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Arthropods associated with carrion in a salt marsh habitat in southeastern Florida

Stephanie L. Richards[1,2,], C. Roxanne Connelly[1], Jonathan F. Day[1], Timothy Hope[1], and Ricardo Ortiz[1]*

Abstract

Few studies have assessed arthropods associated with carrion in salt marsh environments. Arthropods attracted to animal carcasses in a salt marsh environment were surveyed in southeastern Florida during four seasons from September 2009 to July 2010. A total of 2,324 arthropod specimens were collected from carcasses (coyote, opossum, raccoon, bobcat, and otter) and 4 orders, 23 families, 28 genera, and 24 species were identified. Specimens were most frequently encountered from the order Diptera (85% of total), and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) and *Musca domestica* (L.) (Diptera: Muscidae) represented 50% of specimens collected. We observed seasonal variation in abundance of dipterans. The information presented here may be useful for future studies identifying arthropods having wildlife forensic entomology significance within subtropical Florida salt marsh habitats.

Key Words: entomology; Calliphoridae; Diptera; decomposition; *Chrysomya rufifacies*; *Phormia regina*

Resumen

Pocos estudios han evaluado los insectos de importancia forense de ambientes de pantanos salados. Se realizo un sondeo de los insectos atraídos a los cadáveres de animales en un ambiente de pantano salado en el sureste de la Florida durante cuatro temporadas desde septiembre del 2009 hasta julio del 2010. Se recolectaron un total de 2,324 especímenes de insectos de los cadáveres (coyote, tlacuache, mapache, y lince rojo) y se identificaron 4 órdenes, 23 familias, 28 géneros y 24 especies. Los especimenes encontrados con mayor frecuencia fue de la orden Diptera (85% del total). *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) y *Musca domestica* (L.) (Diptera: Muscidae) representaron el 50% de las muestras recolectadas en el Orden Diptera. Observamos variación estacional de la abundancia de dípteros. Esta información puede ser usada para identificar los insectos que tienen importancia forense dentro de los hábitats de pantanos salados subtropicales de la Florida.

Palabras Clave: entomología; Calliphoridae; Diptera; descomposición, *Chrysomya rufifacies, Phormia regina*

Forensic entomology is used in criminal cases to estimate the postmortem interval (PMI) of a corpse (Byrd & Castner 2010; Tomberlin et al. 2011). Time of year can influence decomposition times due to environmental temperature and seasonality of arthropod species (Campobasso et al. 2001; Amendt et al. 2007; Anderson 2010; Benbow et al. 2013); thus, it is important to determine arthropod diversity, succession, and spatiotemporal biodiversity in different geographic regions and habitats as such information may be useful in decomposition studies (e.g., Turchetto & Vanin 2001; Ngoen-klan et al. 2011; Mohr & Tomberlin 2014). Knowledge of the occurrence of arthropods and how they respond to different environmental conditions improves estimation of the PMI and can help determine if a carcass was relocated from another region (Byrd et al. 2010). The subtropical climate of southern Florida allows most arthropod species to remain active year round. Blow flies (Diptera: Calliphoridae) are the first to visit a carcass, followed by flesh (Diptera: Sarcophagidae) and house (Diptera: Muscidae) flies (Kreitlow 2010). Female flies use the carcass as an oviposition substrate and larval flies eat the carcass tissues.

There are few studies of arthropods associated with carrion in coastal areas (e.g., Barros de Souza et al. 2008 [Brazil], Bucheli et al. 2009 [Texas]; Chen et al. 2014 [Malaysia]) and no known studies in salt marsh regions of Florida. Hence, the objective of this study was to characterize arthropods visiting small animal carcasses in a salt marsh habitat in southeastern Florida. Arthropod diversity is discussed with regard to season.

Materials and Methods

STUDY SITE

All experiments were conducted at a single study site in an open area approximately 4 m from the edge of tidal brackish water connecting to the Indian River Lagoon; 27° 35′ 1″ N, 80° 22′ 0″ W. Average daily temperature (ADT) was monitored at the Vero Beach Municipal Airport approximately 10 km from the study site (http://cdo.ncdc.noaa.gov/

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Table 1. Description of carcasses placed in the field.

Carcass	Dates	Days	Average daily temperature (°C)
Otter (fall)	September 15-25, 2009	11	27
Bobcat (fall)	October 13-26, 2009	14	28
Coyote (winter)	December 22, 2009 – February 5, 2010	46	14
Racoon (spring)	April 12, 2010	24	23
Racoon (fall)	November 10-24, 2010	15	29
Opossum (summer)	July 18-26, 2010	9	29

qclcd/QCLCD). At extreme high tide, the cage containing carrion could be subject to flooding, although no flooding was reported during the study periods. Land crab (*Cardisoma guanhumi* Latreille*)* holes were observed near the cage. The area 2 m around the cage was devoid of vegetation growth except for red mangrove (*Rhizophora mangle* L.) seedlings and black mangrove (*Avicennia germinans* L.) pneumatophores. The overstory of the lowland area was populated by black and red mangroves. There were almost no understory plants in the lowland area; however, there were many mangrove seedlings. The upland overstory vegetation, ranging 2-4 m from the cage, included live oak (*Quercus virginiana* Miller) that were small, weathered, and stunted; privet (*Forestiera segregate* [Jacquin], Krug, & Urban) and juvenile sabal palm (*Sabal palmetto* [Walter] Lodd. ex Schult. & Schult). Understory vegetation was predominately beautyberry (*Callicarpa americana* L.) and shiny-leafed wild coffee (*Psychotria nervosa* Swartz). The upland ground had a dense cover of grassy species such as torpedograss (*Panicum repens* L.). Signs were posted indicating that a forensic study was in progress.

CARCASSES

We collected opossum (*Didelphis virginiana* Kerr), raccoon (*Procyon lotor* L.), coyote (*Canis latrans* Say), bobcat (*Lynx rufus* Schreber), and otter (*Lutra canadensis* Schreber) carcasses from roadside locations within a 30 km radius of the study site. Carcasses were transported to the laboratory, examined, and stored in a -20°C freezer until used for experiments (Institutional Animal Care and Use Committee Protocol Number 201003749). Only fresh carcasses (collected *ca*. 1-2 d post-mortem that had not yet entered the bloated stage) with no external wounds were used in this study. Immediately prior to each experiment, the selected carcass was weighed and placed in a cylindrical, heavy, galvanized wire mesh cage (16 gauge, 100 cm diameter, 24 cm height). All carcasses were weighed except for the otter and bobcat. The cage had no wire floor and was secured to the ground with plastic tent stakes. The top had a hinged wire mesh door equal to one half the diameter of the cage. The door provided access for sample collecting and was locked to prevent wildlife from disturbing the carcass. Carcasses were placed in the field as shown in Table 1.

OBSERVATIONS AND ARTHROPOD COLLECTIONS

The cage and carcass status was checked daily for the duration of each experiment. Arthropod collections were conducted for *ca.* 30-45 min. each day using forceps (immature), sweep nets, and mechanical aspirators (adults). No passive trapping methods were used in the current study. Immature and soft-bodied adult arthropods were preserved in 70% ethanol, and other adult arthropods were frozen and pinned. Immature (Stehr 1991; Wells et al. 1999; Byrd & Castner 2010)

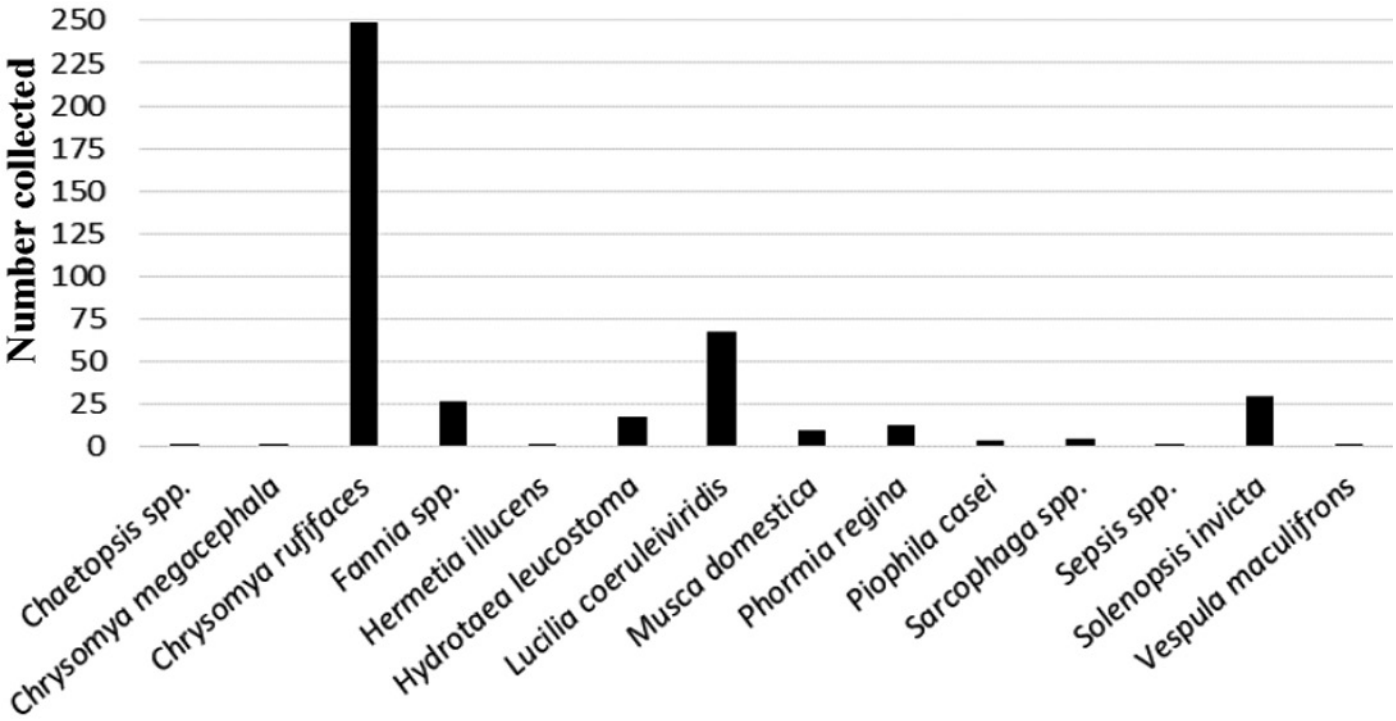


Fig. 1. Arthropod diversity (immature and adult) on bobcat and otter during fall 2009.

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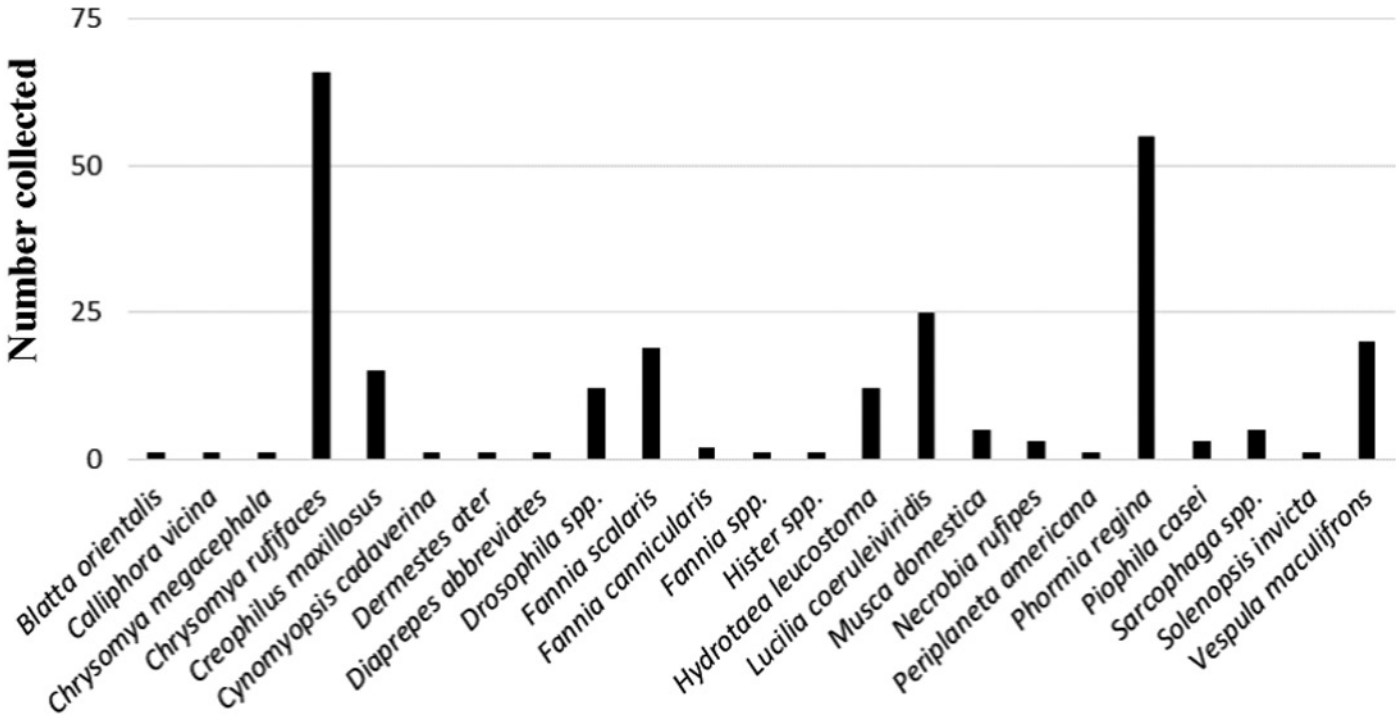


Fig. 2. Arthropod diversity (immature and adult) on coyote during winter 2009.

and adult (Hall 1948; Hall & Townsend 1977; Whitworth 2006) arthropods were identified with taxonomic keys and pictorial keys provided by the Centers for Disease Control and Prevention (http://www.cdc.gov/nceh/ehs/Docs/Pictorial_Keys/Flies.pdf). Because carcass types were not replicated between seasons in this pilot study, only numerical tabulations are reported.

Results

The arthropod species found on respective carcasses are shown in Figs. 1-5 and Table 2. To monitor seasonal presence/absence of arthropods, at least one carcass was placed in the field each season: otter and bobcat (Fall 2009; unknown weights), coyote (Winter 2009,

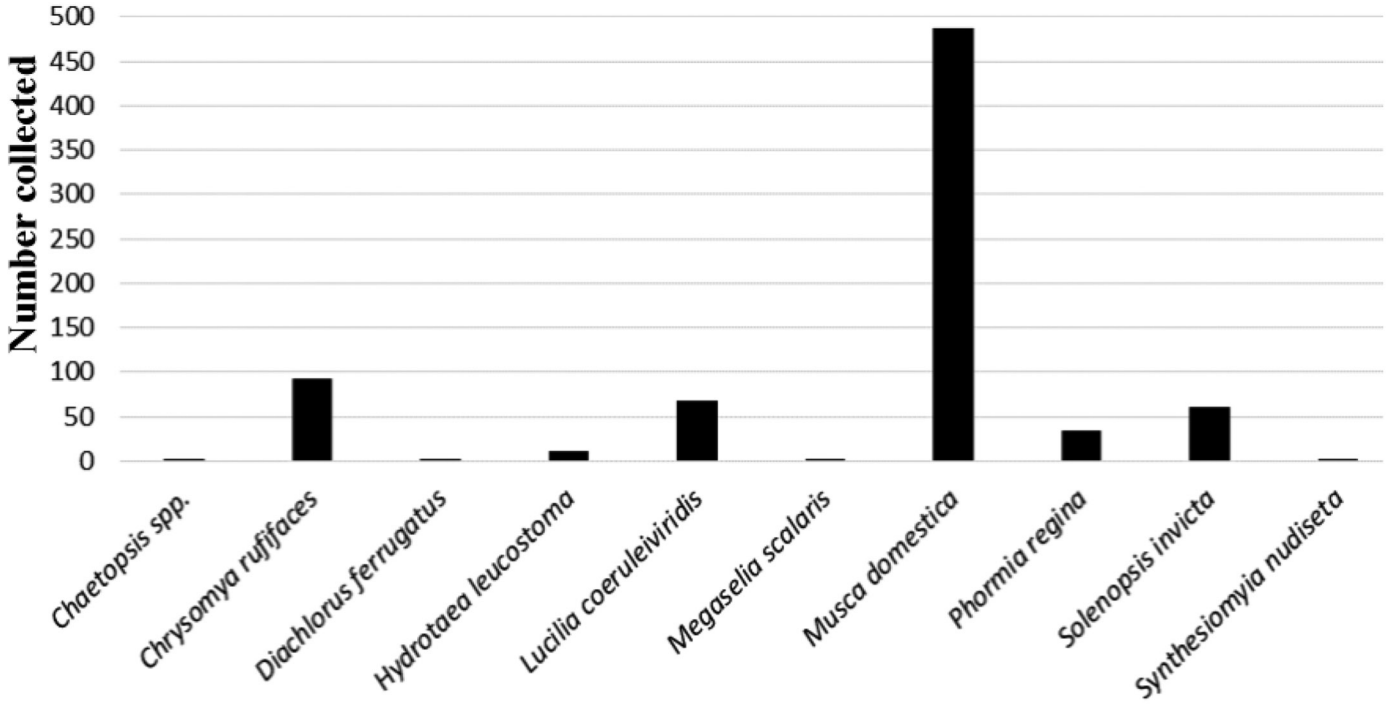


Fig. 3. Arthropod diversity (immature and adult) on raccoon during spring 2010.

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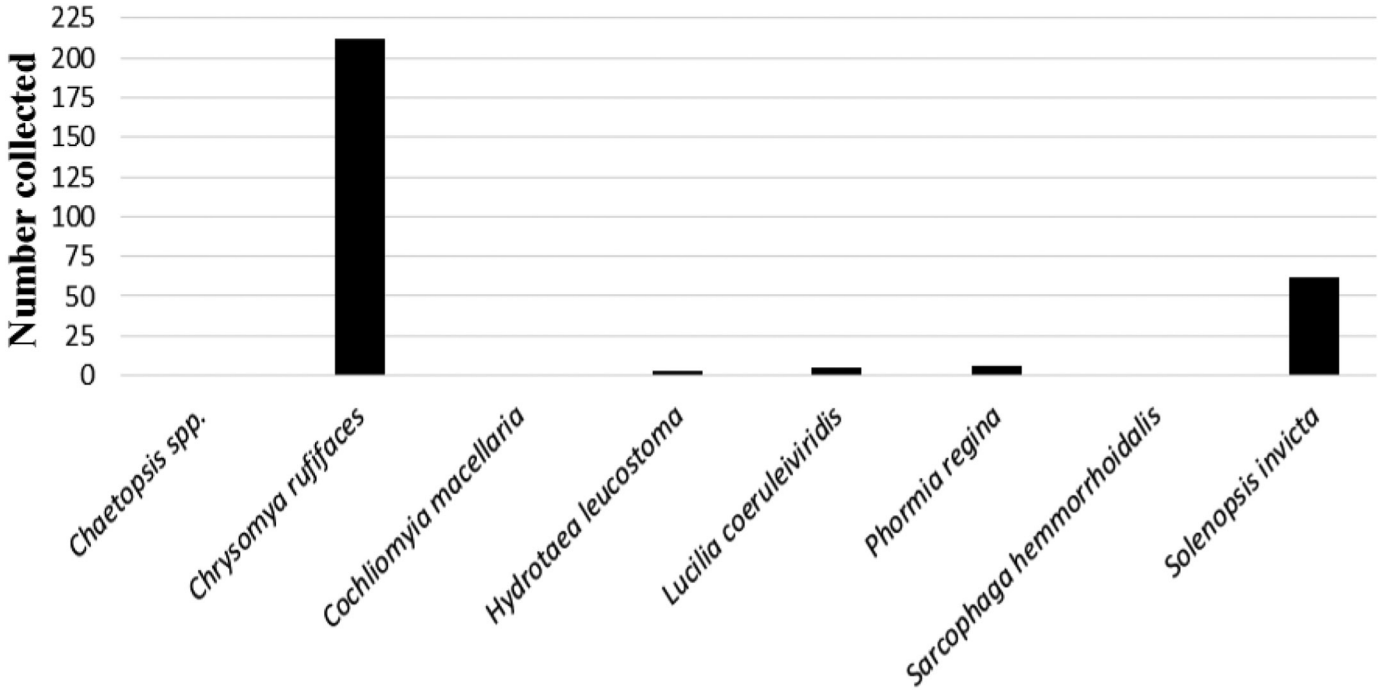


Fig. 4. Arthropod diversity (immature and adult) on opossum during summer 2010.

8.02 kg), raccoon (Spring 2010, 5.22 kg; Fall 2010, 6.37 kg), and opossum (Summer 2010, 4.99 kg). We collected 2,324 specimens from four arthropod orders (Blattodea, Coleoptera, Diptera, and Hymenoptera), including 23 families, 28 genera, and 24 species (Figs. 1-5, Table 2). Specimens were most frequently encountered from Diptera (85% of total), Hymenoptera (8.9%), Coleoptera (1.5%), and Blattodea (0.1%). A total of 1,820 immature and 157 adult Diptera were collected with the majority of collections occurring on the spring (N > 700) and fall (N > 500) raccoon carcasses.

Carcasses were left in the field through all decomposition stages, i.e., fresh to dry remains. Most of the specimens collected were dipteran. Dipterans were collected most frequently from the raccoon carcass (spring; N = 645 larvae, 63 adults) and were observed least frequently from the coyote carcass (winter; N = 72 larvae, 34 adults) (Figs. 1-5,

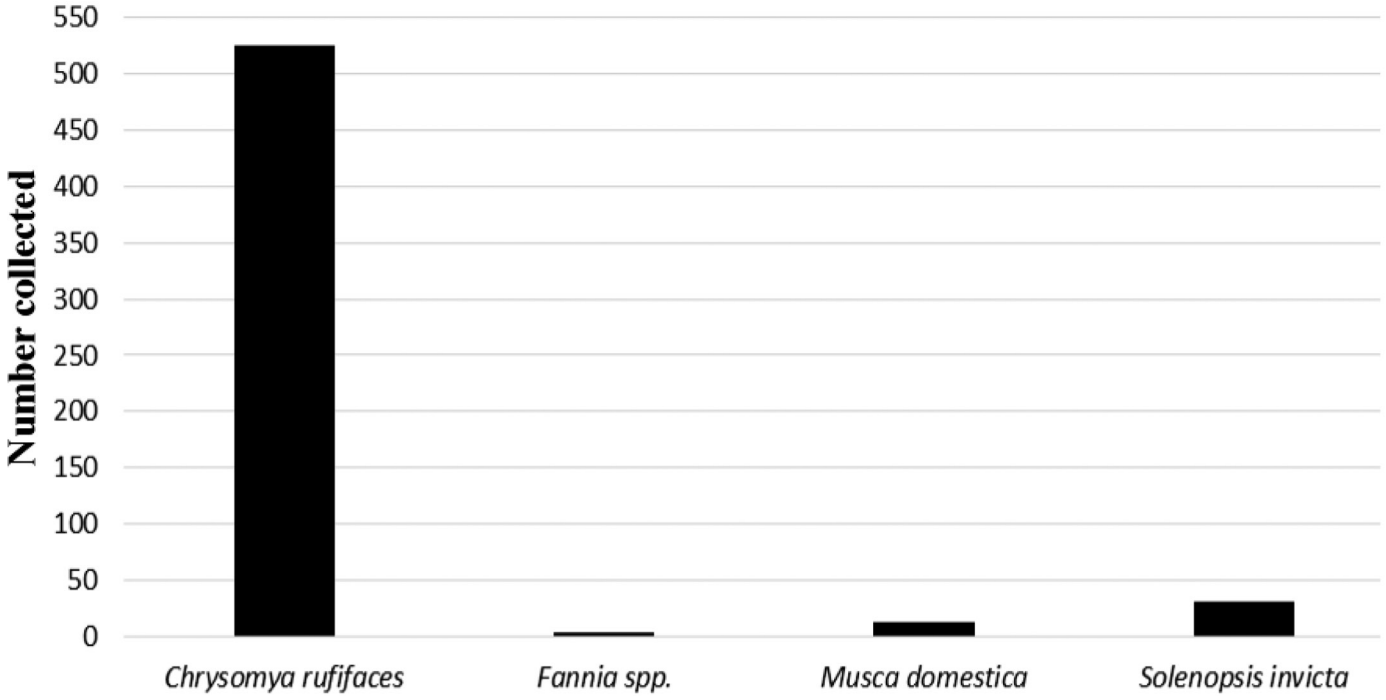


Fig. 5. Arthropod diversity (immature and adult) on raccoon during fall 2010.

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Table 2. Taxonomic list of arthropod collections by carcass type, season, and days observed post-placement.

Order	Family	Genus and Species	Days Post-placement
Fall 2009 – Otter			
Diptera	Sarcophagidae	*Sarcophaga* spp.	1
Diptera	Calliphoridae	*Lucilia coeruleiviridis*	1
Diptera	Piophilidae	*Piophila casei*	2
Diptera	Sepsidae	*Sepsis* spp.	2
Diptera	Ulidiidae	*Chaetopsis* spp.**	2
Diptera	Calliphoridae	*Chrysomya megacephala*	2
Diptera	Calliphoridae	*Chrysomya rufifacies*	2,8
Diptera	Muscidae	*Musca domestica*	2
Diptera	Calliphoridae	*Phormia regina*	2
Diptera	Muscidae	*Hydrotaea leucostoma*	4
Diptera	Stratiomyidae	*Hermetia illucens*	8
Diptera	Fanniidae	*Fannia* spp.	11
Fall 2009 – Bobcat			
Hymenoptera	Formicidae	*Solenopsis invicta*	1,4,14
Diptera	Muscidae	*Hydrotaea leucostoma*	3
Hymenoptera	Vespidae	*Vespula maculifrons*	3
Diptera	Sarcophagidae	*Sarcophaga* spp.	3
Diptera	Calliphoridae	*Phormia regina*	3
Diptera	Calliphoridae	*Chrysomya rufifacies*	3,4
Diptera	Calliphoridae	*Lucilia coeruleiviridis*	3,4
Diptera	Muscidae	*Hydrotaea leucostoma*	4
Diptera	Muscidae	*Musca domestica*	4
Winter 2009 – Coyote			
Diptera	Calliphoridae	*Phormia regina*	3,5,7,9,11,15-16,22
Diptera	Calliphoridae	*Lucilia coeruleiviridis*	3,9,11,34
Diptera	Muscidae	*Hydrotaea leucostoma*	5,9,16
Hymenoptera	Formicidae	*Solenopsis invicta*	5
Diptera	Calliphoridae	*Calliphora vicina*	7
Diptera	Muscidae	*Musca domestica*	7,37
Diptera	Piophilidae	*Piophila casei*	7
Diptera	Calliphoridae	*Chrysomya rufifacies*	7,9,11
Diptera	Calliphoridae	*Cynomyopsis cadaverina*	9
Diptera	Calliphoridae	*Chrysomya megacephala*	11
Coleoptera	Staphylinidae	*Creophilus maxillosus*	15-16,30,34
Coleoptera	Histeridae	*Hister* spp.	15
Coleoptera	Cleridae	*Necrobia rufipes*	16
Coleoptera	Histeridae	*	17
Coleoptera	Dermestidae	*	17
Diptera	Drosophilidae	*Drosophila* spp.**	22
Diptera	Muscidae	*Fannia canicularis*	22
Coleoptera	Staphylinidae	*	22
Hymenoptera	Chalcididae	*	22
Diptera	Sarcophagidae	*Sarcophaga* spp.	22
Diptera	Muscidae	*Fannia scalaris*	25
Hymenoptera	Sphecidae	*	30
Hymenoptera	Vespidae	*	30
Hymenoptera	Vespidae	*Vespula maculifrons*	30
Coleoptera	Curculionidae	*Diaprepes abbreviates***	30
Coleoptera	Dermestidae	*Dermestes ater*	30
Blattodea	Blattidae	*Periplaneta americana***	30
Blattodea	Blattidae	*Blatta orientalis***	30
Blattodea	Blattidae	*	30
Diptera	Muscidae	*Fannia*	37
Diptera	Psychodidae	*	44

*not identified
**not of forensic importance

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Table 2. (Continued) Taxonomic list of arthropod collections by carcass type, season, and days observed post-placement.

Order	Family	Genus and Species	Days Post-placement
Spring 2010 – Raccoon			
Hymenoptera	Formicidae	*Solenopsis invicta*	1-3,11,14-15,17-18
Diptera	Muscidae	*Hydrotaea leucostoma*	2-4,8
Diptera	Muscidae	*Synthesiomyia nudiseta*	2-3
Diptera	Calliphoridae	*Phormia regina*	3-4,7-11
Diptera	Phoridae	*Megaselia scalaris*	3
Diptera	Calliphoridae	*Lucilia coeruleiviridis*	4,8
Diptera	Ulidiidae	*Chaetopsis* spp.**	7,11
Diptera	Muscidae	*Musca domestica*	7-11,14-15,17
Diptera	Calliphoridae	*Chrysomya rufifacies*	7-11
Diptera	Tabanidae	*Diachlorus ferrugatus***	10
Diptera	Piophilidae	*	15
Fall 2010 – Raccoon			
Hymenoptera	Formicidae	*Solenopsis invicta*	1,3,6,9,13
Diptera	Calliphoridae	*Chrysomya rufifacies*	6,8-10,13-15
Coleoptera	*	*	7
Diptera	Muscidae	*Musca domestica*	9-10,14-15
Diptera	Fanniidae	*Fannia* spp.	15
Summer 2010 – Opossum			
Hymenoptera	Formicidae	*Solenopsis invicta*	1-5
Diptera	Sarcophagidae	*Sarcophaga hemmorrhoidalis*	2
Diptera	Muscidae	*Hydrotaea leucostoma*	2-3
Diptera	Calliphoridae	*Lucilia coeruleiviridis*	2-3
Diptera	Calliphoridae	*Cochliomyia macellaria*	2
Diptera	Calliphoridae	*Phormia regina*	2-3
Diptera	Calliphoridae	*Chrysomya rufifacies*	3-5
Diptera	Ulidiidae	*Chaetopsis* spp.**	4

*not identified
**not of forensic importance

Table 2). *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) and *Musca domestica* (L.) (Diptera: Muscidae) represented 50% of dipteran specimens collected. *Chrysomya rufifacies* was collected more than any other dipteran and the diversity of species collected varied between carcasses. *Musca domestica* was abundant in spring while *C. rufifacies* was prevalent in summer and fall. In winter collections, we collected both *C. rufifacies* and *Phormia regina* (Meigen) (Diptera: Calliphoridae). *Solenopsis invicta* (Buren) was collected in every season. Other Hymenoptera (Sphecidae, Vespidae, and/or Chalcididae) were only collected from carcasses in the winter (coyote) and fall (otter and bobcat). Specimens from five families of beetles were collected from the coyote carcass (winter), including Staphylinidae and Dermestidae that are known to be of forensic importance.

Discussion

As expected, longer decomposition times were observed during cooler (e.g., coyote in winter; 46 days) compared with warmer (e.g., opossum in summer; 9 days) periods. A study conducted in southern Ohio placed swine carcasses (14 – 18 kg) in the field and showed significant seasonal differences in abundance of specimens from Calliphoridae (i.e., *P. regina* and *Lucilia coeruleiviridis* [Macquart]), Muscidae, Staphylinidae, and Silphidae (Benbow et al. 2013). The weight range (*ca*. 5-8 kg) of the carcasses used in the current study was similar and likely did not play a major role in species diversity; however, caution is advised when comparing results from similar arthropod succession studies using larger carcasses, as different blow fly species may be attracted to different sized carcasses. Due to the nature of the study's use of carcasses from the roadside, time of death is estimated (fresh carcasses collected 1-2 days post-mortem) and this could impact the PMI.

We observed differences in seasonal distribution (i.e., numbers and species) of dipteran specimens with the highest number collected from the spring raccoon carcass in the field for 24 d. *Chrysomya rufifacies*, the hairy maggot blow fly, was the species collected most frequently in the current study. *Chrysomya rufifaces* larvae are predators of other immature dipterans and are known to suppress and/or eliminate other arthropods on carrion when food is scarce (Cammack & Nelder 2010), thereby potentially affecting estimation of the PMI. In another study in Gainesville in north central Florida, *C. rufifaces* was found to be the dominant species colonizing black bear carcasses, and outcompeting or preying on other arthropods, i.e., *C. megacephala* (Fabricus)*, C. macellaria* (Fabricus)*, Lucilia coeruleiviridis* (Macquart)*, P. regina,* and *L. sericata* (Meigen) (Swiger et al. 2014). A study conducted near Earleton in northern Florida showed that *L. coeruleiviridis* was the most prevalent species of Calliphoridae on 16-30 kg pig carcasses (Gruner et al. 2007). A study on blow flies attracted to 60-80 kg pig carcasses in Texas showed *C. macellaria* and *C. rufifaces* collected frequently in summer (≤ 38.2 °C), while *C. vicina* and *P. regina* were collected most often in the winter (Mohr & Tomberlin 2014). In the current study in a salt marsh environment in southeastern Florida, *L. coeruleiviridis* was collected in each season, but was most prevalent in spring and fall. We observed *P. regina* most frequently in winter collections in Florida, similar to observations in West Virginia where this species was col-

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lected most frequently in spring (Joy et al. 2002), i.e., average winter temperatures in Vero Beach, Florida are similar to spring temperatures in West Virginia. A study in northern Florida showed *P. regina* was also most prevalent in winter (Gruner et al. 2007).

Musca domestica was collected in most seasons and was most abundant on the spring raccoon carcass, compared to other carcasses (Fig. 3). A study in northern Thailand showed similar results with most *M. domestica* collected from traps baited with beef during April-June, although most specimens collected in the aforementioned study were *C. megacephala* (Ngoen-klan et al. 2011). *Musca domestica* was considered forensically important on pig carcasses in Argentina (summer, fall, and spring) (Horenstein et al. 2010) and monkey and pig carcasses (summer) in coastal areas of Malaysia (Chin et al. 2008; Chen et al. 2010). Regional temperature differences should be considered when comparing studies where seasonal differences in collections are observed. The low numbers (N = 35) of specimens from order Coleoptera collected were likely due to our collection methods used, which were not effective for this group. Pitfall traps and/or other passive surveillance methods might be better for future studies. *Solenopsis invicta* were collected for the duration of the study and, since ants are known to eat larval and adult flies and beetles (Campobasso et al. 2009), may have impacted decomposition rates on the carcasses used in the current study. Omnivorous Florida land crabs in the study area are known to eat insects and carrion (Hill 2001); however, this was not directly observed in the current study.

Others have shown no difference in blow fly and beetle activity between pig carcasses frozen (for two months)-thawed and carcasses that were only refrigerated (Bugajski et al. 2011); however, the pig carcasses used in the aforementioned study were smaller (*ca.* 1 kg) than the carcasses used here. Another study showed anaerobic decomposition in freshly killed rats (*ca.* 0.316 kg), while aerobic decomposition was predominant in frozen-thawed rats (Micozzi 1986). Further studies should evaluate the effects of freezing on decomposition in different sized carcasses.

This is, to our knowledge, the first report of arthropods associated with carrion from a salt marsh habitat in southeastern Florida. Further studies are needed to characterize arthropods, including the extent to which ecological differences exist between species found in coastal environments. Biodiversity of arthropods in the salt marsh environment is likely affected by environmental factors and this must also be considered in future studies and forensic investigations.

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