Evaluating the effects of temperature on larval *Calliphora vomitoria* (Diptera: Calliphoridae) consumption

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Abstract: *Calliphora vomitoria* (Diptera: Calliphoridae) are responsible for more cases of myiasis than any other arthropods. Several species of blowfly, including *Cochliomyia hominivorax* and *Cocholiomya macellaria,* parasitize living organisms by feeding on healthy tissues. Medical professionals have taken advantage of myiatic flies, *Lucillia sericata,* through debridement or maggot therapy in patients with necrotic tissue. This experiment analyzes how temperature influences blue bottle fly, *Calliphora vomitoria.* consumption of beef liver. After rearing an egg mass into first larval instars, ten maggots were placed into four containers making a total of forty maggots. One container was exposed to a range of temperatures between 18°C and 25°C at varying intervals. The remaining three containers were placed into homemade incubators at constant temperatures of 21°C, 27°C and 33°C respectively. Beef liver was placed into each container and weighed after each group pupation. The mass of liver consumed and the time until pupation was recorded. Three trials revealed that as temperature increased, the average rate of consumption per larva also increased. The larval group maintained at 33°C had the highest consumption with the shortest feeding duration, while the group at 21°C had lower liver consumption in the longest feeding period. Research in this experiment was conducted to understand the optimal temperature at which larval consumption is maximized whether in clinical instances for debridement or in myiasis cases.

Keywords: *Calliphora vomitoria,* Calliphoridae, myiasis, consumption, debridement

As an organism begins to decompose, necrophagous insects begin to swarm the corpse due to the release of gases during autolysis of cells and the most common of these insects are of the family Calliphoridae, which are among the first to arrive on scene following the death of an organism (LeBlanc 2008). In some instances, species of the order Diptera will infest the tissues of a living organism, otherwise known as myiasis (Failoc-Rojas et al 2016). In instances of primary myiasis, species like that of *Cochliomyia hominivorax* a fly will infest healthy tissue, while others typically target open wounds or necrotic tissue. Blow flies (carrion flies and bottle flies) parasitized animals and communities of low socioeconomic status with cutaneous or intestinal myiasis, typically in tropical and subtropical regions (Failoc-Rojas et al 2016). From a production perspective, they are responsible for severe profit losses due to damage of animal byproducts. Because fly larvae secrete antibiotic enzymes during feeding, the removal of the larvae from wounds can make the host more susceptible to infections (Francesconi and Lupi 2012). Understanding the development of fly

species is important to a wide variety of economic and scientific studies, but their existence is beneficial in medical and forensic fields.

Medical facilities have been taking advantage of larval consumption of necrotic tissues in debridement, also known as maggot therapy (Francesconi and Lupi 2012). In this procedure, larvae, commonly *Lucillia sericata* are placed into patient's wounds to inhibit the spread of necrosis throughout the limbs, while simultaneously cleaning the affected area (Francesconi and Lupi 2012). Forensic entomology relates the study of insects to legal investigations. Once an adult fly lands on a cadaver and oviposition occurs, the developmental stage, or instar, of the offspring can be used to determine an approximate time or location of death (Isaac et al. 2011). A few environmental factors affect the rate of fly development including time of year, temperature, and the substrate that the eggs were laid on. It is critical that researchers consider these factors when applying maggots to their respective field of study. In this experiment, the larvae of *Calliphora vomitoria* will be isolated in controlled environments at different temperatures in an effort to observe the impact temperature has on the rate of consumption by maggots.

Materials and Methods:

The experimental portion of this research was conducted in an enclosed room cooled between 18°C and 25°C at regular intervals. The procedures were divided into three different sections as follows: Calliphoridae egg collection, controlled environment, maggot distribution.

Calliphoridae egg collection:

Beef liver was set outside in an open area to attract Calliphoridae flies. Once an egg mass was oviposited, the egg were removed from the liver onto a damp paper towel and reared until the first larval instar.

Controlled environment:

Three incubators were constructed by inserting the probe of a temperature controller (Zilla Franklin,WI) through a small hole into a foam bucket. A lamp with a reflective shade was plugged into the temperature controller and used as a heat source. The incubator was monitored for 24 hours before proceeding with the experiment, and included a thermometer to monitor temperature at any given time.

Maggot distribution:

Ten newly hatched larvae were added to each of the four sealable containers and a 30g piece of beef liver was provided as a food source. Three of the containers were placed into the temperature regulated incubators, one at 21°C, another at 27°C, and the last at 33°C. The final container was placed in a foam bucket without a heat source to represent the control experiment and to prevent drastic temperature changes. Three trials were conducted using the clutch of Calliphoridae eggs making a total of 120 maggots for the entire experiment. The mass of each maggot and time in hours until each maggot pupated was recorded.

Results:

Calliphoridae egg collection:

Within an hour of leaving the beef liver exposed, a clutch containing over 200 eggs was gathered. The larvae used in the experiment were reared into the adult stage and identified as *Calliphora vomitorea.* The observations per trial were recorded in Table 1.

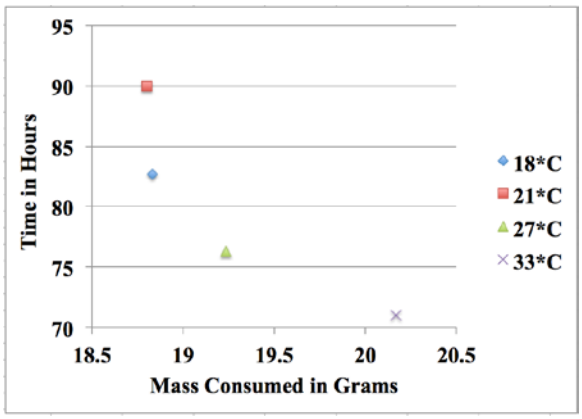
Table-1: Observation of Mass Consumption until Larval Pupation Data from Trails 1-3.

	Control		21°C		27°C		33°C	
	Time (h)	Mass Consumed (g)	Time	Mass Consumed	Time	Mass Consumed	Time	Mass Consumed
Trial 1	**84**	**18.7**	**92**	**18.4**	**76**	**19.4**	**73**	**20.2**
Trial 2	**81**	**19.2**	**89**	**18.9**	**77**	**19.6**	**69**	**19.9**
Trial 3	**83**	**18.6**	**89**	**19.1**	**76**	**18.7**	**71**	**20.4**

Controlled Environment:

The control group maintained at temperatures between 18℃ and 25℃ took an average of 82.6 hours (s~1.53) to enter the pupation stage of all ten larvae. While conducting the five-day observational until Larval pupation period, the thermometer frequently read at temperatures of 23°C and occasionally dropped to 18°C at later times in the day during the first and final trials. 56.5g of beef liver were consumed with an average of 1.83g (s~0.32) per larva. The larval group incubated at the lowest constant temperature of 21°C, had the highest pupation duration of all the groups at an average of 90 hours (s~1.73). At the time of the first experimental trial, two larvae took four hours longer to reach the pupa stage, increasing the average group pupation time and prolonging the feeding time. The 30 larvae consumed a total 56.4g of liver at a1.88g (s~0.36) per larva. At a stable 27°C, all three larvae trials consumed a total of 57.7g of liver at a rate of 1.92g (s~0.47) per larva. The average of time of until pupation was 76.3 hours (s~0.57). All larvae reached pupation at approximately the same time throughout each trial as there were no significant outliers. The final group incubated at an optimal temperature of 33°C, had the shortest larval pupation interval of 71 hours (s~2.0). At this temperature, the pupation between groups varied the most. During first trial, all but two larvae pupated at 68 hours. The second trial showed no significant outliers. However, half the larvae reached the pupa stage a few hours earlier in the final trial. All three trials consumed a total of 60.5g of liver at a rate of 2.02g per larvae. This data was expressed in Figure-1 below.

Figure-1: Average times to pupation vs Average Mass Consumption for 18°C, 21°C, 27°C, and 33°C.



Discussion:

Based on the fact that temperature readily affects the average span of the Calliphoridae lifecycle (Donovan et al 2006), the time of pupation was recorded to incorporate the duration of the larval feeding. From the information presented, the control group revealed that a broad temperature range presents a moderate pupation length. The variability between mass consumed could be a factor of the temperature or the period of feeding before

pupation. In the case of the control group, the second trial consumed the most beef liver in the shortest time frame, while the other groups had no significant difference between mass consumed or pupation length. The average control larva had a rate of consumption of 0.022grams/hour. From the information presented, the control group is a viable standard for this experiment. The larval group maintained at 21°C had the overall lowest consumption rate with the longest average feeding duration. At a low constant temperature, the rate of consumption per larva was 0.020 g/hour. On the opposite end of the spectrum, the group with the highest incubation temperature at 33°C had the highest total consumption in the shortest average time. The average rate of consumption per larvae was calculated to be 0.028g/hour. From the results obtained from the experiment one can conclude that as the temperature increased, the feeding duration decreased while the mass of consumption increased- which lead to an increase in the average rate of consumption per larva.

Taking into account standard deviations, the results obtained from this experiment are not statically relevant to openly declare that increasing the temperature increases the rate of consumption. From a biological standpoint, higher temperatures are optimal for certain bacteria to decompose organic material (Isaac et al 2011). Additionally, an increase in bacterial cultures can assist decomposition enzymes excreted by the larvae during feeding (Isaac et al 2011). In order to more accurately analyze the effects of temperature on larval Calliphoridae consumption, the experiment would have to be recreated in a more controlled and sterile environment.

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