* 2009; Zencak *et al.* 2007). This will predict whether the PAHs originated from a petrogenic or pyrogenic source and the degree of weathering. The alkylated double ratio plots will not be used because our methods lacked sensitivity for alkylated PAHs found in BP petroleum and WAF samples.

## 1.5 COMMUNITIES POTENTIALLY IMPACTED AND ORGANISMAL EFFECTS

### 1.5.1 DEEP SEA COMMUNITIES

Deep sea communities are located more than 200 m in depth and are characterized by cold temperatures, constant salinity, lack of light, and high pressures (Etter and Mullineaux 2001). The bottom is mostly a soft-substrate habitat, mostly covered by coarse particles originally from eroded rocks on land, organism secretions, and fine clays. The sediment composition differs with depth, latitude, flow patterns, topography, and overlying surface production.

The seafloor also has islands of hard-bottom habitat composed of rock, mineral deposits, and biogenic material (Etter and Mullineaux 2001). These habitats can be as small as a few centimeters on a nodule to hundreds of kilometers of undersea mountain ranges. These mountain ranges create their own flow patterns and particulate organic matter (POM) fluxes due to their ridges, seamounts, and escarpments. These patterns affect food supply and dispersal of

organismal larval stages. No primary production occurs due to the lack of sunlight, except for around hydrothermal vents and cold seeps, so organisms rely of POM flow for food. Organisms found in deep sea communities include whales, dolphins, sharks, fish, polycheates, gastropods, shrimp, crustaceans, tubeworms, mussels, clams, and corals (Cordes 2010).

### 1.5.2 SEAGRASS COMMUNITIES

Seagrasses are primarily subtidal, clonal marine flowering plants that survive in shallow soft-sediment habitats along the shores of bays and estuaries (Williams and Heck 2001). Seagrass beds consist of various species of seagrass such as *Thalassia testudinum*, *Halodule wrightii*, *Syringodium filiforme*, *Ruppia maritime*, *Halophia engel mannii*, *Halophia decipiens*, and *Halophia johnsonii*, many types of algae, and animals from every major phylum. Seagrass communities are vital in their roles of trophic support, refuge from predation, recruitment, and nursery areas and have been found to be relatively tolerant to oil spills (Lewis and Devereux 2009).

### 1.5.3 SALT MARSH COMMUNITIES

Salt marshes are an environment in the upper coastal intertidal zone in between land and salt or brackish water that consists of salt-tolerant, halophytic, terrestrial plants (Adam 1990). Salt marshes make up more than 50% of the east and Gulf coasts of the United States (Engle 2011; Pennings and Bertness 2001). Salt marshes are critical to the aquatic food web, sheltering coasts from erosion, filtering sediments and nutrients from the water column, transferring nutrients to other waters, and supporting fisheries (Adam 1990; Pennings and Bertness 2001).

Because salt marshes also serve as protection to shorelines, they are extremely vulnerable to oil contamination from spills. Louisiana's salt marshes alone consist of 14% of the total salt marsh land in the contiguous United States, for a total of $1.63 \times 10^6$ acres (Summers *et al.* 2005). Two U.S. Strategic Oil Reserve Sites are located in coastal Louisiana, involving thousands of miles of pipelines, refineries, and gas production facilities. Due to this large amount of petroleum oil production and transportation occurring just south of Louisiana, numerous studies have been performed prior to the Deepwater Horizon incident to determine effects of oil spills and clean-up methods on the salt marshes. The intensity of the impact of the oil is dependent on abiotic and biotic factors such as the type and volume of oil, the plant species and extent of oil coverage, season of the spill, weather conditions at the time of the spill, and soil composition (Lin and Mendelssohn 1996).

### 1.5.4 OYSTER REEFS

Oysters can form "beds" or "reefs" of hundreds to thousands of individual oysters per square meter in marsh, seagrass, and mangrove habitats (Pennings and Bertness 2001). The oyster beds of the Gulf coast of the United States are one of five remaining wild capture of native oysters regions in the world. Oyster catches are also the highest in the world on the Gulf coast (Beck *et al.* 2011). These reefs have the ability to act as a coastal defense from erosion and storms, filtering the water to deposit nutrients in the sediment, and are known ecosystem engineers, producing food and a habitat for entire ecosystems to survive (Beck *et al.* 2011; Pennings and Bertness 2001). Oysters are filter-feeders, meaning they remove suspended particles from the water column. This can improve water quality, reducing potential for algal blooms and enhancing light penetration for plants. This filter feeding ability allows oysters, or

bivalves in general, to bioaccumulate contaminants, such as oil, from the water column, as well as metabolize or experience adverse effects to these contaminants (Bebianna and Barreira 2009).

Chemical analysis of environmental contaminants in bivalve tissue has become common practice for biomonitoring, and allows for the assessment of the bioavailable amount of contaminant in the water column due to exposure over time. Status and trends of coastal contaminants in mussels and oysters in the United States is monitored through NOAA's National Status and Trends (NS&T) Mussel Watch Program (Summers *et al.* 2005). This program was initiated in 1986 at specified sites throughout the United States by analyzing bivalve tissue for environmental contaminants. A total of 220 sites were chosen based on abundance of mollusk communities, allowing for resampling. Sites of known point source pollution influence were avoided. The goal of the program is to determine status and trends, not effects of the chemicals on the marine life or human seafood consumers. The FDA makes suggestions whether to fish or sell seafood from a certain location based on these results. The status and trends are helpful for determining coastal conditions.

The Eastern oyster (*Crassostrea virginica*) is commonly found in estuaries and coastal areas with low salinities in reef formation in the Western Atlantic Ocean to the Gulf of Mexico, Caribbean, and coasts of Brazil and Argentina (Carriker and Gaffney 1996). Reef formation can occur in intertidal or subtidal areas. The Eastern oyster is important commercially, but due to overfishing, catch rates have decreased. Because Louisiana is a national leader in oyster production, the Eastern oyster is closely monitored as seafood and as a biomonitor for the Northern Gulf of Mexico.

### 1.5.5 EFFECTS OF PETROLEUM CONSTITUENTS ON ORGANISMS

#### 1.5.5.1 PLANTS

Plants are chemically and physically affected by petroleum hydrocarbons (Pezeshki *et al.* 2000). Fouling, an accumulation of unwanted material on a plant, by oil can cause short term effects that range from reduction in transpiration and carbon fixation to plant mortality. The sensitivity of the plant to oil fouling can vary by species, age of the plant, and season of the spill. Physically induced effects usually occur through coating of the plant and soil surfaces. This can cause temperature stress, reduced photosynthesis, plant mortality, and oxygen stress. Chemically induced effects include prevention of leaf and shoot regeneration and damage to the root membrane affecting the ionic balance of the plants to their salt tolerance (Pezeshki *et al.* 2000). The active growing period of a plant is more susceptible to adverse effects of oil than other plant seasons. Effects of clean-up activities on marsh vegetation have also been evaluated (Pezeshki *et al.* 2000). Natural attenuation is the best clean-up response in salt marshes because other methods can be damaging to vegetation, such as uncertainty of dispersant and date of added nutrients (DeLaune and Wright 2011; Pezeshki *et al.* 2000). Lewis and Devereux (2009) found adverse effects, such as decreased photosynthetic activity, and tissue and whole plant mortality, were short-term when they occurred, and plants made rapid recoveries. Direct observations of the effects of an oil spill on seagrass were made after incoming oil reached the seagrass beds of Roscoff (France) (Jacobs 1980). When the oil reached the seagrass, the leaves seemed "burnt," but after a few weeks, normal tissue regrowth occurred. *Z. marina*, a common seagrass species, enhanced biodegradation of PAHs from oil in marine sediments (Huesmann *et al.* 2003). Added

dispersants prevented adverse effects on seagrass photosynthesis by decreasing fouling (Macinnis-Ng C.M.O. and Ralph 2003).

Lin et al (1996) found that *Spartina alterniflora*, a common plant species in salt marshes along the Gulf coast, photosynthetic rates were unaffected by Louisiana crude oil until 3 months after the application of oil, when the rates decreased. Four months after above ground application, biomass production was unaffected except for at the oil concentration of 8 $L/m^2$, in which no regrowth had occurred one year after application. As a response to the Deepwater Horizon incident, Lin et al. (2012) examined the effects of the oil on salt marsh *Spartina alterniflora* and *Juncus roemerianus*. Seven months after the oil landed in the salt marsh of Barataria Bay, Louisiana, oil concentrations were found to be as high as 510 mg/g dry soil, considered heavy oiling, and almost complete mortality of both species occurred (Lin and Mendelssohn 2012). In moderately oiled areas of the marsh *Spartina* was less severely affected than *Juncus*. There was no significant effect on *Spartina* when compared to reference sites, while *Juncus* above ground biomass and stem density was significantly lowered compared to these reference sites. *Spartina* almost fully recovered, while *Juncus* did not (Lin and Mendelssohn 2012).

#### 1.5.5.2 INVERTEBRATES

Populations of microbial petroleum degraders are present throughout the year with peak numbers in April in the Gulf of Mexico (Horel *et al.* 2012). The constant presence of microbial populations allow for the degradation of at least a fraction of spill related petroleum hydrocarbons. Graham et al (2010) examined oil in the plankton food web. By using $\delta^{13}C$ as a tracer of oil-derived carbon, they confirmed that carbon depletion in the food chain coincided

with incoming oil and that subsurface oil carbon had become incorporated into the plankton food web (Graham *et al.* 2010). McCall and Pennings (2012) investigated the abundance of intertidal crabs and terrestrial arthropods during the landing of the Deepwater Horizon oil in 2010, and then a year later to determine recovery. The numbers of intertidal crabs and arthropods, insects and spiders, were found to be suppressed during the influx of oil, even where the marshes seemed unaffected by the oil, but snails were unaffected (McCall and Pennings 2012). One year after the spill, the crab and arthropods had recovered showing both the initial vulnerability and then resilience of crabs and arthropods.

The mysid shrimp, *Americamysis bahia*, native to the Gulf of Mexico was exposed to dispersed Louisiana crude oil (LCO) in lab studies to determine its acute toxicity (Hemmer *et al.* 2010). The Corexit 9500A dispersed LCO LC50 was 5.4 ppm and considered moderately toxic to the mysid shrimp. Alone, the LC50 concentrations of Corexit 9500A were higher than any of the concentrations used for toxicity testing. The LCO alone was found to be moderately toxic to the mysid shrimp suggesting the dispersant did not change the toxicity to invertebrates.

Oyster stressor effects have been studied from the cellular to the community level. When oil is the stressor, oysters have increased susceptibility to disease and decreased reproduction. At the population level, change in age-size structure was seen, because less recruitment occurs, and mortality increases (Soniat *et al.* 2011). Soniat et al (2011) investigated potential lethal and sublethal effects related to the Deepwater Horizon Spill to *Crassostrea virginica* in Louisiana. A higher percent of female oysters (50-80%) occurred at oil sites when compared to unoiled sites, however, this was determined to be a result of differences in salinity. When looking at PAHs accumulated in the oysters, used the QuEChERS method and analyzed for 13 PAHs, the only

PAH detected was B(a)P in oiled, non-oiled, and reference oyster. Ultimately, the authors concluded that the peak was not actually B(a)P, after using fluorescence detection. After six months of capping the Deepwater Horizon well, no PAHs were found in the oysters from oil-exposed sites (Soniat *et al.* 2011).

Natural environmental factors such as temperature, pH, salinity, and dissolved oxygen can stress oysters and compound effects of climate change, ocean acidification and anthropogenic contamination (Chapman *et al.* 2011). Chapman et al (2011) investigated gene expression changes in *C. virginica* due to these natural environmental factors so in the future, observed changes in gene expression can be related to contaminant exposure. Temperature and pH were found to have the largest transcriptional response, while pollution did not cause a large effect.

#### 1.5.5.3 FISH

A major concern during the Deepwater Horizon oil leak, was that it was spawning season for many commercially important species, such as bluefin tuna, groupers, or snappers (Fodrie and Heck 2011; Muhling *et al.* 2012). PAHs can cause acute developmental effects or mortality to fish. Muhling et al (2012) compared oil coverage with spawning habitat models of bluefin tuna. They found that less than 10% of the spawning areas were contaminated with oil and less than 12% of the bluefin tuna larvae were located in contaminated areas per week. This result is considered a preliminary approximation of the effects of the spill because this is considered a short term effect, and it is unknown what long term effects contamination in spawning areas will be. Fodrie and Heck (2011) observed juvenile fishes representing 86 taxa from 2006 until October 2010 to determine if there were decreases in catch rate abundance. Losses were avoided

and no shift in species composition occurred due to the oil spill, catch rates actually increased in 2010. Considerations such as large-scale fishery closures due to unsafe seafood, and unequal risk per species were evaluated.

Studies investigating impact of oil on biota in salt marshes occurring before the Deepwater Horizon incident, examined *Fundulus grandis*, Gulf killifish, *C. virginica*, Eastern oyster, and *Litopenaeus setiferus*, white shrimp, in response to an Alaska North Slope crude oil compared to the dispersed oil, using Corexit 9500 (Liu *et al.* 2006). The adult *L. setiferus* showed acute sensitivity to both the oil and dispersed oil, but showed a survival rate of 83% after 24 hours. The juveniles exhibited toxic effects at 30 mg/L, the same concentration as the adults. Another study investigated the short-term effects of an oil spill in Louisiana on marsh-edge fish and crustaceans (Roth and Baltz 2009). The mobility of the fish allowed them to leave the contaminated spill area, while the less mobile crustaceans remained in the contamination. However, the overall community structure was able to recover. Whitehead et al (2011) examined gene expression in *F. grandis*, Gulf killifish, as compared with oil exposure. Although low concentrations of PAHs were found in the water and *Fundulus* tissues, changes in the gene expression (e.g. downregulation of ARG2, DIO2, and JUNB) were found to be congruent with oil contamination and were consistent with expected changes due to PAH exposure (Whitehead *et al.* 2011).

The nine to 14 day old inland silverside larvae, *Menidia beryllina*, native to the Gulf of Mexico, were exposed to dispersed LCO in lab studies to determine its acute toxicity (Hemmer *et al.* 2010). The Corexit 9500A dispersed LCO LC50 was 7.6 ppm and considered moderately

toxic to inland silverside. The LCO alone was found to be more than moderately toxic in the inland silverside at 3.5 mg TPH/L.

## 1.6 HUMAN EFFECTS

### 1.6.1 SEAFOOD SAFETY

Sampling of the local seafood occurred to determine its safety for consumers. There was difficulty developing methods to determine seafood safety due to the lack of consistency in the literature (Gohlke *et al.* 2011). The sensory testing method performed by 10 trained experts was based on the smell of raw and cooked seafood (Ylitalo *et al.* 2012). According to protocol, 70% of the sensory assessors had to determine subsamples undetectable for petroleum or dispersant odor or flavor (Ylitalo *et al.* 2012). Chemical analysis was executed with liquid chromatography tandem fluorescence detection or gas chromatography tandem mass spectrometry. Once concentrations were determined to be below the FDA's level of concern (LOC) for human health risk, fishing areas were reopened. Total PAH levels of concern were determined for young children (0.6-600 ppb), older children (0.9-86 ppb), adult women (1-119 ppb), and adult men (1-108 ppb) eating contaminated seafood twice a week (Gohlke *et al.* 2011). For specific species, shrimp and crab, oysters, finfish, PAH LOCs were determined to be 61.5, 66.5, and 16.35 ppb, respectively (Gohlke *et al.* 2011).

There remains some controversy as to whether the FDA LOCs were protective of vulnerable populations. Rotkin-Ellman et al (2012) investigated the adequacy of the risk criteria and established LOCs and found that due to failure to account for increased susceptibility of developing fetuses and children, use of regional seafood consumption rates and pertinent health

endpoints, and integrated health protective estimates of exposure duration and acceptable risk, the FDA risk assessment method was inaccurate and out-dated. Rotkin-Ellman et al (2012) claim the FDA's LOCs for benzo(a)pyrene and naphthalene were set 2-4 magnitudes higher than the most recent FDA set level. Approximately 53% of Gulf shrimp samples would have been above LOCs for pregnant women if the lower LOCs were used. Ylitalo et al (2012) oppose the previous argument and defend the FDA assessment by describing the development of the health risk assessment by the FDA and NOAA. They contend that USEPA reference doses considered were made to be protective of sensitive populations, such as children and pregnant women. Regional seafood consumption rates for the Gulf region were unavailable when creating the risk assessment guidelines, which is why the $90^{th}$ percentile consumption data for national seafood was used, but was adjusted for consumption frequency. A five year duration period was chosen because it was thought exposure to the oil spill would not outlast this period due to the type of crude oil spilled, physical conditions of the environment, spill location, and metabolism rates of impacted seafood (Ylitalo *et al.* 2012).

## 1.7 CLEANUP WORKER STUDIES

### 1.7.1 NIEHS STUDY

As a result of the Deepwater Horizon Oil Spill, the National Institute of Environmental Health Sciences (NIEHS) is funding a study on the human health effects of the oil spill cleanup (Schmidt 2011). The study will focus on respiratory, neurological, and hematological effects currently linked to oil constituents as well as psychological effects. Aerosol particles have been investigated to understand air quality that could have adverse effects on the cleanup workers and Gulf Coast population (Avens *et al.* 2011; de Gouw *et al.* 2011; Middlebrook *et al.* 2012).

Aerosol particles were thought to be formed by evaporation and chemical processes of surface oil and from *in situ* oil burns by the U.S. Coast Guard (Middlebrook *et al.* 2012; Schaum *et al.* 2010). Middlebrook *et al* (2012) used a regional air quality model that showed an increase in organic aerosol concentrations along the Gulf Coast that were most likely a result of the Deepwater Horizon Oil Spill. De Gouw *et a*l (2011) found a hydrocarbon plume downwind of the Deepwater Horizon Oil Spill and also a much larger plume of secondary organic aerosols formed by less volatile hydrocarbons. Avens *et al* (2011) investigated the health effects of cleanup workers due to BTEX compounds, volatized from the surface oil. Measurements found that 99% of measurements taken before the well was capped were well under the U.S. Occupational Safety and Health Administration's (OSHA) Permissible Exposure Limits (PELs), and these levels remained unaltered after the well was capped. This study found that BTEX compounds had a primary contributor other than the oil spill (Avens *et al.* 2011).

### 1.7.2 GENERAL PUBLIC

Acute and chronic human health effects of the cleanup workers and general public are also important to understand. In Louisiana, acute human health effects for the Deepwater Horizon Oil Spill were compared to those of previous oil spills, such as the *Sea Empress*, *Prestige, Tasman Spirit*, *Nakhodka,* MV *Braer*, *Erika,* and *Exxon Valdez* oil spills (Diaz 2011; Goldstein *et al.* 2011). Acute symptoms experienced were similar to those of previous oil spills. Post-traumatic stress disorder was found to be a social disruption among Alaskan Natives and European Americans one year after the *Exxon Valdez* spill (Palinkas *et al.* 1992). Compared to a control site, residents near the *MV Braer* spill had significantly higher amount of headaches,

throat irritation, and itchy eyes (Campell *et al.* 1993). Symptoms began on day one, but by day seven of the *MV Braer* spill, 97% of the residents said their symptoms had been resolved.

Schaum et al (2010) estimated cancer risks for the following three exposure routes to the Deepwater Horizon oil spill scenarios: inhalation by workers, inhalation by mainland residents, and fish ingestion to residents. There were no cancer risks higher than $1 \times 10^{6}$, the lower boundary of carcinogenic effects requiring more action to be taken. Increased occurrence of lower respiratory tract symptoms were chronic health effects experienced after both the *Exxon Valdez* and *Prestige* oil spills (Diaz 2011).

### 1.7.3 IMMUNOTOXICITY

During an assessment of the coastal shorelines, many species, such as sea turtles, marine mammals, birds, and estuarine organisms, were found to have health effects due to oil spill exposure (Barron 2011). Immunotoxicity was not evaluated in the wildlife due to its dose dependency, but it was thought likely to have occurred. Due to cleanup workers complaints of acute pulmonary and dermatological adverse health effects, immune response experiments were performed to determine adverse effects of COREXIT 9500A (Anderson *et al.* 2011). Anderson et al (2011) found that COREXIT 9500A did not induce immunosuppression in rats and mice and was classified as an irritant and allergic sensitizer.

### 1.7.4 PSYCHOLOGICAL

Psychological impacts such as distress levels, adjustment mechanisms, and alleged risk to communities due to Deepwater Horizon have also been investigated (Grattan *et al.* 2011). Economic loss was thought to be an impacting factor for the mentioned psychological symptoms.

Grattan *et al* (2011) found that communities in Alabama and Florida impacted by the oil spill were more depressed and more likely to withdraw and keep to one's self as a way to cope as compared to individuals from communities not impacted by the spill. Because these results were short term after the oil spill, it is thought that they could be an underestimation. There was also a study designed to investigate the mental health effects of residents in southeastern Louisiana (Osofsky *et al.* 2011). Negative effects such as anxiety, depression, and posttraumatic stress were found, which were similar to effects found in the local population after Hurricane Katrina. Follow up studies indicated that the community was able to rebound and mental health improved (Osofsky *et al* 2011).

## 1.8 SOURCES AND EXPOSURE PATHWAYS OF PAHs

Because PAHs represent the major toxic constituents of petroleum, their measurement was the focus of this thesis. In addition to petroleum, PAHs are found all around the environment, due to formation during incomplete combustion. PAHs primarily accumulate in sediments and biota, due to their hydrophobic nature and attraction to the organic carbon (Jacob 1996; Meador *et al.* 1995). PAHs can be found in water from particulate matter fallout from the atmosphere, runoff of polluted grounds, municipal and industrial effluents, and natural byproducts of organisms before they enter the food chain (Harvey 1991; Meador *et al.* 1995).

Regulatory guidelines including levels of concern and NOAA's Screening Quick Reference Tables (SQuiRTs) have been formulated for PAHs. SQuiRTs are a compilation of compiled toxicity reference tables of potentially hazardous chemicals (Buchman 2008). They serve as a preliminary assessment of potential hazardous chemical concentrations in water, sediment, and soil involving multiple degrees of adverse effects. Effects Range Low (ERLs)

values represent values at which toxicity may begin to occur and are based on the lower tenth percentile concentrations of the available toxicity data where adverse effects of sensitive species rarely occur. Probable Effects Level (PELs) are representative of levels where adverse effects are commonly expected. Effects Range Median (ERM) is the median value of sediment toxicity data. Neither ERL nor ERM coincide with $LC_{10}$ or $LC_{50}$ values (Buchman 2008).

PAHs have health risks to humans. *In vivo* experiments have resulted in both benign and malignant tumors, while during *in vitro* experiments, cell-transforming properties point to mutagenic and carcinogenic possibilities (Jacob 1996). Environmental exposure to humans can occur through ingestion of fatty foods, such as contaminated processed oils and fats, contaminated seafood, cereals, and margarine (Gilbert 1994). Other exposures include cigarette smoke and dermal contact with soot, tars, and polluted soils; with cigarette smoke being the primary exposure source (Fiala *et al.* 2001). Drinking water, another human exposure source, is estimated to have between 0.1 -23 ng/L of PAHs (Harvey 1997). Occupational exposures, such as coal gasification, coke producers, chimney sweepers, pavers and roofers with coal tar pitch, and aluminum producers, have been categorized by the International Agency for Research on Cancer as carcinogenic to humans (IARC 2009).

Regulations to keep the community informed and workers safe have been formed due to toxicity of PAHs. Regulations and reference doses for 17 PAHs have been developed by the United States Environmental Protection Agency (USEPA), Emergency Planning and Community Right-To-Know (EPCRA), Occupational Safety and Health Administration (OSHA), Clean Water Effluent Guidelines (CWEG), and the Resource Conservation and Recovery Act (RCRA) (Mumtaz and George 1990). Reference doses are developed by the EPA and are the maximum

acceptable oral dose of a toxic contaminant. The references doses for anthracene, acenaphthene, fluoranthene, fluorene, and pyrene are 0.3, 0.06, 0.04, 0.04, 0.03 mg/kg/day, respectively. EPCRA requires operation facilities to report their disposal of PAHs into environmental media ever year. OSHA requires workers exposed to PAHs to use engineering and work practices to keep their inhalation exposure below 0.2 and 5 $mg/m^3$/8 hour work day for coal tar pitch volatiles, and mineral oil mist, respectively. Other regulations deal with amount of PAHs disposed of into the environment.

## 1.9 METABOLIC PATHWAYS OF PAHs

### 1.9.1 CANCER

Metabolism of certain high molecular weight PAHs, once they have entered the body, is required to initiate their carcinogenic effects by facilitating DNA binding (Marczynski *et al.* 2009; Meador *et al.* 1995). One pathway, as described by the "Bay Region Theory," occurs when the PAHs are activated by cytochrome P450-dependant monooxygenases, particularly the CYP1 family (Marczynski *et al.* 2009). By following this pathway, PAHs are activated to arene oxides, which are then converted to phenols or *trans*-dihydrodiols. These compounds are then oxidized to form diolepoxides, which possess tumor initiating properties by reacting directly with DNA. This pathway has been most commonly studied with benzo(a)pyrene (B(a)P). High molecular weight PAHs (HPAH), such as BaP are strong agonists of the aryl hydrocarbon receptor (AhR) (Incardona *et al.* 2006; Safe 2001). Ligand (BaP) binds to AhR causing AhR to move into the nucleus and dimerize with aryl hydrocarbon receptor nuclear translocator (ARNT) (Mimura and Fujii-Kuriyama 2003; Safe 2001). The newly formed heterodimer binds with the xenobiotic responsive element (XRE) sequence on gene promoters which in turn initiates transcription of

multiple genes, including CYP1A. Some other well known AhR agonists are polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzodioxin (TCDD).

### 1.9.2 NARCOSIS

Narcosis is a general mechanism that describes relationships between water-lipid partitioning, the kinetics of bioaccumulation, and the acute lethality of organic compounds (Billiard *et al.* 2008). The non-polar narcosis mechanism of toxicity proposes that all soluble components of oil bioaccumulate in aquatic organisms and play a role in the toxicity of the oil, allowing narcosis to be approximated using the octanol-water coefficient, or lipophilicity (Barron *et al.* 1999; Billiard *et al.* 2008). This is accepted due to the concept that toxicity of a substance correlates to the internal dose received by the organism in question and is important in toxicity modeling (Peterson 1994). Peterson (1994) investigated algal toxicity of gasoline and found that alkanes were twice as acutely toxic as PAHs.

### 1.9.3 IMMUNOTOXICITY

The immune system involves the spleen, lymphoid, thymus, bone marrow, and skin with operative cells such as leukocytes, lymphocytes, T cells, B cells, and macrophages (Kovacic and Somananthan 2008). PAHs, specifically B(a)P and 7,12-dimethylbenz(a)anthracene (DMBA), are recognized immunosupressors. B(a)P is immunosuppressive in both mammals and fish causing reduction in lymphocyte proliferation, phagocyte-mediated superoxide generation, and antibody-forming cells. DMBA decreased the weights of the spleen and thymus by more than half in a wild type mouse (Kovacic and Somananthan 2008). The microsomal epoxide hydrolase was suggested to activate DMBA toxicity in the spleen. PAH toxic mode of actions for the immune system, other than the AhR pathway, include membrane perturbation effects, altered

interleukin (IL) production, interruption of intracellular calcium mobilization, and metabolic activation of PAH metabolites (White Jr *et al.* 1994c).

Due to the planar structure of PAHs and their lipophilic properties, some PAHs interrupt transmembrane signaling by entering the membrane of cells, or altering the receptor conformation in the membrane (White Jr *et al.* 1994b). Alteration of interleukin production is thought to allow PAHs to cause immunosuppressive effects. B(a)P effected the production of IL-1 and IL-2, while DMBA inhibited their high-infinity receptor production. As stimulation of T cell mitogen phytohemagglutinin (PHG) increased, DMBA inhibition of $Ca^{+2}$ mobilization and free cytoplasmic $Ca^{+2}$ increased. DMBA also increased $Ca^{+2}$ in resting B cells, inhibiting B cell activation by anti-IgD antibodies. DNA fragmentation suggests that DMBA is specifically lymphotoxic by an apoptosis mechanism activated by $Ca^{+2}$-activated enzymes (White Jr *et al.* 1994a). DNA fragmentation, however, occurred at concentrations much higher than concentrations required for adverse immune effects.

### 1.9.4 DEVELOPMENTAL

During the early life stages of fish, chronic PAH exposure causes CYP1A induction, edemas, hemorrhaging, cardiac dysfunction, mutations, heritable changes in progeny, morphological deformities, neuronal cell death, anemia, stunted growth, and higher mortality rates (Billiard *et al.* 2008). These adverse effects are not considered additive due to multiple mechanisms of toxicity of PAHs and their rapid metabolism into metabolites. Billiard et al (2008) reviewed six possible mechanisms of developmental PAH-mediated toxicity: narcosis, cardiac-mediated, AHR-mediated, CYP1-mediated, alkylated-PAH toxicity, and oxidative stress.

PAH-induced developmental and cardiovascular toxicity occurs across the taxa of vertebrates, such as birds and murine species. Incardona and coworkers (2004; 2005) found fluorene, phenanthrene, and dibenzothiophenes, all lower molecular weight PAHs, were directly cardio-toxic to zebrafish embryos and suggested that these PAHs are the most toxic component of weathered crude oil. This mechanism of action was independent of narcosis. The effects were similar to atrioventricular conduction block associated with the silent heart mutation of zebrafish, suggesting the cause to be blockage of cardiac ion channels (Incardona *et al.* 2004; Incardona *et al.* 2005). This mechanism was also independent of CYP1A oxygenation or induction and the AHR receptor, because knock down of AHR2 by morpholinos did not reduce toxicity. Larger PAHs, such as chrysene, can cause CYP1A induction but no cardio-toxicity. Pyrene induces CYP1A and acts through the AHR receptor with its toxicity localized in the peripheral vasculature (Incardona *et al.* 2004).

Carls (2008) investigated the difference in embryotoxicity of zebrafish from dissolved PAHs versus oil droplets. Particulate oil was not directly toxic to the fish embryos because it was not bioavailable, as the dissolved PAHs were (Carls *et al.* 2008). The effects from oil droplets would mimic those of hypoxia, which are unlike the cardiovascular deformities from petrogenic PAH exposure. The oil droplets coated the egg thereby blocking the oxygen transport across the chorion. Oil droplets do, however, allow for PAHs to slowly dissolve into the water column, allowing for prolonged exposure. Sublethal effects correlated to petrogenic PAH exposure include impaired swimming abilities, stunted growth, and reduced survival potential. This also implied that use of dispersants increasing solubility would increase sublethal effects.

This thesis focuses on developmental consequences of oil in *F. heteroclitus*. Linden et at (1980) exposed developing *F. heteroclitus* embryos to multiple concentrations of water soluble fraction (WSF) of number 2 fuel oil with respect to different temperature-salinity gradients. The two highest concentrations of WSF, at 20 and 25% dilutions of original mixture, stunted embryo growth and results were unmodified by temperature or salinity. Sublethal effects, such as spinal deformities, occurred reducing chances of survival for later life stages (Linden *et al.* 1980). Clark et al (2010) investigated the developmental effects of PAHs and PCB-126 to *F. heteroclitus* and linked AHR2 as mode of action for cardiac teratogenesis (Clark *et al* 2010). Coulliard and coworkers (2005) exposed *F. heteroclitis* larvae to dispersed oil. Compared to oil in the water column, dispersed oil increased tPAHs and HPAHs two - to five-fold and mortality rates (Couillard *et al.* 2005). The increases in bioavailability of the HPAHs was reflected by an increase in EROD induction (Couillard *et al.* 2005). The authors concluded that dispersant may contribute to increased toxicity to larval fish (Couillard *et al.* 2005).

## 1.10 ASSESSMENTS OF OIL SPILLS

When determining the effects of an oil spill, bioassays and analytical chemistry represent an important two pronged approach. Analytical chemistry determines how much of a contaminant there was and bioassays determine adverse effects, mortality, and modes of action or if there is any biological effect at all at representative exposure concentrations.

### 1.10.1 ANALYTICAL CHEMISTRY

Chemical analysis of different matrices is done to determine the concentrations of specific contaminants in the environment or biota. This is necessary for remediation steps and to later combine with biological assays to determine toxic effects. Common matrices are water

column, sediment, and organism tissue. Since 1984, NOAA has been monitoring environmental impacts through the National Status and Trends (NS&T) program (National Oceanographic and Atmospheric Administration 2005). There are three major components: mussel watch program, bioeffects assessment, and eutrophication studies. The mussel watch project strives to portray a baseline spatial distribution and temporal trends of coastal toxic contamination where there is no point source influence. Bivalves and sediment samples are collected semiannually at over 220 sites around the United States. The data collected is used to determine water quality, environmental health, and human health impacts and to plan resource management. The NS&T bioeffects studies provide environmental toxicity assessments of regional bodies of water, such as sediment toxicity testing and conditions of benthic communities. The data from these studies help coastal managers identify and prioritize areas for remediation. The NS&T eutrophication studies assess eutrophication, impairment causes, and future outlook of estuaries using a Pressure-State Response model. Specific cases showing emerging pollution issues are also undertaken. A current example is evaluating poultry farming effects on the Choptank River Estuary located in Maryland (National Oceanographic and Atmospheric Administration 2005).

### 1.10.2 SEDIMENT CHARACTERIZATION AND C/N RATIOS

Characterization of sediment is useful when determining contamination. Three common classes of sediment are sand, silt, and clay. Contaminants are usually found in the fine-grained silt and clay due to the higher amount of organic content and larger surface area as compared to sands (U.S.Environmental Protection Agency 1994). The carbon to nitrogen (C:N) ratio is one important end point when characterizing the organic content of sediment. The ratios can reflect source of carbon and nitrogen, such as algal or terrestrial origin, hydrocarbon contamination, and

bacteria biodegradation rates (Hoyle *et al.* 1995; Kaushal and M.W.Binford 1999; Meyers 2003; Perez-de-Mora *et al.* 2008). Algae usually have a C:N ratio between 4 and 10, while terrestrial organic matter is greater than 20 (Meyers 1994). This difference comes from algae's lack of cellulose, whereas it is highly abundant in land plants. Changes in C:N ratios have been seen due to microbe degradation and hydrocarbon contamination. Perez-de-Mora et al (2008) found that acid resin produced by mineral oil industries and disposed of in local ponds, increased the total C: total N ratios in affected sediment compared to unaffected. The acid resins were characterized by hydrocarbons and trace elements.

Another useful endpoint for sediment characterization is the use of carbon stable isotopes. $^{13}C$ and $^{12}C$ are the two naturally occurring stable isotopes of carbon and their ratios can be specific to different organic sources (Kim *et al.* 2008). Due to specific physical and biological processes manipulating the oil formation and refinery, oils usually have $^{13}C$:$^{12}C$ ratios unique to specific classes of oils reported in $\delta^{13}C$ parts per thousand (‰) relative to the PeeDee belemnite (PDB) standard (Wang *et al.* 1999). An advantage to this technique is that weathering does not affect the isotopic compositions of the whole oil. This allows to source oils, regardless to the degree that the oil has been weathered to be distinguished by type.

### 1.10.3 BIOASSAYS

#### 1.10.3.1 ETHOXYRESORUFIN-*O*-DEETHYLASE BIOASSAY AND HIGH MOLECULAR WEIGHT PAHs

Ethoxyresorufin-*O*-deethylase (EROD) activity is a fluorimetric measurement quantifying induction of CYP1A (Willett *et al.* 1997). The positive correlation, as EROD activity

increases, CYP1A induction increases, allows this relationship to be a useful biomarker (stegeman and Lech 1991). As described above, HPAHs and halogenated aryl hydrocarbons (HAH) bind to AhR inducing CYP1A gene expression. According to Willett et al (1997), HPAHs can be classified according to their abilities to induce CYP1A. There were seven EROD inducing HPAHs found; benz(a)anthracene, benz(a)pyrene, benzo(k)fluoranthene, benzo(b)fluoranthene, chrysene, dibenz(a,h)anthracene, and indeno(1,2,3-c,d)pyrene. The rest of the PAHs are considered low molecular weight or are not typically considered AhR agonists.

Patterns of PAH metabolism vary between species. High metabolism will leave undetectable traces of PAHs in tissues, which is why biomarkers, such as CYP1A-mediated EROD activity are used to determine environmental exposure to contaminants. Many studies involving the EROD assay have been used to predict petroleum exposure (Couillard *et al.* 2005; dos Anjos *et al.* 2011; Landrum *et al.* 2012; Lyons *et al.* 2011; Pal *et al.* 2011; Ramachandran *et al.* 2004; Schein *et al.* 2009; Willett *et al.* 1995).

## 1.11 AIMS AND HYPOTHESES

The purpose of this study was to assess temporal and spatial impacts on contaminant concentrations from the Deepwater Horizon Oil Spill along the Gulf Coast. Samples were collected biweekly for five months following the initial oil spill, then monthly for two more months. In order to assess concentrations, PAHs were extracted and analytically quantified. PAHs quantitated in this study are shown in Figure 1. Some of the water samples were used to measure developmental effects and EROD induction of *F. heteroclitus*.

Hypotheses to be tested included:

- PAH concentrations would increase temporally as oil invaded the sites, then decrease as the oil biodegraded or left the site.
- Some sites would be more contaminated based on their spatial location to the oil spill.
- tPAH concentrations in the sediment would correlate with tPAH in water.
- tPAH in sediment would be greater than tPAH in the water.
- tPAH in sediments would correlate with increased %C , % silt and % clay, and not correlate with % sand.
- PAH ratios would suggest concentration increases were from the oil spill.
- Gulf water samples would cause developmental effects to *F. heteroclitus* if oil was present.
- Bioavailable PAHs in water samples would induce CYP1A in *F. heteroclitus* causing EROD induction.

**Table 1.** Oil spills that occurred in the Gulf of Mexico and resulted in innovative science and technology. When the spill occurred, how much oil was spilt, and how the spill affected change are noted.

| Incident | Year | Gallons | Location | Other Comments | References |
|---|---|---|---|---|---|
| Ixtoc I | 1979 | $140 \times 10^6$ | Mexican and Texas Coasts | • Initiated new NRDA program assessing effects on offshore benthic environments<br>• Developed us of GC/MS and Stable isotope measurements to identify weathered oil | Boehm 1983 |
| *Burmah Agate* spill | 1979 | $6.3 \times 10^6$ | Texas Coast | | Boehm 1983 |
| *Mega Borg* | 1990 | $3.9 \times 10^6$ | Galveston, Texas | • First field application of dispersants | Payne 1993 |
| Deepwater Horizon | 2010 | $205.8 \times 10^5$ | Louisiana Coast | • Largest spill in U.S. History<br>• First deepwater dispersant application | Schmidt 2010 |

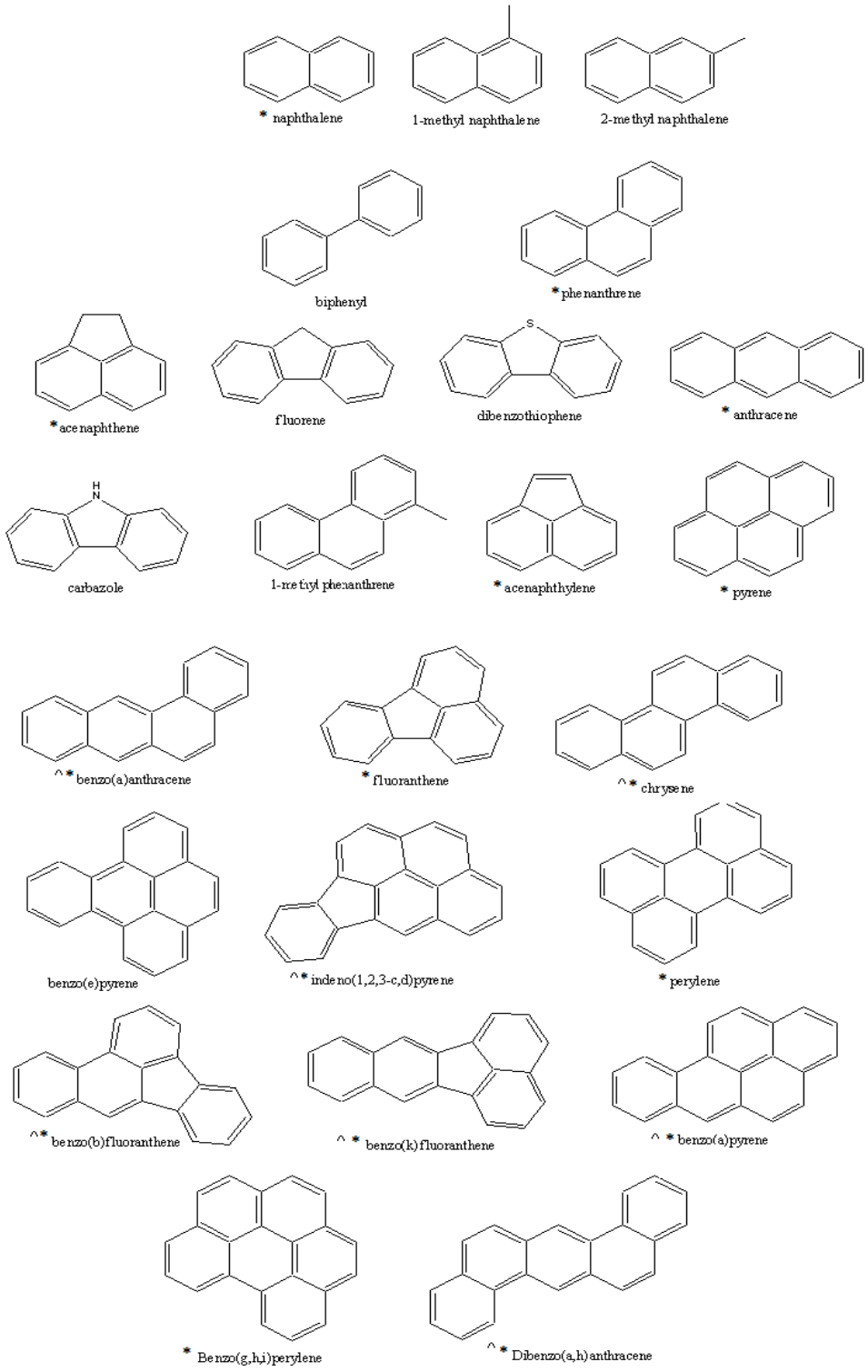


**Figure 1.** Structures of 24 polycyclic aromatic hydrocarbons that were examined during this study. Asterisks denote the 16 priority pollutants as classified by the EPA. Carrots (^) denote HPAHs.

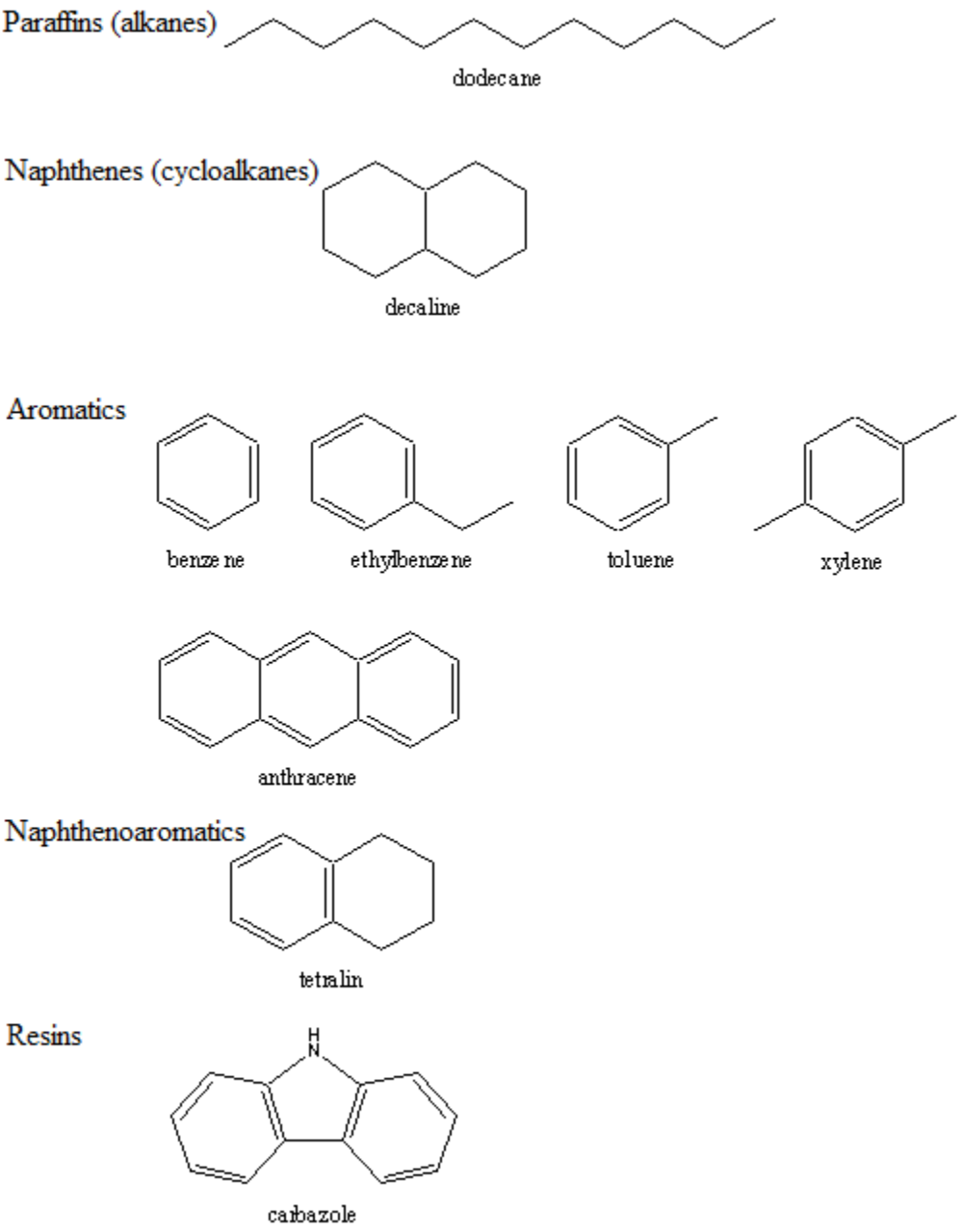


**Figure 2.** The structures of the five major compound classes commonly found in oil mixtures.

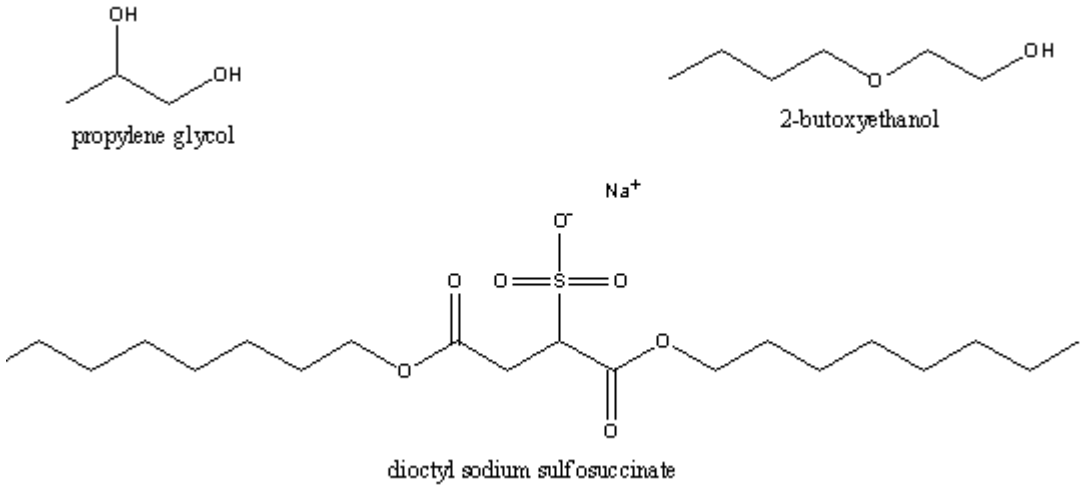


**Figure 3.** The structures of the three major compounds found in Corexit 9500A and 9527.

**Table 2:** Common diagnostic ratios used in oil sourcing. Hansen *et al.* 2007; Dahling and Faksness, 2002; Douglas *et al.* 1996; Kim *et al*. 2008

| **Diagnostic Ratios** | **Why Important** |
|---|---|
| 2-methylphenanthrene/1-methylphenanthrene | Most common: important to middle distillate fuels |
| 4-methyldibenzothiophenes/1-methyldibenzothiophene | Most common: indicates bacteria degradation |
| Methylphenanthrene/phenanthrenes | Ratio >2 indicates a petrogenic source, ratio<2 indicates a pyrogenic source |
| C2-dibenzothiophenes/C2-phenanthrenes | Source indicators: due to variance of dibenzothiophenes between different sources |
| C3-dibenzothiophenes/C3-phenanthrenes | |
| C3-dibenzothiophenes/C3-chrysenes | Weathering indicator: during late stages of weathering, due to low sensitivity |
| C3-naphthalenes/C2-phenanthrenes | Weathering indicator: during early stages of weathering, due to high sensitivity |
| Retene (7-isopropyl-1-methylphenanthrene)/ C4-Phenanthrene | Distinguishes between plant sources and other oils |
| Benzo(a)fluorene/4-methylpyrene | Source indicator between different crude oils |
| Benzo(b+c)fluorene/4-methylpyrene | |
| 2-methylpyrene/4-methylpyrene | |
| 1-methylpyrene/4-methylpyrene | |

# CHAPTER 2 MATERIALS AND METHODS

## 2.1 SAMPLE COLLECTION

### 2.1.1 SITES

The goal of this study was to assess temporal distribution of PAHs in a variety of matrices during and following the Deepwater Horizon oil spill. A total of thirty-four sites from Gulf Island National Seashore, FL, 87°24’25N 30°18’17W, west to Gulfport, MS, 89°4’47N 30°25’33W, and from Steele Creek, AL, 88°2’45N 30°51’39W, south to Fort Morgan, AL, 87°52’8N 30°14’20W, were sampled between May 2010 and February 2011 (Table 3; Figure 3). The sites represent collaboration with five different projects referred to as: Oyster, Manatee, Katrina, Seagrass, and Spatial. The oyster project, a collaboration with Dauphin Island Sea Lab (DISL), focused on the effects of hypoxia and potential oil impact on reef restoration. The manatee project, also with DISL, was focused on the effects of oil on manatees. The Katrina project was a follow up study at the same sites where PAH and trace metal concentrations were quantified after Hurricane Katrina in an east to west fashion to determine storm impacts. The seagrass project, a collaboration with UM Departments of Pharmacognosy and Medicinal Chemistry and the University of South Alabama, was concerned with analyzing protein signatures in *Ruppia maritima, Thalassia testudinum, Halodule wrightii* seagrass species in order to develop and validate biomarkers for monitoring conservation and anthropogenic impacts. The

spatial project focused on gathering PAH concentrations from a wider variety of sites and times after the spill.

Four sites, associated with the oyster project, in Mobile Bay, AL were sampled biweekly from May 26, 2010 through September 24, 2010 and then monthly through November (Figure 3); for seven months post Deepwater Horizon Oil Spill. The four sites were based at two oyster reefs; Denton and Sand Reefs. Two depths (0.1 and 1 meter) were sampled at these reefs to mimic reef restoration heights. The distance between Denton 0.1 m and Denton 1 m was approximately 560 m, while the distance between Sand 0.1 m and Sand 1 m was approximately 200 m. The two sites were approximately 15 kilometers apart. Mobile Bay was an ideal location to study the effects of hypoxia on oysters, because it was subject to periods of sustained low dissolved oxygen (DO) at defined locations in the bay (May 1973). Mobile Bay also included sites that were once commercially important oyster reefs for which data were in demand to support restoration activities (Johnson *et al.* 2009).

Two sites, Perdido, AL (near Orange Beach) and Pointe aux Pines, AL were associated with the manatee project. They were chosen because there were supposed oil sightings at these two sites, and they represented possible manatee habitats. The sites were sampled bimonthly from June 2010 through January 2011, nine months post Deepwater Horizon Oil Spill (Figure 3).

Nine sites, associated with the Katrina project, were located from Gulfport, MS east to Mobile Bay, AL. Weston and coworkers (2010) described these sites in detail. Briefly, the sites were originally chosen to represent a storm surge impact gradient due to Hurricane Katrina; four of the sites were located near boat ramps, four were on sandy beaches, and the last site was near

a seafood processing plant. The sites were only sampled in July 2010, three months post Deepwater Horizon Oil Spill, due to lack of oil sightings (Figure 3).

Six sites, associated with the seagrass project, were located from Gulf Island National Seashore, AL to Pointe aux Chines, MS. Other seagrass sites included were Middle Bay, MS; Grand Bay, AL; Grand Bay, MS; and Jose Bay, MS. The sites were originally chosen to represent a variety of anthropogenic and natural stressor exposures. Sediment samples were collected between July and October 2010, six months post Deepwater Horizon Oil Spill (Figure 3). The remaining thirteen sites were associated with the spatial project. The locations ranged from Palmetto Creek, AL west to the Grand Bay National Estuary Research Reserve, MS. The sites include Palmetto Creek, AL; Wolf Bay, AL; Little Lagoon, AL; Fort Morgan, AL; Fairhope, AL; Steele Creek, AL; Rabbit Creek, AL; Fowl River, AL; Alabama Port, AL; Pass Hotel, MS; and Cedar Point, AL. These sites were sampled in January and February 2011 by collaborators at DISL (Figure 3).

### 2.1.2 WATER COLLECTION

A solvent cleaned 1L amber glass bottle was opened underwater and filled with seawater (Tables 5-7 for specific sites and dates). The bottle was closed with a Teflon cap then brought above the water, dried, labeled, and the stored in a cooler with ice packs. The water samples were shipped overnight. Once water samples were received, they were stored at 4°C and processed within seven days.

### 2.1.3 SEDIMENT COLLECTION

Muffled glass jars (100 mL) were filled with sediment underwater using a metal shovel. The jars were covered with aluminum foil before being sealed with a plastic cap, placed in Ziploc bags and labeled. Sediment samples were stored in a -20°C freezer until shipped overnight in a cooler with icepacks. Once received, the sediments were stored in a -20°C freezer until PAH analysis.

### 2.1.4 OYSTERS COLLECTION

Hatchery-reared juvenile and adult oysters, *Crasseotrea virginica*, acquired from the Auburn Shellfish lab were deployed in aquaculture cages from May 26, 2010 to November, 2010. Oysters were collected roughly biweekly for analysis in a project coordinated by Dr. Ruth Carmicheal and her student Heather Patterson from DISL. The oysters at Sand and Denton Reefs were collected from two depths: 1m and 0.1m above the seafloor. The oysters at Sand and Denton Reefs and Pointe aux Pines were collected from aquaculture cages while SCUBA diving. Oysters collected from Perdido were wild oysters. Once above water, the oysters were flash frozen with liquid nitrogen, placed in Ziploc bags and labeled. The oysters were stored at -80°C and shipped overnight with dry ice. Oysters were then stored at -20°C until PAH analysis.

### 2.1.5 DEEPWATER HORIZON PETROLEUM

Samples containing British Petroleum (BP) oil from the Deepwater Horizon leak were provided by BP (see also section 2.2.4). Professor Joe Griffitt kindly provided a water accommodated fraction (WAF) sample containing both BP oil and COREXIT, as described below. BP provided a sample of oil, as described below. Analyzing these standards provided the

ability to fingerprint the oil and compare its fingerprint to the results from the other mediums and predict whether the contamination found could be from the Deepwater Horizon Oil Spill.

## 2.2 PAH ANALYSIS

### 2.2.1 WATER

The extraction procedure of PAHs from water was described in detail elsewhere (Weston *et al.* 2010). Briefly, PAH surrogate standard, (200 ng of naphthalene-$d_8$, acenapthene-$d_{10}$, phenanthrene-$d_{10}$, chrysene-$d_{12}$, and perylene-$d_{12}$) was added to the ~1000 mL water sample in a separatory funnel. The water sample underwent a liquid-liquid extraction with 50 mL methylene chloride three times. The extract was transferred through a drying column of anhydrous sodium sulfate into a 250 mL flask and then rotary evaporation was used to concentrate sample to 2 mL. The extract was transferred to a GC vial and spiked with the internal standard (200 ng of fluorene-$d_{10}$ and benzo(a)pyrene-$d_{12}$) for a total volume of 2 mL. PAH analysis was performed by gas chromatography (Agilent 6890) coupled with mass spectrometry (Agilent 5973N) using selective-ion (SIM) mode for 24 PAHs, as shown in Figures 3 and 4. The quantitative results were determined by comparing analyte peaks areas to those of the calibration standards (0.02, 0.08, 0.2, 0.8, 2 µg/mL). The $R^2$ of the calibration curve for deuterated compounds (0.2 µg/mL) and PAHs were less than 15% and more than 99%, respectively, with acceptable recoveries ranging from 50-150%. The deuterated compounds remained at the same concentration throughout the five calibration standards, so little to no change was expected which is why less than 15% was acceptable. The reporting limit for each PAH was 20 ng/L, and was calculated based on the lowest calibration standard (0.02 ng/µl), the fact that samples were diluted to 1 ml,

and that 1 L of water was extracted initially. All solvents used were of Optima or pesticide grade (Fisher Scientific; Fair Lawn, NJ) and the standards were purchased from AccuStandard, Inc (New Haven, CT).

### 2.2.2 SEDIMENT

Before performing PAH extractions from sediment, dry weight was calculated to determine the amount of wet weight needed to correspond to 6 g dry weight for the extraction and to convert results into dry weights. Approximately 1 g subsample of sediment was weighed on a pre-weighed aluminum weigh boat and then placed in an oven at 100°C overnight. The sediment was reweighed in the morning to determine dry weight. Dry weight percentage was calculated by dividing the wet weight into the dry weight then multiplying by 100.

The extraction protocol for PAHs from sediment samples was described elsewhere (Weston *et al.* 2010). Wet sediment weight corresponding to 6 g of dry weight, was subsampled and chemically dried using diatomaceous earth (Sigma Aldrich). PAH surrogate standard (200 ng of naphthalene-$d_8$, acenapthene-$d_{10}$, phenanthrene-$d_{10}$, chrysene-$d_{12}$, and perylene-$d_{12}$) and alkane surrogate standard (200 ng of eicosane-$d_{42}$, dodecane-$d_{26}$, triacontane-$d_{62}$) were added to the mixture and extracted using an Accelerated Solvent Extractor (ASE) (Dionex). The ASE was programmed to preheat 0 min, heat 5 min, remain static 5 min, flush 60 s, and purge for 60 s, for two cycles at 1.500 psi and 100°C. Extracts were transferred through an anhydrous sodium sulfate drying column into a 250 mL flat bottom flask. They were then concentrated using a Kaderna-Danish water bath (Fisher Scientific; Fair Lawn, NJ) to near dryness, then solvent exchanged to hexane and concentrated via nitrogen purge to 1 mL. Next, the concentrated extracts were purified with a silica-alumina column (20 g, 10% deactivation and 10 g, 2%

deactivation, respectively). This separated the extract into two fractions: F1, aliphatic fraction (eluted with 45 mL pentane); F2, aromatic fraction (eluted with 250 mL 1:1 mixture pentane:methylene chloride). Both fractions were concentrated to near dryness using a Kaderna-Danish water bath (Fisher Scientific; Fair Lawn, NJ), then solvent exchanged to hexane, and concentrated to 1 mL via nitrogen purge and spiked with the PAH internal standard (200 ng of fluorene-$d_{10}$ and benzo(a)pyrene-$d_{12}$) or the aliphatic internal standard (2 ng of 5α-androstane and hexadecane-$d_{34}$). Analysis was done using a gas chromatograph (Agilent 6890) coupled with mass spectrometry (Agilent 5973N) using SIM mode, as with the water extracts.

Data from the mass spectrometer was analyzed using MSD ChemStation version D.01.02.16. Calibration standards were used to confirm peak retention times and confirmation ion ratios for representative times and ratios (Table 4). Once the peaks were integrated, concentration was calculated by plotting the following formula:

$$\frac{\text{Area}_{\text{Target}}}{\text{Area}_{\text{Internal Std}}} = \text{slope}\left(\frac{\text{Concentration}_{\text{Target}}}{\text{Concentration}_{\text{Internal Std}}}\right)$$

The program broke the above formula into two steps: slope was multiplied by the ratio of the area of the target compound:area of the deuterated compound to give a random variable “y”, because the slope is forced through 0, there was no y-intercept. As is shown below:

$$y = (\text{area ratio}) * \text{slope}$$

Y was then set equal to

$$y = \frac{\text{Concentration}_{\text{Target}}}{0.2\ \text{ng/uL}}$$

in order to determine the concentration of the target compound. The concentration of the deuterated compound was 0.2 ng/µL.

Percent recovery of the deuterated compounds was calculated by taking the concentration of the deuterated compound and multiplying it by the volume within the GC vial, in mL in Excel. This number was divided by 200 ng, the amount of deuterated standard injected into the sample at the beginning of the procedure, and then multiplied by 100%. Acceptable percent recoveries ranged from 50-150%. Non-deuterated samples were calculated by taking the concentration and multiplying by the volume in the GC vial, in mL, then dividing that number by the dry weight. This gave the final concentration in ng/g dry weight. The reporting limit for each PAH in sediment was 3.33 ng/g dry weight (assuming approximately 6 g dry weight for each sample).

### 2.2.3 OYSTERS

Oyster PAH extractions were similar to Willett et al (1997), with modifications. PAH surrogate standard (100-200 ng of naphthalene-$d_8$, acenapthene-$d_{10}$, phenanthrene-$d_{10}$, chrysene-$d_{12}$, and perylene-$d_{12}$) and alkane surrogate standard (1-2 ng of eicosane-$d_{42}$, dodecane-$d_{26}$, triacontane-$d_{62}$) were added to 1.3 to 16.9 g of wet tissue (representing 1 – 3 oysters per sample) prior to the addition of 50 g of anhydrous sodium sulfate followed by homogenization with a mortar and pestle. After adding 100 mL methylene chloride to the homogenate, the sample was vigorously shaken for 30 sec and centrifuged to separate the methylene chloride fraction at 668 × g for 5 min. Another 100 mL methylene chloride was added to the tissue layer and after shaking,

it was centrifuged; this was repeated once more. All three methylene chloride fractions were combined into a 500 mL flat bottom flask via a sodium sulfate drying column. Percent lipid determination was performed by taking a 20 mL subsample from the 300 mL oyster extract before the extract was concentrated. This extract was concentrated to 2 mL in hexane and was purified and separated into two fractions using alumina/silica gel chromatography. The first fraction (F1) containing aliphatic hydrocarbons was eluted and collected using 45 mL of pentane, concentrated to 1 mL hexane and stored for future use. The second fraction (F2) containing PAHs was eluted and collected using 250 mL of a 1:1 mixture of pentane:methylene chloride. The PAH fraction was concentrated to 0.5-1 mL hexane and spiked with the internal standard (100-200 ng fluorene-$d_{10}$ and benzo(a)pyrene-$d_{12}$) or the aliphatic internal standard (1-2 ng 5α-androstane and hexadecane-$d_{34}$), then analyzed by gas chromatography (Agilent 6890) coupled with mass spectrometry (Agilent 5973N) using SIM mode, as with the water extracts. There were 32 total oyster samples analyzed. Nine of the samples had acceptable recoveries after the extraction described above. The remaining samples underwent an additional clean up step (clean-up column consisting of 0.5 g of sodium sulfate and 2.5 g of 5% deactivated alumina where the PAHs were eluted with 15 mL hexane). For ten samples the internal standard was added following the secondary clean up and in 13 samples the standard was added prior to this clean up. The analytical differences are noted in oyster results (Table 15) in the Appendix. Of the 32 samples, 11 were eliminated because of poor recoveries (e.g. not in the range of 40- 150%). The reporting limit for oysters ranged between 6 and 40 ng/g depending on the oyster dry weight.

The percent lipid content in the 15 g of tissue was calculated because PAHs are lipophilic so the amount of lipid in the tissue could affect the concentration of PAHs. The 20 mL extract

was nitrogen evaporated down to dryness in a conical tube. The tube was rinsed with 1 mL of methylene chloride and the 1 mL was transferred to a GC vial. A piece of filter paper was preweighed on a microbalance and 100 µL of lipid extract was transferred onto the filter paper and allowed to dry for 30 minutes. Filter paper was reweighed to determine the lipid weight. The residual weight was multiplied by the final volume (1 mL), this number was divided by 0.0667, accounting for the 20 mL taken from the original 300 mL, the sample weight (tissue weight), and the volume added to the filter paper (0.1 mL). Finally, this number was multiplied by 0.001 to convert from mg to g and then multiplied by 100 to get the % lipid content.

### 2.2.4 PETROLEUM

#### 2.2.4.1 WAF

Oil PAH extractions were performed similar to Wang and coworkers (Wang *et al.* 1994). PAH surrogate standard (200 ng of naphthalene-$d_8$, acenapthene-$d_{10}$, phenanthrene-$d_{10}$, chrysene-$d_{12}$, and perylene-$d_{12}$) was added to glass test tube, which contained an oil dilution or 1 mL WAF (water accommodated fraction) kindly provided by Professor Joe Griffitt at the Gulf Coast Research Labs. The WAF sample was described as being prepared the week of August 23$^{rd}$, 2010 by using 10:1 oil in water and 20:1 COREXIT to oil in 15 ppt ASW (artificial seawater). Professor Griffitt's group made 8 batches and stirred it for 96 hours, let it settle for 6 hours and then drained the water from the bottom. The WAF was transported on ice and stored at 4°C.

Briefly, methylene chloride, 5 mL, was added to a 10 mL test tube, containing 1 mL of WAF, which was vortexed for 30 s. The test tube was then placed in the centrifuge for 7 min at 268 × g. The top organic layer of methylene chloride was transferred into a new test tube. This

was repeated three times, always transferring the top aqueous layer into the new test tube. The methylene chloride extract was concentrated to 2 mL and a scoop of anhydrous sodium sulfate was added. The extract was vortexed for 30 s to pellet the sodium sulfate and then transferred to a conical tube and concentrated to 1 mL. The extract was then transferred to a GC vial and spiked with the internal standard (fluorene-$d_{10}$ and benzo(a)pyrene-$d_{12}$). Analysis occurred using a gas chromatograph (Agilent 6890) coupled with mass spectrometry (Agilent 5973N) using SIM mode, as with the water extracts.

#### 2.2.4.2 DEEPWATER HORIZON OIL

BP oil (item ID A0030W) from the Deepwater Horizon oil spill, provided by BP was diluted as described below and analyzed. This oil was described as source oil, under the reference code, "MASS," and consisted of 100 mL stored in a 125 mL amber glass container. The oil originated from the Massachusetts barge, which was sampled twice for MC252. The barge received oil from the Enterprise Producer which was collecting oil directly from the subsea containment system positioned directly over the leaking well. On July 26, 2010, crude oil was collected from the Massachusetts and then transferred from the tanks of the Massachusetts into a certified clean vac truck. Between the time the oil was offloaded from the Massachusetts and when the drums were ready to be filled, the oil and water had separated into two layers. The water layer at the bottom of the vac truck was gravity fed into drums prior to transferring the oil. Two drums of the oil were collected and shipped to the BP Houma Incident Command Post, Schriever, LA, where they were placed in and maintained in a 7°C refrigerator. The drums were later transported under refrigeration to the long term storage facility. On August 15, crude oil was collected from the Massachusetts docked in Theodore, AL and transferred from the tanks of

the Massachusetts into a certified clean vac truck. Collected samples were transferred to a flatbed truck and shipped at ambient temperature to the long term storage facility, where they were stored in a 7°C refrigerator. It was not specified whether the oil sample received was from the July 26 or August 15 sampling date.

The BP oil was allowed to warm to room temperature for approximately 20 minutes and then sonicated for 5 minutes. Approximately 80 mL hexanes were added to a 100 mL volumetric flask. Then 10 µL BP oil was added to the volumetric flask, which was then brought up to volume and sonicated for 5 minutes. Using a glass pipette, 1 mL was removed from the center of the volumetric flask and transferred to a GC vial with the PAH surrogate standard (200 ng of naphthalene-$d_8$, acenapthene-$d_{10}$, phenanthrene-$d_{10}$, chrysene-$d_{12}$, and perylene-$d_{12}$) and PAH internal standard (fluorene-$d_{10}$ and benzo(a)pyrene-$d_{12}$). Analysis occurred using a gas chromatograph (Agilent 6890) coupled with mass spectrometry (Agilent 5973N) using SIM mode, as with the water extracts.

**2.3 SEDIMENT CHARACTERIZATION**

Sediment samples were characterized similar to Weston and coworkers (2010). Briefly, samples were run on a Horiba model LA-910 laser scattering particle size analyzer to achieve particle size distribution data. Before analysis, 5 g of sediments were freeze-dried. Small portions of sediment were randomly added to the analyzer until laser transmittance was reduced to ~85% within the recommended operating range. Sample material was dispersed in approximately 200 ml water with 5 mL of sodium hexametaphosphate (calgon) surfactant (10% weight:volume) to aid dispersion, and then subjected to thirty seconds of ultrasonic treatment (40W, 39 kHz) by the Horiba LA-910 prior to measurement. Light scattering was measured using both a He-Ne laser

(632.8 mm, 1 mW) and tungsten halogen lamp (50W) with a set of six 18-division, ring shaped silicone photo-diode detectors. Particle sizes were calculated based on Mie scattering theory, using a relative refractive index of 1.32-000. The particle sizes were then classified as sand (>63 μm), silt (63 μm – 2 μm), or clay (< 2 μm) based on size only.

## 2.4 PERCENT TOTAL CARBON (% CARBON)

To determine C:N percentages, 1 g of < 2 mm of sediment sample was subsampled from the 5 g freeze-dried sediment, then analyzed using a Vario Max CNS instrument (Elementar Analysensysteme) in CN mode. A tungsten catalytic tube was used to combust samples and separate gases, and then a thermal conductivity detector was used for measurements. Both %C and size determinations were performed in collaboration with Mr. Sam Testa from the United States Department of Agriculture (USDA) Sediment Lab, Oxford, MS.

## 2.5 BIOASSAYS FOR ORGANISMAL EFFECTS

### 2.5.1 *FUNDULUS* EMBRYO EXPOSURE

A mummichog, *Fundulus heteroclitus*, embryo exposure was performed to determine bioavailability of PAHs in water samples. Water, field sample or autoclaved saltwater, (10 mL) was pipetted into autoclaved scintillation vials. Lab raised *F. heteroclitus* were spawned and eggs were placed in a beaker filled with 100 mL of salt water. After four hours, ten developing fish eggs were placed in each of three vials per treatment. The embryos were transferred to a new vial every other day. On days 5 and 10 the embryos were observed for developmental effects including: cardiac arrhythmia, edema, color, eye development, and deaths. On day 10, the

embryos were either hatched or placed in RNALater. The experimental design of the exposure was similar to Wassenberg and Di Giulio (2004).

Heart elongation was scored as a 0 for no deformity, 1 for slight deformity, and 2 for severe deformity. Effects were normalized into a mean deformity index. Deformities were scored as 0, 1, or 2, during the exposures. To calculate the mean deformity index, the number of embryos scored at a particular level was multiplied by that number. The resulting number for all categories were added together, and then divided by the total number of embryos. For example, out of 10 embryos, if five were scored as 0, three as 1, and two as 2, the category numbers would be 0, 3, and 4, respectively. The numbers would add to seven and after dividing by the total number of embryos, 10, the resulting mean deformity index would be 0.7.

### 2.5.2 EROD ASSAY

The ethoxyresorufin-*O*-deethylase (EROD) *in ovo* assay was performed as described in (Wassenberg and Di Giulio 2004). Briefly, ethoxyresorufin (3.6 µL) was added to embryo exposure vials on day 2 to bring the final concentration of ethoxyresorufin to 21 µg/L. Exposure continued for 48 hrs. Fluorescent intensity was observed through epiflourescent microscope (Axiovert 200) with the computer program Metamorph.

Statistical analysis was described per experiment in the Results and Discussion section. All statistical analyses were done using Graphpad Prism version 5.00 for Windows.

**Table 3.** Example of quantitation and confirmation ions, retention times of PAHs analyzed in this study and SIM detection windows. These ions and retention times were how the compound corresponding with each peak was identified. Actual retention times for each run were based on calibration standards.

| GC/MS Parameters for Target Analytes | | | | | | |
|---|---|---|---|---|---|---|
| **Window** | **Start Time (min)** | **End Time (min)** | **Compound** | **Quantitation Ion** | **Confirmation Ion** | **Retention Time (min)** |
| **1** | **7.00** | **11.1** | **Naphthalene-d8** | **136** | **134** | **9.236** |
| | | | **Naphthalene** | **128** | **127** | **9.299** |
| | | | **2-Methylnaphthalene** | **142** | **141** | **10.51** |
| | | | **1-Methylnaphthalene** | **142** | **141** | **10.685** |
| **2** | **11.1** | **13.00** | **Biphenyl** | **154** | **152** | **11.352** |
| | | | **Acenaphthylene** | **152** | **153** | **12.179** |
| | | | **Acenaphthene-d10** | **164** | **162** | **12.40** |
| | | | **Acenaphthene** | **153** | **154** | **12.494** |
| **3** | **13.00** | **14.50** | **Fluorene-d10** | **176** | **174** | **13.404** |
| | | | **Fluorene** | **166** | **165** | **13.462** |
| **4** | **14.50** | **16.30** | **Dibenzothiopene** | **184** | **152, 139** | **15.038** |
| | | | **Phenanthrene-d10** | **185** | **184** | **15.231** |
| | | | **Phenanthrene** | **178** | **176** | **15.274** |
| | | | **Anthracene** | **178** | **176** | **15.374** |
| | | | **Carbazole** | **167** | **139** | **15.705** |
| **5** | **16.30** | **21.00** | **1-Methylphenanthrene** | **192** | **191** | **16.446** |
| | | | **Fluoranthene** | **202** | **101** | **17.531** |
| | | | **Pyrene** | **202** | **101** | **17.961** |
| | | | **Chrysene-d12** | **240** | **236** | **20.271** |
| | | | **Chrysene** | **228** | **226** | **20.232** |
| | | | **Benz(a)anthracene** | **228** | **226** | **20.304** |
| **6** | **21.00** | **27.25** | **Benzo(b)fluoranthene** | **252** | **253, 125** | **22.650** |
| | | | **Benzo(k)fluoranthene** | **252** | **125** | **22.718** |
| | | | **Benzo(e)pyrene** | **252** | **253** | **23.40** |
| | | | **Benzo(a)pyrene-d12** | **264** | **260** | **23.551** |
| | | | **Benzo(a)pyrene** | **252** | **253** | **23.613** |
| | | | **Perylene-d12** | **264** | **260** | **23.796** |
| | | | **Perylene** | **252** | **253** | **23.756** |
| **7** | **27.25** | **→** | **Indeno(1,2,3-cd)pyrene** | **276** | **277, 138** | **27.629** |
| | | | **Dibenzo(a,h)anthracene** | **278** | **279, 139** | **27.758** |
| | | | **Benzo(g,h,i)perylene** | **276** | **277, 138** | **28.827** |

**Table 4.** GPS coordinates and project associations for the sampling sites used in this study.

| GPS Coordinates of Sites | | | | |
|---|---|---|---|---|
| **Sites** | **Longitude** | **Latitude** | **Institute** | **Project** |
| Denton Top | -88.066 | 30.408 | DISL | Oyster |
| Denton Bottom | -88.071 | 30.412 | DISL | Oyster |
| Sand Top | -88.096 | 30.272 | DISL | Oyster |
| Sand Bottom | -88.096 | 30.274 | DISL | Oyster |
| Perdido (Orange Beach) | -87.514 | 30.308 | DISL | Manatee |
| Pointe aux Pines | -88.315 | 30.377 | DISL | Manatee |
| Mobile Bay | -88.086 | 30.576 | ETRP | Katrina |
| Grand Bay Bayou Heron | -88.402 | 30.413 | ETRP | Katrina |
| Grand Bay Bayou Combust | -88.448 | 30.388 | ETRP | Katrina |
| Pascagoula River | -88.565 | 30.376 | ETRP | Katrina |
| USM Boat Ramp | -88.796 | 30.392 | ETRP | Katrina |
| Gulfport Courthouse | -89.080 | 30.426 | ETRP | Katrina |
| Gulfport Gulf | -89.043 | 30.368 | ETRP | Katrina |
| Biloxi Gulf | -88.868 | 30.391 | ETRP | Katrina |
| Biloxi Back Bay | -88.891 | 30.377 | ETRP | Katrina |
| Middle Bay | -88.405 | 30.386 | ETRP | Seagrass |
| Gulf Island National Seashore | -87.407 | 30.305 | ETRP | Seagrass |
| Grand Bay, MS | -88.397 | 30.362 | ETRP | Seagrass |
| Jose Bay | -88.414 | 30.355 | ETRP | Seagrass |
| Pointe aux Chines | -88.439 | 30.321 | ETRP | Seagrass |
| Grand Bay, AL | -88.366 | 30.369 | ETRP | Seagrass |
| Grand Bay NERR | -88.463 | 30.327 | DISL | Spatial |
| Sandy Bay | -88.312 | 30.383 | DISL | Spatial |
| Alabama Port | -88.110 | 30.367 | DISL | Spatial |
| Cedar Point | -88.138 | 30.310 | DISL | Spatial |
| Palmetto Creek | -87.510 | 30.355 | DISL | Spatial |
| Wolf Bay | -87.603 | 30.350 | DISL | Spatial |
| Little Lagoon | -87.758 | 30.249 | DISL | Spatial |
| Fort Morgan | -87.869 | 30.239 | DISL | Spatial |
| Rabbit Creek | -88.127 | 30.582 | DISL | Spatial |
| Steele Creek | -88.046 | 30.861 | DISL | Spatial |
| Fairhope | -87.918 | 30.515 | DISL | Spatial |
| Fowl River | -88.143 | 30.433 | DISL | Spatial |

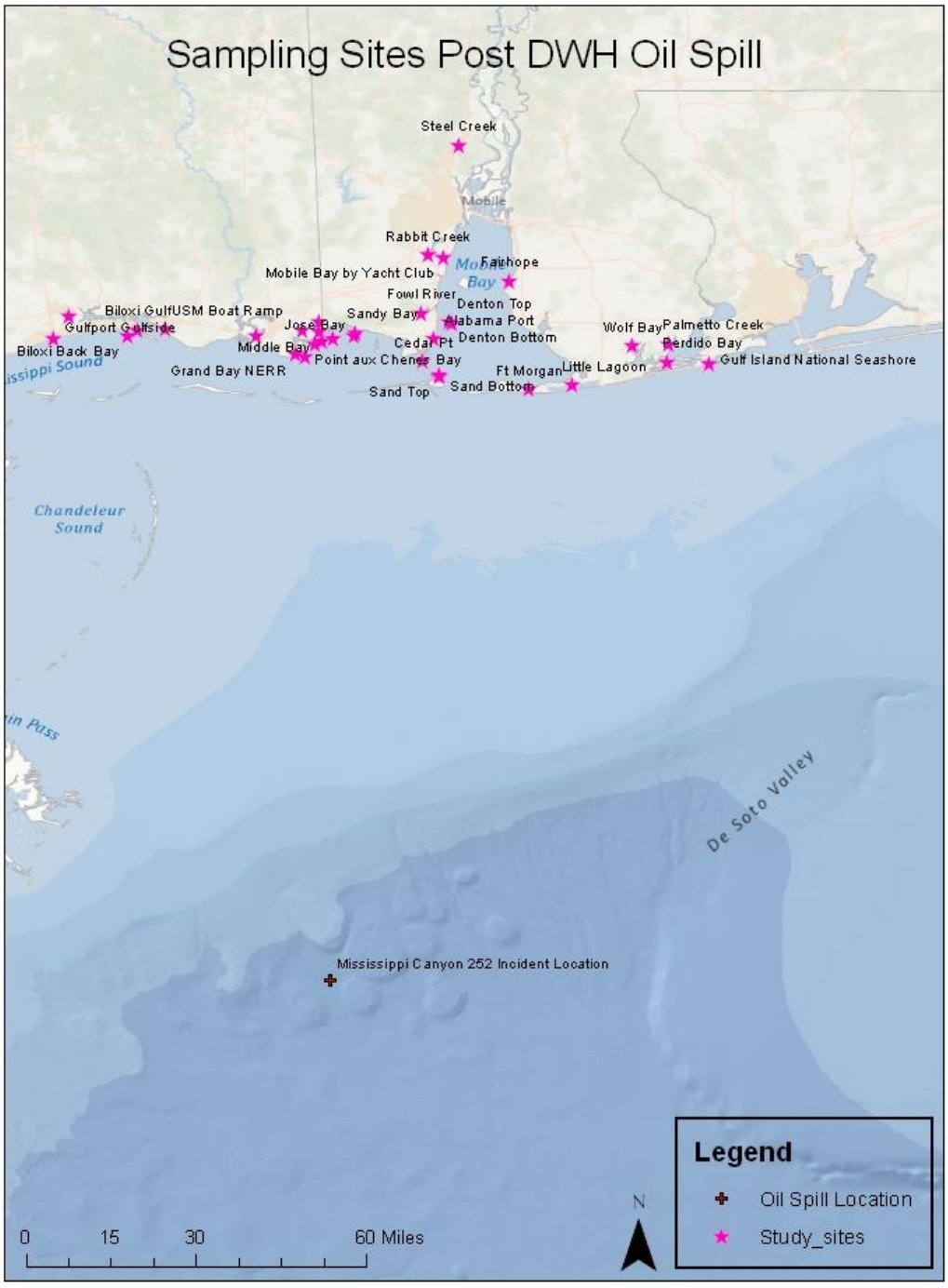


**Figure 4.** Map of sampling site locations compared to the location of the DWH incident.

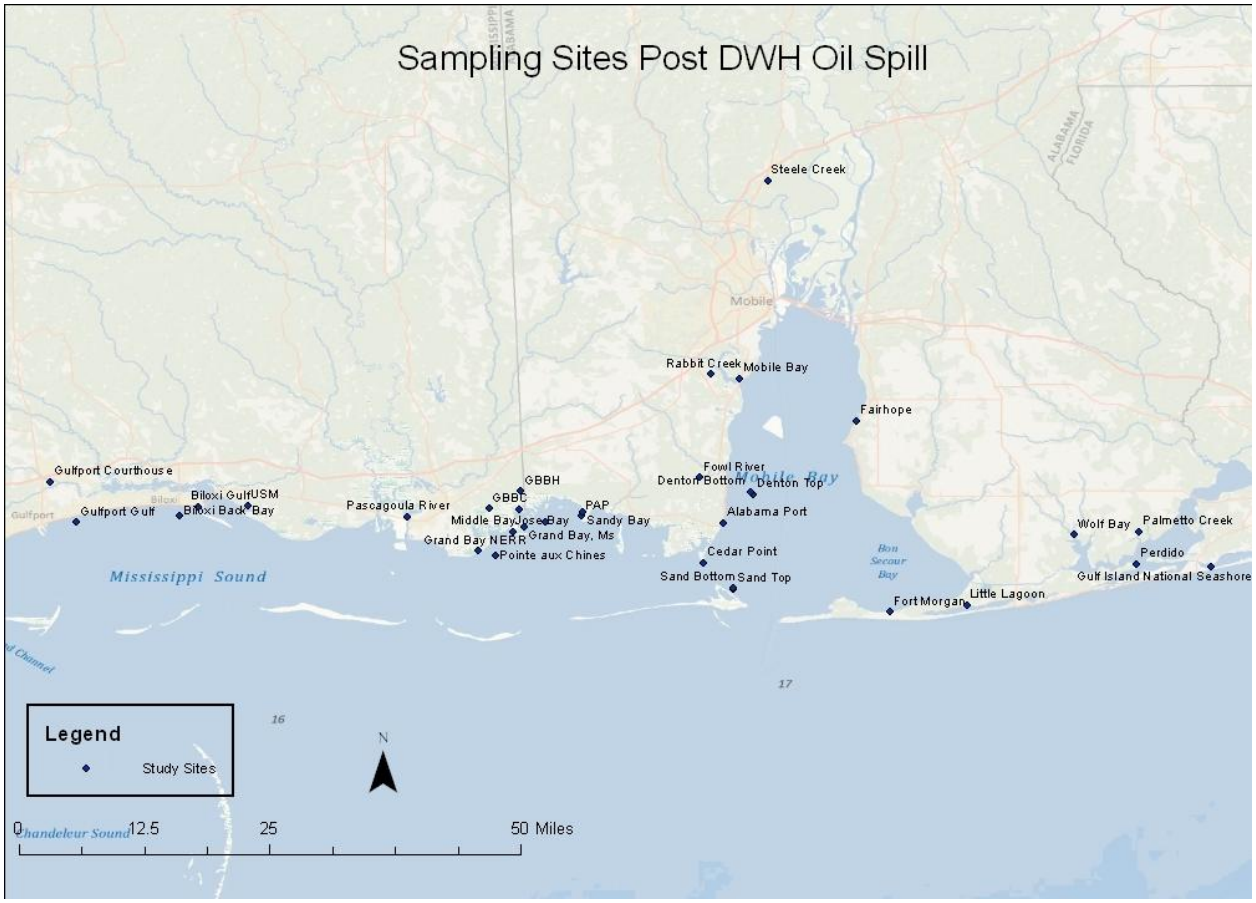


**Figure 5.** Enlarged image map of sampling sites along the Mississippi and Alabama coasts.

**Table 5.** Percent lipid in oysters as dry weight and (wet weight) by collection dates and collection site. A hyphen denotes a lack of sample on that date. An asterisk denotes a % lipid calculated greater than 100%.

| Percent Lipid in Oysters | | | | | |
|---|---|---|---|---|---|
| **Date** | **Sand Top** | **Sand Bottom** | **Denton Top** | **Denton Bottom** | **Pointe aux Pines** |
| **6/9/10** | 54.7 (4.97) | 55.5 (4.76) | 60.7 (5.21) | - | - |
| **6/28/10** | 42.3 (3.61) | 63.7 (7.86) | - | * (10.4) | - |
| **7/1/10** | - | - | - | - | 54.6 (6.87) |
| **7/7/10** | 74.6 (10.9) | * (17.7) | 31.2 (2.78) | * (14.6) | - |
| **7/20/10** | * (9.80) | * (12.6) | 42.9 (4.03) | 17.7 (1.55) | - |
| **8/3/10** | 17.9 (1.63) | 27.1 (2.15) | - | 35.4 (2.90) | - |
| **8/9/10** | - | - | - | - | 38.3 (2.41) |
| **8/19/10** | 14.9 (1.42) | 84.0 (6.40) | 31.8 (10.4) | - | - |
| **9/8-10/10** | 24.1 (2.17) | 68.1 (4.72) | - | - | - |
| **9/20/10** | 18.4 (1.68) | 65.2 (4.16) | - | - | - |
| **10/11/10** | 14.6 (1.40) | 17.9 (1.64) | - | - | - |
| **11/10/10** | 22.0 (2.14) | 15.6 (1.48) | - | - | - |
| **1/18/11** | - | - | - | - | 31.8 (2.96) |

# CHAPTER 3 RESULTS AND DISCUSSION

## 3.1 PAH ANALYSIS

### 3.1.1 PAH ANALYSIS SAND AND DENTON REEFS

#### 3.1.1.1 WATER

As indicated in the Materials and Methods section 2.2.1, Chemstation was used to quantify the concentrations of 24 PAHs in the water samples. PAH concentrations are reported in Table 6 from samples collected at the Denton and Sand sites, and represent two different reporting rationales. Values in 6A are based on the calibration curve, whereas the values in 6B are more conservative and reflect the sum of only PAH concentrations that were above the reporting limit (20 ng/L). Denton Reef, AL was sampled in order to determine if the oil spill affected an ongoing oyster restoration project. Denton 0.1 m, "Bottom" was sampled eleven times for water, while Denton 1 m, "Top" was sampled nine times. During the first sampling event on May 28, 2010 at Denton Reef taken at a depth of 0.1 meters from the bottom, a total PAH (tPAH) water concentration of 15.3 ng/L was measured (Figure 6). The tPAH concentration increased to 299 ng/L on July 21, 2010 then decreased to 9.33 ng/L by August 3, 2010 and did not increase above 50 ng/L through November 2010. The first sampling event at Denton Reef, taken at a depth of 1 meter from the bottom, tPAHs were at a concentration of 14.9 ng/L on May 28, 2010. The tPAH concentration stayed below 65 ng/L until November 11, 2010

when concentrations reached 130 ng/L. There were only five Denton samples with PAH concentrations above the reporting limit (Denton Bottom 6/28, 7/21, and 8/19; Denton Top 9/20 and 11/10).

Based on the oil plume projection maps (Figures 7-13), it could be hypothesized that oil did not impact the Denton Reef site. The oil plume was not shown overlapping the location of Denton Reef (Conservation Biology Institute 2010) so it would not be expected to see a significant spike in PAHs due to the oil spill. The NOAA oil impact map http://docs.lib.noaa.gov/ noaa_documents/DWH _IR/maps/SCAT_SCOS/ for July 22, 2010 shows light to very light oiling observed along the Mississippi and Alabama coasts but no observed oil in Mobile Bay, AL, the location of Denton Reef.

If oil from the Deepwater Horizon oil spill were to have impacted Denton Reef, it could be hypothesized that the source of the PAHs would be petrogenic. However, the sources of Denton water PAHs were estimated to be of pyrogenic origin for every sampling date using the following ratios: Benzo(a)anthracene/(Benzo(a)anthracene+Chrysene) $\geq$ 0.35, Fluoranthene/(Fluoranthene +Pyrene) $\geq$ 0.5, and Anthracene/(Anthracene+Phenanthrene) > 0.1. This ratio is not as robust of a predictor of petrogenic sources for light crudes such as the LA sweet crude from the Deepwater Horizon spill. Better source prediction would have been possible if the more of the alkylated PAHs could have been quantified. Petrogenic PAH origins commonly have a higher percent of HPAHs than pyrogenic PAH origins. HPAHs were only found at Denton Bottom on July 21, 2010 at 42.4% and at Denton Top on July 21 and November 10, 2010 at 46.7 and 28.2%, respectively. The other dates contained no HPAHs (Table 6).

Sand Reef, AL was also sampled for water in order to determine if the Deepwater Horizon oil spill affected oyster restoration. Sand (0.1 m) was sampled ten times for water while Sand (1 m) was sampled nine times. On May 28, 2010, at the first sampling event at Sand Reef Bottom, the tPAH concentration was 12.8 ng/L (Figure 6). The tPAH concentration increased to 79.5 ng/L on August 3, 2010 then decreased to 14.1 ng/L by August 18, 2010. The first sampling event at Sand Reef Top also on May 28th, detected 16.0 ng/L tPAHs. The concentration rose to 268 ng/L on June 28, 2010, and then decreased to 10.4 ng/L on August 18, 2010, and remained below 80 ng/L throughout the remaining sampling dates. None of the Sand Bottom and only two of the Sand Top (6/28 and 8/4) water samples had PAH concentrations above the reporting limit.

To test whether water tPAHs were significantly different at Sand Top and Bottom sites, a 2-way ANOVA test was used. PAH concentrations from the calibration curve (e.g. Table 6A) were used for statistical analysis. At a $p \leq 0.05$ there was no significant difference. Therefore, Sand Top and Bottom waters could be used as duplicates in order to increase the sample size n number. To test whether water tPAHs were significantly different at Denton Top and Bottom sites, a 2-way ANOVA test was again used and similarly no significant differences were detected.

Based on the oil plume projection map (Figure 10), it could be hypothesized that oil impacted Sand Reef on July 2, 2010 due to the loop current patterns in the Gulf, causing an increase in PAHs near this date (Conservation Biology Institute 2010). Unfortunately, Sand Reef was not sampled between June 29 and July 20, 2010, so any spike expected in water PAH concentrations would have been missed. An increase was seen on June 28, 2010, but was insignificant when compared to other dates of PAH concentrations at this site by way of one-way

ANOVA with a $p \leq 0.05$. Using unsubstituted PAH ratios (Section 1.4.2; page 18), Sand Reef PAHs predicted a pyrogenic origin for all sampling dates. This was to be expected because no HPAH were found in any of the Sand Reef samples. Also, there were no samples taken during the peak time frame when oil was expected to impact the site.

Whitehead et al (2011) sampled Mobile Bay, AL May 5, and June 30, 2010 using passive sampling devices and did not find any detectable PAHs in the water column. Some reasons for the differences in tPAH concentration levels between this study and Whitehead et al (2011) could be the use of a passive sampling device versus a grab sample, the sampling depths likely varied, and their collection location within Mobile Bay, AL was approximately 30 km north of Denton Reef, and the dates were not exactly the same (Figure 14). EMAP sampled a site approximately 2 km from Sand Reef, D001-sw-20100101 on May 3, July 1, September 9, 2010 (Environmental Protection Agency 2011). No PAHs were detected on May 3 or September 9, 2010, but on July 1, 2010, 270 ng/L tPAH were detected. This is comparable to the concentration found at Sand Top on June 28, 2010 (234 ng/L) but not Sand Bottom (nd).

To determine if changes in tPAH concentrations across time (temporal differences) were significantly different in the water column between the Denton and Sand Reef sites, a two-way ANOVA across time was performed. There was no significant differences found between replicates (same dates, different depths) but there was a significant difference found across time between Denton and Sand Reef sites. If the PAHs represented a background concentration, no significant difference over time would be expected. This difference indicated the possibility of oil influence at the sites at different times. Looking at the means it was expected that June 28 and July 21, 2010 would be significant spikes in total water PAH concentrations from Sand and

Denton Reefs, respectively. When the sites were analyzed by a one-way ANOVA, separately, across time, no significant differences between dates were found. This was due to an n of 2 per date. With a low n number, the data were not as robust as with a higher n number, making it more difficult to find statistical significant differences. It was possible that the spike in total water PAH concentrations at these dates were influenced by oil, but due to an inadequate alkylated-PAH fingerprint, it was not possible to confirm a petrogenic influence. See also section 3.1.4.

The location of Denton Top and Denton Bottom are approximately 560 m apart and the total depths of the two sites were 4.0-4.4 and 4.0-4.2 m, respectively. The bottom samples being within 0.1 m to the sediment were anticipated to have higher PAH concentrations when oil intrusion was absent. PAHs are lipophilic and are more likely to bind to carbon than to stay in the water column. Additionally, the settling of PAHs throughout the water column and agitation of the sediment can cause local reintroduction of PAHs to the water column. Sand Top and Sand Bottom were located approximately 200 m apart with the overall depths of 3.4-4.2 and 2.2-2.8 m for Sand Bottom and Sand Top, respectively. Therefore the 1 m waters from Sand Top were closer to the surface and potential oil slick then the 1 m Denton Top water samples. To determine if tPAH concentrations in the water were different at Denton and Sand Reefs, a one way t-test with Mann-Whitney post hoc test was performed. At a $p \leq 0.05$, the two average tPAH concentrations at the sites were not significantly different from each other.

### 3.1.1.2 SEDIMENT

Denton Reef was sampled twice per sampling date for a total of 20 sediment samples. During the first sampling on May 26, 2010 the average tPAH sediment concentration was 62.3 ng/g dry weight sediment (Figure 15). This concentration increased to 282 ng/g on June 9, 2010, then decreased and remained below 85.0 ng/g until September 20, 2010 when the average tPAH concentration spiked at 476 ng/g. The average tPAH concentration decreased to 20.1 ng/g on October 11, 2010 and increased again to 347 ng/g on November 10, 2010. These changes in PAH concentration over time were found statistically insignificant due to the high variability by using a one-way ANOVA with a $p \leq 0.05$. Because the sites Denton Top and Denton Bottom were located approximately 560 m apart, high variability was to be expected. When reporting limits were considered (3.33 ng/g), Denton Reef concentrations ranged from 16.9 to 466 ng/g on October 11 and September 20, 2010, respectively. The difference in tPAH concentrations between the two different reporting rationales (calibration curve vs. reporting limits) are shown in Tables 7A and Table 7B. Because of the generally higher PAH concentrations in sediment compared to water, there was a much smaller difference between tPAH concentrations for the two reporting rationales compared to the water results.

Based on the projection maps it could be hypothesized that oil did not impact Denton Reef, and PAHs would not be of petrogenic origin. According to the ratios previously listed, the PAHs originated from a pyrogenic source. The Denton Reef HPAH sediment percentages ranged from 0 to 40% and averaged to 20.7% (Table 7). To test whether sediment tPAHs were significantly different at Denton Top and Bottom sites, a 2-way ANOVA was used. At a $p \leq$

0.05, there were no significant differences, so Top and Bottom samples were considered duplicates.

Sand Reef was sampled twice per sampling date for a total of 19 sediment samples. During the first sampling on May 26, 2010 the average tPAH concentration was 84.2 ng/g. The average tPAH concentrations over time ranged from 8.48 to 86.3 ng/g, taken on August 4, 2010 and July 7, 2010, respectively when using the calibration curve. When Sand Reef tPAH reporting limits (3.33 ng/g dry weight) were considered, tPAH concentrations ranged from 2.32 to 84.2 ng/g on August 4, and May 26, 2010, respectively. The difference in concentrations between comparing to the calibration curve and the reporting limits are shown in Table 7A and Table 7B. By 2-way ANOVA, tPAHs were not significantly different from Sand Top and Sand Bottom.

Based on the oil plume projection map (Figure 10), it could be hypothesized that Sand Reef was impacted by the oil plume on July 2, 2010 and that PAHs found on that date would be of petrogenic origin. The tPAH concentration on June 28, 2010 was the sample collected most close in time to the predicted date of oil impact, July 2, 2010. The possibility that oil settled into the sediment to account for this increase in concentration is feasible, but the PAH ratios indicate that the PAHs originated from a pyrogenic source on June 28, 2010. The HPAHs ranged from non-detectable to 35.8% and averaged 10.8% throughout the sampling dates (Table 7). With a low percentage of HPAHs, a petrogenic origin would not be expected.

In order to determine if there were any significant temporal changes in tPAH concentration over time, Denton and Sand Reef sites were analyzed by a 2-way ANOVA across

time. No significant difference was found between replicates, between the sites, or between the sampling dates. This indicates that oil influence if found would more likely be detected in the water column.

The NOAA SQuiRTs permissible effects level (PEL) is 16,770 ng ΣPAH/g dry weights for marine sediments. Higher tPAH concentrations could be considered toxic to marine organisms. Sand and Denton Reefs did not exceed NOAA's PELs during the 9 months of sampling. NOAA SQuiRTs effects range medium (ERM > 44,792 ng/g) was also not exceeded at Sand and Denton Reefs.

Based on the hydrophobicity of PAHs, it was hypothesized that sediments would contain a higher concentration tPAH than water. In fact, the tPAHs in sediment were higher than tPAHs in water (Figure 16). There was no statistically significant correlation between the total water PAH concentration and total sediment PAH concentration from Denton and Sand throughout the sampling period (Table 8). There was, however, a significant positive correlation found between sediment tPAHs and %C and % Silt (Table 9). This was expected due to the high surface area and amount of organic carbon in silt composition. The negative correlation between tPAHs and % clay was due to the lack of % clay within the study sites.

The sediment samples at Denton and Sand Reefs were composed of mostly silt (Figure 17). The % sand ranged from 0 to 100% across the four sites with the averages ranging from 3.6 to 25%. There was no significant difference found in % sand. The % silt ranged from 0 to 97.6% across the four sites with the averages ranging from 66.4 to 91%. The % clay ranged from 0 to

34.9% across the sites while the averages ranged from 4.9 to 9.1%. There was no significant difference in % clay found between any of the sites.

The %C:N shown over time (Figure 18) peaks on May 26, July 20, September 10, and November 10 at Sand Bottom, Denton Bottom, Sand Top, and Denton Top, respectively. Sand Top consistently had the highest %C:N, while Sand Bottom consistently had the lowest. It was possible that as an increase in PAHs impacted the site, the %C:N would increase. None of the %C peaks corresponded with the same dates as the peaks in %C:N. As shown by Figure 18, the peak in %C:N was actually caused by uniquely low %C values in those particular samples and the ratio is increased only because %N was even smaller.

### 3.1.2 Perdido and Pointe aux Pines Sites

#### 3.1.2.1 Water

Perdido (PER) was a suspected manatee habitat sampled for oil in the water column eleven times. During the first sampling event in Perdido, AL on June 30, 2010 the tPAH was 116 ng/L (Figure 19; Table 10). The concentration was lower on July 14, 2010 (27.2 ng/L) and then gradually increased until a peak concentration of 1100 ng/L was reached on September 9, 2010. TPAH concentrations decreased to 22.6 ng/L by January 18, 2011. The change in concentrations over time was insignificant due to high variability by using a one-way ANOVA with a $p \leq 0.05$.

Based on the projection maps (Figures 8 and 10), it could be hypothesized that the oil plume impacted Perdido on June 24 and July 2, 2010, and that PAHs would originate from petrogenic sources on these dates (Conservation Biology Institute 2010). This does not explain

the peak concentration in September. NOAA maps of where the oil landed for July 12, July 22, September 19, and October 25, 2010 are available at http://docs.lib.noaa.gov/ noaa_documents /DWH _IR/maps/SCAT_SCOS/. The map for September 19 shows no observed oil along the Mississippi and Alabama coasts except for light to negligible tar balls. PAHs detected in the water were of considered of pyrogenic origin, possibly from motor boat usage, oil spill burning, or industrial waste. There were no HPAHs found in any of the water samples at Perdido or Pointe aux Pines (PAP) (Table 10).

PAP, another suspected manatee habitat, was sampled five times. Only the 9/22/10 sample had total PAHs above the limit of reporting. Based on the oil plume projection map (Figure 7), it could be hypothesized that PAP was impacted by oil on June 18, 2010 (Conservation Biology Institute 2010). Unfortunately, there was no June sampling date. PAHs were of pyrogenic origin for all PAP sampling dates.

#### 3.1.2.2 SEDIMENT

Perdido sediment was sampled eight times. During the first sampling of Perdido, the tPAH concentration was 7450 ng/g on July 28, 2010 (Figure 19). The tPAH concentration decreased to 240 ng/g on September 24, 2010 then increased to 1920 ng/g on October 3, 2010 and then again to 3570 ng/g tPAHs on October 19, 2010. The tPAH concentration decreased to 232 ng/g on November 17, 2010, and then increased to 1780 ng/g on November 30, 2010. The tPAH concentration was 87.0 ng/g on January 18, 2011 and remained at a lower concentration (125 ng/g) when sampled February 9, 2011. The change in concentrations over time was insignificant due to the high amount of variability by using a one-way ANOVA with a $p \leq 0.05$.

Using reporting limits (3.33 ng/g dry weight), Perdido ranged from 79.7 to 7450 ng/g on January 18, 2011 and July 28, 2010, respectively (Table 11).

The origins of the PAHs from each date were pyrogenic. The percent HPAH ranged from 0 to 57.0%, averaging to 40.2% (Table 11). With an overall low percentage of HPAHs, pyrogenic origin was to be expected. The National Coastal Assessment-Gulf Coast/Alabama Department of Environmental Management (NCA) sampled sediment at a site in Perdido, AL in 2000 and did not detect any PAHs (USEPA National Coastal Assessment-Gulf 2004). This suggests that pollution has increased at this site within the last ten years.

Pointe aux Pines sediment was sampled four times. During the first sampling of Pointe aux Pines, on May 31, 2010, the tPAH concentration was 231 ng/g (Figure 19). The concentration did not increase until September 22, 2010, when a tPAH concentration of 452 ng/g was found. TPAHs had decreased to 89.1 ng/g by January 18, 2011. There were no significant changes in PAH concentration due to the high amount of variability. Using reporting limits (3.33 ng/g dry weight), PAP ranged from 89.1 to 450 ng/g on January 18, 2011 and September 22, 2010, respectively (Table 11). Based on the oil plume projection map (Figure 7) it can be hypothesized that Pointe aux Pines, was impacted by the oil on June 18, 2010, suggesting a higher PAH concentration to be expected around this time. The site was not sampled during the month of June, but the tPAH concentration did not increase from the month of May to July, so the invasion of oil may have been missed. Furthermore, the PAH ratios from the sediment samples were of pyrogenic origin and there was a low percentage of HPAHs. The HPAHs ranged from 28.0 to 57.2% and averaged 37.4 % (Table 11). Perdido and PAP sediment tPAHs did not exceed NOAA's PELs (16,770 ng/g) or ERM (> 44,792 ng/g) during the 9 months of sampling.

Based on the hydrophobicity of PAHs, it was hypothesized that tPAH sediment concentration would be greater than the tPAH water concentration, but due to accumulation of PAHs in sediment, it would not be hypothesized that there would be a correlation between the tPAH concentrations in sediment and water. There was no correlation between the total water PAH concentration and total sediment PAH concentration from PER and PAP throughout the sampling period (Figure 20).

The %C:N of PER was monitored over time to determine peaks in the ratio (Figure 21). The peaks occurred on August 24 and November 17, 2010. Theoretically, this could indicate an increase in %C, indicating a potential increase in PAHs at that time point. The %C at those time points was actually inverse of this theory (Figure 21). When the raw data of %C and %N were compared, it was found that %C decreased by a magnitude of 10 on those dates, but %N decreased by a magnitude of 1000, causing the %C:N ratio to appear to increase, when the %C did not.

PER and PAP sediments mostly consisted of silt (Figure 22). Because of the higher n numbers (PER n = 12; PAP n = 5), when testing for statistical differences in sediment composition between these two sites statistical differences were found. The % sand composition of PER and PAP was significantly different with averages of 9.8 and 9.0%, respectively. The % silt of PER and PAP was significantly different with averages of 89.9 and 89.3%, respectively. The % clay was also significantly different with averages of 0.32 and 1.67% for PER and PAP, respectively.

### 3.1.3 REMAINING SITES

#### 3.1.3.1 WATER

On July 10, 2010, the Katrina project sites were sampled (Weston *et al.* 2010). The tPAH concentrations ranged from non-detectable to 66.9 ng/L at PR (Table 12). Allan et al (2012) found Gulfport, MS tPAHs to range from 7.3 to 21 ng/L between June 1, 2010 and May 2011. Their reported concentrations were below our method reporting limit. Allan and coworkers' (2012) use of a passive sampling device to determine tPAHs may have contributed to their lower detection limits. The concentration found by Allan and coworkers (2012) was compared to a peak concentration of 170 ng/L at Grand Isle, LA during its peak oiling time. Allan and coworkers (2012) also found a west-to-east trend in the timing of peak PAH concentrations due to distance from the wellhead and the pathway of the loop current. EMAP also sampled approximately 8 km out to sea from Gulfport, MS, nca-1457-1-sw-09142010 and did not detect any PAHs on September 14, 2010 (Environmental Protection Agency 2011).

EMAP sampled in Biloxi, MS, approximately 4 km from the BG site. The site near BG, bch03-sw-201008, was sampled on May 3 and August 20, 2010 and no PAHs in the water column were detected (Environmental Protection Agency 2011). EMAP also sampled a site approximately 4 km downstream of the PR site in this study, bch04-sw020100503, on May 3 and August 19, 2010 and found no detectable PAHs in the water column on either date. Also no PAHs were found at a site approximately 8 km from the MB site in this study, R4-19-A-swa-09172010, sampled by EMAP on September 17, 2010. The lack of PAH concentrations found by EMAP could again be due to different limits of detection between studies.

The remaining sites were sampled in order to temporally and spatially compare concentrations at other sites around the northern Gulf. Fairhope (FH) was the only site sampled for water in June 2010 (Table 12). Located on the eastern coast of Mobile Bay, AL, it had 50 ng/L of tPAHs, which was slightly higher than the average tPAH concentration from the Denton and Sand Reef sites, also located in Mobile Bay, AL. EMAP sampled a site approximately 14 km from FH on September 18, 2010, nca10-1416-b-swa-09182010, and there were no detectable PAHs (Environmental Protection Agency 2011).

In January 2011, FH, RC, FR, and SC were sampled. Steele Creek had the highest concentration of tPAHs with a concentration of 935 ng/L on January 18, 2011 and at Fairhope tPAHs were non-detectable (Table 12). Steele Creek is located north of Mobile Bay, AL. The high concentration of PAHs is most likely from a nearby pollution source. The other three sites are also located around Mobile Bay, AL. In February 2011, Little Lagoon had non-detectable tPAHs. Little Lagoon is located south of Mobile Bay in the middle of the Alabama Gulf Shores. This location is similar to the PER site, which was also had non-detectable PAHs when sampled in February.

EMAP has provided acute and chronic water quality benchmarks for aquatic life for specific PAH congeners. The acute benchmarks for different PAH congeners range from 0.294 to 1280 µg/L, while the chronic benchmarks range from 0.168 to 307 µg/L of C4-chrysene and acenaphthylene, respectively (Environmental Protection Agency 2011). Of the sites sampled within this study, none of the sites had individual PAH concentrations exceeding these acute or chronic benchmarks, meaning none of the individual PAHs were anticipated to be at concentrations toxic to aquatic life. EMAP also provided human health benchmarks for children

swimmers to five PAHs; naphthalene, acenaphthene, fluorene, anthracene, and pyrene (Environmental Protection Agency 2011). The concentrations range from 1800 to 22,000 µg/L. None of the sites in this study exceeded these concentrations for these five compounds.

The majority of the tPAH water concentrations found in this study were low compared to other studies of oiled water samples. Whitehead et al (2012) found 213 µg/L, or 213,000 ng/L, at Grande Terre, LA as a result of the Deepwater Horizon oil spill. During the *Exxon Valdez*, oil tanker spill off the coast of Alaska, two sites were compared for tPAHs in water, both oiled to different degrees (Neff and Stubblefield 1995). The tPAHs of the two Alaskan sites peaked at 420 to 19,380 ng/L. The rest of the samples ranged from non-detectable to 990 ng/L during April and May 1989. Following the *Prestige* oil spill off the northwestern coast of Spain, tPAHs ranged from approximately 900 to 1400 ng/L. Overall, at the northern Gulf of Mexico sites sampled during this study, DB, ST, PER were sites with tPAHs within the above concentration ranges on July 21, June 28, and September 9, 2010, respectively. Steele Creek and Rabbit Creek also were within this concentration range, both sampled on January 18, 2011. When all the water sites that were only collected once were averaged, tPAHs were 98.3 ng/L ± a standard error of 71.

#### 3.1.3.2 SEDIMENT

The sites related to the Katrina project were all sampled in July and ranged from 30.7 (28.3; considering method reporting limit) to 1904 (1900) ng/g total sediment PAH at BG and BBB, respectively (Tables 13 A and B). BBB was expected to be higher than the rest of the sites because it was located in a part of the Bay that does not experience much circulation and had

high PAH concentrations following Katrina (Weston *et al.* 2010). Compared to the average tPAH concentrations from Weston (2010), all of the tPAH concentrations decreased since they were sampled in 2005-2006.

Three of these sampling sites (MB, GBBH, PR) were close to sampling sites of EMAP from 1991-1994. Their site LA91LR06 is located near MB and had a sediment tPAH concentration of 183 ng/g in 1991, which is lower than detected by this study and Weston et al (2010). The EMAP site LA92SR13 is located near GBBH and had a sediment tPAH concentration of 100 ng/g in 1992, which is also lower than both this study and Weston et al (2010). The EMAP site LA94SR15 is located near PR and had a sediment tPAH concentration of 916 ng/g in 1994, which is lower than Weston et al (2010) detected, but higher than detected by this study. Our locations were located closer to the coastline (wading distance) than EMAP, which could account for the difference. EMAP also did sampling during the Deepwater Horizon Oil Spill in 2010 near MB, PR, BBB, BG, and GPG (Environmental Protection Agency 2011). tPAHs ranged from non-detected to 6.9 ng/g in EPA sites sampled on May 3, August 20, September 10, September 14, and September 17, 2010. The peak concentration of this range occurred at BG on August 20, 2010 and the PAH composition consisted of fluoranthene and chrysene. Pass Hotel was also sampled in July and 1435 ng/g tPAHs were detected.

The six sites corresponding with the seagrass project (Figure 23, Table 14) were sampled between July 12 and October 22, 2010. The Grand Bay sites, one in Alabama and one in Mississippi, are on opposite sides of the bay and were sampled September 29, 2010. Grand Bay, AL had a tPAH concentration of 29.3 ng/g, while Grand Bay, MS had 33.5 ng/g. By analyzing with a one way ANOVA with Kruskal-Wallis test and Dunn's multiple comparison post hoc test,

no significant difference between the site concentrations was found. Pointe aux Chines, AL was also sampled once on September 29, 2010 with a tPAH concentration of 20.9 ng/g. The concentration was not significantly different than the concentrations found at the Grand Bay sites with the same date. Jose Bay was sampled on two dates, on September 29, 2010 there was a tPAH concentration of 16.3 ng/g and the concentration increased to 39.3 ng/g on October 22, 2010. Using a one-way ANOVA, at a $p \leq 0.05$, no significant difference was seen at Jose Bay, AL between these two dates. There was no change in tPAH concentrations at the six sites corresponding with the seagrass project when comparing to the calibration curve as opposed to the method reporting limits (3.33 ng/g dry weight).

NOAA published maps of the oil impact between July 12 and October 25, 2010 (http://docs.lib.noaa.gov/noaa _documents/DWH_IR/maps/SCAT_SCOS/). NOAA's map of September 19, 2010 showed very light to no observed oil in the Grand Bay area, where most of the seagrass sites are found. These concentrations are comparable to tPAH concentrations found on the Alabama Gulf Shores by Allan et al (2012). The Gulf Island National Seashore (GINS) had a tPAH concentration of 111 ng/g on July 12, 2010 and the concentration decreased to 21.3 ng/g on October 1, 2010. To test if this change in PAH was significant, an unpaired t-test was used. At a $p \leq 0.05$, this decrease in PAHs was significant. In addition, naphthalenes constituted 93% of the tPAHs on July 12$^{th}$. Based on the oil plume trajectory maps (Figures 8 and 9), it can be hypothesized that oil impacted GINS on June 24 and June 27, 2010. The loop current oil movement could explain the high concentration followed by a large decrease over this time period. NOAA's map of July 12, 2010 shows light to moderate oiling around the GINS area. In

the October 25, 2010 NOAA map, no observed oil was recognized; however, varying degrees of tar balls were shown around the GINS area.

Middle Bay, AL was sampled August 2, 2010 for a tPAH concentration of 41.0 ng/g and non-significantly increased to 118 ng/g on September 29, 2010. Compared to the other sites sampled on September 29, 2010, Middle Bay had the highest tPAH concentration (118 ng/L) but it was not significantly higher than the others.

The only site sampled in June was Fairhope with a tPAH concentration of 240 ng/L (Table 13). This concentration was nearly 3 times larger than the tPAH concentration at Sand Reef (73.9 ng/L), sampled two days earlier. EMAP also sampled a site approximately 14 km FH, nca10-1416-b-sd-0918 on September 18, 2010 (Environmental Protection Agency 2011) and reported that the average tPAH sediment concentration was 87.0 ng/g.

Fairhope (FH), Steele Creek (SC), Fowl River (FR, and Rabbit Creek (RC) were sampled in January 2011 and ranged from 12.3 (nd) to 403 ng/g at FH and FR, respectively (Table 13A and B). Sandy Bay, Little Lagoon, Fort Morgan, Alabama Point, GB NERR, Cedar Point, Palmetto Creek, and Wolf Bay were sampled in February 2011 and ranged from non-detectable to 1940 ng/g at GB NERR and Palmetto Creek, respectively. Palmetto Creek is located west of, but flows into Mobile Bay, AL. The high concentration of PAHs is most likely from a nearby pollution source.

Cedar Point sediment was sampled by NOAA's National Status and Trends (NS&T) in 1996 and 2005 for mussel watch and Katrina special project, respectively. In 1996, 14 ng/g tPAHs were found, but in 2005, 287 ng/g tPAHs were found. In February 2011, we found 76.3

ng/g tPAHs at this site. The oil plume projection map (Figure 10) shows oil impacted Cedar Point July 2, 2010. Whitehead et al (2011) sampled sediment near Fort Morgan on September 12, 2010 and reported 45.4 µg/L. On February 9, 2011 our study sampled Fort Morgan to find 73.5 ng/g. It is difficult to completely compare the results from this study to that of Whitehead et al (2011) because their sediment units are in µg/L, while ours are in ng/g. The conversion between liters and grams is difficult due to varying densities of the sediments. None of the spatial sites exceeded NOAA's PELs or ERMs during the 9 months of sampling. According to EMAP, there was no benchmark sediment concentration for a mixture of PAHs, but each PAH component had its own benchmark concentration.

Whitehead et al (2012) found 5555 µg/L tPAH in sediment samples on October 5, 2010 in Grande Terre, LA, an oiled site due to the Deepwater Horizon Oil Spill. Franco and coworkers (2006) studied sediment in different areas of the Galacia continental shelf after the *Prestige* Oil Spill in November 2002. Sediment samples from their three sites closest to the heaviest oil impact ranged from 14.8 to 89.6 ng/g dry weight (Franco *et al.* 2006). After an oil spill occurred off the coast of Hong Kong, the mangrove swamp sediment was sampled for tPAH contamination (Ke *et al.* 2002). Surface sediment located within oil contamination contained 2135 ng/g tPAH. After the *Exxon Valdez* Oil Spill nearshore sediments had tPAH concentrations ranging from 446 to 665 $\pm$ 2717 ng/g (Page *et al.* 1995). Many of our sediment samples tPAH concentrations fell within the ranges detected following other spills. Denton tPAH sediment concentrations were 280 ng/g, 466 ng/g, and 345 ng/g on June 9, September 20, and November 10, 2010, respectively. Perdido tPAH sediment concentrations ranged from 7450 ng/g, 233 ng/g, 1920 ng/g, 3570 ng/g, 223 ng/g, and 1780 ng/g on July 28, September 24, October 3, October 19,

November 17, and November 30, 2010, respectively. PAP sediment ranged from 228 ng/g, 208 ng/g, and 450 ng/g tPAH on May 31, July 1, September 22, 2010, respectively. Fairhope, Pass Hotel, Mobile Bay, Biloxi Back Bay, GBBH, GBBC, Gulfport Courthouse, PR, and USM all had concentrations within the oiled ranges reported in the literature during June and July 2010. Fowl River, Rabbit Creek, and Palmetto Creek had similar tPAH concentrations during January and February 2011. All the sediment sites collected once were averaged, and tPAHs were 454 ng/L ± 131.

### 3.1.4 PAH ANALYSIS OF OIL

The oil sample received from BP had a tPAH concentration of 44.0 X $10^6$ ng/mL. The PAH components consisted of 19% naphthalene, 23% 1-methylnapthalene, 33% 2-methylnapthalene, 6% biphenyl, 4% fluorene, 6% phenanthrene, 5% 1-methylphenanthrene, 1.2% benz(a)anthracene, and 1.8% chrysene (Figure 24). Compared to the fingerprint performed by B&B Laboratories, of the 24 PAHs analyzed our sample did not have benzo(e)pyrene, benzo(b)fluoranthene, fluoranthene, pyrene, or dibenzothiophenes (OSATF 2010). These five compounds were found in trace amounts (<50 ng/mg) by OSATF (2010). OSATF (2010) also found alkylated fluoranthene/pyrenes, naphthobenzothiophene, alkylated naphthobenzothiophenes, and alkylated dibenzothiophenes, which were not analyzed for during in this study. The most dominate compounds were the alkylated phenanthrene/anthracenes in the OSATF (2010) study. As shown in Figure 24, naphthalene was the most dominant compound we detected. Reddy et al (2011) determined the Deepwater Horizon Oil to consist of 16% aromatic hydrocarbons, 74% saturated hydrocarbons, and 10% polar hydrocarbons with trace amounts of nitrogen and sulfur. This could have accounted for the inconsistencies between the results of our

study and OSATF (2010) results, because they accounted for aliphatics and more alkylated hydrocarbons than our study.

The dispersant enhanced WAF sample provided by Dr. Joe Griffitt at GCRL had a tPAH concentration of 146,000 ng/L. The PAH components consisted of 28% naphthalene, 14% 1-methylnapthalene, 18% 2-methylnapthalene, 5% biphenyl, 7% fluorene, 12% phenanthrene, 7% benzo(a)anthracene, and 5% chrysene (Figure 24). It was expected that the tPAH concentration would be lower than the amount in the pure BP oil because WAF is extremely diluted oil but that the relative percent of some constituents could be different because the dispersant solubilizes some PAHs Figure 25. These results were based on an n of 1, so it was possible that if more samples were analyzed different results would be observed. The fingerprint of the oil versus WAF showed a relatively similar distribution pattern of the components making up the two mixtures (Figures 24 and 25).

Various methods have been used to identify sources for PAHs in the environment. Furthermore, a number of studies have focused specifically on the impacts of oil spills including the *Exxon Valdez* (Burns *et al*. 1997; Page *et al*. 1995 and 1996), the *Aegean Sea* (Pastor *et al*. 2001) and *Prestige* (Franco *et al*. 2006) spills. Allocation methods included principle component analysis (PCA) of the PAH constituents (Burns *et al*. 1997), consideration of the pyrogenic, petrogenic and biogenic PAH composition (Page et al. 1995), and quantitation of steranes and triterpane (Pastor *et al*. 2001; Franco *et al*. 2006). Additional background was provided in the introduction in section 1.4.2 and diagnostic PAH ratios in Table 2. As noted previously, with the exception of 1- and 2- methylnaphthalene and 1-methylphenanthrene, neither alkylated PAHs nor alkane constituents were quantitated in this study. Additionally, many of the water samples

were below reporting limits so ability to do PCA analysis is limited. That said, the sites or dates with the highest tPAH concentrations, water and sediment, were evaluated for their percent compositions in order to compare to the BP oil and the WAF samples, and pie graphs are shown in Figures 26 and 27.

Of the water samples, June 28, July 21, and September 9, 2010 were the dates with the highest tPAHs at ST, DB, and Perdido, respectively. ST was the most similar to the BP oil and WAF samples because it contained the highest percentage of common compounds. While our dispersed WAF had 28% naphthalene, 14% 1-methylnapthalene, 18% 2-methylnapthalene, the ST sample had 36, 22 and 15% of these three compounds respectively. The only other constituents in the ST water sample were phenanthrene and fluoranthene making up 26% together. In contrast to the ST water sample, the highest DB and Perdido samples had a very different PAH makeup. None of the constituents in the DB 7/21 sample were found in the BP sample or WAF. The PAHs in the sample (~14% each) were benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3,c,d)pyrene, dibenzo(a,h)anthracene, and perylene. However, the DB 6/28 water sample was 100% phenanthrene (tPAHs = 20.8 ng/L). The Perdido sample was dominated by anthracene (41%) and phenanthrene (23%). The highest tPAH from the Katrina site water samples was the Pascagoula River sample and it consisted of only naphthalene (60%) and 2-methylnaphthalene (40%). Steele Creek and Rabbit Creek, like the ST and WAF samples were dominated by the naphthalenes. Both of these sites, however, are located near boat ramps and may reflect PAH contamination from sources other than the Deepwater Horizon incident.

As expected the PAH composition found in sediment samples was more varied and complex. Furthermore, constituents of WAF or source oil are not a good comparison because PAHs reaching sediments will likely be weathered and of higher molecular weights (more persistent) (Page *et al.* 1996). Figure 27 shows the percent composition of PAHs for the samples having the highest PAH concentrations for the various studies. The presence of naphthalenes may represent relatively recent contamination and ranged from 32 to 93% composition in the Sand (7/7), Denton (9/20) and GINS (7/12) most contaminated samples. Perdido (7/28), Pass Hotel (7/14), Biloxi Back Bay (7/10), and Palmetto Creek (2/9/11) consisted of high tPAHs and variable percent compositions without a dominant PAH constituent, likely representing an accumulation of multiple PAH sources over time.

## 3.2 *FUNDULUS* EXPOSURES

### 3.2.1 EROD ANALYSIS

PAHs, as well as other environmental contaminants like PCBs and dioxins ellicit AhR-mediated effects. Nacci et al (1998) developed a specific, sensitive and nondestructive bioassay to measure EROD activity in embryonic and larval fish, specifically *F. heteroclitus*. This bioassay involved the measurement of product fluorescence in the urinary bladders of live embryos, *in ovo* (Nacci *et al.* 1998). This assay has been used specifically to investigate PAH and oil CYP1A induction. Couillard and coworkers (2005) used *F. heteroclitus* to observe increased EROD activity as the concentration of HPAH increased, but not with tPAHs (Couillard *et al.* 2005). The bioavailability of the WAF increased once dispersed due to the increase in the amount of hydrocarbons dissolved into the water column and increased EROD induction (Couillard *et al.* 2005; Schein *et al.* 2009). In other fish studies, CYP1A was also induced by

WAF exposure. Schein et al (2009) observed highly alkylated WAF to cause stable CYP1A induction in *Oncorhynchus mykiss,* Atlantic herring, over time. Importantly, EROD induction from WAF has occurred in *O. mykiss* at concentrations below the detection limit of a GC/MS.

*F. heteroclitus* exposure to WAF did not statistically increase *in ovo* EROD relative to control embryos (Figure 28). There was, however, an increasing trend as %WAF increased, so did the EROD response. B(a)P was used as a positive control and caused statistically significant EROD induction. To test if there were any significant differences in EROD induction, a one-way ANOVA was used with a $p \leq 0.05$. Perdido had the highest site response, but the EROD results from embryos exposed to site water were highly variable. If oil was in the water column, especially after being dispersed, EROD induction would be expected, even at low concentrations. Because there were little to no HPAHs found in water samples, it was hypothesized that EROD activity would be low.

### 3.2.2 DEVELOPMENT DEFORMITIES

Wassenberg and Di Giulio (2004) observed deformities in developing *F. heteroclitus* embryos as a result of exposure to PAHs, with heart deformities being the most sensitive. Other deformities scored were pericardial edema, tail shortening, and hemorrhaging (Wassenberg and Di Giulio 2004). Meyer et al (2002) compared embryos from "clean site" fish to $F_1$ generation from the Elizabeth River, a creosote polluted site. The $F_1$ generation did not form abnormalities due to exposure to β-napthoflavone and Elizabeth River sediment pore water, showing adaptive traits. Clark *et al* (2010) knocked down AHR1, AHR2, or both, to determine if AHR had a protective role in teratogenesis due to PAH exposures. AHR2 was found to mediate PAH responses such as cardiac teratogenesis in *F. heteroclitus* (Clark *et al.* 2010).

*F. heteroclitus* embryos were not significantly affected by the water collected from the sites over time (Figure 28). The highest mean deformity score (MDS) occurred at Perdido on September 9, 2010. The peak MDS for DT and SB occurred on September 14 and June 18, 2010, respectively. ST, with the highest number of n values, had MDS range from 0 to 0.133. The peak MDS of 0.133 occurred on both June 18 and September 4, 2010. These MDSs were calculated for deformities found at 10 dpf. Five and ten dpf MDS were compared (Figure 29). The average MDS of DT, PER, and ST increased from 5 to 10 dpf, while SB decreased. There was less than 4% and 2% incidence of edema and blood clot, respectively, and there were no significant differences in deformity index or lethality. The highest deformity index for Perdido and Denton corresponded with peak tPAH water concentrations, 1103 ng/L and 210 ng/L, respectively. The highest Sand deformity index was found two weeks after peak tPAH water concentrations were measured.

Percent mortalities were observed throughout the exposure and ranged from 0 to 60%. The controls averaged 4% mortality. Perdido had the highest average percent mortality of 34%, followed by DT, ST, and then SB (Figure 30). No significant differences were found due to high variance within each site. Because Perdido had the highest mortality, it was expected that the highest MDS, EROD induction, and tPAHs would be found at Perdido, which occurred.

### 3.3 COMPARISON TO OTHER OIL SPILLS

A similar deepwater blowout occurred to the Ixtoc I in 1979 at 160 feet deep in the southern Gulf of Mexico, compared to 5,000 feet deep for Deepwater Horizon (Schrope 2010). Ixtoc I leaked approximately 140 million gallons of crude oil into the Gulf, while Deepwater Horizon leaked approximately 210 million gallons of crude oil. Due to a lack of funding, not

much was known of the long term ecological effects from the Ixtoc I spill. From interviewing local fisherman, fish catch rates improved within 2-5 years in some areas, while in other areas oysters were never seen again. The areas varied from deep sea communities to mangrove communities. Comparing these areas to comparable ones in the northern Gulf of Mexico, it was concluded that areas such as sandy beaches and rocky shores were able to improve quickly, but areas such as salt marshes retain oil indefinitely (Shrope 2010).

The *Exxon Valdez* oil spill occurred in the Prince William Sound in Alaska in 1989. There are obvious differences between the *Exxon Valdez* spill and Deepwater Horizon. *Exxon Valdez* spilled approximately 11 million gallons of Alaskan crude oil onto the surface water of the Sound as opposed to Deepwater Horizon leaking at 5000 feet below the surface (Boehm 1996). The volume spilled was magnitudes less than the 210 million gallons leaked from the Deepwater Horizon well, the type of oil was different, and the depth of the spill was different. Alaskan crude oil (ACO) when compared to Louisiana sweet crude (LSC) oil was composed of heavier molecular weight PAHs, causing it to be less volatile than the LSC. Also, *Exxon Valdez* occurred in a calm sound, allowing for skimmers to be useful to recover the oil. The Deepwater Horizon occurred in the middle of the Gulf of Mexico, with waves and choppy waters making skimmers difficult to use. The remediation methods also differed. In Alaska remediation efforts included the use of fertilizers to stimulate microbial growth to increase oil degradation, while after the Deepwater Horizon spill dispersants were on the surface and at the source of the leak to increase the oil's vulnerability to microbial degradation (Schmidt 2012). It was important to note that years later, the *Exxon Valdez* spill impacts were still being studied (Boehm *et al*. 1998; Short

*et al*. 2004; Taylor and Reimer, 2008).so the ecological effects of Deepwater Horizon will be investigated for years to come (Schmidt 2012).

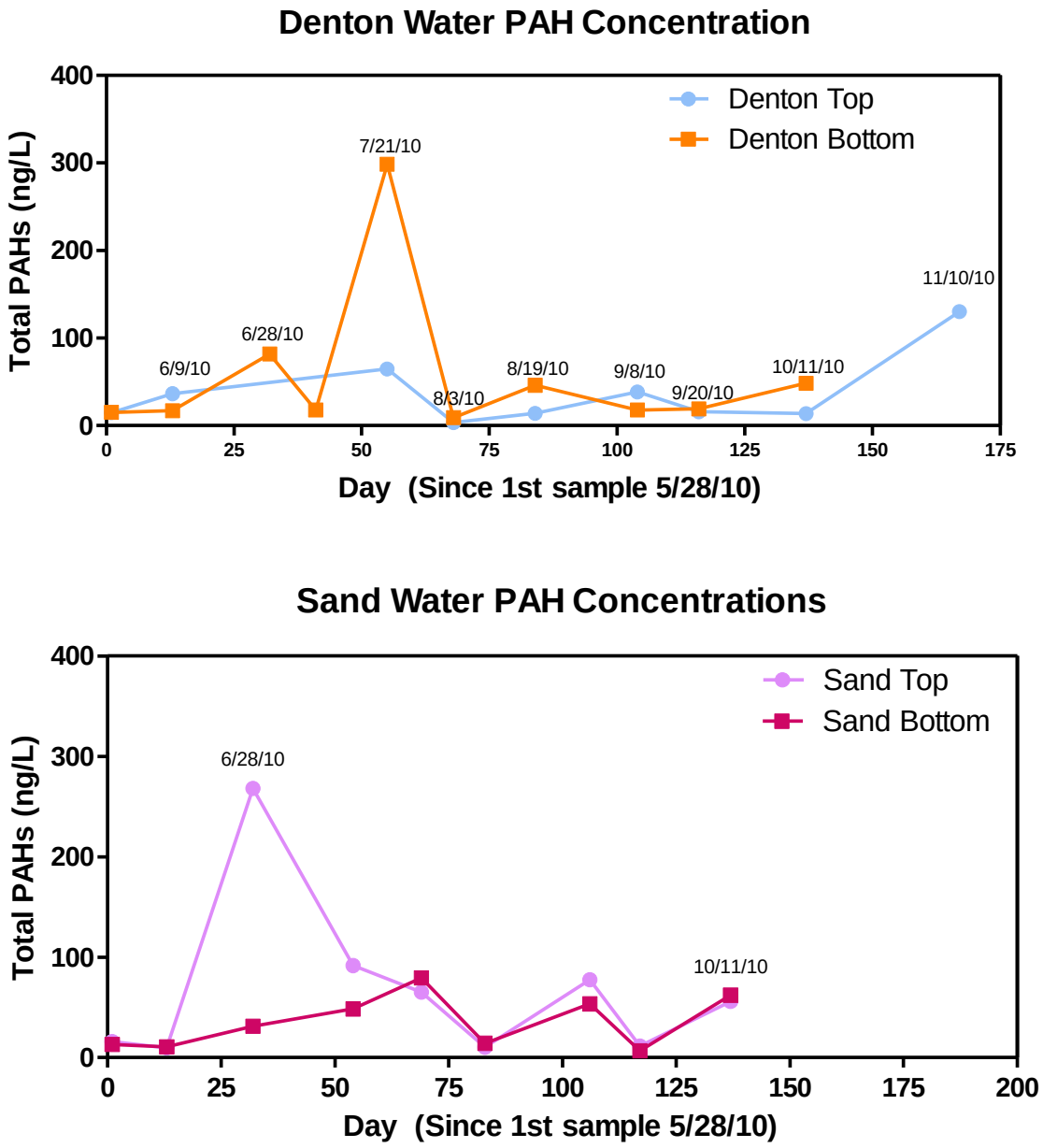


**Figure 6.** Total PAH water concentrations at Denton (top) and Sand (bottom) Reefs across time at top depths of 1 m above the seafloor and bottom depths of 0.1 m above the seafloor (for actual values see Table 6A). To test whether water tPAHs were significantly different at Sand Top and Bottom sites or Denton Top and Bottom sites, a 2-way ANOVA was used. At a $p \leq 0.05$ there was no significant differences at either site. To test whether there was a significant change over time, a 1-way ANOVA was used. At a $p \leq 0.05$, no significant differences were found at any time.

**Table 6A.** Temporal and spatial distribution of water total PAHs ng/L (% HPAHs) at Denton and Sand Reefs based on the calibration curve. nd = non-detectable, "—" = sample not available.

| Date | Denton Bottom | Denton Top | Sand Bottom | Sand Top |
|---|---|---|---|---|
| **5/28/2010** | 15.3 (0) | 14.9 (0) | 12.8 (0) | 16.0 (0) |
| **6/9/2010** | 17.3 (0) | 36.6 (0) | 10.5 (0) | 9.66 (0) |
| **6/28/2010** | 82.0 (0) | -- | 31.0 (0) | 268 (0) |
| **7/7/2010** | 17.72 | -- | -- | -- |
| **7/20/2010** | -- | -- | 48.4 (0) | 91.5 (0) |
| **7/21/2010** | 299 (42.4) | 64.7 (46.7) | -- | - |
| **8/3/2010** | 9.33 (0) | 3.46 (0) | - | - |
| **8/4/2010** | - | - | 79.5 (0) | 64.9 (0) |
| **8/18/2010** | - | - | 14.1 (0) | 10.4 (0) |
| **8/19/2010** | 46.0 (0) | 14.1 (0) | - | - |
| **9/8/2010** | 17.8 (0) | 38.5 (0) | - | - |
| **9/10/2010** | - | - | 53.3 (0) | 77.6 (0) |
| **9/20/2010** | 19.17 (0) | 15.90 (0) | - | - |
| **9/21/2010** | - | - | 6.84 (0) | 11.6 (0) |
| **10/11/2010** | 48.4 (0) | 13.8 (0) | 62.2 (0) | 55.8 (0) |
| **11/10/2010** | 0.00 (0) | 130.19 (28.2) | 0.00 (0) | - |

**Table 6B.** Temporal and spatial distribution of water total PAHs ng/L (% HPAHs) at Denton and Sand Reefs summing only those PAH concentrations that were above the reporting limit of 20 ng/L

| Date | Denton Bottom | Denton Top | Sand Bottom | Sand Top |
|---|---|---|---|---|
| **5/28/2010** | nd | nd | nd | nd |
| **6/9/2010** | nd | nd | nd | nd |
| **6/28/2010** | 20.8 (0) | - | nd | 234 (0) |
| **7/7/2010** | nd | - | - | - |
| **7/20/2010** | - | - | nd | nd |
| **7/21/2010** | 210 (69.9) | nd | - | - |
| **8/3/2010** | nd | nd | - | - |
| **8/4/2010** | - | - | nd | 24.2 (0) |
| **8/18/2010** | - | - | nd | nd |
| **8/19/2010** | 39.9 (0) | nd | - | - |
| **9/8/2010** | nd | nd | - | - |
| **9/10/2010** | - | - | nd | nd |
| **9/20/2010** | nd | 15.9 (0) | - | - |
| **9/21/2010** | - | - | nd | nd |
| **10/11/2010** | nd | nd | nd | nd |
| **11/10/2010** | nd (0) | 130 (28.2) | nd | - |


**Figure 7.** Oil plume projection location for June 18, 2010. The oil plume overlays the sites Sandy Bay and Pointe aux Pines meaning oil was predicted to hit land at these locations, represented by the blue dot.

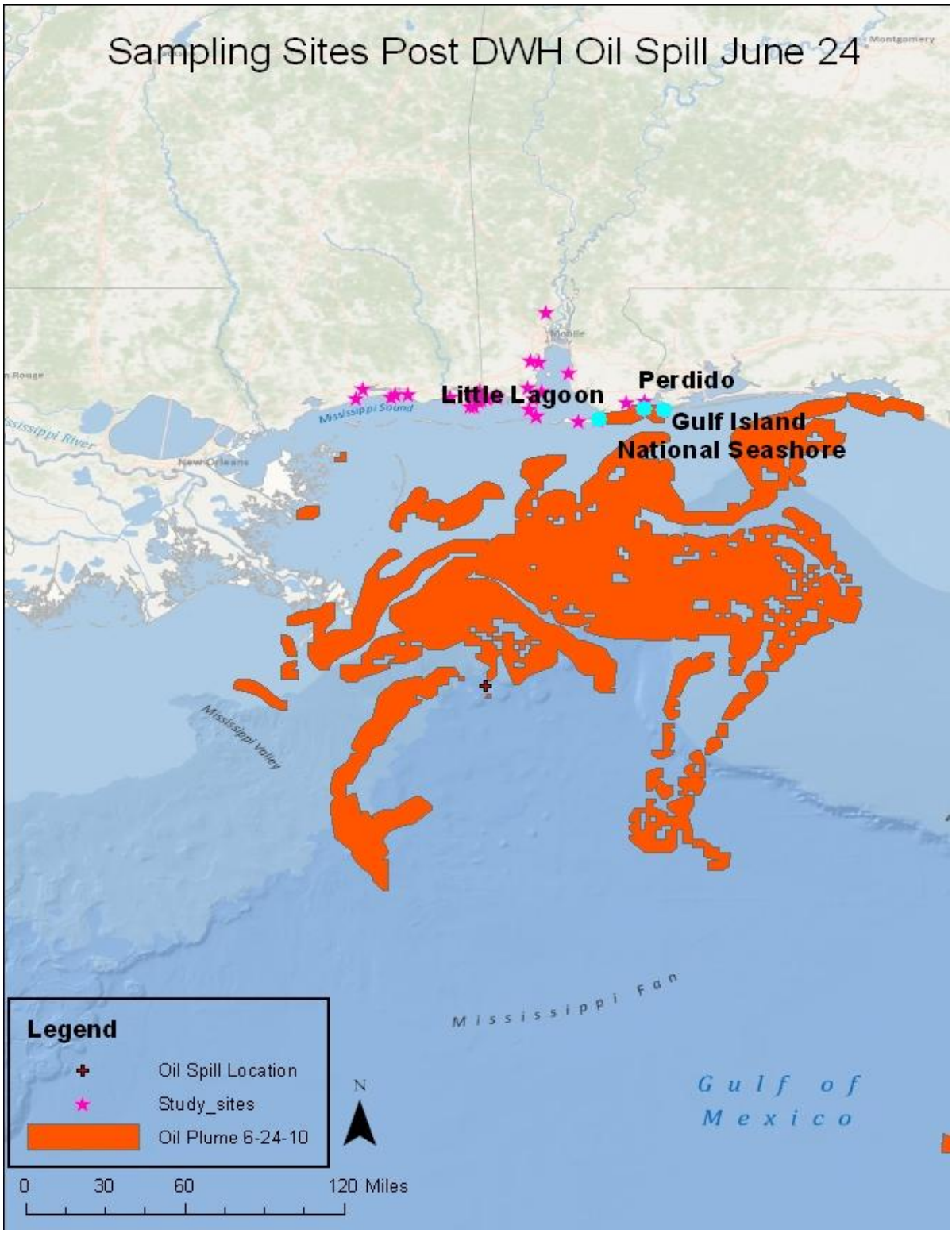


**Figure 8.** Oil plume projection location of June 24, 2010. The oil plume overlays the sites Little Lagoon, Perdido, and Gulf Island National Seashore meaning oil was predicted to hit landfall, represented by the blue dots.

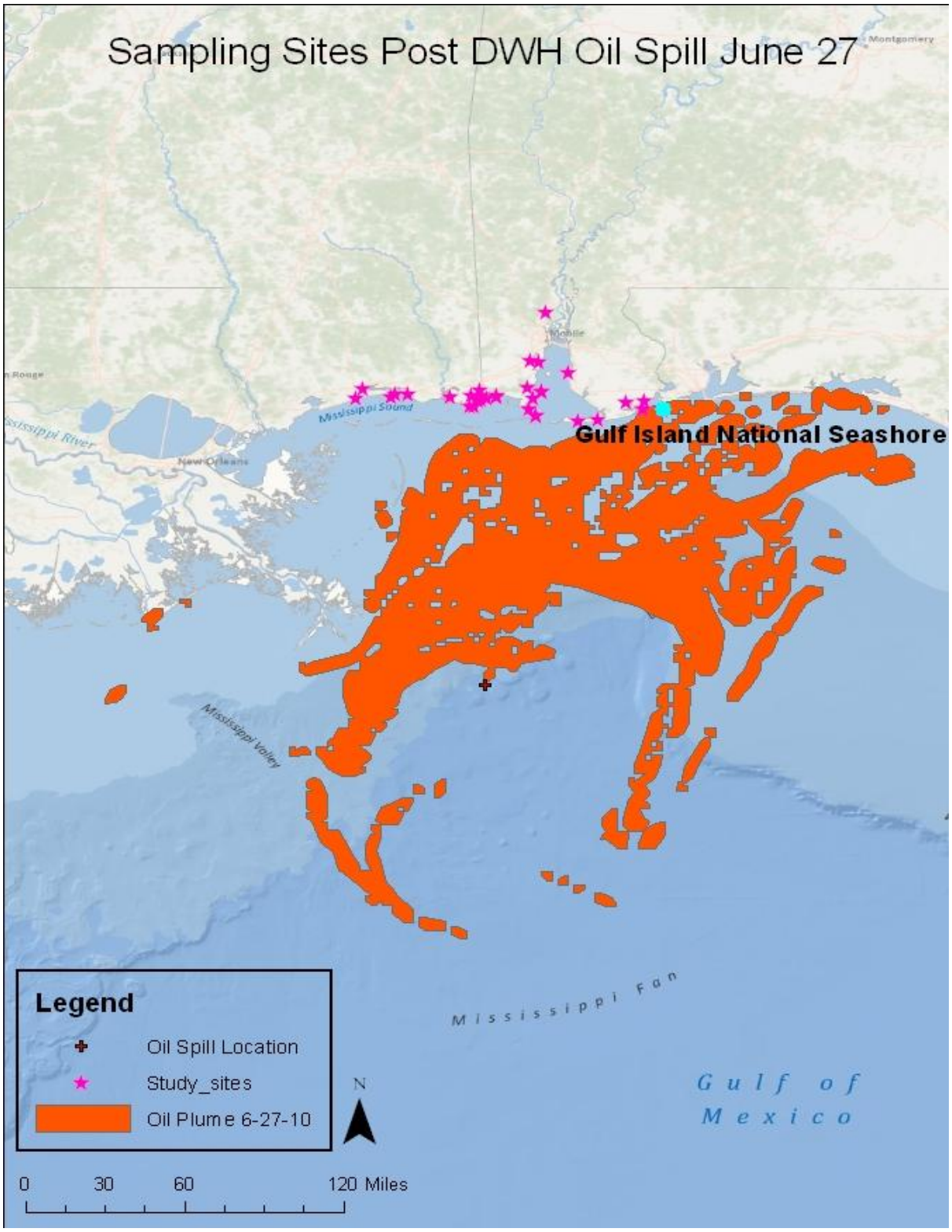


**Figure 9.** The location of the oil plume projection on June 27, 2010. The oil plume overlays Gulf Island National Seashore meaning oil was predicted to hit landfall, represented by the blue dot.

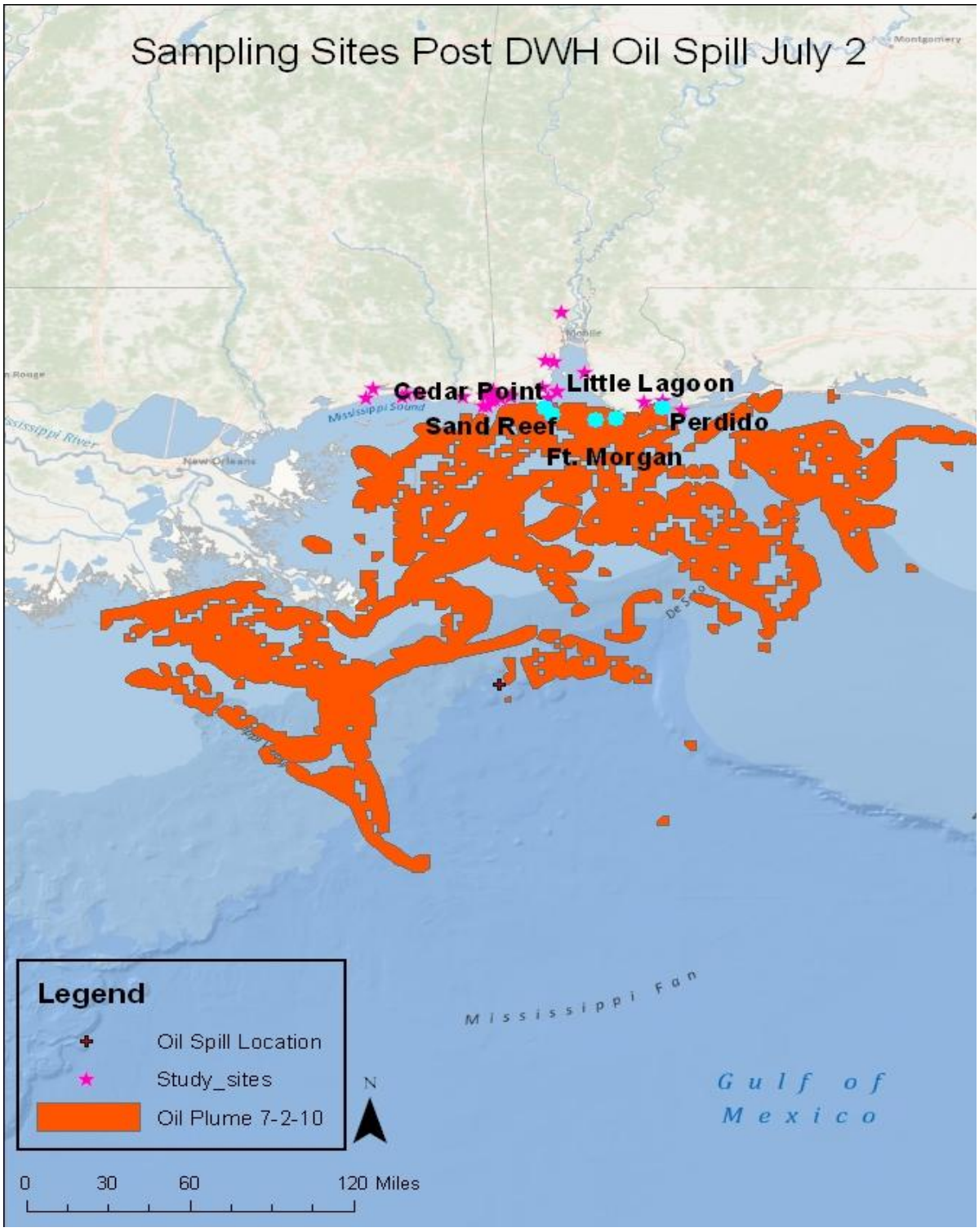


**Figure 10.** The location of the oil plume projection on July 2, 2010. The oil plume overlays Cedar Point, Sand Reef, Little Lagoon, Fort Morgan, and Perdido meaning oil was predicted to hit landfall at these sites, represented by the blue dots.

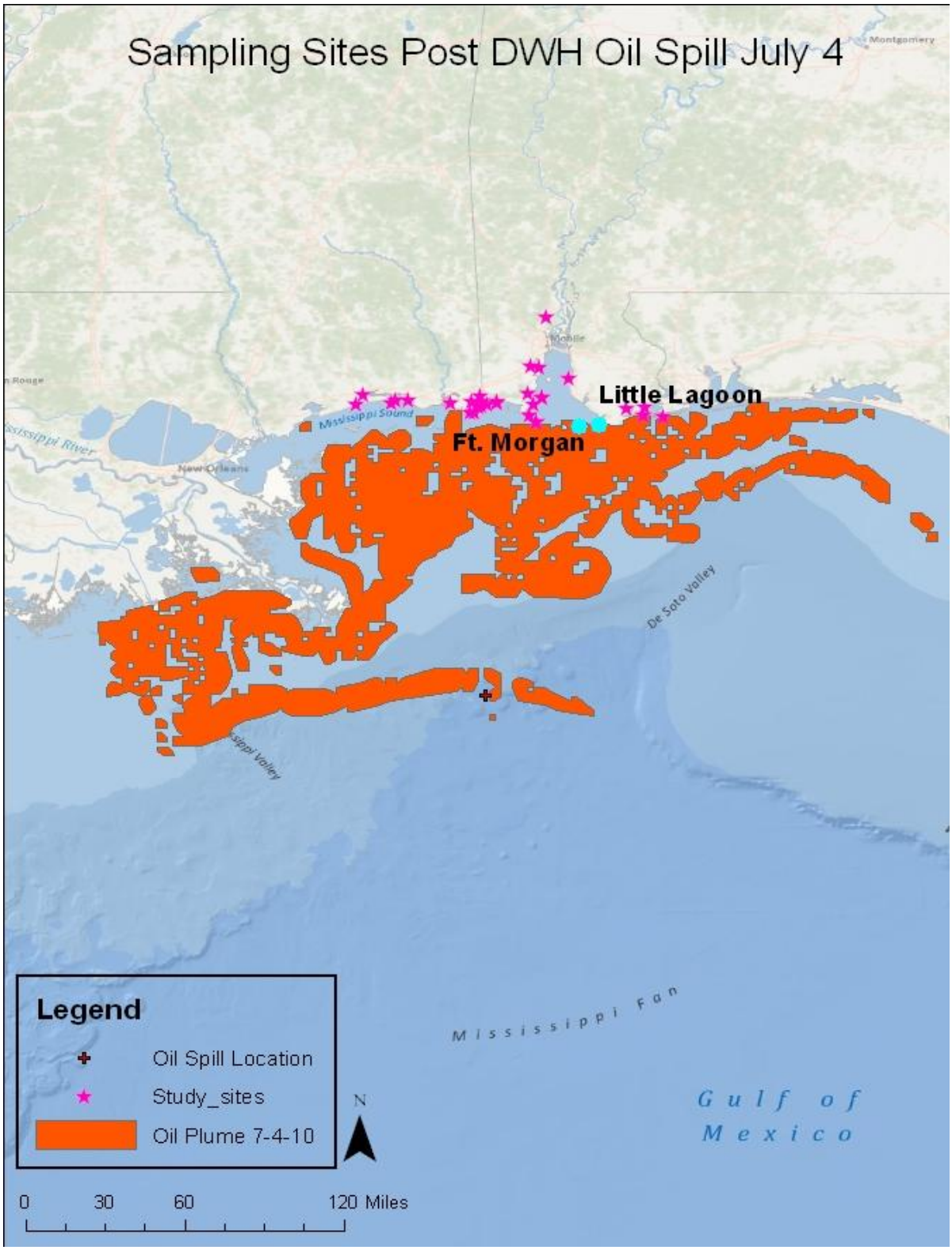


**Figure 11.** The location of the oil plume projection on July 4, 2010. The oil plume overlays Fort Morgan and Little Lagoon meaning oil was predicted to hit landfall at these sites, represented by the blue dos.

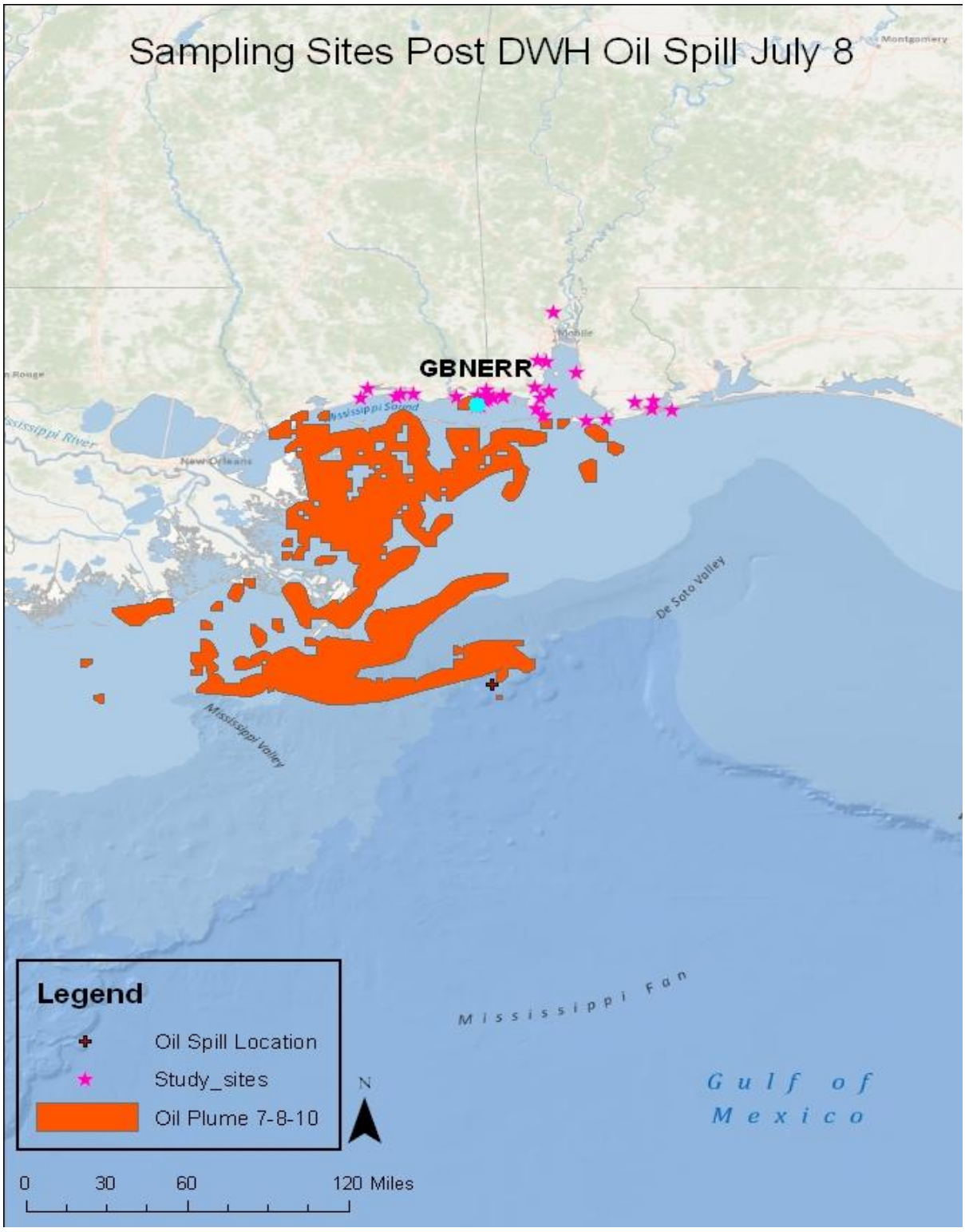


**Figure 12.** The location of the oil plume projection on July 8, 2010. The oil plume overlays Grand Bay National Estuarine Research Reserve meaning oil was predicted to hit landfall at this site, represented by the blue dot.

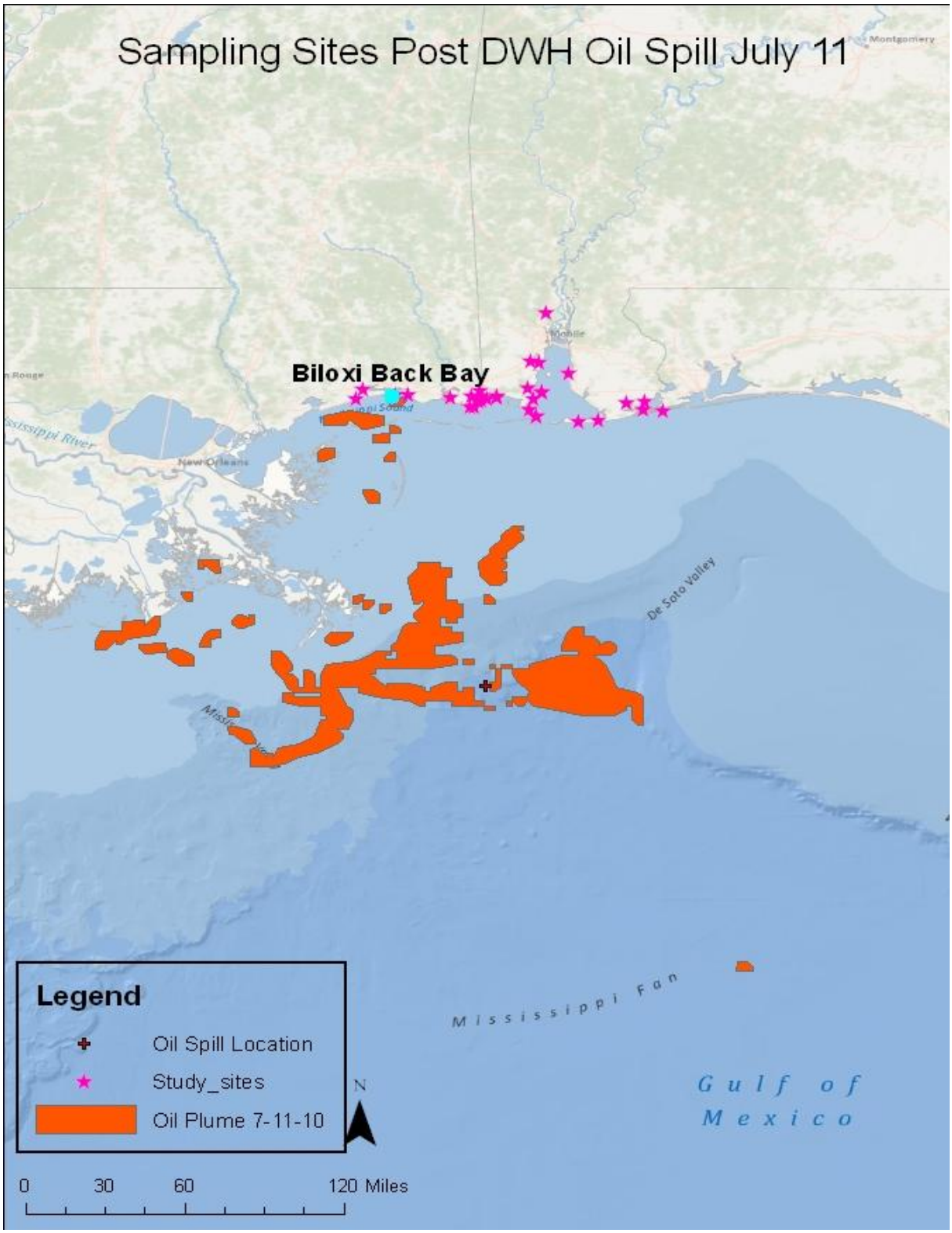


**Figure 13.** The location of the oil plume projection on July 11, 2010. The oil plume overlays Biloxi Back Bay meaning oil was predicted to hit landfall, represented by the blue dot.

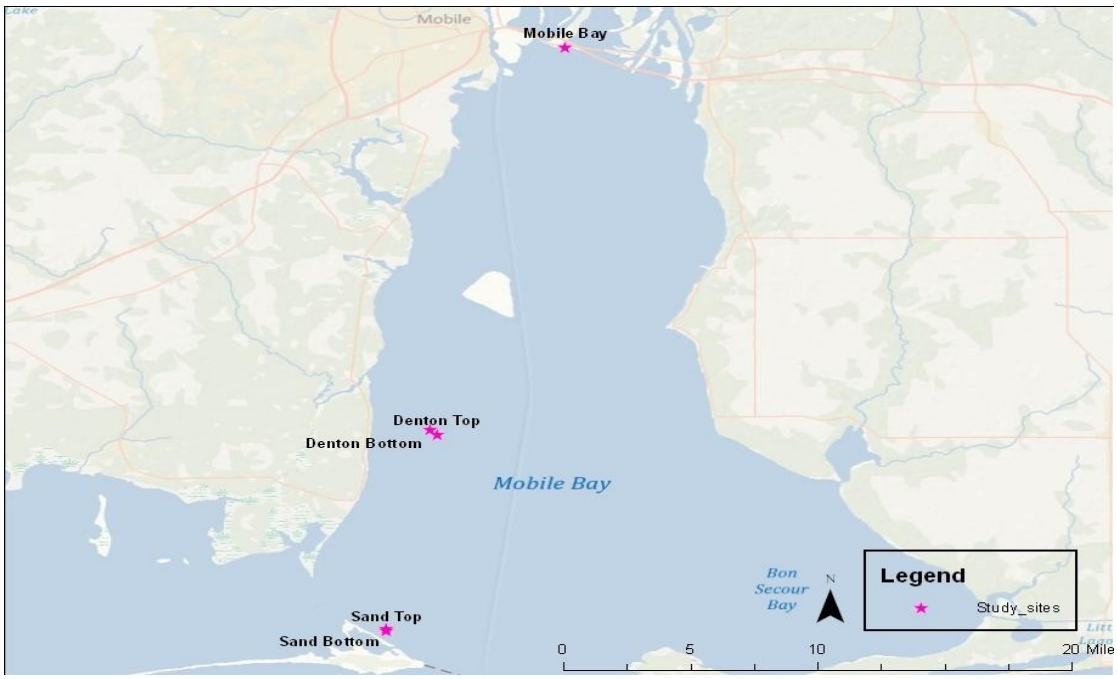


**Figure 14.** Location of Sand and Denton Reefs in comparison to Whitehead (2011) Mobile Bay site.

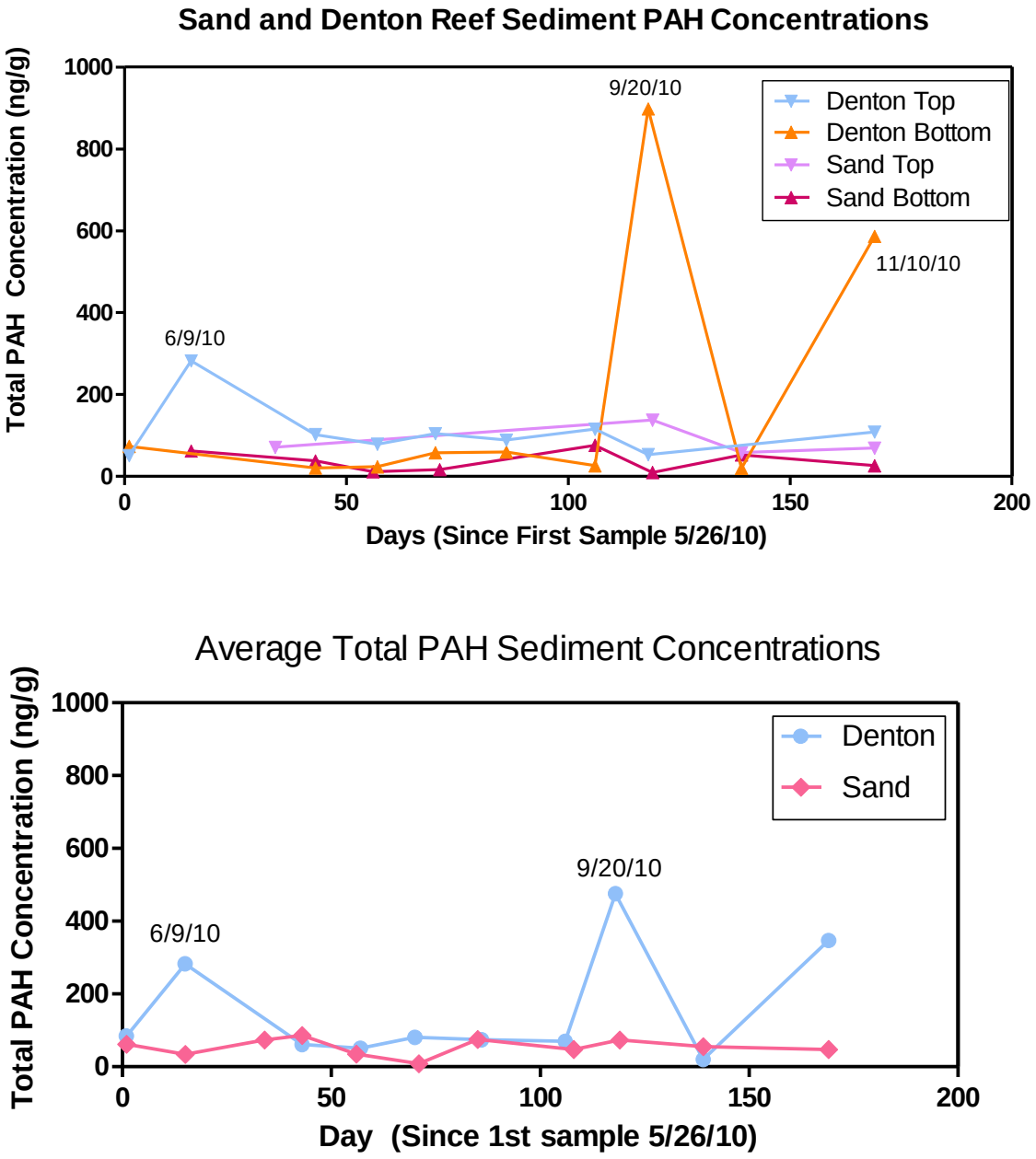


**Figure 15.** Total PAH sediment concentrations at Denton and Sand Reefs at their varying depths ( 0.1 and 1 m) (*top*). Average PAH sediment concentrations at Denton and Sand Reefs (*bottom*) (for actual values see Table 7A). To test whether water tPAHs were significantly different at Sand Top and Bottom sites or Denton Top and Bottom sites, a 2-way ANOVA was used. At a $p \leq 0.05$ there was no significant differences at either site. Then to test if there was a significant change over time, a 1-way ANOVA repeated measures was used. At a $p \leq 0.05$, no significant differences were found across time at either site.

**Table 7A.** Temporal and spatial distribution of sediment total PAHs ng/g (%HPAHs) and Denton and Sand Reefs based on the calibration curve. nd = non-detectable, "-" = sample not available.

| Date | Denton | Sand |
|---|---|---|
| **5/26/2010** | 62.3 (39.7) | 84.2 (0) |
| **6/9/2010** | 283 (31.3) | 34.7± 26.8 (28.7) |
| **6/28/2010** | - | 73.9± 6.29 (35.8) |
| **7/7/2010** | 61.5 (5.43) | 86.3± 57.7 (27.3) |
| **7/20/2010** | - | 35.4± 26.7 (0) |
| **7/21/2010** | 51.4 (27.5) | - |
| **8/3/2010** | 81.3 (34.4) | - |
| **8/4/2010** | - | 8.48± 2.32 (0) |
| **8/18/2010** | - | 75.1 (0) |
| **8/19/2010** | 74.3 (12.7) | - |
| **9/8/2010** | 70.7 (13.3) | - |
| **9/10/2010** | - | 48.1± 41.4 (0.81) |
| **9/20/2010** | 476 (23.7) | - |
| **9/21/2010** | - | 73.8± 65.9 (0) |
| **10/11/2010** | 20.1 (0) | 55.8± 8.56 (15.3) |
| **11/10/2010** | 347 (19.1) | 47.6 ± 21.5 (0) |

**Table 7B.** Temporal and spatial distribution of sediment total PAHs ng/L (% HPAHs) at Denton and Sand Reefs summing only those PAH concentrations that were above the reporting limit (3.33 ng/g dry weight).

| Date | Denton | Sand |
|---|---|---|
| **5/26/2010** | 57.9± 15.8 (39.7) | 84.2 (0) |
| **6/9/2010** | 280 (31.3) | 33.6± 26.8 (28.7) |
| **6/28/2010** | - | 67.1± 15.2 (35.8) |
| **7/7/2010** | 44.9± 27.0 (5.43) | 68.2± 54.2 (27.3) |
| **7/20/2010** | - | 32.2± 23.4 (0) |
| **7/21/2010** | 46.9± 32.0 (27.5) | - |
| **8/3/2010** | 78.9± 25.7 (34.4) | - |
| **8/4/2010** | - | 2.32± 2.32 (0) |
| **8/18/2010** | - | 75.1 (0) |
| **8/19/2010** | 62.5± 10.3 (0) | - |
| **9/8/2010** | 67.7± 44.9 (13.3) | - |
| **9/10/2010** | - | 46.6± 38.4 (43.6) |
| **9/20/2010** | 466± 422 (23.7) | - |
| **9/21/2010** | - | 71.7± 65.9 (0) |
| **10/11/2010** | 16.9 (0) | 51.3± 4.34 (13.5) |
| **11/10/2010** | 345± 242 (19.1) | 47.6± 15.4 (0) |

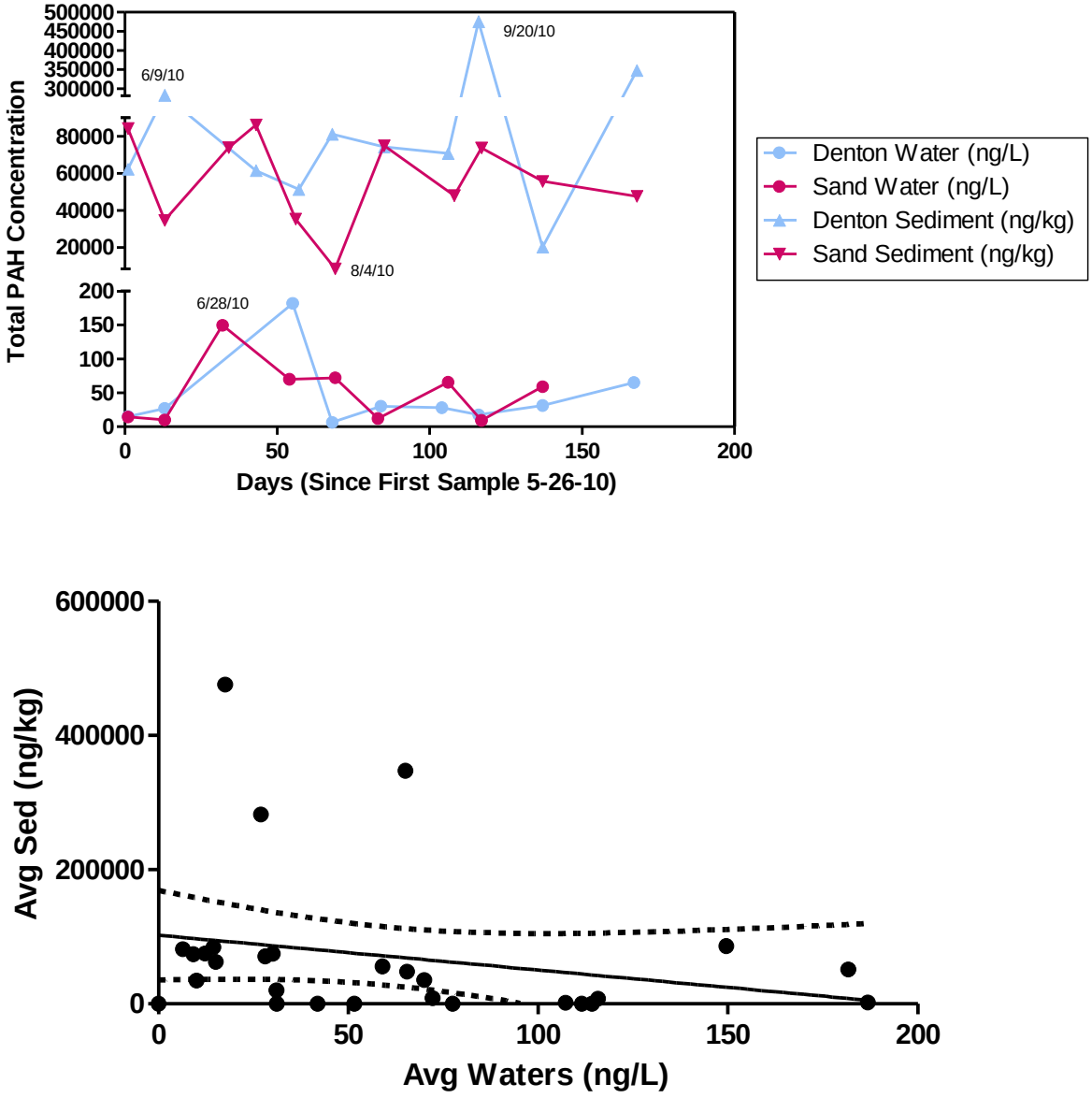


**Figure 16.** Total PAH water and sediment concentrations over time (*top*) Correlation between total PAH sediment and total PAH water concentrations at Sand and Denton (*bottom*). To test whether tPAHs in water were different from tPAHs in sediment, a one sample t-test was used. At a $p \leq 0.05$, there was a significant difference at both Denton and Sand Reefs. To test if there was a correlation between tPAH concentration in water and sediment, a Pearson correlation test was used. At a $p \leq 0.05$, there was no significant correlation.

**Table 8.** Denton and Sand Reef Sites Correlation Coefficient Table. The bottom values are p values and the top values are Spearman r values.

| | tPAH Denton Water | tPAH Denton Sed | HPAH Denton Sed | tPAH Sand Water | tPAH Sand Sed | HPAH Sand Sed |
|---|---|---|---|---|---|---|
| **tPAH Denton Water** | | -0.02381 | -0.6190 | 0.04762 | -0.3095 | 0.1649 |
| **tPAH Denton Sediment** | 0.9768 | | -0.3095 | 0.2619 | -0.2857 | -0.4820 |
| **HPAH Denton Sed** | 0.1155 | 0.4618 | | 0.2619 | -0.2857 | -0.4820 |
| **tPAH Sand Water** | 0.9349 | 0.5364 | 0.5364 | | -0.3571 | -0.3044 |
| **tPAH Sand Sed** | 0.4618 | 0.5008 | 0.5008 | 0.3894 | | -0.3551 |
| **HPAH Sand Sed** | 0.7033 | 0.2431 | 0.2431 | 0.4618 | 0.3894 | |

**Table 9.** Correlation coefficient tables across all sites. Bottom values are p-values and top values are Spearman r values. Significant p-values are in bold.

| | % Sand | % Silt | % Clay | % C | tPAHs | HPAHs |
|---|---|---|---|---|---|---|
| **% Sand** | | -0.416 | -0.5324 | -0.2093 | -0.0204 | -0.0610 |
| **% Silt** | **0.0018** | | -0.2764 | 0.3196 | 0.4915 | -0.1263 |
| **% Clay** | **<0.0001** | **0.0431** | | -0.007366 | -0.3110 | 0.1945 |
| **% C** | 0.1288 | **0.0185** | 0.9578 | | 0.2789 | 0.2360 |
| **tPAHs** | 0.8931 | **0.0005** | **0.0354** | 0.0605 | | 0.1188 |
| **HPAHs** | 0.6907 | 0.2004 | 0.2004 | 0.1186 | 0.4371 | |

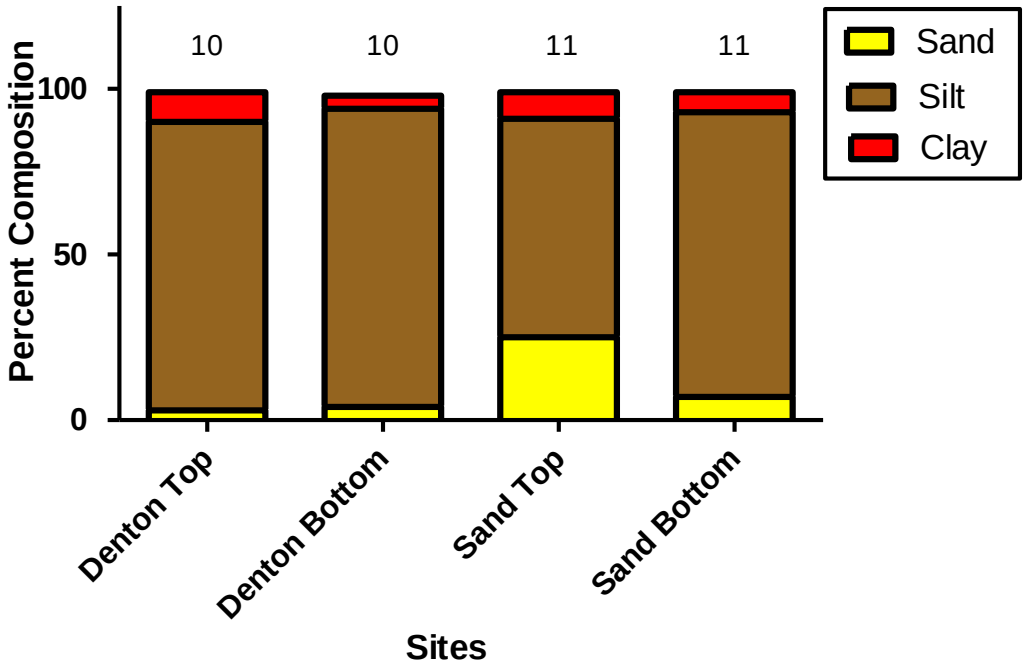


**Figure 17.** Particle size distribution at Denton and Sand Reefs (n = labeled above bars). Freeze-dried samples were analyzed on a Horiba model LA-910 laser scattering particle size analyzer to obtain the data. Particle sizes were classified as sand (2 mm – 64 μm), silt (63 μm – 2 μm), or clay (< 2 μm). To test if there were any differences in % composition between the sites, a one-way ANOVA was performed after the data were arcsin transformed. At $p \leq 0.05$, no significant differences were found.

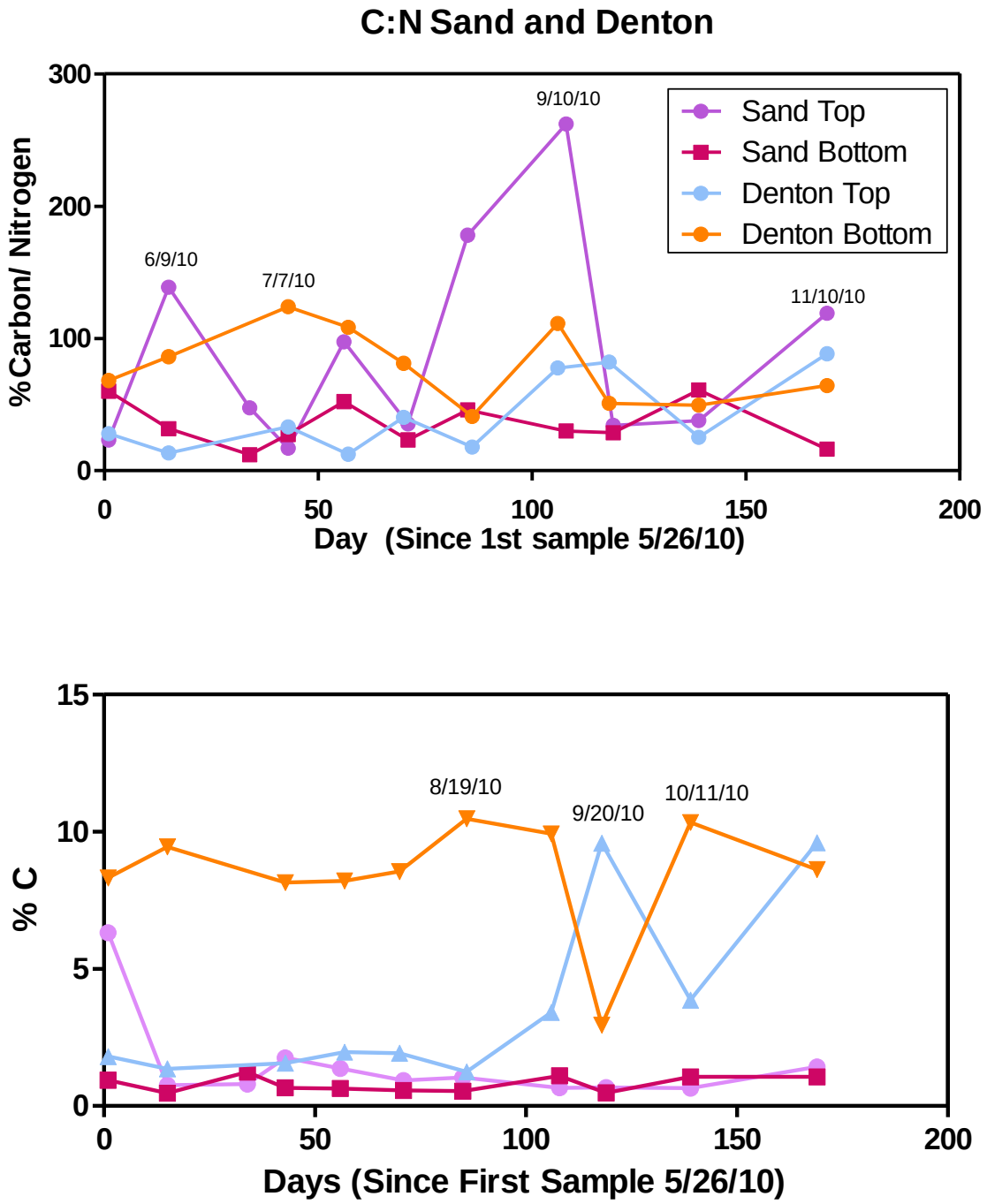


**Figure 18.** %C:N ratio in sediment at Sand and Denton Reefs over time (*top*). %C in sediment at Sand and Denton Reefs over time(*bottom*).

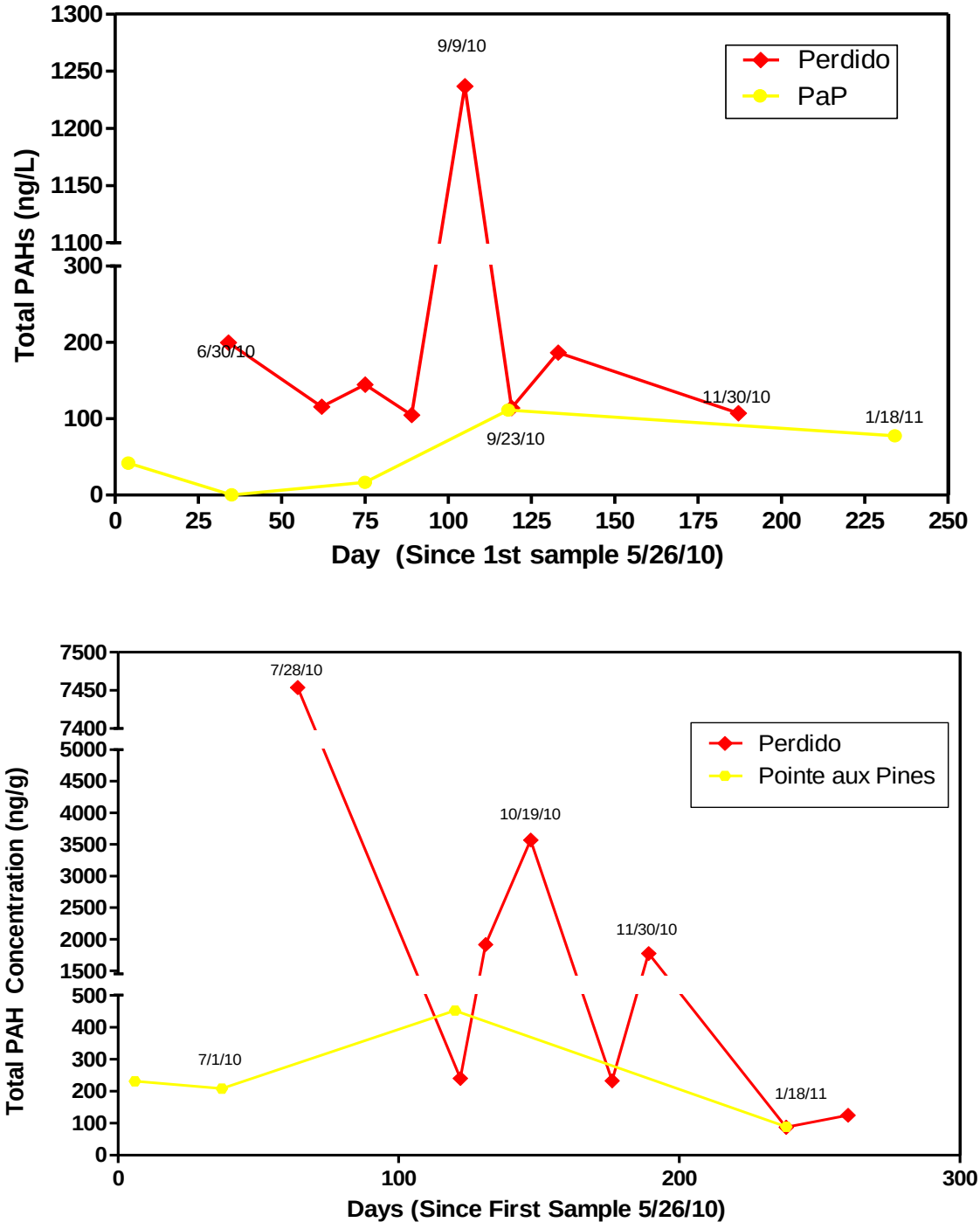


**Figure 19.** Total PAH water concentrations (*top*) and total PAH sediment concentrations (*bottom*) at Perdido and Pointe aux Pines sites over time. To test if there was a statistical change over time, a one-way ANOVA was used. At a $p \leq 0.05$, no statistical difference was found at either site.

**Table 10.** Temporal and spatial distribution of water total PAHs ng/L (% HMW PAHs) at Perdido and PAP summing only those PAH concentrations that were above the reporting limit (20 ng/L). nd = non-detectable, "—" = sample not available.

| Date | Perdido | Pointe aux Pines |
|---|---|---|
| **5/31/2010** | -- | nd |
| **6/30/2010** | 116 (0) | -- |
| **7/1/2010** | -- | nd |
| **7/14/2010** | 27.2 (0) | -- |
| **7/28/2010** | 48.5 (0) | -- |
| **8/10/2010** | 53.7 (0) | nd |
| **8/24/2010** | 78.6 (0) | -- |
| **9/9/2010** | 1100 (0) | -- |
| **9/22/2010** | -- | 28.2 (0) |
| **9/23/2010** | 73.7 (0) | -- |
| **10/7/2010** | 82.5 (0) | -- |
| **11/30/2010** | 107 (0) | -- |
| **1/18/2011** | 22.6 (0) | nd |
| **2/9/2011** | nd | -- |

**Table 11.** Temporal and spatial distribution of total sediment PAH ng/g (%HMW PAH) at Perdido and PAP summing only those PAH concentrations that were above the reporting limit (3.33 ng/g dry weight).

| Date | Perdido | Pointe aux Pines |
|---|---|---|
| **5/31/2010** | - | 228 (28.0) |
| **7/1/2010** | - | 208 (57.2) |
| **7/28/2010** | 7450 (55.8) | - |
| **9/22/2010** | - | 450 (30.8) |
| **9/24/2010** | 233 (23.1) | - |
| **10/3/2010** | 1920 (54.5) | - |
| **10/19/2010** | 3570 (57.0) | - |
| **11/17/2010** | 223 (23.3) | - |
| **11/30/2010** | 1780 (54.2) | - |
| **1/18/2011** | 79.7 (53.6) | 89.1 (53.6) |
| **2/9/2011** | 125 (0) | - |

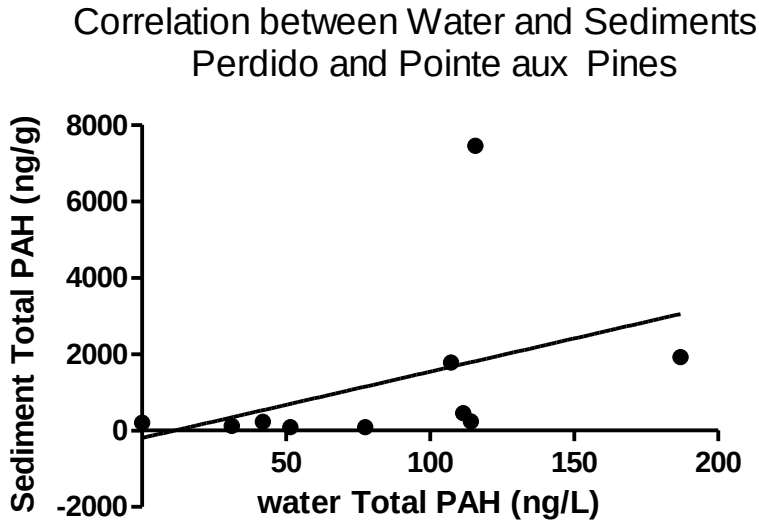


**Figure 20.** Correlation between total PAH concentrations in water and sediment samples at Perdido and PAP. To test whether there was a correlation between tPAHs in water versus sediment, a Pearson correlation was used. At a $p \leq 0.05$, no statistical difference was found.

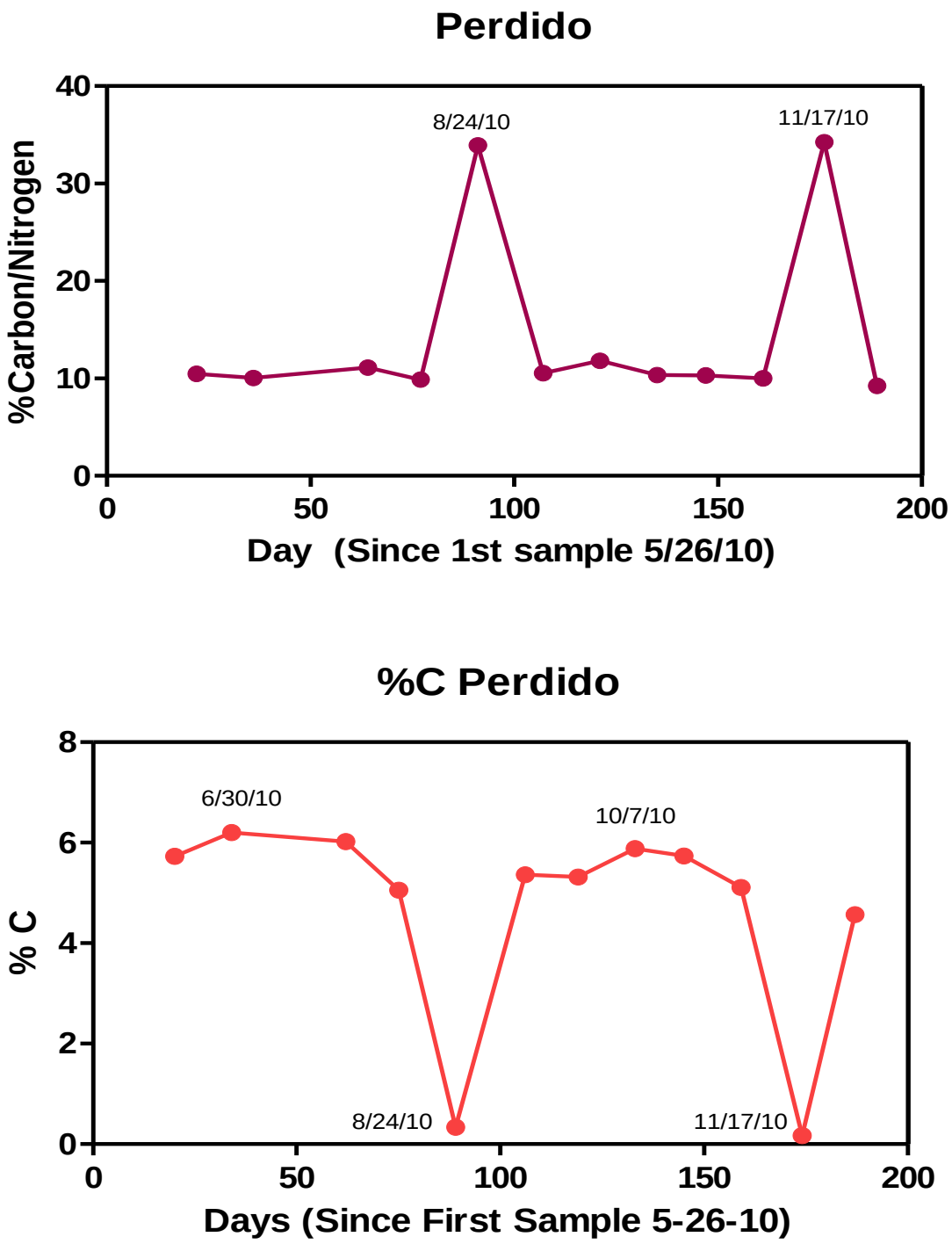


**Figure 21.** The temporal pattern of the %C/N ratio (*top*) vs %C (*bottom*) at Perdido.

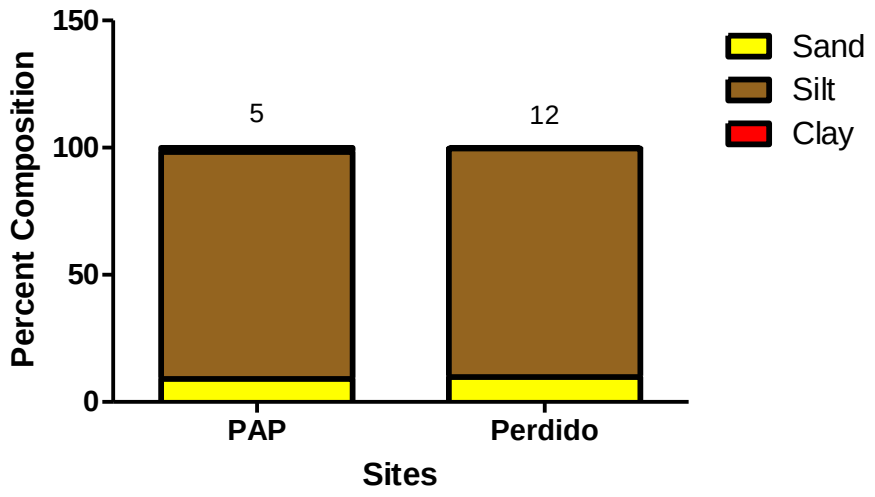


**Figure 22.** Particle size distribution at PAP and Perdido (n = labeled above bars). Freeze-dried samples were analyzed on a Horiba model LA-910 laser scattering particle size analyzer to obtain the data. Particle sizes were classified as sand (2 mm – 64 μm), silt (63 μm – 2 μm), or clay (< 2 μm). To test if there were any differences in % composition between the sites, a one-way ANOVA was performed after the data were arcsin transformed. At $p \leq 0.05$, no significant differences were found.

**Table 12.** Temporal and spatial distribution of water total PAHs ng/L (% HMW PAHs) at spatial sites summing only those PAH concentrations that were above the reporting limit (20 ng/L). nd = non-detectable, "—" = sample not available.

| Site | 6/30/2010 | 7/10/2010 | 1/18/2011 | 2/9/2011 |
|---|---|---|---|---|
| **Fairhope** | 49.9 | - | nd | - |
| **Rabbit Creek** | - | - | 169 | - |
| **Fowl River** | - | - | 85.9 | - |
| **Steele Creek** | - | - | 935 | - |
| **Little Lagoon** | - | - | - | nd |
| **Mobile Bay** | - | nd | - | - |
| **Grand Bay Bayou Heron** | - | nd | - | - |
| **Grand Bay Bayou Combust** | - | nd | - | - |
| **Pascagoula River** | - | 66.9 | - | - |
| **Univ Southern MS-GCRL** | - | 20.7 | - | - |
| **Biloxi Gulf** | - | nd | - | - |
| **Biloxi Back Bay** | - | nd | - | - |
| **Gulfport Courthouse** | - | nd | - | - |
| **Gulfport Gulf** | - | nd | - | - |

**Table 13A.** Temporal and spatial distribution of total sediment PAH ng/g (%HMW PAH) at spatial sites using the calibration curve.

| Site | 6/30/2010 | 7/10/2010 | 7/14/2010 | 1/18/2011 | 2/8/2011 | 2/9/2011 |
|---|---|---|---|---|---|---|
| **Fairhope** | 239.4 (20.8) | - | - | 12.3 (80.7) | - | - |
| **Rabbit Creek** | - | - | - | 342 | - | - |
| **Fowl River** | - | - | - | 402.9 | - | - |
| **Steele Creek** | - | - | - | 49.4 | - | - |
| **Little Lagoon** | - | - | - | - | 23.2 | - |
| **Sandy Bay** | - | - | - | - | 29.4 | - |
| **Fort Morgan** | - | - | - | - | - | 73.5 |
| **Alabama Port** | - | - | - | 342 | - | - |
| **GB NERR** | - | - | - | - | 0.00 | - |
| **Cedar Point** | - | - | - | - | 76.3 | - |
| **Palmetto Creek** | - | - | - | - | - | 1940 |
| **Wolf Bay** | - | - | - | - | - | 42.1 |
| **Pass Hotel** | - | - | 1440 | - | - | - |
| **Mobile Bay** | - | 437 (39.8) | - | - | - | - |
| **Grand Bay Bayou Heron** | - | 433 (53.1) | - | - | - | - |
| **Grand Bay Bayou Combust** | - | 300 (54.1) | - | - | - | - |
| **Pascagoula River** | - | 243 (26.6) | - | - | - | - |
| **Univ Southern MS-GCRL** | - | 867 (49.8) | - | - | - | - |
| **Biloxi Gulf** | - | 30.7 (19.0) | - | - | - | - |
| **Biloxi Back Bay** | - | 1900 (44.0) | - | - | - | - |
| **Gulfport Courthouse** | - | 735 (54.0) | - | - | - | - |
| **Gulfport Gulf** | - | 163 (0) | - | - | - | - |

**Table 13B.** Temporal and spatial distribution of total sediment PAH ng/g (%HMW PAH) at spatial sites using the reporting limits (6.33 ng/g dry weight)

| Site | 6/30/2010 | 7/10/2010 | 7/14/2010 | 1/18/2011 | 2/8/2011 | 2/9/2011 |
|---|---|---|---|---|---|---|
| **Fairhope** | 233 (20.8) | - | - | nd | - | - |
| **Rabbit Creek** | - | - | - | 342 | - | - |
| **Fowl River** | - | - | - | 402.9 | - | - |
| **Steele Creek** | - | - | - | 40.9 | - | - |
| **Little Lagoon** | - | - | - | - | 20.6 | - |
| **Sandy Bay** | - | - | - | - | 23.6 | - |
| **Fort Morgan** | - | - | - | - | - | 73.5 |
| **Alabama Port** | - | - | - | 24.9 | - | - |
| **GB NERR** | - | - | - | - | nd | - |
| **Cedar Point** | - | - | - | - | 76.3 | - |
| **Palmetto Creek** | - | - | - | - | - | 1940 |
| **Wolf Bay** | - | - | - | - | - | 42.1 |
| **Pass Hotel** | - | - | 1440 | - | - | - |
| **Mobile Bay** | - | 428 (39.8) | - | - | - | - |
| **Grand Bay Bayou Heron** | - | 433 (53.1) | - | - | - | - |
| **Grand Bay Bayou Combust** | - | 300 (54.1) | - | - | - | - |
| **Pascagoula River** | - | 243 (26.6) | - | - | - | - |
| **Univ Southern MS-GCRL** | - | 864 (49.8) | - | - | - | - |
| **Biloxi Gulf** | - | 28.3 (19.0) | - | - | - | - |
| **Biloxi Back Bay** | - | 1900 (44.0) | - | - | - | - |
| **Gulfport Courthouse** | - | 732 (54.0) | - | - | - | - |
| **Gulfport Gulf** | - | 163 (0) | - | - | - | - |

**Table 14.** Average PAHs per site (ng/L or ng/g) ± standard error (n value) compared to reporting limit. nd=non-detectable.

| Sites | Total PAH Water (ng/L) | Total PAH Sediment (ng/g) | Total PAH Oyster (ng/g) |
|---|---|---|---|
| Denton Top | 11.1± 33.4 (9) | 147± 157 (10) | 91.3± 6.81 (3) |
| Denton Bottom | 24.6 ± 62.7 (11) | - | 134± 65.9 (3) |
| Sand Top | 28.7± 77.3 (9) | 52.7± 23.9 (11) | 67.4±20.6 (8) |
| Sand Bottom | nd (10) | - | 101± 50.6 (5) |
| Perdido | 163± 332 (11) | 1930± 2550 (8) | - |
| Pointe aux Pines | 5.64± 12.6 (5) | 245± 151 (4) | 278± 235 (2) |
| Fairhope | 24.9± 35.3 (2) | 116± 165 (2) | - |
| Rabbit Creek | 169 (1) | 341 (1) | - |
| Steel Creek | 935 (1) | 40.9 (1) | - |
| Fowl River | 859 (1) | 403 (1) | - |
| Little Lagoon | nd (1) | 20.6 (1) | - |
| Mobile Bay | nd (1) | 428 (1) | - |
| Grand Bay Bayou Heron | nd (1) | 433 (1) | - |
| Grand Bay Bayou Combust | nd (1) | 300 (1) | - |
| Pascagoula River | 66.9 (1) | 263 (1) | - |
| Gulf Coast Research Center | 20.7 (1) | 864 (1) | - |
| Biloxi Gulf | nd (1) | 28.3 (1) | - |
| Biloxi Back Bay | nd (1) | 1900 (1) | - |
| Gulfport Courthouse | nd (1) | 732 (1) | - |
| Gulfport Gulf | nd (1) | 163 (1) | - |
| Sandy Bay | - | 23.6 (1) | - |
| Fort Morgan | - | 73.5 (1) | - |
| Alabama Port | - | 24.9 (1) | - |
| GBNERR | - | ND (1) | - |
| Cedar Point | - | 76.3 (1) | - |
| Palmetto Creek | - | 1940 (1) | - |
| Wolf Bay | - | 42.1 (1) | - |
| Pass Hotel | - | 1440 (1) | - |
| Grand Bay, AL | - | 29.3± 1.80 (2) | - |
| Grand Bay, MS | - | 33.5± 19.2 (2) | - |
| Jose Bay | - | 27.8± 16.2 (2) | - |
| Gulf Island National Seashore | - | 66.1± 52.2 (4)<br>111± 1.20 (2: 7/12/10)<br>21.3± 13.1 (2: 10/7/10) | - |

| | | | |
|---|---|---|---|
| Pointe aux Chines | - | 20.9± 5.04 (2) | - |
| Middle Bay | - | 79.5± 54.4 (2) | - |

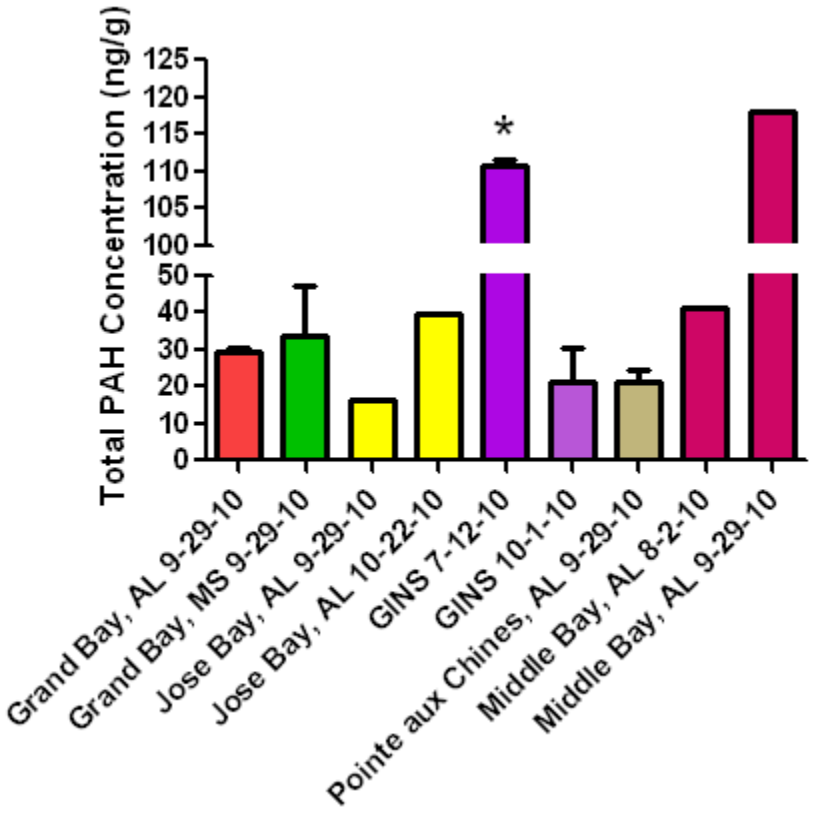


**Figure 23.** Average total PAH sediment concentrations of sites associated with the seagrass project according to date and site. (n values were 1 or 2). To test if there were any differences between all the sites, a one-way ANOVA was used. At a $p \leq 0.05$, no statistical differences were found. To test if the change between GINS 7-12-10 and GINS 10-1-10 was significantly different, an unpaired t-test was used. At a $p \leq 0.05$, the change was significant (*).

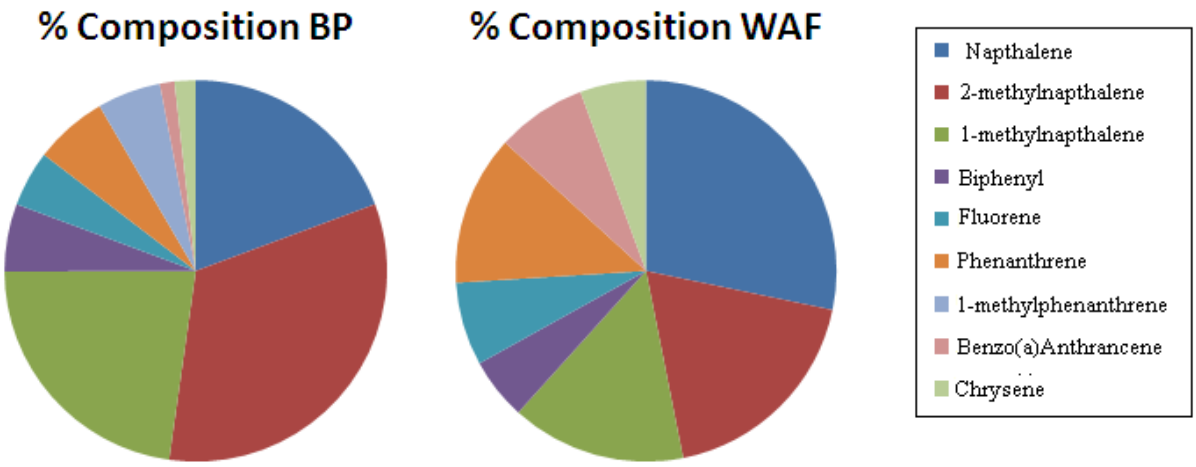


**Figure 24.** Percent composition of PAHs in BP oil sample and in WAF sample. WAF includes dispersant.

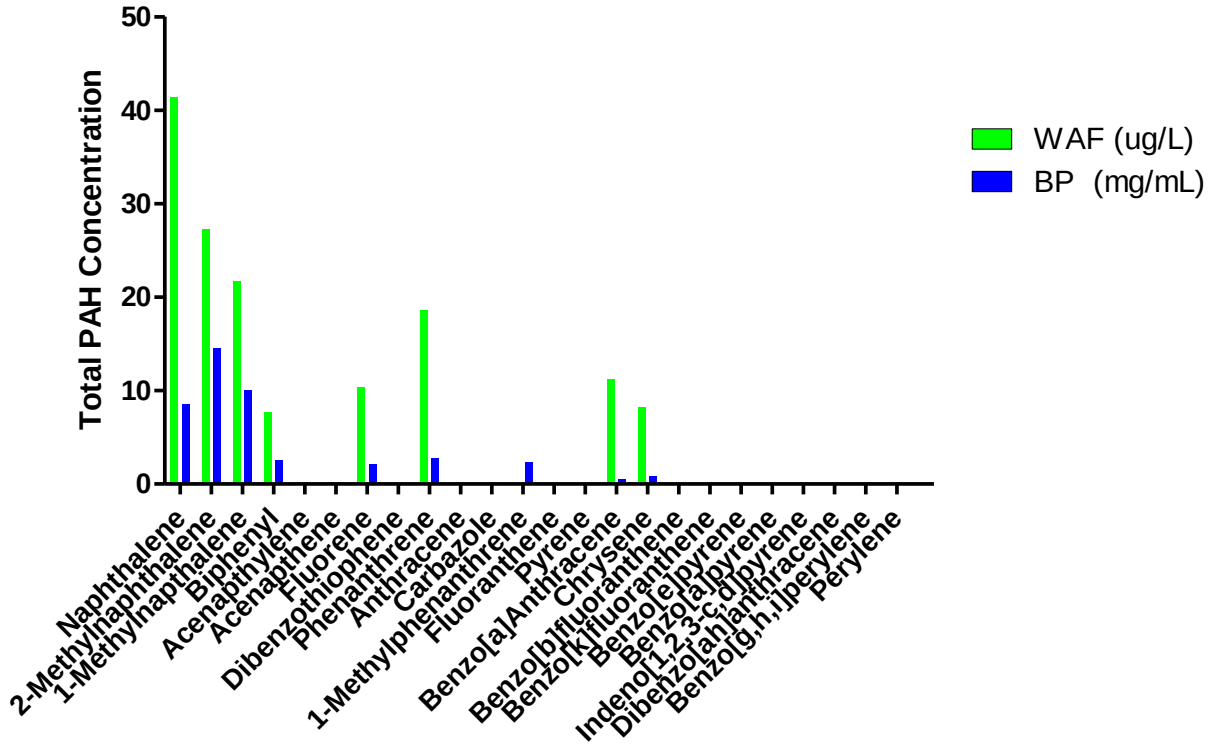


**Figure 25.** Comparative composition of BP Oil sample vs. WAF sample. WAF includes dispersant.

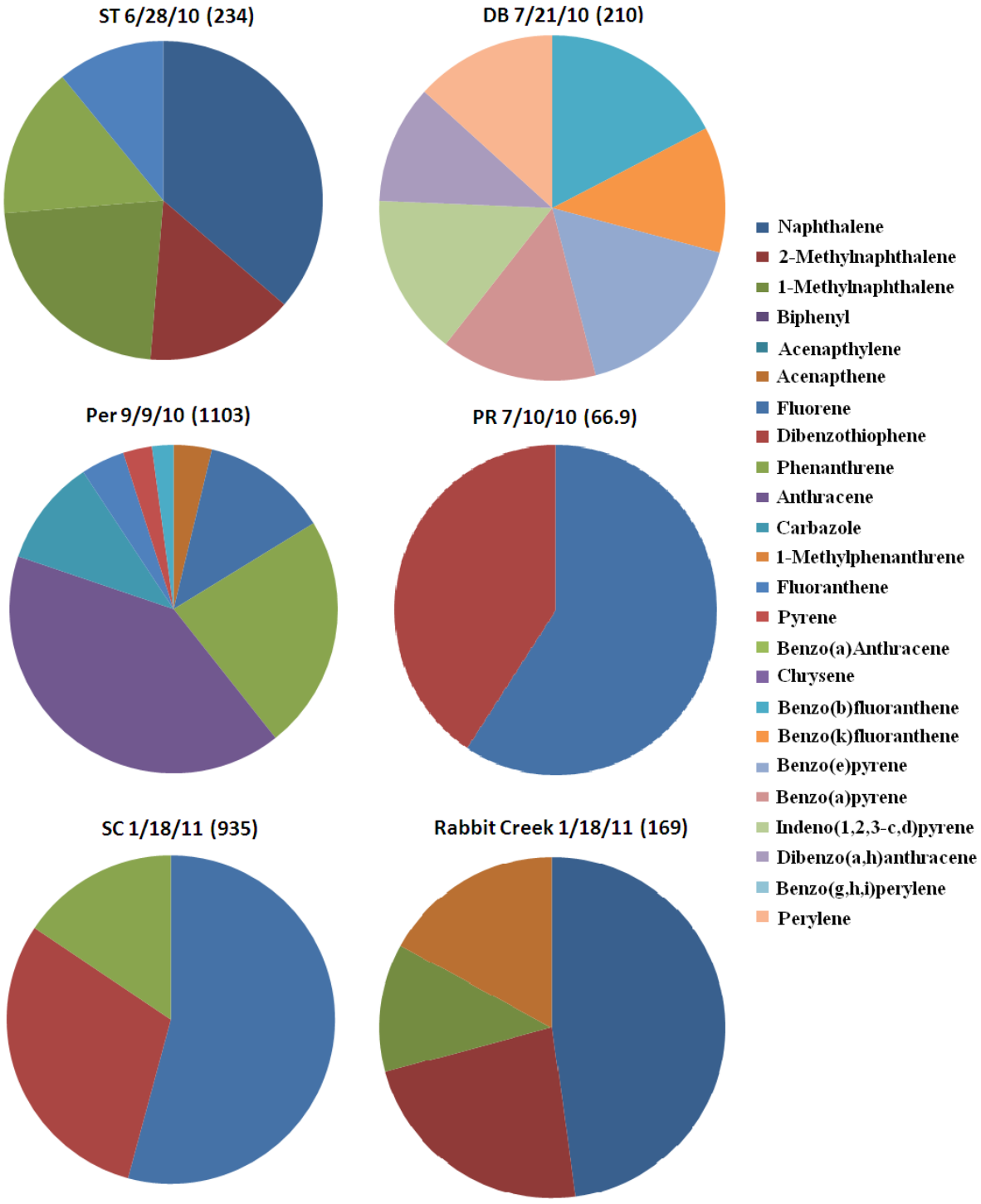


**Figure 26.** Percent composition of individual PAHs in water at ST, DB, Perdido, Pascagoula River, Steele Creek and Rabbit Creek. The actual tPAH concentrations, the highest reported per site, are in (ng/L).

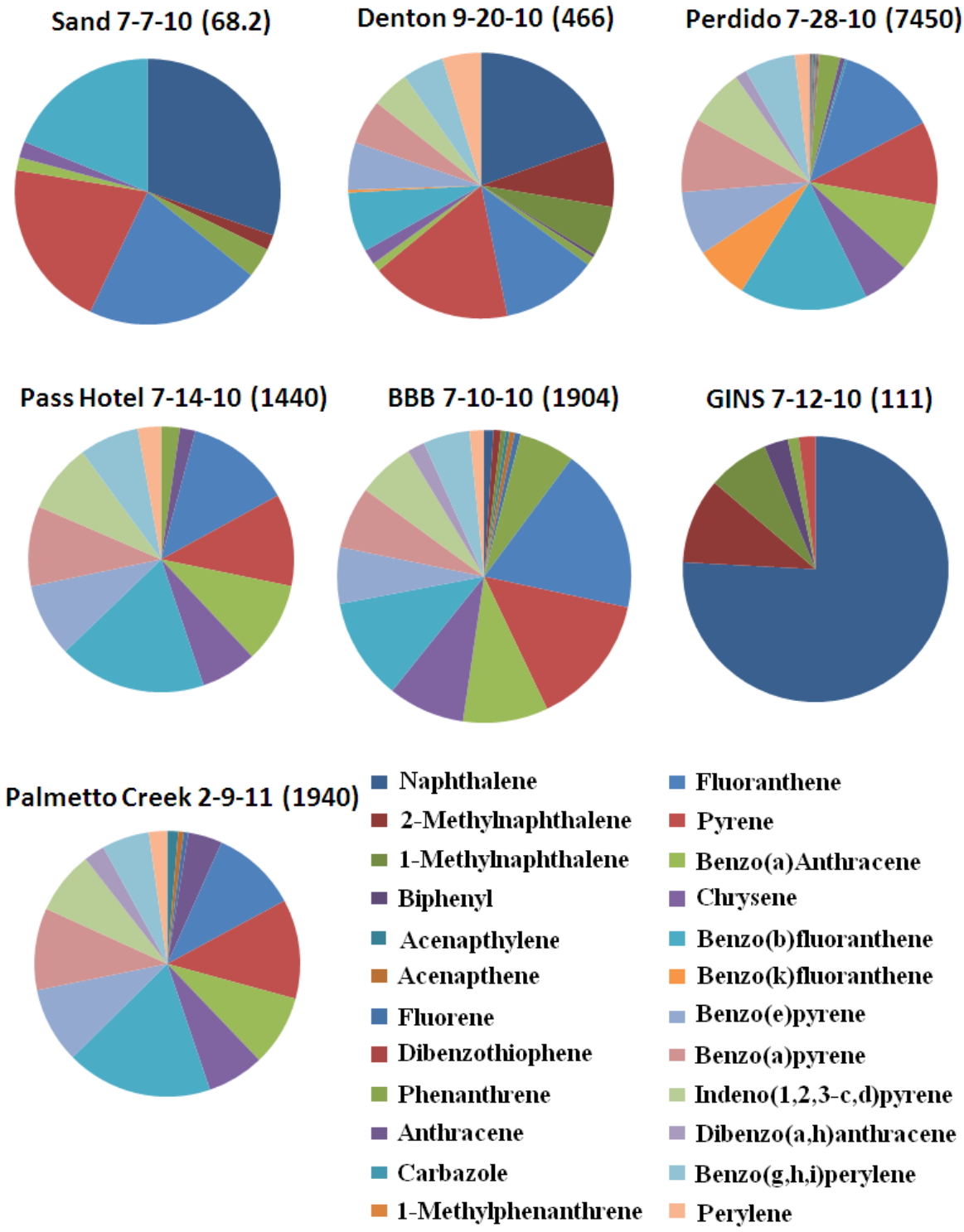


**Figure 27.** Percent composition of individual PAHs in sediment at Denton and Sand Reefs, Perdido, Pass Hotel, Biloxi Back Bay, Gulf Island National Seashore, and Palmetto Creek. The actual tPAH concentrations, the highest reported per site, are in (ng/g dry weight).

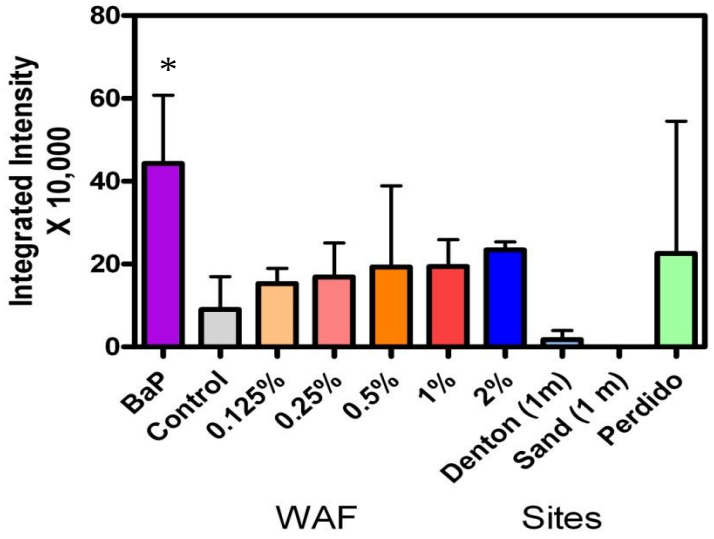


**Figure 28.** EROD activity was used as a biomarker for *in ovo* AhR-mediated CYP1A induction. Percentages represent the percent of WAF + Corexit in water. n values ranged from 2 to 5. BaP (100 µg/L) was used as a positive control. To test if there were any differences between exposures, a one-way ANOVA was used. BAP was statistically different from the control (*), otherwise no statistical significance was found.

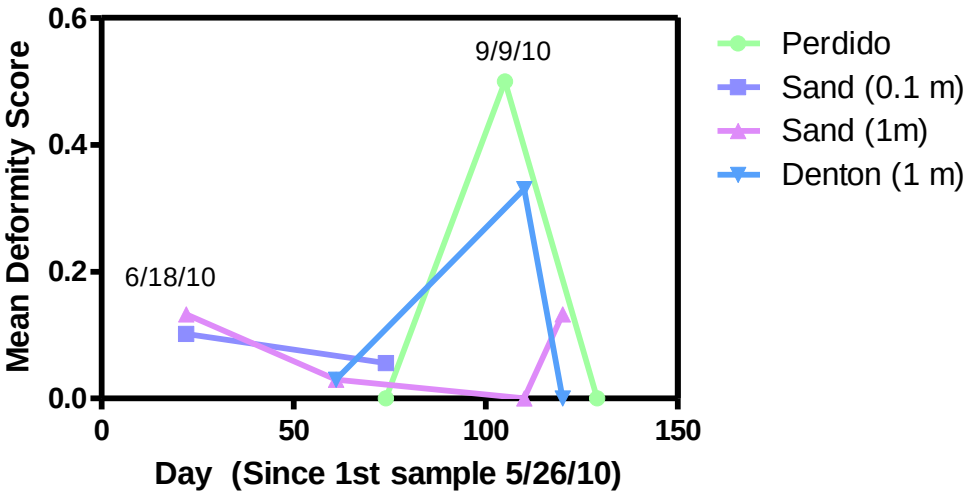


**Figure 29.** *F. heteroclitus* embryo mean deformity score at 10 dpf shown over time (5 dpf not shown). No significant differences were found (ANOVA, n=3-21 vials, 10 embryos/vial).

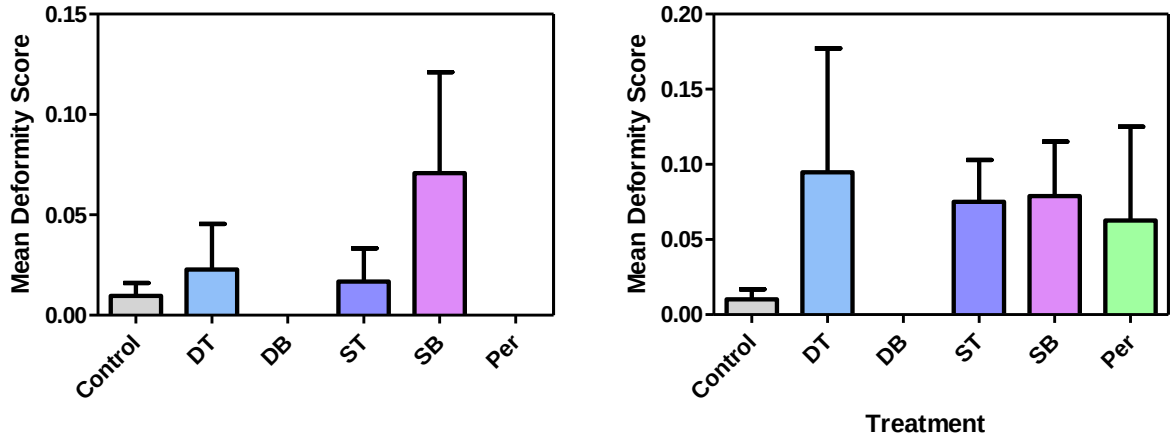


**Figure 30.** *F.heteroclitus* embryo deformity index at 5 (left) and 10 (right) dpf. No significant differences were found (ANOVA, n=3-21 vials, 10 embryos/vial). Bars represent standard error.

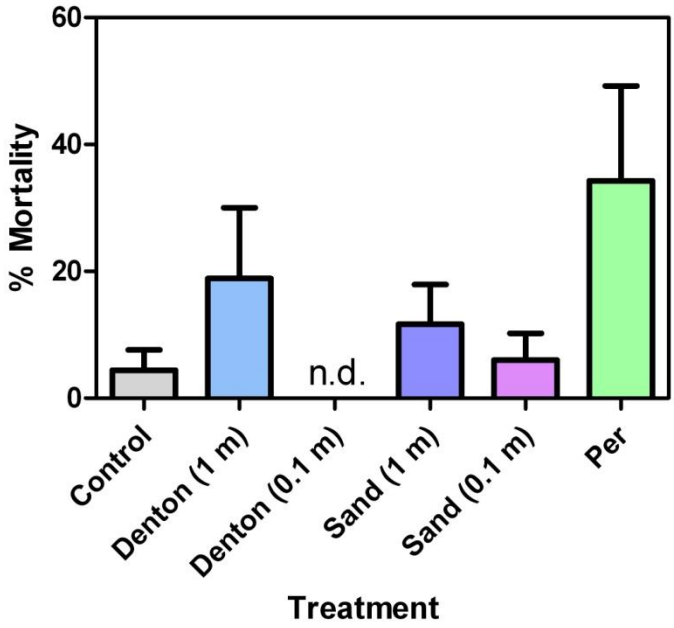


**Figure 31.** *F. heteroclitus* embryo mortality incidence. No significant differences were found (ANOVA, 3 vials per treatment, 10 embryos/vial). Bars represent standard error.

# CHAPTER 4 CONCLUSION AND FUTURE WORK

As with any research undertaking, lessons were learned that had it been done differently the study would be even more robust. Specifically, sample collection could have been linked more closely with oil spill projection maps, quantitation of alkylated PAHs may have improved fingerprinting capabilities, and additional oyster extraction clean-up steps might have enhanced PAH quantitation from that matrix.

Projection maps such as those referenced by the Conservation Biology Institute or NOAA could have been used to guide both collection sites and dates. This would have potentially allowed for a clearer picture of the extent of oil contamination and resolution of peak PAH concentrations. There were several time frames when a site was not sampled (e.g. Pointe aux Pines), but predictions said the sites were impacted by oil. In addition, had samples been taken more frequently it would have been possible to better refine the time course of impact at any given site.

Another inadequacy of this project was the fingerprinting ability. The inability to use alkylated-PAHs to fingerprint hindered the true sourcing of the PAH origins. Ratios of parent PAH compounds were used to determine petrogenic or pyrogenic. If alkylated-PAHs were used, a more specific fingerprint would have resulted in a more definitive ability to determine the origin of the PAHs. Also, if oil was present, an alkylated-PAH fingerprint would have allowed an assessment of the degree of oil weathering. Finally, this fingerprinting would have minimized

the problem of a lack of historical PAH baseline data, by allowing the separation of oiled PAHs from background PAH sources.

The interpretation of the raw oyster data is on-going. Oysters were collected from three sites (Sand, Denton and Pointe aux Pines) and pooled for tPAH determination. Of the 32 samples analyzed, 11 had surrogate standard recoveries of less than 40%. The tPAHs in the remaining 21 samples ranged from 36 to 444 ng/g dry weight. The PAH components within the oysters (Figures 32-34 in Appendix) are being compared to other projects and oil spill information.

This study primarily focused on water and sediment PAHs collected from the northern Gulf coast (Mississippi to western Florida) between May 28, 2010 and February 9, 2011. Sediment concentrations were expected to indicate a more persistent evidence of oil impact, whereas water concentrations were likely to be more transient. At Sand Reef (1 m) and Perdido, there was a peak in water tPAHs that roughly corresponded with projection maps or reported oil present at the corresponding time. At other site and collection times, even though oil impact was projected, relatively low PAH concentrations were detected (e.g. parts per trillon, <20 ng/L reporting limit). While parent PAH ratios predicted a pyrogenic ratio in all the samples, analysis of actual PAH constituents (e.g. naphthalenes and phenanthrene) as related to chemically dispersed WAF did indicate that the 6/28/10 Sand Reef sample may have had Deepwater Horizon oil contamination. In contrast, the Perdido water and sediment percent composition profile across dates was not consistent with a predicted oil profile (Appendix Figure 35). None of the sediment tPAH concentrations exceeded EPA regulatory guidelines, but the concentration range of sediments (non-detectable – 7450 ng/g dry weight) was consistent with those reported at

previous oil spill sites. The Gulf Island National Seashore sample (7/12/10; Seagrass project) was the sediment sample with the highest composition of naphthalenes (> 93%).

While the data presented in this thesis suggest relatively low to background PAH contamination, it is too soon to suggest what the environmental or toxicological consequences of the spill were. Importantly, the highest *Fundulus* deformity score and mortality were found in samples with the highest tPAH. Evidence from other oil spills (e.g. Ixtoc I in the Gulf or *Exxon Valdez* in Alaska) indicate that it will take many years before the true ecological impact of the Deepwater Horizon Oil Spill is understood.

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# APPENDIX A

# Final G.I.S. Project Report For GEOL 500

## Materials and Methods

The data that I collected online was the movement of the oil plume. There were not many sites where this information was available, due to the movement of the oil not being well understood, but I did find what was needed. The website I used was http://databasin.org/; this is a free, online system that connects users with spatial datasets, tools, and expertise. Allowing individuals and organizations to explore and download datasets, upload data, create and publish analysis, utilize working groups, and produce customized maps that can be easily shared. From this site, I downloaded the shape file of the oil plume for May 28, 2010; June 24, 2010; and July 15, 2010. These 3 dates correspond with sampling dates of mine and will look at "pre", "during", and "after" the oil plume hit the Alabama/Mississippi coast. I would have preferred a later date to use as the "after" oil date, but the oil plume shape files stopped in July.

The GPS coordinates of my sites in decimal degree format were:

| Sites | Latitude | Longitude |
|---|---|---|
| Sand Reef | 30.274083 | -88.0959 |
| Mobile Bay by Yacht Club | 30.576667 | -88.086333 |
| Denton Reef | 30.412133 | -88.070917 |
| Perdido | 30.30885 | -87.51488 |
| Grand Bay Bayou Heron | 30.413 | -88.402833 |
| Grand Bay Bayou Cumbest | 30.388333 | -88.4485 |
| Pointe aux Pines | 30.377222 | -88.315277 |
| Pascagoula Boat ramp | 30.376 | -88.565333 |
| USM Boat Ramp | 30.3925 | -88.796833 |
| Biloxi Back Bay | 30.377 | -88.891667 |
| Biloxi Gulf | 30.391667 | -88.868667 |
| Gulfside Courthouse | 30.426667 | -89.043667 |
| Gulfport Gulfside | 30.368633 | -89.08075 |

I have been monitoring total PAH concentrations in the water column at these sites. The following is a table of the concentrations at each date.

The objective of this project was to see if the oil plume encountered my sampling sites and if there may be a PAH concentration correlation. The oil plume did encounter my sampling site, Perdido on June 24, 2010. For this site, the rise and fall of PAH concentrations may be due to the movement of the oil plume. This project also showed that the oil plume did not encounter a majority of my sites, suggesting that other sources of pollution should be considered the culprit.

| Sites | 5-28-10 (ng/L) | 6-24-10 (ng/L) | 7-15-10 (ng/L) |
|---|---|---|---|
| Sand Reef | 62 | 57 | 65 |
| Mobile Bay by Yacht Club | 0 | 44 | 0 |
| Denton Reef | 15 | 59 | 182 |
| Perdido | 0 | 200 | 69 |
| Grand Bay Bayou Heron | 0 | 5 | 0 |
| Grand Bay Bayou Cumbest | 0 | 0 | 0 |
| Pointe aux Pines | 42 | 0 | 0 |
| Pascagoula Boat ramp | 0 | 148 | 0 |
| USM Boat Ramp | 0 | 0 | 0 |
| Biloxi Back Bay | 0 | 37 | 0 |
| Biloxi Gulf | 0 | 0 | 0 |
| Gulfside Courthouse | 0 | 62 | 0 |
| Gulfport Gulfside | 0 | 0 | 0 |

To create my final maps, I started by opening up my oil plume shapefile for May 28, 2010 in ArcGIS. I then added a borders and places shapefile in WGS 84 projection. I had this file saved on my work computer from a previous user. I also added the location of the Deepwater Horizon well head shapefile to the map.

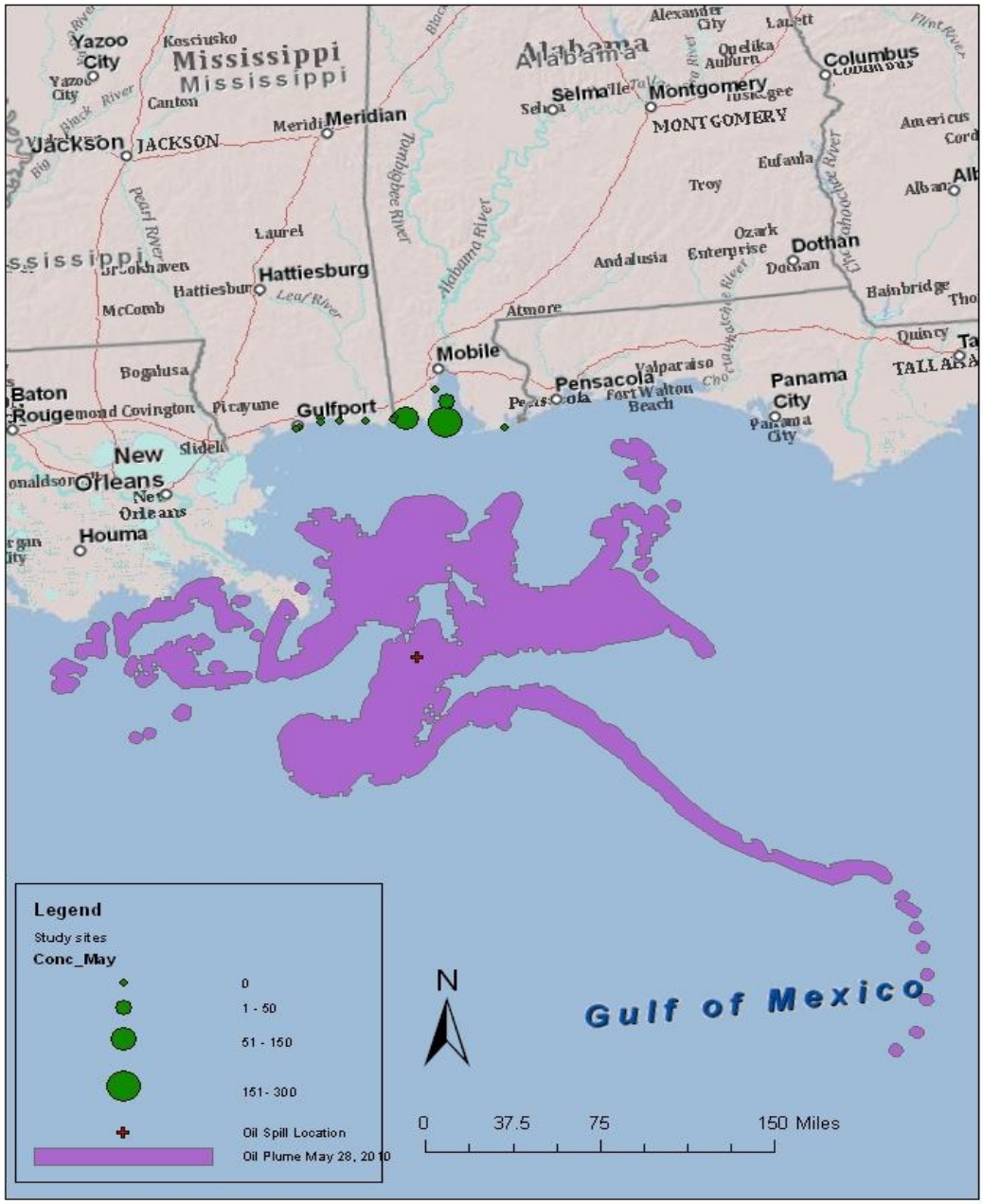
Then due to issues loading my excel file of coordinates for my sampling sites, I added the "editor" toolbar and began editing, to build a new attribute table of my sample sites. I added the points to the map, then labeled them on the attribute table and added the concentrations per date to the attribute table (see results for attribute table). I also labeled the sites according to which research project they corresponded with, Katrina or Oyster. I saved my edits and ended editing. Then I symbolized the sites with graduated symbols. As the concentrations of PAHs increases, the circles increase. I kept the same scale between the three dates in order to make comparison easier.

To make the June and July map, I used the May map as a base, deleted the oil plume shape file and added the oil plume of the appropriate dates. For the sampling sites, I changed the symbolization to be based on the corresponding dates. The appropriate items were added to the final layout. I exported the final layout to a jpg in order to be able to paste the image into this report.

Results

Map 1

Location of Deepwater Horizon Oil Plume May 28, 2010




Meghan Dailey Section 1: Date: November 27, 2011

Table 1

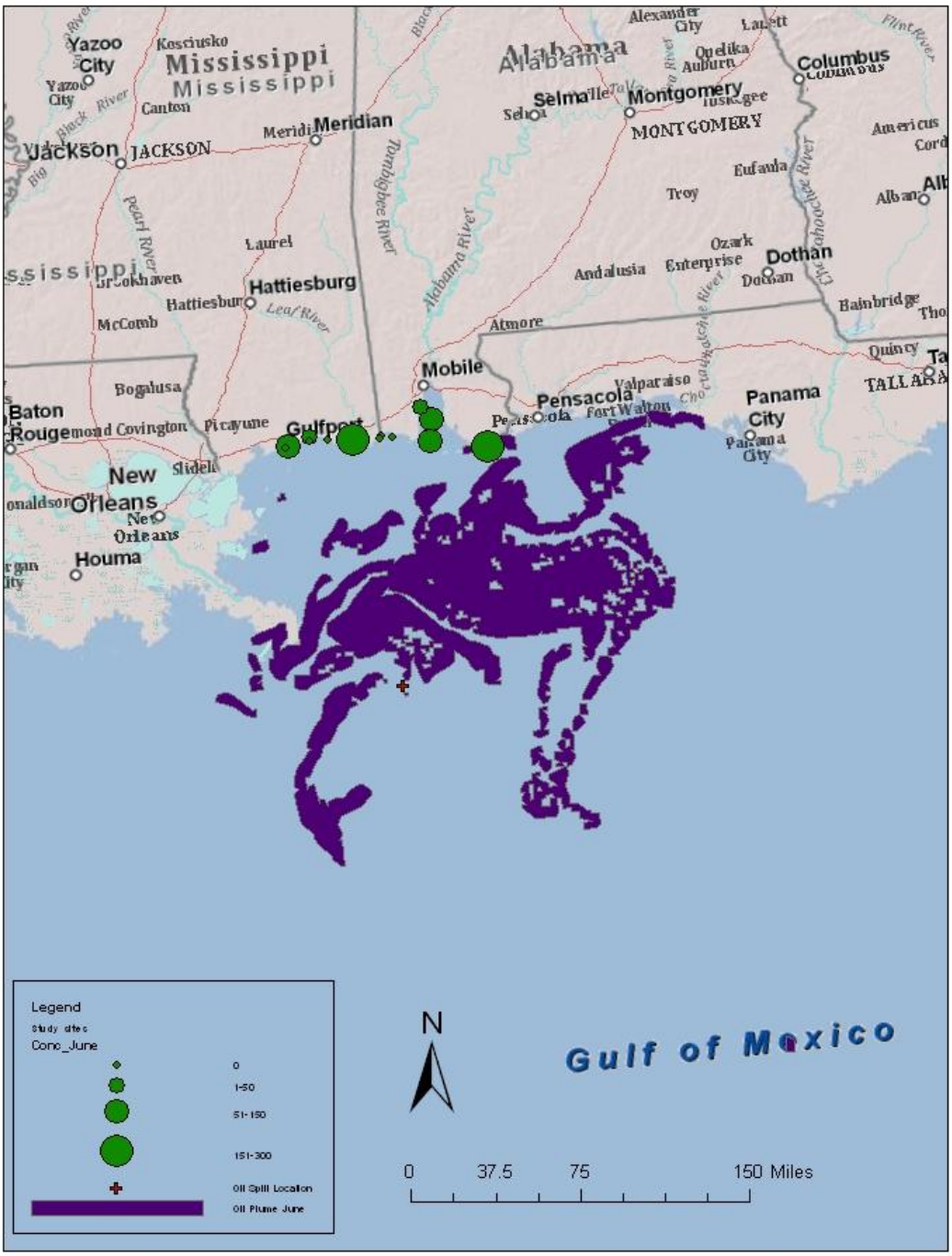
| FID_ | Id | Sample_sit | Site_type | Conc_May | Conc_June | Conc_July |
|---|---|---|---|---|---|---|
| | 0 | Sand Reef | Oyster | 62 | 57 | 65 |
| | 0 | Mobile bay | Katrina | 0 | 44 | 0 |
| | 0 | Denton Reef | Oyster | 15 | 59 | 182 |
| | 0 | Perdido | Oyster | 0 | 200 | 69 |
| | 0 | GB Bayou Heron | Kartina | 0 | 5 | 0 |
| | 0 | GBBC | Katrina | 0 | 0 | 0 |
| | 0 | PaP | Oyster | 42 | 0 | 0 |
| | 0 | Pascagoula Rv | Katrina | 0 | 148 | 0 |
| | 0 | USM | Katrina | 0 | 0 | 0 |
| | 0 | Biloxi Back Bay | Katrina | 0 | 37 | 0 |
| | 0 | Biloxi Gulf | Katrina | 0 | 0 | 0 |
| | 0 | GP Courthouse | Katrina | 0 | 62 | 0 |
| | 0 | Gulfport Gulf | Katrina | 0 | 0 | 0 |

Shown above, are Map 1 and Table 1. Map 1 shows the location of the oil plume due to the Deepwater Horizon oil leak as of May 28, 2010. Mapped along the coastline, in green circles, are the sampling sites, where water was gathered. The sizes of the circles correspond with the concentration of PAHs. As the concentrations increase, the sizes of the circles increase. We are looking to see if oil has invaded any of the sampling sites and determine a correlation between the oil location and PAH concentrations

Table 1, shown above, is the attribute table of my sampling sites. It shows sample site names, site type (based on which project the site corresponds with), and PAH concentrations found at these sites on the corresponding dates. The purpose of separating the sites based on which project they originate from was to normalize the sites so that it would be easy to compare the different concentrations within each project.

Map 2

Location of Deepwater Horizon Oil Plume June 24, 2010

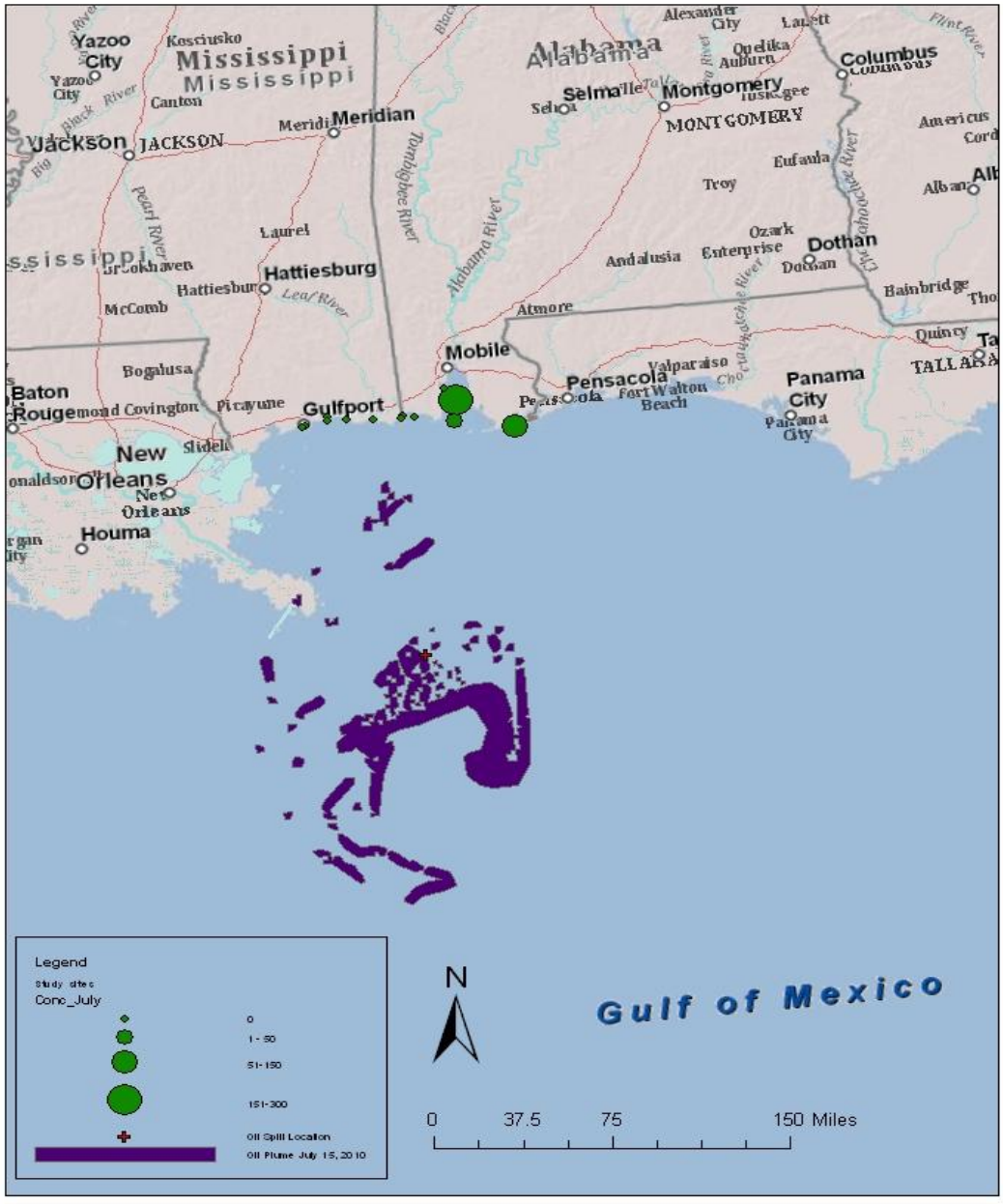



Meghan Dailey Section 1: Date: November 27, 2011

Map 2, shown on the previous page, shows the location of the oil plume on June 24, 2010.

**Map 3**

Location of Deepwater Horizon Oil Plume July 15, 2010




Meghan Dailey Section 1: Date: November 27, 2011

Map 3, as shown above, shows the location of the oil plume on July 15, 2010.

# APPENDIX B

**Table 15.** Temporal and spatial distribution of adult oyster total PAHs (ng/g dry weight) and Sand and Denton Reefs and Pointe aux Pines. The oyster wet weights ranged from 3.05-17.0 g. The reporting limit ranged from 6.02-40.1 ng/g. The reporting limit was calculated using the concentration of the lowest calibration standard (0.02 ng/µL) and multiplying by the final volume (500 µL) then dividing by the original sample dry weight. An asterisk (*) denotes that the internal standard was added to the sample before being transferred through a clean-up column. A carrot (^) denotes that the sample was not transferred through a clean-up column. If no notation was made, the internal standard was added following the clean-up column. ND denotes that surrogate standard recoveries were below 40%, and "-" denotes that no sample was taken at that site on that particular date.

| **Total PAH (ng/g dry weight)** | | | | | |
|---|---|---|---|---|---|
| **Site** | **ST** | **SB** | **DT** | **DB** | **PAP** |
| **Date** | | | | | |
| **6/9/10** | 85^ | 47^ | 117^ | ND | - |
| **6/28/10** | 117^ | 206^ | - | ND | - |
| **7/1/10** | - | - | - | - | ND |
| **7/7/10** | ND | ND | 101* | 220 | - |
| **7/20/10** | 513 | ND | 99* | 71* | - |
| **8/3/10** | ND | 129* | - | 181* | - |
| **8/9/10** | - | - | - | - | 522^ |
| **8/19/10** | 52* | ND | ND | - | - |
| **9/8-10/10** | 61* | ND | - | - | - |
| **9/20/10** | 71* | ND | - | - | - |
| **10/11/10** | 77^ | 94* | - | - | - |
| **11/10/10** | 42* | 94^ | - | - | - |
| **1/18/11** | - | - | - | - | 121^ |

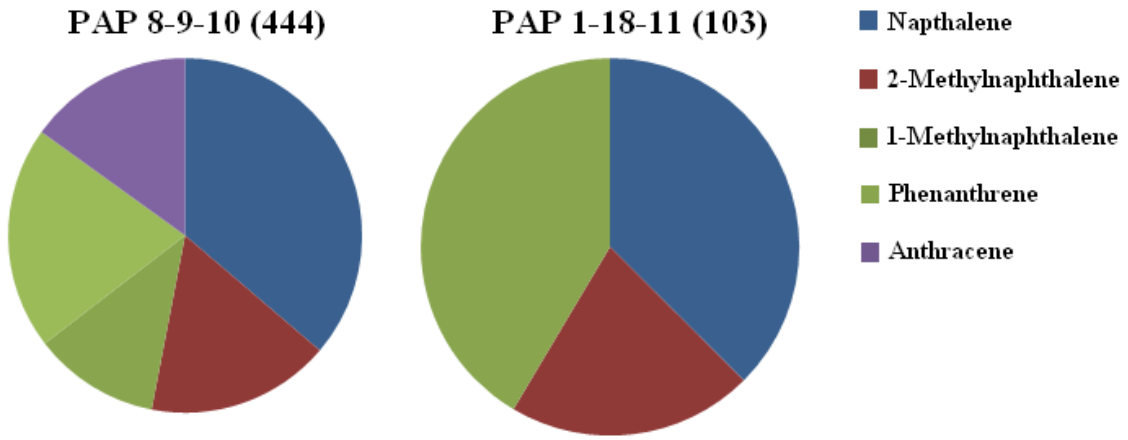



**Figure 32.** Percent composition of individual PAHs from Pointe aux Pines Reef oysters. Sample collection date and (tPAH concentration in ng/g dry weight) are indicated.

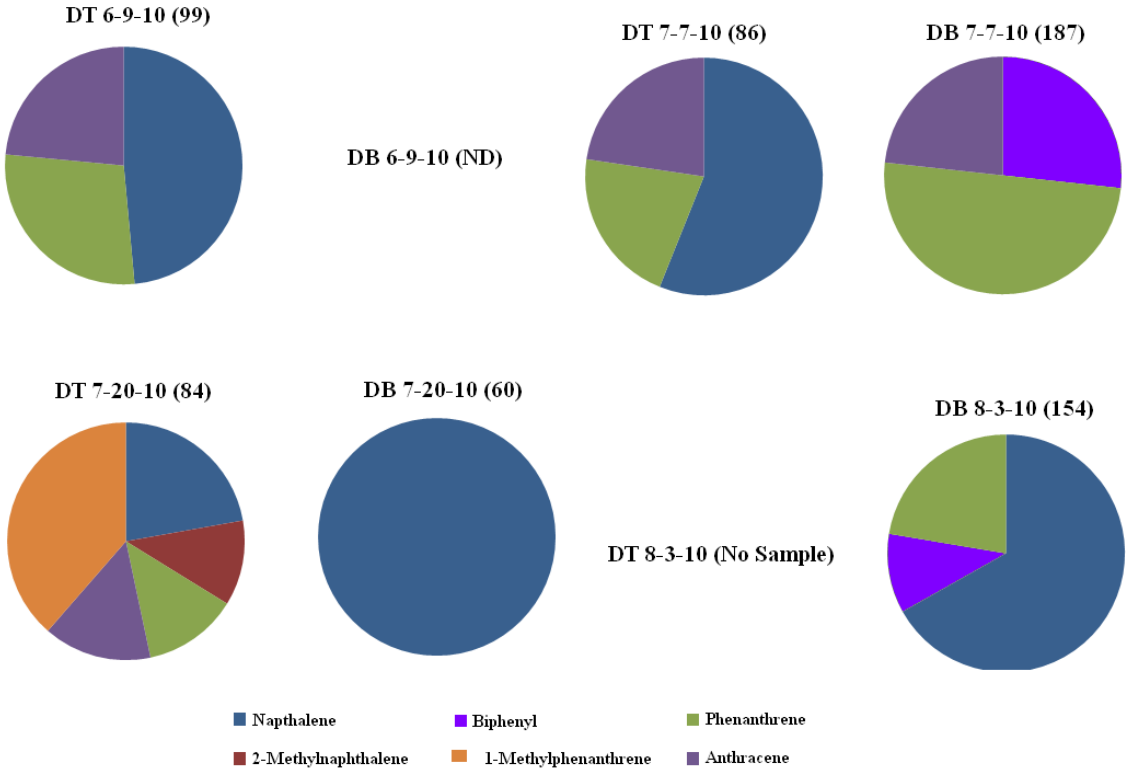



**Figure 33.** Percent composition of individual PAHs from Denton Reef oysters. Sample collection date and (tPAH concentration in ng/g dry weight) are indicated. DT = Denton Top (1 m) and DB = Denton Bottom (0.1 m).

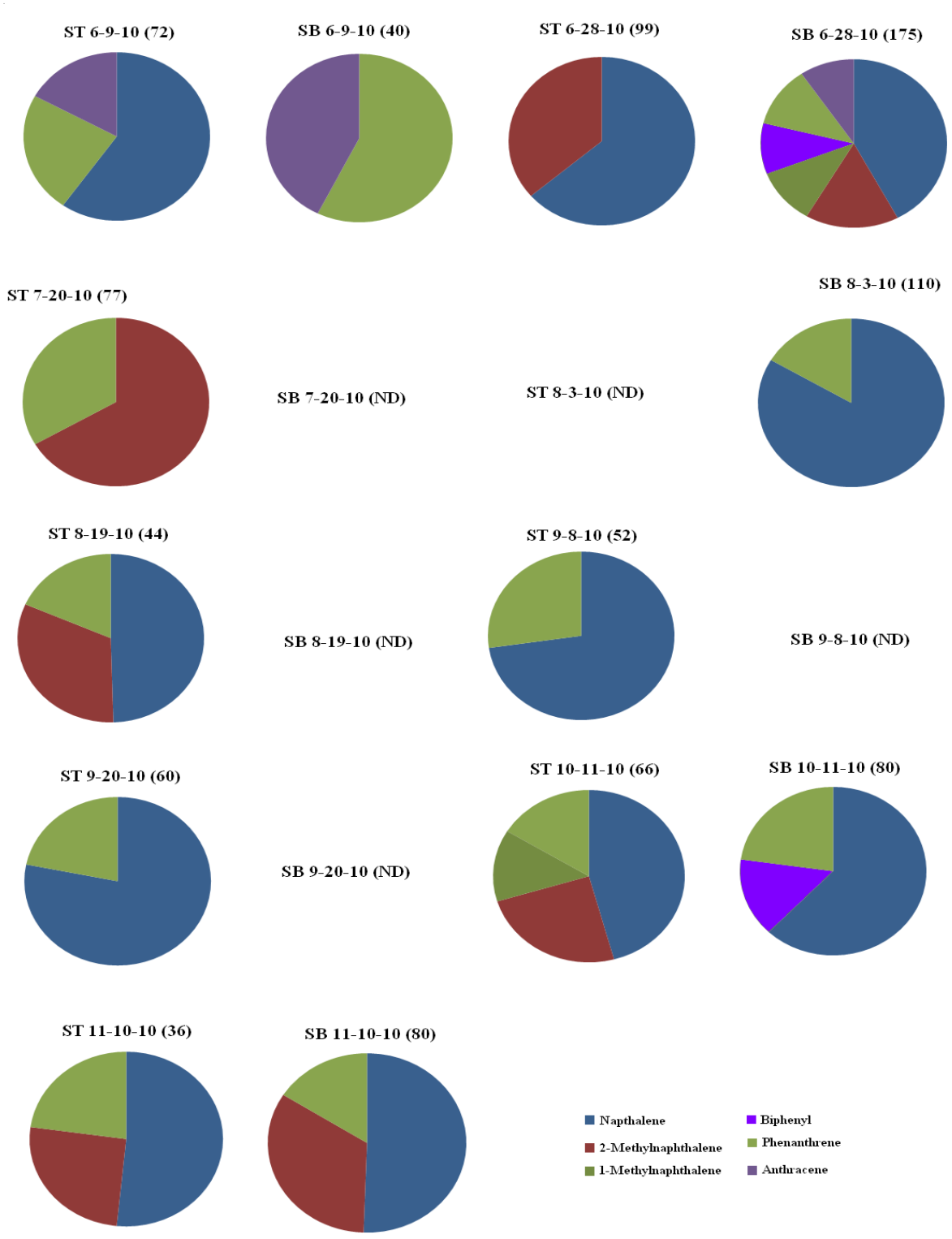



**Figure 34.** Percent composition of individual PAHs from Sand Reef oysters. Sample collection date and (tPAH concentration in ng/g dry weight) are indicated. ST = Sand Top (1 m) and SB = Sand Bottom (0.1 m).

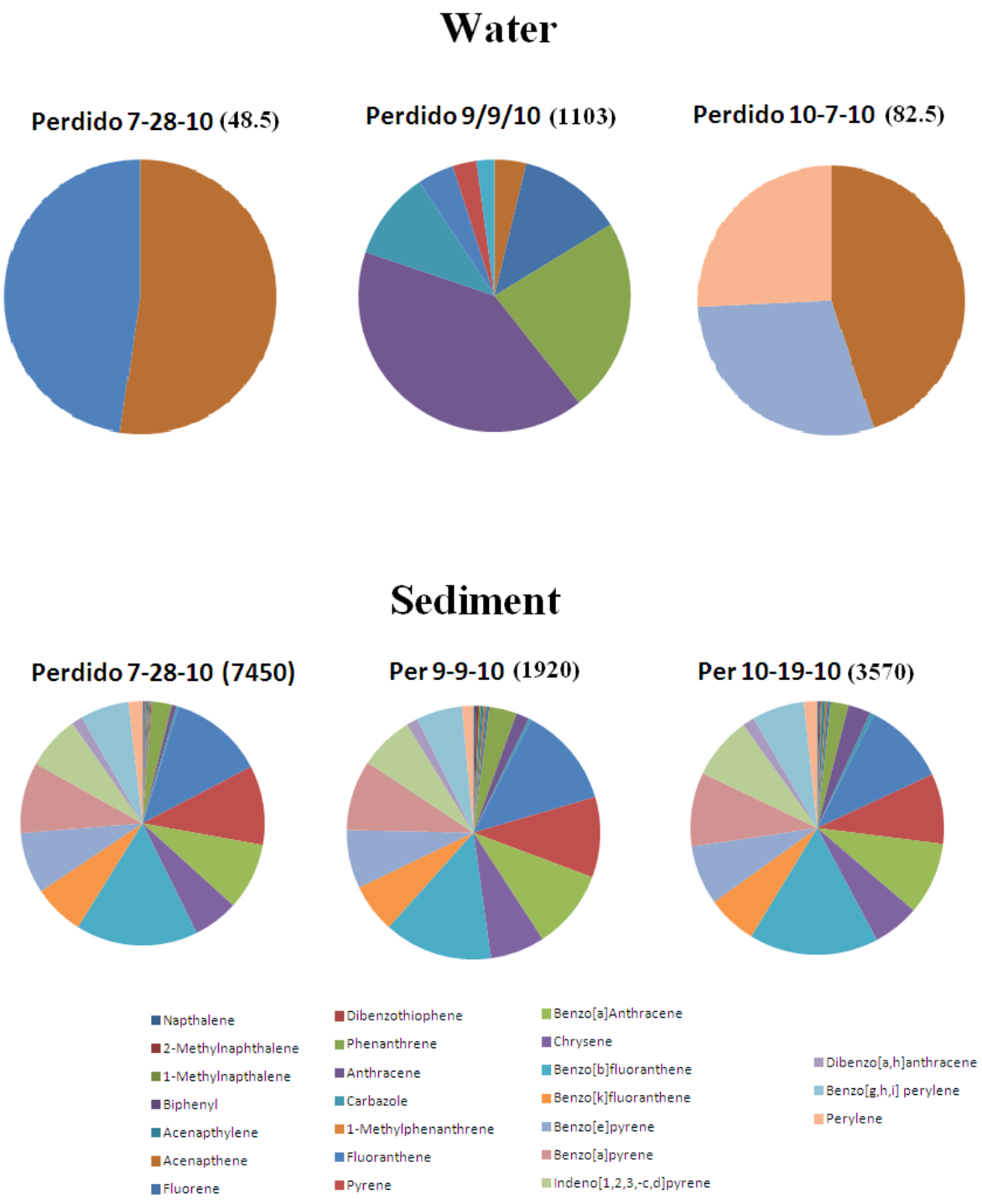



**Figure 35**. Percent composition of individual PAHs in water (ng/L) and sediment (ng/g dry weight) at Perdido before and after the peak tPAH concentrations were detected.

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