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The Collection of Baseline Data on Insect and Plant Communities Across Multiple Salinity Zones Within Louisiana's Tidal Marshes

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**THE COLLECTION OF BASELINE DATA ON INSECT AND PLANT
COMMUNITIES ACROSS MULTIPLE SALINITY ZONES WITHIN
LOUISIANA'S TIDAL MARSHES**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by
Benjamin G. Aker
BS., University of Wisconsin Whitewater, 2017
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Abstract

Tidally influenced coastal marshes provide numerous important ecosystem services, but these habitats are experiencing extreme habitat loss. Louisiana's coastal marshes in particular are vulnerable to land loss resulting from both anthropogenic and natural causes, but especially to subsidence and relative sea-level rise. In response, the Louisiana Coastal Restoration and Protection Authority has outlined and is currently implementing the largest ever attempt at habitat restoration. A major component of this habitat restoration attempt is the use of freshwater and sediment diversions to increase the input of low salinity water, sediment, and nutrients needed to slow marsh loss and build land. Coastal marsh plant communities are primarily stratified by salinity and their distributions and salt tolerances are relatively well understood. However, the insect communities that are associated with these plant communities are less well known. Few inventories of Louisiana's coastal insects have been made, and those that exist did not adequately sample the breadth of the tidally influenced marsh vegetation types. The goals of this study were to create a year-long, family-level inventory of the insects present in the *Spartina* dominated intermediate, brackish, and saline marshes of coastal Louisiana and to determine indicator plant species for each marsh vegetation type for potential environmental monitoring efforts. The objective was to provide baseline data that can be used to both quickly assess marsh type to determine habitat risk to threats such as oil spills or hurricanes and to enable the long-term monitoring of the health of these marshes. Insects and plant ground cover data were collected from July 2018 through June 2019 from 18 sites across Barataria Bay and Caillou Bay in coastal Louisiana. A total of 71 insect families were collected with 61 from intermediate marsh, 64 from brackish marsh, and 39 from

saline marsh sites. Family-insect diversity was negatively correlated with salinity for multiple diversity indices. Salinity, seasonality, total ground cover, and ground cover of four plant species were found to be important factors in determining the distribution of insect families within Louisiana's coastal marshes. Additionally, the abundances of insects belonging to different feeding guilds were found to vary between marsh vegetation types. Families likely to contain useful indicator species were identified to the species level when possible and an Indicator Value Analysis was performed on the species abundance data to determine potential bioindicators. A total of 26 species (from 17 families) were found to be significant indicators for a marsh vegetation type or a combination of marsh habitats. Of these bioindicator species, 11 were indicators of intermediate marsh, 2 were indicators for the combination of intermediate and brackish marsh, 4 were indicators for brackish marsh, 4 were indicators for the combination of brackish and saline marsh, and 5 were indicators for saline marsh.

Introduction

Tidal marshes are unique coastal habitats that predominately develop in areas shielded from heavy wave activity such as in bays and estuaries (Chapman 1977). These marshes are nearly worldwide in distribution excepting Antarctica and near the equator where they tend to be replaced by mangrove swamps. These habitats are interesting because they form a true ecotone between terrestrial and marine environments, and they are heavily stratified by the salinity gradient resulting from frequent inundation by saltwater (Chapman 1977, CPRA 2017). Tidal marshes can generally be broken down into two categories based on the effects of tides. Salt marshes exist where the tides reach their highest point while brackish marshes expand inland to the extent of tidal influence. Within North America, nearly two-thirds of the tidal marsh habitat is contained within the Gulf of Mexico, including 58% of the salt marshes.

This habitat provides numerous ecosystem services such as protecting and promoting the growth of immature nekton such as fish, mollusks, shrimp, and other crustaceans, including many economically important species (Barbier et al. n.d.). It is highly productive habitat that acts a carbon sink, and, along with mangrove swamps, sequester nearly 50,000,000 tons of carbon annually (Schuerch et al. 2018). They additionally protect from erosion and storm surges which are more likely to negatively affect low-income communities.

However, these habitats are being lost at an alarming rate, especially within the Mississippi delta. Over the last 50 years approximately 50% of all salt marsh within North America was lost. To stop land loss and to promote marsh recover the Louisiana Coastal Protection and Restoration Authority has implemented 79 projects to restore tidal habitat in

coastal Louisiana (CPRA 2017). In order to understand the impacts of the contradicting land loss and habitat restoration efforts, detailed knowledge of the taxa present is necessary.

1.1 Tidal marshes

Tidal marshes are unique, coastal ecosystems with a nearly worldwide distribution, except for Antarctica, and are predominantly found in areas protected from heavy wave activity such as bays and estuaries (Chapman 1977). These marshes represent a true ecotone between terrestrial and marine environments, and are highly stratified by the salinity gradient resulting from the frequent inundation by saltwater (Odum et al. 1984, Holland et al. 1990, Greenberg 2006). Tidal marshes vary in salinity from zero parts per thousand (ppt) to in excess of 35 ppt (Greenberg and Maldonado 2006). Tidal marsh habitat with low-growing vegetation can be divided into two general types: salt marshes which exist where the tides reach their maximum height, and brackish marshes which expand inland up to the extent of tidal influence (Chapman 1977). Within North America, the Gulf of Mexico accounts for nearly two-thirds of the approximately 15,000 kilometers of tidal marshes present (including up to 58% of the nation's salt marshes), in addition to having a relatively high proportion of brackish marsh (> 40%). For Atlantic and Pacific coast tidal marshes, brackish marshes make up less than 10% of the total area (Field et al. 1991). In brackish and salt marshes, plant diversity is negatively correlated with salinity, and low-elevation habitat which experiences heavy inundation approach monocultures of either *Spartina* grasses or succulents such as *Salicornia* species depending on geographic location (Chapman 1977, Greenberg and Maldonado 2006). Similar plant communities are found within the Gulf of Mexico and Atlantic coast brackish and salt marshes; low-elevation marsh is typically dominated by *Spartina alterniflora* (Loisel) while higher, less saline marsh is often notably populated by other *Spartina patens* (Aiton), the halophyte graminoid *Distichlis spicata* (L.) Greene, rushes such as *Juncus roemerianus* (Scheele), the marsh

elder *Iva frutescens* (L.) and various other shrubs in the genus *Baccharis*, cattails in the genus *Typha*, and bulrushes in the genus *Schoenoplectus* (Greenberg and Maldonado 2006).

1.2 Marsh Ecosystem Services

Brackish and salt marsh habitats provide numerous critical ecosystem services to coastal populations (Barbier et al. 2011). These marshes protect coastal populations from wave based dangers such as storm surges and erosion (Gedan et al. 2011) which disproportionately affect lower income communities (Mcgranahan et al. 2007) and are likely to worsen in the future due to climate change-induced sea-level rise (Nicholls et al. 1999, Zhang et al. 2004). Coastal salt marsh habitat slows erosion as plant roots help mitigate shearing (Micheli and Kirchner 2002). Furthermore, soil in salt marshes contains more organic material than unvegetated soil due to rotting root biomass which results in increased soil cohesivity and a comparative lessening in erosion (Feagin et al. 2009). Salt marsh habitat similarly decreases the effects of storm surges on coastal populations due to attenuation of waves by marsh plants (Gedan et al. 2011). Additionally, salt marsh habitat is preferable to coastal engineering for protection against erosion and storm surges because not only is the latter costlier, but results in subsidence and the need for further maintenance and expansion (Syvitski et al. 2009, Temmerman et al. 2013). Tidal marshes are important carbon sinks due to their high productivity and their ability to move carbon to the long-term cycle in the form of peat (Mitsch and Gosselink 2008, Mayor and Hicks 2009, Barbier et al. 2011). In total, *Spartina* marshes and mangrove swamps sequester an estimated 49,163,000 tons of carbon yearly (Chmura et al. 2003). Additionally, the ability of *Spartina alterniflora* marshes to sequester carbon is not impacted by increasing global

temperatures, but *Spartina patens* marshes and mangrove swamps appear to lose some sequestration function as a result of warming (Chmura et al. 2003).

Estuarine habitats often take on the role of nurseries, improving the growth and survival of juvenile nekton (Beck et al. 2001). Salt marshes facilitate the growth and protection of motile immatures such as fish, mollusks, shrimp, and other crustaceans from predators before they move back into more open water as adults (Boesch and Estuaries 1984). Refuge in salt marsh habitat from large predators occurs in the form of cover from dense *Spartina* (Vince et al. 1976). It is possible that saltwater marshes produce 66% of the Penaeid shrimp and 25% of the blue crab (*Litopenaeus setiferus* (L.)) that are caught in the United States annually (Zimmerman et al. 2000).

Salt marshes similarly support tourism and recreational based activities. For example, the value of saltwater marsh per acre based solely on recreational fishing for bony and cartilaginous fish is estimated to be \$6,471 for the Eastern coast of Florida and \$981 for the Western coast of Florida (Bell 1997). Furthermore, salt marsh land has shown to be desirable for hunting wildfowl with sites being sold up to £4,000 per acre in England (King and Lester 1995).

1.3 Marsh Loss

However, these coastal marshes that provide immense ecosystem services are in steady decline. Since the 1970's, 50% of the United States salt marsh has been lost to anthropogenic activities (Watzin and Gosselink 1992). For example, coastal engineering such as diverting natural waterways prevents replacement of sediment and nutrients that allow for accretion (Yuill et al. 2009, Kirwan and Megonigal 2013). Vertical accretion is necessary for coastal marsh

habitat to keep up with sea-level rise (Reed 1990) and without it dominant low-marsh plants, such as *Spartina alterniflora*, will migrate inland replacing high-elevation marsh plants before becoming waterlogged, leading to marsh die-backs (Mendelssohn and McKee 1988). While recent research has shown that relative sea-level rise does not pose a severe risk to marsh health in areas with proper sediment deposition, it is still a major threat to marsh habitat where sediment flow has been restricted due to anthropogenic activities such as in the Mississippi delta (Kirwan and Megonigal 2013, Kirwan et al. 2016).

Additionally, pollution resulting from oil spillage poses a large threat to coastal marshes. Oiling of *Spartina patens*, the dominant plant in Gulf and Atlantic coast brackish marshes, and *Spartina alterniflora* and *Juncus roemerianus*, the dominant plants in salt marshes of the same region, results in lowered photosynthetic rates for all three plants (Pezeshki and Delaune 1993, Lin and Mendelssohn 1996, 2012). Numerous coastal oil spills have resulted in decreased above ground biomass for these species often in tandem with severe marsh die-backs and during spillage events in which oiling of the soil and plant roots occurs lasting toxic effects can inhibit plant growth for years to decades (Webb and Alexander 1991, Bergen et al. 2000, Michel et al. 2009, Lin 2015).

Louisiana's coastal marshes, in particular, have been subject to heavy oiling. In 2010 the *Deepwater Horizon* oil spill released 3.19 million barrels of oil, much of which landed in Louisiana's *Spartina alterniflora* and *Juncus roemerianus* dominated salt marshes (Mendelssohn et al. 2012, Michel et al. 2013, Zengel et al. 2014, Malakoff 2015). In heavily-oiled salt marshes within Barataria bay, complete loss of above ground vegetation occurred within months

following oiling (Lin and Mendelssohn 2012, Silliman et al. 2012, Zengel et al. 2015). Loss of below ground plant biomass resulted in undercutting and shearing of the marsh edge, quickening salt marsh loss (Silliman et al. 2012). Lin et al. (2016) found that, while both *Spartina alterniflora* and *Juncus roemerianus* experienced losses in biomass as a result of oil exposure, only *Spartina alterniflora* experienced recovery 42 months following the spill.

While a major driver of subsidence, human activity is not solely responsible for the disappearance of saltwater marshes; multiple additional geological factors play a role in the high subsidence rates seen in Louisiana (Yuill et al. 2009). Movement of tectonic plates resulting in shearing, compaction of sediment deposited by rivers during the Holocene, and the earth returning to a more natural shape after distortion from expanding glaciers in the Pleistocene all lead to subsidence and the reduction of coastal habitat (Yuill et al. 2009).

1.4 Marsh Restoration

To counteract coastal marsh loss and restore habitat, freshwater and sediment diversions are being employed to reroute freshwater flow for salinity control and increase sediment deposits to build land (Gagliano and Van Beek 1973a, Chatry et al. 1983). These diversions are a portion of the largest ever attempt to restore a natural system as outlined by the Coastal Protection and Restoration Authority with 79 restoration projects across 2,000 km of land totaling \$50 billion over the next 50 years (CPRA 2007, 2012, 2017, Elsey-quirk et al. 2019). These diversions greatly impact coastal habitat through alteration of the typical salinity gradient and nutrient additions.

Salinity content limits the growth and productivity of coastal marsh halophytes and is an important factor in the zonation of these species (Flowers et al. 1977, Elsey-quirk et al. 2019). Seasonal increases in salinity constrain the distribution of coastal plant species (Chapin III et al. 1993, Bokhorst et al. 2007), and the high salinity levels reached during the Fall months within Louisiana's marshes can cause tissue damage and death in freshwater and intermediate marsh plants (Pezeshki et al. 1987, McKee and Mendelssohn 1989, Holm and Sasser 2001, Shaffer et al. 2009, Elsey-quirk et al. 2019). Decreasing salinities resulting from freshwater inputs such as those from diversions increase productivity in these habitats, even for the highly saline tolerant plants such as *S. alterniflora* (Nestler 1977, Delaune et al. 2005, Elsey-quirk et al. 2019). However, at these lowered salinity levels, less saline tolerant species can outcompete the more tolerant plants leading to shifts in marsh type in extreme cases (Greiner La Peyre et al. 2001, Elsey-quirk et al. 2019).

Similar to increased freshwater input, sediment diversions will increase the nutrients available to coastal marsh plants, most important of which is nitrogen (N) (Elsey-quirk et al. 2019). Productivity in plants is linked to nitrogen and increases could lead to increased plant growth and above ground biomass (Sullivan and Daiber 1974, Valiela and Teal 1974, Gallagher 1975). However, the Mississippi River Delta is experiencing extensive eutrophication and the effects of increased nutrient loading in these coastal marshes are complex and not well understood (Bandyopadhyay et al. 1993, Howarth et al. 2002, Bricker et al. 2007, Elsey-quirk et al. 2019). Graham and Mendelssohn (2016) noted that different studies undertaken within Louisiana demonstrated varying responses to increased N additions as some sites experienced increased below ground biomass while other sites lost biomass or did not experience significant

changes, likely as a result of differences in environmental conditions such as salinity and inundation effects at these sites (Elsey-quirk et al. 2019). Additionally, N additions have been shown to lead to changes in plant composition with stress-tolerant plants encroaching on the range of more competitively exclusive plants (Bertness et al. 2002): with studies showing *S. alterniflora* expanding into *S. patens* marsh (Levine et al. 1998, Emery et al. 2001), *Distichlis spicata* expanding into habitat previously dominated by *S. alterniflora* (Fox et al. 2012), and both *D. spicata* and *S. patens* becoming more abundant within *Schoenoplectus americanus* (Pers.) dominated habitat (Langley and Megonigal 2010).

1.5 Tidal Marsh Inventories

Vertebrate species have been well documented in brackish and salt marshes. Worldwide most of the vertebrates native to these marshes are North American in distribution including 21 out of the 22 species or subspecies endemic to brackish and salt marshes (Greenberg and Maldonado 2006). Of the 80 mammal species native to these marshes 35 are North American (Greenberg and Maldonado 2006). Birds are the only vertebrate taxa where more native species are found in a continent other than North America (21 endemic in South America versus 19 from North America) (Greenberg and Maldonado 2006). Additionally, the majority of species native to North American marshes are from the East coast and Gulf marshes; for example of the 43 species of reptiles and amphibians native to brackish and salt marshes 41 are North American with 37 species being restricted to the Gulf and Atlantic coasts (Greenberg and Maldonado 2006).

The marine vertebrates and non-insect, invertebrate epifauna that utilize the coastal marshes along the Gulf Coast as nursery habitat are relatively well known. In the Fall, the gobies *Gobiosoma bosc* (Lacépède) and *Gobionellus boleosoma* (Jordan and Gilbert) and the bay anchovy *Anchoa mitchilli* (L.) are abundant and in the Spring the gulf menhaden *Brevoortia patronus* (Goode), the pinfish *Lagodon rhomboides* (L.), and goby *G. boleosoma* are common species present (Minello and Webb 1997). The most abundant crustaceans present in these marshes are grass shrimp, *Palaemonetes* spp., making up 81% of all crustaceans collected in one study (Minello and Webb 1997, Minello 2000). However, multiple commercially important species: the white shrimp and brown shrimp, *Penaeus* spp., and blue crab, *Callinectes sapidus* (Rathbun), are frequently abundant (Minello and Webb 1997, Minello 2000) and during the fall months, thinstripe hermit crabs, *Clibanarius vittatus* (Bosc), are common (Minello and Webb 1997). A single species of snail, the marsh periwinkle (*Littoraria irrorate* (Say)), is the dominant mollusk present in these marshes (Minello and Webb 1997, Minello 2000).

The benthic, non-insect invertebrates present in intertidal marshes are similarly well studied, particularly in marshes of the Southeastern United States. These benthic, non-insect invertebrates are commonly divided into two groups by size; fauna large enough to be captured by 500 μm sieves are referred to as macrofauna and smaller fauna which can usually be collected by 63 μm sieves are referred to as meiofauna (Kneib 1984). Nematodes represent the most abundant non-insect invertebrates found in intertidal marshes for both macrofauna and meiofauna sampling, and the polychaete *Manayunkia aesturina* (Bourne) is additionally commonly represented among both macrofauna and meiofauna in marshes of the Southeastern U.S. (Bell 1979, Kneib 1984). Additionally, copepods are commonly collected from

meiofaunal sampling while oligochaetes, ostracods, hydrobiid snails, and amphipods are often collected macrofauna (Bell 1979, Kneib 1984).

The most thorough inventories of insects within brackish and salt marshes have primarily been conducted in the Eastern United States and Europe (Davis and Gray 1966, Cameron 1972, Irmiler et al. 2002, Gratton and Denno 2005, Pétillon et al. 2008). The use of sweep net and vacuum collection methods are used commonly both in the U.S. and Europe, however many collections in European marshes where much of the lower-elevation marsh is unvegetated are performed with pitfall traps (Irmiler et al. 2002, Greenberg and Maldonado 2006, Pétillon et al. 2008). The most thorough inventories of insects taking place in brackish and salt marshes of the Gulf Coast marshes were the inventories of undertaken by Davis and Gray (1966), Denno (1977), and Gratton and Denno (2005). Davis and Gray (1966) and Denno (1977) both used sweep nets for insect collection while Gratton and Denno (2005) used vacuum collections to sample *Spartina* marsh and sticky traps and the collection of stems to sample *Phragmites australis*. Other collection methods are occasionally used in Gulf and Atlantic coast tidal marshes. However, these have typically been for the purpose of collecting specific taxa. Light traps have been used to sample biting midges in the genus *Culicoides* and mosquitos (Beck 1948, Crans et al. 1976, Kline 1986), canopy traps have been used to collect horse flies and deer flies in the family Tabanidae (Husseneder et al. 2018), and black lights have been used to collect live twisted wing parasites in the order Strepsiptera which are otherwise particularly rare (James and Strong 2018).

Many of the inventories of arthropods of Louisiana's brackish and salt marshes were made in relation to the 2010 BP Oil Spill. McCall and Pennings (2012) sampled marsh insects at

oiled sites within Barataria and Terrebonne bays using a vacuum during August 2010 and again in August 2011. Insects collected were identified to functional feeding groups with the exception of thrips (Thysanoptera), dragonflies (Odonata) and mosquitos (Culicidae) which were excluded. Bam et al. (2018) collected insects from previously oiled sites in Barataria bay and un-oiled sites in Delacroix using a sweep net from April to June in 2013 and for the same time frame in 2014. Aside from four taxa which were identified to family (Miridae, Blissidae, Delphacidae, and Formicidae) their insects were identified to the order. Sokolov et al. (2018) collected insects within a single order, Hemiptera, by sweep net from sites in Barataria bay and Breton Sound in 2011, 2012, and 2013 and identified 44 Hemipteran species within Louisiana's coastal marshes.

1.6 Marsh Vegetation Types

Louisiana's brackish and salt marshes were divided into three marsh vegetation types by Penfound and Hathaway (1938). These vegetation types run in bands along the coast and are stratified longitudinally by the salinity tolerance of the plant communities (Chabreck 1970, 1972). Penfound and Hathaway (1938) originally described these habitats as nearly fresh marsh, brackish marsh, and saline marsh in order of increasing salinity tolerance. While these classifications remain in use today, nearly fresh marsh has since been re-termed intermediate marsh (O'Neil 1949, Chabreck 1970). To avoid confusion, the use of brackish or salt marsh will refer to Chapman's (1977) classification based on tidal influence, while intermediate, brackish, and saline vegetation types will refer to Penfound and Hathaway's (1938) plant community classification. The marsh vegetation types are not defined by single plant species, but instead by communities of plants typical of the marsh type (Chabreck 1970). Additionally, due to varied

salt tolerances among species, many plant species are found across multiple marsh vegetation types (Penfound and Hathaway 1938, Chabreck 1970). Intermediate marsh vegetation type plant communities are typically species rich habitats and fresh and brackish marsh vegetation type plants are frequently encountered (Chabreck 1970). *Spartina patens* is often abundant in the intermediate marsh vegetation type, and *Schoenoplectus* spp., *Vigna luteola* (Jacq.), *Eleocharis* spp., *Phragmites australis* (Cav.), and *Ipomoea sagittata* (Poir.) among other species are common (Penfound and Hathaway 1938, Chabreck 1970, 1972). The brackish marsh vegetation type is typically dominated by *Spartina patens*, but in some areas *Distichlis spicata* is abundant (Penfound and Hathaway 1938, Chabreck 1970, 1972). Also common to the brackish marsh vegetation type are *Juncus roemerianus*, *Bolboschoenus robustus* (Pursh.), *Schoenoplectus americanus*, *Eleocharis parvula* (Roem & Schult.) and *Batis maritima* (L.) (Penfound and Hathaway 1938, Chabreck 1970, 1972). *Spartina alterniflora* is often the dominant plant in Louisiana's saline marsh vegetation type, but *Juncus roemerianus*, *Distichlis spicata*, *Avicennia germinans* (L.), and *Batis maritima* are frequently encountered (Penfound and Hathaway 1938, Chabreck 1970, 1972).

1.7 Bioindication

The Indicator Value Analysis (IndVal) statistically demonstrates species potentials to be indicators for site groups using a measure of specificity and a measure of fidelity followed by a permutation test for significance (Dufrêne and Legendre 1997). Prior to the development of the IndVal which is commonly used today, Two-Way Indicator Species Analysis (TWINSpan) (Hill 1979) was widely used to select indicator species (Legendre and Legendre 2012). Indicator Value Analysis improves on TWINSpan by being able to associate taxa to any group of sites

where as TWINSpan can only be used when sites were selected based on the results of a correspondence analysis or detrended correspondence analysis (Legendre and Legendre 2012). Additionally, with TWINSpan species are biased based on the abundances of other taxa, while with IndVal species are selected as indicators based solely on their own abundances (Legendre and Legendre 2012). Additionally, the use of a partial individual-based correlational index better allows for interpretation of the results using species data than does a presence/absence index (Cáceres and Legendre 2009, Mossakowski and Dormann 2011).

1.8 DNA Barcoding

The idea of DNA barcoding was first proposed by Arnot et al. (1993) and was popularized by Hebert et al. (2003). It is the process of identifying unknown species by sequencing a short fragment of DNA, commonly the CO1 region, and comparing it against a database containing the same data fragment from identified species (Wilson 2012). DNA barcoding can also be used to identify community DNA, which is the DNA of multiple organisms isolated from their environment; this process is known as DNA metabarcoding (Creer et al. 2016). Multiple metabarcoding studies have been conducted in the Gulf of Mexico following the Deepwater Horizon oil spill with the resulting community DNA sequences being uploaded to either the National Center for Biotechnology Information (NCBI) or the Smithsonian Institution National Museum of Natural History database which serves as a resource to compare future DNA sequences against (Bik et al. 2012, Brannock, Ortmann, et al. 2016, Brannock, Wang, et al. 2016, Brannock et al. 2017). It has recommended that DNA barcodes be standardized regions across taxa that and be consistent within a species while still being able to differentiate the

species from closely related taxa (Taberlet et al. 2007). The CO1 and 18S regions are commonly used with dedicated databases (www.blast.ncbi.nlm.nih.gov) (www.arb-silva.de) and are applicable to a wide range taxa (Valentini et al. 2009).

Chapter 2. A MONTHLY INVENTORY OF INSECT AND PLANT COMMUNITIES ACROSS MULTIPLE SALINITY ZONES WITHIN LOUISIANA’S TIDAL MARSHES CONDUCTED OVER ONE YEAR

2.1 Introduction

Salt marshes are important coastal habitats present throughout most of the world; approximately 350,985 individual communities exist worldwide with an estimated combined total of 5,495,089 hectares (Mcowen et al. 2017). These habitats found within tidal estuaries provide important ecosystem services such as erosion and storm surge protection and have been shown to attenuate waves by 63% by the time they have reached seven meters into the marsh (Morgan et al. 2009, Barbier et al. 2011). These marshes are highly productive and play an important role in carbon sequestration due to their ability to move carbon to the long-term cycle in the form of peat (Mitsch and Gosselink 2008, Mayor and Hicks 2009, Barbier et al. 2011). Coastal salt marshes are particularly important for both recreational and commercial fishing as the complex structure of marshes provide nursery for juvenile nekton (Barbier et al. 2011). Salt marshes provide protection of organisms from larger aquatic predators resulting in faster growth rates and overall biomass for important aquatic species (Boesch and Estuaries 1984, Mackenzie and Dionne 2008). For example, there is a positive correlation between the area of interface between water and Louisiana’s coastal estuarine marshes and brown shrimp production (Browder et al. 1989). Additionally, salt marsh habitat has been highly valued for Gulf Coast blue crab production (Freeman 1991).

Unfortunately, coastal marsh habitats and their associated ecosystem services are being lost globally as a result of subsidence, relative sea-level rise, and a decreased ability to accrete

land (Kennish 2001, Crosby et al. 2016). Within the United States, increased periods of inundation as a result of sea-level rise have led to the inland migration of low-elevation marsh plants such as *Spartina alterniflora* and the loss of high-elevation marsh plants such as *Spartina patens*, *Distichlis spicata*, and *Juncus roemerianus* (Donnelly and Bertness 2001, Kennish 2001). Louisiana marshes, in particular, have experienced extensive localized subsidence and land loss. Louisiana has lost approximately 4,833 km² of coastal habitat since 1932 (Couvillion et al. 2017). Numerous processes such as the shift of tectonic plates, sediment compaction, and anthropogenic activities (such as environmental engineering and hydrocarbon withdrawal) are leading to the high level of subsidence in Louisiana (Yuill et al. 2009). Additionally, the lack of the sediment deposition from the Mississippi River, largely due to construction of levees, combined with sea-level rise leads to marsh loss and increased salt water intrusion (Delaune and Pezeshki 1994). These stressors have been compounded on by large-scale disasters such as oil spills and hurricanes which further promote plant death, shearing, and marsh loss (Steyer et al. 2010, Lin and Mendelssohn 2012, Mendelssohn et al. 2012, Lin et al. 2016).

To counteract marsh loss and to promote land accretion, both sediment and freshwater diversions are being employed in Louisiana (Chatry et al. n.d., Gagliano and Van Beek 1973, Delaune et al. 2003, 2013, Elsey-quirk et al. 2019). These diversions bring sediment and nutrients into the marsh and create a salinity gradient slowing saltwater intrusion (Zhang et al. 2012, Nyman 2014) which benefits both low and high-elevation marsh plants. Increased above and below ground biomass as a result of the additional nutrients and decreased salinity associated with diversions has been shown for the common marsh grasses *Spartina alterniflora* and *Spartina patens*. *Spartina patens* benefits more from lower salinity levels than *Spartina*

alterniflora which grows better at salinity levels above 8 ppt (Delaune et al. 2005, Merino et al. 2010, Tobias et al. 2010, Matzke and Elsey-quirk 2018).

While the distribution of coastal plants and their responses to stressors and recovery efforts in Louisiana are well studied, the distribution and responses of the closely related insect community are not. Research has been conducted on the responses of Louisiana's coastal insects to oil spills and hurricanes (McCall and Pennings 2012, Bam et al. 2018, Husseneder et al. 2018) and the insect taxa belonging to the order Hemiptera that are present in these marshes are relatively well documented (Sokolov et al. 2018). Bam et al. (2018) collected insects from oiled sites in Barataria bay and un-oiled sites in Delacroix, LA from April through June in 2013 and 2014 and identified the insects to either order or family. McCall and Pennings (2012) collected insects from Barataria bay, Terrebonne bay, and Bay St. Louis in August of 2010 and 2011 and identified the insects to either order or family. Husseneder et al. (2018) used a single species of Tabanidae from oiled sites in Barataria Bay and un-oiled sites in Cameron and St. Mary's parishes from June through October in 2010 and 2011 for their comparison of population responses to oiling. Sokolov et al. (2018) collected insects in the order Hemiptera from Barataria Bay and Breton Sound in 2011, 2012, and 2013 and identified the insects to the species level. However, a thorough inventory of insects present along coastal Louisiana including their relation to the underlying plant community and salinity gradient is lacking. Therefore, the purpose of this study was to conduct an extensive, year-long inventory of Louisiana's coastal insect families across multiple salinity zones in 2 major estuaries with respect to the underlying plant community.

2.2 Materials and Methods

Site Selection

Sites were selected from low, mid, and high salinity zones within two major Louisiana estuaries: Caillou Bay and Barataria Bay. Monthly salinity data from 2014 until the end of 2017 were obtained from the Coastal Information Management System database (<http://cims.coasta.louisiana.gov>) from six different Coastal Reference Monitoring Stations and were compared using a Welch Test ($F_{(5, 126.26)} = 61.99$, $p < 2.2e^{-16}$) and multiple t tests with corrections for multiple testing (Figure 1). The Welch test results showed that there were significant salinity differences between the low, mid, and high salinity zones, but no significant differences in salinities between the Barataria Bay and Caillou Bay sites for the individual salinity zones. Since there were no differences between the Barataria and Caillou bay sites, they were combined for a total of 18 sites: six within the low salinity zone, six from the mid salinity zone, and six from high salinity zone (Figure 1). Additionally, in order to minimize unseen consequences of the Deepwater Horizon oil spill on coastal plants and inventoried insects, oil landing data collected from the Environmental Response Management Application (<https://erma.noaa.gov/gulfofmexico/>) was used to ensure that no oiling had been observed at the collection sites; the locations of the Coastal Research Monitoring Stations and adjacent collecting sites are provided (Figure 2).

Sites were selected based on accessibility by boat, and by vegetation. Sites were only selected in areas where the low-growing vegetation of brackish and salt marshes was present as opposed to the taller, shrubby vegetation of mangrove swamps. Additionally, small islands

were avoided during site selection to avoid sampling biases. The elevation in Height Above Ellipsoid (HAE) of each site was measured using a Trimble R2 GPS (Trimble, Sunnydale, California) 10 meters into the marsh.

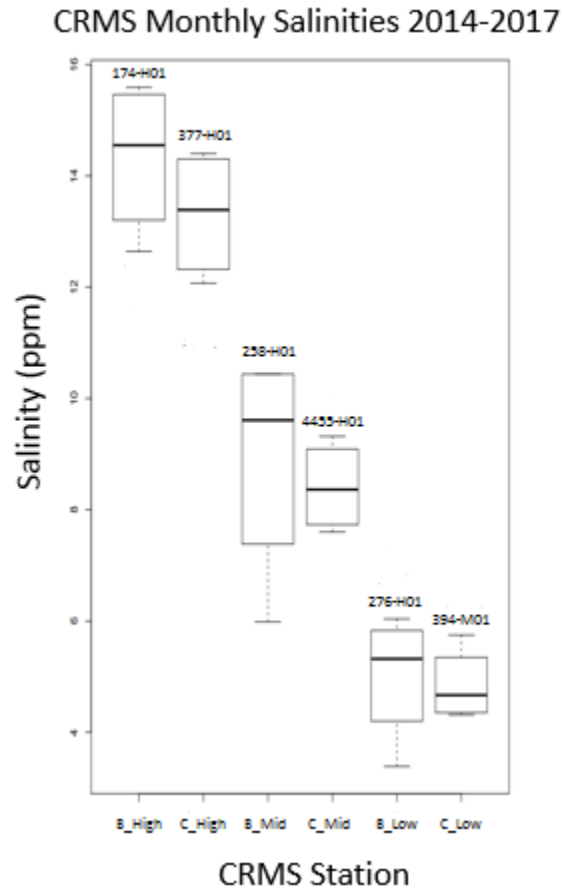


Figure 1. The average monthly salinity recorded over the four years prior to the initiation of the study. There were no significant differences in salinity between the sites in their corresponding salinity zones between Barataria (B) and Caillou (C) bays. The designated code for the individual Coastal Research Monitoring Stations is provided along with the individual boxplots.



Figure 2 The location of low, mid, and high, insect collection sites in Barataria and Caillou bays. The sites were selected based on salinity, proximity to Coastal Research Monitoring Stations, and the presence of low-growing marsh vegetation. There were three collection sites at each point on the map for a total of six low salinity sites (yellow), six mid salinity sites (red), and six high salinity sites (green).

Plant Ground Cover

Sites were visited by boat monthly from July 2018 to June 2019 from both Barataria and Caillou bays. Plant cover was measured 11 times at Caillou bay and 12 times at Barataria bay over the course of the study. At each site plants were identified to the species level the keys of Tiner (1993) within four randomly placed 20 by 50-centimeter quadrats starting at the marsh edge and moving inland with each sample and the percentage ground cover for each plant within the quadrat was estimated. The four quadrats were then averaged to determine the overall plant cover for that site. Voucher specimens for each plant species were collected. Boxplots are displayed showing the average ground cover between bays and salinity zones (Figure 4). However, no formal statistical tests were performed on this data.

Insect Collection and Family-level Identification

Insects were collected monthly by sweep net from July 2018 to June 2019 from both Barataria and Caillou bays. Five sweep net transects were taken at each sampling period. Transects consisted of ten sweeps with one step being taken forward at each sweep. Insects were placed on dry ice for the duration of the trip and placed in a -80 °C freezer until being transferred to 95% ethanol. Subsequently, the adult insects were identified at least to the family level (McAlpine 1981, Triplehorn and Johnson 2005). The family-level abundances of the insects collected from each sweep net transect from a single site were summed. These summed abundances from each site formed six replicates within each salinity zone per month.

Rarefaction and Biodiversity

Sample-based rarefaction was performed using EstimateS (Colwell 2013) for each salinity zone to determine if insect families were adequately sampled. The rarefaction curves were calculated as the average of 1,000 runs with the individuals being replaced after each sample during a run. Chao1 values (Chao 1984) were calculated as an estimate of total family richness for each salinity zone. The estimated percentages of insect families collected within each salinity zone were then calculated by subtracting the upper and lower 95% confidence intervals from the Chao1 value and subtracting the resulting values from 100.

The means and within-group standard deviations of the Fischer's Alpha (Magurran 2004, Hayek and Buzas 2010), Shannon (Magurran 2004), Simpson Inverse (Magurran 1988, 2004, Jost 2006, Hayek and Buzas 2010) biodiversity indices were calculated using EstimateS (Colwell 2013). Multiple diversity indices were used due to biases towards either species richness or evenness. Simpson INV is biased towards evenness while both Fischer's Alpha and Shannon indices are biased towards species richness (Taylor 1978, Magurran 1988). Shannon and Fisher's Alpha biodiversity index is not affected by varying sample size while Shannon index is biased almost completely towards species richness and therefore will not account for high fluctuations in evenness if the richness stays the same (Taylor 1978, Magurran 1988, 2004). ANOVAs were then used to determine if there was a significant difference in insect diversity among the salinity zones.

Analyzing Factors Affecting Family-Level Insect Distribution

The R package "vegan" (Oksanen et al. 2018) was used to perform Canonical Correspondence Analysis (CCA) to determine if selected environmental predictors could explain variation between coastal marsh insect communities and to visually display how the insect families are distributed along important gradients. A CCA was selected over other ordination techniques because it is a method of direct gradient analysis which allows for hypothesis testing as opposed to the exploratory nature of indirect gradient analysis. The inputs for the CCA were a community data set consisting of the monthly, family-level insect abundances from each site and a set of explanatory variables (plant ground cover, salinity zone, month) with values that

correspond to each replicate in the community data set. To avoid introducing too many explanatory variables, only plants that represented above 5 percent of the total ground cover at an individual marsh vegetation type were used in the analysis. Ten predictor variables were used: salinity, seasonality, total percent ground cover, and the percent ground covers of *Spartina alterniflora*, *Spartina patens*, *Distichlis spicata*, *Bolboschoenus robustus*, *Vigna luteola*, and *Schoenoplectus americanus*. An ANOVA was used to test whether or not the CCA model predicts insect family distribution better than random chance. The exploratory variables and axes of the CCA were then tested for being significant predictors of insect distribution using permutation tests with 999 permutations.

Feeding Guilds

Insects were grouped into three trophic levels (Herbivore, Consumer, and Detritivore) and further subdivided into feeding guilds based on the literature, using coastal marsh studies when possible (Marples 1966, Denno 1977, Gratton and Denno 2005, Bam 2015). Herbivores were divided into free-living suckers, free-living chewers, and stem-borers, consumers into predators and parasites, and detritivores into scavengers/shredders and filterers. A PERMANOVA was performed using Bray-Curtis dissimilarities to determine the effect of salinity on the distribution of the feeding guilds. Kruskal-Wallis tests followed by Bonferroni corrections for multiple testing and post hoc Pairwise Wilcoxon Rank Sum Tests were used to determine the effects of salinity on individual feeding guilds.

2.3 Results

Plant Ground Cover

Over the sampling period, 15 plant species were identified, and voucher specimens were collected. There were 12 species present at the low salinity zone, seven were present at mid salinity zone, and four were present at high salinity zone (Figure 3). While species composition varied among salinity zones, *Spartina* species had the highest total ground cover at each of the low (48.7%), mid (56.6%), and brackish (52.3%) salinity zones (Table 1). Additionally, as salinity increased *Spartina* species made up an increasing percentage of the total plant community as estimated by ground cover even though their contribution to ground cover remained similar. *Spartina* species at the low salinity zone made up 60.8% of the plant community by ground cover, while making up 72.7% of the plant community at the mid salinity zone, and 93.2% at the high salinity zone. While *Spartina alterniflora* and *Spartina patens* were abundant at both Barataria and Caillou bays, some common plant species differed (Table 7). *Schoenoplectus americanus* and an *Eleocharis* sp. were present at the low salinity zone in Caillou Bay, but typically absent in Barataria. Additionally, while *Vigna luteola* was present at both bays it was more abundant at Caillou Bay. Similarly, *Batis maritima* and *Juncus roemerianus* were present at the mid salinity zone in Caillou Bay and typically absent at Barataria, while *Distichlis spicata* was present at both bays, but more abundant at Caillou Bay. At the high salinity zone, *Distichlis spicata* was present at Caillou Bay, but not at Barataria Bay. The elevation in HAE for Caillou low sites averaged -0.63 cm, mid at -3.49 cm, and high at -8.21 cm. The elevation in HAE for Barataria low sites averaged -5.17 cm, mid at -9.45 cm, and high at -17.03 cm.

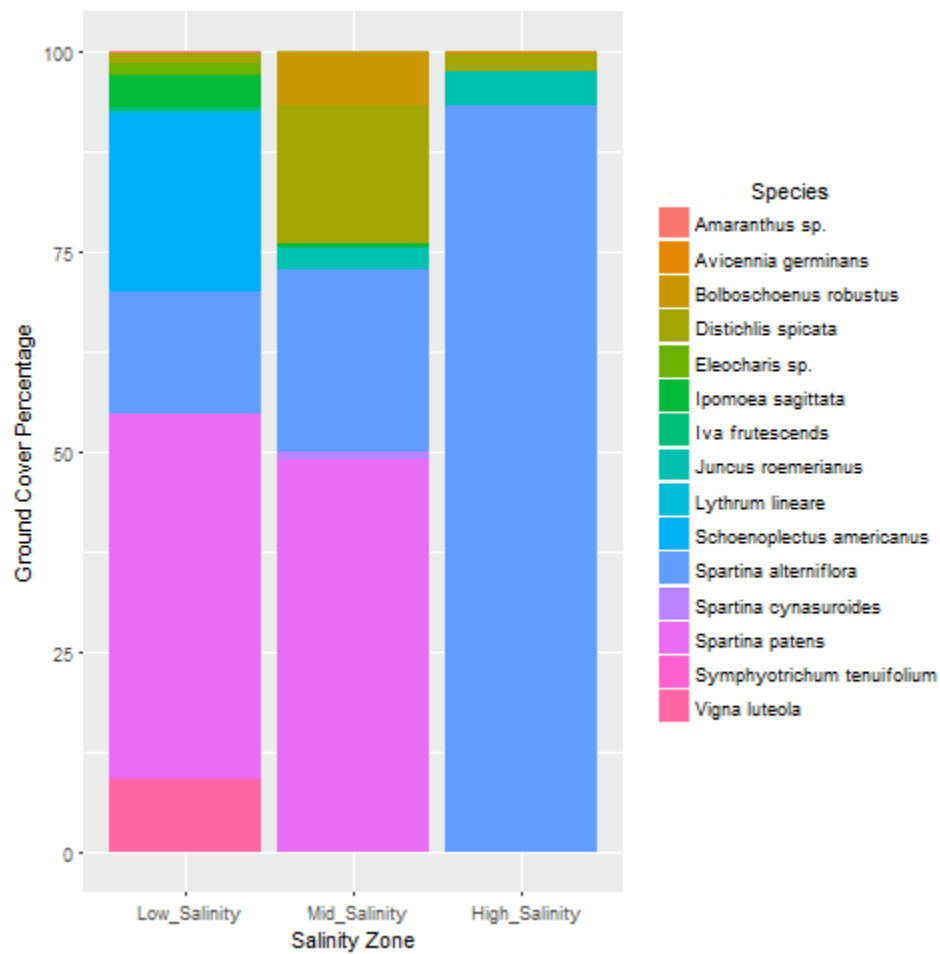


Figure 3 Plant communities present at low, mid, and high salinity zones within Barataria and Caillou bays From July 2018 to June 2019. Plant species were identified in the field and plant ground cover was estimated for each plant species within four randomly placed 20 x 50- centimeter quadrats. Plants are displayed in the bar plots by their percentage of the plant community at each salinity zone as estimated by ground cover

Table 1 The average estimated monthly ground cover for each plant species identified from low, mid, and high salinity zones within Barataria and Caillou bays. Plant ground cover was measured monthly from July 2018 through June 2019 using four randomly placed 20 x 50-centimeter quadrats at a total of 18 sites

Species	Low.Salinity	Mid.Salinity	High.Salinity
<i>Spartina alterniflora</i>	8.6%	13.3%	42.1%
<i>Spartina patens</i>	25.5%	28.6%	0.0%
<i>Juncus roemerianus</i>	0.0%	1.6%	1.9%
<i>Distichlis spicata</i>	0.7%	10.1%	1.0%
<i>Spartina cynosuroides</i>	0.0%	0.6%	0.0%
<i>Bolboschoenus robustus</i>	0.1%	4.0%	0.0%
<i>Vigna luteola</i>	5.0%	0.0%	0.0%
<i>Eleocharis</i> sp.	0.8%	0.0%	0.0%
<i>Ipomoea sagittata</i>	2.3%	0.3%	0.0%
<i>Schoenoplectus americanus</i>	12.5%	0.0%	0.0%
<i>Lythrum lineare</i>	0.1%	0.0%	0.0%
<i>Iva frutescens</i>	0.3%	0.0%	0.0%
<i>Symphytotrichum tenuifolium</i>	0.2%	0.0%	0.0%
<i>Avicennia germinans</i>	0.0%	0.0%	0.1%
<i>Amaranthus</i> sp.	0.1%	0.0%	0.0%

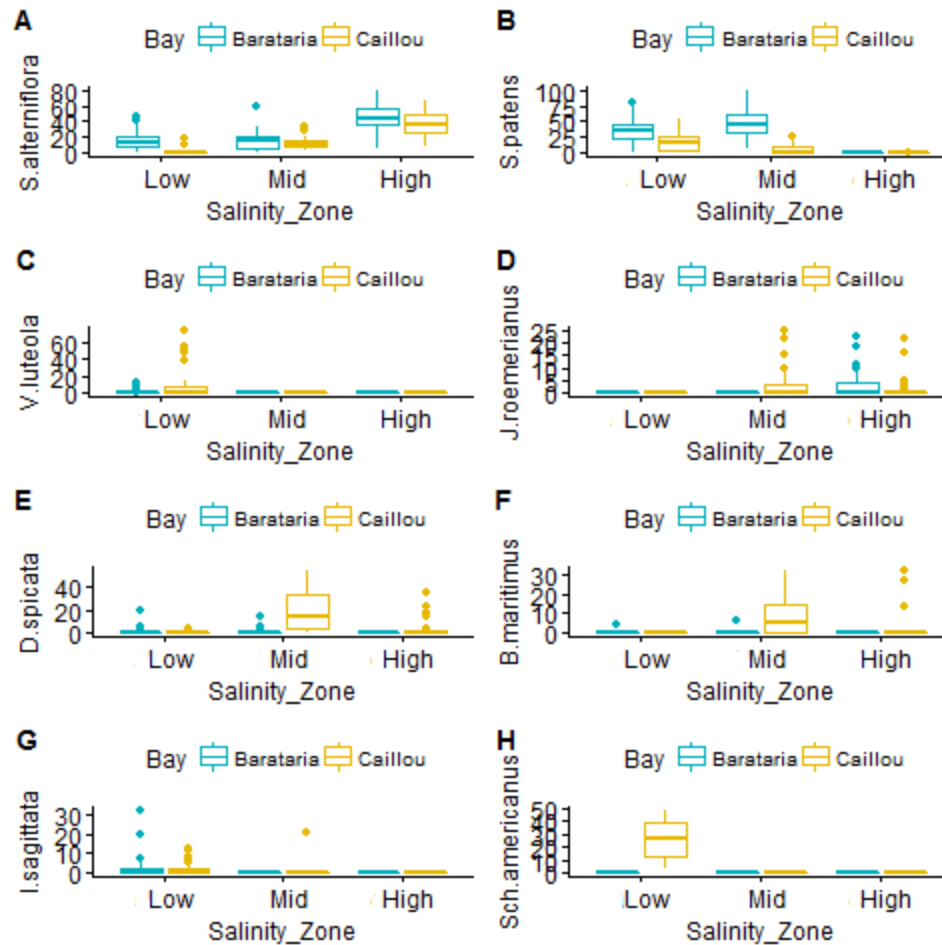


Figure 4 The average monthly estimates of plant ground cover for plant species with over 1% average ground cover compared between low, mid, and high salinity zones and Barataria and Caillou bays in coastal Louisiana.

Rarefaction

Sample-based rarefaction was performed using EstimateS (Colwell 2013) for each salinity zone to determine if insect families were adequately sampled. Between 97.34 – 100% of the total estimated families were collected from intermediate sites, between 97.41 – 100% of the total estimated families were collected from brackish salinity sites, and between 97.06 – 100% of the total estimated families were collected from saline sites as calculated using the Chao 1 values and the Chao 1 upper and lower 95% confidence intervals (Figure 5). As the

rarefaction curves leveled off and the percentages of the total estimated families collected were high, the family-level insect biodiversity was likely adequately sampled across salinity zones.

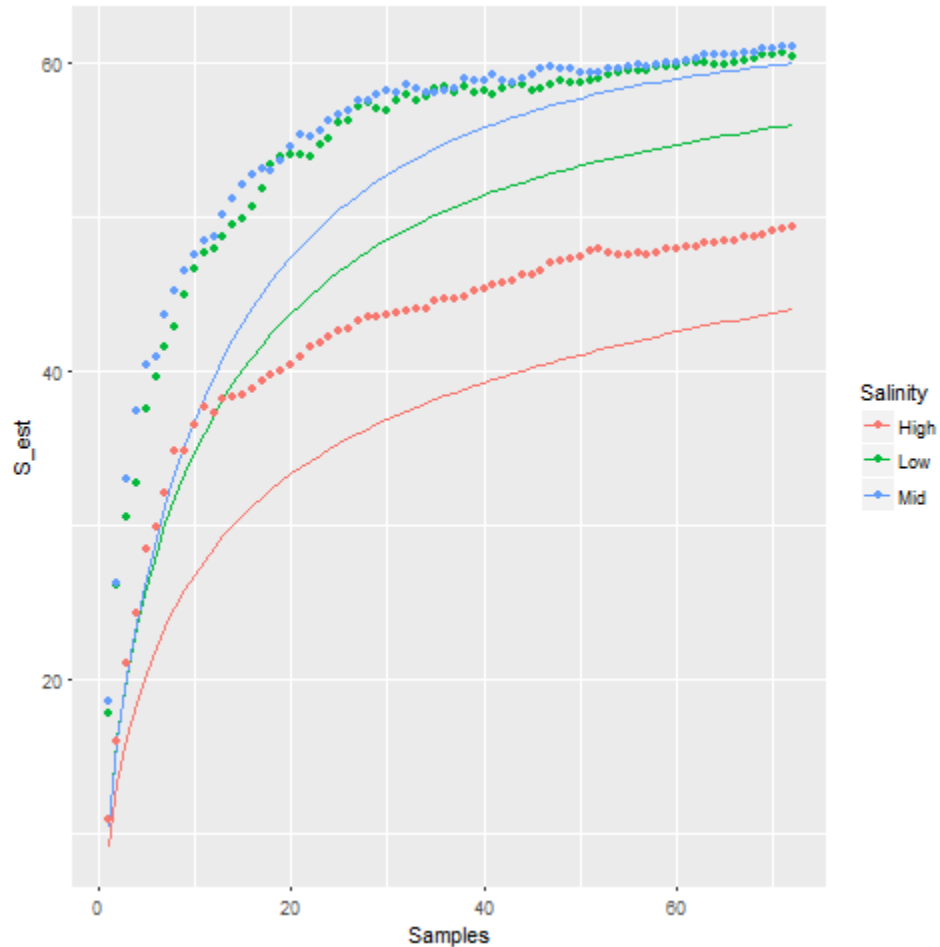


Figure 5 Sample-based rarefaction was performed to view differences in family-level richness between low, mid, and high salinity zones and to determine if the sampling regimen adequately sampled the families present. The rarefaction was performed 1,000 times with sample replacement and the results were averaged. The averaged results are plotted against the number of samples (x-axis) and a rarefied measure of the families observed (y-axis). Unbroken lines represent the rarefaction curve for a given salinity zone while the dotted lines represent the Chao1 values as a measure of the estimated total families for the same salinity zone.

Insect Abundances and Biodiversity

During the study, 71 insect families were collected with 61 families collected from low salinity zone, 64 from mid salinity zone, and 39 from high salinity zone. Total insect family abundances by salinity zone are displayed in Table 2 and by month in Table 3 as well as monthly insect family abundances by salinity zone in Tables 4-6. Across all salinity levels, individuals from the orders Diptera and Hemiptera were the most abundant although individuals from the orders Hymenoptera, Coleoptera, Thysanoptera, and Odonata were additionally common (Table 2). In total, 21 families were collected from the order Diptera, 15 from Hymenoptera, 13 from Hemiptera, 13 from Coleoptera. All of the other orders collected were represented by two or fewer families. While insects were collected year-long, adult abundances were highest in the cooler months from November until May with particularly high peaks in February and March (Table 3). The majority of insects collected from low salinity marsh were the Dipteran families Chloropidae and Chironomidae with the majority of these insects collected December through April (Table 4). At the mid salinity zone the most abundant insect families collected were the Hemipteran family Delphacidae in addition to the Chloropids and Chironomids (Table 5). The Delphacids and Chloropids were most abundant October through June while the Chironomids peaked in February and March. At the high salinity zone individuals from the families Delphacidae, Chloropidae, and Ulidiidae were the most frequently encountered (Table 6). Delphacids and Chloropids abundances were highest in November through April at high salinity marsh, while Ulidiids were most abundant in January through April. Insect family abundances were similar between bays except for a few rare families, and these differences would likely disappear with increased sampling (Table 7).

Table 2 Total number of individuals from each insect family collected by sweep net from low, mid, and high salinity zones within Barataria and Caillou bays from July 2018 to 2019. There were 12 monthly collections made at each of the 18 sites.

Order	Family	Low	Mid	High	Total
Diptera					
	Chloropidae	2130	2123	3493	7746
	Chironomidae	2970	2628	277	5875
	Ulidiidae	73	166	908	1147
	Ceratopogonidae	58	284	437	779
	Ephydriidae	194	114	134	442
	Culicidae	65	48	28	141
	Dolichopodidae	32	57	14	103
	Sciomyzidae	22	35	19	76
	Sepsidae	26	0	0	26
	Sarcophagidae	2	8	4	14
	Tabanidae	5	4	2	11
	Tipulidae	0	8	2	10
	Clusiidae	0	4	6	10
	Muscidae	4	5	0	9
	Sphaeroceridae	5	2	0	7
	Syrphidae	2	0	3	5
	Lauxaniidae	0	0	3	3
	Empididae	1	1	0	2
	Sciaridae	0	1	0	1
	Psychodidae	1	0	0	1
	Drosophilidae	1	0	0	1
Hemiptera					
	Delphacidae	786	4820	4652	10258
	Miridae	23	351	446	820
	Cicadellidae	146	52	8	206
	Blissidae	2	7	189	198
	Derbidae	80	7	0	87
	Membracidae	58	1	1	60
	Issidae	4	13	0	17
	Aphididae	2	3	4	9
	Reduviidae	3	1	4	8
	Clastopteridae	7	0	0	7
	Anthocoridae	6	0	0	6
	Dictyopharidae	3	2	0	5
	Scutelleridae	0	1	0	1
Hymenoptera					

	Formicidae	70	51	32	153
	Eulophidae	27	23	7	57
	Platygastridae	20	25	7	52
	Braconidae	21	20	7	48
	Ichneumonidae	17	17	10	44
	Encyrtidae	10	12	15	37
	Mymaridae	12	19	1	32
	Diapriidae	10	10	4	24
	Trichogrammatidae	10	4	5	19
	Eurytomidae	0	9	2	11
	Pteromalidae	2	5	4	11
	Sphecidae	2	4	1	7
	Eupelmidae	4	1	1	6
	Figitidae	2	2	0	4
	Chalcididae	0	3	0	3
Coleoptera					
	Buprestidae	62	12	0	74
	Staphylinidae	6	62	3	71
	Phalacridae	19	7	24	50
	Cleridae	7	13	29	49
	Mordellidae	2	7	37	46
	Coccinellidae	37	5	2	44
	Melyridae	0	2	20	22
	Curculionidae	6	3	2	11
	Latridiidae	7	1	1	9
	Chrysomelidae	6	1	0	7
	Lampyridae	2	0	0	2
	Corylophidae	0	1	0	1
Thysanoptera					
	Phlaeothripidae	21	150	12	183
	Thripidae	22	24	8	54
Odonata					
	Coenagrionidae	89	7	34	130
	Libellulidae	3	11	6	20
Lepidoptera					
	Elachistidae	6	11	0	17
	Pyrilidae	1	1	1	3
Orthoptera					
	Tettigoniidae	8	9	6	23
	Acrididae	1	2	0	3
Psocoptera					

	Ectopsocidae	0	1	1	2
Trichoptera					
	Hydroptilidae	0	1	0	1

Table 3 The monthly number of individuals from each insect family collected by sweep within Barataria and Caillou bays from July 2018 to 2019. There were 12 monthly collections made at each of the 18 sites.

Order	Family	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Hemiptera													
	Delphacidae	82	120	149	210	1148	1000	870	3559	1325	872	721	202
	Derbidae	11	33	18	17	0	0	0	0	0	0	8	0
	Miridae	37	67	36	24	8	15	5	50	172	79	189	138
	Cicadellidae	9	15	11	12	2	6	13	34	64	27	11	2
	Membracidae	7	22	9	20	0	0	0	1	0	0	1	0
	Anthocoridae	1	5	0	0	0	0	0	0	0	0	0	0
	Issidae	2	4	3	1	0	0	1	1	4	0	1	0
	Clastopteridae	0	1	1	0	0	1	1	0	1	0	1	1
	Dictyopharidae	0	1	2	0	0	0	0	0	0	0	1	1
	Blissidae	20	36	37	75	2	7	1	2	5	3	9	1
	Reduviidae	1	3	1	0	0	0	0	1	0	0	1	1
	Aphididae	1	0	3	0	0	4	0	1	0	0	0	0
	Scutelleridae	0	0	0	0	0	0	0	0	0	0	1	0
Diptera													
	Chloropidae	112	171	169	249	276	396	957	1066	2231	993	586	540
	Ephydriidae	8	33	16	30	39	20	6	62	196	8	10	14
	Ulidiidae	70	54	59	33	34	26	89	240	318	131	52	41
	Chironomidae	34	68	57	19	53	392	453	1672	1833	891	220	183
	Dolichopodidae	6	6	6	0	0	4	2	20	47	8	2	2
	Sarcophagidae	2	0	0	1	1	0	5	1	2	1	1	0
	Tabanidae	5	0	1	0	0	0	0	0	0	2	0	3
	Culicidae	1	0	2	1	31	19	23	44	19	1	0	0
	Ceratopogonidae	9	11	8	2	15	57	10	361	220	66	7	13
	Sciomyzidae	1	2	0	1	2	5	8	22	27	6	1	1
	Syrphidae	0	2	0	0	0	0	0	2	0	1	0	0
	Empididae	0	1	0	0	0	0	0	1	0	0	0	0
	Sciaridae	0	0	0	0	1	0	0	0	0	0	0	0
	Psychodidae	0	0	0	0	0	0	0	1	0	0	0	0
	Sepsidae	0	0	0	0	0	0	0	0	25	0	1	0
	Sphaeroceridae	0	0	0	0	0	1	0	0	2	3	1	0

	Muscidae	0	0	0	0	0	0	1	6	2	0	0	0
	Drosophilidae	0	0	0	0	0	0	0	0	1	0	0	0
	Tipulidae	0	0	0	0	0	0	2	6	2	0	0	0
	Clusiidae	0	0	0	0	0	1	4	2	2	1	0	0
	Lauxaniidae	0	0	0	0	0	0	3	0	0	0	0	0
Coleoptera													
	Chrysomelidae	0	1	2	0	0	0	0	0	0	0	1	3
	Buprestidae	6	9	4	11	3	1	8	7	9	6	3	7
	Coccinellidae	3	12	14	6	0	1	0	2	4	2	0	0
	Curculionidae	1	2	2	0	1	1	0	1	1	1	1	0
	Lampyridae	0	1	0	0	0	0	0	0	0	0	1	0
	Melyridae	0	7	5	1	0	0	0	0	3	5	1	0
	Cleridae	0	8	5	4	0	0	1	5	13	8	3	2
	Mordellidae	1	0	5	10	0	0	0	1	21	7	0	1
	Phalacridae	0	1	4	0	2	7	22	8	4	0	2	0
	Latridiidae	0	1	5	2	1	0	0	0	0	0	0	0
	Corylophidae	0	1	0	0	0	0	0	0	0	0	0	0
	Staphylinidae	1	4	2	0	0	0	2	1	58	3	0	0
Odonata													
	Coenagrionidae	14	6	11	6	0	2	1	1	6	12	17	54
	Libellulidae	1	1	1	0	0	0	0	0	0	3	10	4
Hymenoptera													
	Braconidae	1	4	2	1	7	2	4	4	16	2	4	1
	Ichneumonidae	1	1	0	1	1	6	2	3	22	4	3	0
	Eulophidae	1	5	2	0	4	7	3	3	2	4	6	20
	Formicidae	7	13	53	4	1	3	5	0	18	15	21	13
	Platygastridae	12	4	12	5	1	5	0	0	2	0	4	7
	Chalcididae	0	0	0	1	2	0	0	0	0	0	0	0
	Pteromalidae	4	5	2	0	0	0	0	0	0	0	0	0
	Eupelmidae	0	0	0	0	1	0	0	0	0	4	0	1
	Encyrtidae	2	1	0	0	0	2	2	5	4	9	10	2
	Trichogrammatidae	0	0	0	1	0	0	1	0	2	1	6	8
	Diapriidae	0	0	0	0	0	2	4	0	3	8	5	2
	Mymaridae	0	0	0	0	0	0	0	3	7	9	2	11
	Sphecidae	0	0	0	0	0	0	0	0	2	0	3	2
	Figitidae	0	1	0	0	0	0	1	0	1	1	0	0
	Eurytomidae	0	0	0	0	0	0	1	2	7	0	1	0
Orthoptera													
	Tettigoniidae	3	3	3	3	0	0	0	0	0	4	6	1
	Acrididae	0	0	0	0	0	0	1	0	1	0	0	1
Lepidoptera													

	Pyrilidae	0	0	1	0	0	0	0	0	0	0	1	0
	Elachistidae	0	0	1	0	0	0	2	3	7	3	1	0
Trichoptera													
	Hydroptilidae	0	1	0	0	0	0	0	0	0	0	0	0
Psocoptera													
	Ectopsocidae	0	0	0	0	0	0	0	1	1	0	0	0
Thysanoptera													
	Phlaeothripidae	2	6	5	0	0	4	8	38	14	34	21	51
	Thripidae	0	0	0	0	0	0	0	1	2	0	1	50
Monthly Totals:		479	753	729	751	1636	1997	2522	7244	6728	3238	1959	1384

Table 4 The total number of individuals of each insect family collected by sweep net at the low salinity zone within Barataria and Caillou bays from July 2018 to 2019. There were 12 monthly collections made at each of the 18 sites.

Order	Family	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Hemiptera													
	Delphacidae	9	25	24	10	31	13	104	193	155	63	125	37
	Derbidae	9	30	17	17	0	0	0	0	0	0	7	0
	Miridae	1	0	0	0	0	0	0	0	2	1	8	11
	Cicadellidae	7	14	7	10	0	2	11	21	48	18	8	1
	Membracidae	7	22	8	20	0	0	0	1	0	0	0	0
	Anthocoridae	1	5	0	0	0	0	0	0	0	0	0	0
	Issidae	0	2	1	0	0	0	0	0	0	0	1	0
	Clastopteridae	0	1	1	0	0	1	1	0	1	0	1	1
	Dictyopharidae	0	1	0	0	0	0	0	0	0	0	1	1
	Blissidae	0	0	0	0	1	0	0	0	0	0	1	0
	Reduviidae	0	0	0	0	0	0	0	1	0	0	1	1
	Aphididae	1	0	1	0	0	0	0	0	0	0	0	0
	Scutelleridae	0	0	0	0	0	0	0	0	0	0	0	0
Diptera													
	Chloropidae	35	56	96	114	73	35	132	403	657	185	173	175
	Ephydriidae	4	28	12	23	23	9	3	7	67	3	7	9
	Ulidiidae	10	3	4	1	2	0	5	13	19	8	6	2
	Chironomidae	31	66	55	14	35	277	385	355	752	829	121	104
	Dolichopodidae	4	6	3	0	0	1	0	2	8	4	2	2
	Sarcophagidae	1	0	0	1	0	0	0	0	0	0	0	0
	Tabanidae	1	0	1	0	0	0	0	0	0	0	0	3
	Culicidae	0	0	1	1	24	5	16	14	3	1	0	0
	Ceratopogonidae	0	5	1	1	2	3	2	5	1	33	2	4

	Sciomyzidae	0	0	0	0	2	1	5	4	7	3	0	0
	Syrphidae	0	0	0	0	0	0	0	1	0	1	0	0
	Empididae	0	1	0	0	0	0	0	0	0	0	0	0
	Sciaridae	0	0	0	0	0	0	0	0	0	0	0	0
	Psychodidae	0	0	0	0	0	0	0	1	0	0	0	0
	Sepsidae	0	0	0	0	0	0	0	0	25	0	1	0
	Sphaeroceridae	0	0	0	0	0	0	0	0	1	3	1	0
	Muscidae	0	0	0	0	0	0	0	2	2	0	0	0
	Drosophilidae	0	0	0	0	0	0	0	0	1	0	0	0
	Tipulidae	0	0	0	0	0	0	0	0	0	0	0	0
	Clusiidae	0	0	0	0	0	0	0	0	0	0	0	0
	Lauxaniidae	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera													
	Chrysomelidae	0	1	2	0	0	0	0	0	0	0	1	2
	Buprestidae	4	9	3	10	3	1	8	5	7	5	1	6
	Coccinellidae	2	12	12	6	0	1	0	1	1	2	0	0
	Curculionidae	0	2	1	0	1	0	0	0	1	1	0	0
	Lampyridae	0	1	0	0	0	0	0	0	0	0	1	0
	Melyridae	0	0	0	0	0	0	0	0	0	0	0	0
	Cleridae	0	0	0	0	0	0	0	0	0	4	2	1
	Mordellidae	0	0	0	0	0	0	0	0	0	1	0	1
	Phalacridae	0	0	2	0	0	2	11	2	2	0	0	0
	Latridiidae	0	0	5	2	0	0	0	0	0	0	0	0
	Corylophidae	0	0	0	0	0	0	0	0	0	0	0	0
	Staphylinidae	0	0	0	0	0	0	2	0	3	1	0	0
Odonata													
	Coenagrionidae	14	6	10	6	0	2	1	1	4	11	9	25
	Libellulidae	0	0	0	0	0	0	0	0	0	0	2	1
Hymenoptera													
	Braconidae	1	3	0	0	7	0	4	1	4	1	0	0
	Ichneumonidae	0	0	0	0	0	0	1	2	10	3	2	0
	Eulophidae	0	0	1	0	3	0	3	1	0	2	2	15
	Formicidae	1	0	32	0	0	0	1	0	12	11	7	6
	Platygastridae	3	1	8	3	1	0	0	0	1	0	1	2
	Chalcididae	0	0	0	0	0	0	0	0	0	0	0	0
	Pteromalidae	1	1	0	0	0	0	0	0	0	0	0	0
	Eupelmidae	0	0	0	0	0	0	0	0	0	4	0	0
	Encyrtidae	0	1	0	0	0	1	0	0	0	3	4	1
	Trichogrammatidae	0	0	0	0	0	0	0	0	0	0	3	7
	Diapriidae	0	0	0	0	0	0	1	0	0	5	2	2
	Mymaridae	0	0	0	0	0	0	0	1	4	1	2	4

	Sphecidae	0	0	0	0	0	0	0	0	0	0	0	2
	Figitidae	0	0	0	0	0	0	1	0	1	0	0	0
	Eurytomidae	0	0	0	0	0	0	0	0	0	0	0	0
Orthoptera													
	Tettigoniidae	1	0	0	0	0	0	0	0	0	3	3	1
	Acrididae	0	0	0	0	0	0	0	0	1	0	0	0
Lepidoptera													
	Pyralidae	0	0	0	0	0	0	0	0	0	0	0	0
	Elachistidae	0	0	0	0	0	0	0	0	3	2	1	0
Ephemeroptera													
	Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0
Psocoptera													
	Ectopsocidae	0	0	0	0	0	0	0	0	0	0	0	0
Thysanoptera													
	Phlaeothripidae	0	0	1	0	0	1	2	1	4	3	2	7
	Thripidae	0	0	0	0	0	0	0	0	0	0	0	22

Table 5 The total number of individuals of each insect family collected by sweep net at the mid salinity zone within Barataria and Caillou bays from July 2018 to 2019. There were 12 monthly collections made at each of the 18 sites.

Order	Month	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Hemiptera													
	Delphacidae	24	56	59	114	315	509	537	1305	668	667	431	137
	Derbidae	2	3	1	0	0	0	0	0	0	0	1	0
	Miridae	10	8	21	3	3	7	0	4	44	23	119	109
	Cicadellidae	1	1	3	1	2	3	4	11	15	8	3	1
	Membracidae	0	0	1	0	0	0	0	0	0	0	0	0
	Anthocoridae	0	0	0	0	0	0	0	0	0	0	0	0
	Issidae	2	2	2	1	0	0	1	1	4	0	0	0
	Clastopteridae	0	0	0	0	0	0	0	0	0	0	0	0
	Dictyopharidae	0	0	2	0	0	0	0	0	0	0	0	0
	Blissidae	0	0	4	1	0	0	1	0	1	1	0	0
	Reduviidae	0	1	0	0	0	0	0	0	0	0	0	0
	Aphididae	0	0	2	0	0	0	0	1	0	0	0	0
	Scutelleridae	0	0	0	0	0	0	0	0	0	0	1	0
Diptera													
	Chloropidae	49	44	31	54	62	214	184	403	562	200	152	194
	Ephydriidae	3	1	1	7	10	3	2	15	68	1	0	3
	Ulidiidae	9	5	7	4	3	7	23	40	37	16	4	11
	Chironomidae	1	2	1	2	18	77	116	1270	961	50	72	60
	Dolichopodidae	1	0	3	0	0	3	0	11	37	2	0	0
	Sarcophagidae	0	0	0	0	0	0	5	1	0	1	1	0

	Tabanidae	3	0	0	0	0	0	0	0	0	1	0	0
	Culicidae	1	0	1	0	3	10	4	18	11	0	0	0
	Ceratopogonidae	9	6	6	1	5	13	8	101	100	23	5	9
	Sciomyzidae	1	2	0	1	0	0	2	12	13	3	1	1
	Syrphidae	0	0	0	0	0	0	0	0	0	0	0	0
	Empididae	0	0	0	0	0	0	0	1	0	0	0	0
	Sciaridae	0	0	0	0	1	0	0	0	0	0	0	0
	Psychodidae	0	0	0	0	0	0	0	0	0	0	0	0
	Sepsidae	0	0	0	0	0	0	0	0	0	0	0	0
	Sphaeroceridae	0	0	0	0	0	1	0	0	1	0	0	0
	Muscidae	0	0	0	0	0	0	1	4	0	0	0	0
	Drosophilidae	0	0	0	0	0	0	0	0	0	0	0	0
	Tipulidae	0	0	0	0	0	0	1	6	1	0	0	0
	Clusiidae	0	0	0	0	0	0	0	2	2	0	0	0
	Lauxaniidae	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera													
	Chrysomelidae	0	0	0	0	0	0	0	0	0	0	0	1
	Buprestidae	2	0	1	1	0	0	0	2	2	1	2	1
	Coccinellidae	1	0	2	0	0	0	0	0	2	0	0	0
	Curculionidae	1	0	1	0	0	0	0	0	0	0	1	0
	Lampyridae	0	0	0	0	0	0	0	0	0	0	0	0
	Melyridae	0	2	0	0	0	0	0	0	0	0	0	0
	Cleridae	0	2	3	1	0	0	1	0	5	1	0	0
	Mordellidae	0	0	3	2	0	0	0	0	1	1	0	0
	Phalacridae	0	0	0	0	1	2	1	2	1	0	0	0
	Latridiidae	0	1	0	0	0	0	0	0	0	0	0	0
	Corylophidae	0	1	0	0	0	0	0	0	0	0	0	0
	Staphylinidae	1	4	1	0	0	0	0	1	53	2	0	0
Odonata													
	Coenagrionidae	0	0	1	0	0	0	0	0	1	1	0	4
	Libellulidae	0	1	1	0	0	0	0	0	0	3	4	2
Hymenoptera													
	Braconidae	0	0	0	1	0	2	0	2	12	1	2	0
	Ichneumonidae	0	0	0	0	1	6	1	0	9	0	0	0
	Eulophidae	1	5	1	0	0	7	0	0	2	2	2	3
	Formicidae	3	10	13	2	0	2	4	0	4	3	6	4
	Platygastridae	8	3	3	1	0	2	0	0	1	0	2	5
	Chalcididae	0	0	0	1	2	0	0	0	0	0	0	0
	Pteromalidae	2	2	1	0	0	0	0	0	0	0	0	0
	Eupelmidae	0	0	0	0	0	0	0	0	0	0	0	1
	Encyrtidae	0	0	0	0	0	0	2	0	2	3	5	0

	Trichogrammatidae	0	0	0	0	0	0	1	0	0	0	2	1
	Diapriidae	0	0	0	0	0	2	3	0	1	2	2	0
	Mymaridae	0	0	0	0	0	0	0	2	3	7	0	7
	Sphecidae	0	0	0	0	0	0	0	0	1	0	3	0
	Figitidae	0	1	0	0	0	0	0	0	0	1	0	0
	Eurytomidae	0	0	0	0	0	0	1	1	6	0	1	0
Orthoptera													
	Tettigoniidae	1	3	3	0	0	0	0	0	0	0	2	0
	Acrididae	0	0	0	0	0	0	1	0	0	0	0	1
Lepidoptera													
	Pyrilidae	0	0	1	0	0	0	0	0	0	0	0	0
	Elachistidae	0	0	1	0	0	0	2	3	4	1	0	0
Ephemeroptera													
	Hydroptilidae	0	1	0	0	0	0	0	0	0	0	0	0
Psocoptera													
	Ectopsocidae	0	0	0	0	0	0	0	0	1	0	0	0
Thysanoptera													
	Phlaeothripidae	1	5	3	0	0	2	4	36	8	29	19	43
	Thripidae	0	0	0	0	0	0	0	0	2	0	1	21

Table 6 The total number of individuals of each insect family collected by sweep net at the high salinity zone within Barataria and Caillou bays from July 2018 to 2019. There were 12 monthly collections made at each of the 18 sites.

Order	Month	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Hemiptera													
	Delphacidae	49	39	66	86	802	478	380	2061	502	142	165	28
	Derbidae	0	0	0	0	0	0	0	0	0	0	0	0
	Miridae	26	59	15	21	5	8	13	46	126	55	62	18
	Cicadellidae	1	0	1	1	0	1	1	2	1	1	0	0
	Membracidae	0	0	0	0	0	0	0	0	0	0	1	0
	Anthocoridae	0	0	0	0	0	0	0	0	0	0	0	0
	Issidae	0	0	0	0	0	0	0	0	0	0	0	0
	Clastopteridae	0	0	0	0	0	0	0	0	0	0	0	0
	Dictyopharidae	0	0	0	0	0	0	0	0	0	0	0	0
	Blissidae	20	36	33	74	1	7	1	2	4	2	8	1
	Reduviidae	1	2	1	0	0	0	0	0	0	0	0	0
	Aphididae	0	0	0	0	0	4	0	0	0	0	0	0
	Scutelleridae	0	0	0	0	0	0	0	0	0	0	0	0
Diptera													
	Chloropidae	28	71	42	81	141	147	1220	260	1012	608	261	171
	Ephydriidae	1	4	3	0	6	8	3	40	61	4	3	2
	Ulidiidae	51	46	48	28	29	19	97	187	262	107	42	28

	Chironomidae	2	0	1	3	0	38	11	47	120	12	27	19
	Dolichopodidae	1	0	0	0	0	0	2	7	2	2	0	0
	Sarcophagidae	1	0	0	0	1	0	0	0	2	0	0	0
	Tabanidae	1	0	0	0	0	0	0	0	0	1	0	0
	Culicidae	0	0	0	0	4	4	5	12	5	0	0	0
	Ceratopogonidae	0	0	1	0	8	41	7	255	119	10	0	0
	Sciomyzidae	0	0	0	0	0	4	3	6	7	0	0	0
	Syrphidae	0	2	0	0	0	0	0	1	0	0	0	0
	Empididae	0	0	0	0	0	0	0	0	0	0	0	0
	Sciaridae	0	0	0	0	0	0	0	0	0	0	0	0
	Psychodidae	0	0	0	0	0	0	0	0	0	0	0	0
	Sepsidae	0	0	0	0	0	0	0	0	0	0	0	0
	Sphaeroceridae	0	0	0	0	0	0	0	0	0	0	0	0
	Muscidae	0	0	0	0	0	0	0	0	0	0	0	0
	Drosophilidae	0	0	0	0	0	0	0	0	0	0	0	0
	Tipulidae	0	0	0	0	0	0	1	0	1	0	0	0
	Clusiidae	0	0	0	0	0	1	6	0	0	1	0	0
	Lauxaniidae	0	0	0	0	0	0	6	0	0	0	0	0
Coleoptera													
	Chrysomelidae	0	0	0	0	0	0	0	0	0	0	0	0
	Buprestidae	0	0	0	0	0	0	0	0	0	0	0	0
	Coccinellidae	0	0	0	0	0	0	1	1	1	0	0	0
	Curculionidae	0	0	0	0	0	1	0	1	0	0	0	0
	Lampyridae	0	0	0	0	0	0	0	0	0	0	0	0
	Melyridae	0	5	5	1	0	0	0	0	3	5	1	0
	Cleridae	0	6	2	3	0	0	1	5	8	3	1	1
	Mordellidae	1	0	2	8	0	0	0	1	20	5	0	0
	Phalacridae	0	1	2	0	1	3	15	4	1	0	2	0
	Latridiidae	0	0	0	0	1	0	0	0	0	0	0	0
	Corylophidae	0	0	0	0	0	0	0	0	0	0	0	0
	Staphylinidae	0	0	1	0	0	0	0	0	2	0	0	0
Odonata													
	Coenagrionidae	0	0	0	0	0	0	0	0	1	0	8	25
	Libellulidae	1	0	0	0	0	0	0	0	0	0	4	1
Hymenoptera													
	Braconidae	0	1	2	0	0	0	0	1	0	0	2	1
	Ichneumonidae	1	1	0	1	0	0	2	1	3	1	1	0
	Eulophidae	0	0	0	0	1	0	0	2	0	0	2	2
	Formicidae	3	3	8	2	1	1	0	0	2	1	8	3
	Platygastridae	1	0	1	1	0	3	0	0	0	0	1	0
	Chalcididae	0	0	0	0	0	0	0	0	0	0	0	0

	Pteromalidae	1	2	1	0	0	0	0	0	0	0	0	0
	Eupelmidae	0	0	0	0	1	0	0	0	0	0	0	0
	Encyrtidae	2	0	0	0	0	1	1	5	2	3	1	1
	Trichogrammatidae	0	0	0	1	0	0	0	0	2	1	1	0
	Diapriidae	0	0	0	0	0	0	0	0	2	1	1	0
	Mymaridae	0	0	0	0	0	0	0	0	0	1	0	0
	Sphecidae	0	0	0	0	0	0	0	0	1	0	0	0
	Figitidae	0	0	0	0	0	0	0	0	0	0	0	0
	Eurytomidae	0	0	0	0	0	0	0	1	1	0	0	0
	Tettigoniidae	1	0	0	3	0	0	0	0	0	1	1	0
	Acrididae	0	0	0	0	0	0	0	0	0	0	0	0
Lepidoptera													
	Pyrilidae	0	0	0	0	0	0	0	0	0	0	1	0
	Elachistidae	0	0	0	0	0	0	0	0	0	0	0	0
Ephemeroptera													
	Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0
Psocoptera													
	Ectopsocidae	0	0	0	0	0	0	0	1	0	0	0	0
Thysanoptera													
	Phlaeothripidae	1	1	1	0	0	1	2	1	2	2	0	1
	Thripidae	0	0	0	0	0	0	0	1	0	0	0	7

Table 7 Between bay and salinity differences for insect families collected from low, mid, and high salinity zones in coastal Louisiana's Barataria and Caillou bays

	Low		Mid		High	
	Barataria	Caillou	Barataria	Caillou	Barataria	Caillou
Delphacidae	726	110	4539	283	437	4361
Derbidae	9	71	7	0	0	0
Miridae	26	1	315	36	90	364
Cicadellidae	96	60	23	30	3	6
Membracidae	3	55	1	0	1	0
Anthocoridae	0	6	0	0	0	0
Issidae	0	4	11	2	0	0
Clastopteridae	5	2	0	0	0	0
Dictyopharidae	0	3	0	2	0	0
Blissidae	3	0	0	8	44	145
Reduviidae	1	2	0	1	0	4
Aphididae	1	1	2	1	0	4
Scutelleridae	0	0	0	1	0	0
Chloropidae	1977	232	1435	714	1412	2630
Ephydriidae	117	81	65	49	66	69

Ulidiidae	54	22	40	126	316	628
Chironomidae	1565	1586	1690	940	160	120
Dolichopodidae	14	19	50	7	8	6
Sarcophagidae	1	1	3	5	2	2
Tabanidae	2	3	1	3	1	1
Culicidae	59	6	27	21	1	29
Ceratopogonidae	36	23	183	103	299	142
Sciomyzidae	4	19	30	6	1	19
Syrphidae	0	2	0	0	0	3
Empididae	0	1	1	0	0	0
Sciaridae	0	0	0	1	0	0
Psychodidae	0	1	0	0	0	0
Sepsidae	26	0	0	0	0	0
Sphaeroceridae	7	0	2	0	0	0
Muscidae	3	1	5	0	0	0
Drosophilidae	0	1	0	0	0	0
Tipulidae	0	0	7	1	0	2
Clusiidae	0	0	4	0	5	3
Lauxaniidae	0	0	0	0	0	6
Chrysomelidae	6	0	1	0	0	0
Buprestidae	5	57	2	10	0	0
Coccinellidae	18	21	4	1	0	3
Curculionidae	3	3	1	2	2	0
Lampyridae	1	1	0	0	0	0
Melyridae	0	0	0	2	0	20
Cleridae	10	0	5	8	14	16
Mordellidae	1	1	0	7	15	22
Phalacridae	4	15	4	3	4	25
Latridiidae	2	5	1	0	0	1
Corylophidae	0	0	0	1	0	0
Staphylinidae	3	3	54	8	2	1
Coenagrionidae	16	75	2	5	33	1
Libellulidae	2	1	11	0	5	1
Braconidae	7	15	13	7	6	1
Ichneumonidae	10	8	15	2	8	3
Eulophidae	13	15	17	6	4	3
Formicidae	63	14	16	35	19	13
Platygastridae	15	5	11	14	4	3
Chalcididae	0	0	0	3	0	0
Pteromalidae	1	1	3	2	4	0
Eupelmidae	4	0	1	0	0	1

Encyrtidae	6	4		9	3		5	11
Trichogrammatidae	7	3		3	1		3	2
Diapriidae	7	3		6	4		4	0
Mymaridae	9	3		18	1		1	0
Sphecidae	2	0		4	0		1	0
Figitidae	2	0		0	2		0	0
Eurytomidae	0	0		6	3		0	2
Tettigoniidae	4	4		7	2		0	6
Acrididae	0	1		1	1		0	0
Pyrilidae	0	0		0	1		0	1
Elachistidae	5	1		9	2		0	0
Hydroptilidae	0	0		1	0		0	0
Ectopsocidae	0	0		0	1		1	0
Phlaeothripidae	12	10		140	10		4	8
Thripidae	20	2		23	1		7	1

Significant differences in biodiversity among the salinity zones were found for Fischer's Alpha, Shannon, and Simpson inverse diversity indices (Table 8). For Fischer's Alpha index analysis indicated that biodiversity was significantly different across all salinity zones, the results of Shannon index showed that biodiversity at low salinity zone differed from the mid and high salinity zone, and the Simpson's Inverse index showed that biodiversity at high salinity zone differed from low and mid salinity zones. For all three biodiversity indices, salinity was negatively correlated with biodiversity.

Table 8 Biodiversity indices and standard deviations for insect communities found at low, mid, and high salinity zones in Louisiana's tidal marshes. Biodiversity values with the same letter were not significantly different. Significant differences in

biodiversity between salinity zones were found with all indices and for all three indices biodiversity decreased as salinity increased.

Index	Low.Salinity	Mid.Salinity	High.Salinity
Fischer's Alpha Mean	8.49 a	8.0 b	6.1 c
Fischer's Alpha SD	0.42	0.38	0.32
Shannon Mean	1.87 a	1.73 b	1.63 b
Shannon SD	0.09	0.07	0.11
Simpson INV Mean	3.68 a	3.58 a	3.35 b
Simpson INV SD	0.36	0.35	0.37

Factors Affecting Family-Level Insect Distribution

The Canonical Correspondence model was found to predict the distribution of insect communities better than random chance with 21.02 percent of variation in the family-level distribution of coastal Louisiana insects explained by the model ($F_{9,197} = 5.8265$, $p < 0.001$). Six of the ten predictor variables (Salinity, seasonality, total ground cover, and ground cover by *Spartina alterniflora*, *Spartina patens*, *Distichlis spicata*, and *Vigna luteola*) were found to be significant indicators of insect distribution ($p < 0.001$). Additionally, four of the constrained CCA axes predicted insect family distribution better than chance. The distribution of the insect families relative to the explanatory environmental variables are displayed in a CCA biplot (Fig. 4). The axes of the biplot represent the two constrained axes of the CCA that explain the most variation of the insect distribution.

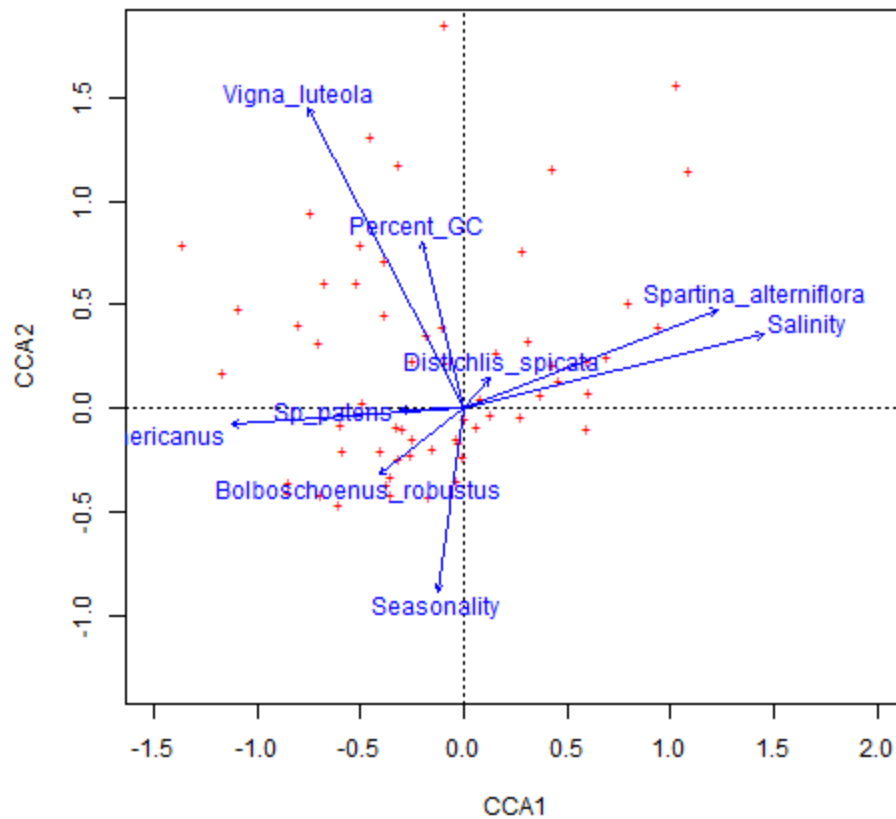


Figure 6. CCA biplot showing the important factors contributing to the distribution of insect families which were collected from sites along the salinity gradient within Barataria and Caillou bays from July 2018 to June 2019. CCA1 and CCA2 are the axis which explain the most variation in the distribution coastal Louisiana's insects. Arrows that point in the same direction are positively correlated while arrows that point in opposite directions are negatively correlated. Individual families are represented by dots.

Feeding Guilds

Of the 71 families collected in this study, 22 were assigned to the herbivore trophic level, 28 to the consumer trophic level and 21 to the detritivore trophic level (Table 9). One family, Miridae, was assigned to both the herbivore and consumer trophic levels as the Mirid *Trigonotylus uhleri* feeds on *Spartina alterniflora* and the Mirid *Tythus vagus* is an egg predator of Delphacid planthoppers (Denno 1977, Denno et al. 2009). The ants, Formicidae, were not

assigned a trophic level as nearly all collected Formicids were individuals of *Crematogaster pilosa*, a species with uncertain feeding habits that resides within dead plant stems in coastal marshes (Loken and Oliver 2016). The herbivores consisted of 12 free-living sucking families, predominately from the order Hemiptera aside from a single Thysanopteran family, 3 free-living chewers within Coleoptera and Orthoptera, and 7 stem-borers consisting of Dipteran, Coleopteran, and Lepidopteran families. The consumer trophic level consisted of 13 predators from Hemiptera, Diptera, Coleoptera, and Odonata and 15 parasites within the order Hymenoptera. The scavenger/shredding families made up 18 of the 21 detritivores. These were predominately Dipteran and Coleopteran families although singular families within the orders Thysanoptera, Psocoptera, and Ephemeroptera were present. The remaining three detritivore families were all Dipterans belonging to the filterer feeding guild.

Table 9 Insects collected from Louisiana's coastal marshes grouped by trophic level (Herbivore, Consumer, and Detritivore) and feeding guild (Sucker, Chewer, Stem-borer, Predator, Parasite, Scavenger/Shredder, and Filterer). Due to the unknown feeding habits of the most abundant Formicid species in these marshes, this family was not placed into a trophic level or guild.

Order	Family	Trophic Level	Feeding Guild
Hemiptera			
	Delphacidae	H	Sucker
	Derbidae	H	Sucker
	Miridae	H/C	Sucker/Predator
	Cicadellidae	H	Sucker
	Membracidae	H	Sucker
	Anthocoridae	C	Predator
	Issidae	H	Sucker
	Clastopteridae	H	Sucker
	Dictyopharidae	H	Sucker
	Blissidae	H	Sucker
	Reduviidae	C	Predator
	Aphididae	H	Sucker
	Scutelleridae	H	Sucker
Diptera			
	Chloropidae	H	Stem-Borer

	Ephydriidae	D	Scavenger/Shredder
	Ulidiidae	H	Stem-Borer
	Chironomidae	D	Filterer
	Dolichopodidae	D	Scavenger/Shredder
	Sarcophagidae	D	Scavenger/Shredder
	Tabanidae	C	Predator
	Culicidae	D	Filterer
	Ceratopogonidae	D	Filterer
	Sciomyzidae	C	Predator
	Syrphidae	C	Predator
	Empididae	C	Predator
	Sciaridae	D	Scavenger/Shredder
	Psychodidae	D	Scavenger/Shredder
	Sepsidae	D	Scavenger/Shredder
	Sphaeroceridae	D	Scavenger/Shredder
	Muscidae	D	Scavenger/Shredder
	Drosophilidae	D	Scavenger/Shredder
	Tipulidae	D	Scavenger/Shredder
	Clusiidae	D	Scavenger/Shredder
	Lauxaniidae	D	Scavenger/Shredder
Coleoptera			
	Chrysomelidae	H	Chewer
	Buprestidae	H	Stem-Borer
	Coccinellidae	C	Predator
	Curculionidae	H	Stem-Borer
	Lampyridae	C	Predator
	Melyridae	C	Predator
	Cleridae	C	Predator
	Mordellidae	H	Stem-Borer
	Phalacridae	D	Scavenger/Shredder
	Latridiidae	D	Scavenger/Shredder
	Corylophidae	D	Scavenger/Shredder
	Staphylinidae	C	Predator
Odonata			
	Coenagrionidae	C	Predator
	Libellulidae	C	Predator
Hymenoptera			
	Braconidae	C	Parasite
	Ichneumonidae	C	Parasite
	Eulophidae	C	Parasite
	Formicidae	Unknown	Unknown

	Platygastridae	C	Parasite
	Chalcididae	C	Parasite
	Pteromalidae	C	Parasite
	Eupelmidae	C	Parasite
	Encyrtidae	C	Parasite
	Trichogrammatidae	C	Parasite
	Diapriidae	C	Parasite
	Mymaridae	C	Parasite
	Sphecidae	C	Parasite
	Figitidae	C	Parasite
	Eurytomidae	C	Parasite
Orthoptera			
	Tettigoniidae	H	Chewer
	Acrididae	H	Chewer
Lepidoptera			
	Pyrilidae	H	Stem-Borer
	Elachistidae	H	Stem-Borer
Ephemeroptera			
	Hydroptilidae	D	Scavenger/Shredder
Psocoptera			
	Ectopsocidae	D	Scavenger/Shredder
Thysanoptera			
	Phlaeothripidae	D	Scavenger/Shredder
	Thripidae	H	Sucker

The percent community composition of each salinity zone by feeding guild is shown in Figure 7. Significant differences in the abundances of the feeding guilds among salinity zones were found for free-living suckers, stem-borers, predators, parasites, and filterers, but not for free-living chewers or scavengers/shredders (Table 10). There were significantly more sucking insects from the mid and high salinity zones than at the low salinity zone. Stem-borers were significantly more abundant at the high salinity zone than the mid or low salinity zones. There were significantly more predators at the low salinity zone than the mid salinity zone, but there were no significant differences between the abundances of predators at the high salinity zone

and the low or mid salinity zones. Similarly, parasites were more abundant at mid salinity zone than high salinity zone, but there was no significant difference between the number of parasites at low salinity zone and the mid or high salinity zones. Filterers were found to decrease as salinity increased for all zones.

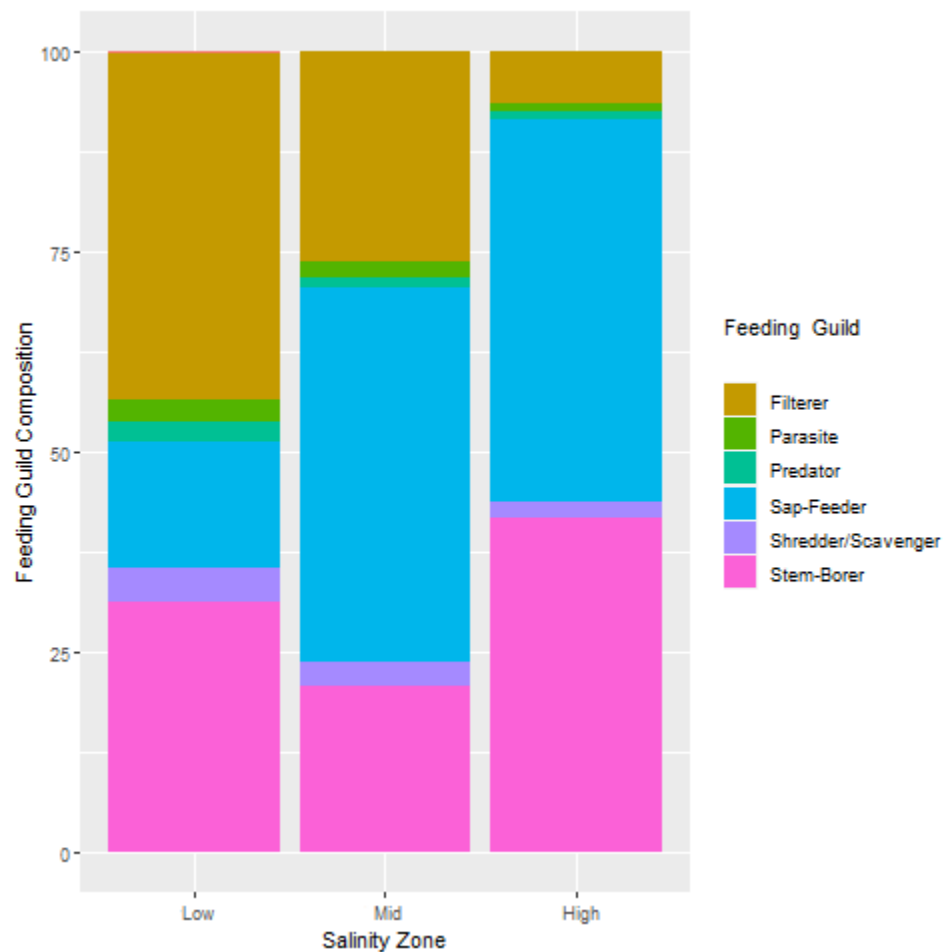


Figure 7 The community composition of insects collected from low, mid, and high salinity zones within Louisiana's coastal marshes are displayed by feeding guild. Chewing herbivores were not included in this graph as they made up less than 0.2% of the insect community at any salinity zone.

Table 10 The average number of insects belonging to each feeding guild collected per site per collection trip from low, mid, and high salinity zones in Louisiana's coastal marshes. Average abundances that are followed by the same letter do not differ between salinity zones.

Guild	Low_Salinity	Mid_Salinity	High_Salinity
Sucker	15.8 A	73.4 B	75.4 B
Chewer	0.2 A	0.2 A	0.1 A
Stem-Borer	31.7 A	32.6 A	66.3 B
Predator	2.5 A	2.0 B	1.7 AB
Parasite	2.9 AB	2.8 A	1.4 B
Shredder/Scavenger	4.3 A	5.0 A	2.9 A
Filterer	43.7 A	41.2 B	10.4 C

2.4 Discussion

In this study, the number of plant species found at the different habitats dropped from 12 species at intermediate sites to 4 species at saline sites (Table 1); these data support previous observations that plant diversity in tidal marshes decreases with increasing salinity (Chapman 1997). The composition of the plants present at the study sites (Figure 3) was consistent with prior descriptions of typical brackish and salt marshes along the Gulf and Atlantic coasts with sites being dominated by *Spartina patens* and *Spartina alterniflora* (Lin et al. 1996; Greenberg and Maldonado 2006; Lin et al. 2016). Additionally, despite differences in the plants present between bays (Figure 4), the plant species in the salinity zones of the 2 bays coincide with the three brackish and salt marsh vegetation types found in Louisiana and are typically stratified by both salinity and latitude (Penfound and Hathaway 1938). The plant community present at the low salinity zone consisted primarily of intermediate marsh type plants such as *Spartina patens*, *Schoenoplectus americanus*, *Eleocharis* spp. *Vigna luteola*, and *Ipomoea sagittata*, the plant community present at the mid salinity zone consisted of brackish marsh type plants such as *Spartina patens*, *Distichlis spicata*, *Bolboschoenus robustus*, and

Juncus roemerianus, and the high salinity zone consisted of primarily of saline marsh type plants such as *Spartina alterniflora*, *Distichlis spicata*, *Juncus roemerianus*, and *Avicennia germinans* (Penfound and Hathaway 1938, Chabreck 1970, 1972). As such, the insects collected in this inventory can be interpreted both in terms of the salinity zones found within the brackish and salt marshes and with respect to the marsh vegetation type found at each zone.

As the underlying plant community has been shown to be important in affecting the abundance and distribution of native herbivores and because insect biodiversity is positively correlated to vegetation complexity (Denno et al. 2009, Denno, Gratton, et al. 2016, Denno, Lewis, et al. 2016), it would be expected that as salinity increases there should be a decrease in the family-level insect biodiversity mirroring the decrease in plant species. This study found that insect biodiversity followed this trend for both dominance and species richness biased diversity indices (Table 8). Additional sampling is unlikely to change this finding as the rarefaction curves for each salinity zone leveled off (Figure 5). Although insect biodiversity decreased as salinity increased, insect abundance increased dramatically from the low salinity zone (7,223 individuals) to the mid (11,287 individuals) and high salinity zones (10,919 individuals) again likely as a result of the underlying plant community (Table 2). The plant communities at both mid and high salinity zones had higher percentages of *Spartina alterniflora* than intermediate salinity sites, and the mid salinity zone also had a greater percentage of *Distichlis spicata* in the plant community when compared to the other zones; both of these plants provide resources that produce larger herbivorous insect abundances (Davis and Gray 1966).

The CCA model output showed that seven of the environmental variables tested explained a significant amount of the variation within the distribution of insect families in the marsh. The Constrained Canonical Analysis results showed that most of the variation in insect distribution in the marsh is explained by the gradient from *Spartina patens*-dominated marsh to *Spartina alterniflora*-dominated marsh as salinity increases (Figure 6). This indicates that there are likely two separate insect communities determined by the dominant *Spartina* species present. This is noticeable for individuals of the most abundant families (Table 2). At higher salinity zones with increasing *S. alterniflora* and decreasing *S. patens* the most abundant families shift from Chironomids and Chloropids to Delphacids and Chloropids. Similar trends can be seen for individuals of less abundant families. For example, Membracids, Derbids, and Coccinellids decrease in abundance salinity increases and the abundances of Blissids, Ulidiids, and Clerids increase as salinity rises.

The explanatory variables seasonality, total plant ground cover, and *Vigna luteola* fall along the constrained axis that explains the second most amount of variation in the distribution of insect families in Louisiana's coastal marshes. *Vigna luteola* and total plant ground cover are positively correlated with each other and negatively correlated with seasonality. This implies that some insect families present in the marsh are associated with plants (such as *Vigna luteola*) that are only present for a short period of the year, but are extremely dense and account for the majority of the ground cover when present.

The abundances of both free-living sucking herbivores and stem-boring herbivores were found to increase as salinity increases (Table 10, Figure 7). However, while the number of abundant free-living sucking families decreases with salinity the opposite is true for the stem-

boring insects. A variety of Auchenorrhynchan hoppers were common from the low salinity zone with individuals from the families Delphacidae, Cicadellidae, Membracidae, and Derbidae frequently being collected (Table 2). While at mid and high salinity zones, individuals primarily belonged to only two families, Delphacidae and Miridae. Only the stem-boring Chloropid flies are abundant at the low salinity zone while the *Spartina alterniflora* associated stem borers, Ulidiidae and Mordellidae, become more common at the mid and high salinity zones. At the low salinity zone, individuals of many free-living sucking families are abundant during the warmer summer months (Table 3), while at the mid (Table 4) and high (Table 5) salinity zones free-living sucking insects are most abundant from November through April. However, the adult stem-boring insects were most abundant from January through April for all salinity zones. The free-living sucking insects and stem-boring insects also differed at the ordinal level (Table 9). Whereas, nearly all free-living sucking insects belonged to the order Hemiptera, the majority of stem-boring insects were Dipterans and Coleopterans. No significant differences were found between the salinity zones for the free-living chewing insects, which were much less commonly collected. Only three families, Tettigoniidae, Acrididae, and Chrysomelidae within the orders Orthoptera and Coleoptera were collected over the year-long inventory.

From the consumer trophic level, the predators were more abundant at low salinities than mid salinities, but there was no significant difference between the abundances of predators at the high salinity zone and the low or mid salinity zones. The parasites were more commonly collected from the mid salinity zone than the high salinity zone, but there was no significant difference in the abundances of parasitic insects collected from the low salinity zone and the mid or high salinity zones (Table 10). While the predatory insects belonged to a variety

of orders (Coleoptera, Hemiptera, Odonata, and Diptera), the parasites all belonged to the order Hymenoptera (Table 9). Lady beetles in the family Coccinellidae, narrow-winged damselflies in the family Coenagrionidae, and pirate bugs in the family Anthocoridae were primarily limited to the low salinity zone, while the Clerid and Melyrid beetles were more abundant at higher salinities (Table 2). The Mirid *Tytthus vagus* and the individuals of the families Reduviidae and Sciomyzidae were present across salinities. The predators collected in this study are primarily generalists with the exception of *Tytthus vagus* which is a specialist predator of the eggs and young of *Prokelisia dolus* (Wilson), a common saltmarsh Delphacid (Denno et al. 2005). Predatory taxa collected in this inventory were highly similar to those collected in a thorough inventory of an Atlantic coast tidal marsh (Davis and Gray 1966) with the exception of robber flies in the family Asilidae which were absent from this inventory likely due to the absence of large stands of *Juncus roemerianus* where it typically rests. However, multiple parasitic families (Eulophidae, Ichneumonidae, Encyrtidae, Mymaridae, Diapriidae and Trichogrammatidae) were commonly encountered in Louisiana's tidal marshes, but not collected by Davis and Gray (1966). Of the Hymenoptera present, only the families Platygasteridae and Braconidae were abundant in both inventories.

Among the detritivorous insects there was no significant difference in the abundances of scavengers/shredders, but the abundances of the filtering insects decreased as salinity increased. The detritivorous insects primarily belonged to the order Diptera, with the exception of the Thysanopteran family Phlaeothripidae (Table 9). Among the scavengers/shredders, Ephydrid and Dolichopodid flies were abundant at all salinity zones, while individuals of Phlaeothripidae were most common at the mid salinity zone. For the

filterers, Chironomid numbers decreased as salinity increased, while the opposite was true for Ceratopogonids. Individuals of Culicidae had similar abundances at each salinity level. Both the scavengers/shredders and filterers were most abundant from December through June (Tables 3-5). While no filterers were mentioned by Davis and Gray (1966), the families Ephydriidae and Dolichopodidae were the most abundant scavengers/shredders encountered in both studies.

The inventory of insects identified to the family level and its division into feeding guilds provided in this chapter should provide a useful baseline for assessing future impacts of both stressors, such as oil spills or saltwater intrusion, and habitat changes resulting from restoration efforts such as freshwater or sediment diversions independent of the time of the year. For example, if a freshwater diversion was implemented to curb the impacts of influxes of highly saline water during winter months, monitoring the abundances of families within the filterer feeding guild would be useful as both shifts in the total abundances of filterers and the composition of the filterer families would result in useful information. If there was an increase in the overall abundance of filterer insects and a shift in the overall composition to more Chironomids and fewer Ceratopogonids, it would indicate that the freshwater diversion was successful in stemming saltwater intrusion. These are only a fraction of the useful application of the data provided in this chapter can serve in future evaluation of habitat changes in coastal Louisiana estuaries. Furthermore, these data provide much needed baselines for possible long term ecological monitoring programs for the Gulf of Mexico.

Chapter 3: INSECT BIOINDICATORS OF DIFFERENT SALINITY ZONES IN LOUISIANA'S COASTAL MARSHES

3.1 Intro

Tidal marshes are unique habitats found in bays and estuaries along the coasts of each continent, with the exception of Antarctica, that form a true ecotone from terrestrial to marine environments (Chapman 1977, Holland et al. 1990, Greenberg 2006). Approximately 15,000 kilometers of tidal marsh exists within North America with over two-thirds found along the Gulf coast (Field et al. 1991). These marshes are highly stratified by the salinity gradient resulting from the frequent inundation by water with salt content ranging from 0 parts per thousand (ppt) to greater than 35 ppt (Odum et al. 1984, Holland et al. 1990, Greenberg 2006).

Brackish and salt marshes are often dominated by either *Spartina* grasses or succulents dependent on their geographic location. Within North America *Spartina alterniflora* and *S. patens* are prominent in Gulf and Atlantic coast marshes while succulent members of the genus *Salicornia* are more abundant along the Pacific coast (Greenberg and Maldonado 2006). Gulf coast and Atlantic marshes overall have similar plant communities with *Spartina alterniflora* being more abundant in lower-elevation marsh with higher salinities and *Spartina patens* being more common in higher-elevation, intermediate marsh (Greenberg and Maldonado 2006). While primarily dominated by these two grasses, other *Spartina* species, black needle rush (*Juncus roemerianus*), saltgrass (*Distichlis spicata*), salt marsh elder (*Iva frutescens*), bulrushes in the genus *Schoenoplectus* and *Bolboschoenus*, and other plants are commonly found in these marshes (Greenberg and Maldonado 2006).

Brackish and salt marshes are among the most important habitats and provide numerous important ecosystem services (Barbier et al. 2011). They are highly productive, sequestering large amounts of carbon relative to marsh area and act as nursery habitat for large populations of commercially important fish and crustaceans (Boesch and Estuaries 1984, Zimmerman et al. 2000, BECK et al. 2001). Additionally, brackish and salt marshes provide protection from storm surges and erosion which can cause extensive damage to coastal human communities and especially affect low-income populations (Mcgranahan et al. 2007, Gedan et al. 2011).

However, coastal marshes and their associated ecosystem services, especially within Louisiana, are at risk due to numerous stressors such as subsidence in large part due to anthropogenic activities such as construction of canals and levees, relative sea level rise, oil spills, and hurricanes (Yuill et al. 2009, Steyer et al. 2010, Lin and Mendelssohn 2012, Kirwan et al. 2016). The impact these stressors and the success of restoration efforts will have on marsh habitat both depend on the effects of salinity on the relevant marsh habitats. Therefore, being able to quickly determine when varying salinity conditions cause shifts in marsh habitat as well as being able to observe the direction of change is crucial for understanding the risk of stressors and effectiveness of habitat restoration.

Census of biological communities known to occur in specific habitats can be used for estimating habitat changes. Insects make up a high percentage of terrestrial biomass of Louisiana's coastal marshes and their species richness and environmental importance make them attractive candidates for monitoring biotic and abiotic effects on the environment, its ecological functioning, and the taxa present (McGeoch 1998). Furthermore, multiple decades of

research have led to robust criteria for the selection and validation of insects as bioindicators (McGeoch 1998, Legendre and Legendre 2012). In this chapter, insect bioindicators of salinity zones will be identified as baseline data to allow future assessments of the effects of changes in salinity. Within Louisiana's marshes in particular, insect bioindicators are promising as the salinity gradient and associated transition from *Spartina patens* to *Spartina alterniflora* dominated marsh was shown to explain the most variance in family-level insect distribution in these marshes (Chapter 2, Figure 6).

3.2 Materials and Methods

A total of 18 sites were selected across three marsh vegetation types within Barataria and Caillou bays based on historical salinity data from the beginning of 2014 until the end of 2017 which was obtained from the Coastal Research Monitoring Stations database (Figure 2) (lacoast.gov). A Welch test ($F_{(5, 126.26)} = 61.99$, $p < 2.2e^{-16}$) found that there were significant salinity differences between the low, mid, and high salinity zones, but no significant differences in salinities between the Barataria Bay and Caillou Bay sites (Chapter 2, Figure 1).

Insects were collected monthly by sweep net along five 10-sweep transects established at each site. Insects were immediately stored on dry ice to preserve DNA and moved to 95% ethanol upon returning to the lab. The insects were then identified to family level. The family-level abundances of the insects collected from each sweep net transect from a

single site were summed. These summed abundances from each site formed six replicates within each salinity zone per month.

An exploratory Indicator Value Analysis (IndVal) was performed to determine which insect families are statistically significant bioindicators for each salinity zone or group of salinity zones using the R package *indicspecies* (Dufrêne and Legendre 1997, De Cáceres and Jansen 2016). The IndVal statistic is a measure of fidelity (the percentage of the target taxa that is found within a specific site group) multiplied by a value of specificity (the percentage of sites within the specific site group from which the target taxa was collected) multiplied by 100 for a maximum value of 1; this output is followed by permutation tests for significance and corrections for multiple testing.

Families that were found to be significant indicators for a salinity zone or groups of salinity zones and had an IndVal statistic above 0.25 were identified to the lowest taxonomic level possible using the relevant keys and species descriptions (Townes Jr 1945, Ross 1963, Kelton 1971, Waugh and Wirth 1976, Blanton and Wirth 1979, Slater 1979, Downes and Wirth 1981, Flynn and Kramer 1983, Steyskal 1987, Ma 1988, Macrae 1991, Hamilton 2000, Dietrich 2005, Leavengood Jr 2008, Spinelli et al. 2009, Henry 2012, Zahniser and Dietrich 2013, Catalano et al. 2014, Grogan et al. 2019). A second IndVal procedure was performed to find which taxa within families are the most useful as indicators and which are most often collected outside of the relevant salinity zones. Identified taxa were imaged and DNA barcoded to facilitate identification and confirmation in future studies as well as other applications.

From each bioindicator species, five individuals were selected for DNA barcoding. DNA was extracted using DNEasy blood and tissue kits (Qiagen, Germantown, MD). Two loci, the CO1 and 18S regions, were amplified for each species. The CO1 locus was amplified using the LepF/LepF-R primer set (Brandon-Mong et al. 2015) or the HCO1/LCO1 primer set (White 1990) using the protocol outlined by Wilson (2012). The ITS2 region of the 18S locus was amplified using the ITS3/ITS4 primers (Earth Microbiome) using protocol of 1 cycle of 3 minutes at 94 °C, 35 cycles of 45 seconds at 94 °C, 60 seconds at 57 °C, and 90 seconds at 72 °C, and 1 cycle of 10 minutes at 72 °C. PCR reagents were submitted to the LSU Genomics Facility for sequencing. Forward and reverse sequences were aligned using the Genious software and the DNA barcodes were blasted against the NCBI database.

3.3 Results

The exploratory Indicator Value Analysis found a total of 25 families to be significant predictors of a single salinity zone or combination of salinity zones (Table 11). Of these families, 17 were selected for further identification: excluding families that were below the Indicator Value cutoff of 0.25 (Sepsidae and Chrysomelidae), families that had both numerous morphospecies with only a few individuals per morphospecies (Staphylinidae), and families that are highly difficult to identify further without prior knowledge of the life history of collected individuals (multiple Hymenopteran families). From the 17 selected families, 6 were found to be indicators for intermediate marsh, 2 for the combination of intermediate and brackish marsh, 2 for brackish, 4 for the combination of brackish and saline marsh, and 3 for saline marsh.

Table 11 Insect families were selected by Indicator Value Analysis to narrow down investigation for possible bioindicator species of differing marsh vegetation types of Barataria and Cal Bays in coastal Louisiana. A total of 17 families were selected and are listed below in order of decreasing IndVal statistic by salinity zone.

Salinity.Zone	Family	IndVal.Statistic
Low	Buprestidae	0.601
Low	Coenagrionidae	0.585
Low	Derbidae	0.518
Low	Coccinellidae	0.432
Low	Membracidae	0.384
Low	Clastopteridae	0.312
Low + Mid	Chironomidae	0.876
Low + Mid	Cicadellidae	0.679
Mid	Phlaeothripidae	0.613
Mid	Issidae	0.326
Mid + High	Uliidae	0.872
Mid + High	Miridae	0.834
Mid + High	Ceratopogonidae	0.694
Mid + High	Cleridae	0.423
High	Blissidae	0.719
High	Mordellidae	0.366
High	Melyridae	0.355

Of the insect taxa that were identified from the selected families, 26 were found to be significant indicators for a salinity zone or groups of salinity zones (Table 12). Out of those 26 species, 11 were indicators of intermediate marsh, 2 for the combination of intermediate and brackish marsh, 4 for brackish marsh, 4 for the combination of brackish and saline marsh, and 5 for the saline marsh.

Table 12 Indicators of different coastal marsh vegetation types and their temporal distribution. Insects were collected by sweep net monthly in Barataria and Caillou bays. Taxa were indicators for a single salinity zone or combinations of more than one salinity zone. A total of 27 species were found to be significant indicators: 11 for intermediate marsh, 2 for the combination of

brackish and intermediate marsh, 4 for brackish marsh, 4 for the combination of brackish and saline marsh, and 6 for saline marsh.

Salinity.Zone	Family	Indicator.Taxa	IndVal.Statistic	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	March	Apr	May	June
Low	Buprestidae	Taphrocerus agriloides	0.612	4	9	1	10	3	1	7	5	7	4	1	5
Low	Cicadellidae	Draeculacephala portola	0.608	2	2	2	8	0	2	11	30	52	20	5	1
Low	Coenagrionidae	Ischnura ramburii	0.578	11	6	11	6	0	2	1	1	5	10	17	50
Low	Chironomidae	Tanytus	0.506	12	13	0	0	0	19	71	26	43	15	2	9
Low	Derbidae	Cedusa obscura	0.489	6	28	17	17	0	0	0	0	0	0	8	0
Low	Coccinellidae	Naemia seriata	0.432	3	12	14	6	0	1	0	2	4	2	0	0
Low	Membracidae	Spissistilus festinus	0.384	7	22	9	29	0	0	0	1	0	0	1	0
Low	Cicadellidae	Deltacephalinae	0.316	1	0	1	1	0	0	1	0	3	0	3	0
Low	Clastopteridae	Clastoptera xanthocephala	0.312	0	1	1	0	0	1	1	0	1	0	1	1
Low	Cicadellidae	Empoasca	0.264	1	13	1	0	0	1	0	0	0	0	0	0
Low	Ceratopogonidae	Palpomyia subaspera	0.264	0	0	0	0	0	0	0	1	0	18	1	0
Low + Mid	Chironomidae	Chironomus sp.	0.660	0	0	0	3	19	42	182	1600	423	785	4	8
Low + Mid	Buprestidae	Taphrocerus gracilis	0.300	0	0	3	1	0	0	0	3	1	1	2	2
Mid	Phlaeothripidae	Phlaeothrips	0.603	2	6	5	0	0	4	8	38	14	34	21	51
Mid	Ceratopogonidae	Dasyhelea grisea	0.547	10	2	8	1	5	7	6	93	29	2	2	9
Mid	Cicadellidae	Spangbergiella vulnerata	0.333	0	0	1	0	0	0	0	2	1	2	2	1
Mid	Issidae	Aphelonema simplex	0.326	2	4	3	1	0	0	1	1	4	0	1	0
Mid + High	Miridae	Trigonotylus uhleri	0.808	36	64	31	23	7	9	5	37	124	69	190	147
Mid + High	Ulidiidae	Chaetopsis aenea	0.685	16	14	5	8	4	2	12	73	134	63	13	11
Mid + High	Ceratopogonidae	Culicoides mississippiensis	0.575	0	0	0	0	5	43	3	268	184	25	0	0
Mid + High	Cleridae	Wolcottia aegra	0.423	0	8	5	4	0	0	1	5	13	8	3	2
High	Ulidiidae	Chaetopsis fulvifrons	0.857	44	26	30	8	28	23	77	165	174	65	24	18
High	Blissidae	Ischnodemus conicus	0.699	19	38	36	77	2	7	1	2	5	3	9	1
High	Ulidiidae	Chaetopsis debilis	0.511	7	15	22	16	0	0	0	0	0	3	15	14
High	Mordellidae	Mordellistena sp.	0.366	1	0	5	10	0	0	0	1	21	7	0	1
High	Melyridae	Collops nigriceps	0.355	0	7	5	1	0	0	0	0	3	5	1	0

The DNA barcode from each individual was compared against other individuals of the same species collected in this study to determine the intraspecific percent identity to ensure the absence of cryptic species. The identified bioindicator species tended to have highly similar intraspecific DNA barcodes (Table 13). The species with the highest amount of intraspecific variability was *Palpomyia subaspera* which still had DNA barcodes that were 99.06% similar for

the CO1 locus and 100% similar for the 18S locus. As such, the chance for cryptic species being present among the indicator species collected is low

Table 13 The closest DNA barcode matches for the bioindicator species as of 04 April 2020 are shown for both the Cytochrome Oxidase 1 and Ribosomal 18S coding regions. The Intraspecific Percent Match column shows how closely each barcoded individual of a species matches to other members of the same species collected in this study. The percent identity column shows how similar the DNA barcode is to the closest GenBank match.

Indicator	Gene	Primer Pair	Accession	Intraspecific Percent Identity	Closest Match	Percent Identity	Match Accession
<i>Taphrocerus agriloides</i>	CO1	HCO1		100%	<i>Taphrocerus fasciatus</i>	87.58%	KM364339.1
<i>Taphrocerus agriloides</i>	CO1	HCO1					
<i>Taphrocerus agriloides</i>	CO1	HCO1			<i>Taphrocerus fasciatus</i>	87.46%	KM364339.1
<i>Taphrocerus agriloides</i>	CO1	HCO1			<i>Taphrocerus fasciatus</i>	87.77%	KM364339.1
<i>Taphrocerus agriloides</i>	CO1	HCO1			<i>Taphrocerus fasciatus</i>	87.52%	KM364339.1
<i>Taphrocerus agriloides</i>	18S	Earth Microbio me		100%	<i>Quedius mesomelinus</i>	98.16%	AJ810738.1
<i>Taphrocerus agriloides</i>	18S	Earth Microbio me					
<i>Taphrocerus agriloides</i>	18S	Earth Microbio me			<i>Quedius mesomelinus</i>	98.15%	AJ810738.1
<i>Taphrocerus agriloides</i>	18S	Earth Microbio me			<i>Trochoideus goudoti</i>	96.91%	GQ302217.1
<i>Taphrocerus agriloides</i>	18S	Earth Microbio me			<i>Tenebrio molitor</i>	97.09%	X07801.1
<i>Draeculacephala portola</i>	CO1	LepF1		99.15-99.57%	<i>Draeculacephala sp.</i>	99.55%	HQ985147.1

<i>Draeculacephala portola</i>	CO1	LepF1			<i>Draeculacephala</i> sp.	100%	HQ985147.1
<i>Draeculacephala portola</i>	CO1	LepF1			<i>Draeculacephala</i> sp.	99.57%	HQ985147.1
<i>Draeculacephala portola</i>	CO1	LepF1			<i>Draeculacephala robinsoni</i>	99.57%	KF920428.1
<i>Draeculacephala portola</i>	CO1	LepF1					
<i>Draeculacephala portola</i>	18S	Earth Microbio me		100%	<i>Philaenus spumarius</i>	97.02%	U06480.1
<i>Draeculacephala portola</i>	18S	Earth Microbio me			<i>Philaenus spumarius</i>	97.02%	U06480.1
<i>Draeculacephala portola</i>	18S	Earth Microbio me			<i>Philaenus spumarius</i>	97.02%	U06480.1
<i>Draeculacephala portola</i>	18S	Earth Microbio me			<i>Philaenus spumarius</i>	97.08%	U06480.1
<i>Draeculacephala portola</i>	18S	Earth Microbio me					
<i>Ischnura ramburii</i>	CO1	HCO1					
<i>Ischnura ramburii</i>	CO1	HCO1					
<i>Ischnura ramburii</i>	CO1	HCO1					
<i>Ischnura ramburii</i>	CO1	HCO1					
<i>Ischnura ramburii</i>	CO1	HCO1			<i>Ischnura ramburii</i>	99.86%	MH450004.1
<i>Ischnura ramburii</i>	18S	Earth Microbio me					

<i>Ischnura ramburii</i>	18S	Earth Microbio me					
<i>Ischnura ramburii</i>	18S	Earth Microbio me					
<i>Ischnura ramburii</i>	18S	Earth Microbio me					
<i>Ischnura ramburii</i>	18S	Earth Microbio me					
<i>Tanypus sp.</i>	CO1	LepF1			<i>Tanypus neopunctipennis</i>	99.16%	HQ582763.1
<i>Tanypus sp.</i>	CO1	LepF1			<i>Tanypus neopunctipennis</i>	100%	HQ582763.1
<i>Tanypus sp.</i>	CO1	LepF1			<i>Tanypus neopunctipennis</i>	98.28%	HQ582763.1
<i>Tanypus sp.</i>	CO1	LepF1					
<i>Tanypus sp.</i>	CO1	LepF1					
<i>Tanypus sp.</i>	18S	Earth Microbio me		100%	<i>Ablabesmyia rhamphe</i>	93.87%	U48384.1
<i>Tanypus sp.</i>	18S	Earth Microbio me			<i>Ablabesmyia rhamphe</i>	94.12%	U48384.1
<i>Tanypus sp.</i>	18S	Earth Microbio me			<i>Ablabesmyia rhamphe</i>	94.25%	U48384.1
<i>Tanypus sp.</i>	18S	Earth Microbio me			<i>Ablabesmyia rhamphe</i>	94.22%	U48384.1
<i>Tanypus sp.</i>	18S	Earth Microbio me					

<i>Cedusa obscura</i>	CO1	HCO1		99.20-100%	<i>Kallitaxila sinica</i>	85.38%	MG099957.1
<i>Cedusa obscura</i>	CO1	HCO1			<i>Kallitaxila sinica</i>	85.69%	MG099957.1
<i>Cedusa obscura</i>	CO1	HCO1			<i>Kallitaxila sinica</i>	85.45%	MG099957.1
<i>Cedusa obscura</i>	CO1	HCO1			<i>Kallitaxila sinica</i>	85.51%	MG099957.1
<i>Cedusa obscura</i>	CO1	HCO1			<i>Kallitaxila sinica</i>	85.73%	MG099957.1
<i>Cedusa obscura</i>	18S	Earth Microbio me		99.43-100%			
<i>Cedusa obscura</i>	18S	Earth Microbio me			<i>Cedusa obscura</i>	99.31%	DQ532517.1
<i>Cedusa obscura</i>	18S	Earth Microbio me					
<i>Cedusa obscura</i>	18S	Earth Microbio me			<i>Cedusa obscurar</i>	99.32%	DQ532517.1
<i>Cedusa obscura</i>	18S	Earth Microbio me			<i>Cedusa obscurar</i>	98.65%	DQ532517.1
<i>Naemia seriata</i>	CO1	HCO1		99.26%			
<i>Naemia seriata</i>	CO1	HCO1					
<i>Naemia seriata</i>	CO1	HCO1			<i>Tytthaspis sedecimpunct ata</i>	87.25%	KU913704.1
<i>Naemia seriata</i>	CO1	HCO1			<i>Tytthaspis sedecimpunct ata</i>	87.16%	KU913704.1
<i>Naemia seriata</i>	CO1	HCO1					

<i>Naemia seriata</i>	18S	Earth Microbio me		100%			
<i>Naemia seriata</i>	18S	Earth Microbio me			<i>Pristonema</i> sp.	100%	FJ687665.1
<i>Naemia seriata</i>	18S	Earth Microbio me			<i>Pristonema</i> sp.	98.27%	FJ687665.1
<i>Naemia seriata</i>	18S	Earth Microbio me			<i>Pristonema</i> sp.	98.22%	FJ687665.1
<i>Naemia seriata</i>	18S	Earth Microbio me					
<i>Spissistilus festinus</i>	CO1	LepF1					
<i>Spissistilus festinus</i>	CO1	LepF1					
<i>Spissistilus festinus</i>	CO1	LepF1					
<i>Spissistilus festinus</i>	CO1	LepF1					
<i>Spissistilus festinus</i>	CO1	LepF1					
<i>Spissistilus festinus</i>	18S	Earth Microbio me		100%	<i>Spissistilus festinus</i>	99.40%	U06477.1
<i>Spissistilus festinus</i>	18S	Earth Microbio me			<i>Spissistilus festinus</i>	99.40%	U06477.1
<i>Spissistilus festinus</i>	18S	Earth Microbio me			<i>Spissistilus festinus</i>	99.40%	U06477.1
<i>Spissistilus festinus</i>	18S	Earth Microbio me					

<i>Spissistilus festinus</i>	18S	Earth Microbio me			<i>Spissistilus festinus</i>	99.40%	U06477.1
Deltocephali nae sp.	CO1	LepF1		99.62-100%	<i>Graminella nigrifrons</i>	99.18%	MG401918.1
Deltocephali nae sp.	CO1	LepF1			<i>Graminella nigrifrons</i>	99.57%	MG401918.1
Deltocephali nae sp.	CO1	LepF1			<i>Graminella nigrifrons</i>	99.18%	MG401918.1
Deltocephali nae sp.	CO1	LepF1			<i>Graminella nigrifrons</i>	99.18%	MG401918.1
Deltocephali nae sp.	CO1	LepF1					
Deltocephali nae sp.	CO1	LepF1					
Deltocephali nae sp.	18S	Earth Microbio me		100%	Cicadellidae sp.	99.39%	KJ461311.1
Deltocephali nae sp.	18S	Earth Microbio me					
Deltocephali nae sp.	18S	Earth Microbio me					
Deltocephali nae sp.	18S	Earth Microbio me			Cicadellidae sp.	99.38%	KJ461311.1
Deltocephali nae sp.	18S	Earth Microbio me					
Deltocephali nae sp.	18S	Earth Microbio me					
<i>Clastoptera xanthocephala</i>	CO1	LepF1					
<i>Clastoptera xanthocephala</i>	CO1	LepF1					
<i>Clastoptera xanthocephala</i>	CO1	LepF1					

<i>Clastoptera xanthocephala</i>	CO1	LepF1					
<i>Clastoptera xanthocephala</i>	CO1	LepF1					
<i>Clastoptera xanthocephala</i>	18S	Earth Microbiome					
<i>Clastoptera xanthocephala</i>	18S	Earth Microbiome					
<i>Clastoptera xanthocephala</i>	18S	Earth Microbiome					
<i>Clastoptera xanthocephala</i>	18S	Earth Microbiome					
<i>Clastoptera xanthocephala</i>	18S	Earth Microbiome					
<i>Empoasca sp.</i>	CO1	LepF1		99.61-100%	<i>Empoasca fabae</i>	92.95%	MK032789.1
<i>Empoasca sp.</i>	CO1	LepF1			<i>Empoasca fabae</i>	92.95%	KR042591.1
<i>Empoasca sp.</i>	CO1	LepF1			<i>Empoasca fabae</i>	93.39%	KR042591.1
<i>Empoasca sp.</i>	CO1	LepF1			<i>Empoasca fabae</i>	93.39%	KR042591.1
<i>Empoasca sp.</i>	CO1	LepF1					
<i>Empoasca sp.</i>	18S	Earth Microbiome					
<i>Empoasca sp.</i>	18S	Earth Microbiome					

<i>Empoasca</i> <i>sp.</i>	18S	Earth Microbio me					
<i>Empoasca</i> <i>sp.</i>	18S	Earth Microbio me					
<i>Empoasca</i> <i>sp.</i>	18S	Earth Microbio me					
<i>Palpomyia</i> <i>subaspera</i>	CO1	HCO1		99.06-99.70%			
<i>Palpomyia</i> <i>subaspera</i>	CO1	HCO1			Ceratopogoni dae sp.	97.87%	HM386311.1
<i>Palpomyia</i> <i>subaspera</i>	CO1	HCO1			Ceratopogoni dae sp.	97.42%	HM386311.1
<i>Palpomyia</i> <i>subaspera</i>	CO1	HCO1			Ceratopogoni dae sp.	97.42%	HM386311.1
<i>Palpomyia</i> <i>subaspera</i>	CO1	HCO1			Ceratopogoni dae sp.	97.45%	HQ582913.1
<i>Palpomyia</i> <i>subaspera</i>	18S	Earth Microbio me		100%			
<i>Palpomyia</i> <i>subaspera</i>	18S	Earth Microbio me			<i>Culicoides</i> <i>imicola</i>	98.16%	AF074019.1
<i>Palpomyia</i> <i>subaspera</i>	18S	Earth Microbio me			<i>Culicoides</i> <i>imicola</i>	98.24%	AF074019.1
<i>Palpomyia</i> <i>subaspera</i>	18S	Earth Microbio me					
<i>Palpomyia</i> <i>subaspera</i>	18S	Earth Microbio me			<i>Culicoides</i> <i>imicola</i>	98.16%	AF074019.1
Ortrhocladii ae sp.	CO1	HCO1		99.71%	<i>Cricotopus</i> sp.	98.12%	KR165280.1
Ortrhocladii ae sp.	CO1	HCO1			<i>Cricotopus</i> sp.	100%	HQ940393.1

Ortrhocladiin ae sp.	CO1	HCO1			<i>Cricotopus</i> sp.	99.85%	HQ984515.1
Ortrhocladiin ae sp.	CO1	HCO1			<i>Cricotopus</i> sp.	99.85%	HQ984515.1
Ortrhocladiin ae sp.	CO1	HCO1					
Ortrhocladiin ae sp.	18S	Earth Microbio me		100%	<i>Cricotopus sylvestris</i>	99.32%	AB704957.1
Ortrhocladiin ae sp.	18S	Earth Microbio me			<i>Cricotopus sylvestris</i>	99.32%	AB704957.1
Ortrhocladiin ae sp.	18S	Earth Microbio me			<i>Cricotopus sylvestris</i>	99.32%	AB704957.1
Ortrhocladiin ae sp.	18S	Earth Microbio me			<i>Cricotopus sylvestris</i>	99.32%	AB704957.1
Ortrhocladiin ae sp.	18S	Earth Microbio me					
Ortrhocladiin ae sp.	18S	Earth Microbio me					
<i>Chironmus</i> sp.	CO1	HCO1		99.56-99.85%	<i>Chironomus</i> sp.	99.39%	HQ944865.1
<i>Chironmus</i> sp.	CO1	HCO1			<i>Chironomus</i> sp.	99.39%	HQ944865.1
<i>Chironmus</i> sp.	CO1	HCO1			<i>Chironomus</i> sp.	99.24%	HQ944865.1
<i>Chironmus</i> sp.	CO1	HCO1					
<i>Chironmus</i> sp.	CO1	HCO1					
<i>Chironmus</i> sp.	18S	Earth Microbio me		100%	<i>Dicrotendipes fumidus</i>	97.52%	AY821866.1
<i>Chironmus</i> sp.	18S	Earth Microbio me			<i>Dicrotendipes fumidus</i>	97.56%	AY821866.1

<i>Chironmus</i> sp.	18S	Earth Microbio me					
<i>Chironmus</i> sp.	18S	Earth Microbio me					
<i>Chironmus</i> sp.	18S	Earth Microbio me					
<i>Taphrocerus</i> <i>gracilis</i>	CO1	HCO1		99.57-100%	<i>Taphrocerus</i> <i>shannoni</i>	88.36%	KM364340.1
<i>Taphrocerus</i> <i>gracilis</i>	CO1	HCO1			<i>Taphrocerus</i> <i>shannoni</i>	88.24%	KM364340.1
<i>Taphrocerus</i> <i>gracilis</i>	CO1	HCO1			<i>Taphrocerus</i> <i>shannoni</i>	87.60%	KM364340.1
<i>Taphrocerus</i> <i>gracilis</i>	CO1	HCO1			<i>Taphrocerus</i> <i>shannoni</i>	88.34%	KM364340.1
<i>Taphrocerus</i> <i>gracilis</i>	CO1	HCO1			<i>Taphrocerus</i> <i>shannoni</i>	88.05%	KM364340.1
<i>Taphrocerus</i> <i>gracilis</i>	18S	Earth Microbio me			<i>Trochoideus</i> <i>goudoti</i>	96.99%	GQ302217.1
<i>Taphrocerus</i> <i>gracilis</i>	18S	Earth Microbio me			<i>Tenebrio</i> <i>molitor</i>	97.04%	X07801.1
<i>Taphrocerus</i> <i>gracilis</i>	18S	Earth Microbio me					
<i>Taphrocerus</i> <i>gracilis</i>	18S	Earth Microbio me					
<i>Taphrocerus</i> <i>gracilis</i>	18S	Earth Microbio me					
<i>Phlaeothrips</i> sp.	CO1	LepF1					
<i>Phlaeothrips</i> sp.	CO1	LepF1					

<i>Phlaeothrips</i> sp.	CO1	LepF1					
<i>Phlaeothrips</i> sp.	CO1	LepF1					
<i>Phlaeothrips</i> sp.	CO1	LepF1					
<i>Phlaeothrips</i> sp.	18S	Earth Microbio me					
<i>Phlaeothrips</i> sp.	18S	Earth Microbio me					
<i>Phlaeothrips</i> sp.	18S	Earth Microbio me			<i>Haplothrips chinensis</i>	98.86%	JQ259052.1
<i>Phlaeothrips</i> sp.	18S	Earth Microbio me					
<i>Phlaeothrips</i> sp.	18S	Earth Microbio me					
<i>Dasyhelea</i> sp.	CO1	HCO1		99.41-99.71%	Ceratopogoni dae sp.	99.70%	JF871649.1
<i>Dasyhelea</i> sp.	CO1	HCO1					
<i>Dasyhelea</i> sp.	CO1	HCO1			Ceratopogoni dae sp.	99.39%	JF871649.1
<i>Dasyhelea</i> sp.	CO1	HCO1			Ceratopogoni dae sp.	99.85%	JF871649.1
<i>Dasyhelea</i> sp.	CO1	HCO1					
<i>Dasyhelea</i> sp.	18S	Earth Microbio me					
<i>Dasyhelea</i> sp.	18S	Earth Microbio me					

<i>Dasyhelea</i> sp.	18S	Earth Microbio me					
<i>Dasyhelea</i> sp.	18S	Earth Microbio me			<i>Culicoides imicola</i>	93.53%	AF074019.1
<i>Dasyhelea</i> sp.	18S	Earth Microbio me					
<i>Spangbergiel la vulnerata</i>	CO1	LepF1		100.00%	<i>Spangbergiella viridis</i>	97.24%	KX809678.1
<i>Spangbergiel la vulnerata</i>	CO1	LepF1			<i>Spangbergiella viridis</i>	97.24%	KX809678.1
<i>Spangbergiel la vulnerata</i>	CO1	LepF1			<i>Spangbergiella viridis</i>	97.13%	KX809678.1
<i>Spangbergiel la vulnerata</i>	CO1	LepF1			<i>Spangbergiella viridis</i>	97.11%	KX809678.1
<i>Spangbergiel la vulnerata</i>	CO1	LepF1					
<i>Spangbergiel la vulnerata</i>	18S	Earth Microbio me		100%	Cicadellidae sp.	100%	KJ461311.1
<i>Spangbergiel la vulnerata</i>	18S	Earth Microbio me					
<i>Spangbergiel la vulnerata</i>	18S	Earth Microbio me			Cicadellidae sp.	100%	KJ461311.1
<i>Spangbergiel la vulnerata</i>	18S	Earth Microbio me					
<i>Spangbergiel la vulnerata</i>	18S	Earth Microbio me					
<i>Aphelonema simplex</i>	CO1	HCO1		100%	<i>Aphelonema simplex</i>	99.54%	KR035340.1
<i>Aphelonema simplex</i>	CO1	HCO1			<i>Aphelonema simplex</i>	99.54%	KR035340.1

<i>Aphelonema simplex</i>	CO1	HCO1					
<i>Aphelonema simplex</i>	CO1	HCO1					
<i>Aphelonema simplex</i>	CO1	HCO1					
<i>Aphelonema simplex</i>	18S	Earth Microbio me		100%	<i>Bruchomorph a</i> sp.	98.06%	DQ532544.1
<i>Aphelonema simplex</i>	18S	Earth Microbio me			<i>Bruchomorph a</i> sp.	98.08%	DQ532544.1
<i>Aphelonema simplex</i>	18S	Earth Microbio me					
<i>Aphelonema simplex</i>	18S	Earth Microbio me					
<i>Aphelonema simplex</i>	18S	Earth Microbio me					
<i>Trigonotylus uhleri</i>	CO1	LepF1			<i>Trigonotylus longipes</i>	92.89%	KR034293.1
<i>Trigonotylus uhleri</i>	CO1	LepF1			<i>Trigonotylus longipes</i>	93.36%	KR034293.1
<i>Trigonotylus uhleri</i>	CO1	HCO1			<i>Trigonotylus longipes</i>	88.19%	KR034293.1
<i>Trigonotylus uhleri</i>	CO1	LepF1					
<i>Trigonotylus uhleri</i>	CO1	LepF1					
<i>Trigonotylus uhleri</i>	18S	Earth Microbio me		100%	<i>Trigonotylus</i> sp.	100%	AY252238.1
<i>Trigonotylus uhleri</i>	18S	Earth Microbio me			<i>Trigonotylus</i> sp.	100%	AY252238.1

<i>Trigonotylus uhleri</i>	18S	Earth Microbio me			<i>Trigonotylus</i> sp.	100%	AY252238.1
<i>Trigonotylus uhleri</i>	18S	Earth Microbio me			<i>Trigonotylus</i> sp.	100%	AY252238.1
<i>Trigonotylus uhleri</i>	18S	Earth Microbio me			<i>Trigonotylus</i> sp.	100%	AY252238.1
<i>Chaetopsis aenea</i>	CO1	LepF1					
<i>Chaetopsis aenea</i>	CO1	LepF1			<i>Scaptodrosop hila sp.</i>	87.55%	KR070840.1
<i>Chaetopsis aenea</i>	CO1	LepF1		99.30-100%	Drosophilidae sp.	88.74%	HM386285.1
<i>Chaetopsis aenea</i>	CO1	LepF1			<i>Scaptodrosop hila sp.</i>	87.65%	KR070840.1
<i>Chaetopsis aenea</i>	CO1	LepF1					
<i>Chaetopsis aenea</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.74%	EU179519.2
<i>Chaetopsis aenea</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.80%	EU179519.2
<i>Chaetopsis aenea</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.82%	EU179519.2
<i>Chaetopsis aenea</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.80%	EU179519.2
<i>Chaetopsis aenea</i>	18S	Earth Microbio me					
<i>Culicoides mississippiensis</i>	CO1	HCO1					

<i>Culicoides mississippiensis</i>	CO1	HCO1					
<i>Culicoides mississippiensis</i>	CO1	HCO1					
<i>Culicoides mississippiensis</i>	CO1	HCO1					
<i>Culicoides mississippiensis</i>	CO1	HCO1					
<i>Culicoides mississippiensis</i>	18S	Earth Microbiome			<i>Culicoides imicola</i>	98.84%	AF074019.1
<i>Culicoides mississippiensis</i>	18S	Earth Microbiome					
<i>Culicoides mississippiensis</i>	18S	Earth Microbiome			<i>Culicoides imicola</i>	98.85%	AF074019.1
<i>Culicoides mississippiensis</i>	18S	Earth Microbiome					
<i>Culicoides mississippiensis</i>	18S	Earth Microbiome					
<i>Isohydnocera aegra</i>	CO1	LepF1		99.93%			
<i>Isohydnocera aegra</i>	CO1	LepF1			<i>Isohydnocera curtipennis</i>	87.42%	KC524594.1
<i>Isohydnocera aegra</i>	CO1	LepF1			<i>Tarsostenodes guttulus</i>	88.25%	KC524666.1
<i>Isohydnocera aegra</i>	CO1	LepF1			<i>Isohydnocera curtipennis</i>	87.58%	KC524594.1
<i>Isohydnocera aegra</i>	CO1	LepF1					

<i>Isohydnocera aegra</i>	18S	Earth Microbio me		100%			
<i>Isohydnocera aegra</i>	18S	Earth Microbio me			<i>Tenebrio molitor</i>	98.25%	X07801.1
<i>Isohydnocera aegra</i>	18S	Earth Microbio me			<i>Tenebrio molitor</i>	98.26%	X07801.1
<i>Isohydnocera aegra</i>	18S	Earth Microbio me			<i>Tenebrio molitor</i>	98.25%	X07801.1
<i>Isohydnocera aegra</i>	18S	Earth Microbio me					
<i>Chaetopsis fulvifrons</i>	CO1	LepF1		100.00%	<i>Pseudogripho neura sp.</i>	91.12%	KT272855.1
<i>Chaetopsis fulvifrons</i>	CO1	LepF1			<i>Pseudogripho neura sp.</i>	91.12%	KT272855.1
<i>Chaetopsis fulvifrons</i>	CO1	LepF1			<i>Ptecticus aurifer</i>	89.26%	KT225297.1
<i>Chaetopsis fulvifrons</i>	CO1	LepF1			<i>Pseudogripho neura sp.</i>	89.57%	KT272855.1
<i>Chaetopsis fulvifrons</i>	CO1	LepF1			<i>Ptecticus aurifer</i>	89.21%	KT225297.1
<i>Chaetopsis fulvifrons</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.80%	EU179519.2
<i>Chaetopsis fulvifrons</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.82%	EU179519.2
<i>Chaetopsis fulvifrons</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.82%	EU179519.2
<i>Chaetopsis fulvifrons</i>	18S	Earth Microbio me					

<i>Chaetopsis fulvifrons</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.82%	EU179519.2
<i>Ischnodemus conicus</i>	CO1	HCO1		99.70-99.85%	<i>Ischnodemus falicus</i>	88.91%	KR032103.1
<i>Ischnodemus conicus</i>	CO1	HCO1					
<i>Ischnodemus conicus</i>	CO1	HCO1			<i>Ischnodemus falicus</i>	88.75%	KR032103.1
<i>Ischnodemus conicus</i>	CO1	HCO1			<i>Ischnodemus falicus</i>	89.06%	KR032103.1
<i>Ischnodemus conicus</i>	CO1	HCO1			<i>Ischnodemus falicus</i>	89.06%	KR032103.1
<i>Ischnodemus conicus</i>	18S	Earth Microbio me			<i>Henestaris oschanini</i>	94.89%	AY324853.1
<i>Ischnodemus conicus</i>	18S	Earth Microbio me					
<i>Ischnodemus conicus</i>	18S	Earth Microbio me					
<i>Ischnodemus conicus</i>	18S	Earth Microbio me					
<i>Ischnodemus conicus</i>	18S	Earth Microbio me					
<i>Chaetopsis debilis</i>	CO1	LepF1		99.56%	Drosophilidae sp.	87.55%	HM386285.1
<i>Chaetopsis debilis</i>	CO1	LepF1			<i>Paraleucophe nga longiseta</i>	86.67%	JF273073.1
<i>Chaetopsis debilis</i>	CO1	LepF1			Drosophilidae sp.	88.05%	HM386285.1
<i>Chaetopsis debilis</i>	CO1	LepF1			Drosophilidae sp.	88.70%	HM386285.1
<i>Chaetopsis debilis</i>	CO1	HCO1					

<i>Chaetopsis debilis</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.80%	EU179519.2
<i>Chaetopsis debilis</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.80%	EU179519.2
<i>Chaetopsis debilis</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.80%	EU179519.2
<i>Chaetopsis debilis</i>	18S	Earth Microbio me			<i>Anastrepha ludens</i>	98.80%	EU179519.2
<i>Chaetopsis debilis</i>	18S	Earth Microbio me					
<i>Mordellisten a sp.</i>	CO1	LepF1					
<i>Mordellisten a sp.</i>	CO1	LepF1					
<i>Mordellisten a sp.</i>	CO1	LepF1					
<i>Mordellisten a sp.</i>	CO1	LepF1					
<i>Mordellisten a sp.</i>	CO1	LepF1					
<i>Mordellisten a sp.</i>	18S	Earth Microbio me			<i>Meloe proscarabaeu s</i>	96.51%	X77786.1
<i>Mordellisten a sp.</i>	18S	Earth Microbio me					
<i>Mordellisten a sp.</i>	18S	Earth Microbio me					
<i>Mordellisten a sp.</i>	18S	Earth Microbio me					

<i>Mordellistena</i> sp.	18S	Earth Microbiome					
<i>Collops nigriceps</i>	HCO1	LepF1					
<i>Collops nigriceps</i>	HCO1	LepF1					
<i>Collops nigriceps</i>	HCO1	LepF1			<i>Collops tricolor</i>	94.39%	HM905916.1
<i>Collops nigriceps</i>	HCO1	LepF1					
<i>Collops nigriceps</i>	HCO1	LepF1					
<i>Collops nigriceps</i>	18S	Earth Microbiome		100%			
<i>Collops nigriceps</i>	18S	Earth Microbiome			<i>Trichodes ornatus</i>	99.37%	KC966562.1
<i>Collops nigriceps</i>	18S	Earth Microbiome			<i>Trichodes ornatus</i>	99.30%	KC966562.1
<i>Collops nigriceps</i>	18S	Earth Microbiome			<i>Trichodes ornatus</i>	99.40%	KC966562.1
<i>Collops nigriceps</i>	18S	Earth Microbiome					



Table 14 (Placeholder) Images of insect taxa that were found to be significant indicators of different salinity zones within Barataria and Caillou bays in Louisiana's coastal marshes.

3.4 Discussion

The mid salinity zone had the least amount of indicator families with potential bioindicator species that did not overlap with another salinity zone (Table 11., which is logical due to the extension of the indicator value analysis which allowed for determining indicators for a combination of classes of sites as opposed to a single class of sites. Since the mid-salinity zone was intermediary to the other salinity zones, more families that would have been assigned to this individual salinity were associated with the combination of low or high salinity zones. Additionally, multiple Hymenopteran families (selected for further identification) for the mid

salinity zone were not used as extensive barcoding and knowledge of the life history of individuals present is often necessary for identification.

Of the families found to have bioindicator species, only five were found to have more than one significant bioindicator species (Table 12), and for only seven families (Buprestidae, Cicadellidae, Coenagrionidae, Chironomidae, Ceratopogonidae, Miridae, and Ulidiidae) were individuals of more than one species present in this collection. A total of two species from Buprestidae, four species from Cicadellidae, two species from Coenagrionidae, four morphospecies from Chironomidae, three species from Ceratopogonidae, and one species from Miridae were not found to be significant indicators for any salinity zone or groups of salinity zones. The families with more than one significant indicator species were Dipteran, except for one Coleopteran and one Hemipteran family. Additionally, the families with multiple significant indicator species tended to be indicators of lower salinity levels, with 8 of the 15 indicating low or the combination of low and mid salinity which was expected as insect family-level biodiversity was found to be inversely correlated with salinity (Chapter 2, Table 8). Similarly, the majority (14 out of 17) of the indicators for the low salinity zone, combination of low and mid salinity zones, and mid salinity zones were broadly distributed outside of coastal habitats while this was true only for two of the nine indicators from the combination of mid and high salinity zones and the high zone.

Of the eleven taxa found to be low salinity zone indicators, three are coastally regulated and are likely particularly useful for positively indicating the presence of healthy intermediate marsh. These species which include a lady beetle, a planthopper, and a non-biting midge have varied life cycles and represent multiple functional feeding groups. The first coastally bound

indicator is *Naemia seriata* (Melsheimer), the seaside ladybug. *N. seriata* (Table 14) is omnivorous; both immatures and adults of this species are known to feed on *Spartina* pollen and are voracious predators of certain *Spartina* associated insects, especially *Duplachionaspis spartinae* (Comstock) a scale insect in the family Diaspididae (Finke 2005, Finke and Denno 2005). The abundances, fecundity, and survival rates of *N. seriata* are all highly correlated to the co-occurrence of both its predominant prey and *Spartina* pollen (Rinehart 2018). Additionally, as individuals of *N. seriata* do not face predation by other insects or spiders common in this environment, the abundance of *N. seriata* is primarily tied to the availability marsh resources (Finke 2005, Rinehart 2018). Specimens of *N. seriata* are highly mobile and seek out both *Spartina* and scale insect prey using chemical cues and have been shown to select habitat most likely to provide individual success (Rinehart 2018). Due to the mobility of *N. seriata*, its ability to select habitat where both *Spartina* pollen and dense populations of *Spartina* associated herbivores are presents, and the strong correlation of its abundances to those factors makes the collection of this species highly indicative of healthy, intermediate, tidal marsh.

The nonbiting midge identified here as *Tanytus* sp. is likely *Tanytus clavatus* (Beck), which is an abundant member of the genus commonly found in Louisiana's coastal marshes (Goldfinch and Carman 2000, Maddi 2003). *Tanytus clavatus* immatures have been shown to temporally rely on *Spartina* detritus as a food source, to represent a relatively large portion of the biomass that results from grazing on benthic microalgae when compared to other meiofauna (fauna small enough to pass through 500 μm sieves, but large enough to be caught by 63 μm sieves), and to be important members at the bottom of the food chain, transferring

energy from primary producers to higher trophic levels (Goldfinch and Carman 2000, Maddi 2003). Since *Tanytus clavatus* immatures and other meiofauna rely on mostly identical food sources in Louisiana's marshes, collection of adults is likely to indicate the presence of intermediate, tidal marsh and both a healthy meiofaunal community and food web.

Draeculacephala portola (Ball) is a sharpshooter endemic to tidal marshes of the Gulf and Atlantic coasts (Dietrich 1994, Sokolov et al. 2018). *Spartina* spp. are the purported but unconfirmed host plants for this species (Dietrich 1994). Individuals were collected from sites where *Spartina patens* dominated, but *S. alterniflora* was present and also from sites where *Spartina patens* was the sole occurring *Spartina* species. The presence of *D. portola* in collections is indicative of intermediate, tidal marsh likely with enough *Spartina* grasses to sustain immature and adult populations.

The remaining species identified as characteristic indicators of the low salinity zone in this study are widely distributed outside of coastal, tidal marshes. As a result, these species are likely better indicators of low salinity habitat as opposed to intermediate, tidal marsh. While these species are less positive identifiers of intermediate salinity marsh habitat, their presence along with intermediate marsh indicators that are coastally regulated helps to confirm that the habitat is correctly identified as intermediate as opposed to brackish or saline marsh. Additionally, some of these species have life cycles closely tied to certain plant species and the presence of these indicators can give additional insight on the plant diversity in sampled marsh habitat. As such, these species play an important role in an assemblage of indicators.

The species with the highest indicator value for the low salinity zone was *Taphrocerus agriloides* (Kerremans). Members of the genus *Taphrocerus* are stem borers of rushes as immatures; *T. agriloides* specimens have been collected in large series outside of tidal marshes from a species of spikerush in the genus *Eleocharis* (Macrae 1991). In this study, specimens of *T. agriloides* were primarily taken both from sites where chairmaker's bulrush (*Schoenoplectus americanus*) and a species of *Eleocharis* were simultaneously present and from the same sites during periods where only *Schoenoplectus americanus* was present. The collection of *Taphrocerus agriloides* from coastal marshes is likely to indicate intermediate marsh habitat in combination with the occurrence of rush species suitable for immatures.

Ischnura ramburii (Selys) is a damselfly that is widely distributed throughout coastal areas of the Southeastern United States ranging from Maine and Indiana as well as south through Mexico and Central America (Paulson 2009). Immatures of this damselfly are aquatic predators and have been known to develop in water with elevated salinities (Pearse 1932). *Ischnura ramburii* appears to reach the upper range of its salinity tolerance at our intermediate marsh sites and collection of this species is primarily indicative of aquatic habitat suitable for immatures with lower salinity content than that present at brackish marsh.

Of the remaining six intermediate marsh indicators, five were Auchenorrhynchan hoppers within the order Hemiptera and the sixth was a dipteran in the family Ceratopogonidae. The remaining taxa are either reported to be broad generalists with many host plants or little is known about their life history; therefore, their presence does not give additional insight into marsh conditions besides salinity. However, collection of these species in combination with coastally endemic species strengthens the identification of intermediate

marsh. As these species are at the likely limited to intermediate marsh due to the extent of their biological salt tolerance, their ranges can be expected to expand in response to freshwater and sediment diversions.

The first of these generalist species is *Cedusa obscura* (Ball), a derbid planthopper, which is widely distributed throughout the United States and is abundant in collections where it is present (Flynn and Kramer 1983, Bartlett and Bowman 2004, Page 2017). However, little is known about the life history of this species. Many members of this genus don't have well defined host plants, although they tend to be associated with trees and shrubs, and the immatures of most species are cryptic (Flynn and Kramer 1983).

The three-cornered alfalfa hopper, *Spissistilus festinus* (Say), is an economically important agricultural pest present throughout North America and Central America (Deitz and Wallace 2012). *S. festinus* has numerous host plants, many of which are legumes and grasses (Dietrich et al. 1999). The presence of this species at intermediate marsh could possibly be explained by the seasonally abundant hairy cowpea (*Vigna luteola*) which is abundant at roughly similar time periods.

The intermediate marsh indicator, Deltacephalinae sp., is likely *Graminella nigrifrons* (Forbes), a species that was collected from similar marshes by Sokolov et al. (2018) and also matched to our Deltacephalinae sp. ($\geq 99.18\%$) in the NCBI BLAST database (Table). This species is present throughout much of North America and feeds on a wide variety of grass species (Stoner and Gustin 1967, Dmitriev 2019).

Clastoptera xanthocephala (Germar), the sunflower spittlebug, is a generalist herbivore that is widely distributed across the United States and Mexico (Hamilton 1982, Soulier-Perkins 2020). While preferring sunflower (*Helianthus* spp.) and ragweed (*Ambrosia* sp.), *C. xanthocephala* has previously been collected from *Baccharis* shrubs in tidal marsh habitat (Ball 1927, Lago and Testa 2000). However, in this study the only shrubs present at sites where *C. xanthocephala* was collected were individuals of *Iva frutescens*, the saltmarsh elder. The low abundances of this species and lack of known host plants at collection sites make it likely that this species is transient in coastal marshes.

The intermediate marsh indicator identified as a member of the genus *Empoasca* in this study is likely *Empoasca solana* (DeLong), the sole member of the genus collected by Sokolov et al. (2018) and which was most abundant in August in both studies. This species has been recorded broadly from North America to northern South America and has a wide variety of host plants including grasses, shrubs, and trees (Ross et al. 1964, Moffitt and Reynolds 1972). The usefulness of this species as an intermediate marsh indicator is unclear since it was collected frequently at sites resembling our brackish and saline marsh sites in both salinity and dominant vegetation by Sokolov et al. (2018).

Palpomyia subaspera (Coquillett), a common predaceous midge in the family Ceratopogonidae, is distributed from Canada south to Argentina and Chile (Borkent and Grogan 2009, Spinelli et al. 2009, Grogan et al. 2010). Little is known about the life history of this species other than it can be found in large numbers on the edges of streams, ponds, and lakes (Spinelli et al. 2009).

Of the two indicators for the combination of intermediate and brackish marsh sites, a *Chironomus* sp. was by far the most common. Nearly, 3,000 individuals of this species were collected during the period of its peak abundance (November through April). Identification past the genus level was not possible and thus is not a likely candidate as there were for the *Empoasca* and Deltacephalinae species. However, no intraspecific variation was found for either loci among any of the individuals of this species that were sequenced, and the use of DNA barcoding ensures that future studies can match collected Chironomids to this species if applicable.

A metallic wood boring beetle in the family Buprestidae, *Taphrocerus gracilis* (Say), was the second indicator for the combination of intermediate and brackish marsh sites. Similar to *T. agriloides*, immatures of this species are leaf miners of Cyperaceae (Macrae 1991). Intermediate marsh sites had a combination of *Schoenoplectus americanus* and an *Eleocharis* sp. as potential host plants, but at brackish marsh sites the only likely host was *Bolboschoenus robustus*, the saltmarsh bulrush.

Brackish marsh had the fewest characteristic indicators of the three different salinity zones; none of the four characteristic indicators are coastally restricted. However, one species is still a particularly strong indicator for brackish marsh habitat. *Aphelonema simplex* (Uhler), a piglet bug in the family Issidae, is a member of both tidal marsh and tallgrass prairie communities resulting from being a specialist on two congeneric host plants, *Spartina patens* and *Spartina pectinata* (Denno 1980, Johnson and Knapp 1993). These communities are separate due to *Spartina patens* being coastally restricted whereas *Spartina pectinata* is unique among the genus in that it is an inland species and is particularly abundant in the Midwestern

United States and Canada (Johnson and Knapp 1993). As such, the collection of *Aphelonema simplex* from costal habitat is positively indicative of both *Spartina patens* and brackish marsh habitat.

Spangbergiella vulnerata (Uhler) is a Cicadellid planthopper broadly distributed from South America north to the Gulf Coast and Eastern states within the United States (Ma 1988, Hamilton 2000). *S. vulnerata* is abundant primarily on a limited number of grass species but is additionally known to feed on the agriculturally important Manila palm, *Adonidia merillii* (Osborn 1926, Tsai and Fisher 1993, Hamilton 2000). Within tidal habitat, this species is highly associated with the high marsh grass *Distichlis spicata* (Davis and Gray 1966), and in this study *S. vulnerata* was exclusively collected at brackish marsh sites where this graminoid was abundant. Therefore, *S. vulnerata* is particularly useful as an indicator since it was the only species associated with this important marsh grass in this study.

The remaining two brackish marsh indicators were identified to species group for *Dasyhelea* and genus for *Phlaeothrips*. However, unlike other indicators that could not be identified to the species level in this study, there were no likely matches in the literature for these morphospecies. As such, life history data for the relevant genera, and species group for the *Dasyhelea* species, were used for interpretation.

While the *Dasyhelea* sp. collected in this study belongs to the biting midges in the family Ceratopogonidae, members of this genus are thought to feed exclusively on honeydew, plant secretions, and nectar (Waugh and Wirth 1976). Adults of this genus are typically found near semi-aquatic to aquatic habitats appropriate for their larvae (Waugh and Wirth 1976). This

species belongs to the *Dasyhelea grisea* species group of which five species occur within the eastern United States; among these one is a tree hole specialist and the other has a distribution that does not approach Louisiana making these species unlikely candidates (Waugh and Wirth 1976).

The final brackish marsh indicator is a tube-tailed thrip in the genus *Phlaeothrips*. This genus belongs within the subfamily Phlaeothripinae which has classically been divided into three lineages based on feeding habits and structural characters (Mound and Marullo 1996, Minaei 2013). While two of the lineages of Phlaeothripinae are well established and discrete, the fungus feeding *Phlaeothrips* lineage is understudied and likely polyphyletic (Buckman et al. 2013). Knowledge of the life history of the genus *Phlaeothrips* is limited to what is typical of the *Phlaeothrips* lineage. Members of this lineage are often subsocial, living in colonies near feeding and oviposition sites associated with plant detritus (Crespi 1986, Minaei 2013). Adults are typically sexually dimorphic and competition between males determines access to oviposition sites (Crespi 1986). As such, the collection of this species is likely highly indicative of the presence of suitable plant detritus in addition to the being indicative of brackish marsh.

A total of four indicator species were found for the combination of brackish and saline marsh. Of these indicators, the dipteran *Chaetopsis aenea* (Weidemann), has two congeneric species that were identified as indicators of saline marsh. As such, it is discussed along with the other *Chaetopsis* spp. below. The indicator with the highest IndVal for the combination of brackish and saline marsh sites, *Trigonotylus uhleri* (Reuter), is native to the Atlantic and Gulf coasts and is a specialist on *Spartina alterniflora* and potentially *S. patens* (Davis and Gray 1966, Kelton 1971, Denno 1977). Denno (1977) expressed doubt that *S. patens* is a host plant of *T.*

uhleri, but both Davis and Gray (1966) and this study found large abundances of *T. uhleri* from *Spartina patens* dominated sites. However, the presence of individuals of *Trigonotylus uhleri* at the brackish marsh sites in this study is potentially explained by the presence of a band of *S. alterniflora* common along the marsh edge among these sites. Outside of *Prokelisia* hoppers, *T. uhleri* is one of the most abundant sap-feeders present on *S. alterniflora* with abundances of up to 3,000 individuals per m²; but, even at these extreme densities, feeding by individuals of *T. uhleri* does not inhibit *S. alterniflora* growth (Daehler and Strong 1995).

Culicoides mississippiensis (Hoffman), a biting midge in the family Ceratopogonidae, was found to be an indicator of the combination of brackish and saline marsh sites. This species is limited to the Gulf Coast where its immatures develop in soil primarily among stands of *Spartina alterniflora* and less commonly *Distichlis spicata* (Blanton and Wirth 1979, Wood and Kline 1989). Additionally, *Spartina alterniflora* has been shown to be a preferred resting place for both male and female adults of *C. mississippiensis* (Lillie and Kline 1986). As the adults prefer resting near immature habitat and are weak fliers, the presence of the adults is likely an indicator of suitable immature habitat.

Relatively little is known of the life history of *Wolcottia aegra* (Newman), a checkered beetle in the family Cleridae that is uncommon in collections and apparently endemic to coastal marshes of the southeastern United States (Wheeler and Stocks 2009). This is the first record of this species occurring in Louisiana and also the first record of its association with *Spartina alterniflora*. Specimens were collected both from sites where plants that were previously associated with this species, *Spartina patens* and *Distichlis spicata*, were present and from sites where none of its known plant associations occurred such as the high salinity zone at Barataria,

which consisted primarily of large stands of *Spartina alterniflora*. Previous collections of this species also have been made from both *S. bakeri* and *Eragrostis curvula*, an introduced grass species (Wheeler and Stocks 2009). This species is both assumed to be predaceous (Davis and Gray 1966) and are believed to be mimics of *Pseudomyrmecine* ants (Wheeler and Stocks 2009). The feeding habits of *Wolcottia aegra* were not observed in this study. However, ants in the genus *Pseudomyrmex* were present at the same sites as *W. aegra*.

The species that were determined to be indicators of saline marsh in this study are all highly associated to *Spartina alterniflora* which was the dominant plant species at all saline marsh sites. The first saline marsh indicator, *Ischnodemus conicus* (Van Duzee), is a chinch bug in the family Blissidae. This species is distributed coastally along the Gulf Coast marshes and in Atlantic marshes from Virginia southward (Slater and Baranowski 1990). The sole host plant for *I. conicus* is *Spartina alterniflora* (Harrington 1972) and Slater and Baranowski (1990) note that that Wood reported in a personal communication the finding of “adults and nymphs in the seed head of the same host”. *I. conicus* is present year-round, although abundances are highly seasonal (Table 12), and has been recorded as a prey species for the seaside sparrow, *Ammodramus maritimus* (Wilson), which is another indicator of healthy salt marsh habitat (Slater and Baranowski 1990). Armitage et al. (2013) found that this species is highly intolerant to disturbance of *S. alterniflora* as many individuals are micropterous with little dispersive ability, with abundances up to 75% lower at disturbed sites and independent of patch size (Armitage et al. 2013). Thus, *I. conicus* populations are particularly useful for identifying and comparing disturbances within saline marsh across varying spatial scales.

The only omnivorous indicator of saline marsh identified in this study was the black-headed Melyrid, *Collops nigriceps* var. *floridanus* (Say). *Collops nigriceps* is common along the Atlantic and Gulf coasts from both tidal marsh and dune-strand habitat, although it does less regularly appear inland in agricultural landscapes (Fall 1912, Richmond 1962, Wagner 1964, Davis and Gray 1966, Johnson et al. 1974, Gardiner et al. 2011, Adams et al. 2017). *C. nigriceps* var. *floridanus* is more southern in distribution than the type species and can be differentiated by the having a less prominent to absent prothoracic spot (Fall 1912). Specimens taken from dune-strand are typically collected from sea oats (*Uniola paniculata*), the pollen of which is the apparent main source of food for *C. nigriceps* in this habitat (Wagner 1964). From tidal marsh environments, this species is highly associated with *S. alterniflora* (Davis and Gray 1966); whether *S. alterniflora* pollen is similarly important in the diet of *Collops nigriceps* is unknown. However, this species appears to be bivoltine in our collection with two distinct population peaks over the course of the year, the second of which was concurrent with the flowering of *S. alterniflora* (Fang 2002). Davis and Gray (1966) observed this species preying on injured flies in tidal marshes. While further study is needed to determine if *Collops nigriceps* var. *floridanus* relies on *S. alterniflora* pollen, the presence of this species could potentially be a strong indicator of a healthy, interconnected food web similar to *Naemia seriata* at intermediate salinities.

The saline marsh indicator *Mordellistena splendens* (Smith) is a tumbling flower beetle in the family Mordellidae. This species is a stem borer of *Spartina alterniflora*, *S. alternifolia*, and *S. cynosuroides* with a relatively well-known life history (Stilling and Strong 1963, Strong et al. 1984). Individuals of *M. splendens* feed on the seed heads and flowers of *Spartina* spp. and

oviposit on the upper portions of the stem; the immatures then bore downwards into the stem where they feed and undergo metamorphosis before emerging (Stilling and Strong 1963). In a study by Gaeta and Kornis (2011), a Mordellid species that was likely *M. splendens* was found to be absent in *Spartina alterniflora* marshes that were experiencing die back. As such, the presence of this species is potentially a useful indicator of healthy saline marsh, especially if used in conjunction with other indicators.

The remaining bioindicators identified in this study, one for the combination of brackish and saline marsh and two for saline marsh are picture-winged flies in the genus *Chaetopsis*. Outside of tidal marshes, immatures of the *Chaetopsis* are commonly secondary invaders of corn, species name, preferring to utilize the holes made by other species, typically corn-boring moths. Within tidal marshes, *Chaetopsis* immatures are stem borers of *Spartina alterniflora*. Adults *Chaetopsis* species in tidal marshes have been observed running their sponging mouthparts across *Spartina alterniflora*, feeding on plant excretions (Davis and Gray 1966, Marples 1966).

The species found indicative of the combination brackish and saline marsh sites, *Chaetopsis aenea*, is endemic to brackish and salt marsh habitat along the Gulf and Atlantic coasts (Weiss 1924, Steyskal 1965). This species is a natural enemy of *Spartina alterniflora* and it is considered a promising potential biocontrol agent in areas where *S. alterniflora* is invasive (Grevstad et al. 2004, Viola et al. 2004). The feeding of a single immature of *C. aenea* on a *Spartina alterniflora* shoot will typically lead to death of the shoot; in contrast, approximately 200 *Prokelisia* hoppers would be required to cause the same amount of damage (Daehler and Strong 1997, Grevstad et al. 2004). Within this study, *C. aenea* was a statistically significant

indicator for both brackish and saline marsh sites, but the non-coastally regulated *Chaetopsis* species were more limited to saline marsh. In brackish marsh sites, *Spartina alterniflora* made up less of the total average ground cover than at saline marsh sites (Chapter 2, Figure 3), and the *S. alterniflora* that was present was limited to the marsh edge. As such, it seems likely that individuals of *Chaetopsis aenea* are more adapted to small patches of *S. alterniflora* than the more widely distributed congeneric species. *Chaetopsis apicalis*, another picture-winged fly that is often compared to *C. aenea* due to similarly being both coastally regulated and a potential control agent of *S. alterniflora*. This species was notably absent in this study and is predominately limited to the Atlantic coast.

Chaetopsis fulvifrons (Macquart), the second picture-winged fly identified in this study, is broadly distributed across the eastern United States from Wisconsin to Rhode Island and across the southern states from Florida to California (Steyskal 1965). Immatures of this species are known to inhabit yellow nutsedge (*Cyperus esculentes*) and barn-yard grass (*Echinochloa crusgalli*) in addition to being a pest of corn and *S. alterniflora* (Davis and Gray 1966, Valley et al. 1969, Langille 1975, Knutson 1987, Phatak et al. 1987).

The final indicator of saline marsh, *Chaetopsis debilis* (Loew), is distributed throughout the southern United States, Mexico, and Cuba (Steyskal 1965). Outside of corn, the host plants of this species are less well known (Langille 1975). It is likely that *Spartina alterniflora* represents an additional host for immatures of this species, but its presence at saline marsh sites could alternatively be transitory in nature as this species has been collected in light traps in the Gulf of Mexico 32 kilometers from the shore line (Sparks et al. 1986).

In this study, 26 bioindicator taxa were identified across 3 marsh types, 11 for intermediate marsh, 2 for the combination of intermediate and brackish marsh, 4 for brackish marsh, 4 for the combination of brackish and saline marsh, and 5 for saline marsh. Using an assemblage of bioindicators as opposed to a singular indicator ensures that species are more readily sampled across temporal scales (McGeoch et al. 2002). Additionally, the use of multiple bioindicators with well understood and potentially different life histories allows for better understanding of how the environment will react to various stressors or habitat management. An example of this application would be changing how varying salinity conditions would likely affect the identified bioindicator taxa and the underlying plant community. In areas where saltwater intrusion resulting from relative sea-level rise is a concern, shifts from lower salinity indicators to indicators of saline marsh would be expected, whereas in monitoring changes in salinity resulting from habitat restoration through freshwater and sediment diversions the opposite would be expected to occur. These shifts would likely be first noticed in indicator species with aquatic immatures (*Ischnura ramburii*) or species with immatures that develop in frequently inundated soil (*Culicoides mississippiensis*). The bioindicator taxa from the filterer feeding guild are particularly suited for monitoring changes in salinity as they are all aquatic or benthic, are not specifically tied to plant species, are indicators across multiple salinity zones. Additionally, due to the different periods of abundance for the adults in this bioindicator assemblage, *Ischnura ramburii* would be more useful for monitoring increases in salinity in the summer months, while *Culicoides mississippiensis* would be more useful in the late Fall to early Spring. Conversely, the sampling of immature populations could also provide a more time sensitive method.

Plant driven insect populations at *Spartina alterniflora* dominated saline marsh are likely particularly useful for monitoring stressors as well as the occurrence of salt marsh dieback. In a study of the stem-borers of *S. alterniflora*, *Chaetopsis* spp. were shown to become more abundant in coastal marsh experiencing dieback resulting from drought and elevated salinity conditions whereas the overall species richness of stem-borers decreased; notably, the stem-boring, saline marsh indicator *Mordellistena splendens* was absent at sites experiencing dieback (Gaeta and Kornis 2011). In the current study, the coastally endemic *Chaetopsis* species, *Chaetopsis aenea*, was found to be a significant indicator for brackish and saline marsh sites, while the two *Chaetopsis* species with a wider distribution were indicators only for the saline marsh sites. As infestation by this genus is highly tied to stressors affecting the host plant, it is possible that the elevated stress levels resulting from increased salinity at our saline marsh sites allow for the two non-endemic *Chaetopsis* species to become abundant, where *Chaetopsis aenea* would be more likely to infest individuals of *S. alterniflora* experiencing relatively less stress. If so, a shift in the relative abundances from endemic to non-endemic *Chaetopsis* species and an overall increase in *Chaetopsis* spp. would indicate increasing stress and potential for dieback in a coastal marsh regardless of the etiology. A monitoring program utilizing the *Chaetopsis* and *Mordellistena* stem-borers that additionally incorporates *Ischnodemus conicus*, a species that is sensitive to disturbance of *S. alterniflora* marsh independent of marsh patch size, and *Collops nigriceps* var. *floridanus*, an omnivore and top predator useful for monitoring the health of the food web, would be highly robust and suited for identifying healthy and at-risk saline marsh.

This assemblage of coastal marsh bioindicators could similarly be useful for identifying vulnerable or resistant habitat in the face of a disaster such as an oil spill. While *S. alterniflora* is negatively affected by oiling, *S. patens* is vastly more susceptible (Lin and Mendelssohn 1996). In the event of an oil spill the bioindicators identified in this study could be used to quickly inform habitat protection decisions. *S. patens* was the most dominant plant species at brackish sites, was less relatively less abundant at intermediate marsh sites, and completely absent from saline marsh (Chapter 2, Figure 3). Through the collection of bioindicator species, a ranking of the most to least vulnerable habitat could be identified. The *S. patens* associated *Apheloma simplex* at brackish marsh sites, the *Spartina* spp. associated *Draeculacephala portola* at intermediate sites, and any of the *Spartina alterniflora* associated saline marsh indicators would be particularly useful for this informing this decision, although other indicators may be more readily collectable for a certain salinity level depending on the time of the year. In the possible wake of an impending environmental disaster, a rapid inventory collection would be useful as an anchor to the data provided in this thesis.

Conclusions

Insect insect families within Louisiana's coastal marsh were inventoried acting as baseline data for future research exploring the effects on both stressors such as subsidence, relative sea level rise, pollution from oil spills, eutrophication, or coastal engineering or habitat restoration projects such as those outlined by the Louisiana Coastal Protection and Restoration Authorities. Comparisons in biodiversity and abundances of insect families were made between multiple salinity zones and marsh vegetation types in this study. Factors that most affect the biodiversity of the insect families were identified and differences in the abundances of insects in differing feeding guilds and trophic levels between salinity zones were explored.

A total of 26 bioindicator taxa were identified over the course of this year long inventory. This assemblage of insects are particularly useful as bioindicators as they are useful independent of the time of the year and because they have varied life histories, which allows for insights beyond just the salinity zone or marsh vegetation type.

Additionally, as these taxa have been imaged and DNA barcoded, facilitating their use for future research. Further studies in the marsh, particularly those using metabarcoding techniques, can easily reference this collection promoting interconnectivity in research.

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Vita

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