The development rate of forensically relevant blow flies (*Lucilia sericata*) based on different food sources

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Abstract:

The study aims to examine the survival of the local blow fly species *Lucilia sericata* (Diptera: Calliphoridae) on different food sources (e.g., beef liver, tuna, spam). The development rate of blow flies and other flies found at crime scenes is the key in estimating the time of colonization (TOC), which refers to the point in time when blow flies first laid eggs on a body. Research conducted on the time of colonization ranges greatly, because TOC is used in various scenarios. This short study seeks to answer how development rates, based on the TOC of different food sources that can be found throughout grocery stores in the United States. Widely accessible food sources that flies will likely thrive in will be priority, such as spam, tuna, canned foods, beef liver, etc. The experiment will use 1st instar larvae from a control colony in the Forensic lab, and then placed into their specific food treatments. The expected outcome of this experiment is that beef liver will have a better survival rate then canned food sources, as other studies have found beef liver to be the preferred food source.

Introduction:

Flies are a common occurrence in everyday life, they are found across the world in grocery stores, commercial farms, and crime scenes. Blow flies (Diptera: Calliphoridae) are particularly important for many forensically important questions related to crime scenes around the world. Research surrounding the study of blow flies (Diptera: Calliphoridae) have been dedicated towards many research topics like nutrition intake (Mackerras, Josephine, & Freney, 1933 and Ribeiro, Silva, & Zuben, 2010), development of various blow fly species (Jeong, Yangseung, et al., 2022), and many other studies throughout the world. Blow flies are consistently used by forensic entomologists to determine a time of colonization (TOC) that can contribute to narrowing down the minimum time the insects have had with a particular resource. Post Mortem Interval (PMI) provides the time elapsed from a person's passing, whether it be natural or under a medico-criminal investigations, and the use of entomological evidence can be a key contributor towards a PMI (Catts & Goff, 1992 and Chen, Hung, & Shiao, 2004).

Blow fly life cycles can take upwards of three weeks, starting from an egg as small as the tip of a pen to the death of the adult fly. A female adult blow fly will oviposit between 150-200 eggs per batch, and upwards of 2,000 eggs in a lifetime (Clark, Evans, & Wall, 2006). The eggs laid will take within a day's time to hatch into the first larval stage, also known as an instar, where they will resemble small moving rice grains. These larvae will begin to feed on the material they were oviposited onto, almost immediately after hatching from the egg casing (Donovan, 2006). The longer the larvae feed on the material provided, the sooner the larvae will make it through the 3 instars before pupation. Once the larva has reached the 3rd instar, then the larvae will leave the feeding material and begin to form the pupal casing. In this time, the pupae will form a shell-like structure around itself and harden the shell, turning the casing to a dark seed-like color. Adults will typically emerge from the pupae casing anywhere from 3-5 days, depending on the time they began pupation. Mating will begin soon after the adults have fully emerged from the pupal casing, and females will then seek the appropriate food source to oviposit their eggs.

Flies typically oviposit their eggs onto decaying material, such as carrion material, as well as many various food sources we dispose of and consume. Blow flies

(Diptera: Calliphoridae) are particularly found on meats, where most of their life cycle will remain. Meats are preferred by female blow flies, because they provide some assurance that their progeny can feed and survive on this food source as soon as they have hatched. Meat is particularly used by flies, because it decomposes relatively quickly which allows for the maggots to feed on the softening food source. The source of meat is also relatively found in large amounts from decaying animals and other resources, so it makes sense that the larvae will have a better shot at survival if given large quantities of a food resource(Matuszewski, 2021). The study of survival rates on various food sources for blow flies has been studied minimally.

Understanding the survival rates of blow flies is important for interpreting how long a person has passed away at crime scenes. Survival rates can then contribute to finding the postmortem interval (PMI), which will help contribute towards investigations by narrowing the time frame of an event. Blow flies will discover the remains of a carcass within minutes of the organism's death, and female blow flies will begin colonizing it with their egg masses. The point of colonization is dependent on temperature, because like all insects flies are poikilotherms. This means that the insect's development rate is completely reliant on the seasonal temperature norms (Hans, LeBouthillier, & VanLaerhoven, 2019). If an experiment is conducted where temperature is controlled, and constant, in a laboratory environment, then the only factor that would impact the survival rates of blow flies would be the food sources used in the experiment.

This study describes the importance of the food source that blow flies develop upon over their lifetime, as well as comparing different types of sources that may, or may not, contribute to the overall development rate of blow flies. A variety of food sources similar to meat material would be used to imitate the typical decaying material that many flies revolve their life stages around. Understanding this concept can help lead future research into how the development rate is impacted by different conditions and the limitations of the food sources available. The food sources in question that would be used may speed up or slow down the development rate of the flies by way of their quality in nutritional value. A mixture of nutrients is vital in the development rate of insects, but within food sources like those found in grocery stores preservatives may have an unknown cost on said development. Foods like spam, beef liver, chicken, cat and dog food, or tuna fish can be used as a variety of sources that can typically propagate blow flies fairly well and we can distinguish between all of these, which food source provides the best opportunity for faster or slower development rates. Development rates will be influenced positively by the high nutritional value of the food source, that contains high amounts of proteins and carbohydrates like fats, whereas it is negatively impacted by the lack there of in some sources.

Methods:

This study used specimens from a laboratory maintained colony of blow fly, *Lucilia sericata*. The laboratory colony was provided water, sugar, and a protein source for an ideal habitat for survival and oviposition. To ensure there were egg masses for all of the treatments, we provided beef liver as the primary source for the female flies to oviposition eggs onto, then following the eggs hatching into 1st instar larvae the beef liver source will be removed. These 1st instar larvae will then be collected and distributed in equivalent amounts (20 larvae per container) amongst the 3 food source treatments, and placed into large sealed clear plastic containers with 150mL of sand (to serve as a pupariation substrate) and 20g of the treatment food resource in 96mL plastic cups. The canned food resource treatments are spam, tuna, and beef liver (control). Each resource is replicated three times, and placed on a lab bench with consistent temperature and humidity (25.4℃). Each replicate was observed every 24 hours for an entire month and data was collected on development time, successful pupariation, and survival to adult and adult size, using wing vein measurements (dm-cu). Wing vein measurements were processed by using imaging software (ImageJ) to trace the length of the dm-cu vein, and this was done for the right wing of each adult fly available by the end of the experiment. The experiment concluded after the end of one month since the larvae were placed into the different trials, at that point all of the entomological evidence for each trial was removed and recorded into petri dishes and placed in a freezer.

Results:

Of the expected 180 adult flies (*Lucilia Sericata*) between all 3 treatments and their subsequent 3 replicates, only 87(48.3%) made it to full adulthood. The remaining 93(52.7%) have been documented as unable to reach adulthood, whether because the pupa never emerged or the specimen drowned in a liquefied food source. The treatment with the highest percentage of adult emergence, came from the spam food source with an average of 63.3% survival to the adult stage of development (Graph 1). In this same graph tuna had 33.3% survival to adulthood and beef liver (our experimental control) had 48.3% survival to adulthood. Take these percentages of adults emerging from pupae and compare them to the percentage of pupae from larvae (Graph 2). There are notable differences as each of the percentages for pupal survival was 75% or above compared to the percentage of adults emerged as stated earlier. Note, the difference in pupae to the adults emerged in the tuna food source (Graph 2) as there is an overall large decrease of adults from pupae. Each of the food sources in this comparison were vastly different as the overall percentage of pupae was larger than the percentage of adults from pupae.

The development time per each stage is represented in Graph 3, which shows the average time in days over a month of observations. The longest development time in this experiment went to the spam food source, as it had the last two adult blow flies of the experiment emerge at the very end of the experiment. Notably, as these were the last two blow flies to emerge in that timeframe, the data for the spam food source may be skewed from these two outliers. Beef liver had the shortest time in the larval stage of development, as well as the longest development time for the pupal stage. Tuna had the shortest pupal development stage, though note that tuna also had the fewest adults emerge from the pupal stage (Graph 2). Expected development for *Lucilia sericata* is typically a range of 2-4 weeks for 25℃, matching the average temperature of the lab this experiment was conducted in.

Table 1, shows the comparison of the final sex ratios and wing vein measurements for each of the various food sources. The expected sex ratio for *Lucilia sericata* is typically close to a 1:1 ratio, and the observed data from the experiment closely resembles what was expected. Notably, each of the sex ratios has some variance, and that can be expected as these results came from the adults of each food source treatment which also had large variance in the amount of adults. The wing vein measurements came from the adult blow flies that emerged from the experiment, and were used to justify the size comparison of the flies in each food source. From these measurements the tuna food source had the largest average wing vein measurement, meaning tuna had the justifiably largest flies in this experiment. The average wing vein measurements of the food sources are listed as; tuna 1.132mm, spam 1.040mm, and beef liver 1.022mm (Table 1).

Discussion:

This study was conducted as one of the first to analyze the link between survival rates versus various food sources, including an assessment of a timeframe for development in this study. This experiment focused specifically on the blow fly, *Lucilia sericata*, from a controlled colony from the Hans Forensic laboratory, where all of the initial first instar larvae were collected for use in this study. *Lucilia sericata* is the common blow fly species found across the Northern United States, and most commonly focused on in studies for Forensic science, veterinary, and medico-legal Entomology. For this study the blow flies were collected from beef liver that was applied to a *Lucilia sericata* colony, by using a wet paintbrush and transplanting 20 first instar larvae to their respective food source treatments for the study. In total, 180 *Lucilia sericata* 1st instar larvae were transplanted to their respective treatments and replicates, where they were then observed and collected as they emerged throughout the month-long experiment.

Lucilia sericata is commonly observed throughout the Northern portion of the United States as a forensically important fly species, but quite often difficult to rear on other protein sources besides beef liver. Commonly larvae were found drowned in the liquefied treatments, or reached the pupal stage without ever emerging into an adult. These varying scenarios influenced the final sample size between all treatments, which is reflected in the compiled data collected over the experiment's timeline. Future studies aiming to observe the correlation of survival rate based on food source treatment, should consider a larger replication of their various treatments for less influence from the survival of the initial sample size of larvae. It should be Considered that using an additive and removing a material that could soak up any residual fluid from the liquefying treatments, a material like dental gauze or allowing the liquid to drain by adding holes to the treatment cups would be preferable to stop larvae from drowning. The unsuccessful emergence of adult blow flies hinders the study greatly, because it may lead to a misrepresentation of the data that is not a true representation of the same events occurring naturally outside of a humidity controlled laboratory.

Development of the individuals for the food sources varied for each source, with notable differences in the amount of time per stage and food source. Spam notably is recorded as the food source that took the longest for adults to emerge, but this is heavily influenced by 2 adult blow flies emerging from pupal casings on the last day of the experiment. This along with other delays in developmental progress, could be attributed to the frequency in which the treatment substrate was searched through for data collection. If the experiment were to be repeated a lighter substrate, like pine shavings, could help make the specimens in the containers stand out and become easier to observe without physically going into each trial. Also, consider that the lab temperature was on average 25.42℃ development was not halted by any inconsistent temperatures that would impact the blow flies development. There are no known external factors that are impacting the development, which brings back questions about the food source for each of the trails and how that is potentially impacting the development. Survivorship of the various food source trials in this experiment is significant when considering that a food source that is typically used in the lab was not as successful compared to the others in this experiment.

Conclusion:

With consistent temperatures, the only factor in the blow flies development is heavily reliant on the food source available. The blow flies, *Lucilia sericata*, used in this experiment had significant survival rates in the spam trials. The hypothesis that the beef liver trials would have significantly better results then the canned food items, was disproven in the case of spam but not in the case of the tuna of this experiment. With an overall survival percentage to adulthood reaching 33.3%, tuna is not an ideal food source as it liquefies quick enough that larvae have no chance to survive and develop. Beef liver is still an ideal source to use as other studies have shown, but the food source has to be maintained and monitored so as not to expire quicker then the larvae can use the resource. For anyone trying to replicate the experiment, it may be helpful to find a way that removes the liquid as the food source decays over time.

Appendices:

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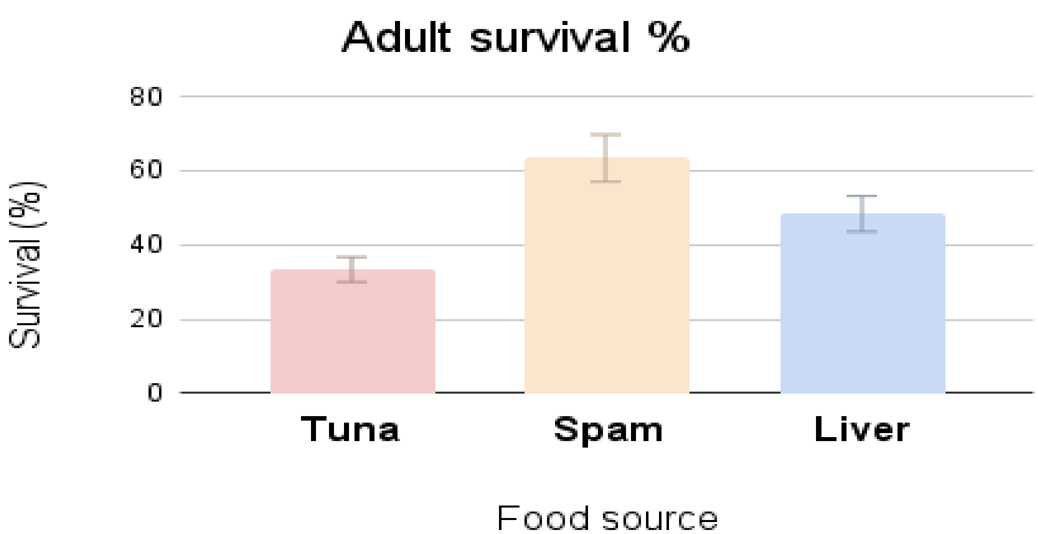
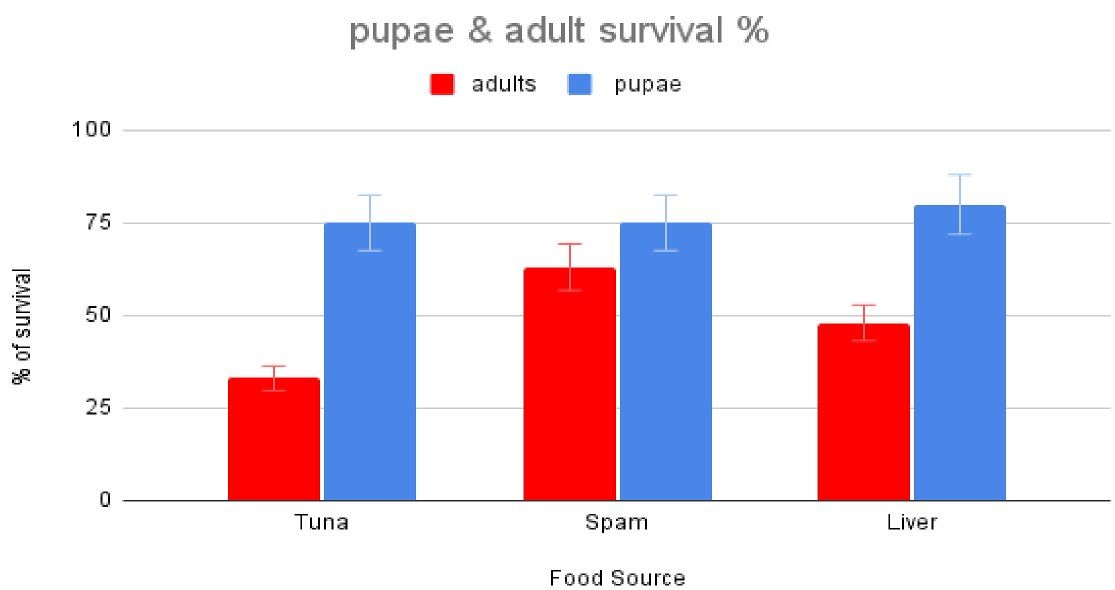
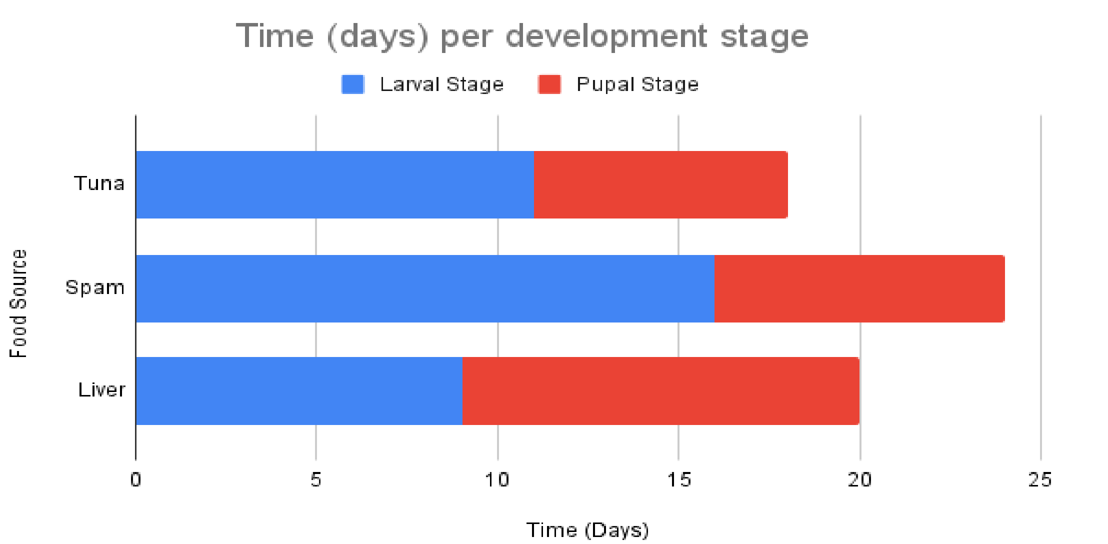


Graph 1., shows the average percentage of adults from pupae in each food source.



Graph 2., compares the % of individuals per stage of development for each of the food sources in the experiment.



Graph 3., conveys the time it took per development stage for each food source. The larval stage refers to going from a 1st instar to pupation, and the pupal stage of this graph represents the time from the start of pupation to an adult emerging.

Food Source	Sex Ratio F:M	Wing Vein Length (mm)
Spam	6:7	1.040
Tuna	8:11	1.132
Control (Liver)	4:3	1.022

Table 1., compares the sex ratio and wing vein measurements per their respective food source.

Budget:

Item	Cost (USD)
Beef liver	$4.50
Canned tuna	$3.50
Spam	$5.00
Large clear cup containers with lids (946 mL)	$75.00
Small plastic deli cups with lids (96 mL)	$30.00
Sand	$10.00
Metal kitchen strainer	$6.00
	Overall cost: $134.00