

# A comparison of two insect collection techniques in oiled and non-oiled salt marshes in Louisiana

Benjamin J. Adams<sup>1,2</sup>, Xuan Chen<sup>1,3</sup>, and Linda M. Hooper-Bùi<sup>1,3,\*</sup>

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## Abstract

Insects are important components in coastal marsh ecosystems and can be used as an indicator of marsh health. Collections of insects in coastal marshes are usually made using either sweep nets or insect vacuums. Differences in these 2 methods have not been compared within the marsh ecosystem. Therefore, we compared collections made using these methods in oiled and non-oiled marshes along multiple transects on the Louisiana coast. We found that sweep net collections accounted for 5.9 times more individual arthropods and more total insect taxa when compared with the insect vacuum collections. Oiled marshes had greater total insect richness but similar abundance and average richness as non-oiled sites; however, we found some changes in insect community composition between sites. These results indicate that sweep net collections are an effective means to sample insects in marsh environments and that oil intrusion can lead to changes in arthropod community structure in coastal marshes.

Key Words: *Spartina alterniflora*; sweep net; insect vacuum

## Resumen

Los insectos son componentes importantes en los ecosistemas de pantanos costeros y se pueden utilizar como un indicador de la salud de los pantanos. Se hacen recolecciones de insectos en los pantanos costeros con frecuencia utilizando redes de barrido o aspiradoras de insectos. No se han comparado las diferencias en estos 2 métodos dentro del ecosistema de pantano. Por lo tanto, comparamos las recolecciones realizadas utilizando estos métodos en marismas aceitosas y no aceitosas a lo largo de múltiples transectos de la costa de Luisiana. Encontramos que las recolecciones utilizando las redes representaron 5,9 veces más artrópodos individuales y más taxones de insectos totales en comparación con las colecciones hechas por aspiradoras de insectos. Las marismas con aceite tuvieron una mayor riqueza total de insectos pero una abundancia similar y un promedio de riqueza como sitios sin aceite; sin embargo, hubo algunos cambios en la composición de la comunidad de insectos entre los sitios. Estos resultados indican que la recolección de insectos con redes es una manera eficaz para muestrear insectos en ambientes de pantanos y que la intrusión de aceite puede resultar en cambios en la estructura de la comunidad de artrópodos en pantanos costeros.

Palabras Clave: *Spartina alterniflora*; red de barrido para insectos; aspiradora insectos

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Marshes play a critical role in coastal and marine ecosystems and human economies, especially in Louisiana, where almost half (approx. 40%) of the coastal marsh area of the United States is located (Erwin et al. 1981; Boesch & Turner 1984; Farber 1987; Bergstrom et al. 1990; Field et al. 1991; Fitz & Wiegert 1991; Turner 1992; Baltz et al. 1993; Peterson & Turner 1994; King & Lester 1995; Möller et al. 2001; Young & Phillips 2002; Minello et al. 2003). The coastal marsh ecosystem is currently threatened by sea level rise, changes in deltaic deposition, hurricanes, ecological disasters such as oil spills, and anthropogenic disturbances including dams, levees, canals, pipelines, and highways (Turner 1997).

Terrestrial arthropods are an important component to marsh and coastal community health and stability (Teal 1962). Insects and spiders quickly move biological material and energy through an ecosystem by acting as primary consumers of plant materials and a major food source for larger arthropods, fish, amphibians, birds, and mammals (Price 1997). They also act as independent detritus consumers, which are crucial to commercial fish stocks (Odum & de la Cruz 1967; Odum & Heald 1972). In addition, insects are accurate bioindicators for evaluating ecological conditions because of their importance to ecosystem

function and sensitivity to environmental changes and disturbances (McGeoch 1998). Although insects have widely been used as indicators in terrestrial and freshwater ecosystems (Duelli et al. 1999; Roy et al. 2003; Schulze et al. 2004), they are often overlooked in coastal wetlands studies (however, see Wimp et al. 2010; McCall & Pennings 2012).

A variety of techniques and equipment exist for collecting insects and other terrestrial arthropods (Triplehorn & Johnson 2005). However, many of these techniques are not suitable for the marsh environment because of flooding, exposure to salt, high winds, the lack of canopy cover, and many other factors. The most common procedures for collecting insects in marshes are sweep net sampling, vacuum sampling, and clip plots (Cameron 1972; Elkaim & Rybarczyk 2000; Finke & Denno 2004; Gratton & Denno 2005; Wu et al. 2009; Harvey et al. 2010; Wimp et al. 2010; McCall & Pennings 2012).

Sweep nets and insect vacuums are both used to collect arthropods on vegetation along transects of set length or during set periods of time (Gratton & Denno 2005; Wu et al. 2009; Wimp et al. 2010; Doxon et al. 2011). Previous studies in mixed grass prairies and pastures have shown that the 2 techniques differ in the particular taxa, mean body

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<sup>1</sup>Department of Entomology, 404 Life Science Building, Louisiana State University, Baton Rouge, LA 70803, USA

<sup>2</sup>Department of Biology, 139 Life Science Building, University of Louisville, Louisville, KY 40292, USA; E-mail: benjamin.adams@louisville.edu (B. J. A.)

<sup>3</sup>Department of Environmental Sciences, 1249 Energy, Coast, and Environment, College of the Coast and Environment, Louisiana State University, Baton Rouge, LA 70803, USA; E-mail: xchen39@lsu.edu (X. C.), lindabui@lsu.edu (L. M. H.-B.)

\*Corresponding author; E-mail: lindabui@lsu.edu (L. M. H.-B.)

size, and overall biomass that they collected but not in total species richness (Doxon et al. 2011). In most marsh invertebrate studies, only 1 of these 2 methods is implemented, and more recent studies use primarily vacuum sampling (Elkaim & Rybarczyk 2000; Finke & Denno 2004; Gratton & Denno 2005; Wu et al. 2009; Wimp et al. 2010; McCall & Pennings 2012). Comparisons of possible differences between these 2 techniques have previously not been tested in salt marshes.

Our objective was to compare 2 techniques, sweep nets and insect vacuums, for collecting arthropods in the *Spartina alterniflora* Loiseleur-Deslongchamps (Poales: Poaceae) marshes of southern Louisiana. In Apr 2010, the Deepwater Horizon (DWH) offshore drilling rig exploded and sank causing a massive oil spill that impacted >1,000 km of the Gulf Coast by Jan 2011. Because our collections occurred after the DWH oil spill, we sampled both oiled and non-oiled sites as part of this comparison. The insects collected during these trials were also used to create a taxonomic list of the entomofauna of the coastal salt marshes of Louisiana.

## Materials and Methods

### LOCATION

We established 8 sites in saline to brackish *S. alterniflora* marshes within Plaquemines Parish (County), Louisiana (Table 1). Each selected site had a >100 m by 20 m section of uninterrupted marsh to ensure that multiple transects could be established at distances far enough apart to reduce interaction between transects. Four of the 8 sites were chosen from areas previously identified as oil contaminated during the DWH spill. Non-oiled or reference sites were located in Bay Sans Bois and Bayou Dulac in Barataria Bay; oiled sites were located in Bay Batiste and Bay Jimmy in Barataria Bay. All collections were made between 8:00 a.m. and 2:00 p.m. in Sep 2011. Environmental factors including air temperature and wind speed were recorded at the time of collections (Table 1).

Six days prior to sampling (3 Sep 2011), tropical storm Lee made landfall in this area. The storm surge from the tropical storm coincided with a high tide event causing the marsh grass to be underwater for >18 h (Brown 2011). The implications of this disturbance are not explored in this study, but we acknowledge that the results discussed here were likely influenced by the storm and as such should be interpreted within that context.

### SAMPLING METHODS

One collection consisting of two 20 m transects for each collection technique was made at each of the 8 sites (i.e., 1 collection for each technique per site; 16 collections in total). Transects ran perpendicular to the edge of the marsh starting at the edge and walking inward to reduce possible edge effects. Transects using the same collection technique were located 10 m apart. Transects using different collection

techniques were separated by >50 m. All collected insects and arachnids were stored in 95% ethanol and later sorted to morphospecies. Insects were identified to genus using a variety of taxonomic keys and lists (Williston 1908; Buren 1958, 1968; Stannard 1968; McAlpine et al. 1981, 1987; Grissell & Schauff 1990; Bolton 1995; Arnett 2000; Needham et al. 2000; Arnett & Thomas 2002; Arnett et al. 2002; Burks 2003; Noyes & Pitkin 2004; Pollet et al. 2004; Triplehorn & Johnson 2005; Wilson 2005; Fisher & Cover 2007; Dash & Hooper-Búi 2008; Merritt et al. 2008; Morgan 2009; Ferro et al. 2010; Johansen 2010; Wimp et al. 2010; Dmitriev 2012; Pape et al. 2012; Walker & Moore 2012). Some morphospecies only contained a single individual that was too damaged to identify to genus. In these cases, taxonomic identification was taken as far as possible. Chalcidoid wasps, in most cases, were only identified to family. Some Cicadellidae and Chironomidae were only identified to tribe.

Sweep net collections were conducted using a 38.1 cm (15 inch) diameter collapsible insect collection net (1,140.1 cm<sup>2</sup>; BioQuip Products, Inc., Rancho Dominguez, California). Vacuum collections were made using a gasoline-powered Agricultural Backpack 2-Cycle Aspirator Model 1612 with a 22.9 cm (9 inch) collection nozzle (411.9 cm<sup>2</sup>; John W. Hock Company, Gainesville, Florida). The vacuum produced a 31 km (19 miles) per h air intake. Collections for both techniques were made by sweeping the net or nozzle in a back-and-forth arching motion through the *Spartina* vegetation along each transect. Therefore, both the sweep net and vacuum collections used the same physical motions but the vacuum had the added benefit of having suction. Collections at all sites were conducted by the primary author in order to create a consistent sampling technique across all sites.

### STATISTICAL ANALYSES

We used 2 mixed-effects linear models to test for differences in arthropod abundance and insect species richness (lme4 package, R Core Team; Bates et al. 2015) and a mixed-effects PERMANOVA (PRIMER 6.1.14 with PERMANOVA+ 1.0.4, 9,999 iterations, PRIMER-E Ltd., Albany, New Zealand; Anderson et al. 2008) to look for difference in insect species composition. All models originally included collection technique and the presence of oil along with their interaction as fixed effects, as well as bay and site nested within bay as random grouping factors. We reduced the models using a backwards stepwise reduction method based on Akaike information criterion (AIC) values to eliminate non-significant terms (Ribas et al. 2003; Johnson & Omland 2004; Adams et al. 2016). A reduced model excluding both of the random grouping factors had the lowest AIC values. We focus our discussion on the results obtained by the reduced model but will include additional information when the results of the full and reduced models did not match. We also created a species accumulation curve for both collection techniques to determine if differences between the 2 techniques were solely due to limited sample size ( $S_{est}$  function in EstimateS<sup>®</sup> software version 9.1.0; Colwell 2009).

**Table 1.** Location and environmental information for the 8 collection sites.

Site #	Date	Time	Location	Approximate coordinates	Air temp. (°C)	Wind (km/h)	Oil
1	9 Sep 11	8:23 a.m.	Bay Sans Bois	29.4671°N, 89.7686°W	21.7	4.8	Absent
2	9 Sep 11	9:40 a.m.	Bay Sans Bois	29.4671°N, 89.7686°W	23.8	9.3	Absent
3	9 Sep 11	10:56 a.m.	Bayou Dulac	29.4561°N, 89.7969°W	25.8	11.9	Absent
4	9 Sep 11	12:18 p.m.	Bay Batiste	29.4728°N, 89.8443°W	27.1	9.1	Present
5	9 Sep 11	1:06 p.m.	Bay Batiste	29.4728°N, 89.8443°W	28.3	12.8	Present
6	10 Sep 11	8:00 a.m.	Bayou Dulac	29.4561°N, 89.7969°W	23.8	4.1	Absent
7	10 Sep 11	9:50 a.m.	Bay Jimmy	29.4561°N, 89.8806°W	25.3	10	Present
8	10 Sep 11	12:01 p.m.	Bay Jimmy	29.4561°N, 89.8806°W	34.4	3.3	Present

We used indicator species analysis to identify insect taxa that were associated with significant terms from the previous analysis (indicspecies package, R Core Team 2015; Dufrêne & Legendre 1997; De Cáceres & Legendre 2009). Indicator species analysis is a permutational approach used to determine the relative strength of the association between individual or groups of species and classifications of habitats or conditions within habitats (Bakker 2008; De Cáceres et al. 2012). An indicator value (IndVal) is produced by multiplying the relative abundance and relative frequency of a species or species group across all habitats or conditions (De Cáceres et al. 2012). The significance of the IndVal test statistic is determined by comparing it to an expected distribution of IndVal obtained through randomly reordering the collection data via permutation as described elsewhere (De Cáceres & Legendre 2009). Specifically, we used indicator species analyses to determine which species or groups of species contributed most to differences detected in sweep net and vacuum collections and between oiled and non-oiled sites.

Two Student's *t*-tests were performed to determine if wind speed or air temperature varied between collections at oiled and non-oiled sites. For insect species richness, the lowest level of known taxonomic identification was used rather than morphospecies to reduce overestimation of species richness (e.g., all morphospecies within a single identified genus were counted as a single species). Arachnids were excluded from species richness and composition analyses due to the difficulty of identifying them beyond order. We calculated similarity among communities using a Bray–Curtis index and used that distance matrix to perform PERMANOVA analyses. We tested the normal distribution of species richness and abundance using the Shapiro–Wilk test. Both species richness and abundance data were log transformed to increase normality in the linear models, and composition data were  $\log(x+1)$  transformed prior to creating the Bray–Curtis distance matrix.

## Results

In total, 1,257 insects and arachnids were collected using both sweep net and vacuum sweeping at the 8 salt marsh sites in Plaquemines Parish. Insects constituted 762 individuals representing 42 genera from 36 families and 7 orders (Table 2). Environmental factors at the time of collection were similar in oiled and non-oiled marsh conditions (air temperature:  $t = 5.38$ ;  $df = 1,6$ ;  $P = 0.07$ ; wind speed:  $t = 0.22$ ;  $df = 1,6$ ;  $P = 0.66$ ).

In total, sweep net collections accounted for nearly 5.9 times more individual arthropods than vacuum sweeping ( $F = 36.04$ ;  $df = 1,12$ ;  $P = 0.0001$ ) and 25 more insect taxa. Oiled sites and non-oiled sites produced similar total numbers of arthropods ( $F = 0.62$ ;  $df = 1,12$ ;  $P = 0.45$ ). Insect species richness followed the same patterns as arthropod abundance with sweep net collections accounting for greater richness than vacuum sweeping ( $F = 17.93$ ;  $df = 1,12$ ;  $P = 0.001$ ) and oil sites having similar richness as non-oil sites ( $F = 1.97$ ;  $df = 1,12$ ;  $P = 0.19$ ). We found no significant interaction effects in any test ( $F < 2.18$ ;  $df = 1,6$ ;  $P > 0.17$  for both tests). Species accumulation curves for both collection techniques failed to reach an asymptote; however, even with low replication ( $n = 8$ ), sweep net collections represented a much richer community sample than vacuum sweeping (Fig. 1).

PERMANOVA tests revealed differences in the species composition of the insects collected using either sweep nets or the insect vacuum ( $Pseudo-F = 4.54$ ;  $df = 1,12$ ;  $P = 0.0005$ ) and in oiled versus non-oiled sites ( $Pseudo-F = 2.77$ ;  $df = 1,12$ ;  $P = 0.01$ ). The interaction term was non-significant ( $Pseudo-F = 0.68$ ;  $df = 1,12$ ;  $P = 0.69$ ). Using the full model, insect species composition only differed as an effect of collec-

tion technique ( $Pseudo-F = 6.02$ ;  $df = 1,6$ ;  $P = 0.0002$ ) and not between oiled and non-oiled sites ( $Pseudo-F = 2.42$ ;  $df = 1,6$ ;  $P = 0.098$ ).

Indicator species analyses showed that sweep net samples were marked by high incidences of the genera *Ischnodemus* (Hemiptera: Blissidae), *Incertella* (Diptera: Chloropidae), *Neohydatothrips* (Thysanoptera: Thripidae), and the beetle species *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae). Individuals in the tribe Diamesiinae (Diptera: Chironomidae) were common in vacuum samples (Table 3). The ant *Crematogaster pilosa* Emery (Hymenoptera: Formicidae) was common in non-oiled sites, and the flies in the tribe Chironomini (Diptera: Chironomidae) were common in oiled sites (Table 4).

## Discussion

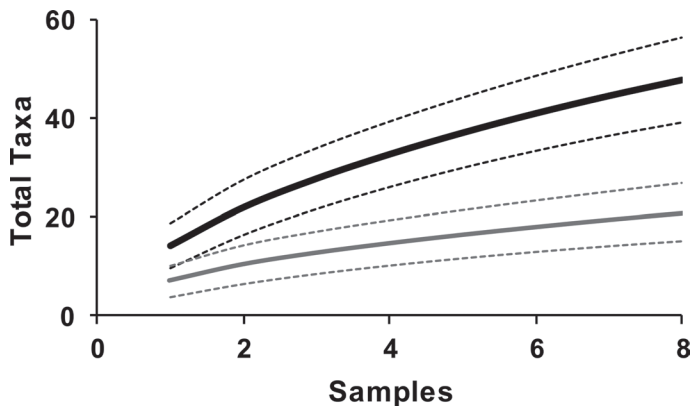
Here we show that sweep net sampling is a more effective and practical means compared with vacuum sampling for collecting insects in salt marshes. The decreased effectiveness of vacuum sampling in this study is likely in part due to the smaller collection area of the vacuum; however, the area alone (2.7 times less with the vacuum) cannot entirely explain the observed difference (>4.5 times more insects in sweep nets). Insect vacuums are particularly heavy and cumbersome to use in salt marshes, where collection is often slowed due to sinking into the soft sediment. The slower movement coupled with the loud noise and exhaust created by the vacuum likely contribute to its decreased effectiveness compared with a sweep net. It is also important to note that indicator species analyses revealed only a single tribe of Diptera as a common component of vacuum collections, whereas multiple genera across multiple orders were all commonly collected in sweep net samples. In combination, these results indicate that it is likely a better choice to use sweep nets than vacuums when addressing large community focused studies in coastal marsh ecosystems.

Our results both contrast and complement a similar study performed in grassland prairies (Doxon et al. 2011). Specifically, in contrast to their findings, we found that sweep net sampling produced higher species richness of insects than using an insect vacuum in marshes. Doxon et al. (2011) showed that sweep net samples had a larger average biomass of insects than vacuum samples. Similarly, our study showed that sweep net sampling produced a higher total abundance of insects, which would likely translate to a larger total biomass although dry weight of insects was not measured in the present study. Both studies showed a tendency to collect Diptera in vacuums and Hemiptera in the sweep nets; however, comparing the relative abundance of orders sampled between these 2 studies is complicated by potential baseline differences in order frequency between the different habitats. Doxon et al. (2011) also showed vacuums were better than sweep nets for collecting small invertebrates (<5 cm). Insect size was not a specific variable of focus in the present study; however, the much higher incidence of mites and thrips (>200 individuals each) in sweep net than in vacuum collections indicates that collection of small invertebrates was not higher in vacuum than in with sweep net collections in this coastal marsh.

Similarities in arthropod abundance and insect species richness between oiled and non-oiled sites have several possible explanations. First, these similarities could indicate that some populations of terrestrial arthropods in the salt marshes of Louisiana were recovering or were unaffected by oil. However, this is unlikely considering the differences detected in community composition and by the indicator species analyses. Specifically, indicator species analyses revealed that the most common ant species (*C. pilosa*) seen in the Louisiana *Spartina* marshes was less abundant in oiled marshes compared with those that were unaffected by the DWH oil spill. Decreases or changes in ant populations

**Table 2.** Taxonomic list of marsh entomofauna including abundance data for sweep net collections (S), vacuum collects (V), non-oiled marsh collections (N), and oiled marsh collections (O). Individuals only identified to tribe are marked with a “” and individuals only identified to subfamily are marked with an “” in the genus/species column. Most parasitic wasps and some damaged specimen were identified to family. Mites were not identified beyond class. Taxonomic ordering is based on Triplehorn & Johnson (2005).

Class /Order	Family	Genus/Species	S	V	N	O
Orthoptera	Tettigoniidae	<i>Conocephalus</i> sp.	0	1	1	0
		<i>Orchelimum</i> sp.	3	0	3	0
Hemiptera	Blissidae	<i>Ischnodemus</i> sp.	131	12	38	106
	Cicadellidae	Chiasmini <sup>†</sup>	2	1	0	3
		<i>Neohecalus</i> sp.	1	0	0	1
		<i>Clastoptera</i> sp.	1	0	0	1
	Clastopterida	<i>Clastoptera</i> sp.	1	0	0	1
	Cymidae	<i>Cymus</i> sp.	0	1	0	1
	Delphacidae	<i>Megamelus</i> sp.	2	0	1	1
		<i>Prokelisia</i> sp.	2	2	1	3
	Membracidae	<i>Ceresa festina</i> Say	1	0	0	1
	Pentatomidae	<i>Chlorochroa</i> sp.	1	0	0	1
	Tingidae	<i>Corythu</i> sp.	1	0	0	1
Thysanoptera	Phlaeothripidae	<i>Phlaeothrips</i> sp.	88	6	43	51
		<i>Williamsiella</i> sp.	0	1	0	1
	Thripidae	<i>Limothrips</i> sp.	10	0	0	10
		<i>Neohydatothrips</i> sp.	130	4	15	119
Coleoptera	Chrysomelidae	<i>Aphthona</i> sp.	1	0	1	0
		<i>Diabrotica</i> sp.	0	0	0	0
		<i>Systema</i> sp.	1	0	0	1
	Coccinellidae	<i>Coleomegilla maculata</i> De Geer	13	0	1	12
		<i>Cycloneda sanguinea</i> (L.)	0	0	0	0
	Melyridae	<i>Collops nigriceps</i> Say	8	0	3	5
	Mordellidae	<i>Glipostenoda</i> sp.	1	0	0	1
	Phalacridae	<i>Stilbus</i> sp.	3	1	1	3
Hymenoptera	Bethylidae	Epyrinae <sup>§</sup>	1	0	1	0
	Dryinidae	Gonatopodinae <sup>§</sup>	1	0	1	0
	Encyrtidae		2	0	0	2
	Eulophidae		2	0	1	1
	Eupelmidae		1	0	0	1
	Eurytomidae	<i>Tenuipetiolus</i> sp.	0	1	0	1
	Formicidae	<i>Crematogaster pilosa</i> Emery	29	25	47	7
		<i>Monomorium minimum</i> (Buckley)	0	0	0	0
		<i>Pseudomyrmex pallidus</i> (Smith)	20	1	0	21
	Mymaridae		4	0	1	3
	Ormyridae		1	0	0	1
	Pteromalidae		1	0	0	1
	Torymidae		3	0	1	2
	Trichogrammatidae		2	0	2	0
Lepidoptera	Pyalidae		1	0	1	0
Diptera	Carnidae	<i>Hemeromyia</i> sp.	7	3	1	9
	Chironomidae	Chironomini <sup>†</sup>	38	27	10	55
		Diamesinae <sup>§</sup>	3	41	30	14
		<i>Thienemanniella</i> sp.	0	1	1	0
	Chloropidae	<i>Chlorops</i> sp.	1	1	0	2
		<i>Incertella</i> sp.	82	2	15	69
	Culicidae		2	0	0	2
	Dolichopodidae	<i>Campsicnemus</i> sp.	1	0	0	1
		<i>Tachytrechus</i> sp.	1	1	1	1
	Lauxaniidae		2	0	0	2
	Muscidae	<i>Caricea</i> sp.	1	0	0	1
	Phoridae	<i>Xanionotum</i> sp.	1	0	0	1
	Sarcophagidae	<i>Senotainia</i> sp.	1	0	0	1
	Ulidiidae	<i>Chaetopsis</i> sp.	13	5	10	8
Arachnida			454	45	315	184
TOTAL			1,075	182	546	711



**Fig. 1.** A species accumulation curve for both the sweep net (black) and the vacuum (gray) collection techniques. Dotted lines indicate the 95% confidence interval around each curve. Neither curve reached an asymptote.

are usually linked to anthropogenic disturbances and environmental stress (Andersen 1997; Andersen et al. 2003; Graham et al. 2004, 2008, 2009). Furthermore, ants have not been included in previous analyses of marsh recovery since the DWH oil spill (McCall & Pennings 2012). Considering that the complete model did not detect a difference between oiled and non-oiled sites, larger and more comprehensive studies including data before and after the DWH oil spill are necessary to draw more certain conclusions.

Storm surge created by tropical storm Lee also provides an explanation for similarities in abundance and species richness. This event likely suppressed insect populations and could have made differences in arthropod abundance and insect species richness undetectable. A follow-up survey of these sites is an important future direction necessary to confirm the observations made here. In either circumstance, our results indicate that *C. pilosa* can act as an indicator of marsh recovery and that further exploration of these communities is needed to determine if the marsh environment is recovering.

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**Table 3.** The indicator species of sweep net and insect vacuum collection techniques for *Spartina* salt marshes in Louisiana. The values included are the indicator value (IndVal) and adjusted *P*-value (*P*).

Technique	Insect taxon	IndVal	<i>P</i>
Net	<i>Ischnodemus</i> sp.	0.957	0.0019
Net	<i>Incertella</i> sp.	0.856	0.0057
Net	<i>Neohydatothrips</i> sp.	0.853	0.0108
Net	<i>Coleomegilla maculata</i>	0.791	0.0240
Vacuum	Diamesinae	0.903	0.0151

**Table 4.** The list of indicator species of oiled and non-oiled marshes from coastal Louisiana with their indicator value (IndVal) and adjusted *P*-value (*P*).

Marsh condition	Insect taxon	IndVal	<i>P</i>
Oiled	Chironomini	0.860	0.0498
Non-oiled	<i>Crematogaster pilosa</i>	0.933	0.0008

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