The Survival of Blow Fly (Calliphoridae) Larvae in Hypoxic Conditions

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Abstract

Flies belonging to the family Calliphoridae are commonly used as forensic evidence as estimators of postmortem interval, due to their close association with carrion and other decomposing material. During certain stages of decomposition, their larvae may become completely submerged in fluids produced by extraoral secretions and microbial activity. In this study, I attempted to find the lethal time to 50% mortality of 3rd instar blowfly larvae (*Calliphora vomitoria*) in hypoxic conditions. The results of the trials were highly variable, with 83% of maggots surviving submergence of 76 hours in spring water or water bubbled with nitrogen, and 67% surviving 76 hours of submergence in water bubbled with carbon dioxide. Mortality rates never remained below 50% for any of the treatments performed, with many trials even showing 0% mortality. Future studies will need to be continued to determine if the type of hypoxic environment plays a role in the survival of *C. vomitoria*.

Background and Significance of Study

Historically, flies have been important to the field of forensic entomology, and the use of insect evidence has increased in recent years (Iancu, Dean, and Purcarea 2018; Madra-Bielewicz, Fratczak-Lagiewska, and Matuszwecki 2017). This is because adult flies and their larvae, known as maggots, are commonly found on or near decaying remains. By observing the succession of insects on carrion and determining the age of larvae present, a postmortem interval can be established, which can be critical information in criminal investigations (Iancu et al. 2018; Lein 2013; Reigada, Giao, Galindo, and Godoy 2011). One of the frequently cited forensically important families of flies is the Calliphoridae, or blow flies. This is because they are often one of the first insects to find and colonize a body (Greenberg 1991; Iancu et al. 2018; Reigada et al. 2011; Singh and Bala 2011).

Decomposing remains go through five general stages of decomposition: fresh, bloat, active decay, advanced decay, and skeletal remains. In the fresh stage, blow flies begin to colonize the body, and bacterial proliferation begins (Iancu et al. 2018; Vass et al. 2002). In the bloat stage, anaerobic activity flourishes and gases are produced, leading to the expansion of the body. The active decay stage begins after the body has ruptured, and fluids from the body accumulate, releasing nitrogen into the surrounding environment. At this point in time, both bacterial and insect activity is prominent (Vass et al. 2002). In the latter stages of decomposition- advanced decay and dry remains- most of the accumulated liquids have seeped into the ground, and maggots have moved away to pupate and complete their life cycle.

Carrion and human remains are presumed to be hypoxic environments (Hoback and Stanley 2001, Lein 2013). Bacterial proliferation leads to the production of nutrient-rich fluids

and gases, as the microorganisms break down bodily tissues (Iancu et al. 2018; Vass et al. 2002). Fluids are also secreted by the maggots themselves, to aid in their extraoral digestion of tissues (Lein 2013). Because of this accumulation of fluids near decaying matter, maggots may become completely submerged in fluids containing little oxygen while feeding.

For the clarity of this paper, hypoxia is defined as a condition in which the oxygen available is below the saturation level; in other words, the partial pressure is below that of environmental conditions, around 21 kPa (Harrison, Greenlee, and Verberk 2018). Anoxia is a condition in which there is a near or total absence of oxygen, and is also referred to as "extreme hypoxia". Hypoxic conditions can often have detrimental long-term effects on insects, such as decreases in growth rate and overall body size (Harrison et al. 2006; Harrison et al. 2018; Houlihan 1973). These effects may lead to discrepancies when attempting to estimate postmortem interval.

A previous thesis observed the survival of forensically important blow flies under anoxic conditions (Lein 2013). *Cochliomyia macellaria*, the secondary screwworm, survived 7 hours on average in an anoxic environment (Lein 2013). Other studies have been performed on other insects in hypoxic environments. One of these studies observed the mortality of *Nicrophorus* beetles in pitfall traps (Cavallaro, Banhart, and Hoback 2017). This study showed that carbon dioxide produced by increased microbial respiration was fatal to some carrion beetles, but water deoxygenated with nitrogen was less fatal (Cavallaro et al. 2017). Similar results were found in cowpea bruchid larvae (*Callosobruchus maculatus*), as carbon dioxide increased the toxicity of the hypoxic environment (Cheng, Lei, Ahn, Wang, Lei, Zhu-Salzman 2013). Another study on

tiger beetle larvae showed that they are able to survive anoxic environments for short periods of time by reducing their metabolic activity (Zerm, Walenciak, Val, and Adis 2004).

Hypothesis and Predictions

Based on previous studies, I predicted that the maggots would last longer on average in a hypoxic environment than an anoxic environment, as the complete absence of oxygen is much more fatal. Because of this, I chose to test a range of times, starting with a couple of hours of submergence, and gradually increasing the length of submergence to up to 76 hours. I hypothesized that regardless of the hypoxic environment the maggots were placed in, there would be no effect on their survival. To test this, I chose conditions similar to previous experiments: submergence in plain spring water, or submergence in water deoxygenated with either carbon dioxide or nitrogen.

Materials and Methods

The set-up and procedure for this experiment was straightforward and somewhat minimal, as all trials needed to be completed during the gaps in my pre-existing schedule. All materials needed for this project were provided by the department or by Dr. Hoback himself.

The maggots used in these trials were 3rd instars of *Calliphora vomitoria*, the blue bottle fly. These larvae were kept in a sealed plastic bag with sawdust substrate in a refrigerator until they were used for trials. Before trials began, the maggots were left out to acclimate to room temperature until signs of activity were seen (movement in the substrate or crawling towards the opening of the bag). Only active larvae were utilized in the trials.

To begin the trials, spring water was poured into a large, plastic container and was bubbled with nitrogen gas, carbon dioxide gas, or left untreated for 3 minutes before larvae were

introduced. A single maggot was placed into a clean, glass snap-top vial, which was then submerged under the water at an angle to eliminate air bubbles. The vials were immediately capped and labelled with the treatment type and time point. Control maggots were kept in clean, glass vials, with a piece of moist paper towel in order to prevent dessication.

Trials were separated by time points, with each trial having 5 to 6 time points being tested; the number of time points tested highly depended on other time constraints. Time points- or hours submerged- began with 4 hours, and increased up to 76 hours of submergence. Each time point contained 6 replicates, for a total of 36 maggots per treatment type for each trial. At the end of their respective times, maggots were removed from their vials, and placed on a piece of paper towel to dry. Their vials were also cleaned and dried, before the maggots were placed back inside for observation. Similar to the control trials, a piece of moist paper towel was placed into the vials as well.

Mortality was determined by observing no response after the larvae were gently prodded with the end of a paintbrush. Initial responses were recorded; however, at times, it took maggots an hour or more to recover after submergence. Multiple observations were made throughout the duration of the trials to ensure that maggots that had a longer recovery time were not accidentally marked as having no response.

Results

The exposure of *C. vomitoria* larvae to conditions of low oxygen showed variable results, regardless of the conditions tested. Despite the maggots being submerged for over 3 days, the survival rates remained high. As seen in Table 1, 83% of larvae survived 76 hours of submergence in water treated with nitrogen gas and in untreated water, and 67% of larvae

survived in water treated with carbon dioxide. However, mortality rates were higher in the initial sets of trials for maggots exposed to nitrogen (33% mortality when submerged for up to 12 hours) when compared to both carbon dioxide or untreated spring water (0% mortality when submerged for up to 12 hours). Despite the survival rates of *C. vomitoria* larvae in nitrogen-treated water being 67% after submergence for 12 hours, this rate increased to 83% survival when submerged for 16 hours; the cause for this change in survival rate despite the longer time of submergence is unknown at this time.

Table 1: Survival and Mortality Rates of Calliphorid Larvae for Each Trial Treatment

	Nitrogen		*Carbon Dioxide*		*Spring Water*	
Time in Hours	Survival	Mortality	Survival	Mortality	Survival	Mortality
4	67%	33%	100%	0%	100%	0%
8	67%	33%	100%	0%	100%	0%
12	67%	33%	100%	0%	100%	0%
16	83%	17%	94%	6%	96%	4%
24	75%	25%	93%	7%	90%	10%
28	83%	17%	89%	11%	77%	23%
32	54%	46%	90%	10%	81%	19%
36	86%	14%	81%	19%	81%	19%
40	92%	8%	83%	17%	83%	17%
48	100%	0%	61%	39%	83%	17%
52	78%	22%	75%	25%	62%	38%
56	71%	29%	62%	38%	71%	29%
60	73%	27%	67%	33%	58%	42%
64	100%	0%	67%	33%	83%	17%
76	83%	17%	67%	33%	83%	17%

When these rates were displayed graphically, the data showed some very interesting trends. The graph makes the variability of the data much more evident, as we can see many large

jumps in survival rates, most notably from the maggots exposed to nitrogen-treated water. At 32 hours of submergence, the mortality rate was only 46%- close to the 50% mortality rate that I was aiming for. However, the survival rate then greatly increased at the 36 hour time point, up to 86%. The only treatment that seemed to show a general downwards trend was carbon dioxide, yet this treatment still had fluctuation in mortality rates.

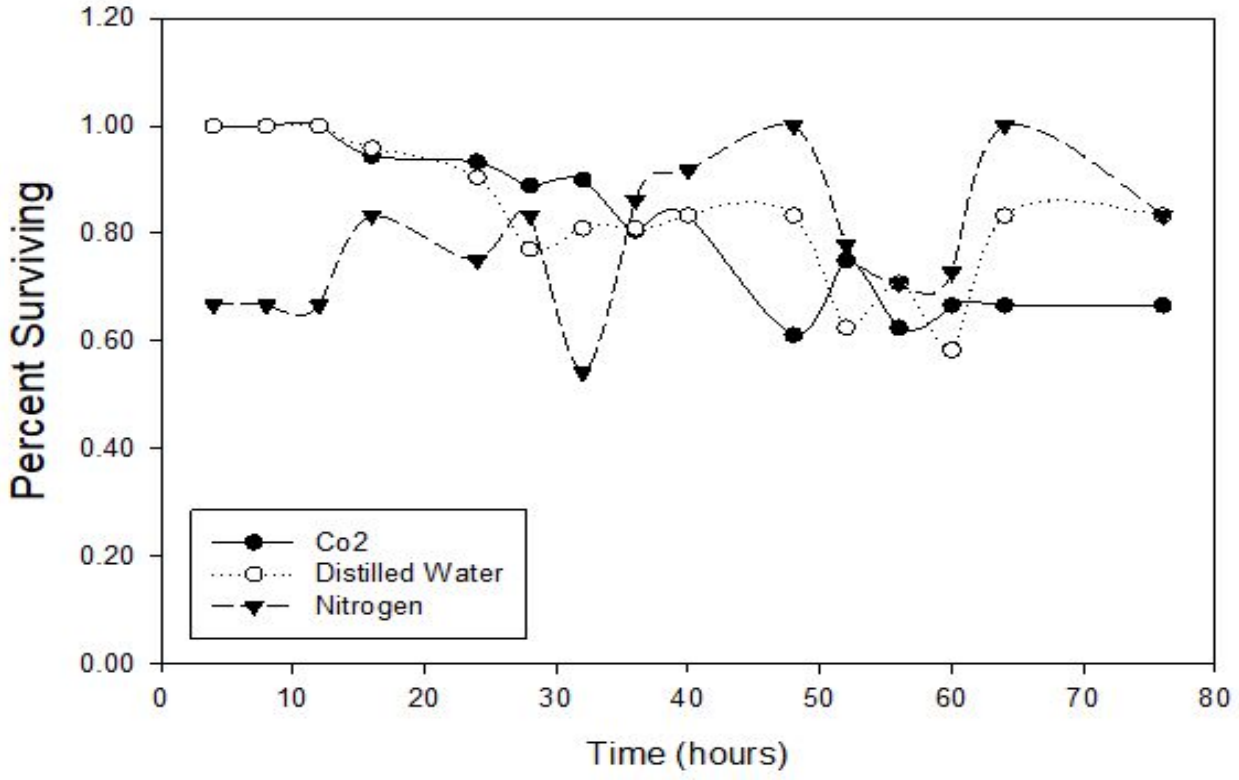

Figure 1: Survival Rates of *Calliphora vomitoria* Larvae in Hypoxic Conditions

Discussion

The results of this experiment were highly variable, which was unexpected. Rather than the data showing a downward trend- that is, as length of submergence increased, mortality rates increased- there are large jumps between mortality rates for different time points. This was because even at long intervals of submergence, relatively few maggots showed no signs of recovery. However, because of the variability of the data, I cannot conclude that the hypoxic environment had any effect on the survival of *C. vomitoria* larvae.

Despite being submerged for over 3 days in certain trials, the mortality rates were never consistently below 50% for any of the treatments. After doing further research into some of my sources, I found that *C. vomitoria* larvae can survive up to 6 days in as little as 1% oxygen (Hoback and Stanley, 2001). Because I did not initially know the species of Calliphoridae I was using in these trials, submergence times were far too low.

Being able to survive in hypoxic or anoxic conditions has also been found to be stage specific for many insects. In a study examining how insect evidence is preserved for forensic investigations, it was found that survival inside an airtight container increased with the age of the insect inside the puparium (Madra-Bielewicz et al. 2017). *C. vomitoria* showed a lower survival rate when placed in airtight containers, when compared to *Lucilia sericata* (Madra-Bielewicz et al. 2017). Other studies with forensically important blowfly larvae showed that 10-hour old larvae could not tolerate submergence in water for over 2 hours; however, 40 to 70-hour-old larvae survived submergence for up to 5 hours (Singh and Bala 2011).

Future studies could greatly improve on this experimental design. First of all, longer time intervals must be tested in order to ensure that 50% mortality is reached. Referencing back to the

thesis paper by Lein, Calliphorid larvae typically survived less than 10 hours in anoxic conditions (2013). I did not initially expect the 3rd instar maggots to survive over 76 hours in deoxygenated water, so the time points I chose were much shorter than they should have been. As mentioned previously, *C. vomitoria* larvae can survive up to 6 days in hypoxic conditions, so trials should start with submergence times of 5 to 6 days and work backward to narrow down the mortality rate (Hoback and Stanley 2001). Another way to improve future experiments would be to test survival at different temperatures, as all of my trials were performed at room temperature. Maggot masses found on carrion are highly exothermic, so testing temperatures that would more accurately mimic the conditions of a maggot mass would be beneficial (Greenberg 1991).

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