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Entomological Observations on a Decomposing Pig (*Sus scrofa*) in Nebraska: Late Spring to Early Summer

Leon Higley^{1*} John Obafunwa¹ and William Belcher²

¹School of Natural Resources, University of Nebraska-Lincoln ²School of Global Integrative Studies, University of Nebraska-Lincoln

*Corresponding Author: Leon Higley, School of Natural Resources, University of Nebraska-Lincoln, Tel: 402-560-6684, E-mail: lhigley1@unl.edu

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Abstract

The determination of the postmortem interval (PMI) towards assisting in medicolegal investigations is a frequent requirement in any developed jurisdiction. Each of the latter has prevailing peculiarities, especially with many operating variables that include seasonal changes. There is a dearth of information about the decompositional pattern and progress of cadavers deposited on the ground in Nebraska, a jurisdiction lying within the Central Great Plains of North America. The present study, using an experimental pig was designed to consider the entomological peculiarities within a geographical area exhibiting taphonomic characteristics, and during a defined season. The porcine study is unavoidable because of ethical and legal considerations. The period of study covered mid-April to the end of July 2023. *Lucilia sericata* was the dominant necrophagous insect during the study period. There were cold periods, especially in the early weeks, with expected limitations of insect activities as to potentially prolong the decompositional period. However, this observation did not fully explain the delayed decompositional progress during these months. The invasion by necrophilous Silphids (*Oiceoptoma novaboracense* and *O. inaequale*) better explains the prolongation of the decomposition process due to the predatory actions of these coleopterans on the necrophagous Diptera. Although decomposition (as indicated by tissue loss and pattern) was delayed, the phenology of blow fly development did not diverge from predicted values. Therefore, any attempt to determine the PMI during this time of the year from stage of decomposition must consider this biotic factor of arthropod scavenging to avoid error, but the developmental models of blow flies remain valid as estimators for PMI.

Keywords: Postmortem Interval; Silphidae; Lucilia sericata

Introduction

After cellular death in humans, the process of decomposition ensues, characterized in part by immediate changes comprising pallor mortis, algor mortis, livor mortis and rigor mortis [1-3]. These changes are noticed within the first 24 – 36 hours depending on many variables that include geographical and physical location, temperature, clothing, nutritional status, pre-existing diseases, cause of death, conduction/convection currents, and radiation among others. These early postmortem changes are gradually terminated by the onset of putrefaction. The decomposition progression has been subdivided in the entomological literature into five stages represented by fresh, bloated, active (early) decay (liquefactive), advanced (late) or post-decay, and drying (skeletonization) [4-8].

The putrefactive stages are characterized by the presence of arthropods which are attracted to the cadaver shortly after death. The type of insects and so-called sequence of invasion (subject to various modifying factors), accessibility, interactions between myriads of abiotic and biotic factors, all affect the rate of decomposition and estimation of the postmortem interval. One of the earliest invaders (necrophagous insects) are the blow flies of the family Calliphoridae [5, 9-11]. The adult flies are chemotactically attracted to the cadaver by the odor of ammonia, hydrogen sulphide, sulphur dioxide, carbon dioxide, methane, ethane, benzene (and its various esters), hexane, ether, etc. [12,13]. Generally, different necrophagous insects respond to specific gases, and this is genetically programmed. The production of these gases is facilitated by the actions of endogenous and exogenous bacteria in and around the cadaver [14].

Studies using human donors, as in taphonomic research or human decomposition facilities, are useful for studying the sequence of entomological invasion, and consequently using the information to attempt to calculate the PMI. The latter is calculated based on observations made by evaluating the stage of growth and development of the early stages of the insect, starting with oviposition and progressing through larva formation (including the three instar stages), pupation, and finally the emergence of the adult form. These developmental and growth cycles are not necessarily as simple; they are modified by abiotic factors like the photoperiod, humidity (moisture), rainfall, temperature, geographical location, accessibility, etc. The biotic factors include food availability, population density (crowding), predation, drug interactions (entomotoxicology), genetic make-up of the arthropods (as it affects olfaction and chemotaxis), and diapause. All these variables ultimately affect the calculated PMI, which is arrived at through the determination of the accumulated degree days (ADD) required for the insect to progress through the stage of oviposition to the emergence of the adult (final eclosion).

In forensic anthropology, Megyesi et al. [15] established the Total Body Score (TBS) as a method for scoring human decomposition stages and promoted the idea of using Accumulated Degree Days (ADD) from forensic entomology. Megyesi et al.'s (ibid.) research was extremely influential in the research that followed its publication albeit based on case studies [16,17]. Wescott (ibid.) has promoted the idea of using experimental studies using either human donors or human proxies such as pigs.

In the absence of human cadavers, pigs (Sus scrofa) have been studied because of certain shared similarities with humans. These include similarities in their skin make-up, muscle types, organ size (anatomy) and function (physiology), as well as some genetic characteristics, thus justifying porcine use in medical experiments for evaluating human diseases, their progression, and even transplantation [18,19]. However, it should be noted that results using human donors and non-human carcasses may not be entirely comparable in terms of decomposition rates and process [20-25].

To estimate the entomological sequence on the human cadaver, a caged (to limit scavenging) pig was studied in the Central Great Plain of Nebraska, utilizing the Reller Prairie Field Station grassland area, a property of the University of Nebraska. Reller Prairie Field Station has humid continental hot summers with year-round precipitation climate (Dfa) [26]. The study period was between late Spring and early Summer of 2023. It is presumed that the identified necrophagous insects can be extrapolated to adequately represent those that will normally invade human remains in the geographical location at that time of the year. This study provides a background for determining key decompositional insects and their interactions, as well as an opportunity to test temperature-development models used for estimating the postmortem interval.

Materials and Methods

A juvenile pig (RP-0002) weighing 65lbs (29.5kg), that died of sepsis following an intestinal obstruction was acquired from a local farm. It had died on the farm at 9:00pm on April 14, 2023; the temperature was 45° F (7° C). The carcass was deposited in a gated cage on Reller Prairie grounds, Martell, Nebraska, at 4:30 pm on April 15, 2023 (Figure 1). Because we were only interested in documenting the occurrence of carrion insects, we did not replicate pigs in this study.



Figure 1: Carcass of Sus scrofa within the cage

The taphonomic setting was an open grassland area: a gated cage (with an open top) was used to preclude the activities of terrestrial (except avian) scavengers. The weather was cloudy and drizzly at the time of deposition of the carcass, and the ambient temperature was 42° F (5.5° C). Daily recordings of the ambient temperatures were made using a thermometer hung on the outside of the cage. The temperature of the decomposing pig was recorded daily using a ThermoPro Dual Laser Infrared Digital Thermometer, Model No. TP450,. Recordings were taken of the head, nose, mouth, chest, abdomen, soil underneath the carcass, and subsequently from any visceral openings in the course of decomposition.

Pictures of different stages of decomposition were made with a cell phone. From hand-written notes, a spreadsheet was maintained for the additional daily recordings of observations relating to the oviposition by the invading flies (their relative density), larval developmental stages (and relative population density), pupation, and other arthropods present, any scavenging activities, and the overall general gross morphological stage of decomposition, including special characteristics like mummification. When appropriate, samples of the larval instars were retained for incubation and subsequent identification. The migratory larvae were reared in the laboratory jar on a substrate of beef (Bos taurus) liver at a constant room temperature of 74° F (23° C). Other documented observations included the presence of odor, disarticulations, and skeletonization. Documentations included notations as to the presence (or absence) of sunshine, rainfall, moisture level, dryness/wetness of the soil, and wind intensity (mild, moderate, and marked). Additional documentations through photographic recordings of the state of the carcass were made. All these recordings were done between April 15 and July 31, 2023. The visits to the cage were reduced to twice weekly after day 50, and then weekly after day 87. Temperature information from the nearest meteorological station in Martell, Nebraska was obtained from Weather Underground (https://www.weatherunderground.com).

Result

The results are as summarized in Figures 2 – 8. Figure 2 shows the pattern of decomposition through time, based on a five-stage scale (fresh, bloat, active decay, late decay, and remains stages). As compared to other examples [e.g., 27] the bloat stage is prolonged in this instance, and tissue loss does not proceed from the head. Figure 3 shows ambient temperatures over the first 30 days postmortem and illustrate some cooler temperatures on days 7 and 8 which subsequently return to moderate (ca. 20° C or higher) levels.



Figure 2: Decomposition stage through time (days postmortem)



Figure 3: Temperature variations (ambient, head and abdomen), through time (days postmortem)

Decompositonal insects were initially blow flies (Lucillia sericata), followed by the occurrence of silphids (*Oiceoptoma novaboracense* and *O. inaequale*) starting 6 days postmortem (Figure 4 and 5). The migratory larvae took a minimum of 5 – 6 days to develop to adult stage following rearing in the laboratory jar. The flies were identified as *Lucilia sericata* (Diptera: Calliphoridae) [9]. The activity of these blow flies drastically reduced to nil whenever the ambient temperature fell below <120C. Other arthropods that invaded the carcass included two carrion beetle species (Coleoptera: Silphidae), *Oiceoptoma novaboracense* and *O. inaequale* [28]. These appeared around day 5 – 6 and their population increased subsequently with an accompanying predation on the eggs and larvae of the blow flies; they co-existed easily with the adult flies and persisted for long with consequent delays in the progression of decomposition due to the consumption of the larvae. There was an incidental presence of Boxelder bug (*Boisea trivittata*) (Order: Hemiptera) on day 4. Rove beetles (Staphylinidae) were seen on day 19 of the decomposition. Ticks appeared around day 22 and their intensity remained for most of the duration of the decomposition (Figure 6). Dermestids were initially noticed on day 77, by which time the carcass had undergone skeletonization with extensive drying of the carcass; they were particularly noticeable underneath the remains, and the blow flies were rarely seen at these times.



Figure 4: Relative density of decompositional necrophagous insects through time (days postmortem)



Figure 5: Decomposition stage and silphid intensity through time days postmortem



Figure 6: Decomposition stage and tick (Dermacenter variabilis) intensity through time (days postmortem)

Based on models of *Lucilia sericata* development [29], Figure 7 illustrates the relative density of maggots on the carcass and the pre-dicted time for developmental events. Although density of maggots is low during the egg to 3rd feeding larvae stages (E-L3f), pre-dicted times for egg to migratory 3rd stage maggots (E-L3m) and for egg to pupae (E-P) correspond to observed events.



Figure 7: Accumulated Degree Days with relative density of Lucillia sericata developmental stages and predicted stage transitions



Figure 8: Accumulated Degree Days for decompositional stages up to 200 days postmortem

Figure 8 relates the occurrence of *L. sericata* to stages of decomposition. Under a typical early summer pattern of decomposition we would expect high densities of blow flies to develop by the bloat phase (stage 2) [27], but in this instance large numbers of maggots do not develop until well into active decay (stage 3). Because substantial tissue loss is absent in the head, the normal pattern of fly infestation, and because eggs were laid in the mouth, presumably predation by silphids resulted in the reduction in maggot numbers initially. Interestingly, the phenology of *L. sericata* is consistent with developmental models (Figure 7), so the reduction in numbers associated with predation did not alter the timing of developmental events.

Generally, disarticulation commenced about day 29, and early skeletonization became apparent after day 39. Avian scavenging was suspected about day 39 because the carcass had been rotated through 1200; the only access (apart from the locked gate) to the cage is through the open top. Occasional vultures and wild turkeys have been observed around the Reller Prairie grassland. Another suspected episode of aerial scavenging occurred on day 60, when the carcass was noticed to have been rotated through 90°.

Representative images of observations made during the study period are shown in Figures 9 - 18. It is noteworthy that no fungal growth (i.e., entomopathogenic fungi) that could have posed a hindrance to the entomological agents was observed during the entire study period. This sequence illustrates an unusual pattern of insect infestation and tissue loss, as well as predation by silphids on *L. sericata* maggots (Figure 12).



Figure 9: Day 4 postmortem. Bloat stage with some greenbottle flies



Figure 10: Day 5 postmortem. Greenbottle flies (*Lucilia sericata*) aggregated around the snout and mouth. A number of 1st and 2nd instars are noted within the nares and the mouth; the whitish granules around the edges of the mouth are particles of sand acquired from the farm prior to death



Figure 11: Day 7 postmortem. With an ambient temperature of 52° F/10.5° C the carcass attracted no flies because of the prevailing cold weather. The carcass is bloated and exhibiting a greenish discoloration around the abdomen



Figure 12: Day 11 postmortem. Silphids (Oiceoptoma inaequale) around the mouth, preying on maggots



Figure 13: Day 13 postmortem. The carcass is bloated and probably showing active decay on the inside with dark greenish-grey discoloration over the chest and abdomen. Some eggs and 1st instar larvae are present in the fold between the left front limb and chest



Figure 14: Day 17 postmortem. Active decay with 2 openings in the chest and another over the abdomen



Figure 15: Day 21 postmortem. A maggot mass of 2nd and 3rd instars is formed around the abdominal opening during the active decay stage



Figure 16: Day 23 postmortem. Much soft tissue loss concentrated in the abdomen in association with the maggot masses



Figure 17: Day 28 postmortem. Early remains stage, drying of the skin and progressing disarticulation. No maggots were observed



Figure 18: Day 42 postmortem. The carcass is becoming skeletonized and has been rotated to the right by about 120° through the activities of avian scavengers

Discussion

The present study reveals entomological observations in respect of porcine decomposition in Nebraska, located in the Central Great Plain region of North America. It also highlights certain aspects of the taphonomic setting of this geographical location.

Though Sus scrofa was used, the result can be extrapolated to what would have been observed in humans [18,19]. However, it is noteworthy that porcine decomposition is slightly faster than that of humans, supporting the opinion in some quarters that certain genetic and anatomical differences still exist between the two species.

There has been a dearth of information on the chronological order of depositional observations in pigs, in Nebraska. Bauer and colleagues [30] addressed the general issue of decompositional entomology and the procedure for its investigation. The latter study, which was done during the summer of 2018 (June – August), considered various taphonomic settings like pigs lying on the surface of the soil, hanging, buried, clothed, underwater, within a car trunk, and another traumatized. The report did not identify specific arthropods. The present study addresses the entomological variable in addition to some relevant taphonomic peculiarities of the environment, represented by Reller Prairie with an 80-acre land space.

The studied pig passed through the five stages of decomposition (Figure 2), which comprise fresh (days 0-2), bloated (days 3-8), active decay (days 9-24), post-decay (days 25-30) and skeletonization (days 39+); there was a lot of overlapping of the post-decay and skeletonization stages during days 30-38. When these periods are compared with earlier observations in Nebraska [30], there is an obvious delay in the decompositional progression. The previous workers recorded for fresh, days 0-3, bloated, days 2-6, active decay, days 5-11, post-decay, days 10-24, and skeletonization, days 24+. At first glance, these observations can probably in part be explained by the fact that while the previous study was in the summer months (June - August), the present one was in the warmer late Spring (April) to early Summer (July) months. Decomposition is generally considered to be slower in the cold seasons spanning late fall/Winter. The temperature expectedly gradually increases from spring to the summer months, however, during the present study, there were episodes of cold weather, with the ambient temperature dropping below 200C (Figure 5). Generally, the temperature around the head and abdomen followed the same trend as the ambient temperature.

Despite the expectation that a temperature increase should occur during the period of transition from Spring to Summer with accompanying reduction in the overall time taken for decompositional progression, a striking prolongation was observed in the present study. This suggests that there has to be other modifiers. There was a delay in oviposition (Figure 6) which can be attributed to the low ambient temperature of 5.5° C at the time of deposition of the carcass at Reller Prairie. The low temperature would naturally delay insect activity, and it is therefore not surprising that the pig remained 'fresh' for a few days; bacterial activity with the generation of volatiles that will chemotactically attract necrophagous insects will be delayed. This will consequently delay the onset and density of oviposition. There was an increase in the temperature around days 3-5 (Figure 3) with a concomitant spike in the density of the eggs (Figure 4), but this sharply declined afterwards, partly due to a slight fall in the temperature.

However, Figure 5 shows a sharp rise in the relative density of Silphids around days 5-6, reaching a maximum about day 8. This coincided with the sharp fall in the density of the eggs; while the population of the larvae had increased about day 5, it plateaued briefly as the Silphid population continued to rise. The larval density fell after day 8 and did not resume its rise until after day 12. The import of all these observations is that the emergence of Silphids coincided with a decline in the population of the eggs and larvae of the necrophagous flies. It is therefore no surprise that the rise in the density of Silphids delayed the progression of decomposition, especially beyond the active decay stage.

Siphids occur in many geographical regions of the world, including Nebraska [7]. These beetles are usually active from April to September, and they usually arrive at the decomposition scene anytime from the bloated stage [23]. These were the observations during the present study period (Figure 6). These arthropods generally feed on dead animals and are thus involved in the recycling of organic materials. Their forensic importance is reflected in the fact that some members of the family Silphidae, are ne-crophilous; they colonize cadavers and scavenge the eggs and larvae of the necrophagous insects. The observation of the Silphids in relatively large numbers in the present study will be the main logical explanation for the sharp decrease in the relative density of eggs and larvae of the necrophagous insect and the consequent delayed progression of decomposition (Figure 5). This will ultimately affect any subsequent attempt at determining the PMI based solely on decompositional stage.

Ticks are known to be present in Nebraska and are generally active between April and October (Spring to Summer). They mostly live on tall grasses near trees, bushes, and decomposing leaves. They are generally important for the diseases they cause in man, such as Lyme disease, through their bites. Their natural ecological habitat will explain their presence at the study site if not their attraction to the carcass. They appeared on day 20 and virtually persisted for most of the study period (Figure 6). However, their presence did not particularly affect the course of decomposition.

The study revealed the dominant necrophagous insect to be Lucilia sericata. This blow fly has been known to exist in Nebraska for over a decade [34], as with many other parts of the Central Great Plains [41]. They are commonly observed with *Phormia regina* during the spring and summer months. *Chrysomya rufifacies* also has been observed in Nebraska. However, the present study revealed only the adult and reared larval forms of *L. sericata*; the reduced diversity is probably attributable to the cold weather that is associated with Spring, permitting only cold-tolerant species to prevail. The relative density of the developmental stages of *L. sericata*, and the decompositional stages, relative to the ADD (Figure 4, 7, and 8), are quite predictable, and while decompositional stages were delayed, developmental events of *L. sericata* were not. Thus, developmental models of blow fly development remain a reliable predictor of PMI even in instances where substantial predation occurs.

Scavengers will naturally hasten the process of decomposition through the consumption of most parts of the carcass, apart from creating openings that will facilitate oviposition. Ultimately, the modifying effect of the scavengers will cause a significant false decrease in the PMI from decompositional staging. The gated cage used for the present study was to exclude the interference due to terrestrial scavengers, except for the aerially travelling avian scavengers. Though there was no functional installed camera at the time of the study, it was logical to conclude that avian scavengers were responsible for the 120° shift on day 39, and another 90° shift on day 60, from the original position of the decomposing pig. Some dispersals of the bones were also observed, but the activities of these scavengers did not significantly affect the course of decomposition.

Of importance for the practical use of these findings, the presence of insect predators did influence the magnitude, and to a lesser degree location, of *L. sericata* maggots. However, the phenology of *L. sericata* development as indicated by existing thermal development models (Figure 7) was unchanged. To the best of our knowledge this is a new finding and offers greater confidence in the use of thermal developmental models for estimating the PMI.

Conclusion

Lucilia sericata was the main necrophagous insect in this example, but its densities were significantly modified by Silphids. The latter are seemingly abundant in the grassland of Nebraska, where they participate in the decompositional process by feeding on the eggs and larval forms of *L. sericata*. Consequently, they cause a delay in the decomposition process with resultant overestimation of the PMI based on decompositional staging but not if PMI is determined by blow fly development.

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