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The Relationship Between Surrounding Temperature and Larval Massing Temperature on Blowfly Growth Rate

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ABSTRACT

Forensic entomologists estimate the postmortem interval (PMI) based on the larvae growth rate in the surrounding temperature where the dead body is found and the temperature within the larval massing. This larvae growth rate can be used to estimate when the dead body is initially colonised by blowfly's larvae based on larvae size that is commonly measured in terms of length, weight and width. The aim of this research is to investigate the relationship between surrounding temperature and larval massing temperature on the blowfly's development rate at three different environmental conditions. Chicken carcasses inserted with temperature logger to record the larval massing temperature were left at three different environmental locations. Another temperature logger was placed near each of the locations to record the surrounding temperature of the locations. Three replicates of chicken carcasses were used for each location. This study shows direct correlation of the two parameters in which as the surrounding temperature rises the larval massing temperature also rises. Among the three locations, jungle site recorded the highest temperature and largest larval masses providing optimal conditions for larval growth. Subsequently, the jungle site exhibited the highest growth rate followed by outdoor site while the abandoned building showed the lowest larvae growth rate. More environmental variables in different locations or surroundings should be included in future research for better understanding and enhance accuracy in estimating PMI.

1. INTRODUCTION

Medico-legal forensic entomology is widely used to estimate post-mortem interval (PMI) [1]. The PMI is the elapsed time between death and the time when the body is discovered [2]. The colonisation of the remains by necrophagous insects mainly by blowflies as a pioneer species generally initiates shortly after death during the first decomposition stages [3], [4]. Adult female blowflies reach the deceased and lays eggs on its openings such as on the head and wounds [5], [6]. The eggs are able to hatch within 8 hours to 3 days in an optimal environment and after hatching, larvae feeds on the decomposing flesh [7], [8]. The larvae would then grow during the feeding stage. Many biotic and abiotic factors that are required to be collected to determine the most accurate PMI estimation because these parameters are known to affect larval growth rate which would subsequently affect the PMI estimation [9], [10].

Temperature is a well-known abiotic factor that affects the blowflies growth rate making larvae development to be temperature dependant [11], [12]. Various studies have shown that any fluctuation in temperatures will affect larvae development rate causing some larvae species to take a longer time to grow into adult blowflies [11], [13]. In a study of *Lucilia cuprina* development by [14] at six constant temperatures, it was found that development of larvae accelerates in higher surrounding temperatures while lower temperatures showed a slower development. On top of that, several other researches have also been focusing on the effect of temperature-controlled environment on larval growth rate such as the study by [15], the significant differences in the

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development stages of larvae between the temperatures of 25 Celsius (°C), 27°C, 30°C, 33°C dan 37°C was studied. It was found that there was significant difference of larvae growth at each instar stage between the temperatures studied. This proves that any fluctuation of temperature will affect the development of the larvae.

Another most frequently discussed factor that influences the blowfly growth rate is massing of larval [6]. Larval massing happens when large number of blowflies' larvae aggregates after four to six days of death [16], [17]. This massing of larval also functions as the temperature buffer which allows each larvae to fully develop [16]. According to a study by [3], the adult females blowflies would oviposit around the same time which is at the early stages of decomposition causing the larval to accumulate together during the first instar stage forming a mass. The increasing of mass volume would increase the temperature produced which would eventually affect the larvae growth rate to speed up [3], [6]. Similar to the study in [18] in which the data findings conclude that as the size of the larval massing increases, the heat emission during larval growth also increases. However, the study by [16] discovered that larval massing controlled the temperature production regardless of the ambient temperature.

Different species would have different growth rate and survivorship depending on the larval massing and surrounding temperature. [6], [18]. However, there is a notable gap in research regarding the influences of temperature and larval massing on each other and subsequently on the larvae growth, particularly in locations with a diverse of environmental conditions. While existing research has focuses on the relationship between temperature and larval growth, this study expands on that knowledge by examining how different environmental conditions would impact larvae development. Therefore, this research aims to investigate the influence of these factors and its correlation on larvae growth rate to enhance forensic investigations thus addressing the current research gap by providing valuable insights for forensic entomology in Malaysia.

2. METHODOLOGY

2.1 Study Site

Three location sites were chosen according to the preliminary research that found one of the most common crime cases that involved a dead body were located at concealed locations to avoid detections such as an abandoned building and jungle. Other than that, jungle and outdoor locations were vulnerable to contamination of evidence making it preferable to criminals to dispose bodies. Moreover, these three locations were chosen to represent a wide range of environmental conditions such as the outdoor location provide natural environment, providing a baseline for how larvae grow in open, fluctuating conditions of temperature, humidity. The jungle location would represent habitat that is characterized by dense vegetation, offering insights into how larvae develop in higher humidity, warmer temperatures. As for the abandoned building, it represents low disturbance, more controlled and stable environment, providing a contrast the other two sites.

Laman Lavenda, Nilai Impian, Negeri Sembilan was chosen as the outdoor location (2°50'35.2"N 101°48'02.8"E) for this research. Next, the jungle location selected for this research was at Laman Lili (2°50'39.0"N 101°47'55.3"E) and as for the abandoned building location, the site chosen was a building in UiTM Shah Alam, Selangor (3°03'54.0"N 101°30'18.3"E). M

2.2 Temperature of the Larval Massing in Chicken Carcasses and the Environmental Locations

Three temperature loggers were inserted in three chicken carcasses to record the changes of temperature as the larvae developed. These loggers were set to record the temperature for every 15 minutes throughout the study period. All three chicken carcasses were set up in three different containers at each of the environmental location. The use of three chicken carcasses represents three replications that were incorporated to reduce the error by calculating the average value and to ensure that the results are reliable and not due to random chance. Three replicates were sufficient to detect consistent trends in the data and were chosen as sample size for practicality in terms of time and resource constraints.

The average mass of all three chickens was 1.42 ± 0.04 kilogram (kg), 1.36 ± 0.11 kg and 1.43 ± 0.08 kg in outdoor site, jungle site and abandoned building site respectively. Another temperature logger was set up to monitor and record the surrounding temperature at each of the study sites. It was placed around 50 centimetres (cm) away from the chicken carcasses to record the immediate surrounding temperature of each site. This logger also recorded the surrounding temperature for every 15 minutes throughout the study period.

2.3 Larvae Development and Data Collection

In this study, the chicken carcasses were used as bait to attract adult female blowflies to come and lay eggs there. Upon the first presence of freshly hatched eggs into the first instar larvae which were approximately 2 millimetres (mm) long, 15 samples of larvae were collected to measure their mass, length and width using electronic balance (Publisi) and metric ruler respectively. Then, these larvae samples were put back inside each of their container to continue their growth. These data were collected and recorded for every 6 hours (h) until larvae reached fully grown third instar stage and started to leave the chicken carcasses for pupariation. All the measurements collected were calculated in average values and growth rate graph of mass against time, length against time and width against time were plotted. The significant difference in larvae growth against time were calculated using Analysis of Variance (ANOVA) that was defined with a threshold of $p < 0.05$. A p-value less than 0.05 indicates that the differences obtained are less than a 5% chance occurred by random chance. Next, a total of 15 samples of third instar larvae was transferred and kept inside a small glass bottle containing 70% ethanol for the morphological identification.

2.4 Larvae Processing for Morphological Identification

Larvae sample preserved in 70% ethanol were placed on a flat and clean surface. The posterior segments of the larvae were half-cut using sterilized scalpel blade and then the larvae were immersed in potassium hydroxide (KOH) solution overnight for muscle softening purposes. Next, the larvae were placed on a petri dish and all of its internal body contents were carefully removed by using soft forceps and a modified applicator stick. Later the larvae were transferred into acetic acid for 10 minutes to neutralize the KOH and later soaked into ascending series of 70%, 80%, 90% and 100% ethanol for 30 minutes in each concentration to dehydrate the larvae.

Then, the larvae were transferred into clove oil for 30 minutes followed by xylene for 30 minutes before being mounted on glass slides with a few drops of Canada balsam. Lastly the slides were kept in the incubator at 40°C for three days before larvae identification under a stereomicroscope at 10X and 40X magnification using taxonomy keys such as posterior spiracles, spines, anterior spiracles and the cephalopharyngeal skeleton [19], [20].

3. RESULTS

3.1 Temperature of Larval Massing at Different Environmental Locations

The age of blowfly larvae was estimated according to their body size and the surrounding temperature to which the larvae were exposed to throughout their development. However, understanding the effect of larval massing in larvae development is also an important factor to consider when estimating the PMI based on larvae growth rate. This is because the larval massing temperature is highly dependent to the environmental conditions. Figures 1, 2 and 3 of surrounding temperature and the larval massing temperature against time indicate that in all these figures, there was a direct correlation between the two variables in which as the surrounding temperature increased, the larval massing temperature within the chicken carcass also increased.

3.2 Outdoor Location

The outdoor location was situated on the outside of a residence building with an overhang roof and not completely enclosed by walls. The site was exposed to the natural elements such as sun and wind. The duration of the research for the outdoor location was for 3 days and 10 hours.

Based on Figure 1, both the larval and surrounding temperatures at the outdoor location showed four peaks. In the first three peaks, the temperature fluctuates over a range of higher temperature around 8AM until 5PM, which then it would decrease to a lower range of temperatures starting around 5PM until 8AM. The lower range of surrounding temperature are 26℃ to 27℃ and the higher range of surrounding temperature are 32℃ to 41℃. As for the larval massing temperature, the lower range of temperature are 24℃ to 28℃ and the higher range of temperature are 30℃ to 34℃. However, the larval temperature on the last peak rose inconsistently compared to the first three peaks while, the surrounding temperature repeated the same trend from the first three peaks.

3.3 Jungle Location

The nature of the jungle location can be described as a field with thick, lush vegetation with a humid climate. This jungle location was subjected to more exposure of natural elements like sun, rain, and wind. The duration of the study in jungle location was for 2 days and 18 hours and the temperature loggers were placed at the jungle around 10AM.

Both the larval temperature data and the surrounding temperature of the jungle location showed three peaks as shown in Figure 2. During the first two peaks, temperatures fluctuated in higher range from 8AM to 4PM and lower range from 4PM to 8AM. The higher surrounding temperatures ranging from 39℃ to 41℃, while the lower surrounding temperatures ranging from 24℃ to 27℃. As for the larval massing temperature, the larval temperature fluctuated between 23℃ to 28℃ in the lower range and 35℃ to 36℃ in the higher range. As for the last peak, the surrounding temperature rose and decreased consistently with the first two peaks but the last peak for the larval temperature was unstable as it rose and dropped inconsistently compared to the first two peaks.

3.4 Abandoned Building Location

As for the abandoned building location, the site mimicked a once-used structure that is now deserted and empty. This site was enclosed by walls and doors of the building and hence, was not exposed to the natural elements like the other two location sites. The duration of study in the abandoned building location was for 4 days and 1 hour.

As for Figure 3, both the larval temperature data and the surrounding temperature of the abandoned building location showed five peaks. The first peak for both surrounding and larval temperature were 30.8℃ at 4PM and 26.9℃ at 8PM respectively. Subsequently for the second until fourth peak, the temperatures decreased to a lower range of temperature from 8PM to 8AM and increased to a higher range of temperature at 12PM to 4PM. The lower range of surrounding temperature are 27℃ to 28℃ and the higher range of surrounding temperature are 30℃ to 31℃. As for the larval massing temperature, the lower range of temperature are 26℃ to 27℃ and the higher range of temperature are 29℃ to 31℃. However, the surrounding temperatures of abandoned building rose and decreased consistently for the last peak but the temperature for larval massing at the last peak showed inconsistency as it rose higher than the surrounding temperature compared to the peaks before.

3.5 Larvae Identification

Based on morphological identification such as anterior spiracle, posterior spiracle, dorsal spine and cephalopharyngeal sclerite on larvae samples collected from chicken carcass models from three different environmental locations showed that two blowfly species *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart) were dominant and formed solid larval masses. This finding correlates well with these two blowfly species known to be dominant in Malaysia [21], [22].

3.6 Growth Rate Based on Larvae Length

All three environmental locations showed ascending growth in larvae length as shown in Figure 4. The highest growth in larvae length of the jungle location recorded was 14.71mm at 48 hours whereas the highest growth in larvae length of the outdoor and abandoned building location recorded was 14.38mm and 13.73mm respectively at 60 hours. The significance difference was detected by ANOVA (F_2 = 27.64, p = 0.0043) as stated in Table 1, the p-value calculated was lower than 0.05 concluding that there was a significant difference in the larvae growth rate at each instar stage across three different environmental conditions.

Larvae developed in jungle location has the highest larvae length growth rate in all of the stages consist of the first instar, second instar and third instar compared to the other two sites. The highest growth rate indicates that the shortest time recorded for larvae in jungle site to develop from one instar to the next measured in length. The larvae developed in outdoor location has the second highest larvae length growth rate for

the first instar and second instar whereas the lowest larvae length growth rate for the third instar. As for the abandoned building, the larvae developed has the lowest growth rate for the first instar and second instar while it has second highest larvae length growth rate for the third instar. As for the overall stage from first instar until fully grown third instar, the larvae length in jungle location has the highest growth rate followed by the outdoor location and then the abandoned building.

3.7 Growth Rate Based on Larvae Width

The larvae width showed increasing growth for all three environmental conditions as shown in Figure 5. The longest larvae width of the jungle location recorded was 3mm at 48 hours whereas the longest growth in larvae width of the outdoor and abandoned building location was 3.5mm and 2.8mm respectively at 60 hours. However, it was calculated that there was no statistically significant difference between the larval growth rate based on larvae width at each environmental condition using the statistical analysis of ANOVA as stated in Table 1 because the p-value calculated was 0.45, value higher than 0.05. This is possibly due to the small differences in each of the larvae width measured during each instar at each environmental location.

3.8 Growth Rate Based on Larvae Mass

Figure 6 showed the growth rates based on larvae mass against time in three environmental locations. The larvae mass showed an ascending growth after the 6-hour mark for the jungle location and 12-hour mark for the outdoor location and abandoned location. The highest larvae mass of the jungle location recorded was 59 grams (g) at 48 hours whereas the highest growth in larvae mass of the outdoor and abandoned building location recorded was 83g and 89g respectively at 60 hours.

Based on the statistical analysis of ANOVA ($F_2 = 18.60$, $p = 0.0093$) in Table 1, the statistical analysis showed a significant difference between the larvae growth rate based on larvae mass at each environmental condition in each of the stages as the p-value calculated was lower than 0.05. The larvae in abandoned building location have the highest growth rate based on individual mass from first instar until fully grown third instar followed by the outdoor location and the jungle location. The growth rate based on mass was different compared to the growth rate based on length and width.

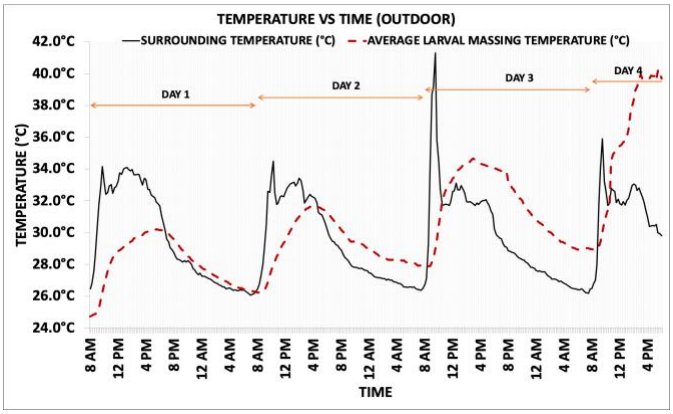

Fig. 1. The surrounding temperature of outdoor site and the temperature of larval massing in chicken carcasses against time.

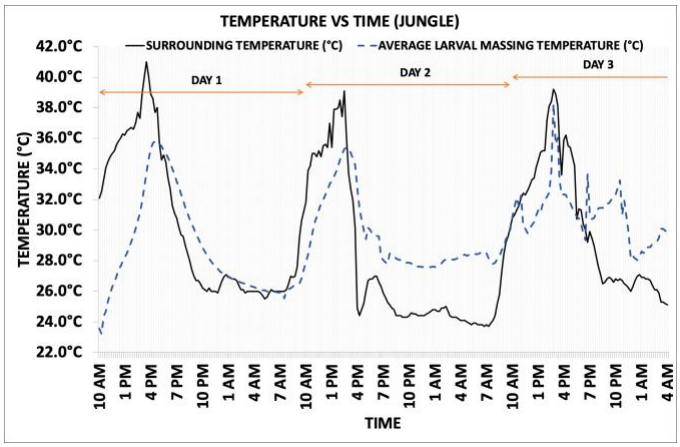

Fig. 2. The surrounding temperature of jungle site and the temperature of larval massing in chicken carcasses against time

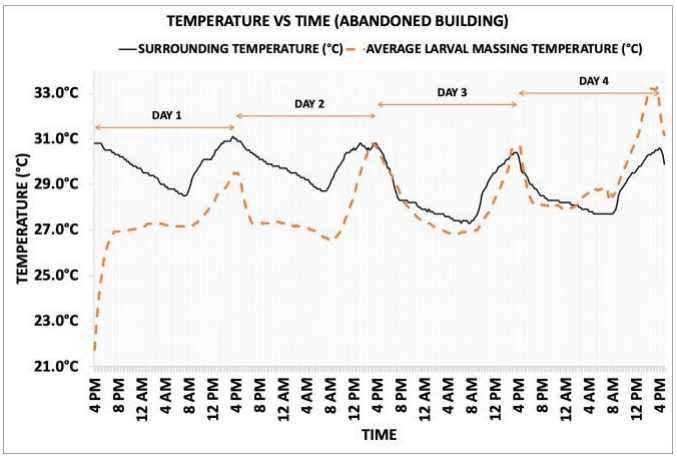

Fig. 3. The surrounding temperature of abandoned building and the temperature of larval massing in chicken carcasses against time.

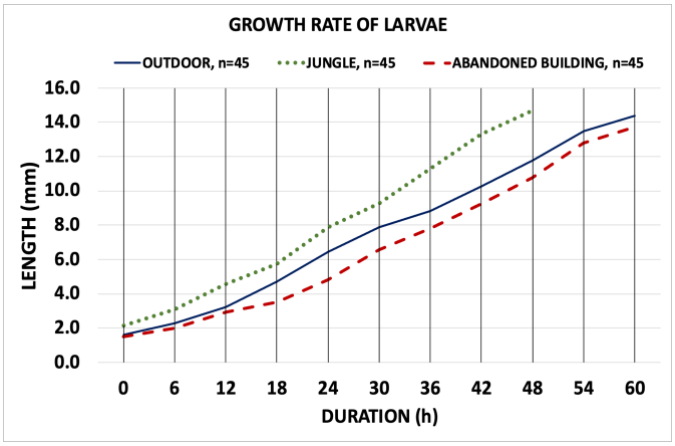

Fig. 4. Growth rates based on larvae length against time in three environmental locations

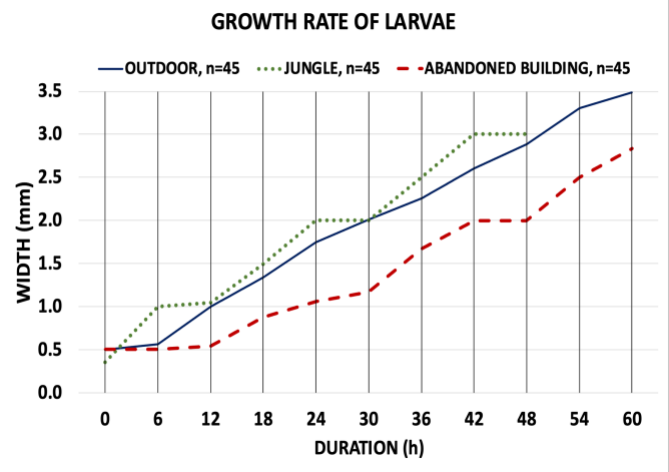

Fig. 5. Growth rates based on larvae width against time in three environmental locations.

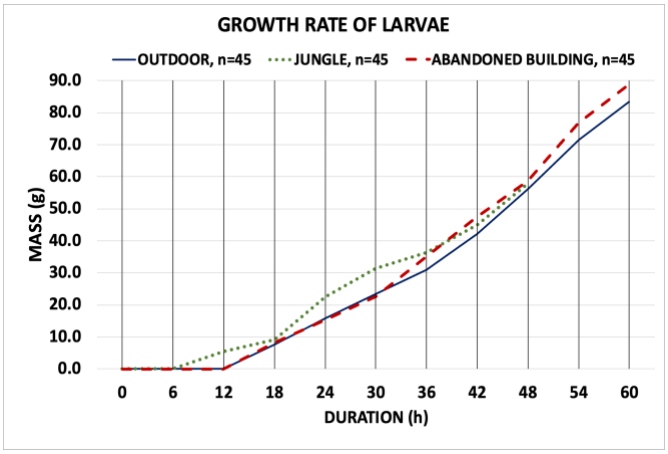

Fig. 6. Growth rates based on larvae mass against time in three environmental locations.

Table 1. Summary of ANOVA of larvae growth rate at each stage.

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Length	0.016011	2	0.00801	28.434	0.004319
Width	0.000295	2	0.00015	0.9866	0.448448
Mass	3.654221	2	1.82711	18.755	0.009286

4. DISCUSSION

4.1 Comparison Between Surrounding Temperature and Larval Massing Temperature at Different Environmental Locations

Understanding the temperature variations of the different environmental locations is essential as it impacted the massing of larval and consequently, affecting its growth rate [16], [17]. In the whole duration of this study, one common trend that can be observed from all three locations which was the similar time frame when the temperature would increase and decrease. Firstly, all three locations showed that the surrounding temperature would start to rise in the morning, reached the highest point of temperature around the afternoon and then, decreased during nighttime after reaching the highest peak. The temperature stayed in the lower range until then next morning in which it will rise again as the trends in Figure 1, 2 and 3 repeats. To summarize, the surrounding temperature is higher in the morning and afternoon meanwhile lower during the night time, this occurrence is due to the presence of sunlight during daytime leading to natural increases of temperature as opposed to nighttime [23].

On the other hand, although the temperature rises and decreases around the same time, the value of the temperatures recorded in the trends of Figure 1, 2 and 3 differs. It can be observed that the surrounding temperature in jungle site showed the biggest difference between the low and high range of temperatures recorded which means that jungle site had warmer temperatures as compared to the other two sites. It is expected for jungle to have a higher temperature due to the nature of its location which was directly exposed to the sun from sunrise to sunset [24], [25]. Even though, the outdoor site was also exposed to the sun from sunset to sunrise, the location was still more shaded due to the overhang roof as opposed to the jungle site that was only shaded by trees. As for the abandoned building, the nature of the location was blocked from sunlight causing lowest range of temperature during daytime recorded.

Figure 1, 2 and 3 revealed the correlation as the surrounding temperature rose, the larval massing temperature within the chicken carcasses also increased. The temperature recorded for larval massing as it rose and decreased almost similar to the temperatures recorded for the surrounding temperature. This occurrence is attributed to the increasing movement of larval masses to enhance their thermal conditions for development, driven by the rising temperatures in their surroundings [26]. Hence, the larval massing temperatures are highly dependent to the environmental conditions especially during the first instar, second instar and the start of the third instar [18], [27].

Many studies have discussed the possible origin of the changes in temperature of larval massing but in the study on heat emitted by larval mass done by [18] found several reasons that affected the amount of heat emission. As shown in Figure 1, 2 and 3, the larval massing temperature in all three environmental locations showed that the third peak reached a higher temperature compared to the second peak. This is because the larvae in second instar in the second peak produced less heat than third instars in the third peak. This finding agreed with the results by [18] that stated the third instar produce more heat than the second instar.

Reference [18] also cited that the amount of available food strongly affected the amount of heat emitted. When the study provided more food for the larvae, the larvae would actively move causing higher heat emission. However, this finding differed from the current study because, even though the same size of chicken used with only a slight difference in mass, the larval mass temperature still differs greatly. It can be observed that the larval massing in jungle location showed the biggest difference as compared to the larval massing on the other two locations.

Based on Figure 1, 2 and 3, one similar trend that can be observed from larval massing in all three different environmental locations was that even though the surrounding temperature remain consistent with the rise and fall of

temperature, the larval massing showed temperature irregularity near the end of the study. This irregularity can be illustrated as the temperature of larval massing was unstable, showing random increases, fluctuations, or decreases due to several reasons. One of the reason why the larval massing temperature is decreasing near the end of the study is because when the larvae reached the fully grown third instar stages, it will start to leave the chicken carcasses for pupariation which will lead to the decrease in the number of larvae present in the chicken carcasses [28], [29]. Another factor that could be the reason for the increase of temperature during the end of the study is the overcrowding of larvae on chicken carcasses. The overcrowding of larvae happened due to odours produced from the continuous decomposition of carcasses causing attraction to blowflies leading to constant oviposition. It is known that overcrowding during larval development leads to a competition for limited food source and overheating [6]. Heat accumulation and development of larval massing study by [30] supported the findings in this study, as it stated that heating increases as larval aggregates, competes for food, or moves away to find another source of food.

4.2 Larvae Growth Rate at Different Environmental Locations

The time taken from the chicken carcasses left on site until pupariation for outdoor, jungle and abandoned building locations was 82 hours (3 days and 10 hours), 66 hours (2 days and 18 hours) and 97 hours (4 days and 1 hour) respectively. Correspondingly, the duration of feeding stages, first instar until fully grown third instar of the larvae in outdoor site, jungle site and abandoned building site was 60 hours (2 days and 12 hours), 48 hours (2 days) and 60 hours (2 days and 12 hours) respectively. In the study by [31], it was stated that in the most optimal condition, the feeding period of larvae will only take as little as two days. The rapid growth of larvae within 3 days in jungle site further proves that the jungle site has the most optimal characteristics for blowfly growth compared to the other two location sites. In addition, another study done by [32] stated that blowfly larvae of *Chrysomya megacephala* (Diptera: Calliphoridae) has an average of 120 hours of larval stage and compared to the findings in this study, the jungle site has an average of only 69 hours of larval stage. This further prove that the growth rate of larvae located in a jungle location has the most rapid and efficient growth rate compared to the other two sites.

The outcome of this study focuses on the growth rate of larvae based on its development characteristics such as length, width and mass. The growth rate of larvae was calculated from the first presence of first instar larvae until fully grown third instar larvae, which is when larvae first leave the chicken carcasses to pupate. Based on Figures 4 and 5, the larvae in jungle location exhibits the highest growth rate based on length and width, followed by larvae growth in outdoor and abandoned building. These results were most likely due to the jungle location having the highest surrounding temperature, the biggest visible larval mass and the highest larval massing temperature. This further indicates that the favourable characteristics of jungle providing optimal conditions in facilitating rapid growth of larvae, supporting the hypothesis that temperature significantly impact larval development [25], [33], [34].

The findings of this study, which show that larvae grow faster in warmer environments, are crucial for PMI estimation in forensic cases. For example, if a body is found in a warm environment, larvae would develop faster and potentially shortening the estimated PMI. Given the influence of environmental conditions, forensic entomologist are required to rear larvae in the laboratory with regard to the environmental conditions found on the scene in order to estimate an accurate PMI of future cases involving bodies found in environments such as jungles or similar habitats [35].

Moreover, the outdoor site showed moderate growth rate based on length and width, while the abandoned building had the lowest larval growth rate. Although the outdoor location was also exposed to sunlight, the place was still more shaded compared to the jungle resulting in cooler temperatures. The overhang roof at the outdoor location also caused lesser ventilation as opposed to the jungle that has an open-air setting which helped more in spreading the odour of carcass. The outdoor site also showed a lower range of temperatures compared to jungle proving that the less exposure to the sunlight caused cooler temperatures [36]. Reference [19] focused on larvae development in a cave and a forest, the study found that the larvae growth in surrounding forest had higher development rate than larval growth in Mount Kapur cave due to several reasons such as fluctuations in environmental condition (ambient temperatures and rain) and limited presence of lights in the cave supporting the findings in this study.

As for the abandoned building, this location has even more confined space that almost obstructs the emission of odour, restricting the accessibility of blowflies and resulting in a smaller number of oviposited eggs along with an even longer time for the eggs to reached third instar stages. In a study on the insect colonisation on carcasses that were confined inside a trunk by [37], showed that the trunk caused a delay of three days from insect colonization because even though the carcass has started to decompose, the smell produced was prevented from spreading which reduced the number of blowflies attracted to it. Moreover, blowflies that had smelt the odour cannot oviposit on the carcass due to being blocked by the trunk. These findings supported the result in this study because the abandoned building also had the longest time recorded for oviposition from the time the chicken were left on the location and the first presence of eggs on chicken.

The larvae growth rate in terms of mass showed a huge difference compared to the larvae growth rate based on length and width. The larvae in abandoned building exhibited the highest growth rate in terms of mass, followed by the larvae in outdoor and jungle locations as opposed to the findings of larvae growth rate based on length and width. The presence of the highest volume of larvae in jungle and largest larval massing on chicken had caused a higher competition for food resulting in slower growth in mass for each larva compared to the other two study sites. Meanwhile for abandoned building, it has the lowest volume of larvae and the smallest larval massing on chicken causing the least competition for food source allowing each of the larvae to grow in mass. Furthermore, the study on larval growth rate by [34], revealed that higher temperatures accelerated the feeding stages, which affected the larval metabolism, causing larval to gain less mass and result in smaller individuals. This finding is agreeable to the current study as the larval in jungle was the fastest to grow in length but smallest in individual mass due to the inability to properly gain mass compared to the larvae in

abandoned building. Hence, calculating PMI depending on larvae growth based on mass alone may not be sufficient for accurate estimation and potentially leading to investigative errors in future cases.

The jungle emerged as the most optimal environment for larval development, followed by the outdoor site while the abandoned building exhibited significant shortcomings. These highlighted that environmental conditions highly affect the growth rates of larvae and plays a crucial role in PMI estimation.

5. CONCLUSION

In conclusion the development of larval massing was directly correlated with the location and its surrounding temperature in which the jungle with the highest surrounding temperature produced the biggest larval massing which accelerates the growth rates. The comparative analysis also revealed significant variations in growth rates based on larval length, width and mass with the jungle site showed the highest growth rate in terms of larvae length and width but lowest based on larvae mass due to aforementioned discussions.

Overall, environmental conditions significantly affected the growth rate of larvae. This study provides valuable data that can serve as a reference for future forensic investigations involving crime cases in environments with similar tropical climates to the jungle site. Moreover, it also emphasizes the importance of examining the environmental conditions to estimate an accurate PMI particularly in forensic investigations of crime cases involving deceased bodies in complex and varied environmental conditions.

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