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Oil-Mediated Mortality and Induced Behavioral Modifications of Coastal Insects

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OIL-MEDIATED MORTALITY AND INDUCED BEHAVIORAL MODIFICATIONS IN
COASTAL INSECTS

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ABSTRACT

The Deepwater Horizon oil spill affected over one thousand kilometers of the southeastern United States' coast in the Gulf of Mexico especially Louisiana's salt marshes. These marshes are a vital part of the state's economy and coastal ecology; however, the insects residing in this area remain largely unstudied. The goal of my project was to answer specific questions arising from two ongoing investigations of the insects in the oiled marshes. I sampled insects using sweep nets and insect vacuums to determine the most efficient technique to use in the marsh, compared insects collected in oiled and non-oiled marshes, and completed a taxonomic list of all of the insects collected in the marsh. I determined the effect of weathered oil on the mortality and foraging behavior of the ant *Crematogaster pilosa*. I used the house cricket, *Acheta domesticus*, to test the effect of direct and indirect oil exposure on insect mortality. I also used colonies of *A. domesticus* to determine if any sub-lethal effects occurred as a result of exposure to oil vapors.

Sweep-net sampling collected four times more insects than vacuuming and collected significantly more insect taxa ($p = 0.0005$). Oiled marshes had increased insect populations compared with non-oiled areas ($p = 0.0495$). Over 108 insect morphospecies were collected in the marsh. *C. pilosa*'s foraging behavior was drastically reduced by oil presence ($p < 0.0001$) though oil did not usually increase mortality. The time for 50% and 100% mortality to occur in *A. domesticus* was significantly less when exposed to direct contact with oil ($p < 0.0001$ for both 50% and 100%) and indirect contact with oil ($p = 0.0053$, $p = 0.0005$ for 50% and 100%, respectively). House crickets exposed to oil vapors showed reduced adult life spans, longer time to maturation, and changes in resistance to parasites when compared with controls. All these data indicate oil exposure can change insect populations, rates of mortality, and behaviors.

CHAPTER 1 - INTRODUCTION

1.1 Justification

The Deepwater Horizon ultra-deepwater oil rig (DWH) exploded on April 20th, 2010 and

sank on April 22nd, 2010, resulting in a three-month-long, active oil spill releasing 4.9 million

barrels of sweet crude into the Gulf of Mexico (Oil Spill Commission 2011). Over one million

gallons of the chemical dispersant Corexit® (Nalco Company) were applied to the oil in order to

prevent the development of a surface slick and applied at the surface to prevent the

contamination of the coast (Place *et al* 2010). As of January 2011, 1046 km of the Gulf of

Mexico's coastline were exposed to the oil emulsion associated with this disaster (Oil Spill

Commission 2011). Investigations of ecological damages and monitoring of recovery of the Gulf

of Mexico and its coastline are currently underway.

1.2 Initial Investigations

Immediately following the DWH explosion but before any oil made landfall, a group of

professors from Louisiana State University (LSU) and the LSU AgCenter established 45 sites

along the gulf coast of Louisiana from Breton Sound to Terrebonne Bay to assess changes in

local flora and fauna due to oil contamination. A variety of data was collected at each site

including information on local plants, mollusks, insects, diatoms, various microbes, soil, and

water (unpublished data). These sites have been maintained and frequently revisited to determine

possible changes to this environment.

In September 2011 a consortium of 26 principle investigators from over a dozen different

institutions and universities led by Nancy Rabalais of Louisiana Universities Marine Consortium

(LUMCON) was funded to address a series of questions created by the DWH spill. The goal of

this consortium is "determining the impacts of the oil, dispersed oil, and dispersant arising from the DWH spill on the ecosystems of the Gulf of Mexico in order to increase the fundamental understanding of the dynamics of such events and the associated environmental stresses" (Rabalais *et al* 2012).

1.3 Personal Contribution

The results described herein are part of the two larger ongoing investigations by LSU/LSU AgCenter and the LUMCON consortium. My project consisted of four separate field and laboratory experiments and observations intended to clarify questions and explain results arising from previous field-based research.

1.3.1 Part One: Sweep versus Vacuum

My objective in part one was to compare two separate techniques for collecting insects (sweep-net sampling and vacuum sweeping) in the *Spartina alterniflora* marshes of southern Louisiana. I sampled in both oiled and non-oiled sites to compare both techniques. The insects collected during these trials and from previous collection trips were used to provide a taxonomic list of the entomofauna of the marsh. Determining the best methodology for collecting insects in the salt marshes of Louisiana is necessary for any future marsh studies in which insects are involved. Furthermore, for ecological conclusions to be drawn from collected insects, proper taxonomic identification is critical. The hypotheses tested in part one were:

a.   The sweep-net collection technique collects significantly more specimens (total number collected, richness) than vacuum sweeping in *Spartina alterniflora* marshes in Louisiana.

b. The sweep-net collection technique collects significantly more species diversity than vacuum sweeping in *Spartina alterniflora* marshes in Louisiana.

c. Sites unaffected by the DWH oil spill will have significantly greater richness (total number) of insects than oiled sites.

d. Sites unaffected by the DWH oil spill will have significantly greater species diversity than oiled sites.

1.3.2 Part Two: *Crematogaster pilosa* Behavior Trials

The objective of part two was to increase our understanding of ecological damages and recovery in the *Spartina* marshes. I investigated what effects exposure to the oil emulsion had on the foraging behavior and rate of mortality of acrobat ants, *Crematogaster pilosa* Emery (Hymenoptera: Formicidae). Ants have previously been used in a variety of disturbance-based ecological studies and thus may also provide important information on the health of the Louisiana salt marshes (Rosenberg *et al* 1986, Andersen 1997, Andersen *et al* 2003, Graham *et al* 2004, Graham *et al* 2008, Graham *et al* 2009). The hypotheses tested in part two were:

a. The presence of DWH-associated crude oil will reduce the foraging behavior of *Crematogaster pilosa.*

b. The presence of DWH-associated crude oil will increase morality in *Crematogaster pilosa*.

1.3.3 Part Three: *Acheta domesticus* Mortality Trials

The objective of part three was to determine if direct and indirect contact with weathered oil emulsions associated with the DWH oil spill caused increased mortality in the house cricket, *Acheta domesticus* Linnaeus (Orthoptera: Gryllidae). The mortality of *A. domesticus*, in *in situ* bioassays is being used to indicate the presence of airborne oil, airborne oil constituents, possible airborne metabolites, and chemical constituents from ultraviolet breakdown or solar insoluation in the salt marshes of south Louisiana since the DWH spill (unpublished results). I investigated the cause of mortality in laboratory trials. I conducted experiments looking at both direct and indirect exposure to the oil emulsion under constant environmental conditions. The hypotheses tested by part three were:

a. Direct contact with weathered oil from the DWH spill will increase mortality in *Acheta domesticus*.

b. Indirect contact with weather oil from the DWH spill through exposure to only vapors will increase mortality in *Acheta domesticus*.

1.3.4 Part Four: *Acheta domesticus* Colonies

The objective of part four was to determine if exposure to indirect contact with the weathered oil emulsions associated with the DWH oil spill caused sub-lethal changes in *Acheta domesticus*. Colonies of *Acheta domesticus* were reared with indirect contact to low doses of the DWH oil emulsions. Observations were made on lethal and non-lethal effects including changes in the rate of mortality, generation time, longevity, and behavior. The hypotheses tested by these experiments were:

a. Exposure to vapors of the weathered oil from the DWH spill will result in higher *Acheta domesticus'* colony failure rates.

b. Exposure to vapors of the weathered oil from the DWH spill will result in shortened generation time of *A. domesticus.*

c. Exposure to vapors of the weathered oil from the DWH spill will result in decreased longevity of *A. domesticus*.

d. Exposure to vapors of the weathered oil from the DWH spill will result in observable changes in behavior of *A. domesticus*.

1.4 Literature Review

1.4.1 General Information

Coastlines can be defined as any area of land directly influenced by marine conditions through direct contact with the marine ecosystem (Davis and Fitzgerald 2004). Coastal Louisiana is composed of a variety of habitats including barrier islands, beaches, dunes, swamps, coastal forests, and marshes (Gomez 2008) and urban sprawl. The natural habitats all show a tendency to undergo rapid changes in response to both natural and anthropogenic stressors (Gosselink *et al* 1998). Marshes are particularly susceptible to the oil from the Deepwater Horizon spill. Therefore, coastal marshes in Louisiana affected by the DWH oil spill were the primary ecosystem on which I focused for this project.

Marshes are described as wetlands with emergent herbaceous vegetation adapted to saturated soils due to frequent or continuous inundation (Mitsch and Gosselink 2007). They can

be divided into four types based on the salinity of the water surrounding the marsh: saline, brackish, intermediate, and fresh (Sasser *et al* 2008).

Marshes play a critical role in coastal and marine ecosystems and economies and provide more inland land masses protection from storms (Boesch and Turner 1984, Farber 1987, Turner 1992, Baltz *et al* 1993, Peterson and Turner 1994, King and Lester 1995, Minello *et al* 2003). A variety of marine and coastal organisms including, bacteria, plants, insects, crustaceans, fish, sea birds and marine mammals rely on marshes to act as spawning grounds, nurseries, adult habitats, and feeding sites (Erwin *et al* 1981, Boesch and Turner 1984, Fitz and Weigert 1991, Young and Phillips 2002). Furthermore, yields from the multi-billion dollar fishing industry, including both commercial and recreational catches, have been directly linked to marsh presence and health (Boesch and Turner 1984, Bergstrom *et al* 1990). Marshes also directly benefit coastal residents by reducing storm surge and wind damages associated with hurricanes and tidal anomalies (Farber 1987, King and Lester 1995, Moller *et al* 2007).

Louisiana's marshes constitute about 40%-41% of the total marsh area on the United States' coasts (Bergstrom *et al* 1990, Field *et al* 1991). This ecosystem is currently threatened due to land loss which is occurring at a rate that ranges from 65.63 km^2/year to 77.4 km^2/year, but has historically been as high as 108.3 km^2/year in Louisiana (Craig *et al* 1979, Gagliano *et al* 1981, Britsch and Dunbar 1993, Barras *et al* 2003). This loss of land is linked to sea level rise, changes in deltaic deposition, hurricanes, and urbanization including dams, levees, canals, pipelines, and highways (Walker *et al* 1987, Gosselink *et al* 1998, Charmbers *et al* 2005).

Insects are important components to marsh health and stability as well as coastal communities for several reasons (Teal 1962). They act as independent detritus consumers which are important to commercial fish stocks (Odum and de la Cruz 1967, Odum and Heald 1972).

Insects also provide a quick means for moving biological material and energy through an ecosystem by acting as primary consumers of plant materials. Insects are often a major food source for larger insects, fish, amphibians, birds, and mammals (Price 1997). Impacts on insects due to toxins may have a cascading effect on the organisms throughout the food web. Increased concentrations of toxins in the food web may lead to increased defects and deformities in alpha predators, reducing their reproductive viability such as in the case of dichlorodiphenyltrichloroethane (Woodwell *et al* 1967, Price 1997). In order to narrow the research areas to those places affected by the spill, insects and all pertinent data were collected from brackish and saline marshes found in Barataria Bay (Plaquemines Parish), and Breton Sound (Plaquemines and St. Bernard Parishes) Louisiana, including both oiled and un-oiled reference areas.

Oil spills have both immediate and long term effects on environments. Exposure to oil and oil components (including breakdown products, dispersants, drilling fluids, and other chemicals associated with oil released during spills) can cause mortality, reduced fecundity, reduced growth, and affect changes in behavior and metabolism in a variety of organisms including bacteria, plants, mollusk, crustaceans, insects, birds, and mammals (Artema and Stein 1974, Krebs and Burns 1977, Vandermeulen *et al* 1982, Michaelis 1983, DeLaune *et al* 1984, Cushman and Goyert 1984, Harrel 1985, Bombick *et al* 1987, Boehm *et al* 1996, Horvath *et al* 1998, Vitaliano *et al* 2002, Peterson 2003). In addition, attempts to clean up oil after a spill using either chemical or mechanical means can also have deleterious effects to local environments and negatively affect invertebrate populations (DeLaune *et al* 1984, de la Huz *et al* 2005).

Insects, specifically, are at a high risk to the impacts of oil and oil components. Oil and the chemicals used to clean up oil spills increase mortality in insects by interacting with the

unique chemical composition of their cuticle. The high metabolic rates of insects have also been implicated to increased mortality due to exposure to toxicants like crude oil (Bombick *et al* 1987). An array of studies have been conducted looking at the effect of oil on various insect populations; however, most of these studies occur in freshwater environments such as rivers or ponds (Michaelis 1983, Crushman and Goyert 1984, Bombick *et al* 1987, Crunkilton and Duchrow 1990, Ort *et al* 1995) with little research conducted on coastal environments.

1.4.2 Sweep versus Vacuum

A variety of techniques and equipment exist for the purpose of collecting insects and other arthropods (Triplehorn and Johnson 2005). Many of these techniques, however, are not suitable for the marsh environment because of the constant inundation of water floods pitfall-traps, exposure to salt destroys the metal frames of some traps, high winds knock over flight-intercept traps and other larger collections equipment, the lack of a canopy cover and large trees limit places hanging traps can be attached, and many other factors. Collection methods in the marsh usually include sweep nets, vacuum sweeps, clip plots, opportunistic collection, and sticky traps. The most commonly chosen collection procedure are sweep-net sampling, vacuum sampling, and clip plots (Cameron 1972, Elkaim and Rybarczyk 2000, Finke and Denno 2004, Gratton and Denno 2005, Wu *et al* 2008, Harvey *et al* 2009, Wimp *et al* 2010, McCall and Pennings 2012).

Clip plot sampling is intrinsically very different from sweep-net and vacuum sampling. It requires that all vegetation be removed from a set area or a set number of plants be collected at each site (Cameron 1972, Gratton and Denno 2005). The vegetation is then sorted through for insects living on or within the collected material. This technique is often better for collecting

internal or concealed insects residing or feeding within a plant but is not the best for collecting active insects that can easily escape during the removal of the plants (Gratton and Denno 2005).

Sweep-nets and vacuums are used to collect insects along transects of set length or during set periods of time (Gratton and Denno 2005, Wu *et al* 2008;). As a result, both methods should collect insects on the outside of plants but have leave behind insects concealed within plants (Wimp *et al* 2010, Doxon *et al* 2011). Previous studies in mixed grass prairies and pastures have shown that the two techniques differ in the particular taxa that they collect, the mean size of the insects collected, and overall biomass of the collection but not in total species richness (Doxon *et al* 2011). In most studies in marsh habitats only one of these two sampling methods is often used with a more recent studies using primarily vacuum sampling (Elkaim and Rybarczyk 2000, Finke and Denno 2004, Gratton and Denno 2005, Wu *et al* 2008, Wimp *et al* 2010, McCall and Pennings 2012). Comparisons of possible difference between these two techniques have not been previously tested in salt marshes.

Correct taxonomic identification of organisms is necessary to ensure clear communication among scientists and accurate assessments of biological experiments and observations (Hunters 1958). Furthermore, improper or a lack of identification can result in confounding environmental signals from species used as biological indicators, especially if sister taxa with differing metabolic or behavioral responses are also present within a study area (Bortolus 2008). Taxonomy has and will continue to play an important role in ecology, conservation, and restoration (Kociolek and Stoermer 2001; Dayton 2003; Dubois 2003; Mace 2004; Kociolek 2005).

Several taxonomic lists of insects residing within salt marshes currently exist in the literature (Teal 1962; Cameron 1972; Williams *et al* 2003; Wu *et* al 2009; Harvey *et al* 2010;

Wimp *et al* 2010). These lists, however, do not provide specific information on the insects in Louisiana's *Spartina* marshes. A project-specific list is an important component of comparisons of insect communities in oiled and un-oiled marshes. Therefore, in order to interpret the results from the on-going investigations in Louisiana's *Spartina* salt marshes, a taxonomic list of the insects living in this habitat was created.

1.4.3 *Crematogaster pilosa* Behavior Trials

The 400+ species of acrobat ants, Myrmicine ants within the monophyletic genus *Crematogaster*, can all readily be identified by the workers' tendency to walk with their heart-shaped gasters arched over the top of their bodies, the dorsal attachment of the postpetiole to the fourth abdominal tergite, the lack of a dorsal node on the petiole, and their spatulate sting (Wheeler 1910; Buren 1958; Buren 1968; Hölldobler and Wilson 1990; Bolton 1995; Longino 2003; Fisher and Cover 2007; Morgan 2009; Mark Deyrup, personal communications). The majority of *Crematogaster* species are associated worldwide with arboreal habitats in the tropics but can also be found in temperate zones usually nesting in the ground. Most species are monogynous although a few tropical species do have polygynous castes. Several species of *Crematogaster* exhibit polydomous behavior, nesting in several separate hollow stems and tree branches. Most species tend to be highly aggressive and very territorial, especially those producing large, polydomous colonies. In Neotropical forests, these ants often play a major ecological role because of their colony size, generalized omnivorous diets, and the tendency to farm Hemiptera (Longino 2003). Six species of the genus are reported in Louisiana: *C. ashmeadi*, *C. atkinsoni*, *C. lineolate*, *C. minutissima*, *C. pilosa*, and *C. vermiculata* (Dash and

Hooper-Bùi 2008). *Crematogaster pilosa* is the most common species of ant found living in the salt marshes of southern Louisiana.

Crematogaster pilosa (Emery) can be identified by its long, propodeal spines, a smooth pronotum, short hairs on the pronotal shoulders, and several erect hairs on the vertex of the head (Morgan 2009). The species is highly variable in its color, hairiness, size, and natural history making it easy to misidentify. It is known to nest generally along the edges of swamps and marshes usually within grass stems, small dead trees, and dead branches on live trees (Deyrup, personal communication). Newly mated queens of *C. ashmeadi* Emery, a closely related species, establish colonies within abandoned Coleopteran and Lepidopteran larval galleries in branches and stems and take about a month to produce a first generation of workers (Baldacci and Tschinkel 1998; Tschinkel 2002). This claustral behavior is most likely similar in *C. pilosa*. In Louisiana's salt marshes, colonies of *C. pilosa* are polydomous living within several hollow, dead stems of *Spartina alterniflora* that are still standing upright and rooted, horizontal dead stems that were unrooted, and few live stems (Hooper-Bui, personal observation).

Investigations from May 2010 to November 2012 have revealed that populations of *Crematogaster pilosa* dramatically decreased in areas affected by the DWH oil spill; whereas, large populations remain in those areas not affected by the spill (190±21.7 colonies/hectare in non-oiled sites; Hooper-Bui, unpublished data, mean ± standard error of the mean). These changes in the ant populations may have significant effects on the marsh ecosystem as *Crematogaster* is known to be ecologically important in other environments (Longino 2003). Therefore, observations of behavioral modifications and mortality induced by exposure to the oil emulsion from the DWH oil spill are an important aspect in elucidating the causes of the population crash in oiled sites.

1.4.4 *Acheta domesticus* Mortality Trials

The use of a variety of organisms or groups of organisms to monitor ecological stability, health, and conditions has a long record in the scientific literature (Hall and Grinnell 1919; Powell and Powell 1986; Noss 1999; Carignan and Villard 2001; Whitfield and Elliott 2002; Rainio and Niemela 2003; Niemi and McDonald 2004; Landres and Verner 2005; Burger 2006; Holt and Miller 2011). Surrogate indicator species are commonly implemented to assess the health of entire ecosystem and/or populations during pollutant- or toxicant-based disturbances much like how canaries were once used coal mines to monitor for the presence of noxious gasses (Landres 1992; Sarkka 1996; Caro and O'Doherty 2001). Invertebrates have specifically been noted as highly effective and efficient bioindicators due to their size, metabolism, abundance, diversity, importance to ecosystems, and relative ease of sampling (Andersen 1997; Andersen *et al* 2003).

Acheta domesticus (Linneaus), an Orthopteran in the family Gryllidae commonly known as the house cricket, was chosen as a surrogate indicator species testing for the presence of oil in the salt marsh. It was chosen due to its general availability, the availability of specific age classes, and ease in rearing (Clifford *et al* 1977). It is also not a native inhabitant of the coastal marsh and therefore should have a very low tolerance to crude oil compared with some coastal and marine organisms (Rossi and Anderson 1976; Moles *et al* 1979; Lin and Mendelssohn 1996; Wheelock *et al* 1999; Hamdoun *et al* 2002). However, similar species of Orthopterans do inhabit coastal marshes (personal observation). *A. domesticus* is a hemimetabolous, non-native generalist omnivore in the United States and is usually found in close association to human habitation. They exhibit continuous breeding behavior without seasonal or environmentally induced

diapause so they are present year round (Alexander 1968). When reared under laboratory

conditions, the expected life span is around 60 days under optimum conditions (Adamo 1999).

Optimum conditions are considered a 12-hour light/dark cycle, temperature at 30.5°C, and food

and water supplied *ab libitum* (Clifford *et al* 1977).

 In situ bioassays conducted from 2010 to 2012 in *Spartina* marshes indicated that under

certain environmental conditions a significant increase in mortality occurred in crickets placed in

known oiled sites versus unoiled reference sites (unpublished data). In order to remove

environmental variation, laboratory trials held under constant conditions were conducted.

Observations were made on changes in mortality and changes in behavior due to either direct

contact with oil or contact with only oil vapors.

 1.4.5 *Acheta domesticus* Colonies

 Toxicant exposure at low doses may not always produce mortality but instead may elicit

changes in an organism's histological, morphological, physiological, or ethological responses to

stimuli (Sprague 1971; Rosenthal and Alderdice 1976; Haynes 1988; Stein *et al* 1992; Elzen

2001; Fleeger *et al* 2003; Desneux *et al* 2007; Whitehead *et al* 2011). Persistent sub-lethal

contact with crude oil, dispersants, and oil emulsions have all been linked to deleterious effects

on various organisms (Swedmark *et al* 1973; Duffy *et al* 1994; Blajeski *et al* 1996; Singer *et al*

1998; Jensen and Carroll 2010; Whitehead *et al* 2011). Insect responses to sub-lethal exposure to

contaminants include changes in behavior, reduced fecundity, failed ecdysis, reduced longevity,

decreased adult size, and changes in metabolism (Moriarty 1969; Simpson 1980; Rosenberg *et al*

1986; Haynes 1988; Jepson 1989; Elzen 2001; Desneux *et al* 2007). Few studies have examined

the sub-lethal effects of crude oil, dispersants, or oil emulsions on insects (Simpson 1980).

Previous observations of *Acheta domesticus* exposed to only the vapors of the oil emulsions associated with the DWH oil spill indicated a potential increase in mortality occurring during ecdysis (Hooper-Bui, unpublished data). Therefore, colonies of house crickets were established to look for possible changes in behavior, increased mortality during molting, and changes in fecundity due to exposure to crude oil vapors.

CHAPTER 2 – MATERIALS AND METHODS

2.1 Sweep versus Vacuum Methods

    2.1.1 Location and Time

    I established eight sites in saline to brackish *Spartina alterniflora* marshes within Plaquemines Parish, Louisiana. These sites had at least a >100m x 20m section of uninterrupted marsh to ensure that multiple transects could be established at distances far enough apart to reduce interaction between transects. Four of the eight selected sites were in areas known to have been oiled during the DWH spill. These oiled sites were either selected from sites used as part of the larger LSU and LUMCON consortium investigations or chosen based on the presence of oil seen on the sediment and large areas of dead *S. alterniflora* along the marsh edge.

    Collections were made in the morning until just after noon on 9 & 10 September 2011. Location, time, water temperature, air temperature, wind speed, and the presence or absence of oil were recorded for each site. All of this information is included in Appendix A – Table 6.

    2.1.2 Collection Procedures

    Two 20m transects for each collection technique (a total of four 20m transects per site) were created at each of the eight sites. The transects ran perpendicular to the edge of the marsh. All sampling was conducted from the edge inward on undisturbed transects. The two transects used for the sweep net technique were placed at least 50m from the two transects used for the vacuum sweeping technique to minimize any possible disturbances caused by the other technique. The pairs of transects with identical collection techniques were spaced at least ten meters apart to prevent collecting from the same area twice. At each site the sweep-net transect was performed first to decrease any disturbance caused by the noise associated with the vacuum.

For each site, insects collected from the matching transects were grouped into the same storage container (two containers per site) and stored in 95% ethanol solution and returned to the lab for identification and further analysis.

Sweep net collections were conducted using a 38.1 cm (15 in) diameter collapsible insect collection net (1140.1 cm^2, Bioquip Products Inc, Rancho Dominguez, CA). Vacuum collections were made using a gasoline powered Agricultural Backpack 2-Cycle Aspirator Model 1612 with a 22.9 cm (9 in) collection nozzle (411.9 cm^2, John W. Hock Company, Gainesville, FL). The vacuum produces a 31 km (19 miles) per hour air intake. Collections were made for each technique by sweeping the net or nozzle in a back-and-forth arching motion through the *Spartina* along each transect. The vacuum and the net were used to collect insects in a 2 m swath – 1 m on each side of the operator. Collections at all sites were conducted by the author in order to create a more consistent sampling technique across all sites.

2.1.3 Identification Procedures

Sweep-net and vacuum samples for each site were sorted in the lab individually to separate insects, arachnids, and crustaceans. The contents of each sample were initially emptied into a large Petri dish (150mm x 25mm, Corning Incorporated) and sorted by eye to separate out the large arthropods. The sample was then placed under a dissecting microscope (Olympus SZX12, Olympus America Inc.) to sort out smaller arthropods. A second search of the entire sample was then conducted by another individual to ensure that all arthropods had been removed from the sample. All collected insects and arachnids from the sample were then identified to order. The insects were then sorted to morphospecies and each morphospecies in each sample was placed in a separate labeled vial containing 95% ethanol.

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After every insect from each sample was identified to order and sorted to a morphospecies, each morphospecies was then identified at least to genus using a variety of taxonomic keys and lists (Williston 1908; Buren 1968; Stannard 1968; McAlpine 1987; Grissell and Schauff 1990; Bolton 1995; Arnett 2000; Needham *et* al 2000; Arnett *et al* 2002; Burks 2003; Noyes and Pitkin 2004; Pollet *et al* 2004; Triplehorn and Johnson 2005; Wilson 2005; Fisher and Cover 2007; Dash and Hooper-Búi 2008; Merrit *et al* 2008; Morgan 2009; Fero *et al* 2010; Wimp *et al* 2010; Johansen 2011; Dmitriev 2012; Pape 2012; Walker and Moore 2012). Some morphospecies only contained a single individual that was too damaged to identify to genus. In these cases, taxonomic identification was taken as far as possible. Chalcidoid wasps in most cases were only identified to family. Some Cicadellidae and Chironomidae were only identified to tribe.

Insects from previously-collected samples (2010-2012) were also identified to create a more complete taxonomic list of all the entmofauna found in Louisiana's coastal *Spartina* marshes. These insects were collected using a variety of techniques including sweep nets, vacuum, clip plots, and opportunistic hand-collecting.

2.1.4 Statistical Analysis

I tested whether sweep-net or vacuum techniques differed significantly in total number of insects and species richness according to hypotheses 1a and 1b. I also determined if the presence of oil significantly affected the total number of insects and the insect species richness. A series of ANOVA statistical analyses were performed on total richness, total insects, and total insect species richness due to the factorial nature of the design of this experiment (comparing two collection techniques across two habitat qualities). P-values were adjusted using a Tukey-Kramer

adjustment. An analysis of richness was also performed on specific taxonomic groups of interest including subclass Acari, orders Araneae, Thysanoptera, Coleoptera, and Diptera, families Blissidae, Formicidae, Chironomidae, and Chloropidae. These groups were chosen based on their high presence in collections and relative importance of to the marsh ecology. Analyses were also performed on functional groups including stem-borers, herbivores, and omnivore/carnivores. The stem-borer group including any specimens that at any point in their life history had a stem boring phase. Herbivores included any insects that fed on plant materials not included in the stem-borer category; also included were fungivores. The omnivore/carnivore group included parasitoids as well. All statistical analysis was performed on SAS 9.3 (SAS Institute Inc.). The programs can be found in Appendix B – Sweep vs. Vacuum Programs.

2.2 *Crematogaster pilosa* Behavior Trial Methods

    2.2.1 Collection and Harborage

    Crematogaster pilosa were collected from the non-oiled salt marshes in Plaquemines Parish between September 2011 and March 2012 to test whether exposure to weathered oil from the DWH oil spill will decrease foraging behavior and increase mortality. *C. pilosa* colonies were located using random search patterns, transects, and grids. Colonies were identified by the presence of workers on the *Spartina* and bored entry holes in the sides of the *Spartina* stems. Once a colony was located, the stems with ants were clipped at the sediment using pruning shears. Because these ants are polydomous, any other stems also containing ants with a 0.5m radius from the original stem were also clipped. All clipped *Spartina* stems were put in a zippered pillow case and brought back to the lab.

Once in the lab, each colony was housed separately in a large cylindrical plastic dish (252.4 mm x 90.5 mm) with a friction-fitting lid to prevent escape. To passively remove the ants from the *Spartina*, the stems were split in half long ways and left in the containers. Small, clear culture tubes (6 mm x 50 mm) with moistened cotton were provided as artificial harborages for the ant colonies. After the ants had moved out of the *Spartina*, the stems were removed from the plastic dishes. This process generally took between one and three days. A 20% sugar water solution, crickets, and Fluker's Cricket Quencher (Fluker's Cricket Farm, Inc. Port Allen, LA) were provided *ad libitum* while the ants were in the lab. All colonies were allowed at least one week to acclimate to the laboratory environment before ant experiments were performed.

2.2.2 Trials and Procedures

I conducted experiments with two controls and five different treatments examining the effect of oil on ant foraging behavior and mortality. A treatment was composed of one cohort of ants, food/water or a food/water dummy, and oil and was replicated five times. The control included one cohort of ants and food/water or a food/water dummy. Food and water consisted of one culture tube with 20% sugar water solution and one piece of 1 cm^3 Fluker's Cricket Quencher. The food and water dummy was one empty culture tube and one 1 cm^3 rock. Each trial of the experiment consisted of a treatment or control with five replications.

To begin each trial, five cohorts of ants were selected from five different colonies. Each cohort was selected by removing a single small culture tube with all ants, larvae, and pupae inside of it from the colony dish. This culture tube was then placed into a separate large plastic dish for the extent of the trial. Cohorts consisted of > 20 and < 100 individual workers. If a

culture tube was selected that did not fit this standard, it was replaced and a new tube was chosen.

Prior to the application of a treatment, the five cohorts in their separate large dishes were placed in an incubator at 33 °C for 24 hrs without food or water. A starvation/dehydration period of 24 hrs was chosen based on preliminary experiments that indicated a greater period caused significant mortality in all cohorts prior to treatment and a lesser period did not elicit foraging behavior from any treatments. Overall temperature and relative humidity (RH) of the incubator was monitored and recorded using a HOBO® LCD logger (Onset Computer Corporation). Data obtained from the logger was graphed and analyzed using HOBOware® Pro software (Onset Computer Corporation) to ensure that environmental variations were constant across all trials.

Any dead ants were counted and removed from the arena after this period of starvation and dehydration then stored in 95% ethanol solution for later analysis. Food, water, and oil in the case of treatments were then added to each of the five replications. When applied, oil was painted onto the bottom of the plastic dish in a 1 cm thick ring using a small paint brush. Afterwards, cohorts were placed back in the incubator until observations of behavior were made. The two controls and five treatments were set up as follows:

1. Control 1 - One cohort of ants and food/water on opposite sides of the dish

2. Control 2 – One cohort of ants and the food/water dummy on opposite sides of the dish

3. Treatment 1 - One cohort of ants and food/water on opposite sides of the dish with oil surrounding the food/water

4. Treatment 2 - One cohort of ants and the food/water dummy on opposite sides of the dish with oil surrounding the dummy

5. Treatment 3 – One cohort of ants and food/water on opposite sides of the dish with oil present but not around the food/water

6. Treatment 4 – One cohort of ants and food/water on opposite sides of the dish with oil surrounding the cohort

7. Treatment 5 – One cohort of ants and the food/water dummy on opposite sides of the dish with oil surrounding the cohort

Twelve observations of foraging behavior were recorded during the first 24 hours after the addition of food, water, and oil to each replicate. Observations were made at 10 min, 20 min, 30 min, 1 h, 1.5 hrs, 2 hrs, 2.5 hrs, 3 hrs, 4.5 hrs, 6 hrs, 7.5 hrs, and 24 hrs. Location and number of individuals at food, water, and dummies were recorded at each observation. Any other behaviors seen during observations were also recorded including movement of brood, movement of queens, and movement of the entire cohort out of the culture tube. The twelve observation times and 24-hour total observation period were decided on based on preliminary experiments. These experiments showed that the most foraging activity happened in the first six hours after the addition of food and water. They showed that after 24 hours, cohorts of ants in control trials had to be starved and dehydrated again to elicit any significant foraging behavior. Furthermore, mortality reached 100% in some preliminary treatments during 24 hours making observations of foraging behavior impossible.

All dead ants were removed from the dishes, counted, and stored in a 95% ethanol solution at the end of the observation period. The remainder of the living ants were also counted and stored in vials with 95% ethanol solution. Total percent mortality of each cohort was then calculated.

All oil emulsions used during these experiments came from a weathered sampled collected at the water's surface at N 28° 44.256' by W 88° 21.704' on July 29th, 2010, by Scott Miles (LSU research associate) according to acceptable methods. This emulsion represents the oil associated with the DWH oil spill in the marshes of coastal Louisiana. This emulsion was obtained through the appropriate channels and a chain of custody form is available.

2.2.3 Statistical Analysis

The effect of oil on foraging behavior was determined by comparing the mean percentage of individuals per treatment recorded at the water/food or water/food dummies during the entire observation period. Comparisons were made using ANOVA with a Tukey-Kramer adjustment. Total mortality, mortality caused by the initial starvation, and mortality only caused by the treatments were all analyzed to determine the effect of oil on mortality. Comparisons were made using ANOVA with a Tukey-Kramer adjustment. All pair-wise comparisons were included for both foraging behavior and mortality. These programs can be found in Appendix B – Ant Behavior Programs.

2.3 *Acheta domesticus* Mortality Trial Methods

2.3.1 Pre-experimentation

House crickets, *Acheta domesticus*, were exposed to three conditions to determine the effects both direct and indirect contact with weathered oil from the DWH spill has on the rate of mortality: indirect exposure through contact with only oil vapors, both direct and indirect exposure through contact with oil and contact with oil vapors, and no contact with oil to act as a control. A fourth condition, exposure to oil through contact without vapors, was not tested as

direct contact with oil will always include some exposure to vapors as well and therefore lacks practical application. Insects exposed to the emulsion in natural environments will also be exposed to the vapors.

House crickets used for these experiments were all obtained from Flukers Cricket Farms, Inc. All crickets were at least 6 weeks old and were either adults or final instar nymphs. Prior to the experiment, the crickets were housed in collapsible mesh rearing cages for at least 48 hrs. During this time crickets were fed *ab libitum* on Fluker's Cricket Quencher and Fluker's Hi-Cal. Cricket Diet (Fluker's Cricket Farm, Inc.). A new cohort of crickets was obtained for each experimental trial to ensure that crickets were the same age across trials.

All experiments were conducted in two model I-36VL Percival incubators (Percival Scientific, Inc.). Both incubators were set on a 12-hour light and dark cycle with temperatures at 37.5 °C during the light and 35.5 °C during the dark and a constant 90% relative humidity (RH). These temperatures and relative humidity were chosen based on the environmental data collected in the marsh during previous field experiments involving *A. domesticus*. A small container with 500 ml of a one molar NaCl solution was included at the bottom of each incubator to act as an insulator of the internal RH. Temperatures and relative humidity of each incubator were measured and recorded using a HOBO® LCD logger. Data obtained from the logger was graphed and analyzed using HOBOware® Pro software to check for temperature spikes. Both incubators were allowed to run for two weeks prior to any experiments to ensure constant temperatures and relative humidity was maintained.

2.3.1 Trials and Procedures

Experiments had to be conducted in trials because of the size limitation of the incubators. A total of 21 replicates of each treatment (control, vapors, and contact; 63 replicates in all) were run over seven trials each composed of nine replicates (three replicates of each treatment). Control replicates were composed of 12 crickets. Both vapor and contact treatments each consisted of six crickets.

During each trial, crickets were housed separately in 70 two-ounce (59.1 ml) portion cups. Each portion cup was provisioned with 5.4 grams to 5.6 grams of Fluker's Cricket Quencher. All cups were ventilated through holes made on both the top and bottom surfaces using a dissecting straight needle. Before adding the crickets, the cups, in groups of twelve, were mechanically connected to six grated cooling racks (25 cm x 20 cm x 2 cm) using staples to elevate the cups several centimeters off the oil. This grouping of 12 cups on a rack will be referred to as one cohort.

At this point, crickets were added to each cohort. Three of the cohorts were treated as controls with no addition of oil. For each of the other three cohorts, half of the crickets (six crickets per cohort) were put in direct contact with the weathered crude oil by applying a small amount to their rear tarsi and abdomen using a number two round artist paint brush. All other crickets used in these experiments had their rear tarsi and abdomen brushed with a number two artist paintbrush without any oil to reduce any variables introduced by possible abrasions caused by brushing the crickets. The oil used in this experiment was the same as that used in the previous ant foraging behavior experiments.

Each cohort was then placed in an aluminum sheet pan (24.13 cm x 33.02 cm x 2.54 cm) once all 70 crickets were each stored in their individual portion cups. The three cohorts and sheet

pans being treated as controls were then stored in plastic containers (38.1 cm x 29.2 cm x 8.3 cm) with lids. The other three cohorts and sheet pans were first treated with weathered oil before being stored in plastic containers identical to the control cohorts. This treatment of oil consisted of 15 grams of weathered crude oil being applied to the surface of the sheet pan using a clean 2.54 cm flat artist paintbrush.

A 200 ml solution of 3M NaCl and water was added to the plastic containers on the outside of the sheet pans to regulate the relative humidity of the environment within each plastic container. For the extent of the trial, the plastic containers were sealed with Parafilm® M Barrier Film (Structure Probe, Inc. Westchester, PA). Temperate and RH were monitored in each sealed container using a HOBO® Micro Station System (Onset Computer Corporation, Bourne, MA). Data from the temperature and RH probe were graphed and analyzed using HOBOware® Pro software.

Each replicate was disturbed by lightly shaking each plastic container to monitor for mortality. Crickets were considered dead or moribund when no movement was observed for greater than 15 seconds after the disturbance.

Preliminary trials indicated that rapid mortality occurred in some treatments within the first 24 hrs of exposure to oil. In order to record this mortality, observations were made every 1.5 hrs for the first 24 hrs after exposure. After the first 24-hour period, total mortality for every replicate was recorded every six hours until all crickets had died.

2.3.3 Storage and Disposal of Materials

All materials used in that trial were discarded after the completion of each trial. Crickets that had direct contact with oil were disposed as biohazard waste. Cooling racks, sheet pans, and

plastic containers exposed to oil were washed with xylenes and soap. Waste materials from that

washing were stored in approved waste containers for future disposal. Washed cooling racks,

sheet pans, and plastic containers were put inside of zipping plastic bags and stored for future

disposal. one incubator and all monitoring equipment (one HOBO® LCD logger and one

HOBO® Micro Station System) were labeled and used only for oil to prevent potential

contamination of both incubators. The other incubator and monitoring equipment were only used

for control treatments. Between trials, both incubators were left open for one week to allow any

accumulated vapors to dissipate.

2.3.4 Statistical Analysis

The effect of exposure to oil through direct contact and contact with vapors on the rate of

mortality over time was analyzed using binomial regression with a Tukey's adjustment. The

three mortality curves were compared using a GLIMMIX command in SAS 9.3. The mean time

it took for 50% (LT50) and 100% (LT100) mortality to occur for each treatment was also

calculated. The LT50s and LT100s of each treatment were compared using ANOVA with a

Tukey's adjustment. These programs can be found in Appendix B − Cricket Mortality Program.

2.4 *Acheta domesticus* Colony Methods

2.4.1 Specimens and Materials

Twenty colonies of the house cricket *Acheta domesticus* were established to test the

effect of prolonged exposure to sub-lethal dosages of the vapors associated with the emulsion

produced by the Deepwater Horizon oil spill. Half (ten) of the colonies were treated with indirect

contact to oil through exposure to vapors; the other half acted as controls with no exposure to oil vapors.

All *A. domesticus* in these colonies were acquired from Flukers Cricket Farms, Inc. Crickets were at least 6 weeks old and determined to be either adults or final instar nymphs prior to the beginning of this experiment. Crickets were kept in collapsible rearing cages for at least 48 hours before starting the cricket colonies to reduce stress and accommodate mortality associated with shipping. During this time crickets were fed *ab libitum* on Fluker's Cricket Quencher and Fluker's Hi-Cal. Cricket Diet (Fluker's Cricket Farm, Inc.).

Two model I-36VL Percival incubators, one for control colonies and one for treatment colonies, were implemented to regulate the environmental condition of the colonies. Both incubators were set on a 12-hour light and dark cycle. Temperatures were set at 32 °C and 30 °C during the light and dark periods, respectively. Relative humidity was set at 90% during the light period and 70% during the dark period. A two-liter reservoir of water was placed at the bottom of each incubator to maintain the high relative humidity in the incubators. Pertinent environmental data was measured and recorded in each incubator using HOBO® LCD loggers. Incubators were allowed to run for two weeks prior to the start of the cricket colonies to ensure constant environmental conditions.

2.4.2 Trials and Procedures

Colonies were started with ten crickets (five male, five female) selected from the collapsible rearing cages. Crickets with any noticeable damage such as missing limbs were not used in this experiment.

Each colony was housed separately in a large circular plastic dish (252.4 mm x 90.5 mm) with a friction-fitting lid to prevent escape. A two-centimeter thick layer of a 50:50 mixture of peat moss and potting soil was blended and added to each colony to act as a substrate. The peat moss and potting soil mixture was placed in a drying oven set to 60 °C for 72 hours to reduce the potential of mite infestation. The mixture was turned over every 12 hours to ensure complete drying. Colonies were also furnished with one medium-sized weigh boat (≈100 ml capacity; 8.4 cm x 8.4 cm x 1.9 cm) filled with a 50:50 mixture of the peat moss/soil mixture and sand. A square (≈15 cm x 15 cm) of recycled-pulp egg crate was placed in each colony to act as a shelter for the crickets.

Ten small holes (0.5 cm diameter) were melted using a soldering iron into the lid of each colony to allow for air exchange within the incubators. Medium-sized weigh boats were attached to the middle of each plastic lid by melting four holes through the lid and the four corners of the weigh boat. Two cable ties (20.3 cm) were then placed through the holes to secure the weigh boat to the lid of the colony. These weigh boats were filled with approximately 5.5g of weathered oil in treatment colonies. Weigh boats were left empty in control colonies. Using a dissecting straight needle, 20 small holes were made in the sides of each weigh boat. The oil used in this experiment was the same emulsion used in the ant foraging behavior trials.

Crickets were loaded into the colonies and colonies were placed in their respective incubators. Observations on mortality, presence of pinheads and juveniles, presence of adults, and any other notable changes in behavior were made three times a week through the life of the colony. All colonies were fed *ab libitum* with Fluker's Cricket Quencher and Fluker's Hi-Cal. Cricket Diet. The weigh boats with the soil and sand mixture were kept moist using deionized water.

2.4.3 Statistical Analysis

I used T-tests to compare difference of adult longevity, time between when adult became present to the emergences of pin heads, total generation time, and colony longevity between treatments and controls. All tests were performed using SAS 9.3. Programs are available in Appendix B - Cricket Colony Programs.

CHAPTER 3 – RESULTS

3.1 Sweep versus Vacuum Results

3.1.1 Difference between Techniques and Environment

A total of 1257 insects, arachnids, and collembola where collected using both sweep-net and vacuum sweeping at the eight salt marsh sites in Plaquemines Parish. Sweep net collection accounted for 1075 individuals. Vacuum sweeping collected a total of 182 arthropods. Insects represented 762 of the collected arthropods with 625 collected using sweep nets and 137 collected using the vacuum. These insects included specimens from seven orders, 42 families, and 50 genera. Of the 50 genera, 47 were collected using sweep nets; 16 were collected using vacuum sweeping. Many genera and several families were represented by only a single individual. The most numerous taxa collected using sweep nets included the genera *Ischnodemus*, *Phlaeothrips,* and *Sericothrips*. Vacuum sweeping produced the most individual specimens in the taxa Diamesinae, Chironomini, and *Crematogaster pilosa*.

Of the total 1257 specimens collected, 546 individuals came from non-oiled marshes and 711 came from oiled marshes. Of the total 762 insects collected 498 came from oiled sites, 264 came from non-oiled sites. Individual insects in non-oiled sites were usually *Phlaeothrips, C. pilosa*, or from the subfamily Diamesinae. Most individuals in oiled sites came from the genera *Sericothrips*, *Ischnodemus*, or *Incertella*.

3.1.2 Total Taxa Identified

Individual insects from over 108 different identified species and morphospecies, 68 genera, 60 families, and nine orders were collected in the *Spartina* marshes of coastal Louisiana

using sweep nets, vacuum sweeping, clip plots, and opportunist hand collecting. A complete list of the entomofauna of the marsh is located in Appendix A – Table 7

3.1.3 Statistical Results

Sweep nets averaged statistically more specimens (insects, spiders, and mites) per site than the vacuum sampling method (ANOVA, 12 df, p = 0.0017). Sweep nets collected a mean of 134.4±27.0 (mean ± standard error of means (SEM)) specimens per site. Vacuum sampling averaged 22.8±2.5 specimens per site. These data support hypothesis 1a. The presence of oil on the marsh and the interaction between technique and the presence of oil did not produce significant differences in collected arthropods per site. These data did not lend support to hypothesis 1c. A complete list of all means and p-values can be found in Table 1.

When only the insect catch was considered, technique, presence of oil, and their combined interaction all had significant effects on the total number of insects collected per site (12 df, p = 0.0006, p = 0.0495, p = 0.0123, respectively). Sweep collections averaged 78.0±17.1 insects per site whereas vacuum collections averaged 18.4±2.9 insects per site supporting hypothesis 1a. Oiled sites produced greater insect abundance with an average of 62.3±19.5 insects compared with an average of 34.1±11.0 insects in non-oiled sites. These data did not support hypothesis 1c.

To analyze species richness, the highest level of identification was considered. Morphospecies were not included in analysis in order to reduce possible over estimates due to multiple morphospecies actually being the same species. ANOVA tests indicated that there was a significant effect on diversity as a result of the method and the interaction between the method and the presence of oil (12 df, p = 0.0005, p = 0.0063). Sweep-nets produced 6.3±0.9 different

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taxa per site whereas vacuum samples collected 2.9±0.4 taxa per site. This supports hypothesis 1b. The presence of oil alone, however, had no significant effect on diversity in samples taken in September of 2011. This does not support hypothesis 1d. All means and p-values are located in Table 1.

Varied results occurred for the effects of collection technique, the presence of oil, and the interaction between the two when I examined more specific taxon separately. The collection of the subclass Acari and the order Araneae were only significantly affected by collection technique (12 df, p = 0.0346, p = 0.0104, respectively). Acari averaged 30.3±13.1 individuals per site using sweep nets versus 0.3±0.2 individuals per site using the vacuum. Sweep-net samples collected 8.8±1.9 Araneae per site versus 2.5±0.7 Araneae per site using vacuum techniques. Similarly, Blissidae catches were significantly different for techniques (12 df, p = 0.0318; 16.4±6.7, 1.5±0.5, means of sweep and vacuum, respectively) but not for the presence of oil or the combined interaction. Thysanoptera also had the same pattern with a significant difference between the techniques (12 df, p = 0.0096; 28.5±9.5, 1.4±0.5, means for sweep and vacuum, respectively) but not between oiled and non-oiled reference sites or as a result of interaction between oil presence and collection technique. Means and p-values for each taxon can be found in Table 1.

The collection technique, presence of oil, and the interaction of the two all significantly affected the catches of the order Coleoptera (12 df, p = 0.0002, p = 0.0095, p = 0.0203, respectively) and the family Chloropidae (12 df, p < 0.0001, p = 0.0002, p = 0.0005, respectively). Coleoptera averaged 3.3±0.9 and 0.1±0.1 individuals per site in sweep and vacuum catches, respectively, and 2.6±1.0 and 0.8±0.5 individuals per site in oiled and non-oiled sites, respectively. Chloropids averaged 10.4±2.8 individuals per site using sweep nets and 0.4±0.3

individuals per site using vacuum techniques. Oiled sites averaged 8.9±3.2 Chloropids per site whereas non-oiled sites averaged 1.9±1.2 Chloropids per site. Insects from the family Chironomidae were unaffected by technique, oil, or the interaction between the two. Table 1 contains a list of all means and p-values.

Diptera catches were unaffected by the collection technique. The presence of oil, however, significantly increased the number of individuals collected per site (12 df, p = 0.0207, p = 0.0125, for presence of oil and the interaction effect, respectively). Diptera catches averaged 20.8±5.4 individuals per site in oiled sites and 8.5±2.9 individuals per site in non-oiled sites. Formicid collections were not significantly different due to the method or the interaction effect of technique and oil; however, the presence of oil did significantly reduce the number of individuals collected (12 df, p = 0.0244). Oiled and non-oiled sites had averages of 0.9±0.4 and 8.5±2.9 individuals per site, respectively. All means and p-values for Diptera and Formicidae collection can be found in Table 1.

I placed insects into functional groups including stem-borers, herbivores, and one group containing both omnivores and carnivores. The collection of individuals from the stem-borer group was not significantly affected by technique or the interaction of technique and oil but did show a significant increase in oiled plots (12 df, p = 0.0231) Oiled sites averaged 19.3±4.6 stem-borers per sample whereas non-oiled sites averaged 8.8±2.8 individuals per site. Herbivore collections were significantly affected by technique (12 df, p = 0.0038) but not the presence of oil. A mean of 25.1±7.8 herbivores were caught using nets compared with a mean 1.8±0.6 herbivores per site collected using the vacuum. Carnivore and omnivore catches were significantly higher using sweep-nets compared with vacuums (12 df, p = 0.0447) but were statistically similar in oiled and non-oiled locations. The sweep-net samples averaged 24.8±8.2

insects whereas vacuums only collected 7.8±1.4 insects per site. The means and p-values

associated with each functional group can be found in Table 1.

Table 1 – All means and p-values associated with the collection of insects made in oiled and non-oiled marshes in Louisiana using sweep-net and vacuum techniques are recorded. Means are presented with standard errors and represent the average number of individuals collected for that group per site. P-values were calculated for collection technique, the presence of oil, and the interaction between the two using ANOVA with a Tukey's adjustment.

Groups	Sweep mean/site	Vacuum mean/site	p-value	Oil mean/site	Non-oil mean/site	p-value	Interaction p-value
Total Specimens	134.4±27.0	22.8±2.5	0.0017	88.9±28.2	68.3±28.3	0.4715	0.3922
Total Insects	78.0±17.1	18.4±2.9	0.0006	62.3±19.5	34.1±11.0	0.0495	0.0123
Total Taxa	6.3±0.9	2.9±0.4	0.0005	5.1±1.2	4.0±0.6	0.1432	0.0063
Acari	30.3±13.1	0.3±0.2	0.0346	7.4±4.8	23.1±14.0	0.2349	0.2212
Araneae	8.8±1.9	2.5±0.7	0.0104	6.6±1.9	4.6±1.7	0.3506	0.636
Thysanoptera	28.5±9.5	1.4±0.5	0.0096	21.6±9.3	8.3±6.6	0.1553	0.1779
Coleoptera	3.3±0.9	0.1±0.1	0.0002	2.6±1.0	0.8±0.5	0.0095	0.0203
Diptera	19.1±5.8	10.1±2.8	0.0742	20.8±5.4	8.5±2.9	0.0207	0.0125
Blissidae	16.4±6.7	1.5±0.5	0.0318	13.3±7.3	4.6±1.3	0.1800	0.1242
Formicidae	6.1±3.2	3.3±1.4	0.3512	0.9±0.4	8.5±2.9	0.0244	0.4872
Chironomidae	5.1±2.9	8.6±2.8	0.3901	8.6±2.8	5.1±2.9	0.3901	0.2058
Chloropidae	10.4±2.8	0.4±0.3	< 0.0001	8.9±3.2	1.9±1.2	0.0002	0.0005
Stem-borer	18.0±4.9	10.0±2.9	0.0707	19.3±4.6	8.8±2.8	0.0231	0.0116
Herbivore	25.1±7.8	1.8±0.6	0.0038	19.8±9.3	7.3±2.0	0.0880	0.0514
Carni/Omnivore	54.8±8.2	4.8±1.4	0.0447	12.9±4.6	16.7±8.7	0.6818	0.8910

3.2 *Crematogaster pilosa* Behavior Trial Results

3.2.1 Foraging Behavior and Times

Preliminary experiments indicated that after a 24-hour starvation and desiccation period in control tests with no oil, workers of *Crematogaster pilosa* took an average 73.2±20.4 seconds (n = 5, mean ± SEM) to locate the food and water source or the food and water dummy. Once food was added to the arena, on average ~10% of the ants within a cohort could be found on the food or water source anytime during the first 24 hours when oil was not present. This average dropped to ~5% when oil was present, but not surrounding the food or colony. The average dropped to 1-2% if oil was present between the colony and the food source. In most cases when oil was between workers and their food and water source or a food and water dummy, workers never made contact with the source or dummy even after 24 hours of observation. These percentages are explained below in more detail. When workers came in contact with the oil, their immediate response was to back away and groom any part of their bodies that had touched the oil. In some cases (n = 3) ants that made contact with the oil while foraging were forcibly removed from the culture tube by other workers in the cohort.

The results of ANOVA tests indicated a highly significant effect on the number of ants at a food or water source because of the presence and location of oil (6 df, p < 0.0001) which lends support to hypothesis 2a. Control one (no oil, food/water present) had the most activity at the food source with a mean of 11.23±1.57% (n = 5, mean ± SEM) of the colony in contact with the food source during any observation. All pair-wise tests indicated highly significant difference from all the other trials (p < 0.0001 for all pair-wise tests). Treatment three (oil present but not around food, food/water present) was also highly significantly different from all other trials (p < 0.0001 for all pair-wise tests) with a mean of 6.06±0.88% (n = 5 mean ± SEM). None of the

other trials were significantly different from each other and mean percentage of active ants

ranged from 0.38±0.14% to as low as 0.02±0.02%. A complete list of mean percentage of ants at

a food source during the foraging trials can be found in Table 2.

Table 2 – Mean percentage of ants at the food source at any observation during the 24-hour foraging trial, the percent mortality after the pre-trial 24-hour starvation period, and the percent mortality after the foraging trial for all treatments and controls are recorded. All percentages include a standard error of means. The letters to the right of each percentage represent statistically similar groups within a column for the mean percent at food, the mortality after starvation, and the mortality after the foraging trial.

Trial	Mean Percent at Food		Mortality After Starvation		Mortality After Trial	
Control 1	11.23±1.57%	a	3.50±2.33%	a	7.16±4.18%	a
Control 2	0.38±0.14%	c	0.42±0.42%	a	25.83±8.03%	a
Treatment 1	6.06±0.88%	b	0.0±0.0%	a	1.20±1.20%	a
Treatment 2	0.02±0.02%	c	1.63±0.69%	a	14.43±4.28%	a
Treatment 3	0.20±0.15%	c	0.0±0.0%	a	7.08±1.65%	a
Treatment 4	0.34±0.18%	c	4.92±01.77%	a	24.61±3.66%	a
Treatment 5	0.08±0.08%	c	6.01±2.40%	a	66.45±14.16%	b

3.2.2 Mortality

I recorded ant mortality at two points during each trial, after the initial 24-hour starvation

period and again after the 24-hour trial was completed (48 hours after the initial addition of ants

to the experimental arena. The ANOVA test on the initial recordings indicated a significant

difference in mortality after the initial 24-hour starvation period (6 df, p = 0.0266). This supports

hypothesis 2b. Pair-wise tests of each of the initial recordings, however, revealed that the mean

percent of mortality in each trial was not significantly different from each other. The percent

mortality after the 24-hour foraging trial took into account the initial mortality in each trial. The

ANOVA test results revealed a significant difference in the final mortalities (6 df, p < 0.0001).

Pair-wise tests for final mortality revealed that only treatment five (no food, oil around the cohort

of ants) had significantly increased mortality then the rest of the trials. The initial and final mean

mortality for each treatment and control are recorded as well as the pair-wise tests groups are

recorded in Table 2.

3.3 *Acheta domesticus* Mortality Trial Results

3.3.1 Viability of Data

I performed seven trials testing the effect of weathered oil from the DWH oil spill on the

mortality of the house cricket *Acheta domesticus*. Of these seven trials, four produced results that

I used in the statistical analyses. Trial one was used as a proof-of-concept for the methodology

and handled differently (including long periods in which cohorts were removed from the

incubators, Parafilm® seals were broken, and crickets were removed or relocated) than all the

following trials. Surrogate oil was also used due to the limited availability of the DWH

weathered-oil emulsion. For this reason, trial one was not included in data analysis. Trials three

and seven were also removed from the analyses due to a temperature spike in the treatment

incubator in trial three and another in the control incubator in trial seven. In total, four trials each

with three replicates of each treatment (12 replicates of each treatment in total) were used for

analysis.

3.3.2 Mortality and Behaviors

The time for an entire cohort to reach 100% mortality ranged from 12 hours to 186 hours.

The average times until all crickets were dead were 152±25.63 hours, 94±13.42 hours, and

41±6.56 hours (n = 12 for each, mean ± SEM) for control, vapor only, and contact and vapor-

exposed cohorts, respectively. I observed that crickets exposed to direct contact with oil moved

less often and groomed more often than those only exposed to vapors or no oil. All (n = 5) crickets that molted while exposed to oil, either contact or vapors only, died during ecdysis.

3.3.3 Statistical Results

I performed a logistic regression on the three mortality curves produced by this experiment. The results of the regression indicated a significant difference in the rate of mortality over time between the treatments (2 df, $p < 0.0001$). Contact treatments caused mortality at a significantly quicker rate than both control and vapor treatments ($p < 0.0001$ for both pair-wise comparisons) which supported hypothesis 3a. Differences in the rate of mortality for vapor and controls trials were not significantly different ($p = 0.2216$). This does not support hypothesis 3b. A graph of these mortality curves is found in Figure 1.

The average LT50s and LT100s were greatly affected by treatment (2 df, $p < 0.0001$). This supports both hypotheses 3a and 3b. Fifty percent mortality occurred in contact treatments at 16 ± 2.60 hours, vapor treatments at 59 ± 8.25 hours, and controls at 90 ± 7.20 hours (n = 12 for each treatment, mean ± SEM). The LT50 of contact treatments varied greatly from both control and vapor treatments ($p < 0.0001$ for both pair-wise tests). This decrease in the mean LT50 time lends further support to hypothesis 3a. Control and vapor LT50's were also significantly different from each other ($p = 0.0053$) which gives some support to hypothesis 3b. These data are summarized in Table 3. The time for 100 percent mortality to occur were 152 ± 25.63 hours, 94 ± 13.42 hours, and 41 ± 6.56 hours for control, vapor, and contact treatments (n = 12 for each, mean ± SEM). The LT100 of contact treatment differed significantly from both control and vapor LT100s ($p < 0.0001$ compared with control, $p = 0.0013$ compared with vapor). These data provide further support to hypothesis 3a. Differences in the LT100s of control and vapor

treatments were also significant (p = 0.0005) which provides more support for hypothesis 3b.

The complete set of data for LT100's can be found in Table 4.

Table 3 – The mean LT50 times plus the standard error of the means for each treatment (Control, Vapor, and Contact) as well as the p-values for each of the pair-wise ANOVA analyses. LT50 times are given in hours.

		LT50 p-values		
Trial	LT50 times (hours)	Control	Vapor	Contact
Control	90±7.20		< 0.0001	< 0.0001
Vapor	59±8.25	< 0.0001		0.0053
Contact	16±2.60	< 0.0001	0.0053	

Table 4 – The mean LT100 times plus the standard error of the means for each treatment (Control, Vapor, and Contact) as well as the p-values for each of the pair-wise ANOVA analyses. LT100 times are given in hours.

		LT100 p-values		
Trial	LT100 times (hours)	Control	Vapor	Contact
Control	152±25.63		0.0013	< 0.0001
Vapor	94±13.42	0.0013		0.0005
Contact	41±6.56	< 0.0001	0.0005	

3.4 *Acheta domesticus* Colonies Results

    3.4.1 Colony Collapse and Statistical Results

I established ten control colonies of *Acheta domesticus* on June 12th, 2012. I also set up ten

treatment colonies on July 18th, 2012. An external plug was left out of the control incubator

resulting in the loss of eight colonies due to desiccation. These eight colonies were replaced on

July 18th, 2012. At the time of this writing, six control colonies have breeding populations of

Figure 1 – The mortality curves for *Acheta domesticus* exposed to three different treatments: control, oil vapors, and oil vapors plus direct contact with oil. Mortality was recorded as a percent of the total number of crickets per trial. Each point represents a mean percent mortality at a given time for each treatment. Each treatment included 12 replication

adults. No treatment colony still contains living individuals. This lends support to hypothesis 4a.

I observed extensive mite infestations in all treatment colonies over the course of the experiment.

Six of the treatment colonies failed within a week of my first observations of the mites. All six

colonies were composed of 2nd generation crickets in 2nd to 4th instar nymph phases. The

increased mite presence may provide support for hypothesis 4d.

I performed T-tests on the amount of time between the first observations of adults to the

first observations of juvenile (pinheads) to compare treatment with control colonies. There was a

significant decrease (24 df, $p < 0.0001$) in the time between adults and pinheads arrival in

treatment colonies and control colonies. Treatment colonies on average took 13.4 ± 1.5 days

between the adult and pinhead arrival whereas controls took 23.5 ± 1.2 days. T-tests revealed that

adult longevity had significantly decreased in treatment colonies compared with the control

colonies (10.7 ± 1.8 days and 17.4 ± 1.1 days for treatment and control, respectively; 30 df, $p =$

0.0026). Crickets in treatment colonies also spent more time as juveniles compared with crickets

in control colonies (12 df, $p = 0.0015$). Crickets in treatment colonies took an average of

47.0 ± 2.5 days to mature from pinheads to adults; crickets in control colonies only took an

average of 34.0 ± 1.7 days to mature. These data supports hypothesis 4c. The time it takes for one

generation of pinheads to grow to adulthood and produce a new set of pinheads was not

significantly different between treatments and controls (2 df, $p = 0.4192$). There was also no

significant difference between the time it took one generation of adults to produce pinheads that

grew into a new generation of adults (7 df, $p = 0.4519$). These data fail to support hypothesis 4b.

The total colony longevity does not currently vary between treatments and controls (18 df, $p =$

0.1103) which does not support hypothesis 4a; however, six control colonies were still active at

the time of writing. All of the comparative data related to the effects of weathered oil from the

DWH oil spill on colonies of the *A. domesticus* can be found in Table 5.

Table 5 – Comparisons of the effect exposure to vapors from weathered oil associated with the Deepwater Horizon oil spill on colonies of the house cricket, *Acheta domesticus*. Comparative categories include the time between observations of pinheads (juveniles) within a colony, the time between two generations of adults in a colony, the time between the first observation of pinheads and the first observation of adults, the time from the first observation of adults to the first observation of pinheads, the time between the first observation of adults and complete adult mortality, and the time a colony maintained a living population of crickets. The mean time period is given in days plus the standard error. P-values were calculated using a T-test.

Category	Oiled (days)	Non-oiled (days)	p-values
Pinhead to Pinhead	64.0±5.0	56.5±5.5	0.4192
Adult to Adult	58.0±2.3	55.6±1.9	0.4519
Pinhead to Adult	47.0±2.5	34.0±1.7	0.0015
Adult to Pinhead	13.4±1.5	23.5±1.2	< 0.0001
Adult to Death	10.6±1.8	17.6±1.4	0.0026
Colony Longevity	52.8±8.7	79.2±13.1	0.1103

CHAPTER 4 – DISCUSSION

4.1 Sweep versus Vacuum

The results for total specimen catch and total insect catch lend support to the hypothesis 1a that sweep-net sampling collects a greater number of specimens in *Spartina* salt marshes in south Louisiana. Sweep-nets also sampled a greater diversity of insect taxa than vacuum sweeping, which supports hypothesis 1b. In total, nets accounted for 488 more individual insects than vacuum sweeping and all but five of the collected morphospecies (all five of which were singletons). Further tests on more specific taxa either indicated that sweep-net sampling was more effective than vacuum collections or there was no discernible difference in the two techniques. Vacuum sweeps did not produce a greater catch than sweep-nets in any tested situation or with any collected group of arthropods.

Sweep-net sampling appears to be a more effective and practical means to collect insects in salt marshes. This is supported by the findings of Doxon *et al* (2011). Sweep net yields more diversity and greater number of arthropods. As a result, previous studies of marsh entomofauna which did not include sweep-net samples (Finke and Denno 2004; Gratton and Denno 2005; Wimp *et al* 2010; McCall and Pennings 2012) may have missed a considerable portion of the insects in the habitat. Furthermore, it is more cost effective to use sweep nets compared with vacuums. A typical sweep net costs $36.95 per net whereas a vacuum costs $726.00 for each vacuum (Bioquip® 38 cm professional sweep model 7325NA net versus Agricultural Backpack 2-Cycle Aspirator Model 1612 with a 22.9 cm nozzle from John W. Hock Company). The sweep net only requires the necessary food and water for a human operator. The backpack vacuum in addition to a human operator also requires gas.

The presence of oil did not significantly reduce total specimen and total insect catch. Instead, it was often the oiled sites that produced a greater quantity of individual insects. When all arthropods collected were considered, the oiled and non-oiled sites did not show a significant difference in total catch. However, when only insects were counted, the oiled sites produced a significantly greater numbers of individuals (a total difference of 234 individuals). The oiled and non-oiled reference sites showed no clear difference in the total insect diversity.

Acari, Araneae, and Blissidae catches were statistically similar between oiled and non-oiled sites. The order Thysanoptera and family Chironomidae were also statistically similar in total insects collected between the oiled and non-oiled locations, but exhibited switches of the major taxa. *Phlaeothrips* was the major Thysanopteran genus in non-oiled sites and *Sericothrips* was the most commonly collected genus in oiled sites. The tribe Diamesinae in family Chironomidae was collected more often in non-oiled sites whereas the tribe Chironomini was more prevalent in oiled site collections. The orders Coleoptera and Diptera, as well as the family Chloropidae, all increased in collections from oiled sites compared with non-oiled site collections. In September 2011 samples, ants were the only group to show a significant decline in oiled collections compared with non-oiled collections. Herbivores and carnivore/omnivore groups did not show a difference in the total catch between the oiled and non-oiled sites, but those insects with stem-boring life stages had significantly increased presence in oiled site collections.

The similar catches of suborder Acari, order Araneae, and family Blissidae in oiled and non-oiled sites may indicate that some populations of arthropods in the salt marshes of Louisiana have been unaffected by or have recovered from the DWH oil spill. This data would support the finds of McCall and Pennings (2012).

Most of the insects that I collected with increased frequency in oiled site are herbivores and, more specifically, herbivores with stem-boring life stages. *Sericothrips* and the Chloropid genus *Incertella*, two of the most numerous insects collected in oiled locations, are both genera composed of mostly phytophagous species with *Incertella* having stem-boring larvae (Stannard 1968; McAlpine *et al* 1987). The increase in the collection of these insects in oiled sites may be due to increased populations of these insects in oiled marshes. An increase in herbivores has previously been linked to increased plant stress (Mattson and Haack 1987; Larsson 1989). In the absence of a viable alternative, the presence of oil in the sediment and water column seems the likely source of stress on these plants. Previous studies have indicated that *Spartina alterniflora*'s above-ground and live biomass are not significantly affected by oil or dispersants (DeLuane *et al* 1970; DeLuane *et al* 1984); the changes in insect populations, however, may indicate non-lethal reductions in plant vitality. Identification to species of both the collected thrips and grass flies should further elucidate the specific feeding habits of the increased populations. These data indicate that the marsh is not recovering but that the populations of insects may instead be changing.

An alternative explanation for the increased collection of insect herbivores in the oiled sites is that these sites naturally had higher populations of these insect herbivores prior to the oil spill. The populations of insects at these sites were not sampled for this particular study prior to the oil spill. Another option is reduced predation pressure from predators such as ants. When ant populations are reduced, their prey are released from the pressure of their predation.

Continued observation of these insect populations as well as future analysis on collections of insects made before and after the oil spill as part of the two larger ongoing

investigations (LUMCON and LSU investigations) may provide better insight into the continued recovery or decline of the marsh in association with the DWH oil spill.

Decreases or changes in ant populations have previously been linked to the changes in an environment associated with anthropogenic disturbances (Andersen *et al* 2003). A decrease in the major ant species is often associated with ongoing environmental stress (Graham *et al* 2008). *Crematogaster pilosa* should be considered a prime indicator of marsh recovery in relation to the DWH oil spill as it is numerous, unaffected by the collection technique, it is easily collected, and collections have negatively been affected by oil presence. Furthermore, since *C. pilosa* acts as an omnivore in the marshes relying heavily on insect prey and bird wastes as a food source, decreasing populations may be an early symptom of some form of trophic cascade or bioaccumulation occurring in higher level predators. Continued monitoring of this ant's populations and recovery should occur.

The taxonomic list of the insects in the marsh created as part of this project will be an important aspect to studying changes in insect populations in Louisiana's salt marshes. This preliminary list will also help assist with future sorting and identification projects involving marsh entomofauna. Comparison of this list with previous lists, like those created by John Teal in 1962 and Gina Wimp *et al* in 2010, reveals a lot of overlap. However, neither of the previous lists included the higher identification in Thysanoptera, which may be important for oil-related marsh studies due to the shift of from a largely predacious or fungivorous genus (*Phlaeothrips*) to an herbivorous genus (*Sericothrips*). The family Formicidae is entirely left out of Wimp's taxonomic list. Future projects on the taxonomic identification of marsh insects should include refining this list to species-level identification for all collected specimens and creating a photographic datebase of collected specimens to aid in sorting and identification.

A noticeable disparity in the number of larger insects exists between the collections made in the sweep and vacuum sampling and the complete taxonomic list of marsh insects. The lack of larger insects including Odonates and Orthopterans in the sweep and vacuum trials is probably due to Tropical Storm Lee, which had made landfall in Louisiana on 4 September 2011. The high winds and storm surge associated with this storm reduced populations below a minimum level needed for sampling. Additionally, Odonata, who are visual and swift flyers, are typically collected by chasing them with a sweep-net rather than transects. Observations during the sweep-net and vacuum tests indicate that dragonflies were present at the time of collection though none were collected. Future analysis of other sweep-net data will clarify the importance of the larger insects residing within the coastal *Spartina* marshes.

4.2 *Crematogaster pilosa* Behavior and Mortality

The presence of oil, whether it directly impedes the ants or is merely in the same vicinity, has a clear, negative impact on the foraging behavior of the acrobat ant *Crematogaster pilosa*. Control trials indicated that, at any time during the first 24 hours after the starvation period, an average of ≈10% of a cohort of ants could be found at an available food source. This foraging behavior was reduced by 50% when oil from the DWH oil spill was present in the same arena but not directly interfering with food and water. When the oil was between the ants and their food source, foraging behavior was indiscernible from trials in which no food was present in the harborage. Immediate grooming behavior elicited by contact with oil, avoidance of oiled areas, and removal of oil-contaminated workers by other workers in a colony all further indicate a negative association with the DWH emulsion.

The reduction of foraging capabilities could potentially be the explanation for decreasing *C. pilosa* populations in oiled marshes. Colonies with oil directly between them and their food source would be unable to forage and would be incapable of surviving for prolonged periods of time. Other colonies not directly impeded by the oil but with oil in the environment would still experience reduced foraging capacities. Vulnerable colony stages, such as incipient colonies, may be at an increased risk to starvation due to low worker numbers and small worker size (Hölldobler and Wilson 1990). In order for this ant to act as an optimum biological indicator of marsh recovery, further research into the life history, biology, ecology of this ant with specific focuses on incipient colonies, responses to external stressors, and colony survival during starvation.

Increased mortality associated with the presence of weathered DWH oil only occurred in a single laboratory treatment. When oil was present immediately around the ant cohort and no food or water was present in the arena over 70% mortality occurred compared with the average 15% with a maximum of 31% in all other treatments. Though a specific cause for the increased mortality was not revealed in these experiments, these results indicate a link between close proximity to the oil in combination with a desiccating environment and lack of food and water. Considering the small size of the experimental arenas used in these experiments, the introduction of Fluker's Cricket Quencher and the 20% sugar water solution may have increased the RH of the laboratory test environment enough to alleviate desiccation stress. Future tests should be conducted with RH probes included in the plastic containers to see if a significant increase in RH is occurring in association with the introduction of Cricket Quencher.

Although not statistically testable due to the nature of the collected data, observational records of the behavior of the ants indicates that a larger number of individuals than in previous

trials interacted with the oil when it was closer to the cohort than when it was around the food. Some ants use a random walk behavior when initially encountering a novel environment (Hölldobler and Wilson 1990). If this is the case with *C. pilosa*, then reducing the distance between the oil and the ants' initial location should increase the chances of interaction, especially if the oil is completely surrounding the cohort as was the case in these tests. More information on the basic behaviors of this ant with a primary focus on foraging search patterns needs to be conducted before a final conclusion can be decided upon.

If both oil exposure and low relative humidity must act together to increase mortality in *C. pilosa* in association with the DWH oil emulsions, then direct mortality as a result of exposure to the weathered oil would not seem a likely cause to explain the decreasing populations of acrobat ants in oiled locations. Marshes are constantly exposed to water and have high relative humidity.

4.3 *Acheta domesticus* Mortality Trials

Direct exposure to oil had a drastic effect leading to 100% mortality in all trials within 90 hours and usually within the first 36 hours after exposure. This is unsurprising because oil, oil components, dispersants, and oil emulsions have all been shown to cause acute reactions in a variety of organisms, including insects, often resulting in death (Artema and Stein 1974; Krebs and Burns 1977; Vandermeulen *et al* 1982; Michaelis 1983; DeLaune *et al* 1984; Cushman and Goyert 1984; Harrel 1985; Bombick *et al* 1987; Boehm *et al* 1996; Horvath *et al* 1998; Vitaliano *et al* 2002; Peterson 2003). The increased grooming behaviors were also expected as many insects will meticulously clean their exoskeleton to prevent abrasions or contamination (Hölldobler and Wilson 1990; Gullan and Cranston 2010). Increased mortality occurring during

molting was also not a surprising result due to changes metabolism, exoskeletons, and cuticles in association with ecdysis (Gullan and Cranston 2010). The exact cause of this increased mortality was not tested in these experiments.

Binomial regression indicated that the vapor and control mortality over time curves were not significantly different from one another. The failure of the regression to detect a difference in the curves may be due to very similar rates of mortality within the first 24 hours and the large number of samples taken within that period. However, LT50s and LT100s both indicated strong significant differences with control individuals surviving much longer than those treated with vapors. On average control LT50s occur 30 hours after vapors and control LT100s occurred nearly 60 hours after vapors. These results indicate that some form of toxic volatile is still arising from the weathered oil used in these experiments and that found on the marsh.

The typical sampling of oil after a spill includes tests of the volatile aromatic hydrocarbons, the alkanes, the total petroleum hydrocarbons, and the polycyclic aromatic hydrocarbons because these are all easily sampled via gas chromatography. Of these sampled hydrocarbons, the volatile aromatics are highly toxic but are relatively short lived (Douglas *et al* 1996; Aeppli *et al* 2012). New findings have indicated that unknown phototoxic compounds and oxygenated residues may compose large portions (greater than 50% in some cases) of weathered oil and remain unaccounted for in present tests (Aeppli *et al* 2012; Incardona *et al* 2012; Incardona *et al* 2012). Oxygenated residues, furthermore, have the potential to become more toxic and bioavailable during weathering. In keeping with these recent findings, the results of these cricket bioassay experiments indicate that potentially toxic unknown volatile compounds are being released from the weathered crude oil associated with the DWH oil spill even two

years after the disaster occurred. Further studies need to be conducted investigating gas samples extracted from oil present in the marshes as well as oil using during incubator tests like these.

4.4 *Acheta domesticus* Colonies

Indirect exposure to a toxicant such as oil at low quantities can potentially produce non-lethal detrimental effects (Moriarty 1969; Simpson 1980; Rosenberg *et al* 1986; Haynes 1988; Jepson 1989; Elzen 2001; Desneux *et al* 2007). Cricket colonies treated with only oil vapors showed significant decreases in adult longevity and the time between when adults were first present to when the next generation of juveniles became present. Changes in reproductive strategy have been linked to biotic and abiotic stress in other insects (Kaitala 1991; Hutchinson and Bale 1994). Results from the previous experiment indicate that the presence of DWH weathered oil increases mortality in *A. domesticus*. Therefore, a decreased period of time between the first arrival of adults and subsequent arrival of juveniles may indicate faster selection of mates and oviposition due to decreased adult longevity or perception of environmental stressors. These non-lethal effects provide insight into potential changes in coastal insect behaviors in response to the DWH disaster. Future monitoring of insect populations should take into account potential changes in reproductive behavior in response to sub-lethal dosages of contaminants.

The lack of changes in generation times can be explained by the temperature and nutrient-driven nature of insect development. For many insects, the most critical factors in development are temperature and the availability of food (Gullan and Cranston 2010). Since

temperatures were maintained near those needed for optimal growth and colonies were fed *ab libitum*, similar generation times between treatment and control colonies is not unexpected.

The similar generation time but decreased adult life span in colonies treated with oil would also indicate an increase in the amount of time spent as nymphs. Nymphs taking longer to reach adulthood may be explained by sub-lethal toxic effects of oil exposure in these experiments. If the same effect is occurring in insect populations in the marsh, a change in the total abundance of insects may not be observed but instead an increase in nymphal and larval forms may be present. Future analysis of insects collected in the marsh should pay special attention to the life stages of the insects.

Although no direct behavioral variations between treatments and controls were observed, the mite infestations in every treatment trial lend support to the hypothesis that oil exposure will produce observable changes in behavior. Infestations became so severe in most colonies that adult crickets appeared to be red from so many mites on their bodies. Since all colonies were given the same diet, environmental conditions, and harborage, induced behavioral changes due to exposure to oil may be responsible for the mite populations in treatments. Changes in grooming could provide mites the necessary conditions to produce massive populations.

4.5 Summary

The four parts of my project are best viewed in the context of the two larger, ongoing investigations being conducted by the team of scientists from Louisiana State University and the 26 principle investigators in the LUMCON consortium. The goal of my project was to answer specific questions arising from these two investigations.

First, I compared two different methods for collecting insects, sweep net and vacuum sweeps, to determine the best method for collecting insects in salt marshes in Louisiana. I also used these data to create a list of insects in the marsh. I found that sweep-net sampling is a better method for collecting insects in both oiled and non-oiled *Spartina* marshes in coastal Louisiana. This is important because previous studies of marsh insects that lack sweep-net samples may be missing large portions of the insect population. Of the 108 morphospecies collected in the marsh, many showed increased populations in known oiled areas compared with non-oiled areas >18 months post-spill. Oil-induced stress in plants of the marsh may be providing increased opportunity for herbivory resulting in these increased populations. This data, coupled with a shift in the major taxa of some groups (*Phlaeothrips* to *Sericothrips* and Diamesinae to Chironomini), indicate a change in insect populations rather than a recovery of the pre-spill populations.

Observations from the two ongoing investigations indicated that populations of the acrobat ant *Crematogaster pilosa* were declining in oiled sites in the marsh. I determined what effect weathered oil from the Deepwater Horizon oil spill had on both the ants' foraging behavior and mortality. *Crematogaster pilosa*'s decreasing populations in the field is probably related to the reduced ability to forage in the presence of oil observed under laboratory conditions. There is little indication that oil is directly causing mortality in this ant species in the field. This is important because it determines a potential cause for the decreased populations of this ant in oiled sites. *C. pilosa* has the potential to act as a biological indicator of marsh recovery in post-oil spill events.

The house cricket, *Acheta domesticus*, is currently being used in *in situ* bioassays in the marsh to determine if oil is present in the environment. I determined if direct and indirect exposure to oil increased the rate of mortality in this cricket. I found that the LT50 and LT100

for contact-exposed crickets were significantly lower than controls. This was an expected result as oil is a known toxicant to many insects. I also found that the LT50 and LT100 of vapor-exposed crickets were significantly lower than the control trials. This provides further evidence to support reports of the presence of phototoxic compounds, oxygenated residues, or other unknown toxic components in weathered oil that might affect living things.

Oil exposure has induced changes in *A. domesticus*'s reproductive strategy and behavior including decreased adult lifespan, increased time for maturity, and a decreased time between the presence of adults and the presence of juveniles of the next generation (which is indicative of the adults laying eggs earlier). These changes in conjunction with those seen in *C. pilosa* and the increased populations of herbivores in oiled marshes provide support for changes in previous behaviors of marsh insects and insect populations as a response to the Deepwater Horizon oil spill.

REFERENCES

Adamo, S. A. 1999. Evidence for adaptive changes in egg laying crickets to bacteria and parasites. Animal Behavior. **57**: 117-124.

Aeppli, C., Carmichael, C. A., Nelson, R. K., Lemkau, K. L, Graham, W. M., Redmond, M. C., Valentine, D. L., & Reddy, C. M. 2012. Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. Environmental Science and Technology. dx.doi.org/10.1021/es3015138

Alexander, R. D. 1968. Life cycle origins, speciations, and related phenomena in crickets. Quarterly Review of Biology. **43**:1 1-41.

Andersen, A. N. 1997. Using ants as bioindicators: Multiscale issues in ant community ecology. Conservation Ecology. **1**:1 8

Andersen, A. N., Hoffmann, B. D., & Somes, J. 2003. Ants as indicators of minesite restoration: Community recovery at one of eight rehabilitation sites in central Queensland. Ecological Management and Restoration. **4**: 12-19.

Arnett, R. H., Thomas, M. C., Skelley, P. E., & Frank, J. H. 2002. American Beetles Volume 1 & 2. CRC Press LLC, USA.

Arnett, R. H. 2000. American Insects: A Handbook of the Insects of America North of Mexico. Second Edition. CRC Press LLC, USA.

Arterna, J. & Stein, L. S. 1974. Effects of crude oil on the feeding behavior of the lobster, *Homarus americauus*. Environonmental Pollution. **6**: 77-86.

Baldacci, J., & Tschinkel, W. R. 1999. An experimental study of colony-founding in pine saplings by queens of the arboreal ant, *Crematogaster ashmeadi*. Insectes Sociaux. **46**: 41-44.

Baltz, D. M., Rakocinski, C., & Fleeger, J. W. 1993. Microhabitat use by marsh-edge fishes in a Louisiana estuary. Environmental Biology of Fishes. **36**: 109-126.

Barras, J., Beville, S., Britsch, D., Hartley, S., Hawes, S., Johnston, J., Kemp, P., Kinler, Q., Martucci, A., Porthouse, J., Reed, D., Roy K., Sapkota, S., & Suhayda, J. 2004. Historic and Predicted Coastal Louisiana Land Changes: 1978–2050. USGS Open File Report 03-334.

Bergstrom, J. C., Stoll, J. R., Titre, J. P. & Wright, V. L. 1990. Economic value of wetlands-based recreation. Ecological Economics. **2**: 129-147.

Blajeski, A., Duffy, L. K., & Bowyer, R. T. 1996. Differences in faecal profiles of porphyrins among river otters exposed to the Exxon Valdez oil spill. Biomarkers. **1**:4 262-266.

Boesch, D. F. & Turner, R. E. 1984. Dependence of fishery species on salt marshes: The role of food and refuge. Estuaries. **7**:4 460-468.

Bolton, B. 1995. A New General Catalogue of the Ants of the World. Harvard University Press, USA.

Bombick, D. W., Arlian L. G., & Livingston, J. M. 1987. Toxicity of jet fuels to several terrestrial insects. Archives of Environmental Contamination and Toxicology. **16**: 111-117.

Bortolus, A. 2008. Error cascades in the biological sciences: The unwanted consequences of using bad taxonomy in ecology. A Journal of the Human Environment, **37**:2 114-118.

Britsch, L. D. & Dunbar, J. B. 1993. Land loss rates: Louisiana coastal plain. Journal of Coastal Research. **9**:2 324-338.

Buren, W. F. 1958. A review of the species of *Crematogaster*, *sensu stricto*, in North America (Hymenoptera: Formicidae) part I. Journal of the New York Entomological Society. **66**:3 119-134.

Buren, W. F. 1968. A review of the species of *Crematogaster*, *sensu stricto*, in North America (Hymenoptera, Formicidae) Part II: Descriptions of new species. Journal of the Georgia Entomological Society. **3**: 91–121.

Burger, J. 2006. Bioindicators: A review of their use in the environmental literature 1970-2005. Environmental Bioindicators. **1**:2 136-144.

Burks, R. A. 2003. Key to the Nearctic genera of Eulophidae, subfamilies Entedoninae, Euderinae, and Eulophinae (Hymenoptera Chalcidoidea). Electronic publication. http://www.faculty.ucr.edu/~heraty/Eulophidae/index.html. 4 September 2012.

Cameron, G. N. 1972. Analysis of insect trophic diversity in two salt marsh communities. Ecology. **53**:1 58-73.

Carignan, V. & Villard, M. A. 2002. Selecting indicator species to monitor ecological integrity: A review. Environmental Monitoring and Assessment. **78**: 45-61.

Chambers, J. L., Conner, W. H., Day, J. W., Faulkner, S. P., Gardiner, E. S., Hughes, M. S., Keim, R. F., King, S. L., McLeod, K. W., Miller, C. A., Nyman, J. A., & Shaffer, G. P. 2005. Conservation, Protection and Utilization of Louisiana's Coastal Wetland Forests. Final Report to the Governor of Louisiana from the Coastal Wetland Forest Conservation and Use Science Working Group. 1-102.

Clifford, C. W., Roe, R. M., & Woodring, J. P. 1977. Rearing methods for obtaining house crickets, *Acheta domesticus*, of known age, sex, and instar. Annals of the Entomology Society of America. **70**:1 69-76.

Colombini, I., Chelassi, L., & Fallaci, M. 2002. Community structure of terrestrial invertebrates inhabiting a tidal marsh inlet in the Mediterranean Sea (Gulf of Gabes, Tunisia). The Scientific World. **2**: 861-868.

Craig, N. J., Turner, R. E., & Day Jr., J. W. 1979. Land loss in coastal Louisiana (U.S.A.). Environmental Management. **3**:2 133-144.

Cushman, R. M. & Goyert, J. C. 1984. Effect of a synthetic crude oil on pond benthic insects. Environmental Pollution. **33**: 163-186.

Crunkilton, R. L. & Duchrow, R. M. 1990. Impact of massive oil spill on invertebrate fauna of a Missouri Ozark stream. Environmental Pollution. **63**: 13-31.

Dash, S. T. & Hooper-Bùi, L. M. 2008. Species diversity of ants (Hymenoptera: Formicidae) in Louisiana. Annals of the Entomological Society of America. **101**:6 1056-1066.

Davis Jr., R. A. & Fitzgerald, D. M. 2004. Beaches and coasts. Blackwell Science Ltd., USA.

Dayton, P. K. 2003. The importance of the natural sciences to conservation. The American Naturalist. **162**:1 1-13.

Deluane, R. D., Patrick Jr., W. H., & Buresh, R. J. 1970. Effect of crude oil on a Louisiana *Spartina alterniflora* salt marsh. Environmental Pollution. **20**:1 21-31.

DeLuane, R. D., Smith, C. J., Patrick Jr., W. H., Fleeger, J. W., & Tolley, M. D. 1984. Effect of oil on salt marsh biota: Methods for restoration. Environmental Pollution. **36**: 207-227.

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.

Dmitriev, D. A. 2012. An Interactive Key to Tribes of Leafhoppers (Cicadellidae). Electronic publication. http://ctap.inhs.uiuc.edu/dmitriev/3i_keys.asp. 20 September 2012

Doxon, E. D., Davis, C. A., & Fuhlendorf, S. D. 2011. Comparison of two methods of sampling invertebrates: Vacuum and sweep-net sampling. Journal of Field Ornithology. **82**:1 60-67.

Douglas, G. S., Bence, A. E., Prince, R. C., McMillen, S. J., & Butler, E. L. 1996. Environmental stability of selected petroleum hydrocarbon source and weathering ratios. Envionrmental Science and Technology. **30**:7 2332-2339.

Dubois, A. 2003. The relationship between taxonomy and conservation biology in the century of extinctions. Comptes Rendus Biologies. **326**: 9-21.

Duffy, L. K., Bowyer, R. T., Testa, J. W., & Faro, J. B. 1994. Chronic effects of the Exxon Valdez oil spill on blood and enzyme chemistry of river otters. Environmental Toxicology and Chemistry. **13**:4 643-647.

Elkaim B. & Rybarczyk, H. 2000. Structure of invertebrate community of the salt marshes of the Bay of Somme (eastern English Channel). Cahiers de Biologie Marine. **41**:3 295-311.

Elzen, G. W. 2001. Lethal and sublethal effects of insecticide residues on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Lygaeidae). Journal of Economic Entomology. **94**:1 55-59.

Erwin, R. M, Galli, J., & Burger, J. 1981. Colony site dynamics and habitat use in Atlantic coast seabirds. The Auk. **98**: 550-561.

Farber, S. 1987. The value of coastal wetlands for protection of property against hurricane wind damage. Journal of Environmental Economics and Managements. **14**: 143-151.

Ferro, M. L., Parys, K. A., & Gimmel, M. L. 2010. Dragonflies and Damselflies of Louisiana. Louisiana State Arthropod Museum, USA.

Field, D. W., Reyer, A. J., Genovese, P. V., & Shearer, B. D. 1991. Coastal wetlands of the United States: An accounting of a valuable national resource. U.S. Department of Commerce, National Oceanic and Atmospheric Administration.

Fisher, B. L. & Cover, S. P. 2007. Ants of North America: A Guide to the Genera. University of California Press, USA.

Fitz, H. C. & Wiegert, R. G. 1991. Utilization of the intertidal zone of a salt marsh by the blue crab *Callinectes sapidus*: Density, return frequency, and feeding habits. Marine Ecology Progress Series. **76**: 249-260.

Fleeger, J. W., Carman, K. R., & Nisbet, R. M. 2003. Indirect effects of contaminants in aquatic ecosystems. The Science of the Total Environment. **317**: 207-233.

Gagliano, S. M., Meyer-Arendt, K. J., & Wicker, K. M. 1981. Land Loss in the Mississippi river deltaic plain. Gulf Coast Association of Geological Societies. **31**: 295-230.

Gomez, G. M. 2008. The Louisiana Coast: Guide to an American Wetland. Texas A&M University Press, USA.

Gosselink, J. G., Coleman, J. M., & Stewart Jr., R. E. 1998. Coastal Louisiana. In: Mac, M. J., Opler, P. A., Haecker, C. E., & Doran, P. D. (eds.) Status and trends of the nation's biological resources. Volume 1. U.S. Department of the Interior, U. S. Geological Survey, USA. 385-436.

Graham, J. H., Hughie, H. H., Jones, S., Wrinn, K., Krzysik, A. J., Duda, J. J., Freeman, D. C., Emlen, J. M., Zak, J. C., Kovacic, D. A., Chamberlin-Graham, C., & Balbach, H. E. 2004.

Habitat disturbance and the diversity and abundance of ants (Formicidae) in the Southeastern Fall-line Sandhills. Journal of Insect Science. **4**:30 1-15.

Graham, J. H., Krzysik, A. J., Kovacic, D. A., Duda, J. J., Freeman, D. C., Emlen, J. M., Zak, J. C., Long, W. R., Wallace, M. P., Chamberlin-Graham, C., Nutter, J. P., & Balbach, H. E. 2008. Ant community composition across a gradient of disturbed military landscapes at Fort Benning, Georgia. Southeastern Naturalist. **7**: 429-448.

Graham, J. H., Krzysik, A. J., Kovacic, D. A., Duda, J. J., Freeman, D. C., Emlen, J. M., Zak, J. C., Long, W. R., Wallace, M. P., Chamberlin-Graham, C., Nutter, J. P., & Balbach, H. E. 2009. Species richness, equitability, and abundance of ants in disturbed landscapes. Biological Indicators. **8**: 866-877.

Gratton, C. & Denno, R. F. 2005. Restoration of arthropod assemblages in a *Spartina* salt marsh following removal of the invasive plant *Phragmites australis*. Restoration Ecology. **13**:2 358-372.

Grissell, E. E. & Schauff, M. E. 1990. A Handbook of the Families of Nearctic Chalcidoidea (Hymenoptera). Entomological Society of Washington (Washington, D.C.) Handbook. **1**:1 1-85.

Gullan P. J. & Cranston P. 2010. The Insects: An Outline of Entomology. Fourth Edition. Wiley-Blackwell, USA.

Hall, H. M. & Grinnell, J. 1919. Life-zone indicators in California. Proceedings of the California Academy of Sciences. **9**: 37–67.

Harrel R. C. 1985. Effect of a crude oil spill on water quality and macrobethos of a southeastern Texas stream. Hydrobiologia. **124**: 223-228.

Harvey, K. J., Britton, D. R., & Minchinton, T. E. 2010. Insect diversity and trophic structure differ on native and non-indigenous congeneric rushes in coastal salt marshes. Austral Ecology. **35**: 522-534.

Haynes, K. F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annual Review of Entomology. **33**: 149-168.

Hölldobler, B. & Wilson, E. O. 1990. The Ants. Harvard University Press, USA.

Holt, E. A. & Miller, S. W. 2011. Bioindicators: Using Organisms to Measure Environmental Impacts. Nature Education Knowledge **2**:2 8.

Horváth, G., Bernáth, B., & Molnár, G. 1998. Dragonflies find crude oil visually more attractive than water: Multiple-choice experiments on dragonfly polarotaxis. Naturwissenschaften. **85**: 292-297.

Hunters, I. J. 1958. Importance of taxonomy in biological investigations. Nature. **182**:4652 1818-1819.

Hutchinson, L. A. & Bale J. S. 1994. Effects of sublethal cold stress on the aphid *Rhopalosiphum padi*. Journal of Applied Ecology. **31**:1 102-108.

Incardona, J. P., Vines, C. A., Linbo, T. L., Myers, M. S., Sloan, C. A., Anulacion, B. F., Boyd, D., Collier, T. K., Morgan, S., Cherr, G. N., & Scholz, N. L. 2012. Potent phototoxicity of marine bunker oil to translucent herring embryos after prolonged weathering. PLOS One. **7**:2 e30116.

Incardona, J. P., Vines, C. A., Anulacion, B. F., Baldwin, D. H., Day, H. L., French, B. L., Labenia, J. S., Linbo, T. L., Myers, M. S., Olson, O. P., Sloan, C. A., Sol, S., Griffen, F. J., Menard, K., Morgan, S. G., West, J. E., Collier, T. K., Ylitalo, G. M., Cherr, G. N., & Scholz, N. L. 2012. Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 Cosco Busan oil spill in San Francisco Bay. Proceedings of the National Academy of Science. **109**:2 E51-E58.

Jensen, L. K. & Carroll, J. 2010. Experimental studies of reproduction and feeding for two Artic-dwelling *Calanus* species exposed to crude oil. Aquatic Biology. **10**: 261-271

Jepson, P. C. 1989. Pesticides and Non-Target Invertebrates. Intercept, USA.

Johansen, K. J. 2010. Creating an interactive and dichotomous key to the world subfamilies of Braconidae. University of Kentucky Master's Theses. Paper 79.

Kaitala, A. 1991. Phenotypic plasticity in reproductive behavior of waterstriders: Trade-offs between reproduction and longevity during food stress. Functional Ecology. **5**:1 12-18.

King, S. E. & Lester, J. N. 1995. The value of salt marsh as a sea defence. Marine Pollution Bulletin. **30**:3 180-189.

Kociolek, J. P. & Stoermer, E. F. 2001. Taxonomy and ecology: A marriage of necessity. Diatom Research. **16**:2 433-442

Kociolek, J. P. 2005. Taxonomy and ecology: Further considerations. Proceedings of the California Academy of Sciences. **56**:10 99-106.

Krebs, C. T. & Burns, K. A. 1977. Long-term effects of an oil spill on populations of the salt-marsh crab *Uca pugnax*. Science. **197**:4302 484-487.

Landres, P. B., Verner, J., & Thomas, J. W. 1988. Ecological uses of vertebrate indicator species: A critique. Conservation Biology. **2**: 316–328.

Landres, P. B. 1992 Ecological indicators: Panacea or liability?. In: McKenzie, D. H., Hyatt, D. E., & McDonald V. J. (eds.) Ecological Indicators: Volumes 1 and 2. Elsevier Science Publishers, USA. 1295-1318.

Larsson, S. 1989. Stressful times for the plant stress: Insect performance hypothesis. Oikos. **56**:2 277-283.

Lin, Q. & Mendelssohn, I. A. 1996. A comparative investigation of the effects of south Louisiana crude oil on the vegetation of fresh, brackish, and salt marshes. Marine Pollution Bulletin. **32**:2 202-209.

Longino, J. T. 2003. The *Crematogaster* (Hymenoptera, Formicidae, Myrmicinae) of Costa Rica. Zootaxa. **151**: 1-150.

Mace, G. M. 2004. The role of taxonomy in species conservation. Philosophical Transactions of the Royal British Society. **359**: 711-719.

Mattson, W. J. & Haack, R. A. 1987. The role of drought in outbreaks of plant-eating insects. Bioscience. **37**:2 110-118.

McAlpine, J. F., Petersen, B. V., Shewell, G. E., Teskey, H. J., Vockeroth, J. R., & Wood, D. M. 1987. Manual of Nearctic Diptera. Volumes 1 & 2. Research Branch Agriculture Canada.

McCall, B. D. & Pennings, S. C. 2012. Disturbance and recovery of salt marsh arthropod communities following BP Deepwater Horizon oil spill. Plos One. **7**:3 e32735.

Merrit, R. W., Cummins, K. W., & Berg, M. B. 2008. An Introduction to the Aquatic Insects of North America. Fourth Edition. Kendall Hunt Publishing Company, USA.

Michaelis, F. B. 1983. Effect of Turoa oil spill on aquatic insects in Mangawhero river system. New Zealand Entomologist. **7**:4 447-455

Minello, T. J., Able, K. W., Weinstein, M. P., & Hays, C. G. 2003. Salt marshes as nurseries for nekton: Testing hypotheses on density, growth and survival through meta-analysis. Marine Ecology Progress Series. **246**: 39-59

Mitsch, W. J. & Gosselink, J. G. 2007. Wetland. John Wiley & Sons, USA.

Moles, A., Rice, S. D., & Korn, S. 1979. Sensitivity of Alaskan freshwater and anadromous fishes to Prudhoe Bay crude oil and benzene. Transactions of the American Fisheries Society. **108**:4 408-414.

Möller, I., Spencer, T., French, J., Leggett, D., & Dixon, M. 2001. The sea-defence value of salt marshes: Field evidence from north Norfolk. Journal of the Chartered Institution of Water and Environmental Management. **1**: 109-116.

Morgan, C. E. 2009. Revision of the Ant Genus *Crematogaster* (Hymenoptera: Formicidae) in North America. ProQuest LLC, USA.

Moriarty, F. 1969. The sublethal effects of synthetic insecticides on insects. Biological Reviews. **44**:3 321-356.

Needham, J. G., Westfall Jr., M. J., & May, M. L. 2000. Dragonflies of North America. Scientific Publishers Inc, USA.

Niemi. G. J. & McDonald, M. E. 2004. Applications of ecological indicators. Annual Review of Ecology , Evolution, and Systematics. **35**: 89-111.

Noss, R. F. 1999. Assessing and monitoring forest biodiversity: A suggested framework and Indicators. Forest Ecology and Management. **115**: 135–146.

Noyes, J. S. & Pitkin, B. 2004. Universal Chalcidoidea Database: A key to the families of chalcidoidea and mymaromatoidea. Electronic publication. http://www.nhm.ac.uk/chalcidoids. 20 September 2012

Odum, E. P. & de la Cruz, A. A. 1967. Particulate organic detritus in a Georgia salt marsh-estuarine ecosystem. In: Lauff, G. H. (ed.) Estuaries: Nutrients and Biological Productions. AAAS Publication 83. 383-388.

Odum, W. E. & Heald, E. J. 1972. Trophic analyses of an estuarine mangrove community. Bulletin of Marine Science. **22**:3 671-738

Oil Spill Commission. 2011. Deep Water: The Gulf Oil Disaster and the Future of Offshore Drilling. Recommendations of the National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, Washington, D.C. Available online at: http://www.oilspillcommission.gov

Ort, M. P., Finger, S. E., & Jones, J. R. 1995. Toxicity of crude oil to the mayfly, *Hexagenia bilineata* (Ephemeroptera: Ephemeridae). Environmental Pollution. **90**:1 105-110.

Pape, T., Dahlem, G., de Mello Patiu, C. A., & Giroux, M. 2012. The World of Flesh Flies (Diptera: Sarcophagidae). Electronic publication. http://www.zmuc.dk/entoweb/sarcoweb/sarcweb/sarc_web.htm. 2 September 2012.

Peterson, C. H., Rice, S. D., Short, J. W., Esler, D., Bodkin, J. L., Ballachey, B. E., & Irons, D. B. 2003. Long-term ecosystem response to Exxon Valdez oil spill. Science. **302**: 2082-2086.

Peterson, G. W. & Turner, R. E. 1994. The value of salt marsh edge vs interior as a habitat for fish and decapods crustaceans in a Louisiana tidal marsh. Estuaries. **17**:18 235-262.

Place, B., Anderson, B., Mekebri, A., Furlong, E., Gray, J. L., Tjeerdema, R., Field, J. 2010. A role for analytical chemistry in advancing our understanding of the occurrence, fate, and effects of Corexit oil dispersants. Environmental Science & Technology. **44**: 6016–6018

Pollet, M. A. A., Brooks, S. E., & Cumming, J. M. 2004. Catalog of the Dolichopodidae (diptera) of America North of Mexico. Bulletin of the American Museum of Natural History. **283**: 1-114.

Powell, G. V. N. & Powell, A. H. 1986. Reproduction by great white herons *Ardea herodias* in Florida bay as an indicator of habitat quality. Biological Conservation. **36**: 101–113.

Price, P. W. 1997. Insect Ecology. Third Edition. Wiley, USA.

Rabalais, N. N., in Moretzsohn, F., Sánchez Chávez, J. A., & Tunnell Jr., J. W. 2012. GulfBase: Resource Database for Gulf of Mexico Research. Electronic publication. http://www.gulfbase.org. 14 September 2012.

Rainio, J. & Niemela, J. 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation. **12**: 487-506.

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

Rosenthal, H. & Alderdice, D. F. 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. Journal of the Fisheries Research Board of Canada. **33**:9 2047-2065.

Rossi, S. S. & Anderson, J. W. 1976. Toxicity of water-soluble fractions of No. 2 fuel oil and south Louisiana crude oil to selected stages in the life history of the polychaete, *Neanthes arenaceodentata*. Bullentin of Environmental Contamination and Toxicology. **16**:1 18-24.

Sarkka, J. 1996. Meiofauna of the profundal zone of the northern part of Lake Ladoga as an indicator of pollution. Hydrobiologia. **322**: 29-38.

Sasser, C. E., Gosselink, J. G., Swenson, E. M., Swarzenski, C. M., & Leibowitz, N. C. 1996. Vegetation, substrate and hydrology in floating marshes in Mississippi river delta plain wetlands, USA. Plant Ecology **122**:2 129-142.

Simpson, K. W. 1980. Abnormalities in the tracheal gills of aquatic insects collected from streams receiving chlorinated or crude oil wastes. Freshwater Biology. **10**:6 581-583.

Singer, M. M., George, S., Lee, I., Jacobson, S., Weetman, L. L., Blondina, G., Tjeerde, R. S., Aurand, D., & Sowby, M. L. 1998. Effects of dispersant treatment on acute aquatic toxicity of petroleum hydrocarbons. Archives of Environmental Contamination and Toxicology. **34**: 177-187.

Sprague, J. B. 1971. Measurement of pollutant toxicity to fish-III: Sublethal effects and "safe" concentrations. Water Research. **5**:6 245-266.

Stannard, L. J. 1968. The Thrips, or Thysanoptera, of Illinois. Illinois Natural History Survey. **29**:4 215-552.

Stein, J. E., Collier, T. K., Reichert, W. L., Casillas, E., Hom, T., & Varanasi, U. 1992. Bioindicators of contaminant exposure and sublethal effects: Studies with benthic fish in Puget Sound, Washington. Environmental Toxicology and Chemistry. **11**:5 701-714.

Swedmark, M., Granmo, Å., & Kollberg, S. 1973. Effects of oil dispersants and oil emulsions on marine animals. Water Research. **7**:11 1649-1672.

Teal, J. M. 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology. **43**:4 614-624

Triplehorn, C. A. & Johnson, N. F. 2005. Borror and Delong's Introduction to the Study of Insects. Seventh Edition. Brooks/Cole, USA.

Tschinkel W. R. 2002. The natural history of the arboreal ant, *Crematogaster ashmeadi*. Journal of Insect Science. **2**:12 Available online: www.insectscience.org/2.12

Turner, R. E. 1992. Coastal Wetlands and Penaeid Shrimp Habitat. In: Stroud, R. (ed.) Stemming the Tide of Coastal Fish Habitat Loss. Proceedings of the 14th Annual Marine Recreational Fisheries Symposium. 97-104

Vandermeulen, J. H., Platt, H. M., Baker, J. M., & Southward, A. J. 1982. Some conclusions regarding long-term biological effects of some major oil spills. Philosophical Transactions of the Royal Society of London: Biological Sciences. **297**:1087 335-351.

Walker, H. J., Coleman, J. M., Roberts, H. H. & Tye, R. S. 1987. Wetland loss in Louisiana. Geografiska Annaler Series A-Physical Geography. **69**:1 189-200.

Walker, T. J. & Moore, T. E. 2012. Singing insects of North America. Electronic publication. http://entnemdept.ifas.ufl.edu/walker/Buzz/. 21 September 2012.

Wheeler, W. M. 1910. Ants: Their structure, development, and behavior. Columbia University Press, USA.

Wheelock, C. E., Wolfe, M. F., Olsen, H., Tjeerdema, R. S., & Sowby, M. L. 1999. Hsp60-induced tolerance in the rotifer *Brachionus plicatilis* exposed to multiple environmental contaminants. Archives of Environmental Contamination and Toxicology. **36**:3 281-287.

Whitehead, A., Dubansky, B., Bodinier, C., Garcia, T. I., Miles, S., Pilley, C., Raghunathan, V., Roach, J. L., Walker, N., Walter, R. B., Rice, C. D., & Galvez, F. 2011. Genomic and physiological footprint of the Deepwater Horizon oil spill on resident marsh fishes. Proceedings of the National Academy of Sciences. doi: 10.1073/pnas.1109545108.

Whitfield, A. K. & Elliott, M. 2002. Fishes as indicators of environmental and ecological changes within estuaries: A review of progress and some suggestions for the future. Journal of Fish Biology. **61**: 229-250.

Williams, R. S., Ellis, M. S., & Fickle D. S. 1995. Insects in the Killbuck Marsh Wildlife Area: 1993 survey. Ohio journal of Science. **95**:3 226-232.

Williston, S. W. 1908. Manual of North American Diptera. New Haven, USA.

Wilson, S. W. 2005. Keys to the families of Fulgoromorpha with emphasis on planthoppers of potential economic importance in the southeastern United States (Hemiptera: Auchenorrhyncha). Florida Entomologist. **88**:4 464-481.

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**:11 3303-3311.

Woodwell, G. M., Wurster Jr., C. F., & Issacson, P. A. 1967. DDT residues in an east coast estuary: A case of biological concentration of a persistent insecticide. Science. **15**: 821-824

Wu, Y. T., Wang, C. H. , Zhang, X. D., Zhao, B., Jiang, L. F., Chen, J. K., & Li, B. 2009. Effects of saltmarsh invasion by *Spartina alterniflora* on arthropod community structure and diets. Biological Invasions. **11**: 635-649.

Young, R. F. & Phillips, H. D. 2002. Primary production required to support bottlenose dolphins in a salt marsh estuarine creek system. Marine Mammal Science. **18**:2 358-375.

APPENDIX A – TABLES AND LISTS

Table 6 – Site locations and environmental information used to compare sweep net and vacuum insect collection techniques.

Site #	Date	Time	Location	Water Temp (C)	Air Temp (C)	Wind (km/h)	Oil
1	9/9/2011	8:23	N 29'27.863 W 89'46.973	24.4	21.7	4.8	no
2	9/9/2011	9:40	N 29'27.225 W 89'46.231	24.6	23.8	9.3	no
3	9/9/2011	10:56	N 29'27.361 W 89'48.087	25.0	25.8	11.9	no
4	9/9/2011	12:18	N 29'82.126 W 89'49.542	25.2	27.1	9.1	yes
5	9/9/2011	13:06	N 29'28.413 W 89'51.534	27.1	28.3	12.8	yes
6	9/10/2011	8:00	N 29'28.214 W 89'43.428	24.9	23.8	4.1	no
7	9/10/2011	9:50	N 29'26.251 W 86'25.568	25.5	25.3	10	yes
8	9/10/2011	12:01	N 29'25.355 W 89'50.010	26.7	34.4	3.3	yes

Table 7 – Taxonomic list of marsh entomofauna. Order names are in bold and underlined. Family names are underlined. Individuals with identification restricting damage are marked with a "*". Taxonomic ordering is based on Triplehorn and Johnson 2005, with minor exceptions for new families.

Odonata

Libellulidae
Erythrodiplax berenice Drury
Pachydiplax longipennis Burmeister

Coenagrionidae
Enallagma sp.
Ischnura ramburii Selys
Ischnura sp.

Orthoptera

Acrididae
Leptysma marginicollis Serville
Orphulella pelidna Burmeister

Tettigonidae
Conocephalus sp.
Orchelimum sp.

Gryllidae
Oecanthus sp.

Hemiptera

Saldidae
Calacanthia sp.

Reduviidae
Doldina sp.

Miridae
Orthotylus sp.
Trigonotylus sp.

Tingidae
Corythuca sp.

Pentatomidae
Chlorochroa sp.
Oebalus pugnax Fabricius

Podisus sp.

Cymidae
Cymus sp.

Blissidae
Ischnodemus sp.

Cercopidae
Clastoptera sp.

Membracidae
Ceresa festina Say

Cicadellidae
Draeculacephala sp.
Neohecalus sp.
Xestocephalus sp.
Tribe Chiasmini
Tribe Erythroneurini*

Delphacidae
Megamalus sp.
Prokelisia spp.

Fulgoridae
Phylloscelis sp.
Rhynchomitra sp.

Thysanoptera

Phlaeothripidae
Phlaeothrips spp.
Williamsiella sp.

Thripidae
Limothrips sp.
Sericothrips spp.

Table 7 Continued

Coleoptera

Staphylinidae
Stenus sp.

Scarabaeidae
Trigonopeltastes delta Förster

Buprestidae
Taphrocerus sp.

Cantharidae
Chauliognathus sp.

Cleridae
Isohydnocera sp.

Melyridae
Collops nigriceps Say
Temnopsophus bimaculatus Horn

Phalacridea
Stilbus sp.

Coccinellidae
Coleomegilla maculate De Geer
Cycloneda sanguinea Linnaeus

Mordellidae
Glipostenoda spp.

Anthicidae*

Chrysomelidae
Acalymma sp.
Aphthona sp.
Diabrotica sp.
Systena sp.

Curculionidae
Sphenophorus sp.

Neuroptera

Mantispidae
Climaciella brunnea Say

Hymenoptera

Braconidae
Vipio sp.

Mymaridae

Trichogrammatidae

Eulophidae

Encyrtidae

Eupelmidae

Torymidae

Ormyridae

Pteromalidae

Eurytomidae
Tenuipetiolus sp.

Bethylidae
Subfamily Epyrinae

Dryinidae
Subfamily Gonatopodinae

Vespidae
Polistes sp.

Megachilidae
Megachile sp.

Formicidae
Camponotus impressus Roger
Cardiocondyla minutior Forel
Crematogaster pilosa Emery
Monomorium minimum Buckley
Pseudomyrmex pallidus F. Smith

Lepidoptera
Pyralidae*

Table 7 Continued

68

<u>Diptera</u>

<u>Chironomidae</u>
Thienemanniella sp.
Subfamily Diamesinae
Tribe Chironomini

<u>Culicidae</u>*

<u>Tabanidae</u>
Tabanus sp.

<u>Dolichopodidae</u>
Campsicnemus sp.
Tachytrechus sp.

<u>Phoridae</u>
Xanionotum sp.

<u>Muscidae</u>
Caricea sp.

<u>Sarcophagidae</u>
Senotainia sp.

<u>Ulidiidae</u>
Chaetopsis sp.

<u>Lauxaniidae</u>*

<u>Chloropidae</u>
Chlorops spp.
Incertella spp.

<u>Carnidae</u>
Hemeromyia sp.

<u>Ephydridae</u>
Notiphila sp.

<u>Unknown Nematocera</u>*
1sp. - site S5

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APPENDIX B – STATISTICAL PROGRAMS

Sweep vs. Vacuum programs

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78
ps=53;
title1 'Sweepnet vs Vacuum';
ods rtf file = 'c:/temp/sweepvsvac.rtf';

data collection;
title2 'All specimens';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method x type;
  LSMeans method type method x type
/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;

data collection;
title2 'Identified Insects only';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;

data collection;
title2 'Ischnodemus only';
input method $ type $ site insect;
Datalines;
```

```
Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;

data collection;
title2 'Thysanoptera';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;

data collection;
title2 'Coleoptera';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;

data collection;
```

```
title2 'Formicidae';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;

data collection;
title2 'Chironomidae';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;

data collection;
title2 'Chloropidae';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;
```

```
data collection;
title2 'Diptera';
input method $ type $ site insect;
Datalines;

Proc glimmix data= collection;
  class method type;
  model insect = method type method*type;
  LSMeans method type
method*type/adjust=tukey pdiff ilink cl;
  output out=resids resid=r;
run;

proc univariate data=resids plot normal; var
r; run;


ods rtf close;
```

Ant Behavior Programs

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78
ps=53;
title1 'Mortality of Ants';
ods rtf file = 'c:/temp/ants.rtf';

data totalmort;
input trial percentmort;
datalines;
;

title2 'Total Ant Mortality';
proc glimmix data=totalmort;
class trial;
model percentmort=trial;
LSMeans trial /adjust=tukey;
run;

data starvemort;
input trial percentmort;
datalines;
;

title2 'Post Starvation Mortality';
proc glimmix data=starvemort;
class trial;
model percentmort=trial;
LSMeans trial /adjust=tukey;
run;

data trialmort;
input trial percentmort;
datalines;
;

title2 'Post Trial Mortality';
proc glimmix data=trialmort;
class trial;
model percentmort=trial;
LSMeans trial /adjust=tukey;
run;

PROC IMPORT OUT=set1
        DATAFILE="C:\Data sheet.xls"
        DBMS=EXCEL2000 REPLACE;
        SHEET='Sheet1';
        GETNAMES=YES;
        MIXED=NO;
        SCANTEXT=YES;
        USEDATE=YES;
        SCANTIME=YES;
run;

proc print data=set1;
run;

Proc glimmix data=set1;
class Trial;
model Active/Total = Trial | Time
/ddfm=KR;
Random Rep;
LSMeans Trial /adjust=tukey pdiff ilink cl;
output out=resids resid=r;
run;


proc univariate data=resids plot normal; var
r; run;

ods rtf close;
```

Cricket Mortality Programs

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78
ps=53;
ods rtf file = 'C:\cricket.rtf';

PROC IMPORT OUT=set1
       DATAFILE="C:\Data sheet.xls"
       DBMS=EXCEL2000 REPLACE;
       SHEET='Sheet1';
       GETNAMES=YES;
       MIXED=NO;
       SCANTEXT=YES;
       USEDATE=YES;
       SCANTIME=YES;
run;

proc print data=set1;
run;

Proc glimmix data=set1;
class Treatment trial;
model mortality/Total = treatment | Hours
/ddfm=KR dist=binomial;
Random Trial rep(treatment*trial);
LSMeans treatment /adjust=tukey pdiff ilink
cl;
output out=resids resid=r;
run;


proc univariate data=resids plot normal; var
r; run;

data criklt50;
input treatment $ lt50 lt100;
datalines;
;

proc glimmix data=criklt50;
title2 "lt 50";
class treatment;
model lt50=treatment;
LSMeans treatment /adjust=tukey;
run;
```

```
proc glimmix data=criklt50;
title2 "lt 100";
class treatment;
model lt100=treatment;
LSMeans treatment /adjust=tukey;
run;

ods rtf close;
```

Cricket Colony Programs

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78
ps=53;
title1 'Cricket Colonies Oil vs Control';
ods rtf file = 'c:/temp/crikcolony.rtf';

data atop;
title2 'Adult to pin times';
input method $ number time;
Datalines;
;

Proc TTest;
Class method;
Var time;
run;

data atod;
title2 'Adult to death times';
input method $ site time;
Datalines;
;

Proc TTest;
Class method;
Var time;
run;

data ptop;
title2 'Pin to pin times';
input method $ site time;

Datalines;
;

Proc TTest;
Class method;
Var time;
run;

data ptop;
title2 'Total colony longevity';
input method $ site time;
Datalines;

Proc TTest;
Class method;
Var time;
run;

data ptop;
title2 'Adult to Adult';
input method $ site time;
Datalines;
;

Proc TTest;
Class method;
Var time;
run;

ods rtf close;
```

VITA

Benjamin Adams was born in Lafayette, Louisiana, USA. Since early childhood he has had an all-encompassing fascination with insects, reptiles, and amphibians. He grew up on the campus of the University of Louisiana at Lafayette where he spent most of his time catching green anoles, yellow-bellied water snakes, and red-eared sliders from the campus swamp or opening red imported fire ant mounds to collect workers for his ant farms. In 2006, he graduated with Honors from Lafayette High School's Gifted and Talented Program.

He was accepted to Louisiana State University's Honors College in 2006 where he pursued a degree in Biology. In 2009, he put his childhood experience with ants to use and began working with Dr. Linda Hooper-Búi on a variety of projects including describing the rafting formation of the red imported fire ant, excavating and mapping Texas leaf-cutting ant nests, examining the kitchen middens of pyramid ants, and looking at the interactions between ant populations, behaviors, and characteristics in response to natural and anthropogenic disturbances. In August of 2010 he received his Bachelors of Science in Biology from Louisiana State University.

He was accepted in 2010 to Louisiana State University's Master of Science program in the Department of Entomology where he worked under Dr. Hooper-Búi observing changes in insect populations, behaviors, and longevity in relation to the Deepwater Horizon oil spill. He graduated in December of 2012. The title of his thesis was "Oil-Mediated Mortality and Induced Behavioral Modifications of Coastal Insects".