# I. Heat production in maggot masses formed by forensically important flies

## II. Abstract

Death of a terrestrial vertebrate animal triggers intense competition among insects for use of the resource. A criminal investigator can use information on the type and timing of insects on a body to estimate the time since death (or post mortem interval) of the corpse. Fly larvae are the most useful insects for forensic investigations because they have relatively predictable development times. However, one major complicating factor is that these flies form large feeding masses on a corpse that can generate an enormous amount of heat. In fact, heat generation has been shown to exceed ambient environmental temperatures by as much as 30-50$^{o}$C, consequently altering fly development and well as subsequent determination of time of death. Surprisingly, no detailed studies have focused on determining the sources of heat production in a maggot mass, an important piece of information needed when attempting to calculate a post mortem interval. This summer I will explore heat production in fly maggot masses by testing the hypothesis that internal heat of a maggot mass is produced by feeding flies in maggot masses. I will also examine whether oxygen consumption rates in these fly larvae are an indicator of heat production.

## III. Project Description (WC 1497)

Many species of flies are attracted to human bodies almost immediately following death. These flies lay eggs or deposit young larvae on the corpse, which then subsequently feed directly on the tissues and fluids of the deceased. By understanding the succession patterns, geographical distribution, food preferences, development, and behavior of flies specific to a cadaver, the insects can be used as forensic tools for determining the time since death or post mortem interval (PMI). Most investigations relevant to forensic entomology have performed ecological succession studies to gain baseline information on the types of insects active on a corpse and when they arrive as the body decomposes. However, few studies have examined any aspect of the physiological interactions that occur among and between insect species while feeding on a cadaver. These interactions undoubtedly alter development of at least one member of the association, and likely impact the usefulness of that species as a forensic tool.

Flies feeding on a corpse form large aggregations (termed maggot masses) that appear to be essential for successful exploitation of the food resource. When the flies reach a critical age of larval development, the masses begin to generate heat that typically exceeds ambient air temperatures by several degrees. In extremely large maggot masses (composed of thousands of larvae and of different species), temperatures can rise by more than 30-50$^{o}$C above air temperatures, producing conditions that accelerate or slow fly development depending on species. The impact persists throughout all subsequent stages of development for the flies. Surprisingly, though maggot mass temperatures are known to alter fly development, no detailed studies have been performed to determine the source of heat production. Many authors have speculated that the metabolic activity of the fly larvae is responsible for elevated mass temperatures. This argument is only weakly plausible considering that all insects are cold blooded (poikilothermic) and thus they normally produce heat to regulate body temperature. As consequence, despite the truly impressive temperatures maggot masses can produce, the source of heat production is unknown. This summer I will explore heat production in fly maggot masses by testing the hypothesis that internal heat of a maggot mass is produced by feeding flies in maggot masses. I will also examine whether oxygen consumption rates in these fly larvae are an indicator of heat production.

### A. Objectives

My long-range goal is to determine the context (when and where) in which insects can be used as forensic tools in criminal investigations. This summer, I plan to focus on the following goals:

1) Assess whether fly larvae developing in maggot mass are the source of heat production in maggot masses.
2) Determine whether rates of oxygen consumption in fly larvae developing in masses reflect heat generation in maggot masses.

### B. Background and Significance

During natural faunal succession of human and other animal carcasses, larvae from several species of flies form dense feeding aggregations that can generate internal heat (Goodbred and Goff, 1990; Anderson and VanLaerhoven, 1996). The heat production can be quite impressive, exceeding ambient air temperatures by several degrees, and in some instances, elevating to >30ºC above environmental conditions. The source of heat production is not known. Heat generation has been attributed to microbial activity (Rodriguez and Bass, 1985; Turner and Howard, 1992) and/or metabolic heat production from the flies, a byproduct of larval activity and high metabolism linked to digestive processes (Campobasso *et al*., 2001; Slone and Gruner, 2007); the latter being essentially associated with rapid rates of food consumption and muscle movement along the fore- and midgut regions (Williams and Richardson, 1984; Greenberg and Kunich, 2002). None of these predications have been experimentally tested. Slone and Gruner (2007) found that the volume of a maggot mass developing on pig carcasses and how ‘tight’ the mass was packed together, but not species composition or age of larvae, profoundly influenced internal mass temperatures. By contrast,_____*et al* (2010) demonstrated that age of the larvae and species did result in differences in mass temperature when flies were reared on bovine liver under laboratory conditions. However, the actual temperatures recorded in laboratory generated maggot masses do not achieve the maximum temperature elevations observed from larval aggregations formed during natural faunal succession (Goodbrod and Goff, 1990; Turner and Howard, 1992; Marchenko, 2001). It is apparent that multiple factors, including several that are abiotic, contribute to heat generation in the larval aggregations (Turner and Howard, 1992; Marchenko, 2001; Slone and Gruner, 2007;____*et al*., 2010). However, no study has yet to decipher the exact contribution of these potential heat sources to the internal mass temperatures.

### C. Experimental Approach

The research will be performed by myself and one undergraduate research student. Below I describe a brief experimental approach and methods for each objective.

<u>Objective 1</u>: *Assess whether fly larvae developing in maggot mass are the source of heat production in maggot masses*: To determine if fly larvae feeding in maggot masses are the main source of heat in the feeding aggregations, measurements of internal and external body temperatures will made at daily intervals throughout the entire duration of larval development. Three sizes (100, 500 and 1000 individuals/mass) of maggot masses composed of either the flesh fly, *Sarcophaga bullata*, or green bottle fly, *Lucilia sericata*, will be used to generate flies for temperature measurements. My previous work has shown that maggot mass sizes of 500 individuals/mass generate heat 5-10$^{o}$C above ambient temperatures, and masses of 1000 result in temperature elevations approaching 15$^{o}$C higher than ambient air temperatures. Small size masses (100 or less) do not generate heat that exceeds ambient conditions. Internal temperatures will be measured by catherizing fly larvae with a 27 gauge needle and threading a thermocouple through the needle into the body cavity. The thermocouple will be attached to a bioamplifier connected to a Macintosh computer and internal temperatures monitored continuously for 1 minute using Chart software (v. 6.0, AD Instruments). Surface temperatures will be recorded for the same larvae (prior to internal measurements) using an Oakton

thermal probe placed against the dorsal abdominal skin for 1 minute. A minimum of 30 larvae per maggot mass size per time point will be monitored for each species, with each experiment replicated at least three times. Data will be analyzed using one and two way ANOVA and Student Newman Keul's multiple comparisons tests. This work is anticipated to take 2 months to complete and will be the first objective initiated.

Objective 2: *Determine whether rates of oxygen consumption in fly larvae developing in masses reflect heat generation in maggot masses*. For endothermic animals, rates of oxygen consumption can be used to calculate basal metabolic rates and thus provide an estimate of heat production. Such measurements are typically not performed on insects since they are ectothermic (poikilothermic). However, some species of insects generate heat during specific development events and for these heterotherms, the rate of oxygen consumption can be used to determine heat production that would be released as a byproduct of aerobic metabolism. If the fly larvae are indeed the source of heat production in maggot masses, then oxygen consumption rates can be used to estimate heat production per individual, which in turn, should closely approximate the body temperature measurements in Obj. 1. Oxygen consumption will be measured using larvae generated from maggot masses described in Obj. 1 placed in respirometers connected to a bioamplifier and using Chart software. A minimum of 30 larvae per maggot mass size per time point will be monitored for each species, with each experiment replicated at least three times.

Data will be analyzed using one and two way ANOVA and Student Newman Keul's multiple comparisons tests. This work is anticipated to take 2 months to complete.

### D. Broader Context of Work

The research proposed for the grant period represents the continuation of a new line of work in Forensic Entomology that I initiated in 2008. The work proposed for this grant period is the first of its kind to use physiological approaches to decipher heat production in fly maggot masses and the resulting data will be extremely useful for forensic entomologists and criminal investigators who use insects to determine post mortem intervals. I anticipate at least one publication to result from this project. Dependent on the outcomes from this research, I plan to determine which fly tissues are responsible for producing the majority of heat released into maggot masses. I also plan to submit a grant application to the USDA focused on maggot mass heat stress impacts on parasitic wasp utility in biological control programs aimed at manure-breeding flies.

I have received one Faculty Development grant to conduct Forensic Entomology research and the completed work has resulted in the development of a manuscript that I anticipate submitting for publication in March 2011. My previous eight faculty development grants have resulted in fourteen publications (eleven with students) and the development of seven external grant applications. The faculty development grants (2) received in the last five years were focused on parasitic wasp venoms and altered fly development resulting from heat stress.

## IV. Cited References


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