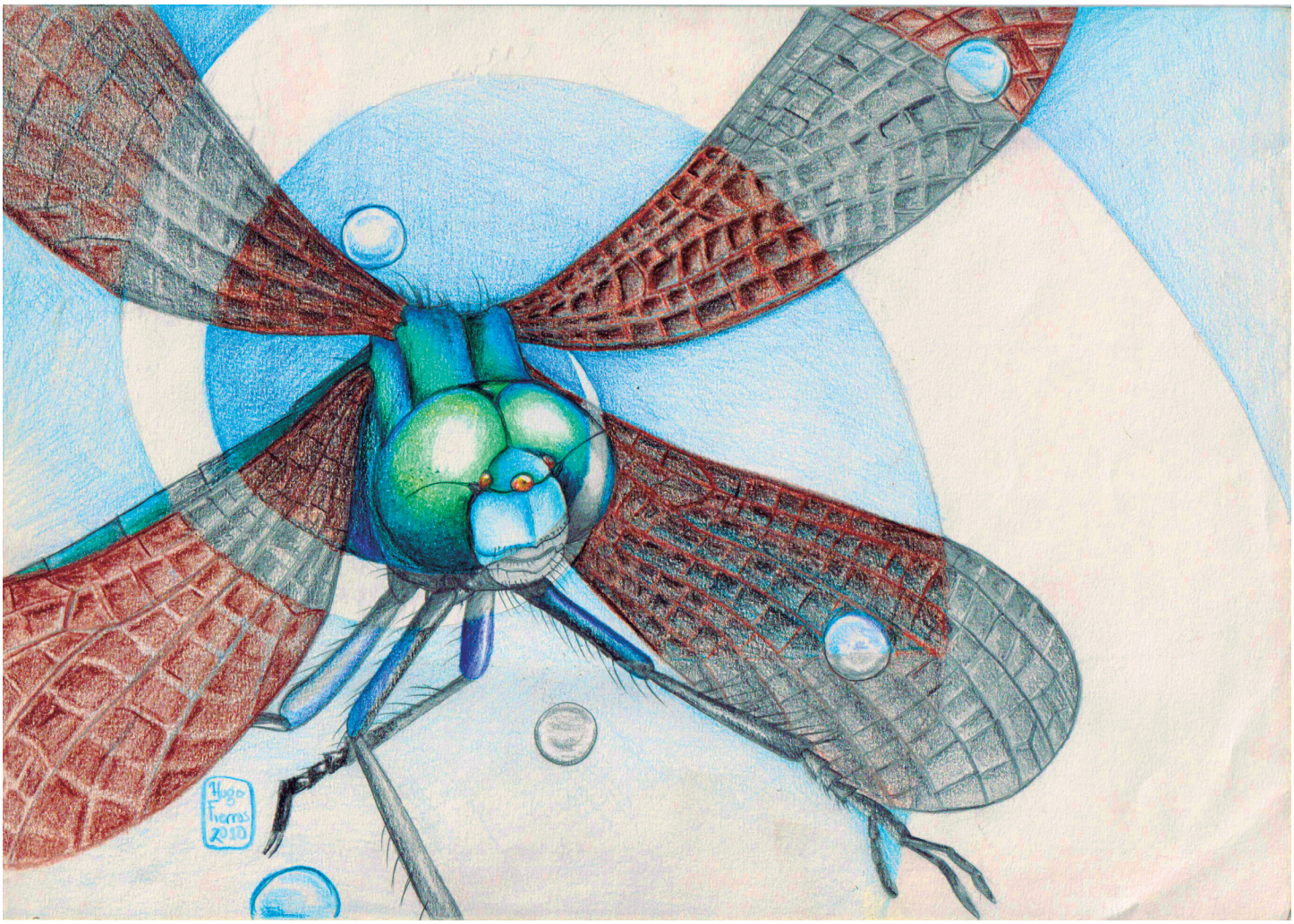

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Diversity of Blowflies (Diptera: Calliphoridae) In A Remnant of Low Deciduous Forest in Yautepec, Morelos, Mexico

Diversidad de moscas carroñeras (Diptera: Calliphoridae) en un remanente de selva baja caducifolia en Yautepec, Morelos, México

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ABSTRACT

A year-long entomological survey was performed in 2016-2017 using carrion-baited traps (chicken, pork, squid, or beef) to assess the diversity of necrophagous flies in the municipality of Yautepec, Morelos, Mexico. A total of 4,765 flies from 18 families were collected, more than 50 percent of the specimens collected (2,644) belonged to Calliphoridae represented by five genera and eight species. Out of these eight species we evaluated if the species presented a preference for a specific type of bait, our results show that *Chrysomya rufifacies* and *C. megacephala* preferred squid*,* while *Cochliomyia macellaria, Hemilucilia segmentaria,* and *Lucilia mexicana* preferred chicken.

Key words Brachycera; exotic species; forensic entomology; necrophagous insects.

RESUMEN

Se llevó a cabo un estudio anual utilizando trampas con carroña (pollo, cerdo, calamar y res) para conocer la diversidad de las moscas necrófagas en el municipio de Yautepec, Morelos, México. Se recolectó un total de 4,765 ejemplares pertenecientes a 18 familias del orden Diptera, más del 50 por ciento (2,644) pertenecen a Calliphoridae representadas con cinco géneros y ocho especies de Calliphoridae. Utilizando estas ocho especies, se analizó si existe la preferencia por algún tipo de cebo, nuestros resultados muestran que *Chrysomya rufifacies, C. megacephala* mostraron una preferencia hacia el calamar*,* mientras que *Cochliomyia macellaria, Hemilucilia segmentaria* y *Lucilia mexicana* prefirieron el pollo.

Palabras clave: Brachycera; especies exóticas; entomología forense; insectos necrófagos.

Calliphoridae is a cosmopolitan family of calyptrate flies with 1,525 described species worldwide (Pape *et al*. 2011). About 130 blowfly species have been recorded in the Neotropical region (Amorim *et al*. 2002) and 100 species in the Nearctic region (Pape *et al*. 2009). There are a few studies in the Neotropical region, mainly focusing in the species descriptions, biological data, synanthropy, and distribution ranges of blowflies (Amendt *et al*. 2004; Beltrán and Villa-Navarro 2011; Byrd and Castner 2007; Sánchez-Álvarez and Cupul-Magaña 2012; Stanford-Camargo *et al*. 2017; Valdes-Perezgasga *et al*. 2010; Vázquez-Saucedo *et al*. 2007;). Despite these studies, there is still a lack of information regarding the Calliphoridae fauna in Latin America. In addition to the importance of the blowflies in medicine-legal areas, some species native of the Neotropical region and others introduced of genera *Chrysomya* and *Lucilia* produce myiasis in different mammals, including humans (Zumpt 1965). Therefore, it is important to conduct entomological surveys to monitor the blowfly populations to prevent potential damages to cattle, to evaluate the introduction of exotic species, and to provide information on their distribution (Harvey *et al*. 2019).

In Mexico, only 30 species have been reported (Jaume-Schinkel and Ibáñez-Bernal 2020). Recently, with the rapidly gaining importance in forensic entomology, some studies have been made aimed to understand the relationship between insects present on cadaveric succession and decomposition Unfortunately, out of the 31 states in Mexico, only 4 or 5 states have been made entomological surveys regarding blowflies (Byrd and Castner 2010).

Adult necrophagous blowflies use olfactory cues to locate food sources and possible ovi/larvi position sites and the composition of species is variable due to species-specific olfactory preferences, therefore, the most common practice during entomological surveys targeting necrophagous Diptera consists in using carrion sources as different baits in different environments to capture all necrophagous species

present in an area to get the best representation of the fly community. (Harvey *et al*. 2019). The goal of this study is to assess the community of Calliphoridae species using four types of carrion baits as we expect some statistical differences between the baits in Yautepec, Morelos, Mexico.

MATERIALS AND METHODS

Study area

This work was conducted in a remnant of low deciduous forest in the facilities of CeProBi-IPN (Centro de Desarrollo de Productos Bióticos–Instituto Politécnico Nacional) on the 6th km in the road Yautepec-Jojutla, Colony San Isidro in the municipality of Yautepec, state of Morelos, Mexico. Yautepec is situated in 18°57' N 98°09' E. (Figure 1). Climate is humid semi-warm with an annual temperature of 20.3 °C, with rains in the summer with an average precipitation of 901.2 mm. The dominant vegetation is characterized by *Ceiba* sp.*, Bursera* spp.*,* and *Ficus* sp. as dominant species (Luna-Reyes *et al*. 2010).

Field and laboratory procedures.

Collections were made each month from September 2016 through August 2017 using eight entomological traps separated by 20 meters (Figure 2), in a 200 meter transect. Each trap was baited with 100 grams of decomposed chicken, pork, squid, or beef (previously decomposition of 10 days). The traps were numbered from one to eight to know the sequence of baits; the first month, traps arrangement was: one-chicken, two-pork, three-squid, four-beef, five-chicken, six-pork, seven-squid and eight-beef; for the following months the number-bait relation was the same, only the order of the traps was determined by a random sequence (www.random.org/sequences/) so the trap order did not influence the survey. Traps were put in place for 72 hrs.

After that time, traps were removed from the site and taken to the facilities in order to sacrifice the insects and process them. All flies were killed with chloroform, then the traps were emptied on a white surface and proceeded to place all the entomological samples in Petri dishes and then stored in a freezer at -20 °C

Identification of Specimens

All specimens belonging to Diptera were examined in a Nikon SMZ800 stereomicroscope and proceeded with the identification using the keys described elsewhere (Brown *et al.* 2009; Dear 1985; Hall 1948; McAlpine *et al.*1981, 1987; Kosmann *et al.* 2013; Mello 1972, 2003; Shewell 1981; Whitworth 2006, 2014). Some specimens were pinned for future studies and for specimen reference collection. Voucher specimens were deposited at Colección Entomológica Instituto de Ecología, Mexico, Veracruz, Xalapa (IEXA). Meteorological Data was obtained from Servicio Meteorológico Nacional-Conagua.

Statistical Analyses

A X^2 Test was used to evaluate the preferences of the blow fly species for each type of bait based on the frequencies that were or not preferred by the species (Carvalho *et al*. 2011, 2013)., additionally, we performed a test to evaluate the bait preferences using a matrix of abundance and frequencies with a X^2 test in Rstudio (Rstudio 2019). Furthermore, we performed a significant species test to evaluate differences

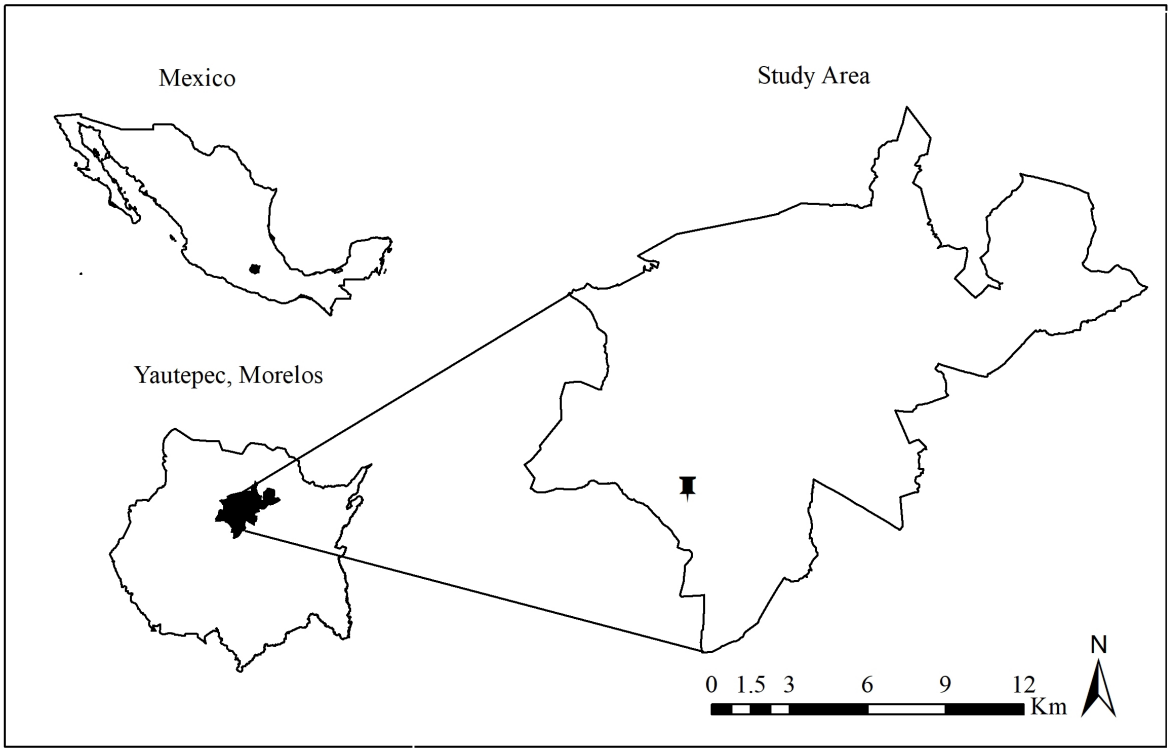

Figure 1. Map showing study area. Pin marking locality of the study.

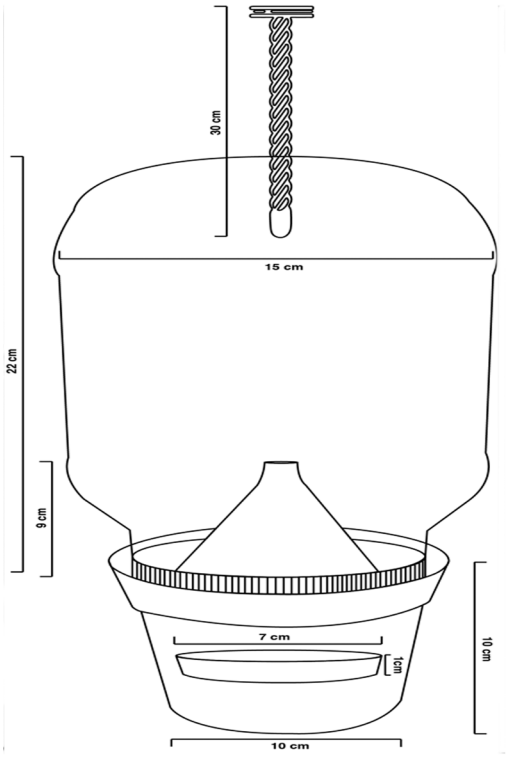

Figure 2. Trap design used in this study.

between baits according to Chao *et al*. (2014) with the iNext online program (Chao *et al*. 2016). This test uses Hill's numbers with three orders (q= 0, q= 1, q= 2), where 0 = species richness, 1 = Shannon diversity, and 2 = Simpson diversity.

RESULTS

A total of 4,765 Diptera specimens were collected belonging to 18 families: *Anthomyiidae, Calliphoridae, Cecidomyiidae, Chironomidae, Culicidae, Drosophilidae, Dryomyzidae, Muscidae, Mesembrinellidae, Neriidae, Pallopteridae, Phoridae, Ropalomeridae, Sarcophagidae, Sciaridae, Stratiomyiidae, Tanypezidae* and *Tephritidae*. The 10 most abundant families are shown in Figure 3. Regarding the Calliphoridae we collected a total of 2644 specimens, the equivalent of 55.74% of the total Diptera, belonging to two subfamilies (Lucilinae and Chrysomyinae), four genera and seven species. The relative abundance of blow flies collected is shown in Figure 4.

The abundances of calliphorid species varied according with the type of bait (Table 1). The X^2 test showed results statistically significant for four species (*Hemilucilia segmentaria* (Fabricius, 1805)*, Cochliomyia macellaria* (Coquerel, 1858), *Chrysomya megacephala* (Fabricius, 1794) and *C. rufifacies* (Macquart, 1843)) ($P < 0.05$), no significance for one species (*Lucilia mexicana* Macquart, 1843*)* ($P > 0.05$), and for the remaining two species (*Hemilucilia semidiaphana* (Rondani, 1850) and *Lucilia cuprina* (Wiedemann, 1830)) we did not perform the test because the low number of specimens ($n < 10$). The cumulative species richness curve (Figure 5) indicates that the sampling effort was good, as the obtained and estimated richness match 100%. The species *Hemilucilia segmentaria* (Fabricius, 1805) and *Cochliomyia macellaria* (Coquerel, 1858) both preferred chicken followed by beef and squid, respectively. *Chrysomya rufifacies,* (Macquart, 1843) which was the most abundant of all species, preferred squid followed by pork, whereas *C. megacephala*, the second most abundant species, also preferred squid followed by beef.

The test performed in Rstudio (Figure 6) showed that *Chrysomya megacephala* and *C. rufifacies* have a strong preference for squid, while *L. mexicana* prefers beef and pork, and *Cochliomyia macellaria* and *H. segmentaria* preferred chicken.

Additionally, in the test performed in iNEXT (Figure 6) with q= 0, we obtained a significative difference between baits as follows: beef and squid, beef and pork, chicken and squid, and no difference between beef and chicken, chicken and pork, additionally when performed with q = 1 there is a significative difference between two groups beef/chicken and pork/squid, this difference is the same when performed with q = 2.

DISCUSSION

We collected a total of 4,765 individual belonging to 18 Diptera families. The ten most abundant families are shown in Figure 3. Being Calliphoridae the most abundant family with more than 50 % (N<2600) of the total captured individuals. Out of these 2,600 individuals, 1800 belonged to a single species *Chrysomya rufifacies*, in other words, 70 % of the specimens of Calliphoridae are *C. rufifacies,* being the most abundant species.

Diptera families with a low representation ($n < 20$) are considered to be an accidental catch with no necrophagous habits, e.g., Culicidae, Chironomidae and Ropalomeridae. Likewise, in the ten most abundant families, some of them are not considered necrophagous per se, but nonetheless some species are attracted to decaying mater, e.g., Neriidae and Tephritidae. Furthermore, families such as Anthomyiidae and Phoridae have a wide variety of habits and might be captured in the traps as a result of predation, phoresy or parasitism or simply because they were attracted to some of the compounds released from the decomposition process. As a final point, the three most abundant families Calliphoridae, Sarcophagidae, and Muscidae are considered as necrophagous.

Inside the Calliphoridae, species such as *Chrysomya megacephala*, *C. rufifacies*, *Cochliomyia macellaria*, *L. cuprina*, *Hemilucilia semidiaphana*, and *H. segmentaria* have been found during entomological surveys attempting to know the Calliphoridae fauna in Argentina (Mariluis and Mulieri, 2003; Olea *et al*. 2012), Brazil (Carmo *et al*. 2017; Oliveira and Vasconcelos 2017), and Venezuela (Nuñez and Lira 2017). Out of these six species, *Chrysomya*

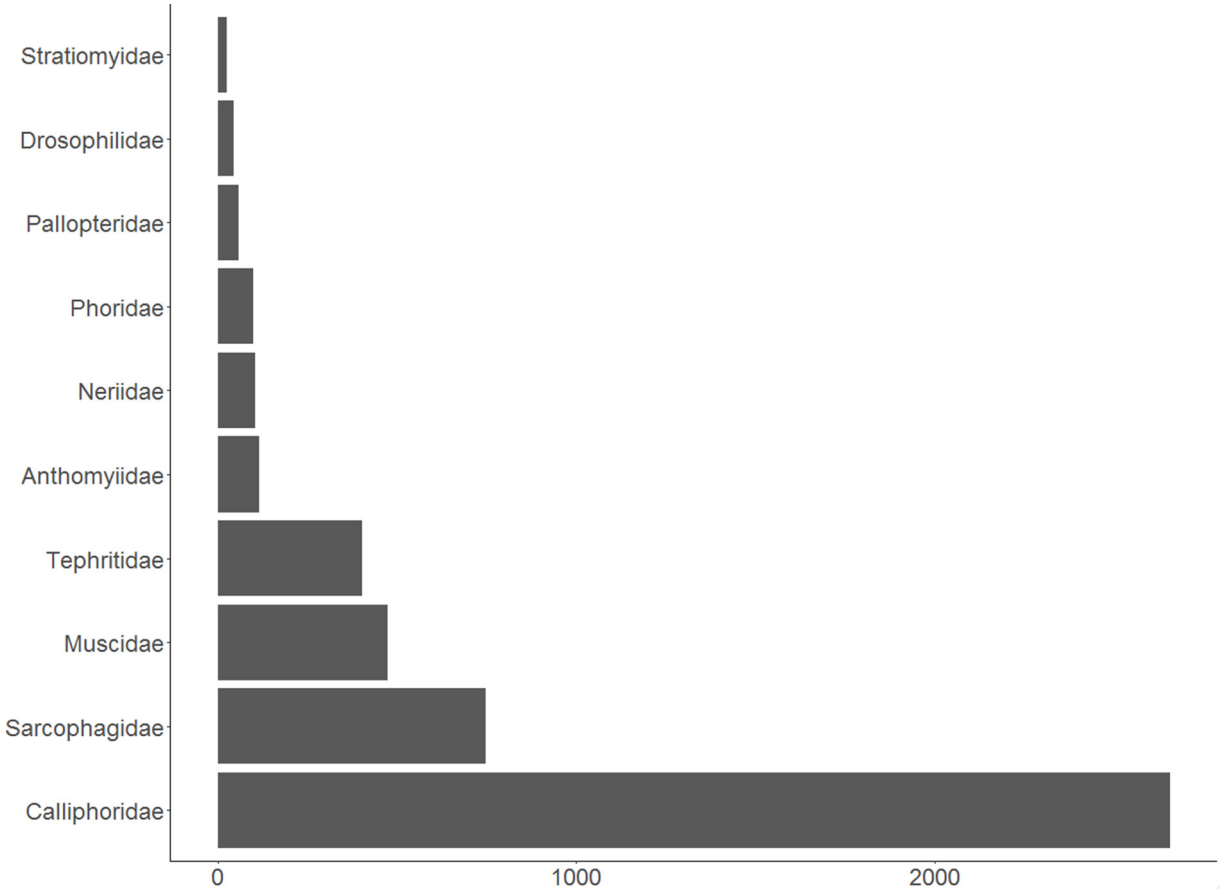

Figure 3. Abundance of the 10 most abundant Diptera families found in the study.

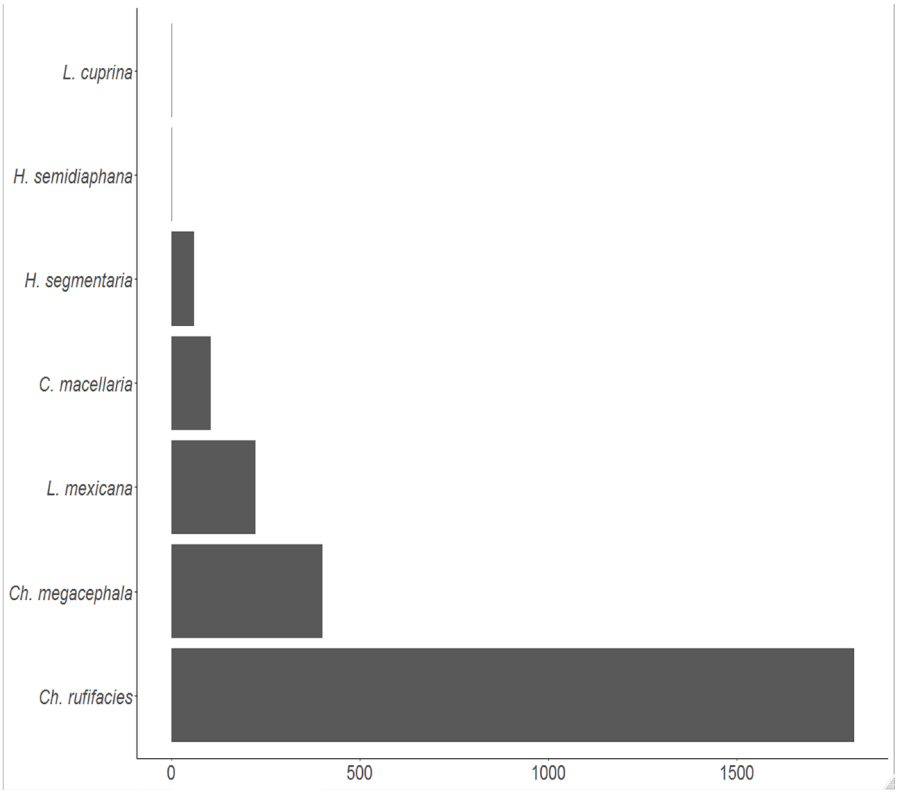

Figure 4. Abundance of Calliphoridae species

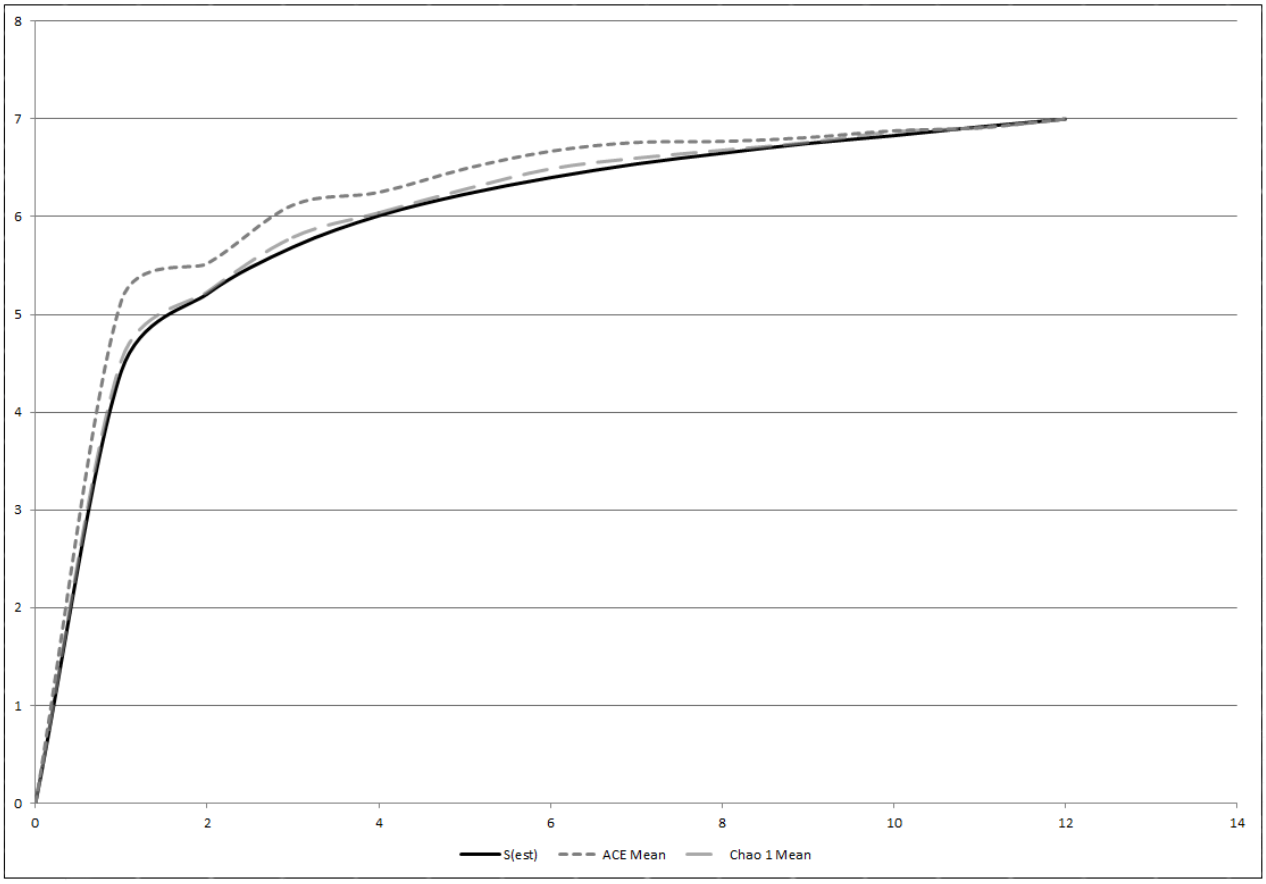

Figure 5. Accumulative species richness curve. Using ACE mean and Chao1 mean as estimators.

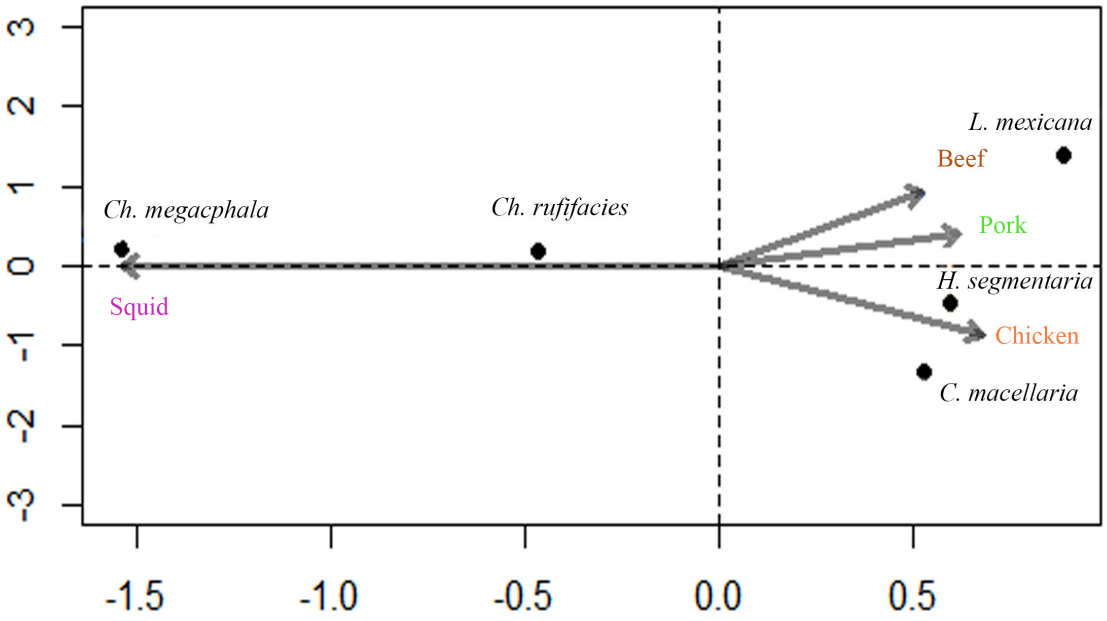

Figure 6. Bait preferences analysis.

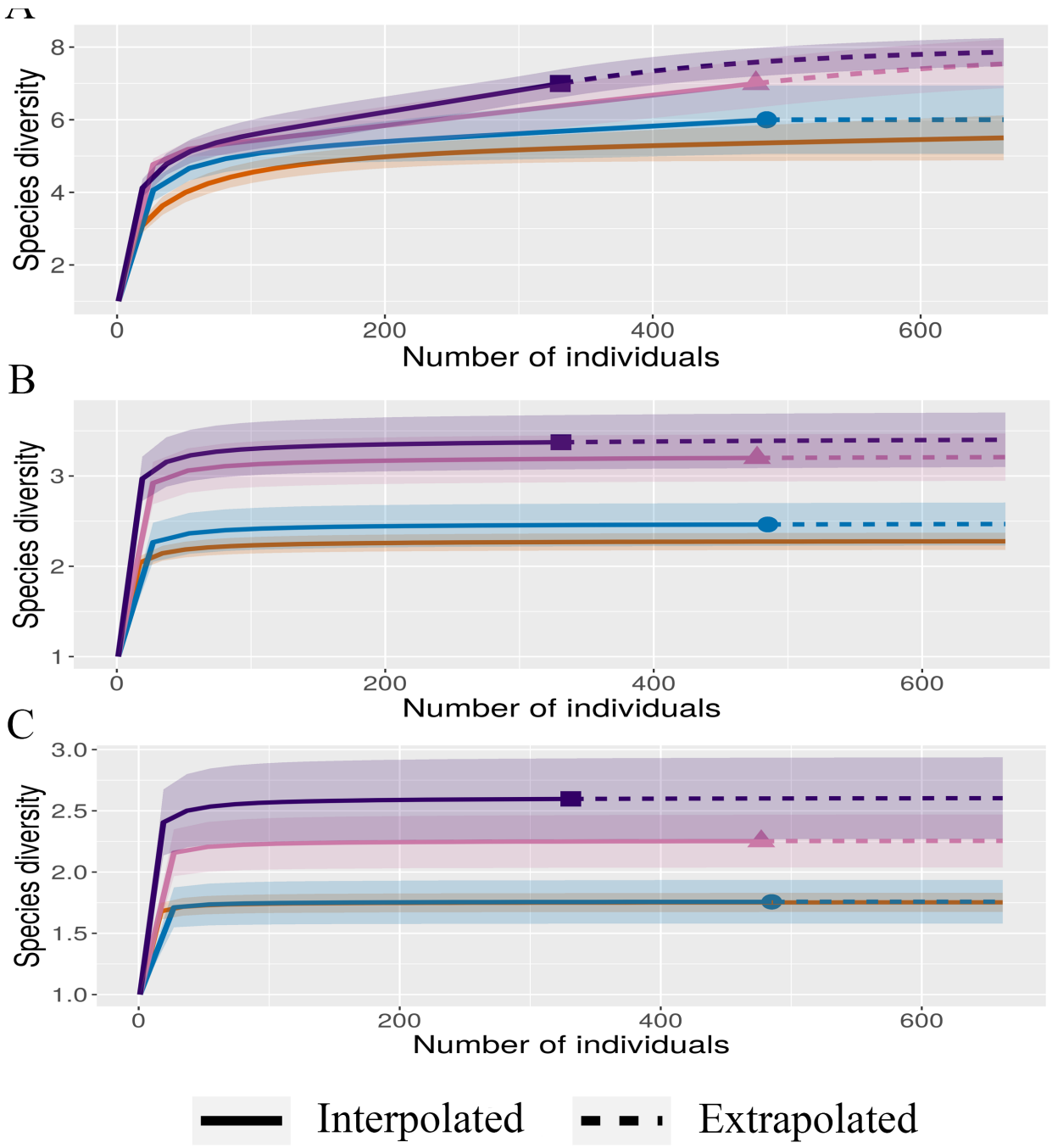

Figure 7. Number of effective species test INext. A using q = 0; B using q = 1; C using q = 2.

megacephala and *C. rufifacies* presented an original distribution in Asia and Africa, and were introduced in the Neotropical Region in the late 1970's (Guimãraes *et al*. 1978). Nowadays, they are stablished in almost all the American continent (South, Central and North America) (Gagné 1981; Baumgartner and Greenberg 1984; Picard 2013).

Both *Hemilucilia segmentaria* and *H. semidiaphana* occur from Mexico to Argentina, having affinities towards warm or template areas, being frequent in forested areas (Ferreira and Barbola, 1998; Marinho *et al*, 2006; Moretti and Godoy, 2013). *Lucilia cuprina* has a distribution almost worldwide in warmer climates of the tropics. In America, it is found from United States to Uruguay and Northern Argentina (Whitworth, 2014). *L. mexicana* has similar distribution raging from United States to southern South America, this species is rather common in some areas (Whitworth 2014).

The abundance of *Chrysomya megacephala* and *C. rufifacies* (Figure 4) may be an indicator that the area where the survey took place is perturbed by human activities, although these flies are present in both forested areas and

urban environments, they can be invasive displacing native species from conserved areas, and both present a high degree of synanthropy, usually found in urban environments (Olea *et al*. 2012; Souza and Zuben 2012; Pereira de Sousa *et al*. 2015). *C. megacephala*, *C. rufifacies* and *L. mexicana* were present all the year, while *L. cuprina* , *H. segmentaria*, and *H. semidiaphana* were present in just a few months; *H. segmentaria* had a higher abundance in the colder months (December and January) when the temperatures were at 21.1 C° and 19.7 C° respectively (Table 2), on the other hand, *H. semidiaphana* is only present in one month (February) that happens to be one of the coldest months with 21.5 C°, but it presented a very low abundance (n = 3). Comparable to *H.* segmentaria, *L. cuprina* is present in three different months (October, February and April) with only one specimen in each month. Calliphoridae species that can be found in a year-long pattern can provide different data which result in a better understanding of the biology of the species sand eventually their use inside forensic investigations.

Squid, pork, and chicken are commonly used as carrion baits to attract several necrophagous insects (Baz *et al*, 2007; Morón and Terrón, 1984; Newton and Peck, 1975). The use of different types of baits is important because some of the species present a statistically important preference (Moretti and Godoy, 2013; Sanchez-Rojas *et al*, 2011). Although species are present in all baits, experimenting with different types can help to identify the best type of bait or baits to attract more necrophagous blow flies helping in achieving better results when doing entomological sampling. In this study, *Chrysomya megacephala* and *C. rufifacies* preferred squid, while *H. segmentaria* and *C. macellaria* preferred chicken. On the contrary, *Lucilia mexicana* showed a significant preference for beef and pork.

Similar studies (e.g. Moretti and Godoy, 2013; Sanchez-Rojas *et al*, 2011) reported differences when using different type of baits, other studies (e.g. George *et al*, 2012) found differences between different aged baits and chemical cues of larval stages, several factors can affect the attraction of the baits, such as temperature, humidity, time of decomposition and these factors are potential subjects of study for further experiments, as for the moment it is unknown the reason why while using different types of baits we found differences. We observed some composition changes in the abundance of flies correlating with the monthly temperature, however this was not analyzed and it gives room for further studies to properly determinate if this is statistically significant and not just speculation. In conclusion, if researchers continue to use different types of baits, with different times of decomposition the more complete the sampling will be, as there is clearly differences in the type of baits. Moreover, if we had extended the sampling periods, seasonal patterns might have become evident. Despite the study area being a small geographical area, we present some relevant information on the biology of the species present in the study.

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Table 1 Abundances of blow flies by type of bait. n=number of specimens per bait. N=total number of specimens per all baits. C=chicken, P=pork, S=squid, B=beef.

	Lucilia mexicana	*Lucilia cuprina*	*Hemilucilia segmentaria*	*Hemilucilia semidiaphana*	*Cochliomyia macellaria*	*Chrysomya megacephala*	*Chrysomya rufifacies*
	n	n	n	n	n	n	n
C	46	1	30	1	41	51	306
P	47	0	8	1	24	46	359
S	67	1	11	1	29	251	961
B	64	1	13	0	12	53	187
N	224	3	62	3	106	401	1813

Table 2 Monthly precipitation (mm), temperature (°C), species richness, and abundance of Calliphoridae species.

Year	Month	Precipitation	Temperature	Richness	Abundance
2016	September	322.8	22	4	65
	October	49.5	22.2	7	69
	November	59.3	20.6	4	42
	December	2.2	21.1	5	104
2017	January	0	19.7	5	214
	February	0	21.5	8	371
	March	13.1	23.4	4	220
	April	13.2	25	5	93
	May	162.1	25.4	3	87
	June	386	23.1	3	397
	July	320.4	22.5	4	874
	August	517.2	22.6	4	120

