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Abstract: Observed to arrive at a body within minutes of death, blowflies are frequently used as a biological clock in criminal investigations, aiding in the estimation of the post-mortem interval (PMI). In forensic entomology, PMI is thus calculated based on the time it takes the larvae recovered from decomposed remains to complete their developmental cycle at a constant ambient temperature under controlled laboratory conditions. However, very few studies take into consideration the mass generated heat produced by larvae co-existing in an aggregation. This study investigates the correlation between the number of blowfly larvae in a maggot mass and the amount of heat generated, as well as identifying the minimum mass size and elapsed time before any differences in accrued temperature achieve significance. Various sized larval masses composed solely of Lucilia sericata were reared in the laboratory at a constant ambient temperature of 22°C. Data loggers were used to record temperatures inside the maggot masses at five minute intervals for the duration of the feeding stage of development. Results showed a strong positive relationship between mass size and the amount of internally generated heat. A minimum mass size of 1200 individuals was required for the microclimate temperature to increase significantly above ambient, with aggregations composed of 2500 larvae producing temperatures that exceeded ambient by as much as 14°C. Comparing accumulated degree hours (ADH) for different sized masses at specific times during development highlighted 26 hours as the point at which the 1200 masses became significantly warmer than ambient. Larger masses had ADH values that diverged from ambient after as little as ten hours into feeding and development. Larvae developing in a mass were also observed to experience two peaks in temperature, one as they progressed from 2nd instar to 3rd instar, and another immediately prior to them entering the post-feeding phase of development. If these localized amplifications in temperature exceed the upper developmental threshold for a species, then larval growth may be disrupted. As a consequence of this, underdeveloped larvae could be mistakenly identified as smaller, younger individuals, which would result in the PMI being underestimated.

Suggested Reviewers:

Quantifying the Temperature of Maggot Masses and its Relationship to Decomposition

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ABSTRACT

Observed to arrive at a body within minutes of death, blowflies are frequently used as a biological clock in criminal investigations, aiding in the estimation of the post-mortem interval (PMI). In forensic entomology, PMI is thus calculated based on the time it takes the larvae recovered from decomposed remains to complete their developmental cycle at a constant ambient temperature under controlled laboratory conditions. However, very few studies take into consideration the mass generated heat produced by larvae co-existing in an aggregation. This study investigates the correlation between the number of blowfly larvae in a maggot mass and the amount of heat generated, as well as identifying the minimum mass size and elapsed time before any differences in accrued temperature achieve significance. Various sized larval masses composed solely of *Lucilia sericata* were reared in the laboratory at a constant ambient temperature of 22°C. Data loggers were used to record temperatures inside the maggot masses at five minute intervals for the duration of the feeding stage of development. Results showed a strong positive relationship between mass size and the amount of internally generated heat. A minimum mass size of 1200 individuals was required for the microclimate temperature to increase significantly above ambient, with aggregations composed of 2500 larvae producing temperatures that exceeded ambient by as much as 14°C. Comparing accumulated degree hours (ADH) for different sized masses at specific times during development highlighted 26 hours as the point at which the 1200 masses became significantly warmer than ambient. Larger masses had ADH values that diverged from ambient after as little as ten hours into feeding and development. Larvae developing in a mass were also observed to experience two peaks in temperature, one as they progressed from 2nd instar to 3rd instar, and another immediately

prior to them entering the post-feeding phase of development. If these localized amplifications in temperature exceed the upper developmental threshold for a species, then larval growth may be disrupted. As a consequence of this, underdeveloped larvae could be mistakenly identified as smaller, younger individuals, which would result in the PMI being underestimated.

INTRODUCTION

Calliphoridae, or blowflies, are recognised within the forensic community as a valuable source of evidence in criminal investigations owing to their predictable behaviour and worldwide distribution [1, 2]. With some species arriving at a corpse within minutes of death [3, 4, 5], their much studied development at controlled temperatures has allowed forensic entomologists to utilise them as a biological clock when estimating the post mortem interval (PMI) [5, 6, 7, 8, 9, 10, 11, 12, 13].

Eggs laid on a suitable ephemeral resource such as a corpse will hatch after between six and 40 hours, depending on environmental conditions and species [1]. These minute larvae will then proceed to feed and develop through three stages known as instars, becoming progressively larger. The general rule amongst forensic entomologists calculating larval age is that the largest individual collected from a corpse is the oldest and most likely to have developed from the first eggs laid. But there are a number of complicating factors, not least the fact that larvae undergo a rapid reduction in body length once they enter the post feeding stage of development [14, 15].

Mature blowflies and their larvae are poikilothermic, meaning body temperature is governed by the ambient temperature, and the warmer the conditions, the faster the metabolic rate and hence the faster the rate of development [8, 13, 14, 16, 17, 18, 19, 20]. Each blowfly population has a particular temperature range wherein successful larval development can occur, and this may differ amongst taxa and geographical location [21].

Development is fastest around the centre of this range, but ceases outside it, in some cases killing the insect [9, 13, 20, 22, 23, 24].

As larvae develop and reach their 2nd instar, they usually aggregate into a maggot mass: an assemblage of feeding larvae in which metabolic heat increases the localised temperature above ambient [4, 25]. A mass is beneficial for larvae since it maximises their feeding efficiency by producing sufficient proteolytic enzymes for tissue breakdown [23, 26, 27, 28, 29]. As larvae in a mass are in a constant state of motion, friction from their bodies repeatedly rubbing against one another, combined with their rapid metabolism, results in a sudden and localized increase in temperature, often reaching highs of 27-35°C [30]. These optimum temperatures provide protection from any sudden and temporary drops in ambient and allow larvae to preserve their own stable environment for accelerated development, even when stored at lows of 4°C [5, 18, 23, 31]. But these temperatures can only be maintained if aggregations exceed a minimum volume, the general rule being that the larger the mass, the greater the amount of heat produced [21, 32, 33]. However, there are cases where large masses have reportedly generated temperatures approaching highs of 50°C [1, 5, 23, 32]. These extreme temperatures lie well above the upper lethal limit for blowfly larvae [9, 10, 13] and one would expect highs such as this to result in increased larval mortality, and at the very least to inhibit development.

Despite the fact that heat generated by a maggot mass is often highlighted in the literature as a significant influence on the larval development rates [1, 5, 7, 18, 26, 28, 31, 32, 33], it

has very rarely been taken into consideration when estimating the post mortem interval. Mass generated heat could affect larval development and any subsequent PMI estimates in two very different ways. Firstly, if the mass increases the local temperature to an optimum level, then the rate of larval development may increase. With individuals becoming larger sooner, entomologists may mistake them for older larvae. This could lead to an overestimation of the post-mortem interval. But in cases involving human cadavers, the opposite could be true. If the larger masses found on these corpses [34, 35, 36, 37] are generating temperatures above the upper limit and hindering development, then the affected larvae may be smaller than expected for their age. If these underdeveloped individuals are mistaken for younger larvae then this might lead to an underestimation in the PMI. Either way, this could have serious implications for criminal investigations.

The aim of this study was to investigate whether the number of larvae in a maggot mass influences the amount of heat generated locally. By identifying the smallest mass size capable of producing a microclimate significantly warmer than ambient, it should be possible to highlight cases where mass generated heat is a factor that may need to be considered in PMI estimates. It is hoped that this will eventually lead towards increasing accuracy when relying on larval development for PMI estimates in criminal investigations.

MATERIALS AND METHODS

Blowfly eggs were harvested from a laboratory reared colony of *Lucilia sericata* (*Phaenicia sericata* in North America), which originated from larval stock purchased from a local fishing tackle outlet. *L. sericata* were used for this investigation since they have a well-studied lifecycle, a global distribution and are commonly located in close proximity to human habitats, which often gives them immediate access to corpses; important characteristics to aid in criminal investigations [38, 39, 40].

Pork liver was introduced into cages housing mature blowflies to stimulate oviposition. Once eggs were harvested they were kept in an incubator with 60% RH, a constant temperature of 22°C and a 16:8h (L:D) photoperiod. The light source in the incubator was supplied by standard 40W fluorescent tubes emitting white light. Eggs were monitored until 1st instar larvae hatched after approximately 24-36 hours. These 1st instar larvae were then counted out and randomly allocated to different sized masses (50, 100, 150, 200, 300, 600, 900, 1200, 1500, 1800 and 2500) before being placed directly onto racks of lamb ribs. Racks of lamb ribs were used for this study, as they were readily available from a local butcher and provided ample food for even the largest experimental masses, allowing them to complete development without facilitating competition, which can reportedly influence mass temperature [1, 4, 6]. The meat and larvae were then stored in 10l plastic containers measuring 27x27x16cm and placed back in the incubator at a constant temperature of 22°C, within the optimum range for this species where maximum heat emission is promoted [33], for the duration of the experiment.

Using data loggers, maggot mass temperatures were recorded every five minutes from the start of the experiment and were verified using a FLIR T425 thermal imaging camera (FLIR Systems Ltd (UK), 2 Kings Hill Avenue, West Malling, Kent, ME19 4AQ UK). Thermocouples with data loggers were also used to take readings at five minute intervals inside the incubator to ensure the ambient temperature remained constant. Once larvae entered the post feeding stage of development and began to migrate away from the food source dispersing the mass, data collection ceased. This usually occurred between 40-48 hours into the experiment and was easily identifiable as migrating larvae were observed escaping sealed containers and incubators with ease. For each of the eleven mass sizes there were three repeats, plus three controls, giving a total of 36 sets of recorded data. Controls for the experiments consisted of ribs with no larvae and were used to represent rib temperature minus larval activity during data analysis.

Statistical Analysis

For each of the 36 experiments, the first two hours of temperature data were discarded, allowing time for both the refrigerated racks of lamb ribs to adjust to ambient and for larvae to recover from any physical disturbance experienced in the initial stages of the experiment. The remaining data were then analysed using the statistical package R [41]. A repeated measures ANOVA was carried out to compare overall temperature in the different mass sizes between the times of 120 and 2880 minutes. The results of this analysis were investigated further with a multiple comparison of means test, used to identify which

masses produced similar or significantly different temperatures. Peak temperatures recorded for each of the 36 experimental masses were examined using linear regression. This was used to determine whether there was a relationship between mass size and the maximum temperature produced as well as identify whether a regression equation could be used to estimate peak temperature based on mass size or vice versa. Using the recorded temperature data it was also possible to calculate accumulated degree hours (ADH) for each of the masses. An ANOVA followed by post-hoc multiple comparison of means using Tukey's Test were used on these data to highlight which masses have significantly different ADH values at specific times during development, with subsets being taken at five hour intervals. Diagnostic plots showed the data to be normally distributed and of uniform variance in every case.

RESULTS

Initial Observations

Condensation was observed on the inside of the plastic containers housing the larger masses (1200+ individuals) along with a substantial amount of liquefied tissue accumulating in the vicinity of the aggregation. Individual larvae appeared more active and frenzied in these masses and on a number of occasions large aggregations of 1800 larvae or more were observed to separate into two smaller masses. Smaller aggregations remained as a single unit and displayed less frenzied behaviour, whilst the conditions produced inside their containers were considerably drier. Masses of all sizes were observed to move around the food source as a single entity and showed preference for more sheltered areas such as the

underside of the meat. Larvae entered the migratory phase of development approximately 40-48 hours into the experiment, with larger masses completing development at the earlier end of the scale.

Maggot Mass Temperatures

Recorded temperatures taken from within the mass ranged from 20.5°C (1.5°C below ambient) to 36°C (14°C above ambient). Figure 1 clearly illustrates that as the larvae in the masses developed they were capable of generating heat greater than ambient, with temperature increasing in conjunction with mass size.   However, this increase in temperature was not a gradual and constant process with the highest temperatures being recorded at the end of feeding when larvae were at their largest. Instead, temperatures were observed to rise and fall throughout development producing two distinct temperature peaks. During the initial hours of development all masses, regardless of their size, showed temperatures that were not only similar to each other, but also to ambient. For the first *ca.* 500 minutes temperatures increased gradually before reaching a plateau and remaining constant for a further *ca.* 500 minutes. After this point mass temperatures began to become more variable.   Larval masses became progressively warmer as development advanced, with larger masses (1200+ larvae) showing more exaggerated increases. However, despite the 2500 mass peaking at approximately 36°C, several degrees higher than the 1800 mass, its temperature curve was initially shallower in comparison. This indicates a slower rate of temperature increase.   After peaking, temperatures dropped, but were maintained several degrees above ambient for the duration of the experiment until

masses experienced a second, smaller peak just prior to the migratory stage of development.

Analysis of the data showed that there were significant differences between masses of different size and the temperatures they produce, with larger masses producing higher temperatures ($F_{11,24}$=12.74, p-value <0.0001). Again, using the control group to represent ambient, the results showed that smaller masses (50-900 individuals) produced temperatures that were similar to one another as well as to ambient (Table 1). Larger masses composed of 1200 larvae or more produced temperatures significantly higher than ambient as well as the majority of the smaller masses. However, despite larger masses producing more heat than ambient, they failed to be significantly warmer than masses of a similar size. For example, the 2500 mass was not significantly warmer than the 1800, 1500 or 1200 masses whilst the 1200 mass was only warmer than the control and the masses of 50, 100 and 150 larvae. This suggests that when comparing the thermal output in two aggregations, there needs to be a difference in mass size of approximately 1000 larvae or more for those temperature readings to differ significantly.

Peak Temperatures

Peak temperatures recorded for each of the 36 experimental masses ranged from 23.5°C in the control experiments to 36°C in the 2500 masses. All temperature peaks occurred after 1200 minutes (20 hours) had elapsed. Figure 2 displays the data points and regression line

for peak temperature against mass size. The regression line through the points produced the simple regression equation of:

$$PEAK = 23.8 + 0.0046 MASS$$

This model fits the data well (r^2=0.95, $F_{1,34}$=684.9, p-value <0.001) and shows that there was a strong positive relationship between the recorded maximum temperature and the size of the maggot mass, with the peak temperature increasing by 0.46°C for every 100 larvae added. 95% of the peak temperature recorded in a mass is accounted for by the size of that aggregation.

Accumulated Degree Hours (ADH)

At 10 hours into development the first differences in ADH values were observed ($F_{11, 24}$=2.44, p-value=0.033) with the 1800 mass producing an ADH value greater than ambient (Figure 3, A). As time progressed and ADH values increased, pairwise comparisons showed that an increasing number of masses began to produce ADH values that differed from ambient as well as from each other. After 40 hours of feeding and development, maggot masses composed of 1200 larvae or more had significantly higher ADH values than ambient ($F_{11,24}$=16.197, p-value <0.0001) (Figure 3, C). Since no smaller masses were capable of producing ADH values greater than ambient, 1200 becomes the critical mass size for heat production.

Twenty-six hours were required before this critical mass was capable of producing ADH values greater than ambient (p-value=0.048). Larger masses composed of 1500, 1800 and 2500 larvae had ADH values greater than ambient after 19, 10 and 22 hours respectively. Despite the 2500 masses producing the maximum recorded temperatures, the amount of time required for them to accumulate heat energy, as reflected in the ADH values, was longer in comparison to smaller masses. Aggregations composed of 1500 and 1800 individuals may not have reached as high a temperature as the 2500 masses, but they were capable of producing ADH values significantly greater than ambient several hours earlier. This was also reflected in the temperature graph (Figure 1) with the 2500 curve appearing shallower in comparison during the first half of the experiment, representing a more gradual increase in temperature.

DISCUSSION

The two temperature peaks recorded in the maggot masses are believed to represent a particular stage of larval development. The first and most prominent peak identifies the point at which 2nd instar larvae metamorphose into larger 3rd instar larvae, whilst the second, smaller, peak occurs just prior to the larvae entering the post-feeding migration phase of development. Increases in temperature at these points can be explained by the increased energy demands required by larvae to pass through these junctures. Moulting from one instar to the next as well as migrating away from a corpse in search of a suitable site to pupate are both energetically expensive stages of development. To ensure they successfully progress through these stages, larvae display more frenzied feeding behaviour, filling their crops within 2-3 minutes [29]. Thus, larvae in this experiment are seen to

increase their basal metabolic rate (reflected in peak heat output) as a means of accumulating and/or storing energy necessary to undertake transition through these phases. As a consequence of this the mass experiences a sudden and localized increase in temperature.

The graph of the linear regression successfully demonstrates the strong positive relationship between the size of the mass and its peak temperature. However, the plotted data points and their associated regression line suggest that, as mass size increases, temperature increases indefinitely with no signs of reaching a maximum level. In reality, this is highly unlikely, especially in large masses similar to those found on cadavers. Temperatures inside a mass can only increase so far. Above the upper lethal limit larvae would be effectively killing themselves with thermal stress. The regression equation proposed in this study is therefore useful in estimating peak temperature or mass size in smaller laboratory based aggregations, but less so for larger masses out in the field. To be able to use linear regression on criminal cases with confidence, more research needs to be carried out on a larger scale and under field conditions with varying ambient temperatures. These studies would enable investigators to substitute larval numbers with mass weight or volume as well as highlight the point at which temperatures begin to plateau.

The 2500 mass may be taking longer to accrue heat in comparison to smaller masses as a result of its larvae altering their behaviour to adapt to an increasingly stressful thermal environment. *Lucilia sericata* are reported to have an upper lethal limit of approximately

35°C [33, 42], which coincides with the maximum temperature recorded in the 2500 mass. If larvae are able to identify the presence of a large number of individuals, this may trigger some form of thermoregulatory behaviour during the initial stages of mass formation when larvae are at their most vulnerable. This early recognition of a potential problem would allow larvae to alter their behaviour accordingly and take control of their microclimate, preventing temperatures becoming too high too soon. By repeatedly moving from the feeding centre of the mass, where temperatures are highest, to the cooler periphery larvae are able to prevent overheating, but at the expense of the amount of time spent feeding. Although unable to stop the inevitable increase in temperature, they have slowed it down. Thermoregulation may also explain the observed splitting of large masses into two smaller masses, another behavioural adaptation perhaps used to avoid critically high temperatures. The fact that the 1800 masses diverged from ambient so early in development (after as little as 10 hours) suggests that this may be the optimum mass size for *L. sericata.* Peaking at approximately 30°C, several degrees below the lethal limit, they were able to produce and maintain temperatures greater than all the other masses throughout 1st and 2nd instar development. This indicates that larvae in these masses are able to feed uninhibited in optimum conditions without having to invest valuable time carrying out temperature avoidance behaviours, since they do not have the capacity to reach the critical temperature.

Internally generated heat in larval aggregations has the potential to impact very significantly on PMI estimates. If the high temperatures produced by larval masses are disrupting feeding and development, then older larvae will be smaller and hence appear younger than they actually are. The problem then arises when these underdeveloped larvae are

compared to laboratory reared larvae and aged without taking into account the mass size or the heat that was generated. This could result in an underestimation when calculating the PMI. Conversely, if the size of the larval mass falls within the optimum range and produces a microclimate more favoured for feeding, then the rate of development may increase and larvae will appear larger, and hence older, sooner than expected. This could then lead to an overestimation of the PMI. These optimum conditions provided by the microclimate could also result in larvae entering the migratory phase of development earlier than expected which may result in evidence being overlooked at a crime scene. When larvae are collected as evidence in a criminal investigation, it is often the largest which are targeted for PMI estimates as it is believed these are the oldest and most developed individuals, hatched from the first batch of eggs. However, this may not always be the case. Larvae in the post-feeding stages of development may have already undergone a reduction in length, but still be in the vicinity of the corpse and be mistaken for younger, smaller larvae. This could lead to further errors in PMI estimates.

This investigation has shown that even small larval masses of approximately 1200 individuals are capable of producing a level of heat significantly greater than ambient after as little as 26 hours. It would be safe to assume therefore that any mass comprised of more than 1200 larvae that has been developing for at least 26 hours is producing a significant amount of heat that may be affecting development of larvae within that mass. This could have consequences for the field of forensic entomology, not just at the crime scene, during the collection of larvae, but also in the laboratory. Very rarely is mass size or volume recorded or even mentioned in reports investigating the use of larval growth and

development for PMI estimates [34, 35, 36, 43]. If the methods used to calculate larval age are derived from studies where individuals were reared in a mass then, depending on the mass size and its potential for heat generation, what we believe to be a "standard" rate of development could actually be non-normative. To avoid any potential inaccuracies in PMI estimates based on larval development it may be necessary to produce a set of standards for the laboratory which factors in mass size. This would ensure that future studies are controlling for the temperature variable, resulting in more consistent developmental data being produced. Further investigations are encouraged to investigate what kind of effect, if any, these mass temperatures have on larval behaviour and development and whether or not it is a variable that should be factored into PMI estimates.

CONCLUSIONS

As larval masses increase in size the amount of heat they produce internally also increases with masses containing 1200 larvae or more producing temperatures that are significantly warmer than ambient. These temperature differences begin to appear after as little as 10 hours with all masses generating excess heat after 26 hours. The microclimate of a maggot mass has the potential to impact hugely on any PMI estimates that are based on larval development. Further research is encouraged in this area to ascertain whether or not mass temperature is a factor which needs to be incorporated into calculations that age larvae. This could therefore increase accuracy when estimating the post-mortem interval in criminal investigations.

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Figure Captions

Fig 1- Mean mass temperatures (°C) vs. time (mins) for each of the mass sizes that displayed temperatures differing from ambient (control).

Fig 2 – Recorded peak temperature (°C) for each of the experimental masses plotted with its regression line and 95% confidence intervals.

Fig 3 – ADH values for each mass size at (A) 10 hours, (B) 30 hours and (C) 40 hours with associated standard error bars.

Table Captions

Table 1 – Comparison of temperatures between maggot masses of different size (** represents significantly different temperatures, N.S represents no significant differences between mass temperatures).

Figure 1

Figure 2

Figure 3

Table 1

Table1 – Comparison of temperatures between maggot masses of different size (** represents significantly different temperatures, N.S represents no significant differences between mass temperatures).
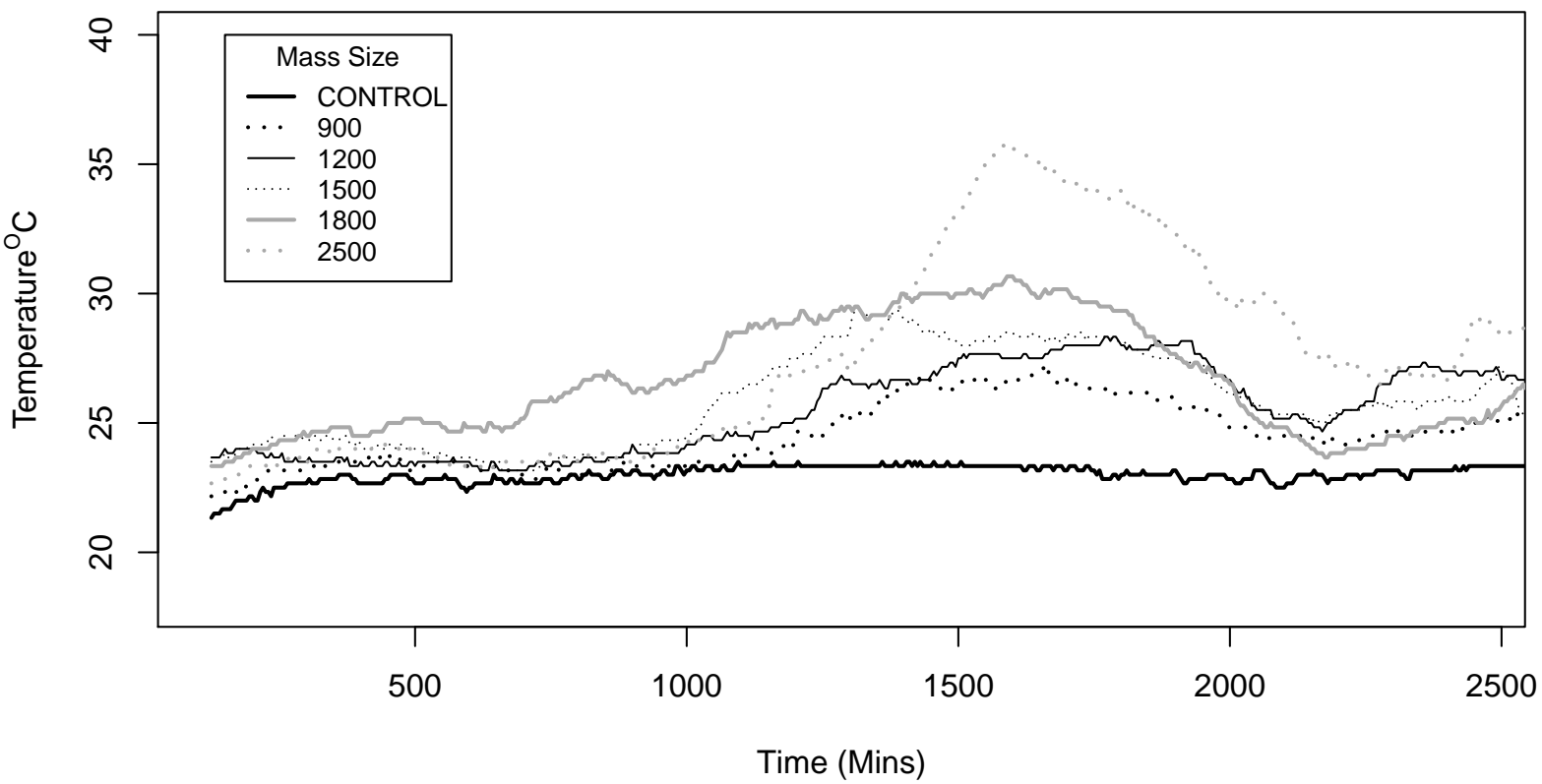
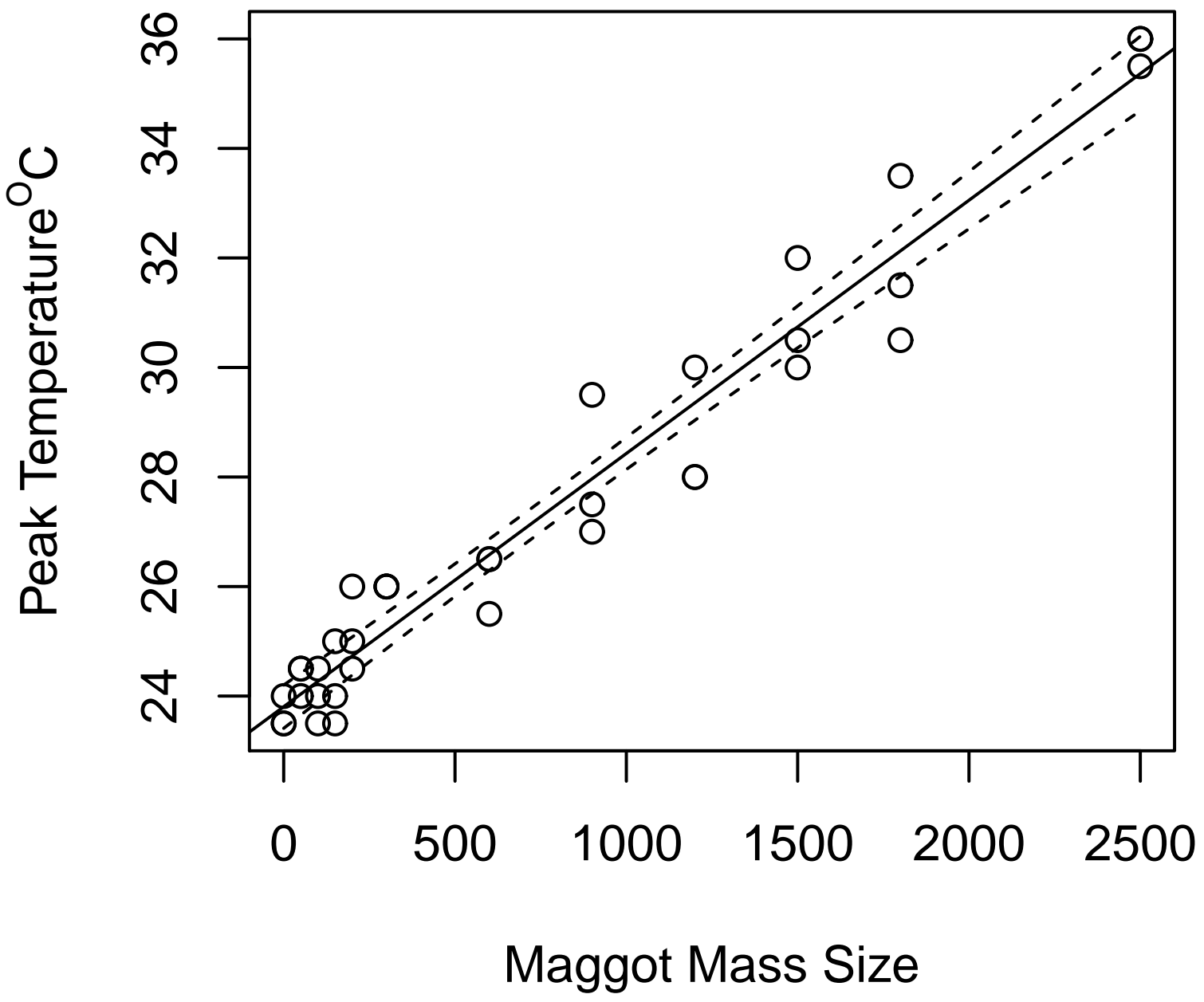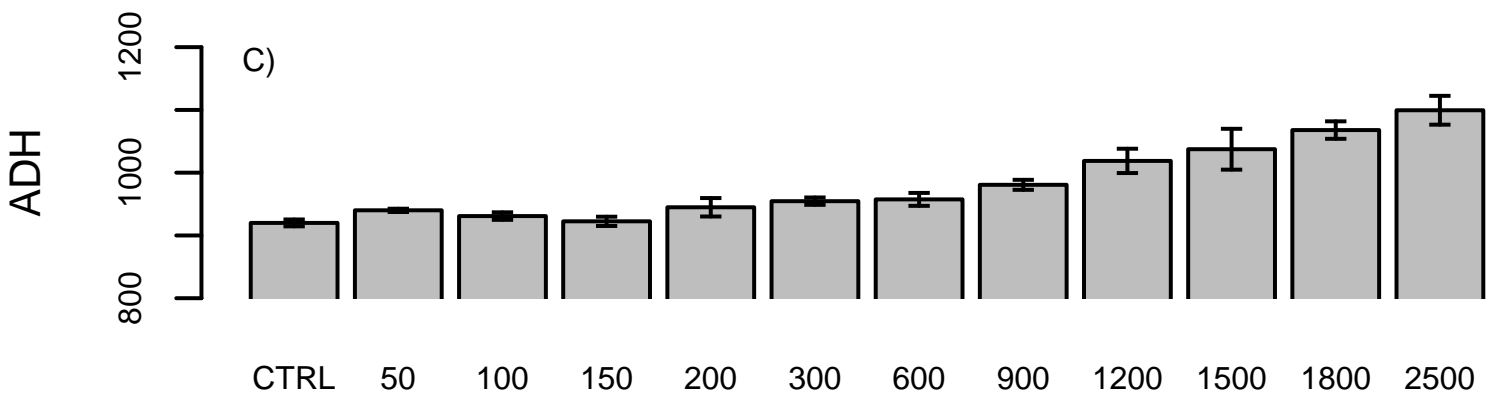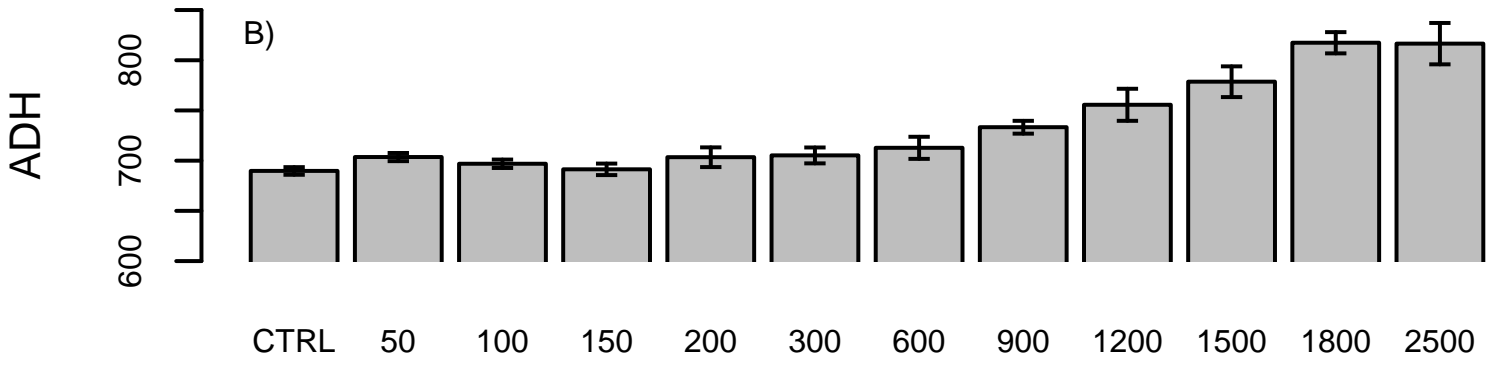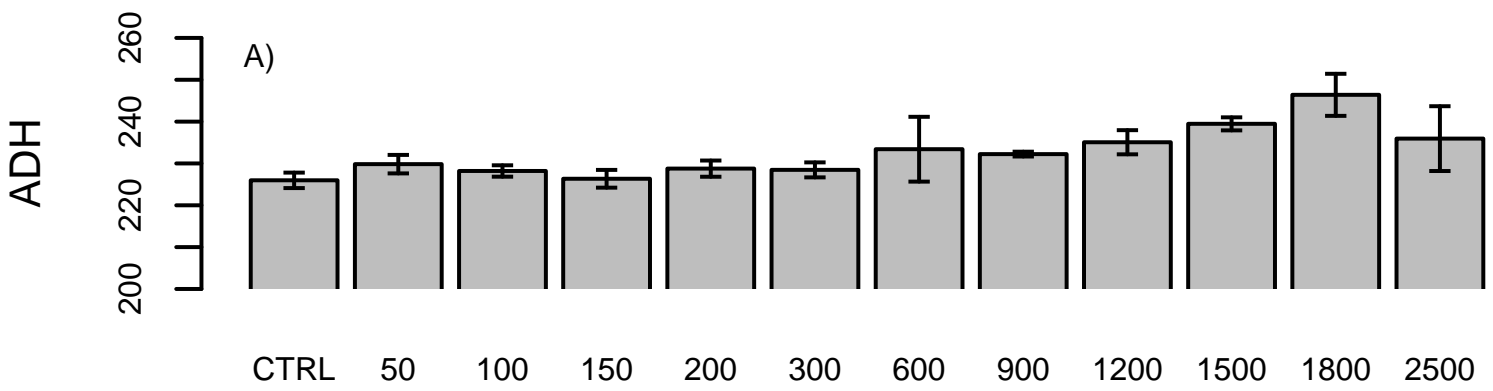|	CTRL	50	100	150	200	300	600	900	1200	1500	1800
2500	**	**	**	**	**	**	**	**	N.S	N.S	N.S
1800	**	**	**	**	**	**	**	**	N.S	N.S	
1500	**	**	**	**	**	**	N.S	N.S	N.S		
1200	**	**	**	**	N.S	N.S	N.S	N.S			
900	N.S	N.S	N.S	N.S	N.S	N.S	N.S				
600	N.S	N.S	N.S	N.S	N.S	N.S					
300	N.S	N.S	N.S	N.S	N.S						
200	N.S	N.S	N.S	N.S							
150	N.S	N.S	N.S								
100	N.S	N.S									
50	N.S										

*Revision Notes

Response to Reviewer Comments re. FSI-D-12-00140

"Quantifying the Temperature of Maggot Masses and its Relationship to Decomposition"

Reviewer 2's Comments	Response
Line 12 abstract - data loggers used to RECORD temps...	Abstract Line 12 – "recorded" amended to "record"
Introduction, para 4, line 7 - I would re-word the end of this sentence. As written it sounds like 27-35C is the actual increase in temp rather than the maximum recorded temp.	Introduction, paragraph 4, line 7 – changed from "commonly in the range of 27-35°C" to "often reaching highs of 27-35°C".
Materials and Methods, para 2: Would it be possible to describe (briefly) the light source used to create the photoperiod and also maybe the wavelength used. There have been reports that certain wavelengths of light affect the growth of insects and this (in theory) could have affected your results.	Materials and Methods, paragraph 2, line 3 – included the sentence "The light source in the incubator was supplied by standard 40W fluorescent tubes emitting white light."
Results - maggot mass temps - line 26. The sentence beginning with "However..." is confusing and should be clarified or re-worded (i.e. a mass of a mass with elevated masses?)	Results, paragraph 2, line 14 – sentence changed from "However, despite the mass of 2500 individuals mass peaking at approximately 36°C, several degrees higher than the 1800 mass, its temperature curve was initially shallower in comparison, indicating a slower rate of temperature increase" to "However, despite the 2500 mass peaking at approximately 36°C, several degrees higher than the 1800 mass, its temperature curve was initially shallower in comparison. This indicates a slower rate of temperature increase."
Please change time since death to PMI throughout the manuscript for consistency	Amended throughout the manuscript
Commas should precede the word "but" throughout the manuscript - sorry about this one, but I have to find something wrong.	Amended throughout the manuscript
Reference 30 - taphonomy is misspelled	Reference 30 – spelling corrected
Results - ADH section. Even though the Figure legend describes this, I would place the letter of the graph next to the number of hours in the text for clarity since hours and ADH are not the same thing. ("At 10 hours (Figure 1, A)...	Results, paragraph 6, line 3 – "(Figure 3)" amended to "(Figure 3, A) Results, paragraph 6, line 7 – "(Figure 3)" amended to "(Figure 3, C)"

Figure 1 caption - I would change the word "against"	This has been changed to "vs." – we cannot think of any other suitable word
The results of the experiments carried out by the Authors are very interesting and a correct statistical approach has been used, despite further improving can be done (it is not clear if a post hoc or a series of anova tests have been performed).	Post-hoc tests after an ANOVA are equivalent to '*unplanned* comparisons'. This means using a test for multiple comparisons (usually Tukey) to make comparisons between groups after the ANOVA has shown you there are differences. This is exactly what was used. The wording for this section reads: "*An ANOVA followed by a multiple comparison of means test was used on these data*" Although we feel this is sufficient, we have changed the wording slight to read : "*An ANOVA followed by post-hoc multiple comparison of means using Tukey's Test were used…*"
The language is not "enough scientific", too poor, with several not necessary sentences and mistakes underlying a poor knowledge of the Insect Nomenclature and Physiology (eg.: "development is fastes around the center of this range....").	In order to make the paper interesting to a wider audience, terminology has been used which is less discipline-specific (jargonised) and you see nowhere where this has compromised the understanding. Since Reviewer 2 found the manuscript to be "*Excellent manuscript - one of the better ones I have read. Very difficult to find even a minor change. Good references. Beautifully written*" – and it is impossible to "satisfy" both reviewers as the comments on our writing are contradictory, we opt to leave our writing style unchanged.