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Seasonal abundance in necrophagous Diptera and Coleoptera from northern Venezuela

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Abstract. A carcass represents an ecosystem for necrophagous insects and other arthropods communities, and the decomposition stages are related to an ecological succession. Several factors are associated with the insects colonization and subsequently, accelerate or retard the decomposition. Some studies confirm the seasonal differences in blowflies and other medicolegal important insects. From 2015 and 2016 during four months was studied the entomofauna associated to a beef biomodel. Insects species abundance from monthly catches was used for richness determination, and the community structure was estimated using diversity and evenness indexes. The monthly precipitation data was used for describing the association between environmental and insects abundance. Were collected 1046 specimens comprises the Order Diptera (97%) and including Calliphoridae, Muscidae, Piophilidae, Sarcophagidae and Otitidae; in Coleoptera (3%): Dermestidae, Cleridae and Staphylinidae. Were found twelve species: *Chrysomya megacephala*, *C. albiceps*, *Lucilia cuprina*, *Cochliomya macellaria*, *Musca domestica*, *Ophyra aenescens*, *O. chalcogaster*, *Atherigona orientalis*, *Piophila casei*, *Dermestes maculatus*, *Necrobia rufipes* and *Belonuchus rufipennis*. The highest insects abundance and richness were registered in May, February and November, while the lowest values were found in August. The Calliphoridae and Muscidae abundance and richness increase between November and May that correspond to ending of rainy season and beginning of the dry season, while the values decrease in August that correspond to the rainy season. Our study represents the first investigation to determine the seasonal variation of medicolegal important insects in Venezuela. However are necessary more studies that consider different habitat type (forests, savannas, etc) to determine the insects occurrence among different decomposition stages.

INTRODUCTION

A carcass represents an ecosystem for necrophagous insects and other arthropods communities, and the decomposition stages are related to an ecological succession. Each stage is characterized by biological and physicochemistry differences that permit the insects arrival in predictable community changes. This succession events permit the knowledge of characteristic species composition for the establishment of Post-Mortem Interval (PMI), in addition to the carcasses decomposition and other environmental factors associated with the crime scene and the medicolegal casuistry. Also, the insect species biology could by contributed to information about the possible death cause and site of the primary (where a crime actually occurred) or secondary (related to the crime but is not where the actual crime took place) crime scene. (Sánchez & Fagua, 2014).

The necrophagous insects are the first to colonized the carcasses and correspond to Calliphoridae and Sarcophagidae (Valdes *et al.*, 2010). In late decomposition stages, between putrefaction and purging, the remaining dipterous are represents by Muscidae, Piophilidae and Phoridae.

(Ramírez, 2012; Zuha & Omar, 2014; Skowronek *et al.*, 2015). Finally, between the post-decay and skeletonized stages, the necrophagous beetles are the common taxa: Dermestidae and Cleridae. However, species of Scarabaeidae, Silphidae, Staphylinidae, Histeridae and Trogidae may occur during different stages because are coprophagous or immature blowfly predators (Jiménez *et al.*, 2013; Bala & Singh, 2015; De Souza & dos Santos, 2016; Pérez *et al.*, 2016).

Nevertheless, the main problem using the insects succession are related with the no coincidence between the colonization beginning and the death time; also in some cases, the medicolegal pathologist opinion displaced to the entomological evidence. Several factors are associated with the insects colonization and subsequently, accelerate or retard the decomposition. The corpse was found on a closed site, was wrapped in cloth or plastic, the body was submerged, among others (Tomberlin *et al.*, 2011).

Rodríguez *et al.* (2015) stated the importance of environmental variables (temperature, relative humidity, precipitation, among others) registered during the medicolegal entomology collections in the crime scene. Several studies confirm the seasonal differences in blowflies and other medicolegal important insects (Serbino & Godoy, 2007; Pires *et al.*, 2008; Koller *et al.*, 2011; Amat *et al.*, 2013); this variation could be used to detect forensically meaningful species and to help inference in applied research and criminal cases (Tomberlin *et al.*, 2011; Zabala *et al.*, 2014). Therefore, we comparing the seasonal abundance of necrophagous insects associated to a beef biomodel in an northern urban area of Venezuela.

MATERIAL AND METHODS

Sampling protocol

The study was realized between August 2015 and May 2016 in an urban area (10°13'78" North Latitude and 68°00'32" West Longitude) of Valencia, Carabobo - Venezuela. Was select beef viscera as biomodel, following the protocols of Nuñez and Liria (2014): 4 Kg *Bos taurus* (Linnaeus, 1758) viscera (liver and lungs); between August, November, February and May the biomodel was placed in a plastic container and exposed during four days, in the fifth day the necrophagous insects were collected using entomological nets (for adults) and/or with specialized forceps (for immature stages). Were selected the fifth exposition day because in this time Nuñez and Liria (2014) reported high insect richness. Finally, the adult blowflies and beetles were preserved in 70% ethanol, while the immature flies were sacrificed in boiled water and then place in 70% ethanol.

Entomofauna identification

The insects associated to the biomodel were taxonomically identified using specialized dichotomous keys: Immature blowflies (Florez & Wolff, 2009; Velásquez *et al.*, 2010), adult blowflies (Amat *et al.*, 2008; Carvalho & Mello, 2008) and beetles (Almeida & Mise, 2009; Aballary *et al.*, 2014). In some immature Diptera larvae was studied the morphology of the cephalopharyngeal skeleton; we follow Sukontason *et al.* (2004) protocol for mouth parts clearing and slide mounting.

Data analysis

Species abundance from monthly catches was used for richness determination, and the community structure was estimated using Simpson evenness and Shanon-Wiener diversity indexes. The monthly precipitation data was obtained in the closest Meteorological station (Arturo Michelena airport: 10°09'04.9" North Latitude, 67°55'58" West Longitude and 430 masl) for describing the association between the insects composition and seasonally. Finally, the species abundance by month was analyzed with Bray-Curtis similarity index and unweighted pair-group cluster method. All statistical analysis were realized with the PAST computer program (Hammer & Harper, 2011).

RESULTS

Were collected 1046 adult specimens in two insect Orders, eight Families, 10 genera and twelve species (Table 1); Diptera was the most abundant taxa (97%), including the Families: Calliphoridae (57.5%), Muscidae (34.6%), Piophilidae (3.6%), Sarcophagidae (1.2%) and Otitidae (0.1%). In Coleoptera (3%) was reported: Dermestidae (2.2%), Cleridae (0.6%) and Staphylinidae (0.2%). The highest abundance (total specimens collected) and species number (=richness) were registered in May (305 specimens / 14 spp.), February (327 / 11 spp.) and November (312 / 8 spp.), while the lowest values were found in August (102 / 5 spp.). The most abundant Diptera species was *Chrysomya megacephala* (Fabricius, 1794) collected in February (156 specimens) and November (131), followed by *Musca domestica* (Linnaeus, 1758) collected in May (121) and February (119), and finally *C. albiceps* (Wiedemann, 1819) was most abundant in November (67); others dipterous species were found between February and May: *Ophyra aenescens* (Wiedemann 1830), *O. chalcogaster* (Wiedemann, 1824), *Atherigona orientalis* (Schiner 1868) and *Piophila casei* (Linnaeus 1758). In Coleoptera the most abundant species was *Dermestes maculatus* (De Geer, 1774) collected in August (9 specimens) and November (7), followed by *Necrobia rufipes* (Fabricius, 1781) in February (4), and finally *Belonuchus rufipennis* (Fabricius 1801) was collected in May (2 specimens). The Shannon-Wiener diversity index was high in May (1.685) and November (1.585) and low in August (1.344) and February (1.337), while high evenness index was found in August (0.767) and November (0.610), and low in February (0.346) and May (0.385). In relation to the immature stages, *C. magacephala* was collected in August and November, and *C. albiceps* in February and May.

Table 1. Taxonomic adult insect composition and species monthly abundance, richness, Shannon-Wiener diversity index, and Simpson evenness index

Order / Family	Species	Aug	Nov	Feb	May	TOTAL
Diptera						
Calliphoridae	*Chrysomya megacephala* (Fabricius, 1794)	37	131	156	87	**411**
	Chrysomya albiceps (Wiedemann, 1819)	16	67	9	30	**122**
	Lucilia cuprina (Wiedemann, 1830)	3	39	6	4	**52**
	Cochliomyia macellaria (Fabricius, 1775)	0	14	0	2	**16**
Muscidae	*Musca domestica* (Linnaeus, 1758)	37	44	119	121	**321**
	Ophyra aenescens (Wiedemann, 1830)	0	7	13	5	**25**
	Ophyra chalcogaster (Wiedemann, 1824)	0	0	1	2	**3**
	Atherigona orientalis (Schiner, 1868)	0	0	4	9	**13**
Piophilidae	*Piophila casei* (Linnaeus, 1758)	0	0	6	32	**38**
Sarcophagidae	Sarcophagidae sp1	0	3	6	3	**12**
Otitidae	Otitidae sp1	0	0	0	2	**2**
Coleoptera						
Dermestidae	*Dermestes maculatus* (De Geer, 1774)	9	7	3	4	**23**
Cleridae	*Necrobia rufipes* (Fabricius, 1781)	0	0	4	2	**6**
Staphylinidae	*Belonuchus rufipennis* (Fabricius, 1801)	0	0	0	2	**2**
	Abundance	**102**	**312**	**327**	**305**	**1046**
	Richness	**5**	**8**	**11**	**14**	
	Shannon-Wiener index	**1.344**	**1.585**	**1.337**	**1.685**	
	Evenness index	**0.767**	**0.610**	**0.346**	**0.385**	

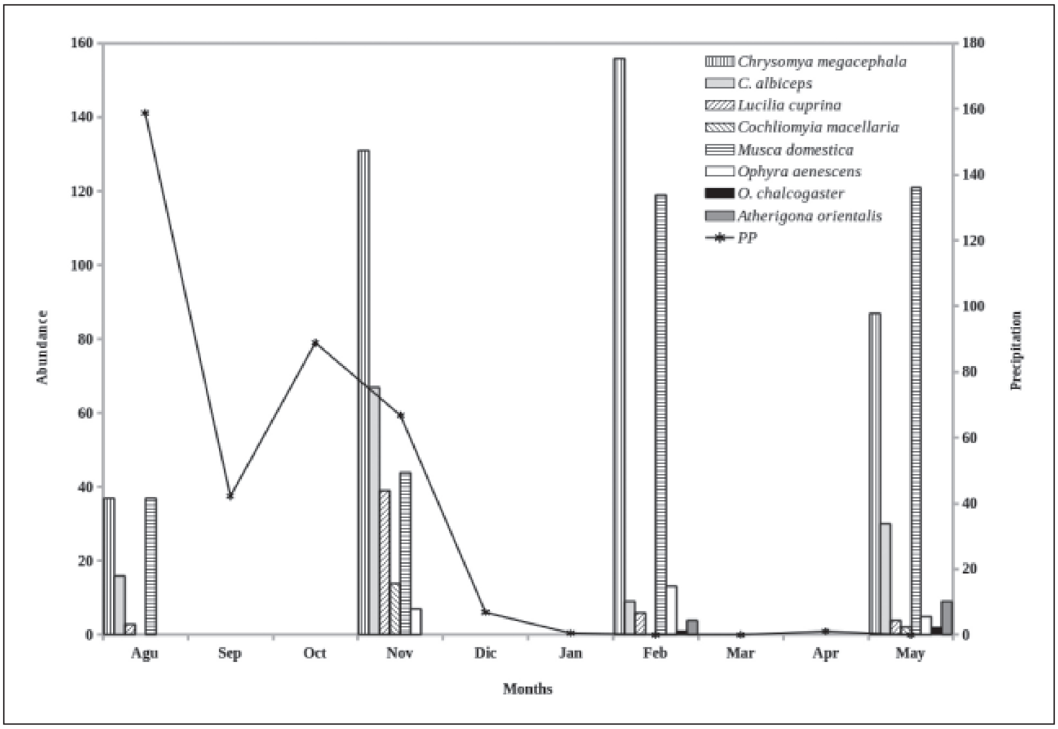

Figure 1. Calliphoridae and Muscidae monthly adult species abundance (bars) in relation with the precipitation (line).

Figure 1 shows the monthly adult Calliphoridae and Muscidae species abundance in relation with the precipitation; the abundance and richness increase between November and May that correspond to ending of the rainy season and beginning of the dry season, while the abundance and richness decrease in August that correspond to the rainy season. Figure 2 shows the cluster analysis based on Bray-Curtis similarity. In August, the necrophagous insect community is separate from the remaining months by the presence of three Calliphoridae species and *Musca domestica*. In November, the insects community is differentiated by the abundance of *C. albiceps* and *L. cuprina*; and February and May are grouped by the abundance of four Muscidae species, *Piophila casei* and *Necrobia rufipes*. Finally, Figure 3 shows the biomodel monthly stage decomposition; in the rainy season (August-November) the biomodel was in chromatic stage, with a mild mummification similar to dehydrated leather (Fig. 3AB); the larvae were distributed between fold viscera and cavities. Later in the dry season (February-May), the biomodel was in advanced liquefaction period with larvae distributed at random (Fig. 3CD).

DISCUSSION

Various authors report differences among necrophagous insects composition in relation to climatic factors. In the rainy season decreases the microbial and insects decomposition activity, extending the biophysicochemical changes (Ahmad *et al.*, 2011). Sanchez and Fagua (2014) argue that species abundance decrease with the precipitation increasing because the rain retards adult colonization and when the carcasses are colonized the water causes immature mortality. However, some studies demonstrated inverse relationship, Amat *et al.* (2013) encountered non-correspondence between the climatic data and the expected and consider those variations could be

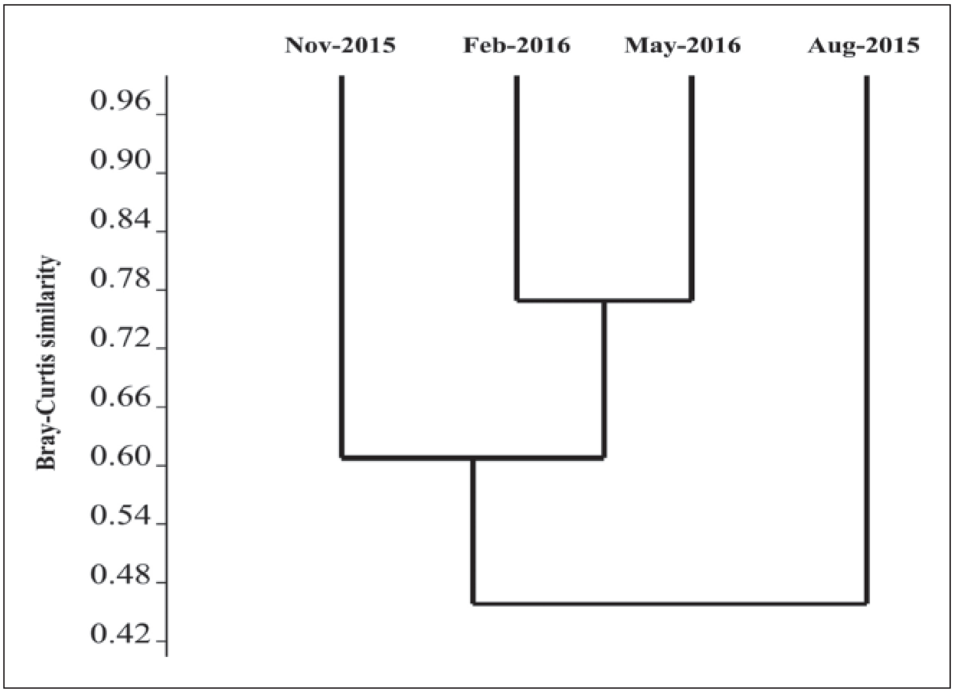

Figure 2. Cluster analysis for necrophagous monthly insects based on Bray-Curtis similarity and unweighted pair-group method.

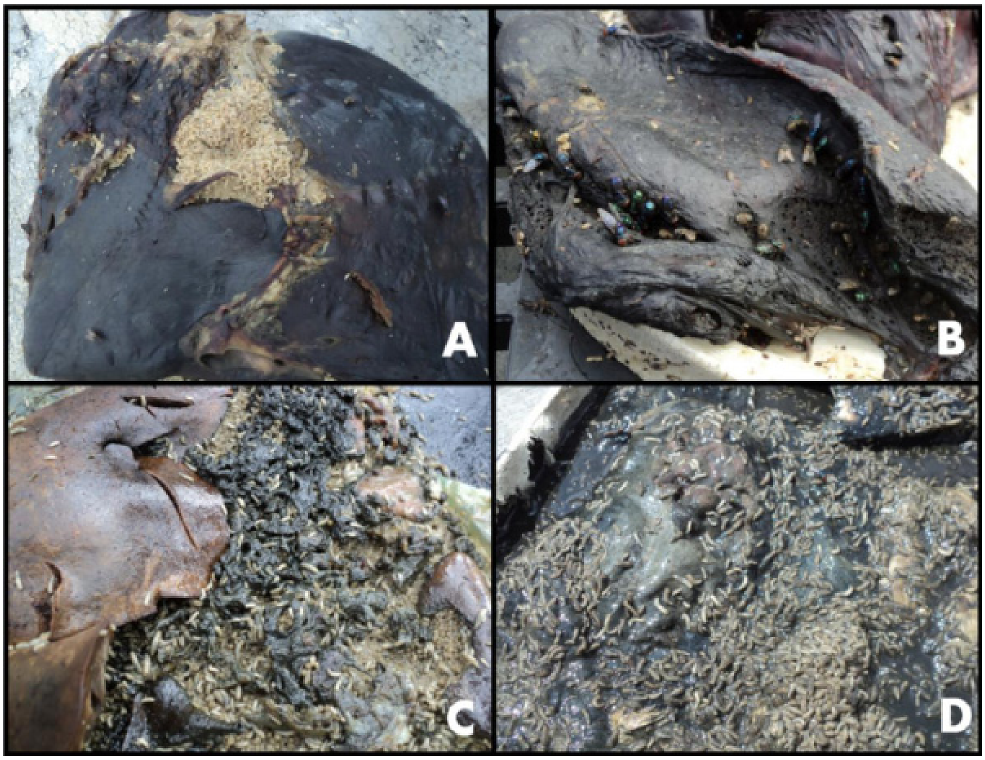

Figure 3. Biomodel monthly decomposition aspect: August 2015 (A), November 2015 (B), February 2016 (C) and May 2016 (D).

attributed to La Niña. Also, they didn't found significant differences between species abundances and seasonality, due to the similarity in the atmospheric conditions. Ramos *et al.* (2014) determined the insects associated to pig carcasses (*Sus crofa* Linnaeus, 1758); in the early decomposition stages, the common species were represented by *Cochliomya macellaria*, *C. albiceps* and *L. eximia*. Contrary, in Venezuela several investigations reported *C. megacephala* and *C. albiceps* as primary colonizers (Mavárez-Cardozo *et al.*, 2005; Magaña *et al.*, 2006; Nuñez & Liria, 2014; Nuñez *et al.*, 2016) and due to this, the three species are considered important medicolegal indicators for the country. However, the presence of *C. albiceps* could by a problem related to PMI estimation because the larvae displace other primary colonizers (Ortloff *et al.*, 2013).

Valdes *et al.* (2010) studied the necrophagous insects associated to pig carcasses, finding species of Cleridae and Piophilidae from early decomposition stages. In our study, *Necrobia rufipes* and *Piophila casei* were found in May that corresponds to dry season and the biomodel was in liquefaction period. Recently, Dos Santos and Firmino (2016) reported the beetles species composition in Brazil; they encountered that succession patterns could by changed according to the climatic conditions. In arid zones, the carcasses could be mummified causing the early colonization of necrophagous Coleoptera. Our investigation finds high *Dermestes maculatus* abundance in the rainy season; In particular, the biomodel was dehydrated and subsequently mummified, an ideal niche for Dermestidae species (Charabidze *et al.*, 2014; Sukchit *et al.*, 2015).

Hewadikaram and Goff (1991), studied the effect of pig carcase size on rate of decomposition and arthropod succession; they obtained no size relation among taxa composition, postmortem interval arthropods indicators or succession patterns. However, 15.1 kg pigs showed a greater number of adult flies, with the results of high immature instars abundance during the decay stage; and the physical parameters of decomposition rate and internal temperature differ between carcasses weight. In our study, we used a nonhuman model that are according with three premises (Catts & Goff, 1992): a) relative easy to obtain, b) be inexpensive, and c) not tend to arouse public objections. Recently, Nuñez & Liria (2014) determined the arthropod succession patterns in a beef viscera as biomodel; they argued that the biomodel was useful because permits recreated the progressive decomposition stages in different organs and the insects succession.

Finally, we reported for first time in Carabobo-Venezuela the occurrence of *O. chalcogaster* and *B. rufipennis*. Patitucci *et al.* (2010) studied the status of *Ophyra* species in Argentina, and mentioned that *O. chlacogaster* is a cosmopolitan species associated with urban or suburban environments that colonizing different substrates: human and dog faeces, fish, chicken viscera, mouse carcass. By the other hand, Almedia and Mise (2009) proposed taxonomic keys for South American forensically important Coleoptera; in this revision, the authors reported *B. rufipennis* associated to squid carcasses. More recently, Salazar and Donoso (2015) prepared a list of forensically important insects for Ecuador, and stated the presence of *B. rufipennis* in the Galapagos Province.

Our study represents the first investigation to determine the seasonal variation of medicolegal important insects in Venezuela. However, are necessary more studies that consider different habitat type (forests, savannas, etc) to determine the insects occurrence among different decomposition stages. From a forensic entomologist point of view, the investigation provides relevant information when the body was found with more than 48 hours. And in several complexes cases, it requires an additional statistical and multidisciplinary support.

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