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Secondary screwworm (Cochliomyia macellaria). Photo courtesy of Matthew Bertone, Cary, NC. See pp. 947–954.

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Research Article

Comparison of Techniques for Sampling Adult Necrophilous Insects From Pig Carcasses

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Abstract

Studies of the pre-colonization interval and mechanisms driving necrophilous insect ecological succession depend on effective sampling of adult insects and knowledge of their diel and successional activity patterns. The number of insects trapped, their diversity, and diel periodicity were compared with four sampling methods on neonate pigs. Sampling method, time of day and decomposition age of the pigs significantly affected the number of insects sampled from pigs. We also found significant interactions of sampling method and decomposition day, time of sampling and decomposition day. No single method was superior to the other methods during all three decomposition days. Sampling times after noon yielded the largest samples during the first 2 d of decomposition. On day 3 of decomposition however, all sampling times were equally effective. Therefore, to maximize insect collections from neonate pigs, the method used to sample must vary by decomposition day. The suction trap collected the most species-rich samples, but sticky trap samples were the most diverse, when both species richness and evenness were factored into a Shannon diversity index. Repeated sampling during the noon to 18:00 hours period was most effective to obtain the maximum diversity of trapped insects. The integration of multiple sampling techniques would most effectively sample the necrophilous insect community. However, because all four tested methods were deficient at sampling beetle species, future work should focus on optimizing the most promising methods, alone or in combinations, and incorporate hand-collections of beetles.

Key words: biodiversity, ecology and behavior, forensic entomology, Calliphoridae, Sarcophagidae

Forensic entomology links the science of entomology and the judicial system, most notably in death investigations. Forensic investigators use entomological evidence (e.g., insect species and developmental stages) from a crime scene and biotic and abiotic characteristics of the environment (e.g., plant cover, soil type, and temperature) to make inferences about the time since death, the postmortem interval (PMI) (Goff 1993). A better understanding of these factors and other parameters, such as geographic location and level of concealment, may enable the prediction of necrophilous insect species succession on a decomposing body, which is a pivotal element in PMI determination (Tomberlin et al. 2011a).

The characterization of ecological succession in general, and of necrophilous insects in particular, depends on accurate documentation of temporal and spatial changes in species diversity, richness, and abundance. To document the complete successional pattern of insects arriving to ephemeral ecological resources, such as small carcasses that are rapidly colonized by flying insects, the sampling methods used to collect newly arriving adults must be relatively unbiased and easy to implement without disrupting the successional process. The aerial sweep net (sweep net, henceforth) is the most common sampling tool in forensic entomology research and, in combination with hand-collections of larvae on and around the body, is the approved technique for investigators to collect necrophilous insects (Byrd et al. 2010). While this combination of methods adequately samples the fauna on large decomposing bodies, it has proven inadequate for succession ecology studies on small pigs (Schoenly et al. 2007). Pigs are the most common model used in forensic studies in place of human cadavers because they are easy to acquire and decompose much like humans (Catts and Goff 1992, Carvalho et al. 2000). Medium-sized 23-kg pigs (weaned 'feeder' pigs) are most commonly used in such studies, but researchers often choose to use smaller pigs (Catts and Goff 1992, Archer 2004, Zimmerman and Wallace 2008). Although smaller sized pigs have been shown to adequately represent the local species composition dynamics throughout succession, they tend to attract fewer insects than larger carcasses (Kuusela and Hanski 1982, Hewadikaram and Goff 1991). Sampling small carcasses, such as neonate pigs, is challenging because they represent small targets and arriving insects are readily disturbed by active sampling methods like sweep nets and do not readily return to such a

small body (AC personal observations). Nevertheless, neonate pigs are easy to acquire and much less costly than larger pigs, so it is important to identify unbiased and less disruptive sampling methods for documenting their associated fauna.

Several passive sampling techniques, such as baited emergence traps, sticky traps (typically baited with odor or tissue), and pitfall traps, have been used by researchers to sample necrophilous insects with fair success (Schoenly 1981, Ashworth and Wall 1994, Hall et al. 2003, Schoenly et al. 2007, e Castro et al. 2009). The lack of direct and quantitative comparisons of active and passive sampling methods motivated us to compare two active and two passive approaches to assess the diversity and number of insects trapped near neonate pigs, as well as the relative ease of use of these methods. The sweep net was chosen as one of the active methods because of its widespread use in forensic research and practice. Because no other active sampling methods could be found in the literature for sampling flying adult necrophilous insects, we adapted a suction trap that targets flying mosquitoes (Vazquez-Prokopec et al. 2009). An emergence trap and sticky traps were selected as the two passive methods, with the whole pig itself serving as the lure, unlike several previous studies that used only specific tissues or synthetic odor blends (Ashworth and Wall 1994, Hall et al. 2003). Of these methods, only the sweep net is commonly used in the practice of forensic entomology, both by investigators at the crime scene and by researchers in the field (Schoenly et al. 2005, Byrd et al. 2010, Goff 2011, Matuszewski et al. 2014). To our knowledge, none of the methods used in our investigation have ever been applied to the neonate pig model. Additionally, we compared the diel pattern of trapping with the four methods to identify the optimal time of day for sampling to achieve maximum diversity and abundance.

Materials and Methods

Study Site and Experimental Design

Experiments were conducted at North Carolina State University's Lake Wheeler Road Field Lab in Raleigh, NC (35.729310, -78.667451). Fully frozen stillborn pigs were acquired from the University's Swine Educational Unit. Because four sampling methods were compared concurrently, four pigs were used in each replicate of the experiment, with each pig assigned a different collection method. Frozen pigs were placed in the field at 06:00 hours on the day of the first sampling (day 0). They were spaced 50 m apart in full sunlight and were sampled four times daily—at 09:00, 12:00 (noon), 15:00, and 18:00 hours—over a 3-d period to examine both the succession process and its diel periodicity. The spacing between pigs ensured experimental independence of the pigs, and the full sunlight conditions reduced any potential variability caused by shading or vegetative differences at the four locations (Shean et al. 1993, Sharanowski et al. 2008, Perez et al. 2015). Freezing the pigs prior to field placement is not known to affect the successional pattern (Bugajski et al. 2011), and pigs at −12°C reached ambient temperature in ~4.5 h in full sunlight (AC personal observations). Because pigs were frozen within minutes of death, we are equating 'decomposition day' and 'days since placement in the field' for all analyses. The experiment was replicated a total of five times during June and July 2012.

Sampling Methods

Two active and two passive insect sampling methods were used. For the purposes of this work, a modified Prokopack aspirator (suction trap) (Vazquez-Prokopec et al. 2009) and sweep net were considered active collection methods, while an emergence trap and sticky traps were considered passive. All methods in this experiment focused on arriving adult necrophilous insects of orders Diptera and Coleoptera.

Sampling on all four pigs was performed simultaneously over a 10-min period. Passive sampling methods remained uninterrupted for the full 10-min interval. Preliminary work revealed, however, that both active methods were highly disruptive to insects at the pig body. Therefore, active sampling was performed for two interrupted 1–1.5 min intervals on the pigs to allow insects to return to the pigs. Thus, suction trap sampling was conducted for 1–1.5 min on pig #1, then sweep net sampling was conducted on pig #2, and this process was repeated once during the 10-min interval, allowing disturbed insects to return to the pig. Meanwhile, passive sampling on pig #3 (emergence trap) and pig #4 (sticky traps) remained uninterrupted for 10 min.

Modified Prokopack Aspirator

The Prokopack aspirator was developed as a portable, cost-effective way to sample adult mosquitoes (Vazquez-Prokopec et al. 2009). Like the original design, our suction trap featured an in-line blower motor, rubber coupling, and a DC battery (Fig. 1A). Unlike the Prokopack however, our trap was not attached to a telescoping pole and did not have a built-in collection cup or backpack. Instead, wire mesh window screening was attached to a 6 cm long, 8 cm diameter section of PVC. The mesh allowed insects to be captured and prevented them from being pulled into the motor's blades. The PVC 'trap' was connected to a 6.5 m^3/min in-line blower (Attwood Turbo 4000, Item #1747-4, Lowell, MI) with a 10-to-8 cm rubber coupling. The blower was powered by a 12V 3ampHR sealed-electrolyte battery (XTREME, XTAX4L-BS, Batteries Plus, Bethel, CT) with an in-line manual switch.

Sampling with the suction trap was limited to the area on and above the pig and did not include any local vegetation. At the end of each 1–1.5 min sampling interval, insects were transferred into 70% ethanol

Sweep Net

A 38-cm-diameter sweep net with fine mesh netting was used to sample necrophilous insects (Fig. 1D). Aerial sweeping was performed in a zig zag motion with 180° twists as described by Wayne and Wallace (Byrd et al. 2010). As with suction sampling, sweep net sampling was limited to the area above the pig and did not include any sweeps of local vegetation. The contents from each 1–1.5 min of active sweeping were emptied into 70% ethanol.

Emergence Trap

A 96 × 26 mesh, 60 × 60 × 60 cm soil emergence trap (Bugdorm Item #BT2003, BioQuip, Rancho Dominguez, CA) was elevated 10 cm from the ground by stakes and placed over a decomposing pig (Fig. 1B), which allowed insects to enter the trap. The collection bottle in the emergence trap contained 70% ethanol as the killing agent. This passive trap was deployed over the pig for 10 min, after which it was removed and the collection bottle retrieved.

Sticky Traps

Sticky traps were chosen as a passive method because of their documented use as successful baited traps (Hall et al. 2003, Cork and Hall 2007). To maximize the surface area of the sticky traps, they were mounted on a wooden frame that allowed for six unscented insect monitoring sticky traps (LoLine, B&G, Jackson, GA) to be simultaneously placed at several heights around the pig (Fig. 1C). To allow for easy placement and removal of the traps, cork stoppers were attached to the wooden frame with wood glue at various points, and traps were attached with standard pushpins. The frame

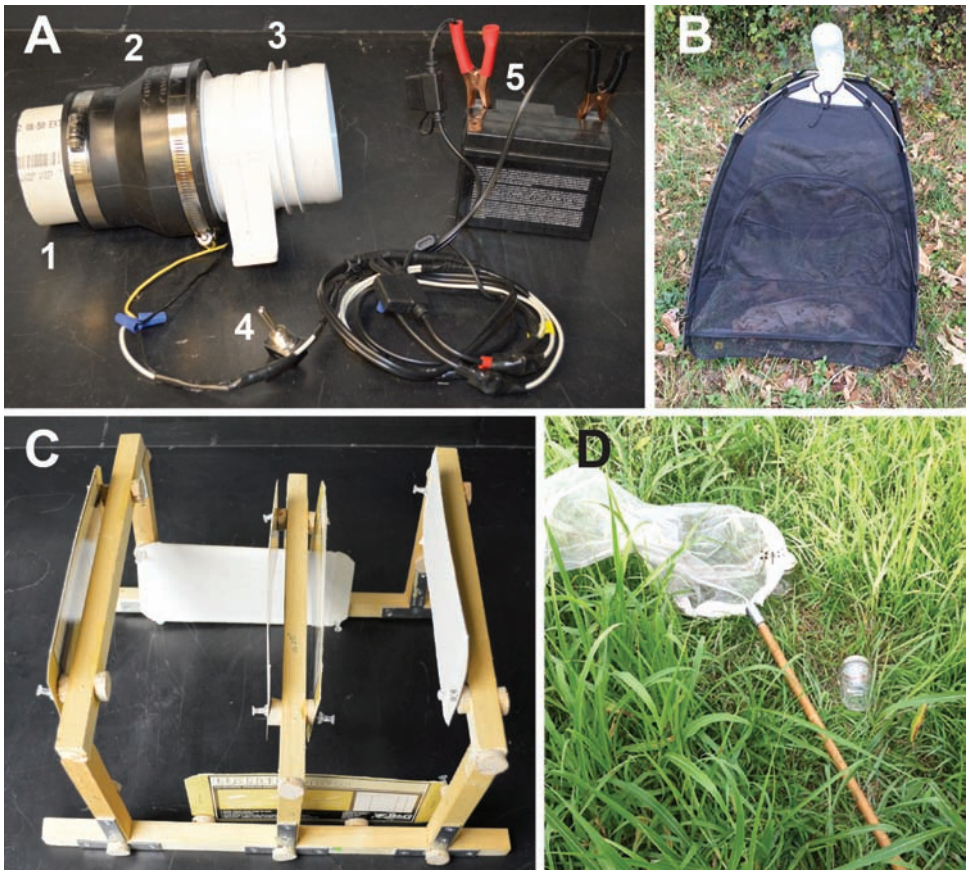

Fig. 1. Trapping methods used in this study. (A) Modified Prokopack aspirator. (B) Emergence trap elevated 10 cm off the ground to ensure insect arrival was not interrupted. (C) Six sticky traps on a wooden frame that was placed over the pig lengthwise. (D) Aerial sweep net. In (A), 1) 8-cm-diameter PVC; 2) 10-to-8 cm rubber coupling; 3) in-line blower motor; 4) in-line on/off switch; and 5) 12V 3ampHR battery.

with six sticky traps was placed over the pig and left undisturbed for 10 min. The traps were collected in plastic cling wrap.

Identifications

Orders Diptera and Coleoptera were the main sampling targets, with emphasis on those necrophilous families commonly used in forensic entomology for PMI determinations. Dipteran families were identified using keys in Whitworth (2006), and Coleopteran families were identified with Triplehorn et al. (2005) and Almeida and Mise (2009). Because of their significant role as primary colonizers of decomposing bodies, the blow flies were further identified to species level using Whitworth (2006). Some specimens were identified at NC State's Plant Disease and Insect Clinic by Dr. Matthew Bertone. Insects on sticky traps were identified in situ.

Analysis

Sampling method, time of day, and day of decomposition were recorded for all samples. Statistical analyses were performed in R, version 3.4.0 (R Development Core Team 2016). Total counts of insects were analyzed to assess the effect of sampling method, time of day and decomposition day with negative binomial generalized linear mixed-effect models (NB glmm) with experimental blocks and individual pigs as random effects using the lme4 package (Bates et al. 2015). *P*-values of comparisons between treatment levels were calculated based on the *z*-distribution. Significance of each variable in the model was assessed by comparing models with and without respective variables or interactions using a χ^2 test (Crawley 2012).

Diversity by method was assessed through overall counts by method, relative percentage of each taxon by method and time, and with three ecological indices (richness, evenness, and Shannon diversity). This allowed us to assess whether there were sampling time (diel effects) or sampling method effects on the taxonomic composition of samples. When considering diversity by sampling method, we subdivided diversity into two components: species richness and species evenness (Clarke and Warwick 1994). Species richness refers to the number of forensically relevant taxa collected respectively by each of the four sampling methods (Spellerberg and Fedor 2003). Species richness (*S*) was represented by the number of taxa that were collected by each sampling method across the five replicates. Species evenness (*J′*) is the consistency in the number of individuals across taxa. We calculated richness and evenness indices, as well as a Shannon diversity index, which considers both richness and evenness as factors in its calculation (Clarke and Warwick 1994, Schüpbach et al. 2016). For the species evenness index and the Shannon diversity index, we included all taxa sampled within the community. Therefore, taxa that were not trapped at all by some methods were represented by zero.

Results

Main Effects: Sampling Method, Sampling Time, and Decomposition Age

All three main effects significantly affected the number of insects trapped (Table 1), and these patterns are shown in Fig. 2. Only some of their interactions, however, were significant (Table 1), and these are

Table 1. Test statistics of model comparisons after stepwise removal of interactions and variables

Model[a]	Removed term[b]	χ^2	df	*P*
1 (Saturated model)	-	-	-	-
2	Method:Time:Day	18.008	18	0.4551
3	Method:Time	14.901	9	0.0937
	Day:Time	70.451	6	<0.001
	Method:Day	39.652	6	<0.001
4	Day	92.325	2	<0.001
	Time	64.287	3	<0.001
	Method	22.401	3	<0.001

Statistical analysis was performed using negative binomial generalized linear mixed model and comparison between models was performed using a χ^2 test (see Material and Methods for details). Models were compared to the immediate preceding model following the steps in model simplification. χ^2 = Chi-square value; df = degrees of freedom used by each model; *P* = *P*-values of model comparisons.

[a]Order of stepwise model simplification.

[b]Interactions or variable removed from preceding model. Method = sampling method; Time = sampling time of day; Day = day of decomposition.

shown in Fig. 3. The emergence trap collected significantly fewer insects than the other three methods (Fig. 2A). There were no significant differences in the numbers of insects sampled by the suction trap, sweep net, and sticky traps; these methods were equally effective in terms of the total insects sampled, disregarding their taxonomic affiliations.

Sampling counts across all methods increased as the day progressed (Fig. 2B). Insect counts at 18:00 hours were significantly higher than at any other sampling time. The day of decomposition also affected the overall number of insects sampled (Fig. 2C) with significant differences among all 3 d of decomposition.

Effect of Decomposition Day and Sampling Time

The total number of insects collected across all methods was significantly affected by the interaction of sampling time and decomposition day (NB glmm, Table 1, Fig. 3A). During the first 2 d of decomposition, afternoon sampling resulted in significantly larger samples than sampling at or before noon. On the second day of decomposition, this effect was continuous, with the number of insects trapped significantly increasing at each sampling time. The 18:00 hours sampling time on this day collected the highest number of insects from any sampling time or day of decomposition. It is important to note that pigs were thawing on day 1 of decomposition, so the ineffectiveness of early sampling times on this day may relate to the slow decomposition of the pigs. Refrigeration is not known to alter the arrival times of insects, but the effect of freezing (without a thawing period) is unknown (Bugajski et al. 2011). On the third day of decomposition, the time at which sampling was performed did not affect the overall number of insects trapped across methods; all four sampling times were equally effective for trapping insects.

Effect of Sampling Method and Decomposition Day

Day of decomposition had a significant effect on the number of insects trapped by method (NB glmm, Table 1, Fig. 3B). All sampling methods collected their lowest respective number of insects on day 1 of decomposition. On this day, the sweep net and sticky trap methods were most effective, although the total numbers of insects sampled by these two methods were significantly lower than for any other day. The distinction among methods was less obvious on day 2 of decomposition, when the suction trap, sweep net, and sticky traps were equally effective for sampling insects. By day 3 of decomposition, clear differences emerged among the methods, with the suction trap sampling more than twice as many insects as any other method.

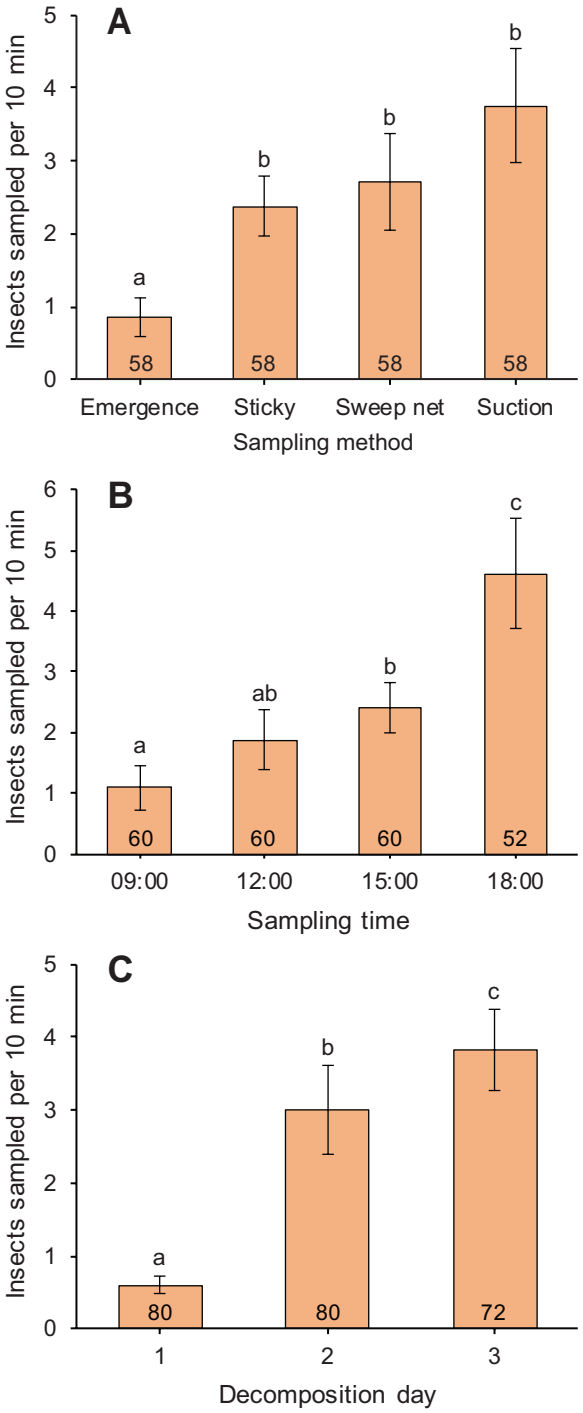

Fig. 2. Number of insects sampled, showing main effects of (A) each sampling method (NB glmm, χ^2 = 22.401, df = 3, $P < 0.001$), (B) sampling time of day (NB glmm, χ^2 = 64.287, df = 3, $P < 0.001$), and (C) decomposition day (NB glmm, χ^2 = 92.325, df = 2, $P < 0.001$). Bars show mean values ± SE. Numbers within the bars denote number of 10 min sampling sessions. Bars labeled with the same letter are not significantly different ($P < 0.05$).

The sampling method most influenced by decomposition day was the suction trap. On decomposition day 1, the suction and emergence traps sampled the fewest insects. By day 2 of decomposition,

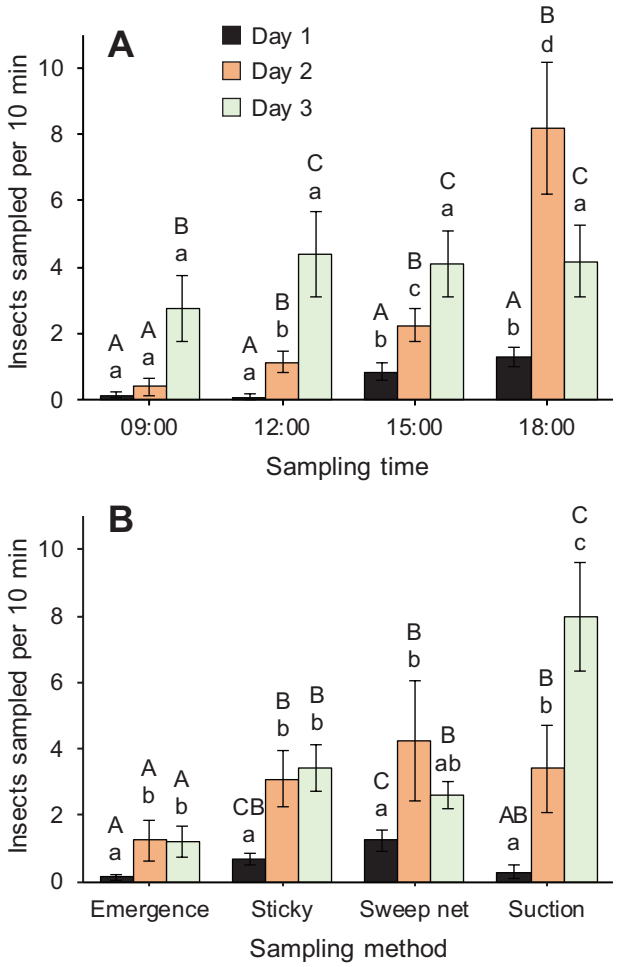

Fig. 3. Number of insects sampled, showing only significant interaction effects of (A) sampling time by decomposition day (NB glmm, $\chi^2 = 70.451$, df = 6, $P < 0.001$), and (B) sampling method by decomposition day (NB glmm, $\chi^2 = 39.652$, df = 6, $P < 0.001$). Other interactions (sampling method by sampling time; sampling time by decomposition day; and sampling method by sampling time by decomposition day) were not significant (Table 1). Bars show mean values ± SE. In (A), bars labeled with the same capital and lower case letters, respectively, are not significantly different within the same hour and day ($P < 0.05$). In (B), bars labeled with the same capital and lower case letters, respectively, are not significantly different within the same sampling method and day ($P < 0.05$).

the suction trap, sweep net, and sticky trap were equally effective. On day 3 however, the suction trap significantly surpassed any of the other methods for the number of insects trapped. All other methods remained steady in their rankings on days 2 and 3, whereas the efficacy of the suction trap increased on each successive day.

Taxonomic Patterns by Sampling Method

Only forensically relevant insects of the Orders Diptera and Coleoptera were included in the analyses, and calliphorids were further identified to species. Our collections included seven calliphorid species (Fig. 4) that overlapped with collections by Cammack et al. (2016), who also sampled in the same location.

Insect counts were compared across sampling methods (Fig. 4A) and by the relative representation of each taxon by each of the four sampling methods (Fig. 4B). Calliphoridae was the most abundant family trapped, followed by Sarcophagidae (Fig. 4A). Sarcophagid flies and the four most abundant species of calliphorid flies collected (*Lucilia illustris* Meigen, *Phormia regina* Meigen, *Lucilia coeruleiviridis* Macquart, *Cochliomyia macellaria* F.), were trapped by all four sampling methods, but few beetles were trapped by each of the four methods (Fig. 4B).

Species richness was highest with the suction trap, with a total of 13 taxa trapped, followed by sticky traps (10 taxa). The suction method collected at least one insect from each taxon, with the exception of dermestid and staphylinid beetles (Fig. 4). It was the only method that captured *Lucilia cuprina* Wiedemann and *Chrysomya megacephala* F., which were the rarest calliphorid species sampled overall (Fig. 4). Additionally, the suction trap was the only method that collected *Phaneus vindex* Macleay (Scarabeidae) and *Necrophila americana* L. (Silphidae). The traditional sweep net method captured eight taxa, including all calliphorids except the two rarest species; but it only captured representatives of one beetle family, Histeridae. The emergence trap captured only five taxa and failed to capture the three least abundant calliphorid species and any beetles; the emergence trap was also the only method that did not sample *Musca domestica* L. Sticky traps captured all calliphorid species except the two rarest, and these traps also captured species in three beetle families. Sticky traps were the only method that sampled staphylinid beetles, which, along with histerid beetles, were the most abundant beetle families collected overall across all methods. Overall, none of the methods collected all the beetle taxa, and only the suction trap sampled all fly taxa (Fig. 4).

Taxonomic Patterns by Diel Periodicity

No taxa were unique to the 09:00 hours sampling time, but several taxa were unique to each of the other sampling times (data not shown). Dermestid beetles and *C. megacephala*, a rare blow fly in this study, were trapped only at 12:00 hours. *Lucilia cuprina*, another rare blow fly in this study, was trapped only at 15:00 hours, and *N. americana*, a rare silphid beetle, was trapped only at 18:00 hours. Several other taxa were trapped during two of the four sampling times, including histerid beetles at 12:00 and 15:00 hours, *Lucilia sericata* Meigen at 12:00 and 18:00 hours, and scarab beetles at 15:00 and 18:00 hours. *Musca domestica* was trapped at all sampling times except 09:00 hours.

Diversity Indices

Species evenness is a measure of each species' numerical representation in the community. Given the relatively small number of insects trapped per species, with some species not represented at all in some sampling methods, this index may be strongly affected by both highly and poorly represented species. Indeed, the emergence trap, which had the lowest taxonomic representation had the highest evenness index ($J' = 0.782$). The relative percentages of each taxon sampled by method are shown in Fig. 4B.

The Shannon index is an overall diversity index that considers both species evenness and species richness, with larger values representing greater diversity. Shannon index values indicated that the sticky trap collections were the most diverse ($H' = 1.786$), followed by the suction trap ($H' = 1.700$). Emergence trap collections were the least diverse ($H' = 1.258$).

Discussion

A National Research Council report critiqued various forensic disciplines, recommending the need for more sound basic biological research to strengthen foundational disciplines and increase the credibility of findings in the court system (National Research Council 2009). In response, research in forensic entomology has moved toward ecological and evolutionary genetics frameworks

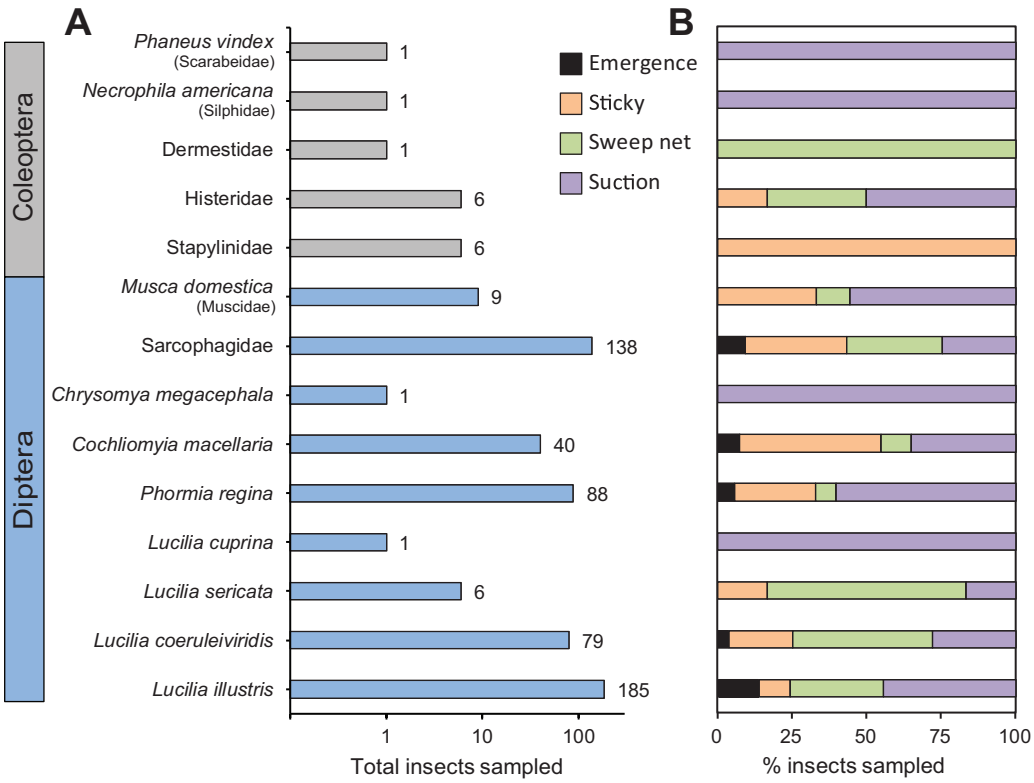

Fig. 4. Representation of each necrophilous insect taxon across all replicates. (A)Total abundance of all taxa (note log scale). (B) Relative representation of each taxon sampled by each of the four sampling methods. Dipterans without family noted are all Calliphoridae. The five most abundant taxa (*L. illustris, P. regina,* Sarcophagidae, *L. coeruleiviridis,* and *C. macellaria*) were sampled with all four methods. Several rare taxa, including *L. cuprina, C. megacephala, N. americana,* and Dermestidae, were trapped only with the suction method. Staphylinid beetles were only sampled with the sticky traps.

(Tomberlin et al. 2011a, Tomberlin et al. 2011b). Investigations of the mechanisms driving the attraction of necrophilous insects to carrion, ecological succession, and other aspects of the pre-colonization interval, or the time from death to the first insect colonization, have been highlighted as particularly lacking (Tomberlin et al. 2011a). In addition, there is a need to better document ecological succession patterns and growth rates of necrophilous insects in various geographic locations to gain a better understanding of their ecological variation (Souza et al. 1997, Turchetto and Vanin 2004, Amendt et al. 2011). Pivotal to all these endeavors is the efficient, unbiased, and representative sampling of the necrophilous insect community.

Sampling Methods

The emergence trap sampled significantly fewer insects than the other three methods, both in an overall analysis of all sampling methods and in each of the three decomposition days. This sampling method should not be used when large insect samples are required over short sampling intervals. The other three methods—the suction trap, sweep net, and sticky trap—displayed similar efficacy when the total numbers of insects were considered across all decomposition days and times of day (Fig. 2A). However, the relative efficacy of these three methods varied significantly by decomposition day, a rough approximation of PMI. Thus, experiments designed to maximize daily insect collections during ecological succession would need to integrate several complementary methods. Both the aerial sweep net and sticky trap were most effective on the first day of decomposition, when the decomposing pigs attracted relatively few insects (Fig. 3B). Of these, the sweep net was considerably easier to use because the sticky trap design required constructing a frame, the use of inconvenient sticky glue, and retrieval of insects from the traps without damaging them was difficult. The two active methods and sticky traps were equally effective on day 2, but the suction trap was clearly superior on day 3, when the decomposing bodies attracted the largest numbers of insects.

The suction trap and sticky trap methods outcompeted the other sampling methods in representing the greatest diversity of the necrophilous insect community. The suction trap was the only sampling method that trapped all nine fly taxa in this study (Fig. 4B). Flies are the most numerous insects on decomposing bodies and are commonly used in PMI estimates, so accurately documenting their diversity, and especially first arrival on the body, is of particular importance (Catts and Goff 1992, Goff 1993). The sweep net and sticky traps failed to trap the two rarest fly species (*L. cuprina* and *C. megacephala*), indicating that perhaps these methods could be improved—the sweep net by altering the motion so that it is less disruptive, and the sticky traps by better utilizing the pig's decomposition odors, known factors in blow fly host finding (Ashworth and Wall 1994, Stensmyr et al. 2002).

Beetle diversity was critically under-represented. All four sampling methods collected few beetles, yet many beetles were observed on the carcasses. The suction trap sampled the greatest beetle diversity, three of the five taxa, with the sticky trap and sweep net collecting two of the five beetle taxa, including Staphylinidae and Dermestidae, respectively. The insufficiency of all selected methods for trapping beetles echoes Schoenly et al.'s (2007) findings when assessing various methods on larger, 23–27 kg pigs—aerial and sticky trap sampling mostly target flies, with hand collections or pitfall traps representing better methods for sampling beetles. Either hand collections or pitfall traps should be included in a

sampling protocol. Nevertheless, several of our attempts to use pitfall traps failed because the soil was highly compacted, requiring disproportionate labor relative to other sampling methods to sample the 20 pigs.

Taxonomic identifications of many necrophilous insects, especially flies, depend on setal and antennal characteristics. From a practical perspective, it is important to note that the two most promising methods for documenting diversity, the suction trap and sticky traps, either severely damaged the insect specimens or were cumbersome, as noted previously for sticky traps (Nelder et al. 2008). Wings and antennae were frequently damaged in the suction trap samples, especially in small insects that often were not well retained by the screen mesh. Design modifications however, such as soft mesh nylon stockings as the collection vessel, should remedy these shortcomings of the suction trap. Although insects could not readily be removed from sticky traps despite several attempts with various oils and solvents, insects remained whole and could be identified in situ. Almost all flies landed on the trap such that characteristic setae on the wings and body were undamaged and visible. If insects do not need to be removed or DNA-based identifications are implemented, the use of sticky traps is not problematic, although these traps are cumbersome to transport and store. Nevertheless, a major advantage of the sticky traps is that they can sample insects for longer durations, and collections can be readily compared between trials or experimenters because experimenter bias is minimal.

Overall, the suction trap outcompeted the other methods for sampling insect diversity, but it, the sweep net, and sticky traps sampled complementary taxa. Both active methods were disruptive to insects on the pig body, so multiple interrupted sampling sessions were required to ensure that all target insects were captured.

Diel Periodicity

In general, the abundance of necrophilous insects increased with time of day, so sampling later in the day (i.e., after noon) yielded larger samples than in the morning (Fig. 2B). This was particularly apparent on day 2 of decomposition, when fourfold more insects were sampled at 18:00 hours than at 15:00 hours. On day 3 however, all sampling times were statistically equivalent in terms of number of insects trapped. In a repeated sampling design with a consistent sampling time each day, after noon sampling should be performed, as it will ensure the largest samples across decomposition days. Such a design, however, only targets large samples and does not consider diversity of samples.

Identifying an ideal sampling time, when both overall abundance and diversity are maximized, depends largely on the aims of the experimental design. We used relatively short 10-min sampling intervals in our design, so the latest sampling time, 18:00 hours, was feasible. This may not be possible in experiments that use longer sampling durations. Nevertheless, our results highlight that large numbers of insects are trapped near dusk (sunset during experiments ~19:30 hours), even though many species are not active at night (Wooldridge et al. 2007, Zurawski et al. 2009). The length of the sampling interval and season should be considered when sampling late in the day. While the afternoon times yielded larger overall samples, specific taxa varied greatly with time. The noon sampling time was the only period during which dermestid beetles and *C. megacephala*, a rare blow fly in this experiment, were captured, even though noon samples were relatively small. Other rare species, including *L. cuprina* and *N. americana* were collected only at 15:00 and 18:00 hours, respectively. Therefore, it is impossible to identify one ideal sampling time that will capture the diversity and abundance of the necrophilous insect community. Repeated sampling during the interval from noon to 18:00 hours, however, would account for all the observed diversity, as no unique species were found before noon.

Repeated sampling of communities is a pivotal strategy to infer reliable estimates of ecological indices and succession. The goal of this work was to identify sampling methods that would result in large and species rich samples of necrophilous insects from neonate pig carcasses. We found significant differences in the number of insects trapped by method, time of day, and day of decomposition. Not surprisingly, a combination of sampling methods deployed for long sampling durations would appear to be most desirable. However, most studies are constrained by limited work force and time. Moreover, sampling for long durations might interfere with the decomposition process and ecological succession. Therefore, several general observations and recommendations emerged from our analysis:

1. Studies seeking to maximize the abundance of necrophilous insects over decomposition should sample in late afternoon (in mid-summer), but a combination of sampling methods should be used to sample across days of decomposition because the effectiveness of sampling methods changes over time. In ecological succession studies that focus on diversity over time, the suction trap should be used each day, but sampling should be repeated several times between noon and dusk. When labor is limited, the sticky traps might perform well at representing the local diversity, but sticky traps are difficult to store, and it is difficult to isolate undamaged voucher specimens without damage. Nevertheless, in combination with periodic use of the suction trap or sweep net, this sampling design would be effective.
2. Our sticky trap design required multiple traps to surround the pig because necrophilous insects are adept at avoiding traps as they navigate to the decomposing pig. An alternative approach might be to reduce trap number, handling time, and storage capacity by directing the pig odors over a single sticky trap. Indeed, this approach is highly effective (Cruise 2017), but requires manipulation of the pig carcass and therefore is limited to small pigs.
3. The sweep net, the established methodology for sampling large pigs, was effective at sampling insects. Notably however, it was inconsistent at representing the local species diversity across the decomposition process. The need to sample for maximal diversity is highlighted by the observed significance of individual species in the decomposition process, independent of their abundance (Crippen and Singh 2015). With several modifications, as discussed above, the suction trap, which captured the greatest species richness from the pigs, might be more effective than the forensic standard, the sweep net.
4. Sampling of beetles was relatively ineffective in this study. An important goal of future work should be to improve sampling of beetles. Both hand-collections and pitfall traps (active and passive sampling methods, respectively) are labor-intensive. Zanetti et al. (2016) found that combining both methods yielded abundant beetle samples with less bias for certain taxa over others. Perhaps ground-touching sticky traps should be considered (Cruise 2017).

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