Teaching forensic entomology with common grocery items: decomposition and insect succession studies

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Abstract: Forensic science and entomology programs may benefit from using deceased pigs as teaching resources due to anatomical similarities to humans. The use of such animals provides opportunities for students to learn the effects of geographical location and climate on insects; understand insect succession; practice insect collection and identification; and learn post-mortem interval procedures, taphonomic principles of decomposition, and crime scene investigation and management techniques. Forensic entomology and taphonomic studies fit well with educational interests in experiential learning. Pigs may be readily available through animal science departments or local producers, and many institutions may have property where this research can be conducted. However, due to regulatory requirements and cost, smaller entities such as community colleges, K-12 schools, criminal justice agencies, students conducting independent research or taking lab courses, and private consultants may have difficulty obtaining suitable materials and space for entomological forensic studies.

The purpose of this study was to evaluate alternative, physically small meat products that are easily obtained from a grocery store or butcher shop and could be used in lieu of a pig or other large animal. Such meats are relatively inexpensive, readily available, and would need little space when setting up an experiential, immersive learning exercise. In many cases the decomposition experiment could be carried out in a closed container such as a fish tank and in less time than with a larger animal source.

Keywords: Forensic entomology, taphonomy, experiential learning, forensically important insects, crime scene investigation.

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Introduction

Forensic entomology is the study and use of arthropods and insects in legal investigations and has also been used as a teaching tool (1,2). Considerable forensic laboratory studies and teaching exercises in taphonomy and entomology have been conducted using pigs as a suitable substitute for human cadavers (3). Pig carcasses have been successfully used at university level instruction for forensic entomology, crime scene investigation, trace evidence, lab safety, insect collection, and insect succession and identification (4).

Taphonomy has been defined as the study of organic remains in all spheres (5), and forensic taphonomy as the study of the change of human bodies from a living organism to deceased remains with judicial and legal purposes (6). Groen and Berger commented on the analytical reasoning application to crime scene investigation, archaeology, and taphonomy in the form of restructuring the past from the present (7).

The authors' institution embraces experiential learning, defined as participating in new experiences and applying observations to the real world (8). It provides students with opportunities to practice what they've learned in settings beyond the classroom. Experiential learning exercises using decomposing meat can provide a good method for teaching scientific principles, report writing, ecology, entomology, taphonomy, biochemistry, crime scene investigation, and other related forensic science topics. Reasoning from the present to the past, as an example, is illustrated by noting the stages of decomposition of a meat and correlating with time since death. The interrelationships of the entities in the natural world are illustrated by the interpenetration of decomposition with the air, soil, microbes, vegetation, and other living and non-living things. In short, using meats for taphonomy and entomology lab experiments is an experiential and fun way to teach many subjects, and associate them with real-world scenarios.

When circumstances support the use of pigs, they have been documented as a good analogue for human cadavers because of similar body structure (organ placement, skin, and hair) and systems (organ function) (9). However, it is not always feasible, and particularly so in smaller school settings, to set up a scene using a pig carcass. A review of the literature suggests other surrogate protein sources.

The researchers Gerard (10), Perez (11), Byrd and Castner (12), and O'Brien (13) have varied recommendations about which protein sources to use in insect and decomposition studies. These include cats, dogs, pigs, porcupines, and meats such as liver. In addition, the literature seems to indicate that unadulterated sources are more representative of natural decomposition and insect succession.

While many forensic insect succession studies testing meat products have used bait traps (14) or been conducted within indoor lab settings, information about testing and ranking various meat products for use in educational or classroom decomposition studies seems to be lacking in the literature. This study seeks to address some of these gaps.

Our study demonstrates a small and inexpensive outdoor decomposition scenario using different commercially available meats arranged in the environment. The idea was to mimic an outdoor scene that would be easy and practical for educators to set up, especially those involved in K-12 instruction or smaller colleges and universities. This would also be feasible for students engaged in independent studies or online class labs.

We used unadulterated materials purchased from grocery stores and offered for human consumption.

In this study we discuss what types of meat (poultry, beef, pork, fish, and processed) we tested and make recommendations for which of these will likely yield the best results when conducting a forensic entomology classroom exercise. This will save the educator time, resources, and cost of running such experiments on their own. Instead, they can focus more on teaching about forensic entomology with known attractive meats at the outset.

Methods

Hazards and Safety Precautions

Although the materials used were generally regarded as safe, outdoor laboratory classrooms can present unique challenges to a successful student experience. Dirkmatt and Cabo describe a forensic taphonomy scene, the processes for such a scene, and subsequent analysis (15). These may involve removing brush or foliage, collecting soil samples, catching and preserving insects, collecting tissue samples, and performing numerous tasks. Using appropriate attire and personal protective equipment (PPE) for field work (long pants, boots, gloves, etc.), including bug spray, can mitigate these challenges. If insect samples are to be collected and preserved for future analyses, ethanol can be effectively used as a preservative.

Materials and Experimental Design

This study was conducted over a 7-day period from June 17-23, 2022. The location was a secured outdoor location on the University of Nebraska—Lincoln campus. The day before the study, eight varieties of meat were purchased from a local supermarket. Meats were chosen based on ease of access from the grocery store (easily available from shelves or in the freezer section). We also wanted to test a range of meats in different categories: (i.e., beef, chicken, fish, pork, etc.) and extent of processing (i.e., whole, thick, thin, canned). Due to some predatory activity, an additional five meat products were purchased and placed out on Day 4. Meats used in the study included:

- 340 g (12 oz) can of Spam®
- 340 g frozen whole filet wild pacific salmon
- Two .45 kg pkgs of lean all-natural ground beef (75% and 80%)
- .31 kg Angus beef ribeye steak
- .69 kg chicken boneless chicken thighs
- .77 kg pork loin
- 1 Tyson® Cornish hen
- Four pkgs (each 74 g) chunk light premium tuna in water
- 1 pkg turkey livers
- 1 pkg. hot dogs (turkey, beef, pork mix)
- 1 pkg. chicken necks
- 1 pkg. pork ends

In addition, prior to beginning the study, several supplies were gathered:

- Ethyl acetate charged glass kill jar
- 70% ethanol for preservation
- KAA (kerosene, ethanol, acetic acid) for preserving maggots
- Labels
- Vials, Spoons, Forceps
- Latex gloves, face masks
- White garden plastic tags and pen to label meats
- Small and large plastic plates to place meat on
- Wire netting, plastic ties, and stakes used for barriers to protect meat from predators
- Thermometer and HOBO® device for environmental records such as humidity and temperature
- I-Phone cameras and standard scales

At 9:00 a.m., June 17, 2022, the meats were placed above ground, labeled with tags to track data for each product, and laid out in three rows of approximately 28 sq. meters.

Each meat item was placed on a plastic plate and placed inside a fenced area. Materials used for fencing were either wire netting (available at most hardware stores) or old dish drying racks. These items were used because they were already available on the research property, but such items could easily be obtained by educators from their homes or purchased inexpensively for classroom experiments. The fencing was then staked and zip-tied to keep it secure. Cinder blocks were used to enclose the ends of the drying rack fencing to reduce movement. These fencing procedures were established to deter predatory animals like racoons, foxes, hawks, cats, dogs, and opossums from taking the meat. All items were in a full sun, outdoor environment (see **FIGURE1**).

FIGURE 1 Meat experiment was laid out in three rows using wire netting, drying racks, stakes, and zip ties to deter predators. Photo credit: Erin Bauer

On Day 4, June 20, 2022, around 12:00 p.m., additional meats were purchased and introduced to the project. They were placed near the initial meat selection and surrounded in wire netting. The purpose of this addition was to replace some of the meat stolen by predators within the first two days of the experiment and to add additional meat types than those used earlier. For example, liver was not in the earlier set of meats, but because it is so often used in literature, the researchers wanted to test it.

Like the meats obtained on the first day, researchers tagged and labelled the new meats, which were fenced near the original study area and laid directly on the ground rather than on plastic plates. This was to compare their attractiveness with that of the meat samples on plates.

Results

TABLE 1 denotes general insect observations on each of the eight meat types the first three days. The Angus ribeye steak and two of the chicken thighs were stolen the first night by predators, despite fencing. As the fencing was not covered, a bird may have been responsible. Persistent ground animals may also have been able to pull pieces of meat through the openings of the dish drying racks.

TABLE *1* *Observations of meats Day 1-3.*

Meat	**Day 1 Observation June 17 9:00 a.m.**	**Day 2 Observation June 18; 11:50 a.m.**	**Day 3: June 19, 2022 1:00 p.m.**
1 Spam	Ants arrived immediately	No activity	No activity
2 Tuna	Ants	Ants	A few blow flies
3 Salmon	Ants	Blow fly and ants	a few blow flies
4 Chicken thighs	Cricket nymph	2 thighs stolen; blow fly	Second instar larvae; many blow flies.
5 Cornish hen	Ants and unknown fly	Ants	Second instar larvae; many blow flies.
6 Pork loin	Ants and flesh fly	Blow fly	Fly eggs; a few blow flies.
7 Ground beef	Ants and flesh fly	Fly eggs	Half of ground beef stolen; hatched first instars on plate.
8 Angus ribeye	Ants	Stolen by predators	N/A

Upon placement of the initial 8 meats out on Day 1, we observed insect activity after a few minutes. Most meats had ant activity, which is to be expected both because of environmental presence and predatory behavior; a few flies made an appearance. By Day 2, some fly eggs were observed, in addition to flies and ants. Some of the meats either had no activity (i.e., Spam) or had been stolen so were excluded from the project (i.e., Angus ribeye). On Day 3, more fly activity, including adult blow flies on all meats other than the ground beef (in addition to half of the ground beef being stolen), hatched first instar maggots on ground beef (see **FIGURE 2**) and fly eggs on pork loin (see **FIGURE 3**) were observed.

FIGURE 2 On Day 3, we found first-instar maggots on the purple plate containing ground beef. Photo credit: Erin Bauer

FIGURE 3: On Day 3, the pork loin was laden with fly eggs. Photo credit: Erin Bauer

On Day 4, the researchers did not record observations for the original meats but added several new meats to the experiment. Experiments on all meats were then recorded for Day 5-7 (see **TABLE 2**)

TABLE 2 *Observations of all meats, Day 5-7.*

Meats	Day 5 Observation June 21	Day 6 Observation June 22	Day 7 Observation June 23
1 Spam	No insect activity; poked hole in meat to see if having a place to burrow would make a difference.	No insects, predator feeding	Consumed by predators
2 Tuna	Unchanged	Stolen, no insects	Unchanged
3 Salmon	A few smaller maggots	2-3 blow flies, no maggot activity	Unchanged
4 Chicken thighs	Pupated blow fly maggots underneath the left corner of the meat; blow flies flying around.	Dried out on top, unchanged from yesterday; pupae remained in dried out fluid.	Unchanged
5 Cornish hen	Lots of maggot activity…some significant sized third instars (flesh flies); different than what we've observed on piglets in past studies.	Migration from food source, a few maggots still present.	Unchanged
6 Pork loin	Numerous third instar flesh fly maggots (other sizes as well) like what was found on the Cornish hen.	2 flesh flies; many third instar maggots underneath meat. Underside very moist; top dried out. When underside exposed, blow flies attracted; evidence of maggot migration away from food source; some seen next to pork loin site, some close to the "new meats" enclosure in front row.	Unchanged
7 Ground beef	Small maggots, second and first instars; a few third	1 flesh fly; a few first instar larvae	Unchanged
8 Angus Ribeye	N/A	N/A	N/A
9 Ground beef (80%)	Broken in half, no activity	Ants	Unchanged
10 Turkey livers	Extensive blowfly activity; obviously the preferred source of food in the area; some eggs already laid.	Massive maggot mass underneath the meat; continued blow fly activity. Meat mostly consumed and dry on top.	Unchanged
11 Hot dogs	No activity	No activity	Unchanged
12 Chicken necks	A few blow flies, but little activity; no maggots	Small sized and number of maggots underneath meat. Several blow flies still interested and flying around or landing.	Unchanged
13 Pork ends	No activity and one was stolen	Some blow fly activity (around 5 flies); small instar (1-2) maggots underneath and small number present.	Unchanged
14 Hot dogs	No activity: poked holes/ tore apart to see if this encouraged activity.	Stolen by predator	Unchanged

The Spam and tuna had very little to no activity. By day 7 both had been stolen by predators. The salmon and ground beef contained a few small maggots and flies, but overall had little activity. On Day 5, the plate with chicken thighs had a few fly pupae in the corner near the meat where residue from meat juices had settled, and a small number of flies were also still landing near or on the meat.

Observations we made during this time were that some of the plastic plates (pork loin, see **FIGURE 4**; and Cornish hen, see **FIGURE 5**) contained predatory insects (ants) and maggots that were larger than those found in other original studies where the authors have used pigs that were laid on the ground surface. We suspected these were Sarcophagidae (flesh fly) maggots. This was later verified through microscopy. The researchers had conducted a preliminary exercise previously during early summer to determine viability of placing meat in the open research area. In that exercise, none of the meat items were on plates with edges and lips, and therefore no substrates had a collection of liquid. We did not see Sarcophagidae maggots under these conditions.

We also observed that some maggots had difficulty migrating from the plastic plates. Meat defrosting and condensation plus the decomposition process contributed to fluid presence on the plates. Maggots were found in the fluid as well as some puparia, indicating that some flies simply pupated on the plate without ever reaching the soil. Byrd and Caster noted that maggots can drown in liquids (16).

FIGURE 4 On Day 5, we observed large maggots on pork loin. Under the microscope, we determined the family to be Sarcophagidae (flesh flies). Photo credits: Pork and maggots, Erin Bauer; Spiracles, Larry Barksdale.

FIGURE 5 On Day 5 we noted Sarcophagidae maggots, ,under the Cornish hen. Photo credit: Erin Bauer

Other highlights from Day 5 included extensive blow fly colonization of the turkey liver (see **FIGURE 6**) with a few flies on the chicken neck. The hot dogs, pork ends, and ground beef had no fly activity.

FIGURE 6 On Day 5, numerous blow flies were seen covering the turkey liver. Photo credit: Erin Bauer

On Day 6, the turkey liver was covered with a large maggot mass (see **FIGURE 7**). Some flies and a few maggots were also observed on the pork ends and chicken liver, but not to the same degree as with the turkey liver.

FIGURE 7 On Day 6, a large maggot mass was observed beneath the turkey liver. Lesser maggot activity was observed under the chicken necks. Photo credit: Erin Bauer

In addition to the newer meats, the authors flipped over the pork loin to look at the underside. It was still moist and was sustaining a significant number of maggots (see **FIGURE 8**). A few flies were still visiting the meat as well, likely because of the continued moisture.

FIGURE 8 On Day 6, the underside of the pork loin showed continued maggot activity, and flies were still attracted to the moisture. Photo credit: Erin Bauer

Day 7 was the last day of observations. Activity remained unchanged or was decreasing on the meats and much of the tissue was beginning to desiccate. It is important to note that we did not observe any late decomposition insects visiting the meat, such as dermestid beetles, nor did we see any carrion beetles during the decomposition process. We theorize that due to the small size of the meats, resource competition may have played a role, unlike in a larger carcass where many different insect species can be sustained.

On Day 11 we followed up and noted that most meats were dried up or stolen with no further insect activity. We broke down the fencing and left any remaining meat out for predators.

Local temperatures during the research time frame ranged from a high of 35 °C to a low of 17 °C (17).

Discussion and Conclusion

Although larger university forensic science programs may have access to research animals such as pigs for use in decomposition studies, many smaller institutions don't have this availability and need a suitable alternative. This study aimed to test various easily accessible grocery store or butcher-bought meats that could be used by K-12 or college educators for experiential learning activities in science courses such as entomology or forensics. The information from our study would also be applicable to student independent studies and online labs that need to be conducted in small home or school spaces and thus would require purchase of meat sources that are inexpensive, physically manageable, and offer a good potential for observable results.

Although students did not participate directly in this project, we have had considerable experience with outdoor forensic teaching exercises or field training programs in the past. These were conducted with law enforcement, students, teachers, researchers, and consultants who have used ½ chickens, chicken breasts, hamburger, steak, roadkill, dead mice, fish, and donated human blood. As an example, students in the university level *Bloodstains as Evidence* course (18) laid out meat products in an effort to record insect artifacts that mimic human bloodshed. Several hundred students have completed this exercise and one predominant issue was the use of material. Students most often used chicken breast, hamburger, or a slice of ham; overall, we learned that these meat products do not appear to produce good results, and that predation is an important issue. Birds and other animals steal the students' samples.

In addition, we have participated in numerous individual research projects involving insect succession and decomposition (19) Several of these were with grocery store meat products and others were with pigs. For example, the *Crime Scene Investigation* course at the University of Nebraska-Lincoln (20) has laid out a pig to simulate human remains as one of 8 crime scenes to be processed during the semester. During the years of the Covid-19, we did not use pigs for exercises due to zoonotic unknowns about viruses. Those two years' experience gave us additional insight on the difficulties identifying products to use for entomological, taphonomic, and crime scene investigation teaching and experiential exercises. Instead, we used various meat products purchased from grocery stores. It is from these experiences that we determined a need for further testing of products that would perform well for insect composition studies.

In this current study, we concluded that several meats were more attractive than others to common forensically important insects and would be a better choice for educators wanting to conduct their own classroom experiment. Biopsy samples are easily obtained and stored for additional research in the fields of toxicology and nutrition. Additionally, preserved samples can be examined later using a variety of microscopic methods.

It is well known that both Calliphoridae (blow flies) and Sarcophagidae (flesh flies) are associated with decomposition scenes. The meats most successful in attracting one or both of these fly families were the turkey liver, Cornish hen, and pork loin, with turkey liver being the most attractive to blow flies and the Cornish hen and pork loin being attractive to both. The turkey liver was removed and placed directly from its container, which resulted in the turkey liver immersed in fluid. Processed meat like Spam and hot dogs seemed to be the least attractive choice to insects, followed closely by ground beef. In general, the fish, both the tuna and salmon, also didn't attract many insects compared to the other choices. The pork ends, chicken necks, and chicken thighs all had activity, but were less preferred than the liver, Cornish hen, and pork loin.

The Angus ribeye steak was stolen the first night, so we were unable to get any data. This is consistent with the authors' past projects and reports from online lab students in which predators would take items.

Based on our observations in this study, the authors would recommend that instructors use turkey liver if the main goal is to encourage rapid arrival and colonization by forensically important flies, especially blow flies. It is a simple and reasonably inexpensive experiment that demonstrates many basic forensic entomology principles. Liver in a container that has obvious blood and has been unprocessed would be the best selection for small, outdoor forensic insect succession experiments.

Other types of livers, such as beef liver, may be a viable choice based on the literature, although it was not tested in this study. In addition, much of the literature using beef liver and other meats, such as that done by Neideregger et. al, have been conducted using indoor lab settings, rather than an outdoor environment, and may show success using meat types (i.e., processed) where we saw little activity (21). One reason for this difference may be that in a natural outdoor environment flies can choose oviposition sites. This is opposed to lab researchers determining which substrates to use for larval rearing. Livers of all types were not available, but analyzing the difference between liver types would be an interesting experiment for future study. In addition, further studies using pork loin or Cornish hens may be warranted, since the authors also saw success in attracting forensically important flies with these products. Pork muscle used in bait traps has shown good results (22).

Overall size of the subject meat appears to affect succession. Insect succession is not as robust on smaller meat masses. The biggest factor impacting this is likely less resource availability over time of the study. From personal observations of the authors during forensic classroom activities, larger intact pig carcasses attract more carrion beetles and a larger number of other insects than piglets or commercial meats. However, the small sizes we used in the outdoor scene with grocery store items seemed to attract sufficient flies and subsequent maggots for a successful general experiment.

No replications were conducted in this study as it was intended to be a quick comparison that would indicate initial insect preferences. This type of simple study may be all that is required or preferred in elementary or middle school levels where the concept of forensics is first being introduced. For high school or college populations, a more detailed experiment may be desired. In this case, testing meats that appeared from our anecdotal observations to be more attractive (i.e., livers, pork loin, etc.) in various settings (ground vs. on plate, in aquariums, etc.) and with different animal types (e.g., turkey, beef, pork) would greatly enhance the experience for these older students. For example, using more or larger meat samples may result in greater diversity of forensically important insects, especially those that colonize later in the decomposition process.

Although we did not pursue this area, the collection of flies and maggots may also lend themselves to post-mortem interval calculations using developmental stages of the insects. We have made time since death calculations and conclusions part of crime scene investigation courses in which students can determine values using entomological and taphonomic techniques and compare with known values.

The plastic plates the meats were placed on also affected results because moisture was maintained on the underside of the meat and, in some cases, pooled outward. This enabled maggots to thrive longer underneath areas where the meat had not yet dried out and where they were protected from predators. However, maggots were observed migrating away from the food source and attempting to scale over the lip of the plate, some struggling with the height of the lip and any liquid that had spread to the area. Thus, the plates may have prevented some maggots from escape. Additionally, pools of liquid may have prevented egg hatching or resulted in drowning. Although beyond the scope of this study, effects of excessive liquid pools may be examined at a later date. We recommend that exercises using meat products not be placed on substrates with constraining edges.

Modifications in experimental design could alleviate the challenges we faced, such as predator activity. Although sample meats were surrounded by staked fencing, animal predators still managed to remove some of the materials. This was especially apparent in the areas where the meat was fenced in on all sides but not at the top, indicating an avian predator. In other cases, such as the dish drying baskets, the meat was pulled toward the side and in some cases removed through the larger openings by ground predators.

One suggestion for future experiments is to utilize fish tank like structures, each holding a different kind of meat. The tanks could be covered with wire mesh that would allow insect access yet prevent predator interference. Studies could be done with different meats having the same substrate on the bottom of the tanks or repeating each meat in a tank with different substrates (i.e., glass bottom, sand or dirt bottom, or fabric bottom) and comparing results of which produces the most flies. This would also serve to keep migrating maggots and pupae contained within the environment so that they can be counted. A successful design would be a great way to teach insect succession and decomposition within a small outdoor space with ease for collecting specimens.

Testing grocery store products for nutrient content would also be worthy research. It would be interesting to know if products with greater nutrient density facilitated greater maggot masses.

We suggest that our research adds to the literature for using entomology and decomposition to teach science, forensic science, taphonomy, and entomology. Products can be obtained at minimal expense. The exercises add to experiential learning for students. They offer the opportunity to practice writing lab reports and to apply logic and reasoning to natural behaviors. They also are intriguing and open doors for many other skill exercises. As an example, calculating degree days and time since death offers practicing mathematical skills, considering error rates, and constructing uncertainty budgets. Students know the time the item was first put out and the times of appearance of first, second, third stage instars, and the immigration of pupae. Students can compare calculations with known times**.** Students can study the process of decomposition such as autolysis, check soils for pH levels during the decomposition, and observe effects of soil texture on decomposition (23).

In summary, the teaching of forensic entomology with associated subjects offers a fun and challenging journey for students of all ages and interests. It offers the opportunity to teach many topics, practice collecting and preserving insects and samples, learn good lab practices, and investigate research ideas. We recommend the use of fresh, unfrozen turkey livers for teaching lab exercises.

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