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Evaluation of bait traps as a means to predict initial blow fly (Diptera: Calliphoridae) communities associated with decomposing swine remains in New Jersey, USA

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ABSTRACT

Information about blow fly (Diptera: Calliphoridae) species distributions can be valuable for criminal investigations, with regards to determining movement of remains from one location to another and time of colonization estimates, making these data extremely useful. Past work has been conducted on initial species community structure across New Jersey, USA using traps baited with beef liver; however, if these same species frequent vertebrate carrion remains unclear. In order to evaluate these data, piglet carcasses were placed out once every two weeks for a year in New Brunswick, New Jersey, USA. The same methods were implemented as those used for traps baited with beef liver, with length of collections being based on ADD values. Seven calliphorid species, *Calliphora vicina* (Robineau-Desvoidy), *Lucilia sericata* (Meigen), *Lucilia coeruleiviridis* (Macquart), *Phormia regina* (Meigen), *Pollenia pediculata* Macquart, *Pollenia rudis* (F.) and *Protophormia terraenovae* (Robineau-Desvoidy) were collected from the carcasses. During this experiment *L. sericata, L. coeruleiviridis* and *P. regina* were the dominant adult blow flies captured, totaling 38.2%, 29.2% and 29.2% respectively of all adults caught. All three species colonized the carcasses as well, although not all were dominant colonizers. *C. vicina* was recorded ovipositing in December, while the piglet was submerged in approximately 5 cm of snow. All species that totaled at least 1% of the total collection (adults captured and larvae reared) were the same across baited traps and carcasses. This study supports the use of beef liver baits for surveying forensically important blow flies and the application of such information to forensic investigations.

1. Introduction

Recently, the field of forensic entomology has grown due to its importance in legal investigations. Typically, insect development in association with temperature can be used to determine a time of colonization (TOC) of human remains, which can then be used to infer a time since death [10]. Insects can also be used to provide information pertaining to body movement based on the geographical distribution of the insects collected from the remains [12] and can aid in cases of abuse or neglect in animals, including humans [7]. Blow flies (Diptera: Calliphoridae) are used often in forensic investigations because they are primary colonizers of remains and can arrive at a carcass within minutes [4]. For entomological evidence to be useful in a given area, it is crucial to have data on the species present there over time. Sanford [35] discusses the importance of baseline survey data in relation to casework, especially in different geographic locations.

There are several different methodologies for collecting forensically important insect taxa, including aerial sweep netting, traps (baited, sticky, or pitfall), and manual collections from remains (most often larvae, pupae or adult beetles). Depending on the research question being asked (e.g., initial colonization verse insect succession) different methodologies can be more applicable for collection. For instance, studies have surveyed forensically important Diptera with the use of baited traps and have used different food sources within each trap including pork liver/muscle, beef liver, and wet cat food [24,8,20,18,42]. Either adult dipterans and/or larvae can be collected from traps to analyze both the species arriving to the trap and species that have successfully

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oviposited. Brundage et al. [8] also confirmed that blow fly species collected in traps baited with beef liver corresponded to species found to colonize human remains in Santa Clara, California, USA. Beef liver is commonly used in baited traps because it is attractive to blow flies, inexpensive, and easy to obtain [1,8,42].

Manual collections or combined collection techniques are more common in literature where insect succession is being analyzed across the decomposition process [22,34]. For example, Gruner et al. [22] showed that adults collected in the vicinity of a carcass (aerial sweep collections) corresponded to the species that colonized remains located east of Gainesville, Florida, USA. Sticky traps are not as commonly used due to the time and effort required to remove specimens carefully and still be able to identify them successfully. Recently, Sanford [36] compared species composition on passive sticky traps to manual collection from human remains and found that only 65% of cases had at least one species match across these two methods, indicating that manual collections may be more useful in a casework situation.

When examining insect colonization and community structures, vertebrates of different sizes have been widely used, including mice, rabbits, swine, and various wildlife including black bear and white- tailed deer [26,38,11,39,34,22]. Human remains are difficult to obtain for research purposes, however several studies have compared species composition on human remains to other animals and baited traps used in research [11,18,39]. Carvalho et al. [11] compared insects on human remains to pig carcasses (~10 kg) and found that of the eight calliphorid species collected from swine remains, six were also collected from human remains. Similarly, Farinha et al. [18] found that traps baited with pork muscle caught six of the eight calliphorid species found on human remains in Portugal. Further, in China, Wang et al. [39] showed that the same calliphorid species occurred on a human carcass, big and small pig carcasses (45 and 48 kg, 23 and 25 kg, respectively), as well as rabbit carcasses (n = 2) (excluding one species which only occurred on one rabbit).

Weidner et al. [42] conducted a two-year survey for forensically important blow flies in New Jersey, USA using traps baited with beef liver, and collected twelve species within six different genera. The goals of the present study were to evaluate the use of beef liver baited traps by comparing the results from Weidner et al. [42] using those obtained with piglet carcasses, as well as to add to the baseline data available on forensically important blow flies in the area. Because many blow flies appear to be generalists with regards to food source, we expected that species composition would be similar between beef liver and piglet carcasses.

2. Methods

One study site (New Brunswick, New Jersey) used by Weidner et al. [42] was chosen for the present study. This site was selected because all blow fly species comprising more than 1% of the total abundance found by [42] were collected there. The methods of Weidner et al. [42] for trap placement and duration were followed, with piglet carcasses replacing beef liver.

From September 2013 to August 2014, one piglet carcass (*Sus domesticus* L.) was deployed once every two weeks, excluding May 2014, during which only one piglet was placed out, totaling 23 piglets. Piglet placement followed the trap placement methods from Weidner et al. [42]. Briefly, the duration the piglet was exposed ranged from one to seven days depending on ambient temperature (approximately 15-16 ADD, Base 10 °C). Each carcass was placed out between the hours of 1300 and 1530, next to a building surrounded by trees, which was within 10 m of a trap location from Weidner et al. [42]. Prior to placement, the piglet was thawed in a sealed bag at room temperature for approximately 24 h. It has been shown that previously freezing then thawing vertebrate carcasses does not appear to have a significant effect on the initial arrival times of calliphorids or their colonization patterns as compared to refrigerated carcasses [9]. The piglet was then sexed and weighed before placement. Piglet weights ranged from 1.12–2.18 kg, with a mean weight of 1.48 ± 0.07 kg. Piglets were placed in one of five locations in the area, and were never placed in the same area within the same month. This approach was to reduce the chance of previous decomposition remnants/fluids being present. A $61 \times 61 \times 61\ cm^3$ cage constructed of chicken wire was placed over the carcass and held in place with stakes to prevent vertebrate scavengers from accessing it.

The piglet was visited daily, and adult flies were collected for five minutes using sweep nets to collect any flying adults over/on the carcass and an ~1 m radius around the carcass. The adults collected were killed by being placed in a kill jar charged with ethyl acetate, and then brought back into the laboratory and placed into a freezer at −20 °C. If the carcass remained out for only one full day, eggs and/or larvae were collected on the second day, and placed on beef liver and brought back to the laboratory where they were reared to adulthood. If the carcass remained out longer than one day, the previously mentioned sweep collections occurred daily along with egg/larvae collections on either day two or the first day eggs/larvae were observed. Eggs and larvae were collected with a plastic spoon and moved onto approximately 15 g fresh beef liver in a 29 mL SOLO® cup. Collections were made from each region on the carcass colonized. Approximately half of the eggs or larvae were collected, excluding carcasses where small amounts of eggs were present (low temperatures or heavy precipitation), in these cases, more than 50% were collected. Eggs and/or larvae within the 29 mL cup were placed onto pine wood shavings within a 946 mL Extreme Freeze™ Reditainer™ Freezable deli container. Reared larvae were given beef liver continuously to ensure survival, and kept at ~23 °C, 14:10 (L:D) photoperiod and ~50–60% RH.

Blow fly species were identified using morphological characteristics from the keys of Whitworth [43], Marshall et al. [30] and Jewiss-Gaines et al. [27]. The specimens were catalogued, and categorized on whether they were caught as adults at the carcass or were reared out. Blow fly species from this study were then compared with adults and larvae of blow fly species collected using traps baited with beef liver from the New Brunswick site of Weidner et al. [42]. Comparisons were broken down by month and season, and both years of the survey were combined and seasons were predetermined by characteristics in Weidner et al. [42]. Using the Shapiro Wilks normality test, temperature and precipitation values were determined to not be normally distributed ($W = 0.9791$, $P = 0.0002286$, $W = 0.4215$, $P = <2.2e{-}16$ respectively), and therefore Kruskal–Wallis one-way analysis of variance (ANOVA) was conducted to determine experimental (bait vs. carcass) and seasonal differences. Wilcoxon Rank-Sum tests were conducted when Kruskal–Wallis results were significantly different ($P < 0.05$).

3. Results

From September 2013 to August 2014, 114 blow fly adults were collected and 1445 larvae reared out successfully, and identified. Seven species spanning five genera were collected from the piglet carcasses (Table 1). From September 2011 to August 2013, 3256 adult blow flies were collected and 6039 larvae reared out successfully and were identified from the traps baited with beef liver within this site. Of the total twelve species collected during the initial survey, ten species spanning six genera were collected from the baited traps in the location of the current study (Table 2). Adults were collected from the piglet carcasses during May through October, and eggs/larvae were collected April through December, in comparison to baited traps where adults were

Table 1
Total number and percentage of collected Calliphoridae species both as adults and eggs/larvae (reared) collected from piglet carcasses between September 2013–August 2014.

Genus	Species	Total adults (%)	Total reared (%)
Calliphora	*vicina*	2 (1.4%)	198 (13.7%)
Lucilia	*coeruleiviridis*	42 (29.2%)	39 (2.7%)
	sericata	55 (38.2%)	1108 (76.7%)
Phormia	*regina*	42 (29.2%)	100 (6.9%)
Pollenia	*rudis*	1 (0.7%)	–
	pediculata	1 (0.7%)	–
Protophormia	*terraenovae*	1 (0.7%)	–

Table 2
Total number and percentage of collected Calliphoridae species both as adults and eggs/larvae (reared) collected from baited traps between September 2011–August 2013.

Genus	Species	Total adults (%)	Total reared (%)
Calliphora	*livida*	9 (0.3%)	1042 (17.3%)
	vicina	92 (2.8%)	531 (8.8%)
	vomitoria	14 (0.4%)	–
Cochliomyia	*macellaria*	5 (0.2%)	–
Cynomya	*cadaverina*	9 (0.3%)	–
Lucilia	*coeruleiviridis*	450 (13.8%)	142 (2.4%)
	illustris	2 (0.1%)	25 (0.4%)
	sericata	1870 (57.4%)	3171 (52.5%)
Phormia	*regina*	799 (24.5%)	1127 (18.7%)
Pollenia	*pediculata*	6 (0.2%)	1 (<0.01%)

collected February through December, and eggs/larvae collected April through October (Table 3). Mean (±SE) seasonal temperatures ranged from 0.56 ± 1.13 to 22.65 ± 0.59 °C during the piglet carcass study, and 3.32 ± 0.48 to 23.39 ± 0.50 °C during the baited trap study (Table 4). Significant differences were found across seasons both during the use of baited traps ($H = 116.3$, $P < 0.001$) and carcasses ($H = 61.9$, $P < 0.001$). Within each trial period (baits, carcasses) significant differences ($P < 0.001$) were found across seasons, except fall and spring for baits ($P = 0.548$) and carcasses ($P = 0.948$). Mean seasonal temperatures were not found to differ significantly ($P > 0.050$) within season across trial periods except in winter ($P = 0.011$), with temperatures being colder when the piglet carcasses were placed out. Mean precipitation amounts did not significantly differ ($P > 0.050$) across trials (Table 4).

For the piglet carcasses, the dominant adult species collected were *Lucilia sericata* (Meigen), *Lucilia coeruleiviridis* (Macquart), and *Phormia regina* (Meigen), accounting for 38.2%, 29.2%, and 29.2% of total adults collected, respectively. Two *Calliphora vicina* (Robineau-Desvoidy) were collected, along with single individuals belonging to *Pollenia pediculata* Macquart, *Pollenia rudis* (F.), and *Protophormia terravenovae* (Robineau-Desvoidy) (Table 1). Of these species, *C. vicina, L. sericata, L. coeruleiviridis,* and *P. regina* laid eggs that successfully enclosed, with *L. sericata* being the dominant colonizer (76.7%), followed by *C. vicina* (13.7%) (Table 1). For the baited traps, the dominant species collected as adults were *L. sericata, P. regina* and *L. coeruleiviridis,* accounting for 57.4%, 24.5%, and 13.8%, respectively (Table 2). Individuals belonging to *Calliphora livida* Hall, *Cochliomyia macellaria* (F.), *Calliphora vomitoria* (L.), *Cynomya cadaverina* Robineau-Desvoidy, *Lucilia illustris* (Meigen), and *P. pediculata* were collected as adults but each made up less than 1% of total adult captures. Of these species, eggs were collected from *C. livida, C. vicina, L. coeruleiviridis, L. illustris, L. sericata, P. regina,* and *P. pediculata,* with *L. sericata* (52.5%), *P. regina* (18.7%), and *C. livida* (17.3%) being the dominant colonizers (Table 2).

When examining species composition across seasons the dominant adult species collected during the spring in baited traps were *P. regina, L. sericata* and *L. coeruleiviridis* (39.5%, 28.8%, and 14.9% respectively), while on piglet carcasses they were *P. regina* (87.5%) and *L. sericata* (12.5%). The main colonizers of baited traps in the spring were *L. sericata* (31.1%) and *C. livida* (47.3%), while the piglet carcasses were colonized by *L. sericata* (66.1%) and *P. regina* (21.8%) (Table 3). During the summer, the dominant adult species collected from baited traps were *L. sericata* (70.2%) and *L. coeruleiviridis* (21.6%), while the dominant adult species collected from the piglet carcasses were *L. sericata, L. coeruleiviridis,* and *P. regina* (39.4%, 23.4% and 34.0% respectively). The main colonizers of baited traps in the summer were *L. sericata* (67.8%) and *P. regina*

Table 3
Blow fly species collected from baited beef liver traps (B) and piglet carcasses (P) in New Brunswick, NJ. Baited traps were placed out from September 2011 to August 2013 and piglet carcasses were placed out from September 2013 to August 2014. If species are present the first number represents total numbers of adults, followed by number of larvae successfully reared out.

	Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Species													
	C. livida (B)	–	–	–	2, 878	7, 163	0, 1	–	–	–	–	–	–
	(P)	–	–	–	–	–	–	–	–	–	–	–	–
	C. vicina (B)	–	1, 0	–	13, 98	63, 321	1, 4	3, 0	–	6, 1	4, 107	–	1, 0
	(P)	–	–	–	–	0, 14	–	–	–	–	2, 124	0, 7	0, 53
	C. vomitoria (B)	–	–	–	6, 0	8, 0	1, 0	–	–	–	–	–	–
	(P)	–	–	–	–	–	–	–	–	–	–	–	–
	C. macellaria (B)	–	–	–	–	–	–	–	–	5, 0	–	–	–
	(P)	–	–	–	–	–	–	–	–	–	–	–	–
	C. cadaverina (B)	–	–	1, 0	2, 0	1, 0	–	–	–	–	–	5, 0	–
	(P)	–	–	–	–	–	–	–	–	–	–	–	–
	L. coeruleiviridis (B)	–	–	–	1, 0	94, 0	74, 4	122, 43	59, 32	92, 63	8, 0	–	–
	(P)	–	–	–	–	0, 19	5, 3	13, 6	4, 9	13, 2	7, 0	–	–
	L. illustris (B)	–	–	–	–	1, 0	–	1, 25	–	–	–	–	–
	(P)	–	–	–	–	–	–	–	–	–	–	–	–
	L. sericata (B)	–	–	–	8, 118	176, 567	44, 231	670, 1335	114, 506	847, 414	11, 0	–	–
	(P)	–	–	–	0, 145	1, 34	16, 356	14, 104	7, 123	6, 88	11, 258	–	–
	P. regina (B)	–	–	–	–	252, 58	26, 836	54, 40	9, 0	457, 193	–	–	1, 0
	(P)	–	–	–	0, 1	7, 58	17, 23	15, 0	0, 1	3, 17	–	–	–
	P. pediculata (B)	–	–	–	–	3, 0	2, 0	0, 1	–	–	–	–	1, 0
	(P)	–	–	–	–	–	1, 0	–	–	–	–	–	–
	P. rudis (B)	–	–	–	–	–	–	–	–	–	–	–	–
	(P)	–	–	–	–	–	–	1, 0	–	–	–	–	–
	P. terraenovae (B)	–	–	–	–	–	–	–	–	–	–	–	–
	(P)	–	–	–	–	–	–	1, 0	–	–	–	–	–

Table 4
Mean (±SE) temperature (°C) and precipitation (cm) amounts by season and month across trials (baited traps and piglet carcasses).

Season & month	Baited traps		Piglet carcasses	
	Mean temp. (°C)	Mean precip. (cm)	Mean temp. (°C)	Mean precip. (cm)
Spring	12.97 ± 0.87	0.21 ± 0.06	10.17 ± 1.22	0.46 ± 0.24
Mar	8.94 ± 1.12	0.22 ± 0.09	4.95 ± 1.90	0.82 ± 0.57
Apr	12.17 ± 0.73	0.08 ± 0.05	12.45 ± 0.64	0.15 ± 0.11
May	17.65 ± 0.91	0.38 ± 0.10	17.64 ± 0.66	0.39 ± 0.22
Summer	23.39 ± 0.50	0.60 ± 0.26	22.65 ± 0.59	0.83 ± 0.50
Jun	21.21 ± 0.70	0.60 ± 0.31	21.89 ± 1.41	0.42 ± 0.20
Jul	24.54 ± 0.52	0.69 ± 0.39	23.82 ± 0.46	1.48 ± 1.05
Aug	23.30 ± 0.33	0.48 ± 0.28	21.25 ± 1.33	0.03 ± 0.01
Fall	13.63 ± 1.01	0.25 ± 0.11	10.97 ± 1.36	0.05 ± 0.03
Sep	21.31 ± 0.56	0.66 ± 0.28	19.72 ± 2.29	0.04 ± 0.04
Oct	13.72 ± 0.56	0.16 ± 0.10	11.90 ± 1.06	0.01 ± 0.01
Nov	7.09 ± 0.65	0.04 ± 0.03	5.09 ± 1.30	0.08 ± 0.06
Winter	3.32 ± 0.48	0.37 ± 0.10	0.56 ± 1.13	0.29 ± 0.10
Dec	4.70 ± 0.56	0.52 ± 0.14	3.69 ± 1.82	0.13 ± 0.07
Jan	3.57 ± 0.67	0.36 ± 0.12	−5.97 ± 2.28	0.10 ± 0.08
Feb	2.78 ± 0.54	0.16 ± 0.05	1.15 ± 1.10	0.55 ± 0.25

(28.6%), while *L. sericata* (93.3%) was the main colonizer of the piglet carcasses (Table 3). During the fall, the baited traps collected mainly of *L. sericata* (59.8%) and *P. regina* (31.8%) adults, while the adults caught around the carcasses were *L sericata, L. coeruleiviridis* and *P. regina* (39.4%, 23.4% and 34.0% respectively). The main colonizers of the baited traps were in agreement with the dominant adult species caught, *L sericata* (53.2%) and *P. regina* (24.8%), while the dominant colonizers for the carcasses were *L. sericata* (69.8%) and *C. vicina* (26.4%) (Table 3). Lastly, winter had extremely low numbers of adults captured (four on baited traps, zero from carcass). Of the four adults captured from baited traps, *C. vicina* comprised 50% (2 individuals) and *P. regina* and *P. pediculata* accounted for 25% each (1 individual), and no colonization occurred within the traps. No adults were collected from the piglet carcasses while they were out, however colonization by *C. vicina* (100%) did occur. Season determination is specified with clarification in Weidner et al. [42] and consisted of Spring (March–May), Summer (June–August), Fall (September–November), and winter (December–February).

4. Discussion

This is the first study to compare blow fly species composition data from piglet carcasses to trap data in New Jersey. *L. sericata, L. coeruleiviridis*, and *P. regina* were the dominant species attracted to the bait in both trials (traps and carcasses), and all species that accounted for at least 1% of the total adult collections were found across both trials, supporting that beef liver baited traps do provide a representative sample of species that would arrive to a carcass. When investigating blow fly adults captured, *L. sericata* was found in relatively high numbers, and was a dominant colonizer in spring, summer and fall in both baited traps and piglet carcasses. *P. regina* comprised over 31% of adults collected in spring and fall from baited traps and spring and summer from piglet carcasses. *P. regina* was a main colonizer (28.6%) of baited traps during the summer when adult collections were less than 10% of individuals collected during that season. This indicates that high numbers of adults are not necessarily needed for substantial colonization to occur. Although *L. coeruleiviridis* was a dominant species attracted to baits (14.9% in spring and 21.6% in summer) and piglet carcasses (23.4% in summer and 47.6% in fall), when looking at colonization (eggs/larvae collected and successfully reared to adulthood), this species' numbers were very low. *L. coeruleiviridis* has been shown to be a dominant colonizer of vertebrate carrion [37,28,5]; however, this species does very poor in laboratory settings, with a high mortality rate [41]. Overall, the dominant blow fly colonizers of both the baited traps and piglet carcasses also appear to be consistent with the main species colonizing human remains located within the state of New Jersey (LMW unpublished data).

Interestingly, *P. terraenovae* was collected from an aerial sweep over a piglet carcass, but not collected from beef liver baited traps over the two-year period. *P. terraenovae* is a forensically important blow fly species that is Holarctic in distribution and is common in cooler regions [21]. This species has been collected from animal [15,3] and human [25] remains. Kamal [29] observed that *P. terraenovae* did not readily oviposit on beef liver, but would oviposit in the corner of the cage. This study, along with Kamal's [29] support the notion that this species may be attracted to a specific chemical cue or a chemical combination not released from decomposing beef liver alone. This type of behavior should be considered when surveying and selecting bait choice if carcasses are not available or a practical option. Other species were attracted to the carcass, such that adults were collected, however colonization did not occur. When looking at the piglet carcasses, if a species was collected that totaled less than 1%, colonization was not observed with that species. When examining adults captured in the baited traps, of the six species that totaled less than 1%, three colonized the bait; *P. pediculata* with one individual, *Lucilia illustris* with 25 individuals, and *C. livida* with 1,042 individuals surviving.

No *C. livida* adults were captured from the piglet carcasses, and no larvae were reared out successfully either. Baumgartner [6] collected 1165 blow fly adults in the month of May from traps baited with rat carrion in northeastern Illinois, USA and *C. livida* made up 1% of the total capture. In northern Florida, USA, Gruner et al. [22] only collected 11 *C. livida* adults during the winter trials (22nd December–21st March), and this species was excluded from analysis due to its rarity at the carcasses. Thus, piglet carcasses may not represent the most attractive resource available in nature to *C. livida*. Although low numbers of *C. livida* adults (9 individuals) were collected during a two-year period with traps baited with beef liver, a substantial amount of eggs were laid within those traps (1042, 17.3% of total larvae reared out successfully), indicating that if the species in confined to an area with a possible food source, they will oviposit. However, the lack of adult *C. livida* collection and colonization on piglet carcasses suggests that further comparisons of forensically important Diptera abundance between baited traps and vertebrate carrion should be conducted.

In December 2013, eggs were collected from the left ear of a piglet, and 53 individuals were successfully reared out to adults and determined to be *C. vicina.* When the piglet was placed out there was approximately 5 cm of snow on the ground, and temperatures during that 7 day period ranged from −1.10 °C to 11.95 °C. *C. vicina* is a widespread species in North America [16,33] and Europe [19,2,17]. This species is common in cooler regions or at cooler times of the year such as spring, fall or winter, depending on geographic location [40,33]. Minimum threshold development temperature for this species has been widely debated. Davies and Ratcliffe [13] found that in a laboratory setting eggs hatched at 3.5 °C and larval development was possible at 5 °C, while Deonier [14] noted that *Calliphora* spp. were active at 1.7 °C. Since daily temperatures can range drastically depending on geographic location, a few warmer days in colder seasons could allow for blow fly flight, mating, and oviposition, which could in turn aid in estimating m-PMI determinations, and therefore minimum thresholds should be investigated further in blow flies species that are active during cooler months. Based on the findings of the present study, beef liver baited traps are an informative way to assess blow fly species composition. Although carcasses are larger in size and may be a better proxy for human remains, beef liver baited traps are more affordable and practical for use when surveying large geographical areas.

Two limitations of the present study need to be discussed. First, the collection technique produced overall low numbers of adult Calliphoridae. The baited trap design referred to in the previous study allowed for less blow flies to escape, which facilitated higher collection numbers (as was seen when compared to the carcass). Even though the carcass trial did attract a species not found with baited traps, only a single individual was caught. This could be attributed to the fact that it could freely leave the carcass when disturbed but the carcass was only colonized by the dominant species, which were captured when using beef liver baited traps. Piglets were not contained within a trapping apparatus, which could also explain the comparatively low numbers of adults collected during the present study. Longer collection times (minimum of 10 min), multiple collections per day or combined collection techniques could enable for higher collection totals and should be considered if logistically possible. In addition to combining collection methods, traps incorporating the entire carcass, such as the modification made by Prado e Castro et al. [34] should be considered for future studies to increase the number of individuals captured.

Another limitation of the present study was that the carcasses used (1.12–2.18 kg) were very small in comparison to human remains. For logistical purposes, larger swine carcasses could not be used during this study. However, Hewadikaram and Goff [23] found that there was no difference in arthropod composition or patterns of succession between pig carcasses weighing 8.4 kg and 15.1 kg. On the contrary, Matuszewski et al. [32] found that there were differences in insect assemblages across carcass mass, with smaller carcasses underrepresenting late-colonizing insects. In addition to these findings, it was shown that swine carcasses of larger mass had higher abundances of adult and larval Diptera, although there was no significant difference on the pre-appearance interval of the dominant calliphorid species based to mass [32]. Matuszewski et al. [32] also found that the dominant calliphorid species, *Lucilia caesar* (L.), and *L. sericata*, were collected as both adults and third instar larvae across all carcasses every sampling time. Meanwhile, *P. regina* were collected two of the three times on small carcasses and one medium carcass, and all three times one the medium/large carcasses and large carcasses [32]. The small carcasses ranged from 5–15 kg in the Matuszewski et al. [32] study, and while they were still larger than the carcasses used in the present study, it should be noted that Matuszewski et al. [31] found that there was no significant difference in the onset of the bloat phase across carcass sizes. Since the present study focused on calliphorids that were arriving and colonizing early in the decomposition process, small carcass sizes were sufficient for our purposes. Further work is being conducted to compare both baited traps and piglet carcass findings with casework involving human remains in New Jersey, USA. If extensive studies on faunal succession are to be conducted, larger carcass size should be used. However, when carcasses are not readily available or large areas are being surveyed at once, traps baited with beef liver do provide a predicative sample of forensically important calliphorid species, and should be implemented in more geographical areas to allow for baseline data to be available on blow fly species composition that could later be used in legal investigations.

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References

[1] A. Aak, G.K. Knudsen, A. Soleng, Wind tunnel behavioural response and field trapping of the blowfly *Calliphora vicina*, Med. Vet. Entomol. 24 (2010) 250–257.
[2] C. Ames, B. Turner, Low temperature episodes in development of blowflies: implications for postmortem interval estimation, Med. Vet. Entomol. 17 (2003) 178–186.
[3] G.S. Anderson, Comparison of decomposition rates and faunal colonization of carrion in indoor and outdoor environments, J. Forensic Sci. 56 (2011) 136–142.
[4] G.S. Anderson, S.L. VanLaerhoven, Initial studies on insect succession on carrion in Southwestern British Columbia, J. Forensic Sci. 41 (1996) 61–625.
[5] M.E. Benbow, A.J. Lewis, J.K. Tomberlin, J.L. Pechal, Seasonal necrophagous insect community assembly during vertebrate carrion decomposition, J. Med. Entomol. 50 (2013) 440–450.
[6] D.L. Baumgartner, Spring season survey of the urban blowflies (Diptera: Calliphoridae) of Chicago, Illinois, Great Lakes Entomol. 21 (3) (1988) 119–121.
[7] M. Benecke, R. Lessig, Child neglect and forensic entomology, Forensic Sci. Int. 120 (2001) 155–159.
[8] A. Brundage, S. Bros, J.Y. Honda, Seasonal and habitat abundance and distribution of some forensically important blow flies (Diptera: Calliphoridae) in Central California, Forensic Sci. Int. 212 (2011) 115–120.
[9] K.N. Bugajski, C.C. Seddon, R.E. Williams, A comparison of blow fly (Diptera: Calliphoridae) and beetle (Coleoptera) activity on refrigerated only versus frozen-thawed pig carcasses in Indiana, J. Med. Entomol. 48 (6) (2011) 1231–1235.
[10] Forensic Entomology: The Utility of Arthropods in Legal Investigations, in: J.H. Byrd, J.L. Castner (Eds.), CRC Press, 2009.
[11] L.M.L. Carvalho, P.J. Thyssen, A.X. Linhares, F.A.B. Palhares, A checklist of arthropods associated with pig carrion and human corpses in Southeastern Brazil, Memórias do Instituto Oswaldo Cruz 95 (1) (2000) 135–138.
[12] E.P. Catts, M.L. Goff, Forensic entomology in criminal investigations, Annu. Rev. Entomol. 37 (1992) 253–272.
[13] L. Davies, G.G. Ratcliffe, Development rates of some pre-adult stages in blowflies with reference to low temperatures, Med. Vet. Entomol. 8 (1994) 245–254.
[14] C.C. Deonier, Carcass temperatures and their relation to winter blowfly populations and activity in the Southwest, J. Econ. Entomol. 33 (1940) 166–170.
[15] G.D. De Jong, An annotated checklist of the Calliphoridae (Diptera) of Colorado, with notes on carrion associations and forensic importance, J. Kansas Entomol. Soc. (1994) 378–385.
[16] R.J. Dicke, J.P. Eastwood, The seasonal incidence of blowflies at Madison, Wisconsin (Diptera: Calliphoridae), Wis. Acad. Sci. Arts Lett. 41 (1952) 207–217.
[17] S.E. Donovan, M.J.R. Hall, B.D. Turner, C.B. Moncrieff, Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures, Med. Vet. Entomol. 20 (2006) 106–114.
[18] A. Farinha, C.G. Dourado, N. Centeio, A.R. Oliveira, D. Dias, M.T. Rebelo, Small bait traps as accurate predictors of dipteran early colonizers in forensic studies, J. Insect Sci. 14 (77) (2014) 1–16.
[19] J. Faucherre, D. Cherix, C. Wyss, Behavior of *Calliphora vicina* (Diptera, Calliphoridae) under extreme conditions, J. Insect Behav. 12 (1999) 687–690.
[20] K.A. George, M.S. Archer, T. Toop, Abiotic environmental factors influencing blowfly colonisation patterns in the field, Forensic Sci. Int. 229 (2013) 100–107.

[21] M. Grassberger, C. Reiter, Effect of temperature development if the forensically important Holarctic blow fly *Protophormia terranovae* (Robineau-Desvoidy) (Diptera: Calliphoridae), Forensic Sci. Int. 128 (2002) 177–182.
[22] S.V. Gruner, D.H. Slone, J.L. Capinera, Forensically important Calliphoridae (Diptera) associated with pig carrion in rural north-central Florida, J. Med. Entomol. 44 (2007) 509–515.
[23] K.A. Hewadikaram, M.L. Goff, Effect of carcass size on rate of decomposition and arthropod succession patterns, Am. J. Forensic Med. Pathol. 12 (3) (1991) 235–240.
[24] C. Hwang, B.D. Turner, Spatial and temporal variability of necrophagous Diptera from urban to rural areas, Med. Vet. Entomol. 19 (2005) 379–391.
[25] F. Introna, C.P. Campobasso, A. Di Fazio, Three case studies in forensic entomology from southern Italy, J. Forensic Sci. 43 (1998) 210–214.
[26] J. Isiche, J.E. Hillerton, F. Nowell, Colonization of the mouse cadaver by flies in southern England, Med. Vet. Entomol. 6 (2) (1992) 168–170.
[27] A. Jewiss-Gaines, S.A. Marshall, T.L. Whitworth, Cluster flies (Calliphoridae: Polleninae: *Pollenia*) of North America, Can. J. Arthropod Identif. 19 (2012) 1–19.
[28] J.E. Joy, N.L. Liette, H.L. Harrah, Carrion fly (Diptera: Calliphoridae) larval colonization of sunlit and shaded pig carcasses in West Virginia, USA, Forensic Sci. Int. 164 (2006) 183–192.
[29] A.S. Kamal, Comparative study of thirteen species of sarcosaprophagous Calliphoridae and Sarcophagidae (Diptera). I. Bionomics, Ann. Entomol. Soc. Am. 51 (1958) 261–270.
[30] S.A. Marshall, T. Whitworth, L. Roscoe, Blow flies (Diptera: Calliphoridae) of eastern Canada with a key to Calliphoridae subfamilies and genera of eastern North America, and a key to the eastern Canadian species of Calliphorinae, Luciliinae, and Chrysomyiinae, Can. J. Arthropod Identif. 11 (2011) 1–93.
[31] S. Matuszewski, S. Konwerski, K. Frątczak, M. Szafałowicz, Effect of body mass and clothing on decomposition of pig carcasses, Int. J. Legal Med. 128 (6) (2014) 1039–1048.
[32] S. Matuszewski, K. Frątczak, S. Konwerski, D. Bajerlein, K. Szpila, M. Jarmusz, M. Szafałowicz, A. Grzywacz, A. Mądra, Effect of body mass and clothing on carrion entomofauna, Int. J. Legal Med. 130 (1) (2016) 221–232.
[33] R.M. Mohr, J.K. Tomberlin, Environmental factors affecting early carcass attendance by four species of blow flies (Diptera: Calliphoridae) in Texas, USA, J. Med. Entomol. 51 (2014) 702–708.
[34] C. Prado e Castro, A. Serrano, P. Martins da Silva, M.D. García, Carrion flies of forensic interest: a study of seasonal community composition and succession in Lisbon, Portugal, Med. Vet. Entomol. 26 (4) (2012) 417–431.
[35] M.R. Sanford, Insects and Associated Arthropods Analyzed During Medicolegal Death Investigations in Harris County, Texas, USA: January 2013–April 2016, bioRxiv, 2016, pp. 071027.
[36] M.R. Sanford, Comparing species composition of passive trapping of adult flies with larval collections from the body during scene-based medicolegal death investigations, Insects 8 (2) (2017) 36.
[37] K.L. Tabor, C.C. Brewster, R.D. Fell, Analysis of the successional patterns of insects on carrion in Southwest Virginia, J. Med. Entomol. 41 (2004) 785–795.
[38] J.K. Tomberlin, P.H. Adler, Seasonal colonization and decomposition of rat carrion in water and on land in an open field in South Carolina, J. Med. Entomol. 35 (5) (1998) 704–709.
[39] Y. Wang, M.Y. Ma, X.Y. Jiang, J.F. Wang, L.L. Li, X.J. Yin, M. Wang, Y. Lai, L.Y. Tao, Insect succession on remains of human and animals in Shenzhen, China, Forensic Sci. Int. 271 (2017) 75–86.
[40] E.J. Watson, C.E. Carlton, Insect succession and decomposition of wildlife carcasses during fall and winter in Louisiana, J. Med. Entomol. 42 (2005) 193–203.
[41] L.M. Weidner, J.K. Tomberlin, G.C. Hamilton, Development of *Lucilia coeruleiviridis* (DipteraCalliphoridae) in New Jersey, USA, Florida Entomol. 97 (2014) 849–851.
[42] L.M. Weidner, D.E. Jennings, J.K. Tomberlin, G.C. Hamilton, Seasonal and geographic variation in biodiversity of forensically important blow flies (Diptera: Calliphoridae) in New Jersey, USA, J. Med. Entomol. 52 (2015) 937–946.
[43] T. Whitworth, Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico, Proc. Entomol. Soc. Wash. 108 (2006) 689–725.