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Effects of Oil Spill and Recovery of Terrestrial Arthropods in Louisiana Saltmarsh Ecosystem

Wokil Bam
Louisiana State University and Agricultural and Mechanical College

EFFECTS OF OIL SPILL AND RECOVERY OF TERRESTRIAL ARTHROPODS IN LOUISIANA SALTMARSH ECOSYSTEM

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ABSTRACT

Saltmarshes are under continuous multiple stressors such as, land loss, erosion, climate change, environmental pollutions and oil spills, which affect the ecological communities inhabiting saltmarshes. Terrestrial arthropods play an important role in the ecology of saltmarshes, affecting primary production and decomposition. Arthropods are often found in the gut contents of Seaside Sparrows and fishes making them an important trophic link to terrestrial and marine vertebrates. Insects and spiders have the potential to be a good indicator of overall marsh health and environment as they are differentially sensitive to oil exposure. Oil pollutants may have significant long-term negative impacts on the terrestrial arthropods and consequently the food web. Ten sites along the coast of Louisiana were sampled: 3 lightly-oiled, 4 heavily-oiled sites in Barataria Bay, and 3 reference unoiled sites in Delacroix, St. Bernard Parish northeast of Barataria Bay, to determine the impacts of the Deepwater Horizon oil spill on the Louisiana saltmarsh terrestrial arthropods. Insects were collected via sweep net, 20m inland from the shoreline monthly between April and June of 2013 and 2014. Orthoptera, Hemiptera, Diptera, Thysanoptera, and Araneae were the most abundant groups of arthropods found at most sites. Species richness was significantly higher ($P < 0.05$) in references sites than lightly-oiled and heavily-oiled in both years. Shannon Weaver Index was similar in all sites, but higher in 2014 than 2013, suggesting a positive recovery of terrestrial arthropods' communities. Higher number of arthropods were observed in 2014 than 2013. Odonata were significantly higher in reference sites in both years. Orthoptera significantly increased in 2014 at all sites. Herbivores, Delphacidae populations increased in 2013 in response to the stress on plants due to Hurricane Isaac. Araneae were higher in oiled sites in 2013, but in 2014 the Araneae increased in reference sites, whereas they decreased in oiled sites. Overall, the terrestrial arthropods were affected by

the oil and Hurricane Isaac. The oil contamination effects still persist today. Although the slow recovery of certain terrestrial arthropods was observed, long term monitoring of arthropod communities would help better understand the recovery and succession of the marsh ecosystem.

INTRODUCTION

Background

Saltmarshes comprise most of the coastal ecosystems in the upper coastal intertidal zone and open brackish or salt water. Common along the coasts of the mid-latitudes, saltmarshes occupy a small area globally compared to other wetlands (Greenberg 2006); yet they contribute a wide range of ecosystem services that have significant global economic and societal value (Spencer and Harvey 2012). The Atlantic coast and Gulf of Mexico contain the greatest land area of saltmarshes in the world (Greenberg and Maldonado 2006). They are important transitional habitats found between marine and terrestrial ecosystems carrying vital ecological functions. Saltmarshes carry out a wide range of ecosystem services via process and function: they support a high abundance of saltmarsh species (Adam 1990) and contain one of the most productive ecosystems (Crooks and Turner 1999). Saltmarshes not only provide habitat for wildlife and plants, but also perform multiple ecosystem services such as improving water quality by filtering, nutrient cycling, hydrological functions, atmospheric regulation, as well as providing nursery habitat for fish and attenuation of storm surge (Granek et al. 2009). Carbon sequestration is high in coastal saltmarshes, thus they act as the most powerful carbon sinks (Macreadie et al. 2013). Many saltmarsh birds such as the Seaside Sparrow, Common Loon and other migratory birds depend on saltmarsh ecosystems for breeding, foraging and food sources (Weller 1999; Greenlaw and Woolfenden 2006).

Regardless of the enormous benefits provided by saltmarshes and brackish marshes, they are under continuous multiple stressors and highly vulnerable to loss and deterioration (Greenberg 2006). Human activities such as, dredging canals (Bass and Turner 1997), increasing coastal populations (Jackson et al. 2001), oil drilling and constructing gas pipelines (Boesch et

al. 1994), and the resulting oil spills are negatively impacting the saltmarsh ecosystem and the services saltmarshes provide (Mitsch and Gosselink 2000). Aside from human activities, saltmarshes are vulnerable and facing degradation due to natural processes, which include climate change, sea level rise, subsidence and compaction (Bass and Turner 1997), erosion, and tide wave interaction. Saltmarshes are integral parts of the Louisiana coastal ecosystem. The Louisiana coast is experiencing the largest loss of saltmarsh area in the United States (Jorgensen 2009). In the last 50 years, the Louisiana coast has lost more than 4920 square kilometers of coastal land with an average rate of 88 square kilometers of land lost per year (Couvillion et al. 2011). The Louisiana saltmarshes not only provide ecosystem services and functions (Barbier and Heal 2006), but also support the state economically by generating billions of dollars per year in revenues from tourism, recreational and fisheries industries (Engle 2011).

The arthropod community is influenced by many abiotic and biotic factors such as wind, rainfall, temperature, salinity, competition, predation and vegetation (Speight et al. 2008). The effects can either be direct or indirect, such as, wind which can carry arthropods many miles from their original habitat or heavy rain that can kill the insects or host plants (Harrison and Rasplus 2006). These abiotic and biotic factors also impact reproduction, growth, abundance and distribution of arthropods (Speight et al. 2008). Arthropods are an important part of the ecosystem as primary and tertiary consumers (Speight et al. 2008). Leaf-shredding insects such as Diptera and Lepidoptera convert leaf and litter (coarse particulate organic matter) into fine particulate organic matter (Meyer and O'Hop 1983; Wallace et al. 1991). According to Hunter (2001), herbivorous insects influence nutrient dynamics in the soil in different ways: (a) fecal material deposition - adding more nutrients, (b) insect cadavers - source of nutrient return, (c) change in nutrient content due to defoliation – dissolution of foliage and other excreted

materials, (d) change in plant community influenced by insects, and (e) root and insect symbiont interaction – myccorhize.

Insects are essential in the decomposition of organic matter in the ecosystem as they feed on detritus (decomposers and detritivores). Decomposers play a key role in the carbon cycle as they feed on dead and decaying plants and animals thus processing the organic carbon. Due to their significant role in ecosystem functions, some species of insects are known to be ecosystem engineers (Jones et al. 1994; Jouquet et al. 2006; Hastings et al. 2007). Insects not only have an impact on plants and animals, but also important effects on soil systems. A study by Werner (1975) found that terrestrial arthropods such as Coleoptera, Araneae, Heteroptera, and Hymenoptera were the most significant food source for the birds residing in saltmarshes. Velando et al. (2005) indicated food sources availability influences higher trophic level organisms within the arthropod food web and vertebrate food web. Arthropods are an important food source for birds (Werner 1975) and estuarine fishes (Pfeiffer and Wiegert 1981; Pennings et al. 2014), thus linking the different trophic levels.

Members of terrestrial arthropod communities have potential as useful indicators of the overall health of saltmarshes. Certain species of terrestrial arthropods, such as ants (Hooper-Bui et al. in prep), spiders and beetles (McGeoch 1998), are very sensitive to the changes in the environment and can be regarded as strong ecological indicators (Holloway and Stork 1991). Habitat destruction and fragmentation, (Hafernik 1992; Jennings and Tallamy 2006), abiotic and biotic environmental changes (Schowalter 1981) and change in use of lands (Young et al. 2005) poses various threats to arthropod communities. Few studies have been done on the ecology and influences of environmental changes on terrestrial arthropods in Louisiana saltmarshes. Terrestrial arthropods are found in both great richness and abundance in saltmarshes and

brackish marshes (Davis and Gray 1966). Arthropods have a main role in food webs, which affect ecosystem functions as arthropods inhabit high diversity of micro-habitats and niches (Longcore, 2003). De la Huz et al. (2005) observed a decrease in the richness and abundance of macro-fauna as a result of the effects of the oil spill in Bay of Biscay, Spain. The Exxon Valdez oil spill in Prince William Sound had significant long-term negative impacts on the saltmarsh birds, intertidal arthropods and their habitat (Day et al. 1997). Organisms residing in oil-affected areas might be affected directly by exposure to oil and oil pollutants due to toxicity (Votier et al. 2005) or indirectly by reducing the availability of key food sources such as plants and amphipods (Velando et al. 2005). The studies conducted by McCall and Pennings (2012), Pennings et al. (2014) and Hooper-Bui et al. (in prep.) have indicated a decline in the insects' populations in oil effected saltmarshes after the Deepwater Horizon (DWH) oil spill. It is important to have an understanding of influences of oil contaminations and abiotic and biotic changes on the saltmarsh ecosystem. The dysfunctional food webs in saltmarshes could result in die-offs of the marshes (Silliman and Bertness 2002).

Problem Statement

The Deepwater Horizon (DWH) oil spill in April 2010, is one of the largest oil spills in history releasing an estimated 4.9 million barrels of oil into the Gulf of Mexico (Crone et al. 2010). The oil reached the Louisiana coast (approximately 70 kilometers from the Macondo well) on July 15, 2010 (Turner et al 2014a), which has affected the coastal ecosystem and its components directly and indirectly. Approximately 1773 km of Gulf of Mexico shoreline, which makes up 45% of coastal marsh, was significantly oiled (Turner et al. 2014b) endangering the coastal habitat and ecosystem. Louisiana coastal ecosystems were exposed to the most extreme oiling (Turner et al. 2014 and Bergeon-Burns et al. 2014). According to the National

Commission (2010) on the BP Deepwater Horizon Oil Spill and Offshore Drilling, the oil spill immediately threatened a rich productive marine ecosystem due to toxicity of crude oil. Some of the studies conducted have shown the negative impact of oil on the ecosystem and the major threat that oil poses to the saltmarsh ecosystem. McCall and Pennings (2012), Pennings et al (2014), and Hooper-Bui et al. (in prep) show the decrease in insect populations. McCall and Pennings (2012) reported a 50% reduction of the insect and spider community on the oiled saltmarshes in Louisiana in 2010. Also, McCall and Pennings (2012) found that in 2011, the populations of arthropods on oiled sites were similar to those on control sites. Due to short-term life cycle and generation times, most arthropods can be considered to be ideal indicators for monitoring the ecosystem for both short-term and long-term control (Rosenberg et al. 1986; Kremen et al. 1993). The large population size, high reproductive rates, short life cycle and relatively easy methods of sampling, provide statistically significant sample size (Longcore 2003) as well as fewer chances of diminishing the population. The oil was redistributed by Hurricane Isaac, thus contaminating more of Louisiana's saltmarshes (PAH DATA). Research was conducted by McCall and Pennings in 2010 - 2011 to assess the immediate impacts of oil contamination on the arthropod community. I focused on the aftermath of oil redistribution and recontamination due to Hurricane Isaac.

Objectives

There is no comprehensive study on the effects of oil, and other associated pollutants and cleanup efforts on the invertebrates of the banks and shores associated with oil spills in North America. The overall objective of the research project is to determine impacts of Macondo Oil (Deepwater Horizon oil spill) on terrestrial arthropods in Louisiana saltmarshes by comparing oil-spill affected sites to reference sites. The specific objectives are:

1. To study the community structure, abundance, diversity and distribution pattern of terrestrial invertebrates inhabiting Louisiana saltmarshes;
2. To determine the impacts of DWH oil spill on terrestrial arthropods in Louisiana saltmarshes 2013 and 2014;
3. To understand the recovery of terrestrial arthropods post oil spill;

I hypothesized that there would be differences in the terrestrial arthropod communities among the reference, lightly-oiled, and heavily-oiled sites in Louisiana saltmarshes. This study will provide an outlook on the trophic effects of oil pollution on saltmarsh ecosystems.

MATERIALS AND METHODS

Study Area

Barataria Bay, Plaquemines Parish and Delacroix, St. Bernard Parish in Breton Sound (northeast of Barataria Bay) (Figure 1) were selected as primary research sites. Seven study sites were selected in Barataria Bay of which three sites were lightly oiled (Macondo oil circulated and transported via tropical storms or hurricanes) and four sites were heavily oiled with Macondo Oil from the Deepwater Horizon platform disaster. The sites were classified based on total polycyclic aromatic hydrocarbons (PAHs) concentrations (Appendix A). The NOAA NRDA Workplans and Datasheet for PAHs (http://www.gulfspillrestoration.noaa.gov/oil-spill/gulf-spill-data/, Appendix B, Appendix C) in 2010 and 2011 showed no Macondo oil in Delacroix, St. Bernard Parish, which are regarded as reference sites in this study. In addition, the same datasheet showed patchy distribution of concentration of oil in Barataria Bay, and the sites are classified as lightly-oiled and heavily-oiled with reference to sediment PAHs (Appendix A).

Saltmarshes predominate Barataria Bay which has a low tidal range. Dominant vegetation in these sites are *Spartina alterniflora, Juncus roemarianus, Distichlis spicata* and *Avicennia germinans* (Sasser et al. 2014). Three reference or unoiled sites were selected in Delacroix, St. Bernard Parish where the oil was not suspected at the beginning of the study. Breton Sound is a brackish marsh with low tidal range and freshwater inputs from the Mississippi River. During summer, water levels drop below the elevation of the marshes and marshes are often dry. *Spartina patents, Spartina alterniflora, Juncus roemarianus* and *Bolboschoenus robustus* are the dominant vegetation in the Breton Sound (Sasser et al. 2014). Even though the study sites vary in salinity, the dominant organisms from the plants to the insects to the birds are similar. Each study site extends 100m inland from the coast shoreline and is 500m wide because my sampling was in

conjunction with a project to study Seaside Sparrows, *Ammondramus maritimus*, first described by Wilson 1811 (Adriaens 2014). *Ammondramus maritimus* are found exclusively in tall *Spartina* stands along larger estuaries where they have ample foraging mudflats available (Post and Greenlaw 2009). *Ammondramus maritimus* inhabiting saltmarshes, feed on seeds, insects, amphipods, spiders and mollusks, foraging primarily on open stands of grass and shallow pans and ponds (Post et al. 1983).

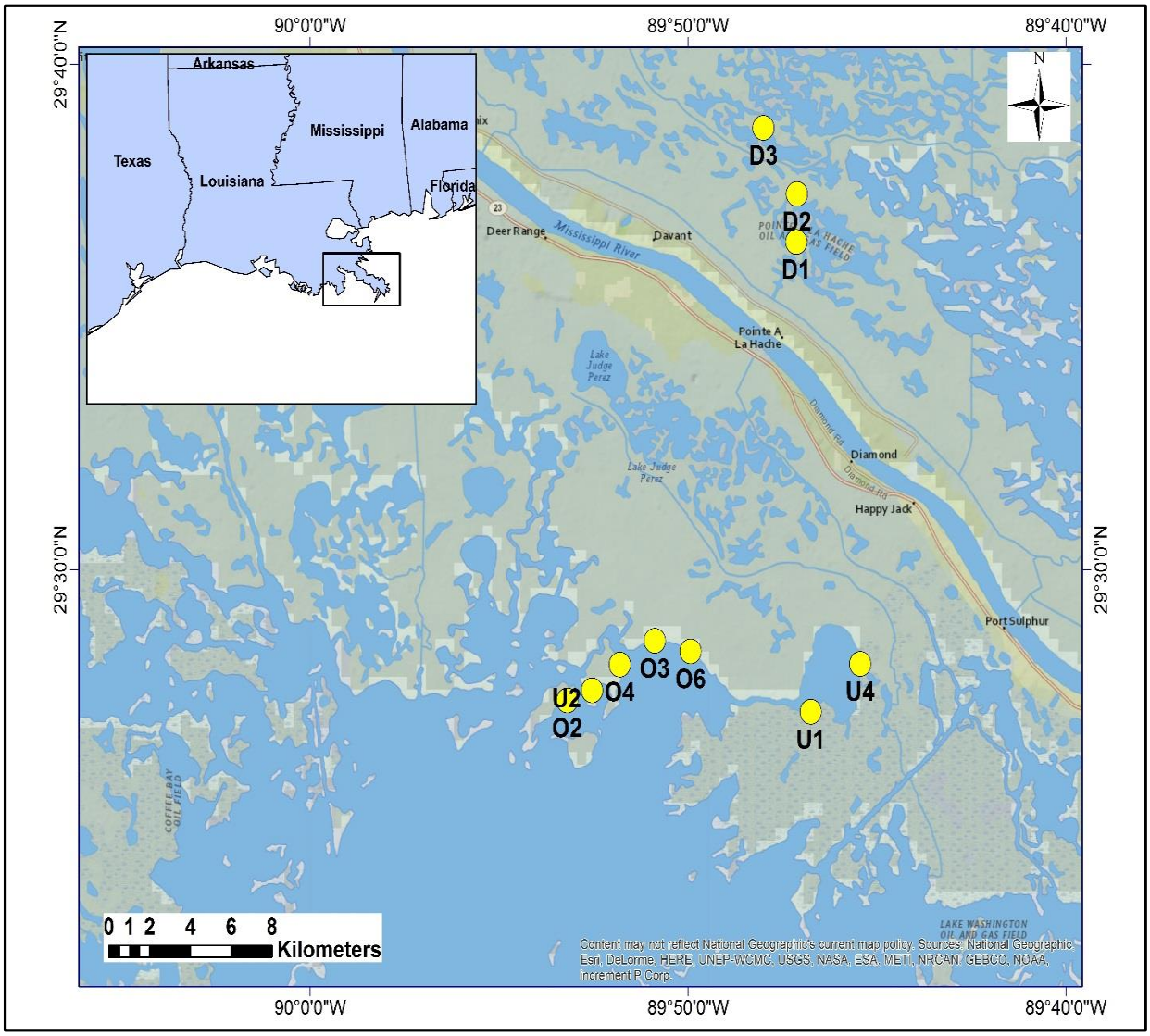


Figure 1. Study sites in Barataria Bay, U indicates lightly-oiled sites, O indicates heavily-oiled sites and Delacroix, D indicates reference sites in Louisiana (Source: ESRI-Data, 2015).

Analysis for Sediments PAHs

The top 5 cm of the surface sediment samples for polycyclic aromatic hydrocarbons (PAHs), adjacent to shoreline were collected from Barataria Bay and Delacroix in April 2013 (Figure 1) and stored at 4 ˚C until analysis. I followed the protocol used Turner et al. (2014b) and Adhikari et al. (2015) for the extraction and analysis PAHs (Appendix D). Briefly, weighted sediment samples were placed at -80 ˚C for ~12 hours, transferred to a freeze drier for ~3 days and then ~10g of the dry samples were extracted using a Buchi Speed Extractor (New Castle, Delaware, USA). This method is similar to an automated solvent extractor (ASE) which provides percent moisture values; however, concentrations reported are on a dry weight basis. The sample extracts were nitrogen blowdown to 1 ml, added with internal standards and analyzed using an Agilent 7890A GC interface with an Agilent 5975C inert XL mass selective detector (MSD) (Modified EPA SW-846 method 8270, US EPA 2000). The MSD was operated selective ion monitoring (SIM) mode, targeting for 43 PAHs (18 parent PAHs and 25 associated alkyl homologues) (Table 1). The commonly used biomarkers, hopanes, steranes, and triaromatic steroids compounds were also analyzed for source fingerprinting. The Macondo Oil, MC252, was used for source oil identification. Appropriate quantitative analysis and quality control (QA/QC) (Modified SW-846 methods, US EPA 2000) procedures for sample extraction were maintained (Turner et al. 2014b; Adhikari et al. 2015). The concentrations of all 43 individual PAHs identified and measured are presented in Appendix A.

Table 1. Oil Analytes of Interest

Naphthalene	Anthracene	Benzo (k) Fluoranthene
C1-Naphthalenes	Fluoranthene	Benzo (e) Pyrene
C2-Naphthalenes	Pyrene	Benzo (a) Pyrene
C3-Naphthalenes	C1- Pyrenes	Perylene
C4-Naphthalenes	C2- Pyrenes	Indeno (1,2,3 - cd) Pyrene
Fluorene	C3- Pyrenes	Dibenzo (a,h) anthracene

(Table 1 continued)

C1-Fluorenes	C4- Pyrenes	Benzo (g,h,i) perylene
C2-Fluorenes	Naphthobenzothiophene	
C3- Fluorenes	C-1 Naphthobenzothiophenes	
Dibenzothiophene	C-2 Naphthobenzothiophenes	
C1-Dibenzothiophenes	C-3 Naphthobenzothiophenes	
C2-Dibenzothiophenes	Benzo (a) Anthracene	
C3- Dibenzothiophenes	Chrysene	
Phenanthrene	C1- Chrysenes	
C1-Phenanthrenes	C2- Chrysenes	
C2-Phenanthrenes	C3- Chrysenes	
C3-Phenanthrenes	C4- Chrysenes	
C4-Phenanthrenes	Benzo (b) Fluoranthene	

Sample Collection

The arthropod population was collected during the 2013 and 2014 breeding season of the Seaside Sparrow, which occurs in April, May, and June. Quantitative sampling of terrestrial arthropods (insects and spiders) was done using sweep nets, on plots measured from the edge of the marsh to 20m inland (20m X 5m linear transects) at each site. Sites were marked using vertical PVC pipes. The sweep net is considered as one of the most effective methods for sampling terrestrial arthropods (Larson et al. 1999; Doxon et al. 2011; Adams et al in internal review). Insects and spiders were collected between 6:30 am and 12:00 pm, once per month from all the sites. Sites in Barataria Bay and Breton Sound were visited on consecutive days. Collected terrestrial arthropods were transferred into plastic ziplock bags with 95% ethanol. The samples were transported to the lab at Louisiana State University and were sorted to morphospecies and stored in vials of 95% ethanol. The insects and spiders were classified to order and family using appropriate taxonomic keys and the morphospecies approach (Triplehorn and Johnson 2005; Wimp et al. 2010; Pennings et al. 2011). A morphospecies approach was established by Oliver and Beattie (1993) to group taxa of insects based on morphological characteristics when

estimating diversity using higher taxon. Diversity measurement with higher taxon such as beetles and flies were found to be good substitutes for species-based measures (Oliver and Beattie 1996; Baldi 2003). All types of spiders were counted and grouped as one group for all calculations and statistical analyses. The quantity of each type of insect and all types of spiders was recorded. Temperature, wind speed, wind direction and sampling time were recorded in the field, immediately before sample collection. All the sample collections were done in between 6:30 am to 12:00 pm, temperature ranged from 22ºC to 34ºC.

Diversity Measurement

The total number of species present in an area provides the simple calculation of species diversity (Sanders 1968) but fails to describe the abundance and frequency of occurrence of species (MacArthur 1965). Species richness estimates the numerical number of different species represented in the sample area or ecosystem. Menhinick's Index (D_{Mn}) is one the best known species richness indices (Whittaker 1972):

$$D_{Mn} = \frac{S}{\sqrt{N}}$$

Where S = number of species recorded and N = total number of individuals in the samples.

Shannon and Weaver in 1949 purposed an index to express the species diversity of ecological systems.

$$H' = -\sum_{i=1}^{s} (p_i) \log p_i$$

Where $p_i = n_i/N$, N = total number of individuals on sample and n_i = the number of individual species in the sample, where i = species number from 1, 2….. 16.

Statistical Analysis

Data were analyzed using multivariate analysis of variance (ANOVA), in which treatment/ independent variables included references sites, lightly-oiled sites and heavily-oiled sites (oiled) and the dependent variables were number of terrestrial arthropods per sweep. The purpose of this analysis was to calculate the interaction between treatments and years on insect species. Parameters were set to use Tukey's adjustment to separate differences at error rate of 0.05. Differences in family/order level for all samples from all sites were compared using PROC MIXED and PROC GLM model on SAS9.4 software (SAS Institute, 2013, Cary NC) Shapiro-Wilk test was performed for the test of normality and the distribution was highly skewed. The data were log transformed to normalize the individuals and dataset. Convergence criteria was met for all individual arthropods during statistical analysis.

The species richness was estimated for each site and each month based on the samples counted for families. One-way ANOVA followed by Bonferroni (Dunnette) tests for Richness, Tukey's Studentized Range (HSD) Tests and t-tests for Fishers Least Significant Difference (LSD) Test for Richness were used to determine differences between study sites for species richness of inhabiting terrestrial arthropods. The t-grouping as well as p-values (<0.05) were used for comparisons among the sites and years. Treatments and year were compared. One-way ANOVA was used to compare the diversity measurement Shannon and Weaver Index among treatments and years. All statistical tests were performed in SAS 9.4 software (SAS Institute, 2013, Cary NC). Linear regression was performed for number of individuals for each arthropod and PAHs values associated with the sites and months.

Community structures of the study sites was determined using Principal Component Analysis (PCA) on SAS Enterprise Guide 6.1 software (SAS Institute, 2013, Cary NC), with the

ordination axes compared with arthropod groups. The distribution of trophic levels of collected terrestrial arthropods was calculated on the percentage of total terrestrial arthropods collected with treatment and each year. The comparison was done using descriptive statistics and no statistical test was performed, as it was with total overall calculation of percentage.

$$\%\ distribution\ of\ trophic = \frac{(total\ no.\ of\ indv\ of\ trophic\ level\ )}{total\ no.of\ indivials\ in\ sample} X\ 100\ \%$$

RESULTS

Arthropod Data

A combined total of 60 sweep net samples in all the treatment sites for both years resulted in collection of 21,732 individuals. Figure 2 shows the mean (±SEM) number of specimens collected per sweep across all three treatment sites in both 2013 and 2014. The high standard deviation and high standard errors suggest that the data are not normal, as earlier indicated by Shapiro-Wilk statistic test.

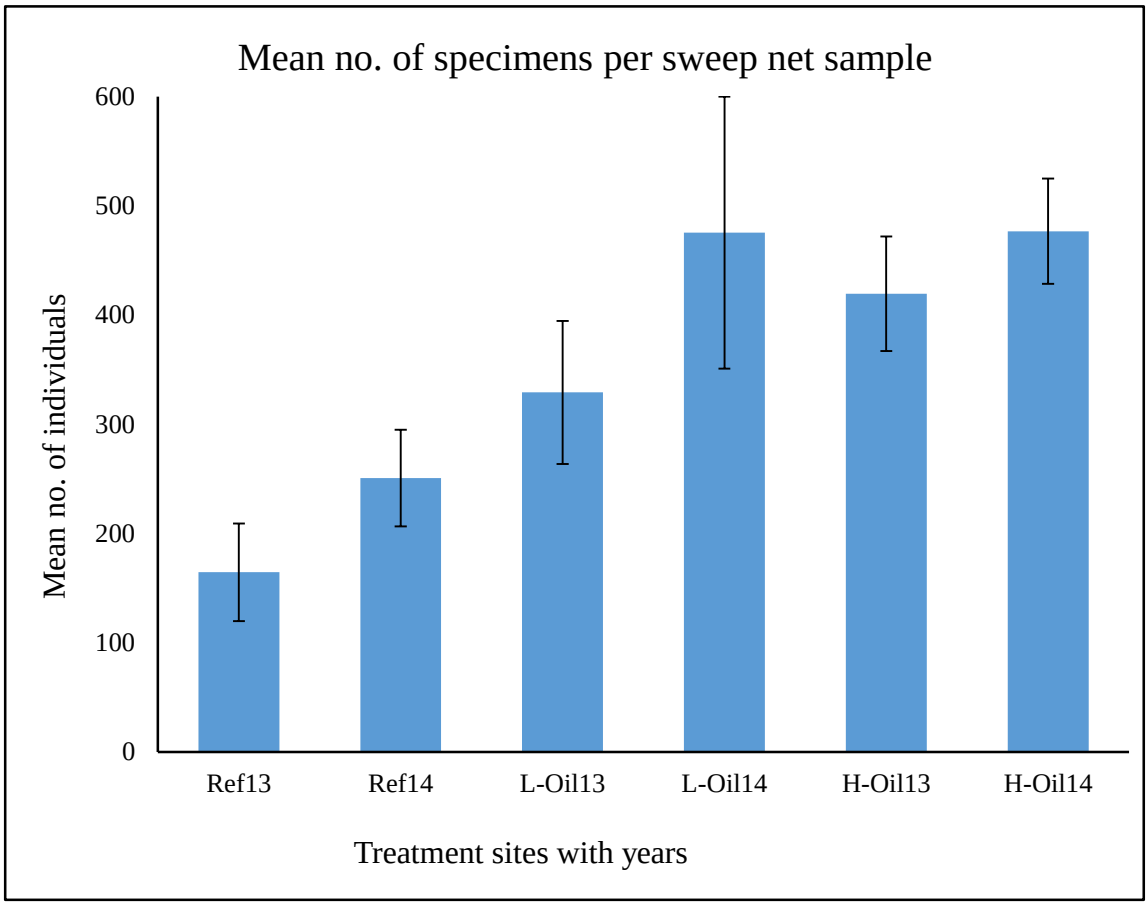


Figure 2. Mean (±SEM) individuals sorted per sweep net sample in all treatment sites in 2013 and 2014.

The number of terrestrial arthropods collected in 2013 were 165 ± 134 (mean ± SEM), 329 ± 217 and 420 ± 199 in reference (Ref13), lightly-oiled (L-Oil13) and heavily-oiled sites (H-Oil13) respectively. In 2014, 251 ± 133, 476 ± 374 and 477 ± 167 individuals/specimens were

collected in reference (Ref14), lightly-oiled (L-Oil14) and heavily-oiled (H-Oil14) sites, respectively. The numbers of total arthropods in 2013 (9,476) were lower than 2014 (12,256), but the difference was not statistically significant ($P > 0.05$). However, the numbers of arthropods in 2013 were significantly different ($P< 0.05$, $f=3.37$, $df = 54$) among the treatment sites (Figure 2). Heavily-oiled sites had more arthropods followed by lightly-oiled and reference sites. In 2014, heavily-oiled and lightly-oiled sites had a similar number of arthropods and were significantly higher than reference sites ($P < 0.01$, $f=5.36$, $df = 54$).

Species Richness

A total of 28 families of insects were recorded in the collected samples. Hemiptera, Diptera, Orthoptera, Thysanoptera, and Araneae were the most abundant groups of terrestrial arthropods found in most of the sites. For the purpose of calculating species richness, some of the families were combined together within orders as some of them were singletons or doubletons throughout the sample. Some of the species/families were removed from the analyses as the individuals were extremely rare and not repeated in samples. A total of 15 families and orders were used for calculation of species richness, which were obtained in the sampling over all the sites and both years. Figure 3 represents the mean species richness (± SEM) with respect to sites and years.

The references sites had significantly higher ($P<0.05$) species richness both in 2013 (0.76 ± 0.06) and 2014 (0.75 ± 0.05) than lightly-oiled and heavily-oiled sites (Figure 3). Lightly-oiled and heavily-oiled sites had similar species richness, 0.57 ± 0.05 (L-Oil13), 0.52 ± 0.04 (L-oil14),

0.48 ± 0.04 (H-Oil13) and 0.52 ± 0.04 (H-Oil14) in both years. The species richness did not vary significantly (P>0.05) between the years for all the treatment sites.

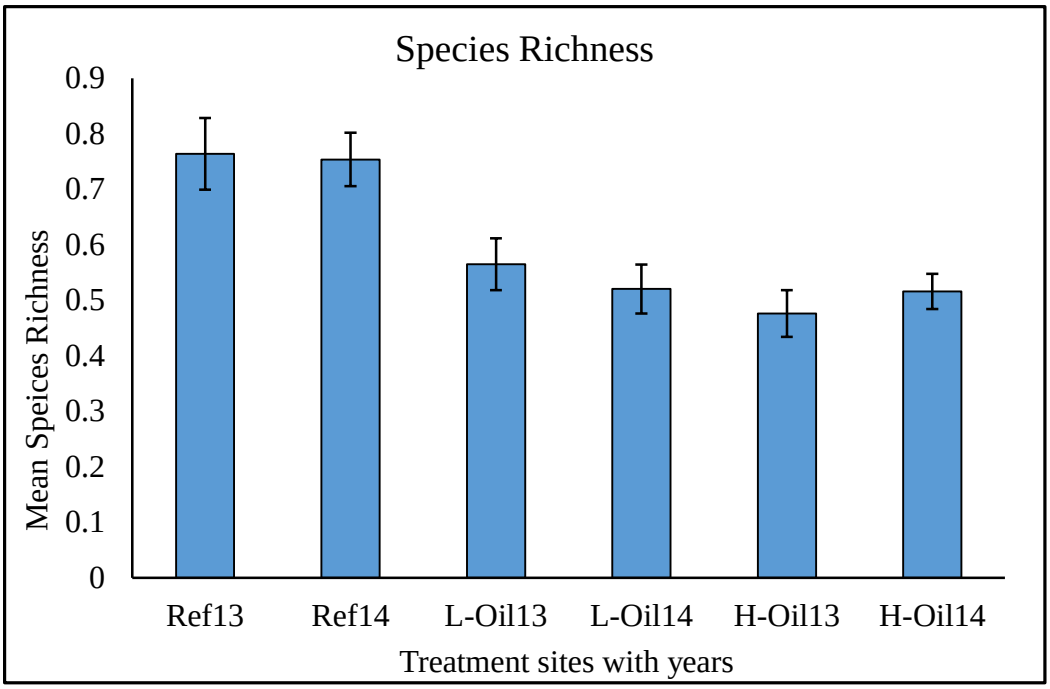


Figure 3. The mean species richness across the treatment sites and years.

Diversity Measurement

According to the Shannon and Weaver Index, the mean diversity was 1.4 ± 0.14 (Ref13), 1.73 ± 0.08 (Ref14), 1.50 ± 0.1 (L-Oil13), 1.67 ± 0.05 (L-Oil14), 1.53 ± 0.05 (H-Oil13) and 1.77 ± 0.04 (H- Oil14, Figure 4). In 2013, the reference sites had lower diversity than the lightly-oiled and the heavily-oiled sites, however, the difference was not significantly different (P> 0.05) among the diversity index for all the sites. The lightly-oiled sites had a lower diversity index than the reference and the heavily-oiled sites in 2014, however, they were not significantly different (P> 0.05). All the sites had significantly higher mean diversity in 2014 compared to 2013 (P < 0.05, t= 3.071, df = 54). Overall, the Shannon and Weaver Index diversity measurement showed the temporal variation on diversity for all the sites whereas no spatial variation was observed (Figure 4).

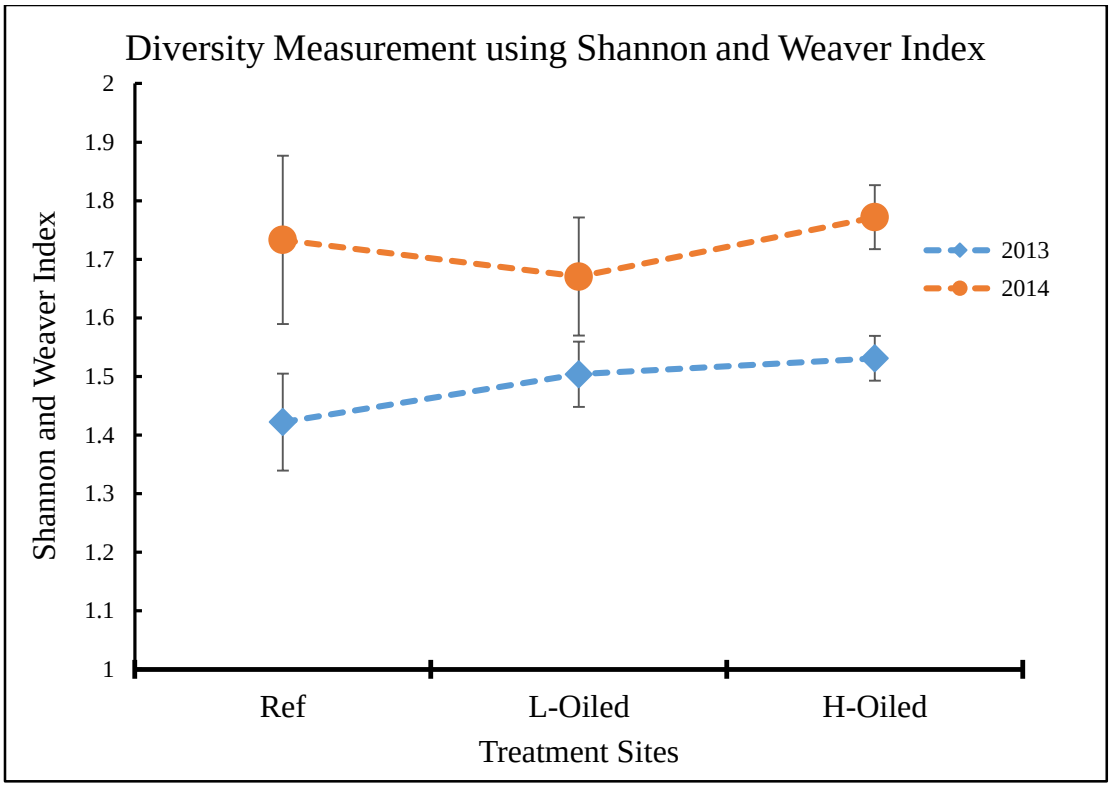


Figure 4. Mean Shannon and Weaver Index with SEM for treatments and year 2013 (diamonds) and 2014 (closed circle).

Arthropod-Treatments Relationship

Figures 5, 6, and 7 show the mean number of each arthropod collected per sweep across all three treatment sites in both 2013 and 2014. All the data represent the mean number of individuals collected per sweep. The table 2 shows the effect of treatment, year and their interaction on insects and spiders. The p-value ($p<0.05$) represents at least one model (treatment, year and interaction) is significantly different with respective values. Comparison among the arthropods were not made, which is no comparison or letter indication of significant differences horizontally on the table. Dragonflies and damselflies were included in the order Odonata and both were present in the samples. In 2013, Odonata were significantly higher ($P = 0.04$, $t = 2.94$, $df = 47$) in the reference sites (6 ± 2) than the lightly-oiled (1 ± 1) and heavily-oiled (1 ± 1) sites. The same trend was observed in 2014, whereas the reference sites (14 ± 3) had a significantly

higher numbers of Odonata than the lightly-oiled (3 ± 1) and heavily-oiled sites (4 ± 2, Figure 5a). Odonata increased significantly ($P < 0.01$, $t = -4.42$, $df = 47$) in 2014 across the treatment sites, however no significant difference was seen in the interaction (Table 2). Ground-dwelling Orthoptera (crickets), were not significantly different ($P = 0.9$, $t = -0.01$, $df = 7$) across the sites in 2013, although the reference sites (9 ± 3) had a higher number than the heavily-oiled (4 ± 4) and lightly-oiled sites (2 ± 5, Figure 5b). Orthoptera populations significantly differed ($P < 0.05$, $t=1.8$, $df = 47$) among all treatment sites with the highest number of them being observed in the lightly-oiled (41 ± 7), followed by heavily-oiled (28 ± 5) and reference sites (19 ± 4) in 2014. The number of Orthoptera were significantly increased ($P < 0.01$, $t = -8.84$, $df = 47$) in 2014 compared to 2013 (Table 2) for all the sites.

The Miridae population significantly differed among all the treatments sites in 2013 and 2014. Heavily-oiled sites (60 ± 15) had significantly higher ($P <0.01$, $t = 3.79$, $df =47$) population of Miridae than lightly-oiled (41± 4) and reference (12 ± 6) in 2013. Whereas, in 2014, both heavily-oiled (71 ± 19) and lightly-oiled (129 ± 56) were significantly more ($P = 0.02$, $t = 3.87$, $df = 47$) populated by Miridae than reference sites (18 ± 8, Figure 5c). Overall there was no significant difference ($P = 0.98$, $t=-0.95$ $df =47$) in the interaction among the treatments and years. Delphacidae, (planthoppers) were one of the largest groups of arthropods collected in the samples in terms of abundance. In 2013, reference sites (30 ± 8) had a significantly lower ($P < 0.01$, $t = 2.96$, $df = 47$) number of Delphacidae than at lightly-oiled sites (142 ± 68) and heavily-oiled sites (144 ± 37), which had a similar population of plant hoppers. However, in 2014 there was no significant difference ($P = 0.45$, $t = 0.76$, $df = 47$) in the Delphacidae population across all sites, heavily-oiled (57 ± 11), lightly-oiled (46 ± 15) and reference (60 ± 24). Heavily-oiled and lightly-oiled sites experienced significant decreases ($P < 0.05$, $t = 2.47$, $df=47$) in the population

of Delphacidae in 2014, but the Delphacidae' population increased in reference sites although it was not significant (P= 0.1, t= -0.99, df = 47, Fig 5d).

The Blissidae populations were similar in heavily-oiled sites (3 ± 1) and lightly-oiled sites (3±1), but significantly larger (P< 0.01, t= 4.8, df= 47) than reference sites (1 ± 0) in 2013. However, in 2014, Blissidae were significantly higher (P<0.01, t= 3.84, df= 47) in heavily-oiled sites (10 ± 3) than lightly-oiled (5 ± 2) and reference sites (2±2, Figure 5e). Heavily-oiled and reference sites experienced a significant increase (P < 0.01, t= -3.61, df= 47) in Blissidae in 2014. The number of Blissidae increased in lightly-oiled sites in 2014, but no significant increase (P=0.43, t= -0.79, df =47) was noted. Rhopalidae were rare in occurrence both in abundance and frequency in the sample collected (Figure 5f). There was no significant difference among the treatment (P = 0.1, t= -1.56, df = 47) and year (P = 0.2, t= -1.06, df = 47) but their interaction showed a significant difference (P = 0.03, t= 2.47, table 2).

Table 2. Effect of treatment, year and their interaction on the insects and spider *(*p values). Different letters indicate the significant differences horizontally. Treatment with same letter group are not statistically different for a given arthropod.

Arthropods	p value			Heavily-oiled		Lightly-oiled		Reference	
	Treatment	Year	Interaction	2013	2014	2013	2014	2013	2014
Odonata	0.042	0.002	0.211	A	B	A	B	B	C
Orthoptera	0.990	0.001	0.034	A	B	A	C	AD	D
Miridae	0.002	0.349	0.980	A	A	B	AC	D	D
Delphacidae	0.000	0.001	0.137	A	B	A	BC	C	BC
Blissidae	0.019	0.004	0.154	A	B	A	AB	C	A
Rhopalidae	0.103	0.207	0.035	A	A	A	A	A	B
Thysanoptera	0.048	0.017	0.070	A	B	BC	C	BC	C
Coleoptera	0.025	0.058	0.397	AB	A	AB	AB	B	AB
Formicidae	0.029	0.001	0.242	A	B	AB	C	N/A	A
Pompiloidea	0.003	0.001	0.315	A	A	A	B	C	A
Lepidoptera	0.939	0.176	0.289	A	B	AB	AB	AB	AB
Diptera	0.001	0.802	0.938	A	A	ABC	C	B	B
Culicoidea	0.287	0.009	0.247	A	B	A	BC	AC	B
Araneae	0.447	0.148	0.001	AC	AB	AB	AB	B	C

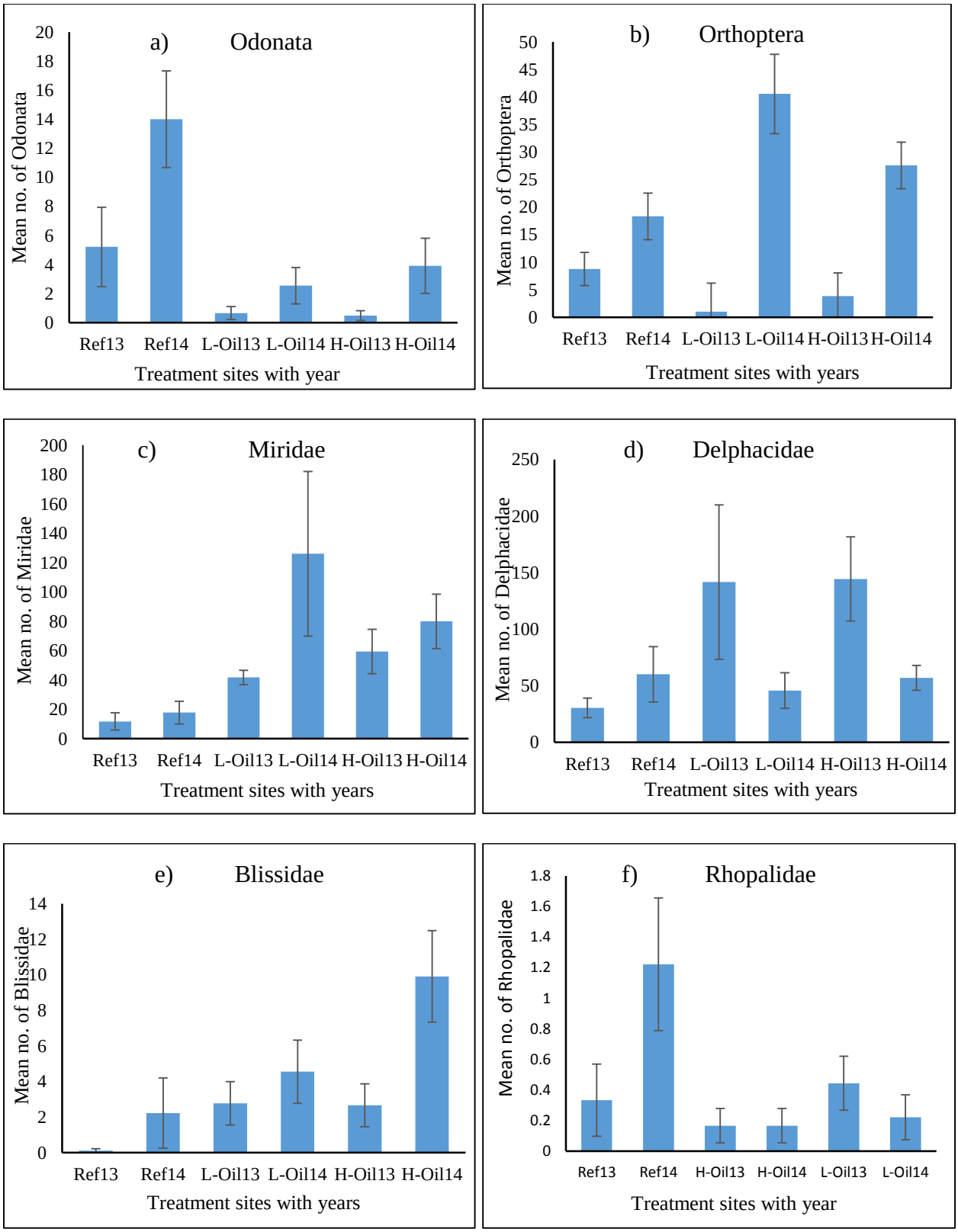


Figure 5. Mean (±SEM) abundance of arthropods collected per sweep: a) Odonata, b) Orthoptera c) Miridae, d) Delphacidae, e) Blissidae, f) Rhopalidae with respect to treatment sites and years.

Thysanoptera (thrips), were highly abundant and significant (P <0.01, t= 3.94, df =3.94) in heavily-oiled sites (40 ± 22, 2013) compared with heavily-oiled (4 ±2, 2014), lightly-oiled (4 ± 2, 2013; 1 ± 1, 2014) and reference sites (2 ± 2, 2013; 1 ± 1, 2014; Figure 6a). Reference sites (1 ± 1) had significantly lower (P= 0.02, t= 4.05, df 47) number of Coleoptera (beetles) than lightly-oiled sites (4 ± 1) and heavily-oiled sites (6 ± 3) in 2013. Whereas, in 2014 the number of beetles were not different (P=0.56, t= 0.59, df= 47) across the sites heavily-oiled (8 ± 6), lightly-oiled (5 ± 5) and reference (6 ± 4, Figure 6b) sites. There was no significant difference (P =0.06, t= -1.94, df =47) in the number of beetles in 2013 and 2014.

The mean number of Formicidae (ants) were similar (P= 0.79, t=-0.29, df =47) in both lightly-oiled sites (2 ± 0.5) and heavily-oiled (1 ± 0.5) sites in 2013. Formicidae were absent in the sample collected in reference sites in 2013. In 2014, the mean number of ants was significantly higher (P< 0.01, t= 2.88, df = 47) in lightly-oiled sites than heavily-oiled sites (5 ± 2) and reference sites (2 ± 1) per sweep (Figure 6c). There was a significant difference in the number of ants across the treatment (P = 0.02, t=3.52 df = 47) and years (P <0.01, t= -3.16, df =47), however no significant differences were observed among the treatment and year interaction (P = 0.24, t=-1.19, df =47, table 2). All the treatment sites had significantly more (P =0.02, t= 3.16, df= 47) ants in 2014 than 2013. Lightly-oiled sites in 2014 had a significantly higher number of ants among all treatment sites. The superfamily Pompiloidea – the spider wasps, included all families of wasps in the study. Wasps were significantly higher (P <0.01, t= 3.63, df = 47) in reference sites (9 ± 1) than in heavily-oiled (4 ± 1) and lightly-oiled (3 ± 1) sites in 2013. However, in 2014, the lightly-oiled sites (12 ± 2) had significantly higher (P <0.01, t=4.81, df = 4.81) number of wasps than reference sites (4 ± 1) and heavily-oiled sites (3 ± 1, Figure 6d).

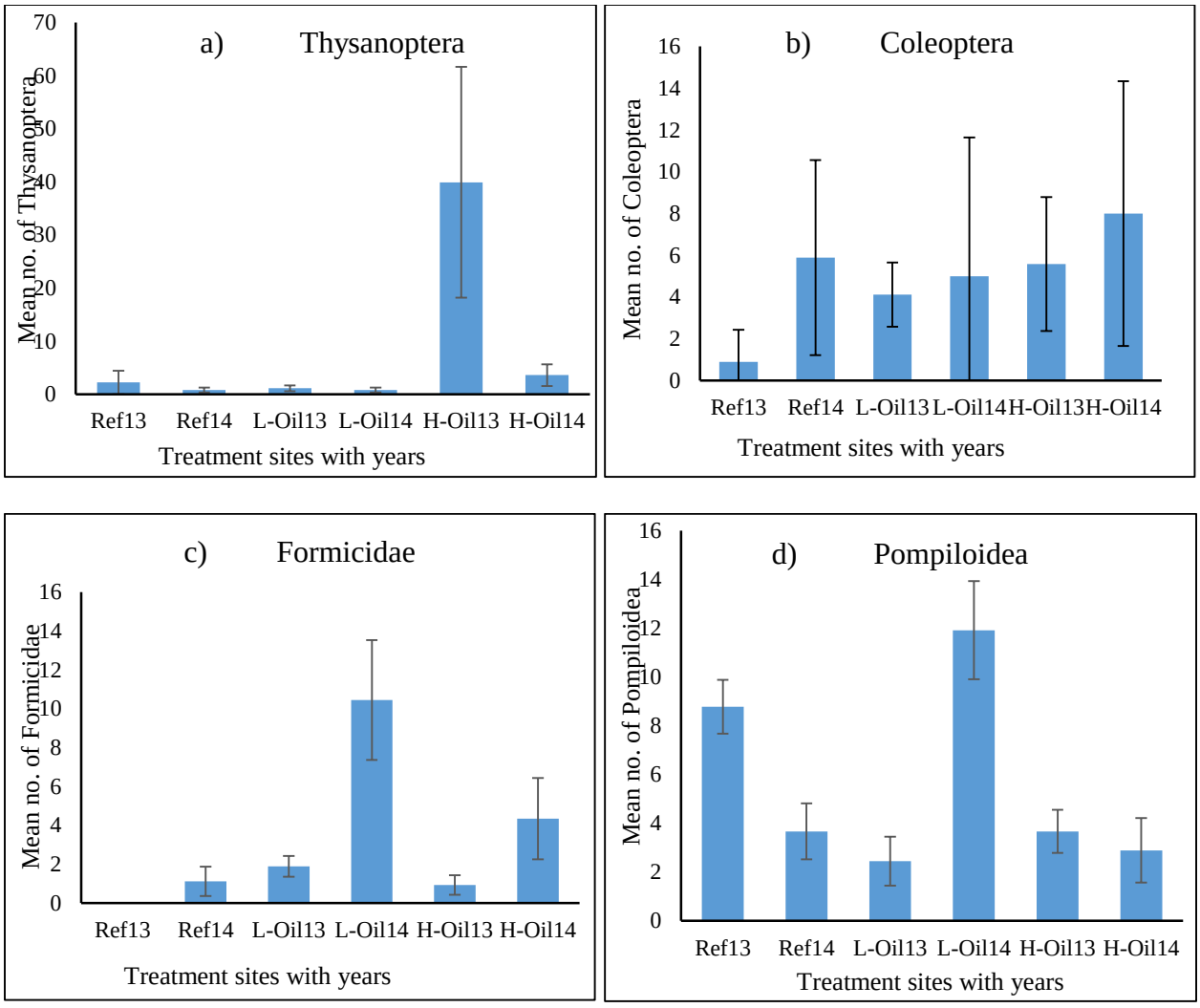


Figure 6. Mean (±SEM) abundance of arthropods collected per sweep: a) Thysanoptera, b) Coleoptera, d) Formicidae, and e) Pompiloidea with respect to treatment sites and years.

Lepidoptera (moths) were also rare in occurrence in the collected samples with a mean value of less than one (Figure 7a). There was no significant difference in treatment, years and interaction among the all sites (Table 2). Diptera (flies) included the families Ulidiidae and Chloropidae. Diptera were significantly lower ($P < 0.01$, $t = 4.04$, $df = 47$) in reference sites (22 ± 4) in 2013 and (30 ± 7) 2014 than lightly-oiled (67 ± 18, 2013; 62 ± 10, 2014) and heavily-oiled sites (86 ± 14, 2013; 81 ± 8, 2014). Lightly-oiled and heavily-oiled sites showed a decrease in the number of flies in 2014, but the reference sites showed an increase in 2014, however, the change in numbers was not significantly different ($P > 0.2$, $t = 1.06$, $df = 47$) for any of the sites

(Figure 7b). There were significant differences only in treatments but not significant difference with years and interaction (P= 0.80, t= -0.25, df = 47, table 2).

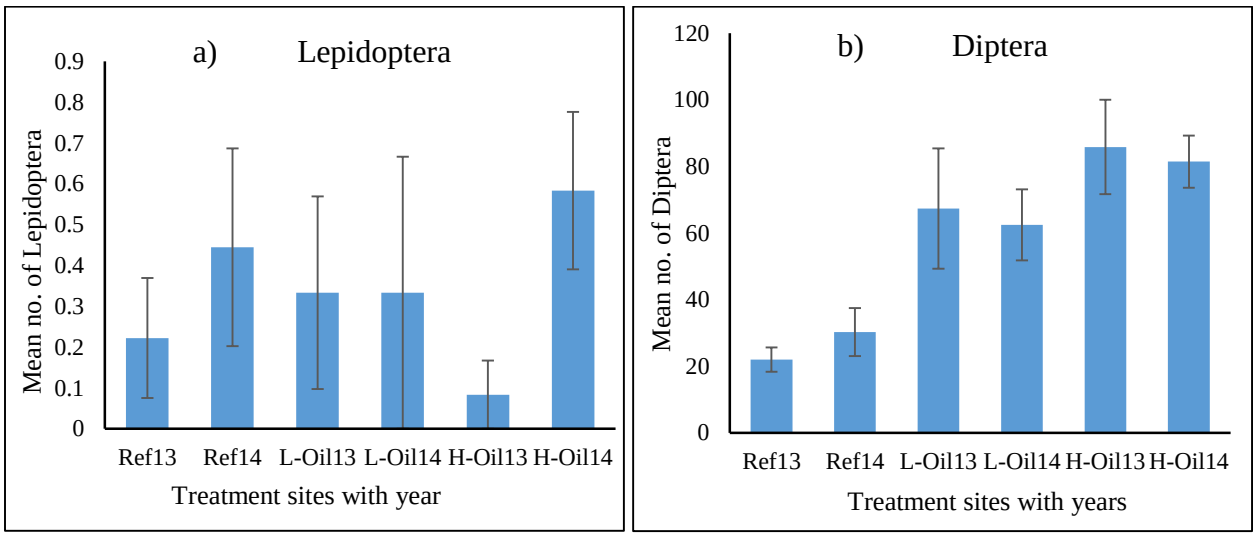


Figure 7. Mean (±SEM) abundance of arthropods collected per sweep: a) Lepidoptera and b) Diptera with respect to treatment sites and years.

The midges and mosquitoes were grouped together under a superfamily Culicoidea following the morphospecies approach. The reference sites (69 ± 35) had higher a numbers of Culicoidea followed by heavily-oiled (48 ± 15) and lightly-oiled (44± 10) sites in 2013, but were not significantly different (P = 0.83, t= 0.21, df =47). But in 2014, reference sites (64 ± 36) had significantly lower (P < 0.02, t= 2.39, df= 47) numbers of Culicoidea than lightly-oiled (153 ± 57) and heavily-oiled sites (170 ± 34, Figure 8a). The Culicoidea population was significantly higher (P < 0.01, t = -2.69, df= 47) in heavily-oiled and lightly-oiled sites in 2014 than 2013 (Table 2) but not significantly different (P > 0.20) between the two years in reference sites.

Araneae (spiders) were not significantly different (P = 0.43, t= -0.78, df = 47) between heavily-oiled (24 ± 2) and lightly-oiled (20 ± 4), but significantly fewer in reference sites (12 ± 4) in 2013. Spiders in reference sites (32 ± 4) in 2014 were significantly increased and higher (P < 0.01, t= -4.15, df= 47) when comparing with reference sites (2013), as well as heavily-oiled

sites (18 ± 4, 2014) and lightly-oiled sites (15 ± 3, 2014; Figure 7b). There was no significant differences in the number of individuals of arthropods and the PAHs concentration of sediments (Appendix E).

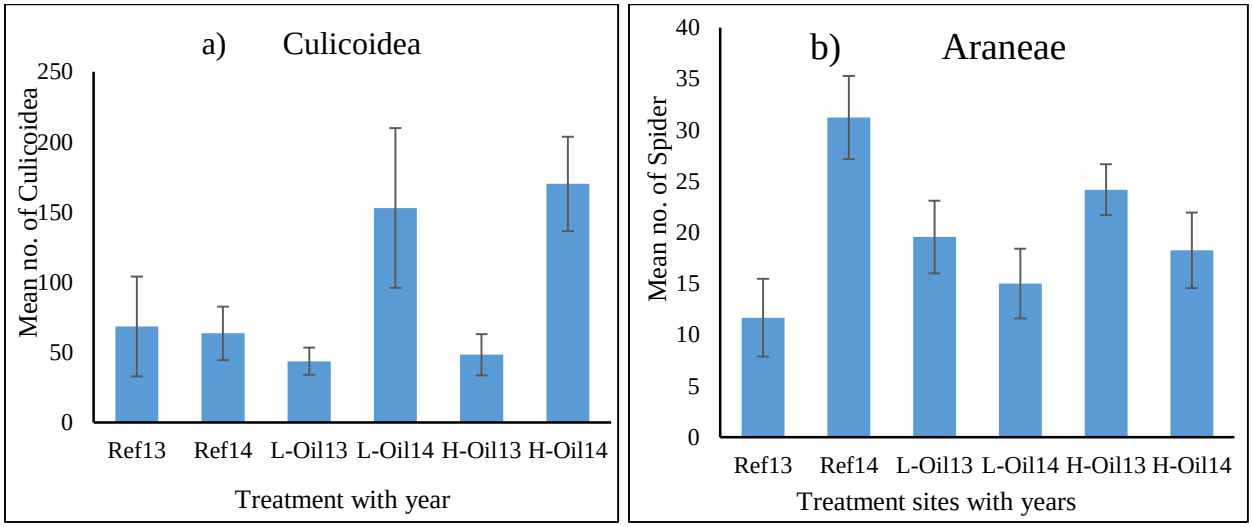


Figure 8. Mean (±SEM) abundance of arthropods collected per sweep: a) Culicoidea, b) Araneae, with respect to treatment sites and years.

Trophic Distribution

The terrestrial arthropods collected during the study were categorized into three trophic levels: herbivores, omnivores and predators. Trophic levels were categorized based on the literature, online sources (bugguide.net) and personal communication with respective experts. In 2013, reference sites, arthropod community consisted of 32.5% herbivores, 55.5% omnivores and 12 % predators, whereas in 2014 the arthropod community was made up of 40% herbivores, 40% omnivores and 20% predators. The lightly-oiled sites community consisted of 58% herbivores, 35% omnivores and 7% predators in 2013, and 48% herbivores, 46.4% omnivores and 5.6% predators in 2014. The heavily-oiled sites community was comprised of 59.9% herbivores, 33.3% omnivores and 6.8% predators in 2013 whereas 38.4% herbivore, 54.4% omnivore and 7.2% predators in 2014. Trophic levels for treatment and year are shown on figure 9, expressed on the composition percentage.

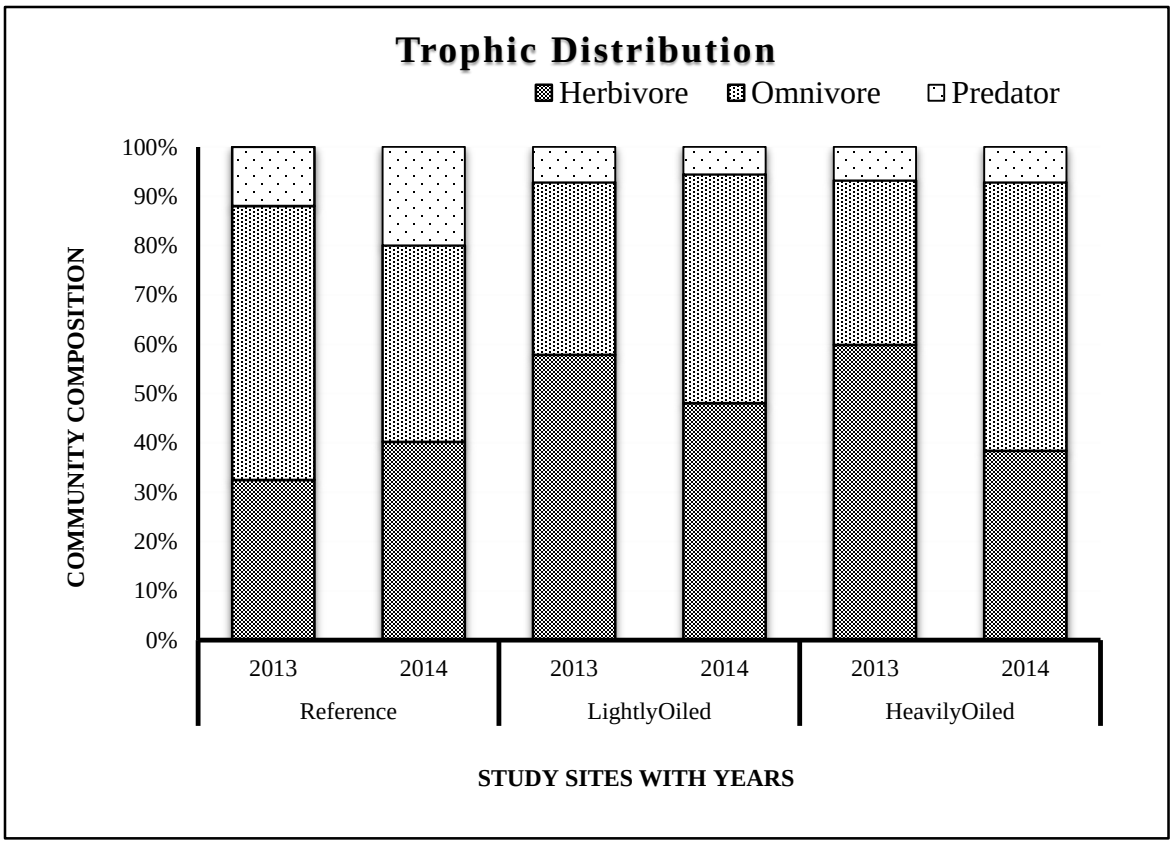


Figure 9. Trophic community composition of terrestrial arthropods

Community Structure

The principal component analysis explained 94% of variation (76.82% component 1 and 17.2% component 2) of the community structure as shown on figure 10. The reference sites, community structures were distinctive in both 2013 and 2014, as well as lightly-oiled and heavily-oiled sites. The lightly-oiled and heavily-oiled sites community composition in 2013 overlaps with each other and grouped together. In 2014, in the lightly-oiled and heavily-oiled sites community composition is similar but not overlapped. Although the lightly-oiled and heavily-oiled sites community composition is similar to each other in respective years, there is a clear distinction in community structure in 2013 and 2014 as shown on Figure 10. The reference sites community composition in 2013 is similar to lightly-oiled and heavily-oiled sites community composition of 2014, but not overlapped. The high abundance of the Delphacidae

and Culicoidea mainly influence the community structure of the treatment and reference sites (Figure 11). Most of the insects are grouped together beside the Delphacidae and Culicoidea, which were scattered toward positive axis of component 1 with high value. Second PCA was run after ignoring the Delphacidae and Culicoidea.

The second PCA (PCA II) explained 92% variation (Fig 12). In PCA II, reference sites in both years are placed closer to each other in a positive axis, but different and the high value of both component I and component II. The lightly-oiled and heavily-oiled sites in both years 2013 and 2014 are clustered together in the positive axis of component I and negative axis of component II. After removing the leafhopper and midges, the community structure of reference sites resulted in difference with the community structure of heavily-oiled and lightly-oiled sites in both years.

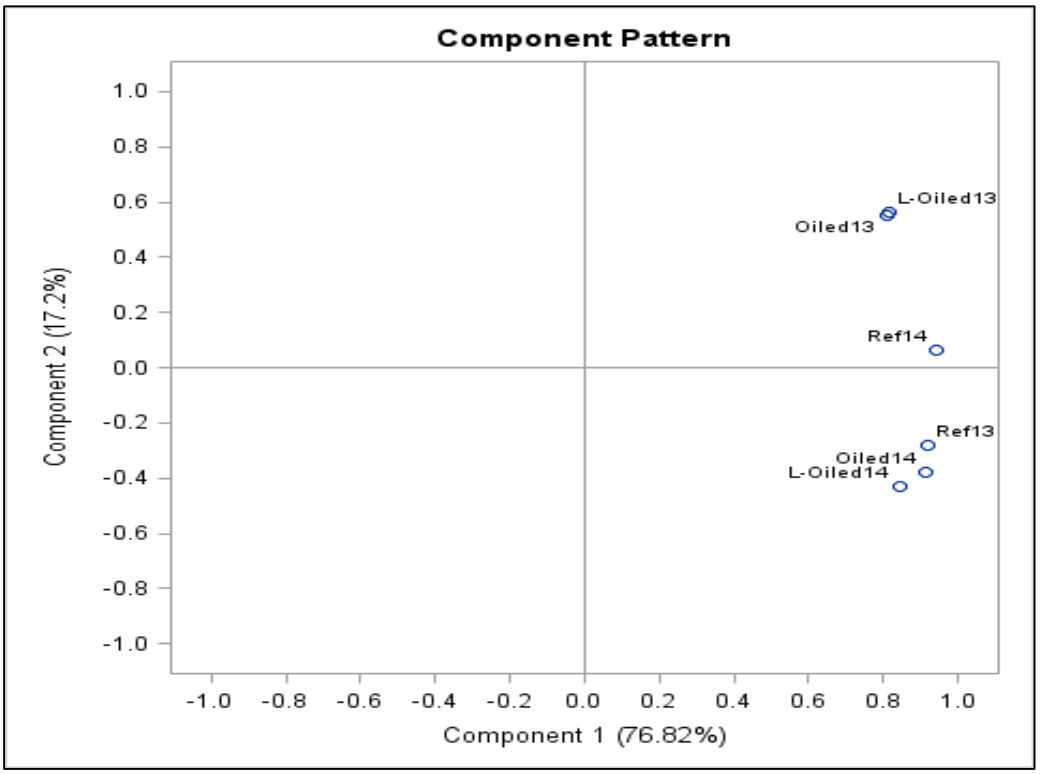


Figure 10. Community Structure Analysis using Principal Component Analysis (PCA) – Covariance for the treatment sites and year.

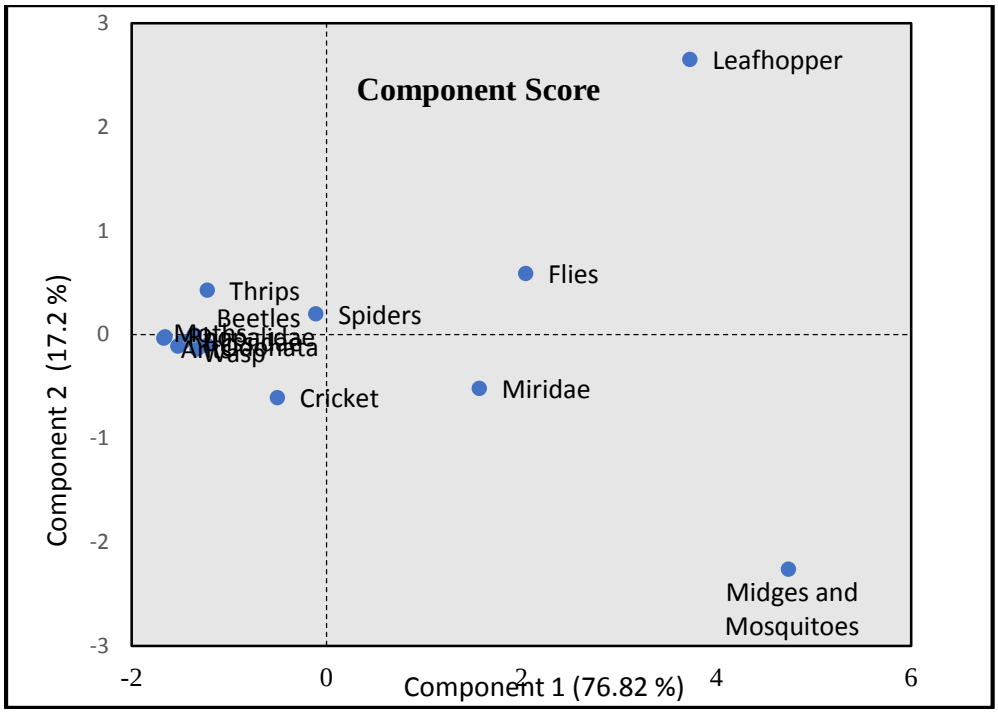


Figure 11. Component score for each individual family/order obtained using Principal Component Analysis (PCA) – Covariance for the treatment sites and year.

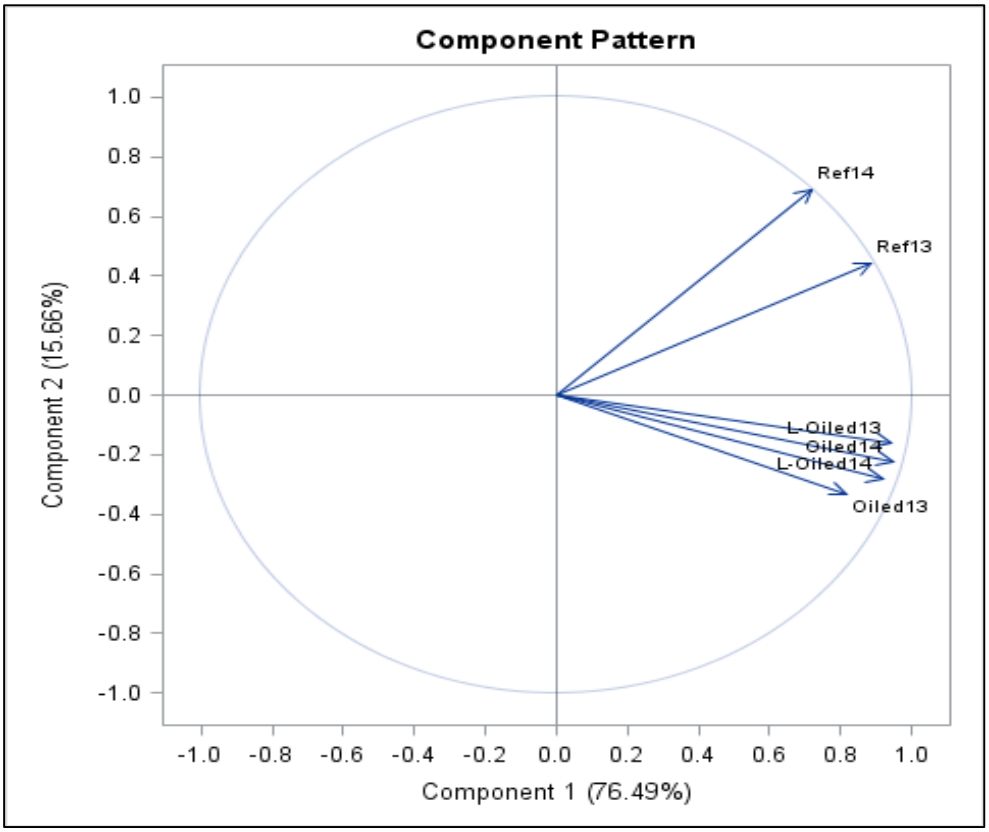


Figure 12. Community Structure Analysis using Principal Component Analysis II (PCA II) – Covariance for the treatment sites and year.

DISCUSSION

Oil pollution had various effects on different terrestrial arthropods in my study. Each group I measured responded differently over the treatments, years and interactions. Some of the arthropod groups were affected in years three and four after the disaster, whereas some didn't show any response at this time. It should be noted that this study was conducted several months after Hurricane Isaac spent ~72 hours over south Louisiana covering the marsh with storm surge. Hurricane Isaac landed and passed through the study sites from 29th August to 3rd September 2012 (Breg 2013), and created disturbance (storm surge) to the coastal ecosystem including the arthropod community, while redistributing the Macondo oil (Turner et al 2014a and Hooper-Bui and E Overton pers. comm.). Sample collections in lightly-oiled and heavily-oiled sites were done on the same day, whereas samples were collected in reference sites the following day. The effect of temperature was ignored for this study as Speight et al. (2008) explained there are minimal effects of change in temperature from 20ºC to 30ºC in insects' response. Rainy days were excluded from sampling to avoid any potential in sampling error; salinity was not factored into the results. The terrestrial arthropod communities can have seasonal changes in trophic level depending upon the availability of resources (Cameron 1972, Ali-Shtayeh et at. 2010). Maximum abundance of terrestrial arthropods were observed in summer and spring with minimum in winter (Ali-Shtayeh et al. 2010). I assumed that the species richness, abundance of arthropods and seasonal changes over three months (April – June) spring/summer was minimal. Hence, I considered the three months sample collection as replicated samples and mean of the samples was used in species richness and diversity index.

This study was done during April – June of 2013 and 2014 when terrestrial arthropods are present as food for the nesting Seaside Sparrow. Summer is regarded as best sampling time for

insects and spiders in saltmarshes (Davis and Gray 1966). Some terrestrial arthropods might be opportunistically present in the saltmarsh, which might have been randomly found in the samples or, they were simply rare in the marsh. The rare species influences the species richness slightly depending upon their evenness and total abundance (Wisley and Stirling 2007). When competition regulates the communities, evenness and richness are weakly related (Wisley and Stirling 2007). So such extremely rare arthropods in both evenness and abundance in samples were not included in the species richness and diversity measurements.

The abundance and species richness of terrestrial arthropods is also influenced by immigration from adjacent habitats (Desender and Maelfait 1999). Some of the study sites were fragmented saltmarshes and the overall land area of saltmarshes was not considered for species richness and diversity measurements. The number of species available in an island is influenced by the area of the island, which is represented by the species-area curve equation;

$$logS = logc + zlogA$$

where S is the number of species, c and z are constants and A is the area of habitat or island (Lomolino 2000). For this study, only the sampling area size was considered, not the total area of the island or saltmarsh. All our sampling area were of same size, but not essentially the islands/marsh area, hence species richness are not explained by the area of marshes rather than by the transect (20m X 5m). A study conducted by Desender and Maelfait (1999) reinforced the species-area curve equation with findings that marshes with larger area had higher number of species abundance. Also, all the samples were collected near the edge of the salt marshes and did not count for gradient changes along the marshes.

It was observed that the overall abundance in 2014 (Figure 2) were significantly higher than 2013 for all the reference, lightly-oiled and heavily-oiled sites. This provides evidence of whole ecosystem recovery in the 6-18 months after Hurricane Isaac in August 2012. I will discuss the possible reasons for the higher arthropod individual numbers when I discuss each group examined. The main points are: (1) individuals that are expert fliers and colonizers are in higher numbers in 2014 than 2013; (2) individuals who feed on detritus or stressed plants are in higher numbers in oiled sites than reference sites.

Halobinotic/Halophilic species of terrestrial arthropods dominate the saltmarshes and brackish marshes (Desender and Maelfait 1999). Species richness is one of the simplest measure to represent the number of species present in certain area (Cameron 1972) and with two basic components: number of species, and evenness of distribution (Huston 1994; Purvis and Hector 2000, Magurran 2004). Magurran (1988) suggested that species richness could be regarded as good indicator of the health of an ecosystem and is often used to conduct the environmental assessment studies (Bechtel and Copeland 1970; Egloff and Brakel 1973; Wu 1982; Roth et al. 1994; Karr and Kimberling 2003; Bowser and Morton 2008). Species richness was higher at nearly 0.8 in reference sites in both years as opposed to oiled sites, which ranged from 0.5 to <0.6. This shows that even after four years and several tropical storms and two hurricanes, that species richness is negatively affected by presence of Macondo oil. Shannon Weaver Index was significantly higher for all sites in 2014 than in 2013, which suggests positive recovery of the insect and spider communities after Hurricane Isaac. Because the Shannon Weaver Index takes into account the relative abundance of the measured taxa, a highly abundant taxa will mask differences in evenness and richness. For example, large numbers of Diptera were present

because of large amounts of dead material; additionally, herbivorous insects that capitalize on the stress of plants were numerous.

My results showed the mixed effects of oil contamination, however McCall and Pennings (2011) found saltmarsh invertebrate communities to be strongly affected - suppressed by 50% - by oil contamination within the first year. This can perhaps be attributed to the timing of the experiment. McCall and Pennings (2011) showed that oiled sites recovered within the first year and were similar to the control sites. McCall and Pennings (2011) found that in 2010 oiled sites had fewer arthropods than control sites. All the groups of terrestrial arthropods were not affected homogeneously. Some of the arthropods groups show treatment effects, whereas some arthropods groups have temporal differences or differences in the years and interaction. After initial recovery from Isaac - Odonata and Araneae - some of the most abundant arthropods were significantly higher in reference sites than lightly-oiled and heavily-oiled sites. They were comparatively more affected than Coleoptera and Diptera in 2014. The dragonflies and damselflies may have had lower numbers on oiled sites because of decreased prey availability for these fast-flying predaceous insects. The dragonflies and damselflies are predators mainly feeding on smaller insects and spiders. Orthoptera and Miridae were more plentiful in lightly-oiled sites in 2014 compared to lightly-oiled in 2013 and heavily-oiled and reference sites for both years. The reduction of predators might have led to an increase of herbivores (Figure 9). In 2013, Delphacids increased in oiled sites, which were increased due to the combined stress of oil and Hurricane Isaac. Delphacidae were similar across all reference, lightly-oiled and heavily-oiled sites in 2014, suggesting the population of Delphacidae might be back to normal. Blissidae in 2014 increased in all sites compared to 2013, however highly-oiled sites had higher increase in

numbers of Blissidae than other sites. Blissdae in heavily-oiled sites might have migrated from adjacent marshes because of lowered defenses in stressed plants.

The herbivores in both lightly-oiled and heavily-oiled sites were higher in 2012 compared to 2014, as demonstrated by the spike in Thysanoptera populations in heavily-oiled sites in 2013. Mattson and Haack (1987) and Larsson (1989) has explained the linkage between increasing herbivory with an increase in plant stress. Herbivores might have increased in response to the stress and disturbance caused by Hurricane Isaac combined with the effects of Macondo oil. Lightly-oiled and heavily-oiled sites are located in Barataria Bay, which was the predominant path of Hurricane Isaac. All the sites had almost similar numbers of Coleoptera across all sites, except the reference sites in 2013. After 3-4 years of oil contamination, Coleoptera might be able to recover and being omnivorous, they had enough food sources available to recover.

Ground-dwelling crickets are interesting because some of members of their group lives in close contact with the sediment. The crickets increased in abundance in all sites reference sites in 2014 but significantly in lightly-oiled and oiled-sites. Having both omnivores (crickets) and herbivores (long-faced katydids), Orthoptera populations increased in oiled areas possibly indicating an increase in dead forage and increase in plant stress. Orthoptera were clearly affected by Hurricane Isaac and made a resurgence across the ecosystem in 2014. Ants were greatly affected by Hurricane Isaac (Hooper-Bui pers. comm.) and my data from 2013 support that. However, my data show ants present in all areas in 2014, presumably because of mating flights that occur in April (Hooper-Bui pers. comm.). Ants were present in all sites through June 2014. Spider wasps are strong predators on highly mobile prey – the spiders. They increased along with the spiders on the plots.

Lepidoptera, which play important role in pollination in plants, were rare in the samples collected from all sites for both years and it was hard to establish any effects of oil and the hurricane on Lepidoptera. Diptera is reported to be highly abundant in saltmarshes by Kubatova-Hirsova (2005), which is supported by my study. Diptera are likely increased because of abundance of dead and decaying material at the oiled sites and because they are swift fliers able to colonize compromised habitats quickly. Culicoidea, which are filter feeders on detritus as larvae and predatory as adults, had increased population in oiled sites in 2014 but reference sites had similar numbers in both years. However the total proportion of predator didn't change for oiled sites in 2013 and 2014.

After the disturbance in the saltmarshes, it was assumed that predators would increase over time as the result of increases in herbivores (Petchey et al. 2004). Predators can alter abundance of herbivores in the ecosystem (Schmitz 1992). Herbivorous insects' outbreak are associated with different abiotic/environmental stress or disturbances (Larsson and Tenow 1984, Larrson 1989, Spiller and Schoener 2007, Menge and Sutherland 1987, Power et al. 1996, Wootton et al. 1996, Preisser and Strong 2004, Logan et al. 2003). An outbreak of herbivores can be explained for various reasons due to stress in plants (Larsson 1989), absence of topdown control by parasitic wasps (Stireman et al. 2005), density-dependent responses (Abrams 1995, Siemann 1998), shift of predators/parasitoids feeding preferences (Murdoch 1969), and overall reduction in predation (Menge and Sutherland 1987; Spiller and Schoener 2007).

Hunter and Forkner (1999), Hirsh and Marler (2002), Spiller and Agrawal (2003), and Nakamura et al. (2005) have found increases in herbivores population after hurricanes. Spiller and Schoenor (2007) explained predators such as web spiders may affect herbivores by direct predation (decrease herbivores population) or by feeding on predators/parasitoids that feed on

herbivores (increase herbivores population). Increase in consumption of plants by herbivores (terrestrial arthropods) was observed following hurricane disturbance (Spiller and Schoener 2007). The increase in predators such as Araneae would reduce the abundance of herbivores such as Delphacidae (Schmitz 1998). My results support Schmitz's (1998) statement as there is decrease in Delphacidae whereas there were increases in Araneae in reference sites. Araneae are predators and eat whatever comes their way.

The reference sites were comparatively less disturbed by Hurricane Isaac than light-oiled and heavily-oiled sites. The reference sites had a lower herbivore percentage in 2013 than in 2014 whereas, it was opposite for lightly- and heavily-oiled sites; this is good evidence for plant stress. Higher percentages of herbivores in lightly- and heavily-oiled sites in 2013 supports the previous studies mentioned above. In 2014, the percentage of omnivores was lower compared to 2013 in reference sites, but it was higher in both lightly-oiled and heavily-oiled sites in 2014 giving credence to the idea that stress will increase numbers of some omnivores. For lightly- and heavily-oiled sites, the percentage of predators in both years was similar. Predators were reduced in the oiled sites in 2014 compared to reference sites. Predators were in similar percentage in lightly-oiled and heavily-oiled sites in both years in my study.

Previously and at different sites in the same basin, McCall and Pennings (2011) had significant increase in the number of predators in control and oiled sites over time. McCall and Pennings suggested that the effect was similar across the different trophic levels, but my results suggested the effect was not similar four years after the disaster. This demonstrates that predators showed a lag in the differential and indirect effect of the oil.

The proportion of increase in herbivores is similar in lightly-oiled and heavily-oiled sites in 2013, which might suggest increases of herbivores in response to stress. Overall the

percentage of herbivores was greater than omnivores and predators, which partially supports the concepts of Speight et al. (2008) that more than 50% of existing arthropods in the ecosystem are herbivores and feed on plants. This study found the majority of trophic distribution was herbivores, supporting Davis and Gray (1966), but not essentially 50% of total arthropods were herbivores. The significant increase of Araneae (predators) in reference sites in 2014 might be one of the reasons for decreases in herbivores and omnivores, which was not observed in lightly-oiled and heavily-oiled sites.

The study also reported total number of arthropods were similar in control (~1,500 arthropods per $0.5m^2$) and oiled sites (~1,600 arthropods per $0.5m^2$) in 2011 but higher than 2010 (~1,100 arthropods per $0.5m^2$ control sites and 500 arthropods per $0.5m^2$ oiled sites) (McCall and Pennings, 2011). My study also found higher numbers of arthropods in 2014 (mean ± SEM) (251 ± 133 reference, 476 ± 374, 477 ± 167 heavily-oiled) than in 2013 (165 ± 134 reference, 329 ± 217 lightly-oiled and 420 ± 199 heavily-oiled). McCall and Pennings (2011) used Dietrick Vaccum sampling whereas I collected samples using sweep net. Though there is a difference in the total number of arthropods collected, more importantly both the results suggest in the increase of arthropods the following year in reference sites as well as oil contaminated sites. McCall and Pennings' (2011) results added to my higher species richness in reference sites, higher diversity index in 2014 of this study, and some of the results of this study can suggest that the oil contamination effects still continue but there is also a slow recovery of the ecosystem.

Some of the differences in responses of certain arthropods may be associated with the sampling technique. The insects that reside inside the stems of plants or tightly attached to plants might rarely get in the sweep net, whereas other arthropods might have higher chances of getting into the net. Also, the highly active arthropods such as Orthoptera, Odonata and Lepidoptera

have lower chances of being captured in the net compared to others such as Araneae. The results of this study cannot be completely compared with the results of McCall and Pennings (2011) study as they sampled each site once per year. This study partially supports the overall findings and conclusion of McCall and Pennings (2011), i) the impacts of oil contamination cannot be overlooked and ii) the terrestrial arthropod communities are capable of recovery from ecological or chemical disturbance though the rate of recovery for specific arthropods might be different. Additionally, iii) to quantify and explain the effects of oil spills and pollution on recovery and succession of marsh ecosystems, baseline and long-term data are needed.

REFERENCES

Adam, P., 1990. *Saltmarsh Ecology.* Cambridge University Press. New York, NY 10011. 461pp.

Adhikari, P.L., Maiti, K., Overton, E.B., 2015. Vertical fluxes of polycyclic aromatic hydrocarbons in the northern Gulf of Mexico. *Marine Chemistry* 168, 60-68.

Abrams, P.A., 1995. Monotonic or unimodal diversity–productivity gradients: what does Competition theory predict? *Ecology* 76, 2019–2027.

Adriaens, P., 2014. *Ammodramus maritimus* (Wilson, 1811). Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=15939.

Ali-Shtayeh, M.S., Ali, W.M.D., Jamous, R.M., 2010. Ecological investigations on terrestrial arthropods biodiversity under different grassland ecosystems in el-Faraa area (Palestine). *Biodiversity and Environmental Sciences Studies Series* 5, 19-34.

Baldi, A., 2003. Using higher taxa as surrogates of species richness: a study based on 3700 Coleoptera, Diptera, and *Acari* species in Central-Hungarian reserves. *Basic and Applied Ecology* 4, 589–93.

Barbier, E.B., Heal, G.M., 2006. Valuing ecosystem services. *Economists' Voice* 3, 1-6.

Bass, A.S., Turner, R.E., 1997. Relationships between salt marsh loss and dredged canals in three Louisiana estuaries. *Journal of Coastal Research* 13, 895-903.

Bechtel, T.J., Copeland, B.J., 1970. Fish species diversity indices as indicators of pollution in Galveston Bay, Texas. *Contributions in Marine Science* 15, 103-132.

Boesch, D.F., Josselyn, M.N., Mehta, A.J., Morris, J.T., Nuttle, W.K., Simenstad, C.A., Swift, D.J., 1994. Scientific assessment of coastal wetland loss, restoration and management in Louisiana. *Journal of Coastal Research* i-103.

Bowser, M.L., Morton, J.M., 2008. Monitoring and modeling terrestrial arthropod diversity on the Kenai National Wildlife Refuge. In *McWilliams, W., Moisen, G., Czaplewski, R., (Comps.), Proceedings of the Forest Inventory and Analysis (FIA) Symposium*.

Berg, R., 2013. Tropical cyclone report: Hurricane Isaac (AL092012). *National Hurricane Center*, *NOAA.*

Burns, C.M.B., Olin, J.A., Woltmann, S., Stouffer, P.C., Taylor, S.S., 2014. Effects of oil on terrestrial vertebrates: Predicting impacts of the Macondo blowout. *BioScience* 64, 820-828.

Cameron, G.N., 1972. Analysis of insect trophic diversity in two salt marsh communities. *Ecology* 53, 58-73.

Couvillion, B.R., Barras, J.A., Steyer, G.D., Sleavin, W., Fischer, M., Beck, H., Trahan, N., Griffin, B., Heckman, D., 2011. Land area change in coastal Louisiana from 1932 to 2010: US Geological Survey Scientific Investigations Map 3164, scale 1: 265,000.

Crone, T.J., Tolstoy, M., 2010. Magnitude of the 2010 Gulf of Mexico oil leak. *Science* 330, 634-634.

Crooks, S., Turner, R.K., 1999. Integrated coastal management: sustaining estuarine natural resources. *Advances in Ecological Research* 29, 241-289.

Davis, L.V., Gray, I.E., 1966. Zonal and seasonal distribution of insects in North Carolina salt marshes. *Ecological Monographs* 36, 275-295.

Day, R.H., Murphy, S.M., Wiens, J.A., Hayward, G.D., Harner, E.J., Smith, L.N., 1997. Effects of the Exxon Valdez oil spill on habitat use by birds in Prince William Sound, Alaska. *Ecological Applications* 7, 593-613.

De la Huz, R., Lastra, M., Junoy, J., Castellanos, C., Vieitez, J.M., 2005. Biological impacts of oil pollution and cleaning in the intertidal zone of exposed sandy beaches: preliminary study of the "Prestige" oil spill. *Estuarine, Coastal and Shelf Science* 65, 19-29.

Desender, K., Maelfait, J.P., 1999. Diversity and conservation of terrestrial arthropods in tidal marshes along the River Schelde: a gradient analysis. *Biological Conservation* 87, 221-229.

Doxon, E.D., Davis, C.A., Fuhlendorf, S.D., 2011. Comparison of two methods for sampling invertebrates: vacuum and sweep-net sampling. *Journal of Field Ornithology* 82, 60-67.

Egloff, D. A., Brakel, W.H., 1973. Stream pollution and a simplified diversity index. *Journal Water Pollution Control Federation* 45, 2269-2275.

Engle, V.D., 2011. Estimating the provision of ecosystem services by Gulf of Mexico coastal wetlands. *Wetlands* 31, 179-193.

Granek, E.F., Polasky, S., Kappell, C.V., Reed, D.J., Stoms, D.M., Kochm, E.W., Kennedy, C.J., Cramer, L.A., Hacker, S.D., Barbier, E.B., Aswani, S., Ruckelshaus, M., Perillo, G.M.E., Silliman, B.R., Muthiga, N., Bael, D.,Wolanski, E., 2009. Ecosystem services as a common language for coastal ecosystem-based management. *Conservation Biology* 24, 207-216.

Greenberg, R., 2006. Tidal marshes: home for the few and the highly selected. *Studies in Avian Biology* 32, 2-9.

Greenberg, R., Maldanado, J.E., 2006. Diversity and endemism in tidal marsh vertebrates. S*tudies in Avian Biology* 32, 32-53.

Greenlaw, J.S., Woolfenden, G.E., 2007. Wintering distributions and migration of saltmarsh and Nelson's Sharp-tailed Sparrows. *The Wilson Journal of Ornithology* 119, 361-377.

Hafernik, J.E.J., 1992. Threats to invertebrate biodiversity: implications for conservation strategies. In *The Theory and Practice of Nature Conservation, Preservation and Management* (eds P.L. Fielder & S.K. Jain) 172–195. Chapman & Hall, London.

Harrison, R.D., Rasplus, J.Y., 2006. Dispersal of fig pollinators in Asian tropical rain forests. *Journal of Tropical Ecology* 22, 631–639.

Hastings, A., Byers, J.E., Crooks, J.A., Cuddington, K., Jones, C.G., Lambrinos, J.G., Theresa S. Talley, T.S., Wilson., W.G., 2007. Ecosystem engineering in space and time. *Ecology letters* 10, 153-164.

Hirsh, H., Marler, T., 2002. Damage and recovery of Cycas micronesica after Typhoon Paka. *Biotropica* 34, 598–602.

Hunter, M.D., 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Agricultural and Forest Entomology* 3, 77–84.

Hunter, M.D., Forkner, R.E., 1999. Hurricane damage influences foliar polyphenolics and subsequent herbivory on surviving trees. *Ecology* 80, 2676–2682.

Huston, M.A., 1994. Biological Diversity. Cambridge University Press, Cambridge, UK.

J.B., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J. et al., 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293, 629-637.

Jennings, V.H., Tallamy, D.W., 2006. Composition and abundance of ground-dwelling Coleoptera in a fragmented and continuous forest. *Environmental Entomology* 35, 1550-1560.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. *Oikos* 69, 1-14.

Jorgensen, S.E., 2009. Ecosystem Ecology. Academic Press (390). Amsterdam, Netherlands.

Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P., Lepage, M., 2006. Soil invertebrates as ecosystem engineers: intended and accidental effects on soil and feedback loops. *Applied Soil Ecology 32*, 153-164.

Karr, J.R., Kimberling, D.N., 2003. A terrestrial arthropod index of biological integrity for shrubb-steppe landscapes. *Northwest Science* 77, 202-213.

Kremen, C., Colwell, R.K., Erwin, T.L., Murphy, D.D., Noss, R.F., Sanjayan, M.A., 1993. Terrestrial arthropod assemblages: their use in conservation planning. *Conservation Biology* 7, 796-808.

Kubatova-Hirsova, H., 2005. Temporal patterns in shore fly (Diptera, Ephydridae) community structure in a salt marsh habitat. *Ecological Entomology* 30, 234-240.

Larson, D.P., O'Neill, K.M., Kemp, W.P., 1999. Evaluation of the accuracy of sweep sampling in determining grasshopper (Orthoptera: Acrididae) community composition. *Journal of Agriculture and Urban Entomology* 16, 207-214.

Lomolino, M., 2000. A call for a new paradigm of island biogeography. *Global Ecology and Biogeography 9*, 1-6.

Larsson, S., 1989. Stressful times for the plant stress: insect performance hypothesis. *Oikos* 56, 277-283.

Larsson, S., Tenow, O., 1984. Areal distribution of a Neodiprion sertifer (Hym., Diprionidae) outbreak on Scots pine as related to stand condition. *Ecography* 7, 81-90.

Leibold, M. A. 1996. A graphical model of keystone predation: effects of productivity on abundances, incidence and ecological diversity in communities. *American Naturalist* 147, 784–812.

Logan, J.A., Regniere, J., Powell, J.A., 2003. Assessing the impacts of global warming on forest pest dynamics. *Frontiers in Ecology and Environment* 1, 130–137.

Longcore, T., 2003. Terrestrial arthropods as indicators of ecological restoration success in coastal sage scrub (California, U.S.A.). *Restoration Ecology* 11, 397-409.

MacArthur, R.H., 1965. Patterns of species diversity. *Biological Reviews* 40, 510-533.

Macreadie, P.I., Hughes, A.R., Kimbro, D.L., 2013. Loss of 'blue carbon' from coastal salt marshes following habitat disturbance. *PloS one 8*, e69244.

Magurran, A.E., & Magurran, A. E. (1988). *Ecological diversity and its measurement*, Vol, 168. Princeton, NJ: Princeton University Press 179p.

Magurran, A.E., 2004. Measuring Biological Diversity. Blackwell Publishing, Oxford, U.K

Mattson, W., Haack, R., 1987. The role of drought in outbreaks of plant-eating insects. *Bioscience* 37, 110-118.

Menge, B.A., Sutherland, J.P., 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. *American Naturalist,* 130, 730-757.

Meyer, J.L., O'Hop, J., 1983. Leaf-shredding insects as a source of dissolved organic carbon in headwater streams. *American Midland Naturalist* 109, 175–83.

McGeoch, M.A., 1998. The selection, testing and application of terrestrial insects as bio-indicators. *Biological Reviews of the Cambridge Philosophical Society* 73, 181-201.

Mitsch W.J, Gosselink, J.G., 2000. The value of wetlands: Importance of scale and landscape setting. *Ecological Economics* 35 25-33.

Murdoch, W.W., 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecological Monographs* 39, 335–354.

National Commission, 2011. National Commission on the BP Deep Ocean Horizon Oil Spill and Offshore Drilling (2011). Deep water: The Gulf Oil Disaster and the Future of Offshore Drilling.

Nakamura, M., Utsumi, S., Miki, T., Ohgushi, T., 2005. Flood initiated bottom-up cascades in a tritrophic system: host plant regrowth increases densities of a leaf beetle and its predators. *Journal of Animal Ecology* 74, 683–691.

Oliver, I., Beattie, A.J., 1993. A possible method for the rapid assessment of biodiversity. *Conservation Biology* 7, 562-568.

Oliver, I., Beattie, A.J., 1996. Invertebrate morphospecies as surrogates for species – a case-study. *Conservation Biology* 10, 99-109.

Pennings, S.C., McCall, B.D., Wimp, M.G., 2011. Basic guide to identifying *Spartina alterniflora marsh arthropods.* Accessed on at http://nsmn1.uh.edu/steve/CV/Spartina%20Arthropod%20Guide%2012%202011.pdf.

Pennings, S.C., McCall, B.D., Hooper-Bui, L., 2014. Effects of oil spills on terrestrial arthropods in coastal wetlands. *BioScience* 64, 789-795.

Petchey, O.L., Downing, A.L., Mittelbach, G.G., Persson, L., Steiner, C.F., Warren, P.H., Woodward, G., 2004. Species loss and the structure and functioning of multitrophic aquatic systems. *Oikos* 104, 467-478.

Pfeiffer, W.J., Wiegert, R.G., 1981. Grazers on Spartina and their predators. In *The ecology of a Salt Marsh* (pp. 87-112). Springer New York.

Post, W., Greenlaw, J.S., 2009. Seaside Sparrow (*Ammodramus maritimus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America http://bna.birds.cornell.edu/bna/species/127.

Post, W., J. S. Greenlaw, T. L., Wood, L.A., 1983. Comparative ecology of northern and southern populations of the Seaside Sparrow. Pages 123-136 *in* The Seaside Sparrow, its

biology and management. (Quay, T. L., J. B. Funderburg, Jr., D. S. Lee, E. F. Potter, and C. S. Robbins, Eds.) Occas. Pap. North Carolina Biol. Surv. Raleigh, NC.

Power, M.E., Parker, M. S., Wootton, J.T., 1996. Disturbance and food chain length in rivers. In *Food Webs* pp.286-297. Springer US.

Preisser, E.L., Strong, D.R., 2004. Climate affects predator control of an herbivore outbreak. *American Naturalist* 163, 754–762.

Purvis A., Hector, A., 2000. Review article: getting the measure of biodiversity. *Nature* 405 212–219.

Rosenberg, D. M., Danks, H.V., Luhmkuhl, D.M., 1986. Importance of insects in environmental impact assessment. *Environmental Management* 10, 773–783.

Roth, D.S., Perfecto, I., Rathcke, B., 1994. The effects of management systems on ground-foraging ant diversity in Costa Rica. *Ecological Applications* 4, 423-436.

Sanders, H.L., 1968. Marine benthic diversity: a comparative study. *American naturalist* 102, 243-282.

Sasser, C.E., Visser, J.M., Mouton, E., Linscombe, J., Hartley, S.B., 2014. *Vegetation Types in Coastal Louisiana in 2013* (No. SIM-3290). United States Geological Survey.

Schmitz, O.J., 1998. Direct and indirect effects of predation and predation risk in old-field interaction webs. *The American Naturalist* 151, 327-342.

SAS Institute Inc., SAS9.4 Software, Cary, NC: SAS Institute Inc., 2013.

Schmitz, O.J., 1992. Exploitation in model food chains with mechanistic consumer-resource dynamics. *Theoretical Population Biology* 41, 161-183.

Schowalter, T.D., 1981. Insect herbivore relationship to the state of the host plant: biotic regulation of ecosystem nutrient cycling through ecological succession. *Oikos* 37, 126-130.

Siemann, E., 1998. Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology* 79, 2057–2070.

Silliman, B.R., Bertness, M.D., 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the national Academy of Sciences* 99, 10500-10505.

Speight, M.R., Hunter, M.D., Watt, A.D., 2008. Ecology of insects: concepts and applications. Wiley-Blackwell Science Ltd, Oxford UK.

Spencer, K.L., Harvey, G.L., 2012. Understanding system disturbance and ecosystem services in restored saltmarshes: Integrating physical and biogeochemical processes. *Estuarine, Coastal and Shelf Science* 106, 23-32.

Spiller, D.A., Schoener, T.W., 2007. Alteration of island food-web dynamics following major disturbance by hurricanes. *Ecology 88*, 37-41.

Stireman, J.O.III., Dyer, L.A., Janzen, D.H., Singer, M.S., Lill, J.T., Marquis, R.J., Ricklefs, R.E., Gentry, G.L., Hallwachs, W., Coley, P.D., Barone, J.A., Greeney, H.F., Connahs, H., Barbosa, P., Morais, H.C., Diniz, I.R., 2005. Climatic unpredictability and parasitism of caterpillars: implications of global warming. *Proceedings of the National Academy of Sciences* 102, 17384-17387.

Triplehorn, C.A., Johnson, N.F., 2005. *Borror and DeLong's Introduction to the Study of Insects*. Belmont, CA: Thomson Brooks/Cole.

Turner, R.E., Overton, E.B., Meyer, B.M., Miles, M.S., Hooper-Bui, L., 2014a. Changes in the concentration and relative abundance of alkanes and PAHs from the Deepwater Horizon oiling of coastal marshes. *Marine Pollution Bulletin* 86, 291-297.

Turner, R.E., Overton, E.B., Meyer, B.M., Miles, M.S., McClenachan, G., Hooper-Bui, L., Engel, A.S., Swenson, E.M., Lee, J.M., Milan, C.S., Gao, H., 2014b. Distribution and recovery trajectory of Macondo (Mississippi Canyon 252) oil in Louisiana coastal wetlands. *Marine Pollution Bulletin* 87, 57-67.

US EPA (United States Environmental Protection Agency), 2000. Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW-846. USEPA, Office of Solid Waste and Emergency Response. Washington, D.C. http://www.epa.gov/epawaste/hazard/test methods/index.htm>.

Velando, A., Munilla, I., Leyenda, P.M., 2005. Short-term indirect effects of the 'Prestige' oil Spill on European shags: changes in availability of prey. *Marine Ecology Progress Series* 302, 263-274.

Votier, S.C., Hatchwell, B.J., Beckerman, A., McCleery, R.H., Hunter, F.M., Pellatt, J., Trinder, M., Birkhead, T.R., 2005. Oil pollution and climate have wide-scale impacts on seabird demographics. *Ecology Letters* 8, 1157-1164.

Wallace, J.B., Cuffney, T.F., Webster, J.R., Lughart, G.J., Chung, K., Goldowitz, B.S., 1991. Export of fine organic particles from headwater streams: effects of season, extreme discharges, and invertebrate manipulation. *Limnology and Oceanography* 36, 670-82.

Werner, H.W., 1975. The biology of the Cape Sable Sparrow. Project completion report prepared for the U.S. Nat. Park Serv. Everglades Nat. Park, FL.

Whittaker, R. H., 1972. Evolution and measurement of species diversity. *Taxon* 21, 213-251.

Wimp, G.M., Murphy, S.M., Finke, D.L., Huberty, A.F., Denno, R.F. 2010. Increased primary production shifts the structure and composition of a terrestrial arthropod community. *Ecology* 91, 3303-3311.

Wilsey, B., Stirling, G., 2007. Species richness and evenness respond in a different manner to propagule density in developing prairie microcosm communities. *Plant Ecology* 190, 259-273.

Wootton, J.T., Parker, M.S., Power, M.E., 1996. Effects of disturbance on river food webs. *Science* 273, 1558–1560.

Wu, R.S.S., 1982. Periodic defaunation and recovery in a subtropical epibenthic community, in relation to organic pollution. *Journal of Experimental Marine Biology and Ecology* 64, 253-269.

Young, J., Watt, A., Nowicki, P., Alard, D., Clitherow, J., Henle, K., Johnson, R., Laczko, E., McCracken, D., Matouch, S., Niemela, J., Richards, C., 2005. Towards sustainable land use: identifying and managing the conflicts between human activities and biodiversity conservation in Europe. *Biodiversity and Conservation* 14, 1641–1661.

APPENDIX A
PAHs CONCENTRATION IN THE SEDIMENT SAMPLES

Field ID#: 2013	D1 April	D2 April	D3 April	D1 May	D2 May	D3 May	D1 June	D2 June	D3 June
Aromatic Analyte:	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**
Naphthalene	20	15	15	22	21	18	0.19	0.20	0.12
C1-Naphthalenes	27	17	21	42	33	23	0.19	0.18	0.23
C2-Naphthalenes	16	15	16	38	29	19	0.40	0.31	0.36
C3-Naphthalenes	7.2	7.6	9.5	23	17	10	0.83	0.54	0.51
C4-Naphthalenes	6.7	6.2	8.6	16	14	9.1	1.6	1.0	0.68
Fluorene	3.1	2.1	2.4	4.0	3.1	2.3	0.30	0.18	0.12
C1-Fluorenes	10	9.9	11	25	18	15	2.2	1.4	0.93
C2-Fluorenes	29	30	39	100	82	46	8.9	5.4	3.1
C3- Fluorenes	39	39	57	130	110	53	15	9.5	4.8
Dibenzothiophene	0.50	0.51	0.67	1.2	0.95	0.56	0.09	0.05	0.06
C1-Dibenzothiophenes	2.9	3.1	4.4	10	7.1	4.3	0.90	0.52	0.26
C2-Dibenzothiophenes	5.5	6.0	9.0	20	16	9.3	3.0	1.7	0.82
C3- Dibenzothiophenes	3.0	3.5	5.0	11	9.0	5.4	3.5	2.0	0.99
Phenanthrene	15	12	15	24	19	9.9	1.7	1.4	0.72
C1-Phenanthrenes	37	40	58	150	120	75	7.7	4.5	2.6
C2-Phenanthrenes	48	54	76	190	130	84	18	10	5.6
C3-Phenanthrenes	19	19	27	76	57	35	21	12	6.7
C4-Phenanthrenes	4.4	4.5	6.7	19	14	8.2	7.2	5.3	2.2
Anthracene	19	4.4	3.3	3.9	7.2	1.4	1.8	2.0	0.73
Fluoranthene	100	45	18	23	49	13	22	25	9.0
Pyrene	100	60	46	72	94	38	63	41	16
C1- Pyrenes	48	30	20	73	57	30	47	33	15
C2- Pyrenes	25	16	7.8	21	20	11	34	28	8.4
C3- Pyrenes	11	9.7	4.0	7.3	7.5	5.5	24	19	5.1
C4- Pyrenes	7.1	6.0	1.8	2.3	3.1	1.9	18	15	3.9

Naphthobenzothiophene	0.05	0.03	0.01	0.03	0.04	0.01	0.06	0.05	0.02
C-1 Naphthobenzothiophenes	0.06	0.04	0.02	0.03	0.05	0.03	0.13	0.14	0.04
C-2 Naphthobenzothiophenes	0.05	0.03	0.01	0.02	0.03	0.02	0.14	0.18	0.03
C-3 Naphthobenzothiophenes	0.03	0.03	0.01	0.01	0.02	0.01	0.19	0.16	0.02
Benzo (a) Anthracene	47	16	0.00	2.5	8.4	0.72	3.3	6.1	1.9
Chrysene	43	25	0.51	5.4	12	2.6	34	25	4.2
C1- Chrysenes	31	19	2.0	3.0	13	3.4	54	29	4.6
C2- Chrysenes	15	13	5.2	4.7	7.2	5.2	43	30	6.1
C3- Chrysenes	8.0	8.2	3.0	2.5	3.1	3.0	19	17	0.00
C4- Chrysenes	0.00	7.3	2.9	0.00	0.00	0.00	27	22	0.00
Benzo (b) Fluoranthene	41	20	0.72	3.1	12	1.9	5.3	15	3.7
Benzo (k) Fluoranthene	18	9.2	0.11	2.8	7.3	0.74	4.9	9.4	2.4
Benzo (e) Pyrene	22	14	0.56	2.8	8.7	1.6	10	8.1	2.5
Benzo (a) Pyrene	38	16	0.27	2.4	12	0.78	3.5	5.9	2.0
Perylene	13	15	4.7	3.0	8.9	3.7	14	8.1	8.0
Indeno (1,2,3 - cd) Pyrene	23	12	0.51	1.5	7.4	1.1	3.3	6.2	2.9
Dibenzo (a,h) anthracene	5.6	3.2	0.00	0.48	1.6	0.00	1.5	0.45	0.78
Benzo (g,h,i) perylene	17	9.0	0.00	1.2	4.0	1.1	3.0	3.7	2.1
Total Aromatics	**924**	**643**	**501**	**1,139**	**1,045**	**555**	**531**	**404**	**130**

Field ID#: 2013	U1 April	U1 May	U2 April	U2 May	U2 June	U4 April	U4 May	U4 June
Aromatic Analyte:	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**
Naphthalene	0.16	0.19	0.28	0.19	0.26	0.18	0.19	0.15
C1-Naphthalenes	0.18	0.26	0.31	0.27	0.25	0.22	0.20	0.24
C2-Naphthalenes	0.41	0.54	0.56	0.58	0.51	0.64	0.68	0.50
C3-Naphthalenes	0.43	0.64	0.62	0.71	0.63	0.96	0.51	0.62
C4-Naphthalenes	0.57	0.86	0.79	0.69	0.82	1.8	0.83	0.71
Fluorene	0.18	0.21	0.59	0.36	0.32	0.27	0.17	0.17
C1-Fluorenes	0.94	1.4	1.4	1.2	1.2	2.2	0.99	1.1
C2-Fluorenes	3.1	3.9	3.8	3.1	3.7	8.9	5.0	3.7
C3- Fluorenes	5.7	8.2	6.5	5.6	6.3	15	7.3	6.9
Dibenzothiophene	0.03	0.05	0.04	0.03	0.04	0.09	0.03	0.03
C1-Dibenzothiophenes	0.34	0.50	0.40	0.29	0.33	0.97	0.39	0.33
C2-Dibenzothiophenes	1.2	1.7	1.3	1.0	1.0	3.0	1.4	1.1
C3- Dibenzothiophenes	1.5	2.1	1.5	1.2	1.3	3.8	1.9	1.5
Phenanthrene	0.78	1.2	2.1	1.1	1.1	1.8	0.88	0.83
C1-Phenanthrenes	3.0	4.3	3.9	3.0	3.3	7.9	3.5	2.9
C2-Phenanthrenes	6.9	10	7.9	6.8	6.7	17	8.9	7.3
C3-Phenanthrenes	8.4	12	8.4	8.0	8.1	21	14	10
C4-Phenanthrenes	2.7	3.3	2.6	3.2	3.4	8.1	5.2	3.5
Anthracene	1.3	1.5	6.0	3.2	2.4	1.2	1.6	1.2
Fluoranthene	1.9	21	25	24	48	2.8	20	11
Pyrene	40	50	47	36	60	73	45	26
C1- Pyrenes	27	33	50	31	39	46	36	22
C2- Pyrenes	20	16	19	29	28	27	22	21
C3- Pyrenes	7.9	8.6	11	17	13	13	7.2	6.1
C4- Pyrenes	0.00	3.0	5.9	12	11	5.6	2.1	3.0
Naphthobenzothiophene	0.04	0.04	0.11	0.05	0.07	0.05	0.04	0.03

C-1 Naphthobenzothiophenes	0.17	0.12	0.12	0.16	0.24	0.13	0.14	0.05
C-2 Naphthobenzothiophenes	0.07	0.00	0.08	0.26	0.15	0.07	0.04	0.03
C-3 Naphthobenzothiophenes	0.03	0.00	0.06	0.25	0.12	0.03	0.02	0.02
Benzo (a) Anthracene	4.6	4.5	13	8.0	15	2.9	6.2	3.4
Chrysene	11	9.4	55	24	30	8.8	12	6.7
C1- Chrysenes	7.7	6.5	13	28	22	8.7	6.4	5.9
C2- Chrysenes	0.00	0.00	9.9	29	19	5.6	4.4	4.4
C3- Chrysenes	0.00	0.00	5.7	19	9.9	2.3	2.0	2.1
C4- Chrysenes	11	12	14	29	15	9.9	17	14
Benzo (b) Fluoranthene	9.5	7.7	11	18	27	5.3	9.7	6.7
Benzo (k) Fluoranthene	8.8	4.3	6.9	11	18	3.5	9.0	3.6
Benzo (e) Pyrene	6.0	5.3	6.8	15	16	4.5	6.4	3.9
Benzo (a) Pyrene	5.0	5.3	6.0	10	12	3.7	5.4	4.1
Perylene	17	13	26	42	27	16	12	14
Indeno (1,2,3 - cd) Pyrene	4.0	5.1	5.5	14	11	4.4	6.6	5.0
Dibenzo (a,h) anthracene	1.4	1.5	2.1	5.0	2.9	1.2	1.8	1.2
Benzo (g,h,i) perylene	3.8	4.3	4.2	13	8.2	3.6	5.3	2.8
Total Aromatics	**224**	**262**	**384**	**458**	**475**	**344**	**290**	**211**

Field ID#: 2013	O2 April	O2 May	O2 June	O3 April	O3 May	O3 June	O4 April	O4 May	O4 June	O6 April	O6 May	O6 June
Aromatic Analyte:	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**
Naphthalene	0.22	0.58	2.6	1.0	0.27	1.0	0.35	0.23	0.18	0.37	1.6	0.36
C1-Naphthalenes	0.18	0.29	0.59	0.81	0.13	0.44	0.25	0.19	0.11	0.26	0.32	0.33
C2-Naphthalenes	0.28	0.48	1.0	1.2	0.25	0.70	0.40	0.33	0.24	0.43	0.55	0.47
C3-Naphthalenes	0.39	0.49	0.99	1.1	0.65	0.78	0.75	0.36	0.29	0.56	0.86	0.53
C4-Naphthalenes	0.46	0.75	1.5	1.4	1.7	1.1	1.6	0.52	0.54	0.74	1.3	0.69
Fluorene	0.27	0.27	1.0	1.0	0.27	0.59	0.26	0.20	0.18	0.32	0.55	0.30
C1-Fluorenes	0.83	1.0	2.4	1.8	0.45	1.5	1.2	0.72	0.75	1.1	1.9	1.0
C2-Fluorenes	0.00	4.3	8.0	5.1	1.0	4.6	4.5	2.5	2.6	3.6	5.8	3.1
C3- Fluorenes	5.3	6.3	13	8.8	3.3	7.4	7.7	4.7	4.8	6.0	12	4.8
Dibenzothiophene	0.02	0.02	0.04	0.03	0.01	0.04	0.03	0.02	0.02	0.03	0.06	0.03
C1-Dibenzothiophenes	0.19	0.29	0.64	0.40	0.08	0.41	0.35	0.27	0.27	0.35	0.71	0.24
C2-Dibenzothiophenes	0.59	1.0	2.1	1.4	0.42	1.4	1.1	0.90	0.90	1.0	2.2	0.78
C3-Dibenzothiophenes	0.55	1.4	2.6	1.9	0.45	1.9	1.1	1.2	1.2	1.2	2.5	0.95
Phenanthrene	1.3	0.96	3.2	2.3	0.51	1.8	1.1	0.94	0.67	1.1	1.9	1.1
C1-Phenanthrenes	0.00	3.1	6.4	4.5	0.59	3.9	3.9	2.6	2.3	3.1	6.2	2.5
C2-Phenanthrenes	2.2	7.6	16	11	2.9	10	10	7.4	6.9	6.6	13	5.5
C3-Phenanthrenes	4.3	9.8	20	15	2.4	13	9.3	8.9	8.8	6.8	14	7.1
C4-Phenanthrenes	2.8	3.7	9.2	4.7	0.92	4.2	3.8	3.4	3.4	2.5	4.4	3.0
Anthracene	2.9	2.2	6.5	5.5	1.1	4.2	2.0	1.4	1.4	2.1	3.3	2.7
Fluoranthene	26	17	56	50	4.9	35	14	11	15	24	39	34
Pyrene	31	45	100	87	6.9	70	29	27	35	42	75	41
C1- Pyrenes	33	39	110	65	6.7	54	25	26	28	29	54	33
C2- Pyrenes	40	36	110	41	5.7	33	20	16	15	21	35	24
C3- Pyrenes	38	26	89	24	3.8	15	14	9.5	11	12	18	21
C4- Pyrenes	29	22	70	18	2.5	11	11	7.2	7.5	7.2	9.0	17
Naphthobenzothiophene	0.03	0.04	0.07	0.05	0.00	0.05	0.04	0.03	0.03	0.04	0.07	0.06

C-1Naphthobenzothiophene	0.14	0.11	0.30	0.14	0.03	0.12	0.12	0.08	0.10	0.09	0.15	0.22
C-2Naphthobenzothiophene	0.25	0.18	0.51	0.00	0.03	0.16	0.15	0.09	0.12	0.09	0.13	0.31
C-3Naphthobenzothiophenes	0.26	0.25	0.63	0.17	0.02	0.16	0.11	0.08	0.11	0.07	0.10	0.24
Benzo (a) Anthracene	7.4	5.5	12	13	0.70	9.6	3.9	2.2	2.0	6.5	11	7.3
Chrysene	45	33	110	32	4.3	18	12	7.7	7.6	14	22	42
C1- Chrysenes	85	60	200	35	4.7	17	18	9.4	9.6	12	20	63
C2- Chrysenes	77	54	180	30	3.7	16	15	11	11	13	14	54
C3- Chrysenes	38	31	92	21	3.1	14	12	7.9	7.9	7.3	8.7	27
C4- Chrysenes	36	43	91	35	6.8	30	26	18	12	16	15	30
Benzo (b) Fluoranthene	12	6.2	24	21	1.6	19	7.2	3.6	3.4	11	22	16
Benzo (k) Fluoranthene	6.1	4.4	16	12	2.0	7.3	4.6	2.5	6.4	8.6	14	6.8
Benzo (e) Pyrene	18	11	34	14	1.9	8.2	4.4	2.7	2.6	7.2	14	13
Benzo (a) Pyrene	5.1	4.8	15	11	1.3	8.8	3.7	2.1	2.1	8.0	16	6.2
Perylene	16	22	46	68	11	43	20	17	7.9	27	39	23
Indeno (1,2,3 - cd) Pyrene	5.3	5.4	13	13	1.6	10	4.4	3.9	2.6	7.7	15	7.1
Dibenzo (a,h) anthracene	1.8	2.1	5.0	3.0	0.45	2.6	1.5	1.1	0.76	1.9	3.9	2.2
Benzo (g,h,i) perylene	4.7	4.4	14	8.2	1.2	6.4	3.2	2.7	1.6	5.7	9.4	5.5
Total Aromatics	**577**	**518**	**1487**	**673**	**92**	**489**	**300**	**225**	**225**	**320**	**528**	**513**

Field ID#:2014	D1 April	D2 April	D3 April	D1 May	D2 May	D3 May	D1 June	D2 June	D3 June
Aromatic Analyte:	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	ng/g
Naphthalene	0.39	0.54	0.23	0.16	0.22	0.16	0.19	0.38	0.31
C1-Naphthalenes	3.4	4.5	1.5	0.46	1.5	0.60	1.0	0.48	0.79
C2-Naphthalenes	7.1	9.3	3.1	1.1	3.3	1.1	2.1	0.81	1.5
C3-Naphthalenes	4.8	5.7	1.3	1.1	1.7	0.87	1.0	0.67	1.3
C4-Naphthalenes	4.3	4.4	1.3	1.1	1.6	0.80	0.71	0.67	0.97
Fluorene	0.92	1.1	0.36	0.31	0.50	0.19	0.41	0.21	0.42
C1-Fluorenes	5.8	6.9	2.9	2.8	3.0	1.7	1.5	1.4	3.8
C2-Fluorenes	19	21	6.7	9.5	8.2	4.6	5.8	4.6	7.6
C3- Fluorenes	32	28	7.9	12	12	6.4	9.2	5.2	11
Dibenzothiophene	0.33	0.36	0.10	0.16	0.20	0.06	0.09	0.06	0.13
C1-Dibenzothiophenes	2.1	2.2	0.64	1.0	1.1	0.45	0.58	0.40	0.83
C2-Dibenzothiophenes	5.7	6.5	1.6	3.3	3.1	1.3	2.0	1.0	2.6
C3- Dibenzothiophenes	4.7	5.5	1.4	2.1	1.9	1.2	1.4	0.78	2.0
Phenanthrene	4.5	5.9	1.6	2.1	2.4	1.1	2.5	0.91	2.1
C1-Phenanthrenes	22	23	5.9	12	10	4.1	8.1	3.3	11
C2-Phenanthrenes	39	43	12	18	18	9.0	13	7.0	14
C3-Phenanthrenes	28	30	9.3	15	12	6.2	9.4	3.8	12
C4-Phenanthrenes	14	15	4.6	5.9	6.1	3.9	4.5	1.5	5.9
Anthracene	2.3	5.5	1.2	1.7	3.2	1.7	14	1.4	2.9
Fluoranthene	26	60	11	13	24	11	300	11	20
Pyrene	69	110	23	55	50	19	290	18	41
C1- Pyrenes	75	92	25	39	43	22	140	23	40
C2- Pyrenes	39	52	15	31	28	13	77	16	27
C3- Pyrenes	12	16	3.2	13	8.9	3.9	38	0.00	13
C4- Pyrenes	4.5	0.00	0.00	0.00	0.00	0.00	16	0.00	0.00
Naphthobenzothiophene	0.08	0.13	0.03	0.05	0.07	0.03	0.26	0.00	0.04
C-1 Naphthobenzothiophenes	0.13	0.16	0.04	0.14	0.11	0.07	0.24	0.00	0.14

C-2 Naphthobenzothiophenes	0.06	0.07	0.02	0.06	0.04	0.00	0.20	0.00	0.06
C-3 Naphthobenzothiophenes	0.02	0.04	0.00	0.00	0.00	0.00	0.15	0.00	0.00
Benzo (a) Anthracene	4.4	16	1.5	2.0	5.1	1.9	130	3.9	2.5
Chrysene	14	24	6.1	13	26	7.5	150	17	14
C1- Chrysenes	8.1	12	3.6	7.9	27	4.3	76	15	9.1
C2- Chrysenes	11	0.00	0.00	0.00	0.00	0.00	30	0.00	0.00
C3- Chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	23	0.00	0.00
C4- Chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (b) Fluoranthene	6.9	15	2.6	4.4	16.4	2.7	82	0.00	4.7
Benzo (k) Fluoranthene	5.2	12	1.5	2.3	8.4	0.00	57	0.00	1.8
Benzo (e) Pyrene	3.6	8.7	1.2	2.4	6.6	0.00	45	0.00	2.7
Benzo (a) Pyrene	7.2	15	2.0	2.8	4.5	0.00	68	0.00	3.3
Perylene	7.5	14	7.8	8.7	6.1	8.8	19	6.1	15
Indeno (1,2,3 - cd) Pyrene	3.5	7.6	0.00	1.4	2.8	0.00	45	0.00	0.00
Dibenzo (a,h) anthracene	0.00	0.00	0.00	0.00	0.00	0.00	12	0.00	0.00
Benzo (g,h,i) perylene	2.7	6.0	0.00	1.1	2.0	0.00	32	0.00	0.00
Total Aromatics	**500**	**680**	**166**	**286**	**350**	**139**	**1,708**	**143**	**274**

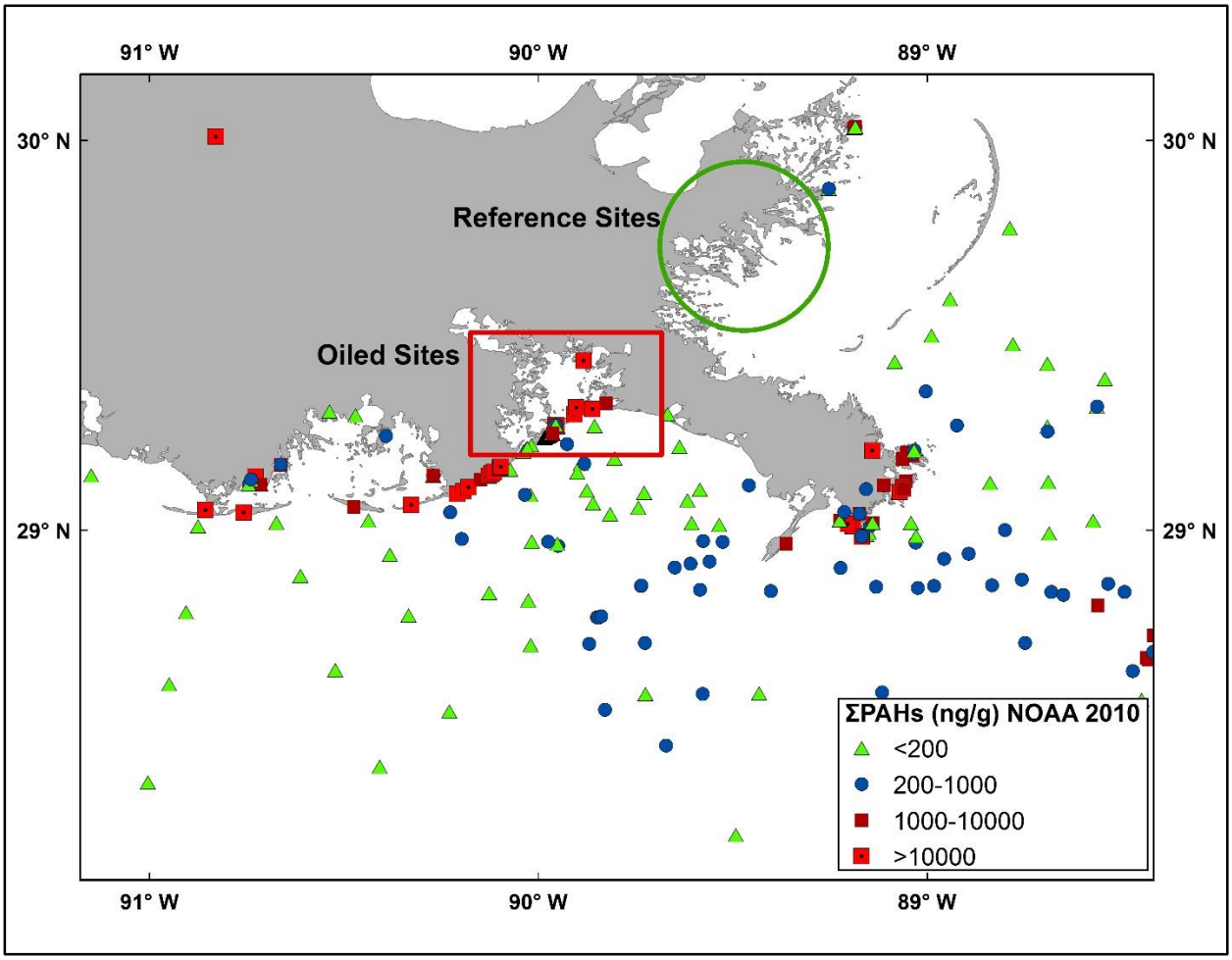
Field ID#:2014	U1 April	U2 April	U1 May	U2 May	U4 May	U1 June	U2 June	U4 June
Aromatic Analyte:	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**
Naphthalene	0.46	0.76	0.29	0.91	0.46	0.37	0.42	0.46
C1-Naphthalenes	3.8	4.3	0.98	1.4	1.9	0.66	1.8	1.7
C2-Naphthalenes	7.9	8.6	1.9	1.9	4.3	1.2	5.1	4.9
C3-Naphthalenes	4.0	4.8	1.6	1.3	2.1	1.0	2.3	1.8
C4-Naphthalenes	3.2	2.9	2.7	1.2	1.7	0.95	1.9	1.5
Fluorene	0.81	1.0	0.49	0.94	0.59	0.49	0.61	0.56
C1-Fluorenes	7.6	5.9	3.5	2.5	3.9	2.4	5.6	3.1
C2-Fluorenes	17	15	8.0	6.3	11	5.8	7.4	10
C3- Fluorenes	19	21	14	7.9	18	9.0	16	12
Dibenzothiophene	0.26	0.32	0.13	0.12	0.18	0.14	0.18	0.12
C1-Dibenzothiophenes	1.5	1.7	0.85	0.57	1.2	0.65	1.3	0.85
C2-Dibenzothiophenes	4.3	4.5	3.1	1.6	4.3	1.9	4.0	3.1
C3- Dibenzothiophenes	2.9	3.6	2.4	1.3	2.4	1.6	2.6	1.8
Phenanthrene	3.4	4.7	2.3	2.4	3.2	2.0	2.7	2.1
C1-Phenanthrenes	15	12	10	6.3	14	9.1	15	11
C2-Phenanthrenes	28	32	19	10	23	15	22	21
C3-Phenanthrenes	21	23	15	8.0	14	11	15	13
C4-Phenanthrenes	15	11	8.8	3.7	6.3	4.5	6.5	6.5
Anthracene	3.5	6.3	6.2	9.2	5.2	3.1	4.1	4.1
Fluoranthene	37	41	28	46	56	30	38	22
Pyrene	72	64	41	55	77	89	61	40
C1- Pyrenes	68	68	41	55	50	61	55	19
C2- Pyrenes	53	57	44	39	55	39	52	24
C3- Pyrenes	13	20	12	17	12	0.00	19	13
C4- Pyrenes	0.00	15	0.00	14	0.00	0.00	13	0.00
Naphthobenzothiophene	0.12	0.11	0.05	0.12	0.08	0.03	0.06	0.04

C-1 Naphthobenzothiophenes	0.24	0.34	0.14	0.21	0.21	0.52	0.18	0.00
C-2 Naphthobenzothiophenes	0.16	0.16	0.06	0.16	0.10	0.23	0.15	0.00
C-3 Naphthobenzothiophenes	0.00	0.11	0.00	0.08	0.00	0.00	0.00	0.00
Benzo (a) Anthracene	4.4	10	6.9	21	7.7	4.5	3.9	4.5
Chrysene	16	27	19	62	31	17	34	20
C1- Chrysenes	8.8	22	11	29	12	0.00	35	0.00
C2- Chrysenes	0.00	29	0.00	32	0.00	0.00	0.00	0.00
C3- Chrysenes	0.00	18	0.00	16	0.00	0.00	0.00	0.00
C4- Chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (b) Fluoranthene	15	12	13.3	28	15	0.00	6.0	11
Benzo (k) Fluoranthene	8.9	12	7.0	17	6.5	0.00	5.5	8.3
Benzo (e) Pyrene	4.1	8.1	5.5	12	6.5	0.00	5.4	5.4
Benzo (a) Pyrene	5.6	6.6	7.0	15	8.0	0.00	3.7	6.9
Perylene	11	13	18	25	23	9.02	21	22
Indeno (1,2,3 - cd) Pyrene	0.00	4.4	4.1	6.1	0.00	0.00	0.00	0.00
Dibenzo (a,h) anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (g,h,i) perylene	0.00	4.1	3.0	5.3	0.00	0.00	0.00	0.00
Total Aromatics	**474**	**596**	**362**	**562**	**479**	**321**	**466**	**297**

Field ID#:2014	O2 April	O3 April	O4 April	O6 April	O2 May	O3 May	O4 May	O2 June	O3 June	O4 June	O6 June
Aromatic Analyte:	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**	**ng/g**
Naphthalene	0.37	0.56	0.43	0.90	0.34	1.7	0.35	0.00	0.50	0.36	1.5
C1-Naphthalenes	0.73	0.77	1.6	4.3	0.85	7.9	0.57	0.00	0.76	1.6	0.78
C2-Naphthalenes	1.4	1.5	4.5	7.9	1.7	19	1.5	0.00	1.4	3.9	1.4
C3-Naphthalenes	1.2	1.2	2.4	3.9	1.0	9.6	2.6	0.00	1.7	1.5	2.1
C4-Naphthalenes	1.1	1.2	1.6	4.5	0.93	9.0	4.8	0.00	1.1	1.2	1.4
Fluorene	0.44	0.72	0.82	1.2	0.57	2.2	0.40	0.00	0.51	0.45	0.77
C1-Fluorenes	5.4	3.3	7.2	1.6	1.8	12	2.4	0.00	2.0	2.6	2.8
C2-Fluorenes	7.0	7.8	14	13	4.5	43	9.4	0.00	6.4	6.3	7.1
C3- Fluorenes	11	11	20	22	6.4	62	19	0.00	10	13	15
Dibenzothiophene	0.11	0.11	0.17	0.26	0.08	0.82	0.11	0.00	0.10	0.11	0.16
C1-Dibenzothiophenes	0.71	0.66	1.2	1.5	0.50	4.6	0.70	0.00	0.69	0.80	0.95
C2-Dibenzothiophenes	2.2	1.9	4.6	3.9	1.5	13	2.5	0.00	2.1	2.5	3.2
C3- Dibenzothiophenes	1.7	1.8	3.0	3.5	1.0	10	2.3	0.00	1.7	1.7	2.5
Phenanthrene	2.1	2.3	4.0	5.3	1.7	10	1.8	0.00	1.9	2.0	2.8
C1-Phenanthrenes	8.2	8.4	15	15	5.2	44	10	0.00	7.2	7.7	11
C2-Phenanthrenes	13	13	31	28	9.1	88	25	0.00	13	16	20
C3-Phenanthrenes	12	10	21	19	6.7	69	23	0.00	10	11	14
C4-Phenanthrenes	5.4	0.56	18	11	4.9	36	15	0.00	5.0	3.6	7.0
Anthracene	5.0	6.3	5.4	6.2	4.3	8.9	7.4	0.00	3.5	2.8	5.3
Fluoranthene	44	35	30	47	38	81	17	0.00	36	26	62
Pyrene	61	54	65	72	44	200	48	41	55	41	85
C1- Pyrenes	45	67	61	73	35	200	53	0.00	22	40	55
C2- Pyrenes	40	70	67	61	33	140	82	440	37	29	39
C3- Pyrenes	13	48	27	21	22	46	110	490	17	0.00	22
C4- Pyrenes	0.00	38	13	0.00	15	18	56	0.00	0.00	0.00	15
Naphthobenzothiophene C-1	0.07	0.05	0.11	0.09	0.05	0.22	0.12	0.00	0.05	0.04	0.07
Naphthobenzothiophenes	0.15	0.16	0.22	0.14	0.14	0.44	0.69	0.00	0.17	0.00	0.18

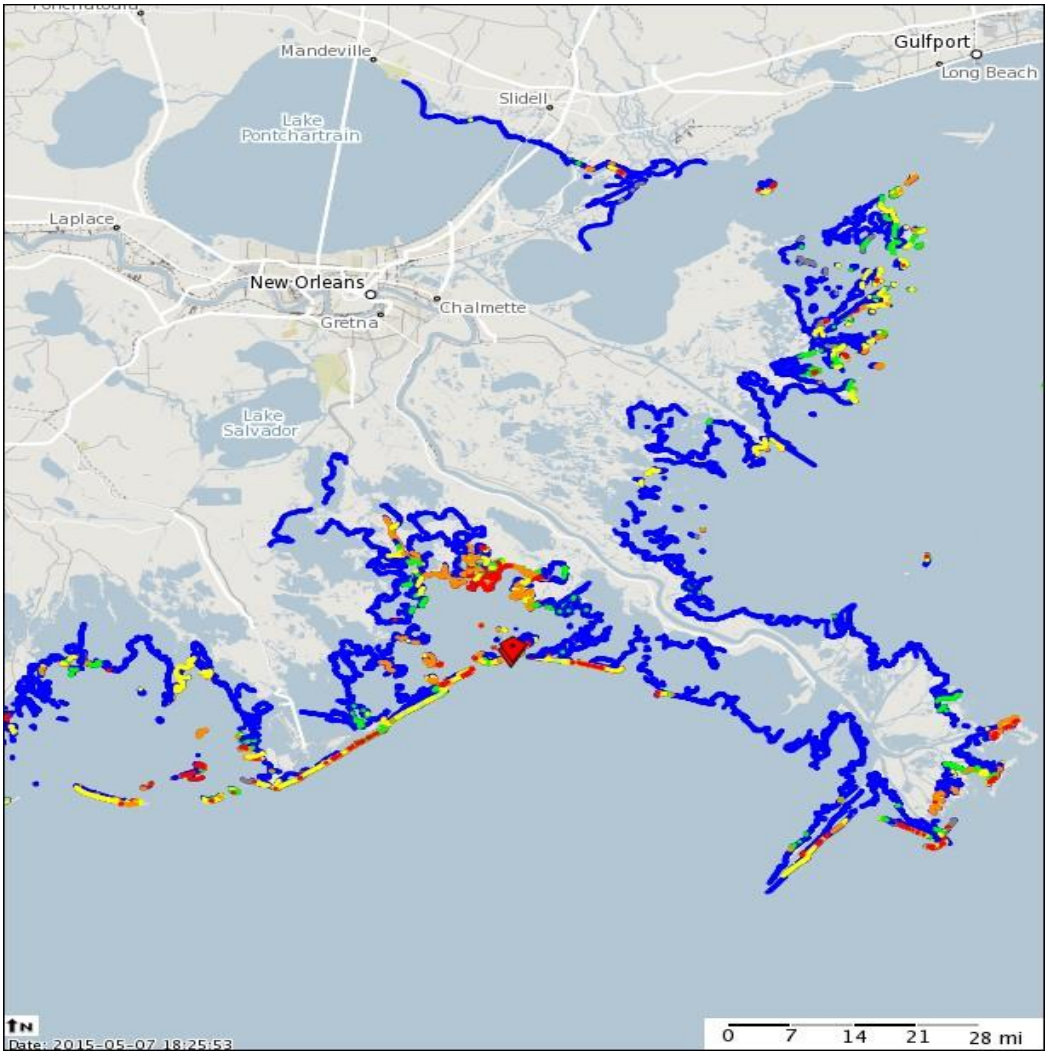
C-2 Naphthobenzothiophenes	0.09	0.28	0.20	0.06	0.10	0.19	0.90	0.00	0.11	0.00	0.11
C-3 Naphthobenzothiophenes	0.04	0.28	0.13	0.05	0.09	0.15	0.76	0.00	0.00	0.00	0.08
Benzo (a) Anthracene	9.2	10	3.7	17	5.1	14	5.15	160	5.3	3.2	9.6
Chrysene	26	70	29	23	34	38	79	720	22	13	30
C1- Chrysenes	11	88	26	16	41	33	150	0.00	22	0.00	22
C2- Chrysenes	0.00	93	12	22	37	45	200	1200	0.00	0.00	7.2
C3- Chrysenes	0.00	46	0.00	0.00	19	20	130	620	0.00	0.00	0.00
C4- Chrysenes	0.00	35	0.00	0.00	17	0.00	91	0.00	0.00	0.00	0.00
Benzo (b) Fluoranthene	13	16	13	12	9.9	15	12	0.00	10	4.6	15
Benzo (k) Fluoranthene	10	11	7.6	7.8	5.7	8.9	6.2	0.00	6.5	3.7	9.2
Benzo (e) Pyrene	6.5	19	5.6	6.8	8.5	12	15	160	7.1	2.4	8.9
Benzo (a) Pyrene	7.7	9.2	3.0	9.8	5.6	12	0.00	0.00	6.4	0.00	7.2
Perylene	18	23	11	15	29	35	14	130	26	11	29
Indeno (1,2,3 - cd) Pyrene	0.00	0.00	0.00	4.4	0.00	6.6	0.00	0.00	0.00	0.00	4.0
Dibenzo (a,h) anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzo (g,h,i) perylene	0.00	0.00	0.00	3.5	0.00	5.2	0.00	0.00	0.00	0.00	3.0
Total Aromatics	**383**	**806**	**532**	**566**	**452**	**1,382**	**1,200**	**3,961**	**342**	**254**	**522**

APPENDIX B
PAHs CONCENTRATION IN SEDIMENT SAMPLES NOAA/NRDA DATA 2010



(Data Source: NOAA/NRDA, Graphic Source: Puspa Adhikari, Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, Louisiana).

APPENDIX C
OIL OBSERVATION ON GROUND NOAA/NRDA DATA 2010/2014



30-Sep-14 SCAT All Oiling Ground Observations

- Heavy
- Moderate
- Light
- Very Light
- No Oil Observed
- Trace < 1%

Maximum Oiling Observed

30-Sep-14 Houma SCAT Maximum Oiling

- Heavy
- Moderate
- Light
- Very Light
- No Oil Observed
- Trace < 1%

August 2010

08-03-2010 SCAT Latest Ground Observations (Milestone Date)

- No Oil Observed
- Tar Balls (< 1% cover)
- Very Light
- Light
- Moderate
- Heavy

08-03-2010 SCAT Observed Today (Milestone Date)

APPENDIX D
STANDARD OPERATING PROCEDURE FOR OIL SPILL SOURCE IDENTIFICATION

Louisiana State University, Department of Environmental Sciences Response & Chemical Assessment Team: LSU- RCAT SOP#001 – 15

1. SCOPE

1.1 The following procedure details the extraction methodologies used to determine the concentration of oil analytes of interest in extracts prepared from many types of matrices for oil spill source identification. The oil analytes of interest are given in Table 1.

Table 1. Oil Analytes of Interest.

Naphthalene	Anthracene	Benzo (k) Fluoranthene
C1-Naphthalenes	Fluoranthene	Benzo (e) Pyrene
C2-Naphthalenes	Pyrene	Benzo (a) Pyrene
C3-Naphthalenes	C1- Pyrenes	Perylene
C4-Naphthalenes	C2- Pyrenes	Indeno (1,2,3 - cd) Pyrene
Fluorene	C3- Pyrenes	Dibenzo (a,h) anthracene
C1-Fluorenes	C4- Pyrenes	Benzo (g,h,i) perylene
C2-Fluorenes	Naphthobenzothiophene	
C3- Fluorenes	C-1 Naphthobenzothiophenes	
Dibenzothiophene	C-2 Naphthobenzothiophenes	
C1-Dibenzothiophenes	C-3 Naphthobenzothiophenes	
C2-Dibenzothiophenes	Benzo (a) Anthracene	
C3- Dibenzothiophenes	Chrysene	
Phenanthrene	C1- Chrysenes	
C1-Phenanthrenes	C2- Chrysenes	
C2-Phenanthrenes	C3- Chrysenes	
C3-Phenanthrenes	C4- Chrysenes	
C4-Phenanthrenes	Benzo (b) Fluoranthene	

1.2 This method can be used to quantitate certain classes of organic compounds that are soluble in methylene chloride or hexane and are capable of being eluted, without derivatization, as sharp peaks from a gas chromatograph fused-silica capillary column coated with a slightly polar silicone.

1.3 The extraction procedures are different for each of the sample matrices; however, the instrumental analysis and report generation procedures are the same, regardless of matrix.

1.4 The internal standard mixture referred to in each extraction procedure is naphthalene- d8, acenaphthene-d10, chrysene-dl2, and perylene-dl2 (usually at a concentration of 10 ng/uL). The surrogate standard mixture referred to in each extraction procedure is 5-alpha androstane and phenanthrene-d10.

1.5 Good laboratory practices are utilized for each of the extraction procedures. This procedure does not address safety concerns associated with its use. It is the responsibility of the user to determine and execute proper safety and health practices. Use of this method should be restricted to trained and experienced personnel.

2. REFERENCE DOCUMENTS

2.1 ASTM Standards

D 5739-00 Standard Practice for Oil Spill Source Identification by Gas Chromatography and Positive Ion Electron Impact Low Resolution Mass Spectrometry

2.2 EPA Methods

SW-846 3510C Separatory Funnel Liquid-Liquid Extraction
SW-846 3540C Soxhlet Extraction
SW-846 3541 Automated Soxhlet Extraction
SW-846 3550B Ultrasonic Extraction
SW-846 3611B Alumina Column Cleanup and Separation of Petroleum Wastes
SW-846 3630C Silica Gel Cleanup
SW-846 8000B Determinative Chromatographic Separations
SW-846 8270D Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)

2.3 Other Methods

Restek, QuEChERS Methodology, AOAC Method

3. SIGNIFICANCE AND USE

3.1 The methodology provided herein is used for general qualitative oil characterizations and quantitative analysis of oil analytes of interest.

3.2 This SOP can also be used for assessing if spilled oil samples are a match or non-match to a source oil based on oil biomarkers that are source-specific chemical compounds and relatively resistant to environmental degradation. Extracted ion chromatograms, or ion fingerprints, from a “spill” sample can be compared to the same ion fingerprints from the “source” oil and the match/non-match determination is made by a qualitative comparison. If further justification is required, the ion fingerprints can be used to calculate source fingerprint indexes (SFIs) for the “spill” and “source” sample. Match/non-match determinations using the SFIs are made by plotting the average of replicate analyses of each SFI in a histogram with standard deviation error bars displayed.

3.3 The instrumental analysis and data processing aspects of this SOP focus directly on the generation of data using a list of target compounds (listed in Table 1) applicable to petroleum oil identification and includes petrogenic and pyrogenic sources of polycyclic aromatic hydrocarbons (PAHs) as well as, straight chain alkanes in the range of nC10 - nC35.

3.4 This SOP provides a means of analyzing oils from light fuel oils through and including heavy fuel oils.

4. APPARATUS

4.1 Gas chromatograph interfaced to a mass spectrometer with a 70-eV electron impact ionization source. The system also includes a computer to control the instrumentation and an auto sampler for consistent injection of samples into the instrument system.

4.2 Capillary GC column, low bleed, fused silica, 5% diphenyl/95% dimethyl polysiloxane (e.g. J&W DB-5ms), 30 meters long, 0.25 inner diameter, and 0.25 micron film thickness.

4.3 Computer with data processing software for extracting oil fingerprints and for quantitative analysis.

5. REAGENTS AND STANDARDS

5.1 Only pesticide/reagent grade solvents will be used in all analyses and dish washing procedures.

5.2 Standards

5.2.1 *Calibration Standards*

A commercially-prepared oil analysis standard, available through Absolute Standards, is used to prepare the five-point calibration standards. Calibration standard solutions are stored in amber colored vials with PTFE-lined caps. The calibration standards are checked frequently for signs of degradation or evaporation and are replaced if the quality control check sample indicates a problem. In some applications, the calibration standard may contain additional analytes not included in the standard oil analysis mix.

5.2.2 *Internal Standard Solutions*

The internal standards are typically naphthalene-d8, acenaphthene-d10, chrysene-d10, and perylene-d12. The internal standards are bought and stored individually until they are mixed to make the internal standard solution. In some applications, the internal standard mix may contain additional standards not included in the typical internal standard mix.

5.2.3 *Reference Oil Standard*

The usual laboratory reference oil is Alaska North Slope Crude Oil (ANSCO). The ANSCO standard is prepared by extracting 1 gram of pure oil in 40-mL of solvent (or equivalent ratio of 1g: 40mL, e.g. 0.50g: 20mL). The laboratory reference oil is analyzed in each sample batch as an additional QA/QC sample (a laboratory control sample).

5.2.4 *Surrogate Standards*

The surrogate standards are typically 5-alpha androstane (alkanes) and phenanthrene- d10 (aromatics). The surrogate standards are purchased and stored individually until they are mixed to make the needed concentration of surrogate standard. In some applications, the surrogate standard may contain additional standards.

6. QUALITY CONTROL

6.1 The GC/MS must be tuned to meet the recommended criteria prior to the initial calibration and for each 12-hour period during which analyses are performed.

6.2 A five-point calibration curve is performed quarterly. A continuing calibration standard (one point of the initial five-point calibration standard) is analyzed in each batch of samples or each 12-hour period during which analyses are performed. The acceptance criterion for the continuing calibration standard is ±20% of the average relative response factor calculated from the initial five-point curve. If the acceptance criterion is not met, all analyses are stopped until the instrument is performing at optimum conditions. Any instrument maintenance or troubleshooting may require a new five-point calibration curve to be performed.

6.3 If surrogate standards are used, extraction efficiency for each sample is evaluated based on the percent recovery of the surrogate standard. The acceptable percent recovery range is 70 – 120%.

6.4 LSU-RCAT has a separate QA/QC document which outlines other necessary procedures for ensuring data quality and is available upon request.

7. PROCEDURES

7.1 *Pure Oil Extraction Procedure*

Pure oil samples are usually extracted in a weight to volume manner. A ratio of 1-gram of oil to 40-mL of solvent (some extracts may be 0.25 grams oil in 10-mL of solvent or 0.50 grams oil in 20-mL solvent). Solvents commonly used include high purity hexane and dichloromethane (DCM). The pure oil sample is usually transferred into the extraction vial with a disposable pipette, if possible, or with clean, solvent-rinsed stainless steel spatulas. The weight of the oil sample is recorded and the solvent is then added. The vial is capped and then shaken to dissolve the oil in the solvent. At this point, it may be necessary to add pre-cleaned, granular anhydrous sodium sulfate to absorb any water from the extract. The vial with the extract is then placed in an ultrasonic bath for 15 minutes to settle out any particulates or asphaltenes. One milliliter of the

extract is then transferred with a clean graduated, gas-tight syringe into an autosampler vial. If a dilution of the extract is necessary, the appropriate volume of extract to solvent ratio to achieve the correct dilution factor is added to the autosampler vial with a graduated, gas-tight syringe. Internal standard is added, then the vial is capped and ready for analysis.

7.2 *Sediment/Soil Extraction Procedure*

Sediment/soil samples are first decanted of any water, homogenized by vigorous stirring, and any gross debris is removed. Sub-samples are then taken from the homogenized sample for analysis. If the samples are frozen prior to sample extraction, the samples are transferred to a refrigerator until defrosted. For each sediment/soil sample, 15-20 g of material is accurately weighed (nearest 0.01g) into a pre-cleaned 500-mL beaker. Hydromatrix (drying agent) is added and mixed into the sample until a "dry" sand-like matrix is created. One milliliter of surrogate standard is spiked into the sample. Samples are then transferred into pre-cleaned stainless steel tubes to be extracted on the pressurized solvent extractor (Buchi Speed Extractor E-916). Extracts are then concentrated to a 1-mL final volume using the Buchi Syncore instrument. If further concentration is necessary, extracts are placed under a gentle stream of purified nitrogen. All extracts are transferred with a clean graduated, gas-tight syringe into a 2-mL autosampler vial. Internal standard is added, then the vial is capped and ready for analysis.

In some instances (grossly contaminated samples), Soxhlet extractions may be performed on sediment/soil samples. The sediment/soil samples are mixed with Hydromatrix, spiked with surrogate standards, placed in a pre-cleaned extraction thimble and are extracted for at least 12 hours in boiling extraction solvent. At the completion of the extraction procedure the extraction solvent is concentrated, if necessary, to 1 to 2-mL. Soxhlet extractions ensure very rapid intimate contact between the sample matrix and solvent and rapid extraction of the organic analytes of interest. If a dilution of the extract is necessary, the appropriate volume of extract to solvent ratio to achieve the correct dilution factor is added to the autosampler vial with a graduated, gas-tight syringe. Internal standard is added, then the vial is capped and ready for analysis.

Sample results are calculated based on dry weight; therefore, a portion of the sediment/soil sample is prepared for drying in an oven overnight. Five to ten grams of sample are weighed in a pre-weighed aluminum weigh boat. The weigh boat with the sample is placed in an oven set for 105°C overnight. The sample is then removed and allowed to cool in a desiccator before determining the final, oven-dried weight of the sample. Percent dry weight is then calculated.

7.3 *Water Extraction Procedure*

Water samples are serially extracted with DCM in a separatory funnel. A measured volume of the water sample is quantitatively transferred to a clean separatory funnel with a PTFE stopcock in place and closed. One milliliter of surrogate standard is then added to the sample. If the entire water sample is used for the extraction, the original sample container is rinsed with 10 to 20-mL of DCM. If only a portion of the water sample is used for the extraction, the graduated cylinder used to measure the volume of the sample is rinsed with

10 to 20-mL of DCM. The DCM is poured into the separatory funnel containing the surrogate-spiked sample. The funnel is sealed and vigorously shaken and periodically vented for 1-2 minutes. The venting allows for the release of excess pressure in the funnel. The funnel is then placed on a ring stand to settle for a minimum of 10-minutes which allows the DCM to separate from the water. After the settling period, the DCM is drained from the bottom of the funnel, through a granular, anhydrous sodium sulfate filter, and into a rotary evaporator flask. The extraction procedure is repeated two more times using fresh portions of DCM. At the end of the extraction procedure, all three extracts are collected in the one rotary evaporator flask. Concentration of the final sample extract is achieved by rotary evaporation and nitrogen "blowdown". The nitrogen blowdown is achieved by transferring the rotary evaporated extract with a disposable pipette into a graduated tube. The sample can then be further concentrated to one to 10-mL. After concentration is complete, one milliliter of the extract is quantitatively transferred to a 2-mL autosampler vial and internal standard is added prior to capping the vial. The extract is now ready for GC/MS analysis.

7.4 *Tissue Extraction Procedure*

Individual bivalves in each sample are rinsed with distilled water thoroughly (including the shell and the tissue) to remove any material not associated with the tissue itself. If the bivalve samples are frozen prior to tissue extraction, the samples are transferred to a refrigerator until defrosted. All samples are shucked and the combined sample weight is recorded before homogenization. The organisms are then homogenized using a tissuemizer and stored in pre-cleaned jars. In most instances, the QuEChERs AOAC extraction method is used. If it is determined that QuEChERs is not sufficient, approximately 5-10 grams of the homogenized tissue is removed from the sample and placed into a pre-cleaned 50-mL beaker. The weight of the homogenized tissue to be extracted will be determined by the actual quantity of bivalve specimens collected. Specimens from the same sampling site may require compositing to achieve the desired extraction weight. Previous DES/RCAT research has indicated no significant differences in the analyte recovery between the digestion and non-digestion methods; therefore, no digestion is performed. Granular, anhydrous sodium sulfate is added to the tissue in quantities of 15-25 grams depending upon the amount of water within the tissues or until a paste consistency is obtained. The sample is spiked with surrogate standard and then 35-mL of DCM is added to the paste. The beaker is covered with two layers of aluminum foil and sonicated for 15 minutes. After soniciation, the solvent extract is filtered through additional anhydrous sodium sulfate and pre-cleaned glass wool into a round bottom flask. The entire extraction procedure is repeated an additional two times with fresh aliquots of DCM.

To concentrate the solvent extract, the sample is rotary evaporated to approximately 2-mL final volume in DCM. The sample can then be split: 1-mL for lipid analysis; and 1-mL for GC/MS analysis. The GC/MS sample is solvent exchanged from DCM to hexane by adding 40- mL of hexane to the 1-mL GC/MS fraction of the extract. The sample is concentrated again by rotary evaporation and nitrogen blowdown to 2-mL in hexane. The sample is fractionated on an alumina/silica gel column by placing the 2-mL hexane aliquot on the aluminum/silica gel column, which is then rinsed with high purity hexane. The flow of hexane is stopped prior to

exposing the silica gel to air. This fraction contains the normal alkanes. The alumina/silica gel column is then rinsed with 50% DCM and 50% hexane. The solvents are allowed to elute completely. This fraction contains the PAHs. The alkane and PAH fractions are combined and concentrated to 0.1-mL under a gentle stream of nitrogen and stored until GC/MS analysis.

For the determination of dry lipid weight, the 1-mL lipid sub-sample is filtered through a clean, 0.1 micron filter into a clean, pre-weighed scintillation vial. The scintillation vial is then loosely covered and the solvent allowed to evaporate. The dry lipid weight is recorded and the final lipid weight calculated and reported.

8. INSTRUMENTAL ANALYSIS (GAS CHROMATOGRAPHY/MASS SPECTROMETRY)

8.1 *GC Operation*

All GC/MS analyses use either an Agilent 6890 or 7890A GC system configured with a 5% diphenyl/95% dimethyl polysiloxane high resolution capillary column (30 meter, 0.25 mm ID, 0.25 micron film) directly interfaced to an Agilent 5973 or 5975 inert XL MS detector system. An Agilent 7693 or 7638B series Auto Injector is used for sample introduction into the GC/MS system. The GC flow rates are optimized to provide a required degree of separation, particularly n-C17 and pristane should be near baseline resolved, and n-C18 and phytane should be baseline resolved. The injection temperature is set at 280°C and only high-temperature, low thermal-bleed septa are used in the GC inlet. The GC is operated in the temperature program mode with an initial column temperature of 60°C for 3 minutes then increased to 280°C at a rate of 5°C/minute and held for 3 minutes. The oven is then heated from 280°C to 300°C at a rate of 1.5°C/min and held at 300°C for two minutes. Total run time is 65.33 minutes per sample. Ultra High Purity (UHP) Helium is the carry gas for the GC/MS system.

8.2 *MS Operation*

The MS is operated in the Selective Ion Monitoring (SIM) to maximize the detection of several trace target constituents unique to crude oil. The interface to the MS is maintained at 300°C, and source and quad temperatures are maintained at 230 and 150°C, respectively. The instrument is operated such that the selected ions for each acquisition window are scanned at a rate greater than 1.4 scans/sec with a dwell time of 60 milli-seconds. At the start of each analysis period or every twelve hours, the MS is tuned to PFTBA, an internal instrument standard. Laboratory reference standards such as a reference oil and a continuing calibration standard are also analyzed prior to the analysis of the unknown sample extracts. This standard operating procedure ensures quality assurance/quality control of the instrument conditions prior to sample analysis.

8.3 *Quantitative Analysis*

Spectral data is processed by Chemstation™ Software using a customized data analysis method developed by DES. The analysis method is run on each sample and results in raw integration data that is transferred to a spreadsheet program for quantitative analysis. A macro printout is also

generated and contains the extracted ion chromatography data in addition to raw integration data. Each macro printout is carefully reviewed and reintegrated as required.

Analyte concentrations are calculated based on the internal standard method. Therefore, an internal standard mixture, composed of naphthalene-d8, acenaphthene-d10, chrysene-dl2, and perylene-dl2 (usually at a concentration of 10 ng/uL) is spiked into the sample extracts just prior to analysis.

The concentration of specific target oil analytes is determined by a 5-point calibration and internal standard method. Standards containing parent (non-alkylated) hydrocarbons are used in the calibration curve. Alkylated homologues are quantified using the response factor of the parent, and are therefore, only semiquantitative. This is the standard procedure since alkylated standards are not available.

8.3.1 *Calculations*

8.3.1.1 CONCENTRATION OF ANALYTES IN A SAMPLE:

Conc (ng/mg or ng/mL) = (Ax * Is * Vt * DF * 1000) / (Ais * RRF * Vi * M or V)

Ax	=	area of analyte
Ix	=	concentration of internal standard injected (ng)
Vt	=	final volume of the total extract (mL)
DF	=	dilution factor
Ais	=	area of internal standard
RRF	=	average relative response factor
Vi	=	volume injected (µL)
M or V	=	mass if solid (mg) or volume if liquid (mL)

8.3.1.2 RELATIVE RESPONSE FACTOR:

RRF = (Ax * Cis) / (Ais * Cx)

Ax	=	area of analyte in calibration standard
Cis	=	concentration of the internal standard
Ais	=	area of the internal standard
Cx	=	concentration of calibration standard

8.4 *Surrogate Corrections*

Recovery of all trace level samples is estimated using a two aromatic hydrocarbon surrogates: 5 alpha androstane and phenanthrene-d10. Sediment samples are never corrected for recovery, but a 70%-120% surrogate recovery acceptance criteria does apply. Tissue samples are corrected for

recovery using the same two surrogate standards and similar surrogate recovery acceptance criteria (70%-120%).

9. REPORT GENERATION

9.1 Spectral data is processed by Agilent Chemstation™ Software using a customized data processing method and macro developed by DES. Each data file is carefully reviewed and re-integrated as needed after the initial processing. The macro prints a specified set of chromatograms that are used for qualitative comparison. The customized data processing method creates a custom report that contains the raw integration data which is then exported to a spreadsheet for quantitative analysis.

9.2 The concentrations of specific target alkanes and PAHs are determined by response factors that are calculated from commercially available internal and calibration standards. The internal standards used in all analyses are naphthalene-d8, acenaphthene-d10, chrysene-d12, and perylene-d12. The calibration standards are prepared at five different concentrations (5-point calibration curve) and contain saturate alkanes in the range of nC10 through nC35 and each parent aromatic hydrocarbon. The calibration curve results in response factors that are used to calculate the individual analyte concentrations in the samples. It is important to mention that the alkylated homologues in the extracted samples are quantified by response factors generated by the unalkylated parent (e.g. the response factor generated for naphthalene (C-0) is used to calculate the C-l through C-4 naphthalene homologues). Therefore, the results of the quantified alkylated homologues are only semi-quantitative since alkylated homologue standards are not available. Recovery and extraction efficiency of all trace level samples are estimated using two aromatic hydrocarbon surrogate standards: 5-alpha androstane for the alkanes and phenanthrene-d10 for the PAHs. Acceptable surrogate recoveries are in the range of 70%-120%.

9.3 Results for all analytical methods are reported as a function of volume, wet weight, or dry weight values depending on the circumstances and sample. The final results of the quantitative analysis are reported at two significant figures. The standard LSU-RCAT report usually includes a project name, investigator name, field and laboratory IDs, sample extraction information (i.e., initial weights or volumes, final extract volumes, percent moisture), individual alkane and aromatic analyte concentrations, and total alkane and aromatic concentrations.

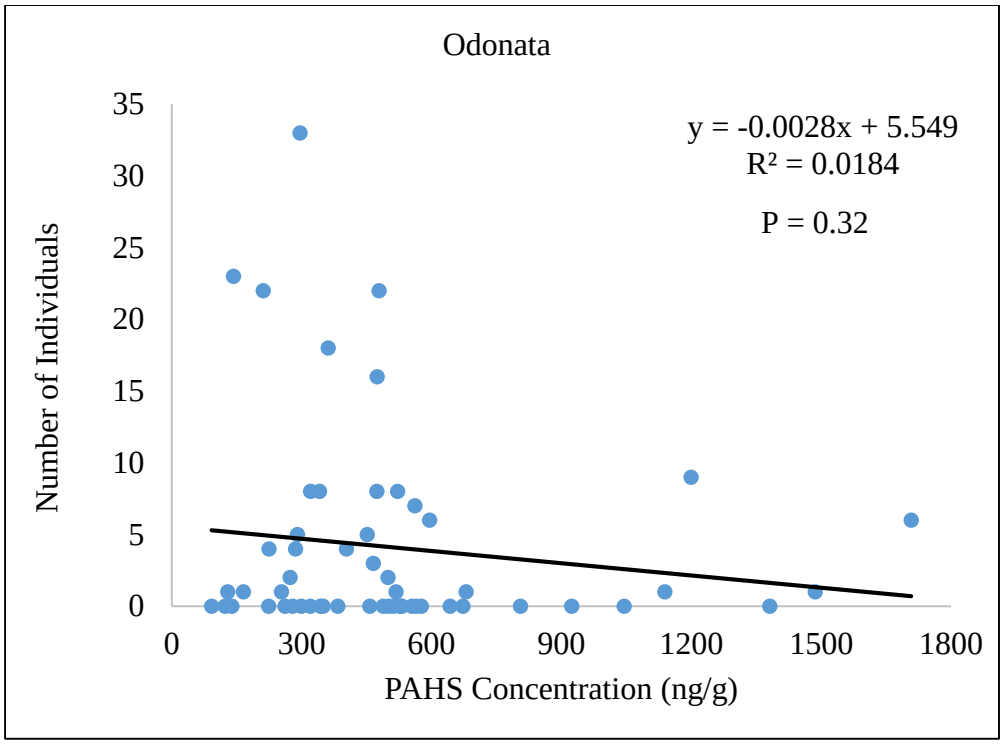
10. SOURCE FINGERPRINTING CORRELATIONS

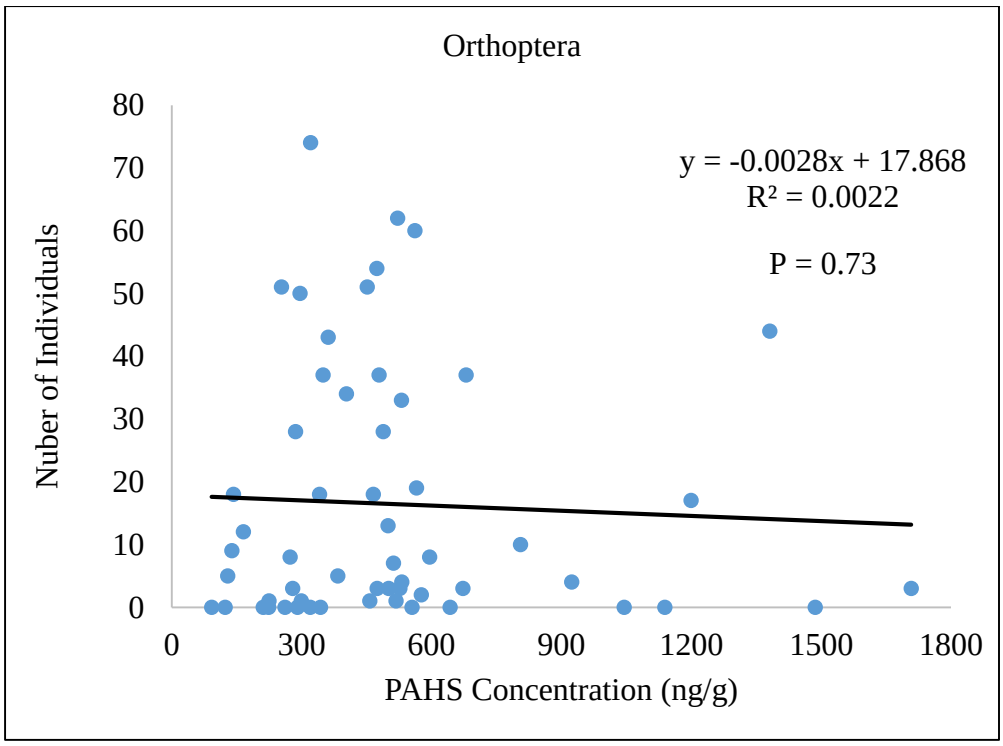
10.1 Source-fingerprinting is an environmental forensics technique that utilizes analytical chemistry to compare samples of spilled oil to a suspected source to assess if the oil is a positive match. Since biomarker compounds are more resistant to environmental weathering processes, compared to most other oil compounds, they can be utilized as conserved reference compounds against which the loss of less stable oil components can be quantitatively estimated by calculating certain ratios. These ratios may be useful in differentiating unknown spill samples from a suspected source. Furthermore, the distributions of oil biomarkers is unique for different types and blends of petroleum products and represent an oil-specific fingerprint to which distinct

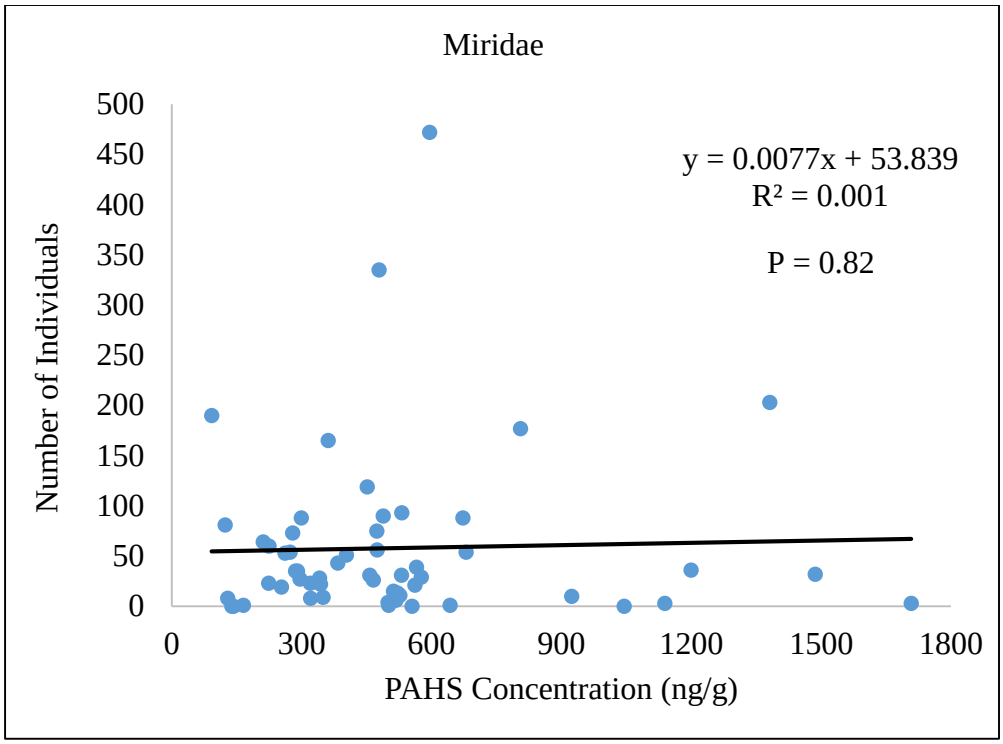
oil samples can be correlated. Match/nonmatch determinations can be achieved qualitatively through visual comparison of ion chromatogram patterns, and quantitatively from calculating the ratio of one biomarker to another. Ratios of certain biomarkers are referred to as source fingerprint indexes (SFI).

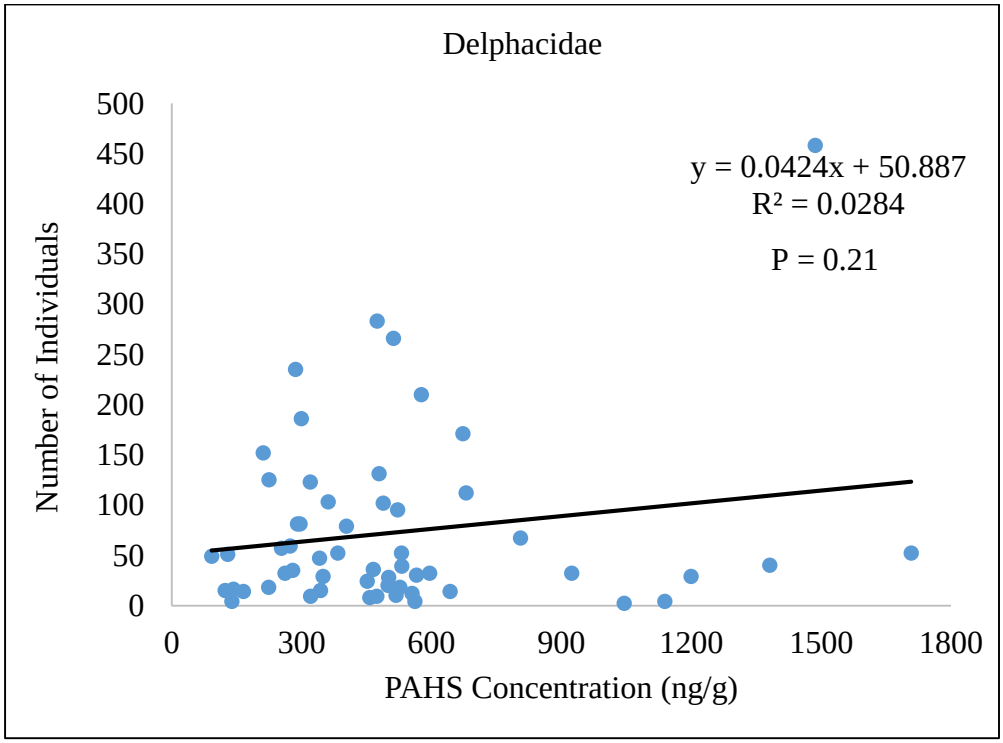
10.2 SFI are calculated by using the ratio of different peaks within the same isomer having similar retention times and identical mass to charge ratios. Choosing isomer groups that have similar water solubilities, vapor pressures, and parent masses will result in potentially useful SFI and contribute to the reduction of instrumental variance effects. As instrument conditions change because of matrix effects, column degradation, sensitivity, or tune degradation, both integers used to calculate the index (assuming they are similar in molecular weight, chemistry and quantitation ion) will be affected by the same relative degree of change; therefore, the index or ratio of the two integers, should remain constant. After a corrected base line value and peak heights have been determined, the SFI are calculated by dividing peak a by peak b in the isomer group. The goals of the SFI approach are to reduce investigator bias through the use of improved quantitative fingerprint techniques; and allow investigators to distinguish subtle differences in actual spill samples that can be easily missed by standard qualitative approaches.

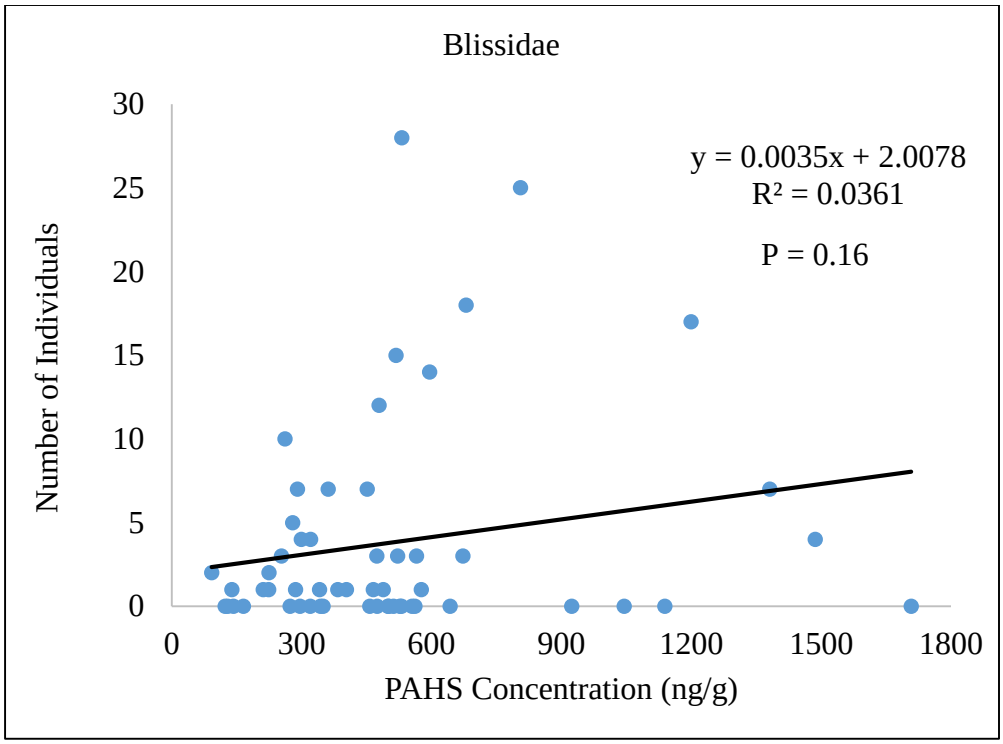
APPENDIX E
NUMBER OF INDIVIDUALS OF ARTHROPODS AND PAHS CONCENTRATION (ng/g) OF SEDIMENTS, REGRESSION PLOTS

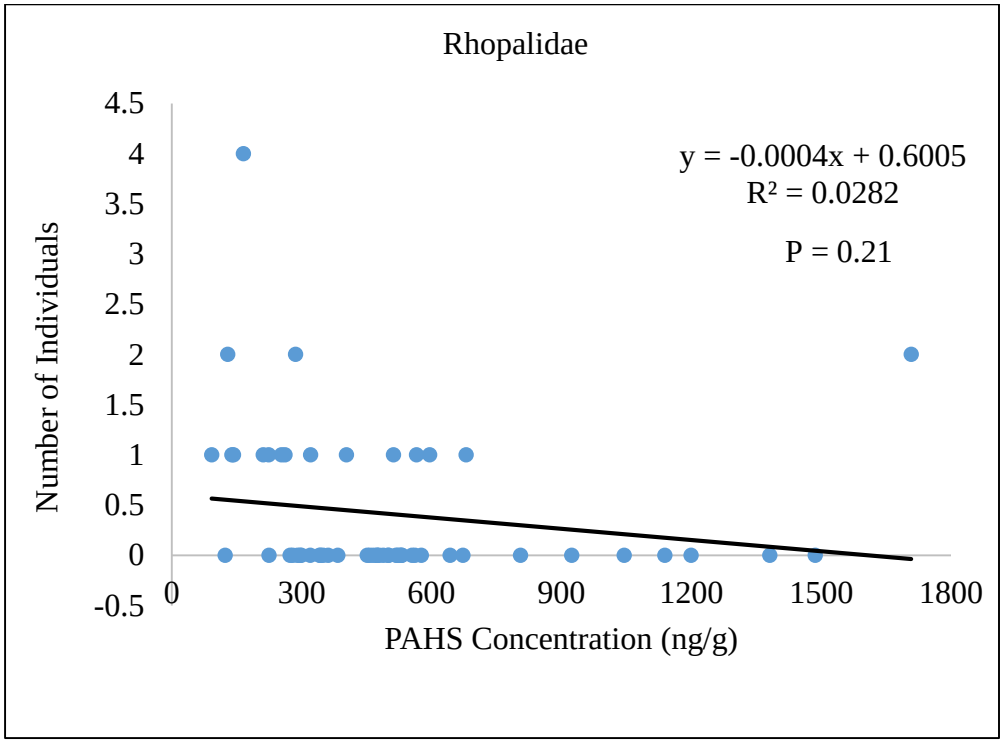


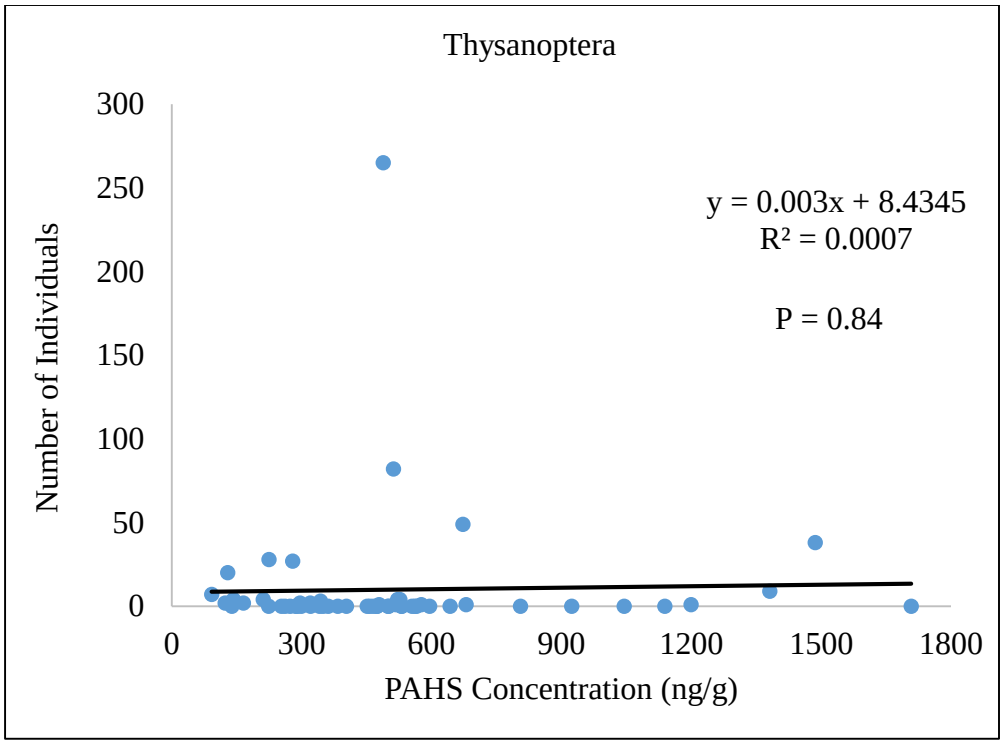


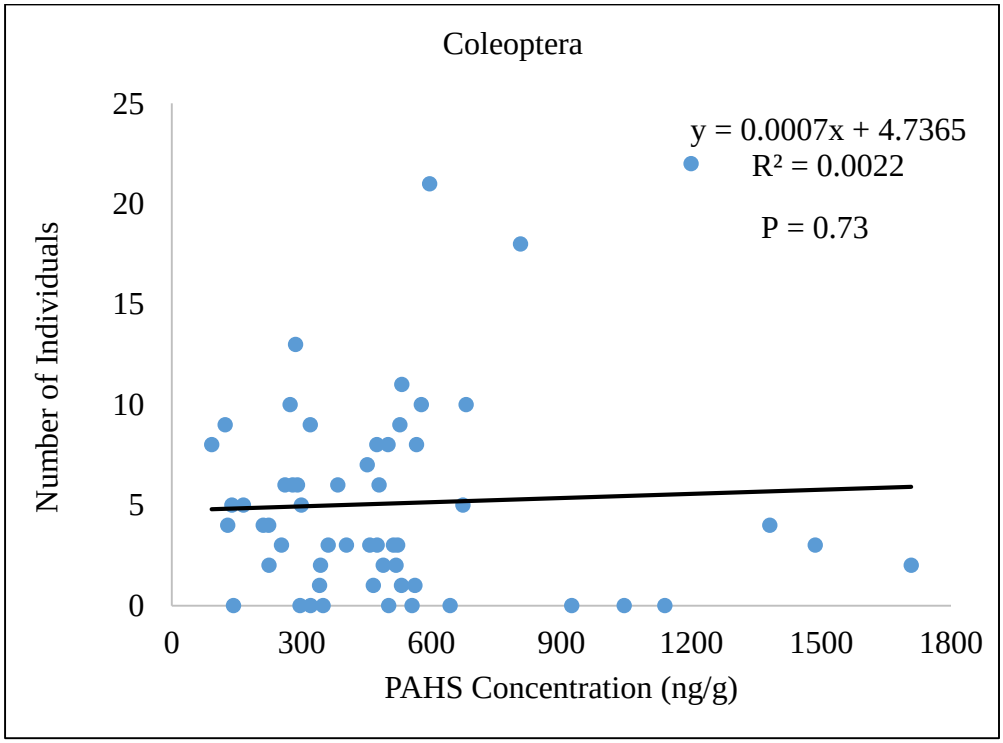

Miridae
Number of Individuals
500
450
400
350
300
250
200
150
100
50
0
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)
y = 0.0077x + 53.839
R² = 0.001
P = 0.82

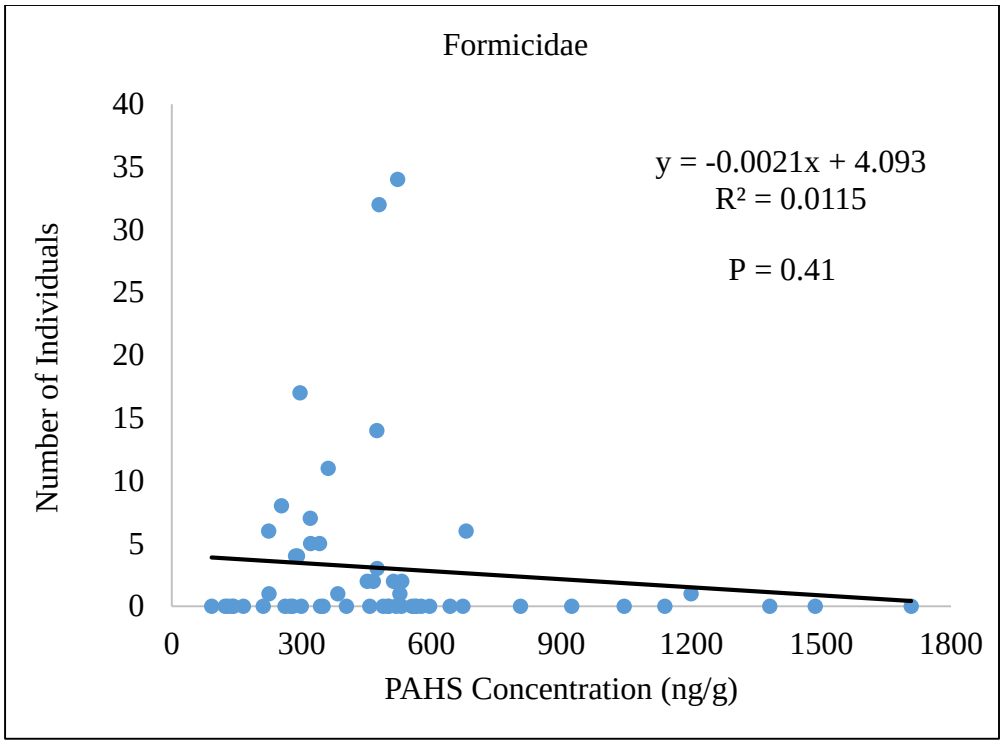

Delphacidae
Number of Individuals
500
450
400
350
300
250
200
150
100
50
0
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)
y = 0.0424x + 50.887
R² = 0.0284
P = 0.21

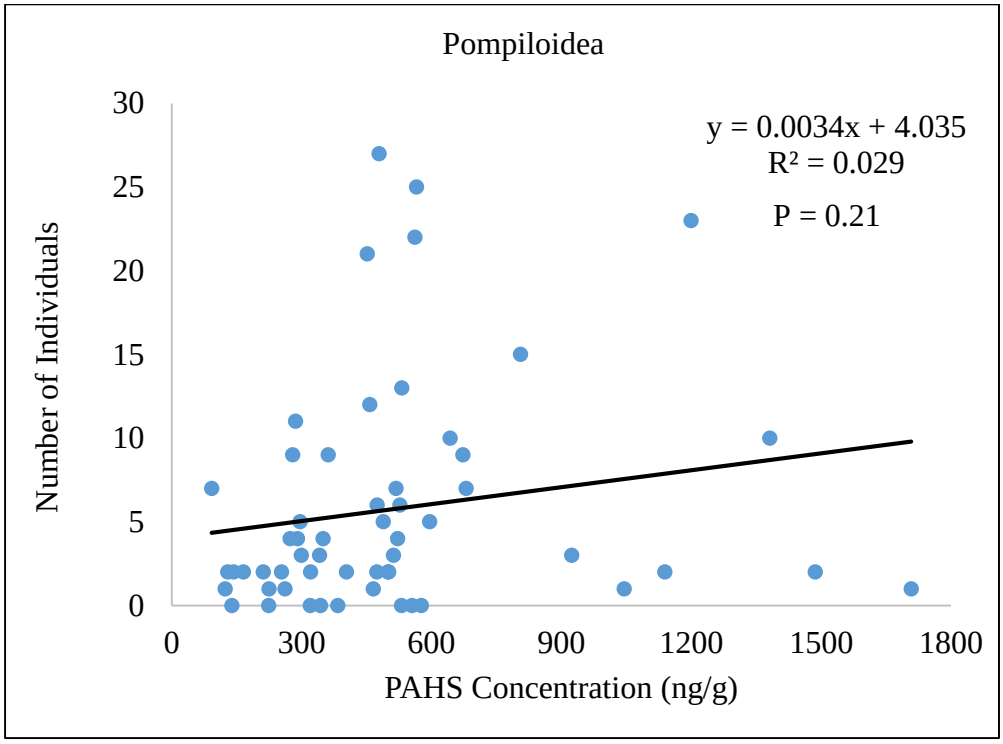

Blissidae
Number of Individuals
PAHS Concentration (ng/g)
y = 0.0035x + 2.0078
R² = 0.0361
P = 0.16
0
5
10
15
20
25
30
0
300
600
900
1200
1500
1800

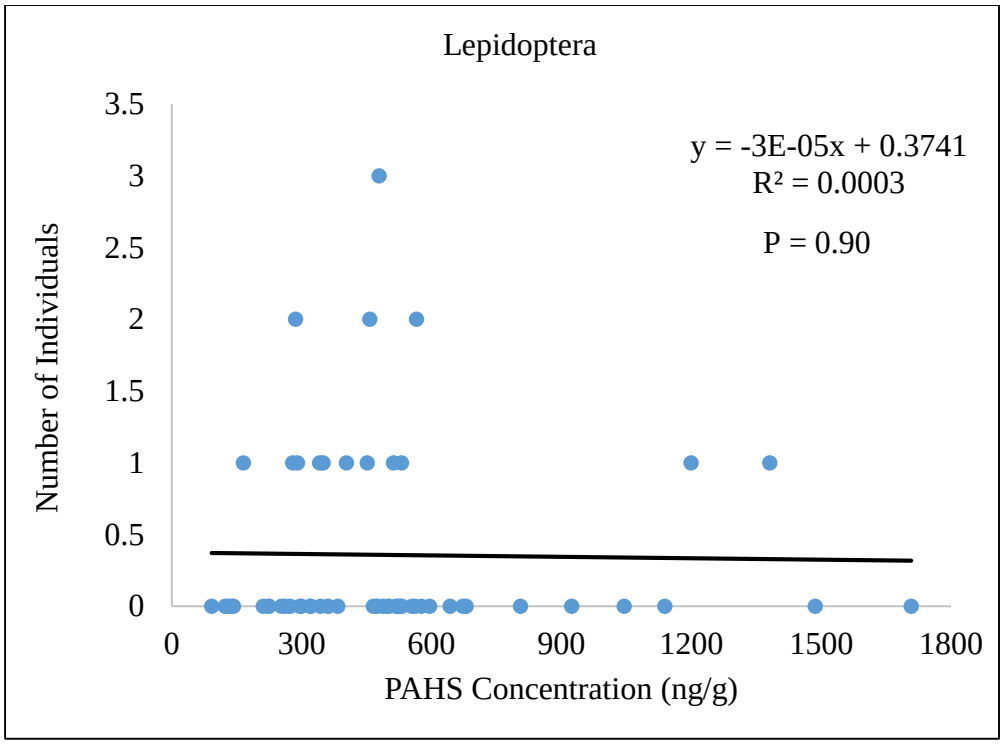

Rhopalidae
Number of Individuals
PAHS Concentration (ng/g)
y = -0.0004x + 0.6005
R² = 0.0282
P = 0.21
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
300
600
900
1200
1500
1800

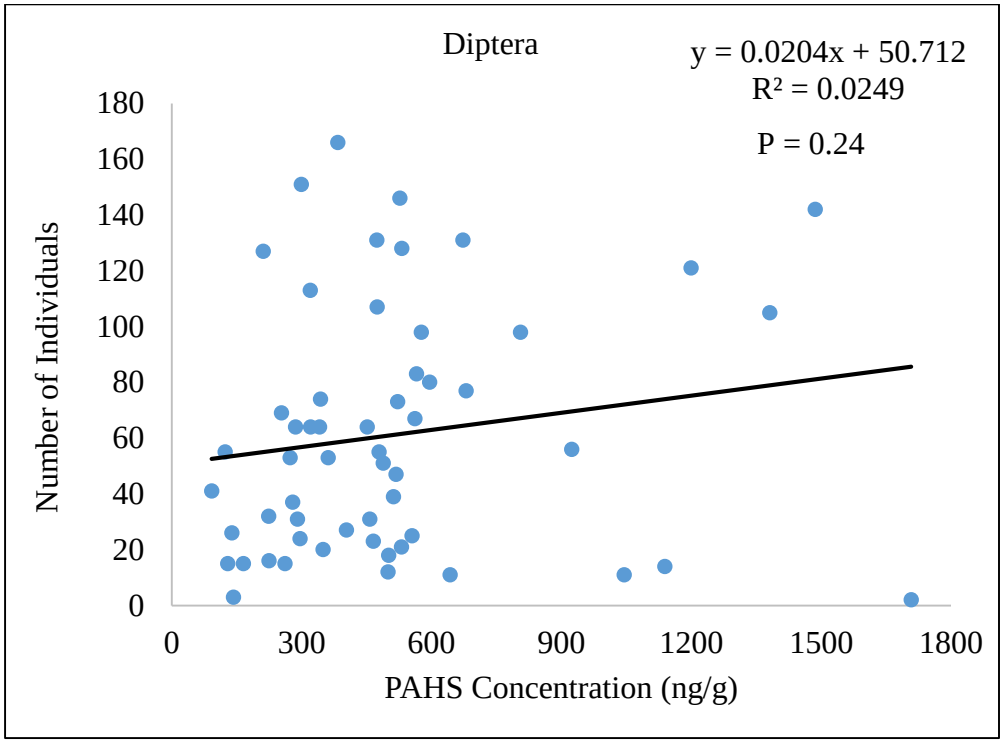

Thysanoptera
Number of Individuals
300
250
200
150
100
50
0
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)
y = 0.003x + 8.4345
R² = 0.0007
P = 0.84

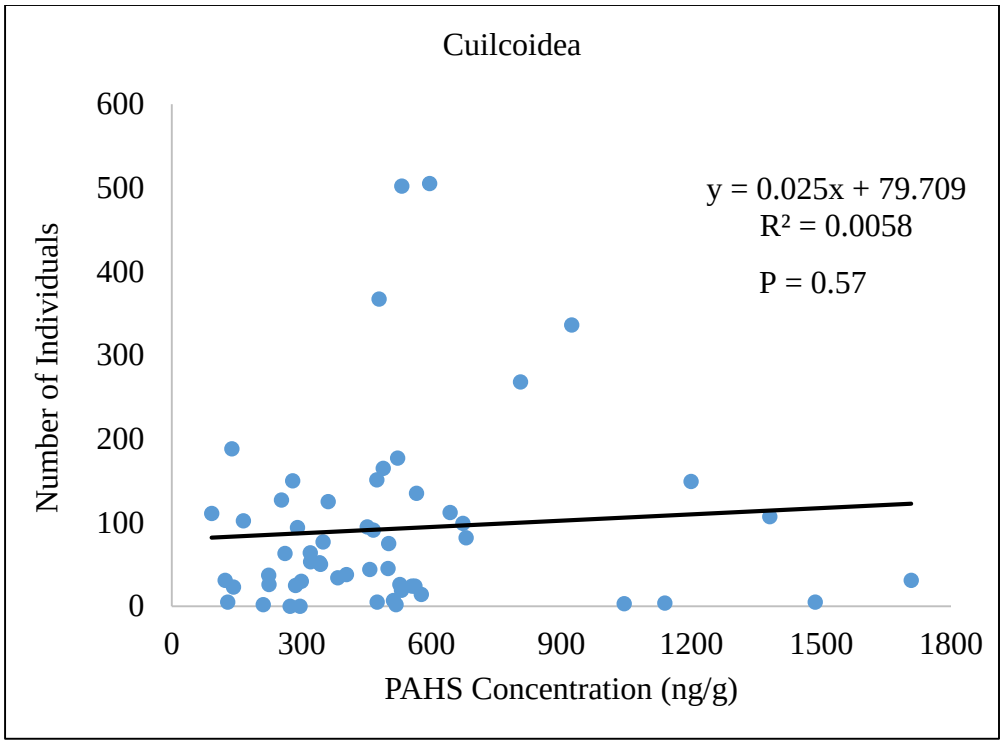

Coleoptera
Number of Individuals
25
20
15
10
5
0
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)
y = 0.0007x + 4.7365
R² = 0.0022
P = 0.73

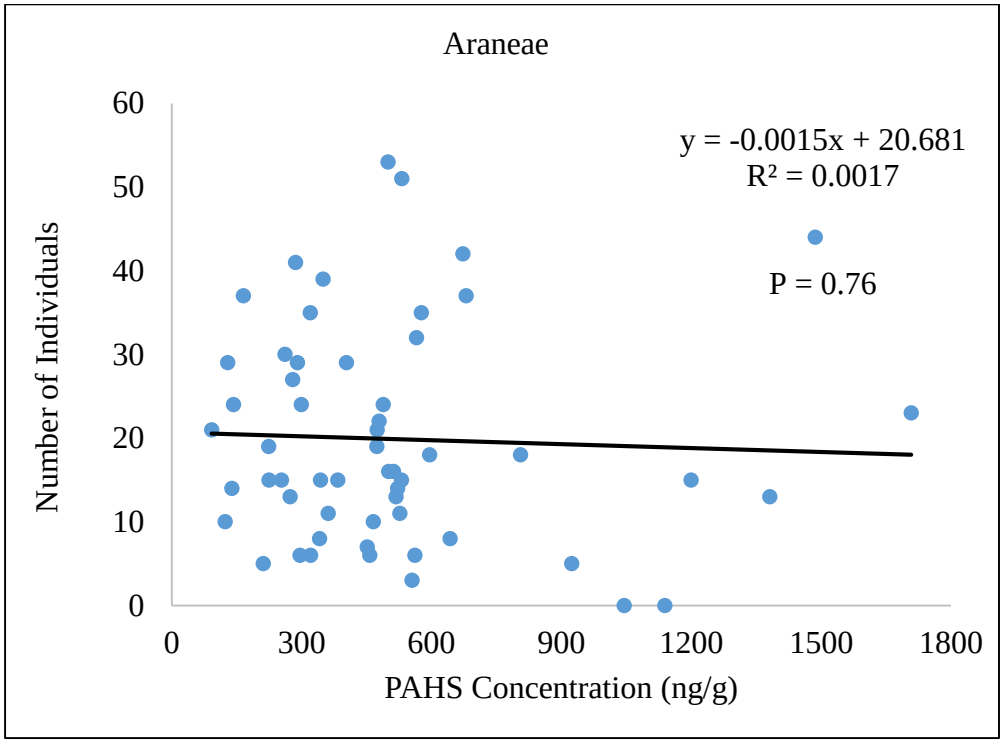

Formicidae
y = -0.0021x + 4.093
R² = 0.0115
P = 0.41
Number of Individuals
PAHS Concentration (ng/g)
0
5
10
15
20
25
30
35
40
0
300
600
900
1200
1500
1800


Pompiloidea
y = 0.0034x + 4.035
R² = 0.029
P = 0.21
Number of Individuals
PAHS Concentration (ng/g)
0
5
10
15
20
25
30
0
300
600
900
1200
1500
1800


Lepidoptera
y = -3E-05x + 0.3741
R² = 0.0003
P = 0.90
Number of Individuals
3.5
3
2.5
2
1.5
1
0.5
0
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)


Diptera
y = 0.0204x + 50.712
R² = 0.0249
P = 0.24
Number of Individuals
180
160
140
120
100
80
60
40
20
0
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)


Cuilcoidea
600
500
400
300
200
100
0
Number of Individuals
y = 0.025x + 79.709
R² = 0.0058
P = 0.57
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)


Araneae
60
50
40
30
20
10
0
Number of Individuals
y = -0.0015x + 20.681
R² = 0.0017
P = 0.76
0
300
600
900
1200
1500
1800
PAHS Concentration (ng/g)

VITA

Wokil Bam was born in Baitadai, Nepal and grew up in Mahendranagar, Nepal. After completing his high school, he moved to Marshall, Minnesota in 2008 to continue his education. He completed his undergraduate degree in Environmental Science and Chemistry with Biology Minor at Southwest Minnesota State University (SMSU), Marshall, MN in December 2012. During his senior year, he worked on capstone project titled "Sodium Analysis in Redwood River Water in Southwest Minnesota" and presented the research at the 7th Annual Undergraduate Research Conference on Dec 5th, 2012 at SMSU. He enrolled into a Master's Program in the Department of Oceanography and Coastal Sciences at Louisiana State University in Jan 2013. He is a candidate for the degree of Master of Science in the Department of Oceanography and Coastal Sciences. He will continue his studies in PhD program in the Department of Oceanography and Coastal Sciences at Louisiana State University.