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Accelerating *Chrysomya Megacephala* Maggot Growth for Forensic Entomology Cases

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ABSTRAK

Entomologi forensik dapat ditakrifkan sebagai pengetahuan mengenai serangga yang berkait rapat dengan bangkai terutamanya manusia, dengan tujuan ia dapat digunakan dalam penentuan jangka masa pascakematian. Jangka masa pascakematian (PMI) dapat ditentukan dengan mengambil kira spesies serangga dan peringkat perkembangan setiap spesies tersebut. Oleh kerana penentuan jenis spesies memerlukan serangga berkembang ke peringkat dewasa dan ini mengambil masa yang lama, maka objektif utama kajian ini adalah untuk mengoptimumkan suhu dan kelembapan terhadap perkembangan serangga dengan menggunakan larva Chrysomya megacephala sebagai spesimen. Larva C. megacephala dipindahkan ke dalam bekas khas, kemudiannya dimasukkan ke dalam pengeram yang telah dilaraskan suhunya kepada 27, 30, 33, 36 dan 39°C. Selepas menentukan suhu optimum perkembangan larva, aras kelembapan relatif ditentukan. Ini dilakukan dengan menentukan tempoh masa yang diperlukan untuk mengembangkan telur C. megacephala hingga ke peringkat dewasa. Untuk itu aras kelembapan relatif dalam pengeram tersebut dilaraskan kepada 54.2, 57.6, 76.0 dan 67.5% (kawalan). Peringkat perkembangan C. megacephala direkodkan. Hasil yang diperolehi menunjukkan perkembangan C. megacephala lebih pantas pada suhu 33°C berbanding suhu-suhu lain yang digunakan. Aras kelembapan relatif yang optimum juga telah dikenal pasti iaitu pada 76.0%. Dengan menggunakan kedua-dua data didapati keseluruhan peringkat perkembangan C. megacephala iaitu daripada peringkat telur hingga dewasa dapat dipendekkan daripada 8 hingga 9 hari kepada 5 hari.

Kata kunci: Chrysomya megacephala, suhu, kelembapan

ABSTRACT

Forensic entomology is defined as knowledge about insect and its relationship with a decomposed human body. With this knowledge, post-mortem interval

(PMI) can be estimated. PMI can be determined by taking into consideration the insect species and the developmental stage of the insects. Identification of the insect species requires the insect to develop into adulthood. Since this will take a relatively long time, the objectives of this study were to optimize temperature and humidity for the growth of Chrysomya megacephala larvae to adults. C. megacephala larvae were transferred into a rearing container and put inside a special incubator with temperature adjusted to 27, 30, 33, 36 and 39°C separately. Once optimum temperature for larvae growth was determined, optimum relative humidity was determined then for the length of time taken for C. megacephala larvae to develop into adults. To achieve this, the larvae of C. megacephala were incubated in a special incubator and the relative humidity set at 54.2, 57.6, 76.0 and 67.5% (control) separately. The developmental stages of C. megacephala for both temperatures and humidity levels were recorded accordingly. Results obtained indicated that C. megacephala developmental stages grew much faster in 33°C than other temperatures. The optimum relative humidity level for the species was 76.0%. By utilizing the appropriate temperature and relative humidity the development of C. megacephala, from eggs to adults could be reduced from 8 to 9 days to 5 days.

Key words: Chrysomya megacephala, temperature, humidity

INTRODUCTION

Forensic entomology or medico-legal entomology can be defined as the study of insects associated with a human corpse in an effort to determine elapsed time since death (Catts & Goff 1992; Hall 2001; Zehner et al. 2004). Scope of medico-legal forensic entomology not only includes arthropod involvement in criminal events such as murder, suicide and rape, but also physical abuse (Gill 2005). When an unexpected death occurs without any witness or superficial evidence, estimation time of the death becomes a major concern.

The major contribution normally made by forensic entomologists in death investigations is the estimation of time between death and corpse discovery. This is referred as post-mortem interval (PMI) (Amendt et al. 2006). The PMI estimate is based on the time needed for the fly larvae to develop to the oldest growth stage after they are collected from a corpse (Zoe & Martin 2003). Based on the age and species of larvae collected from a corpse, the PMI can be determined easily (Catts & Goff 1992). Additional data that can also be obtained include whether the body has been moved to a second site after death or the body has been disturbed at some time either by animals or by the killer returning to the scene of the crime.

Larvae of carrion flies, especially blowflies are by far the most common type of insect evidence collected during a death investigation (Catts & Goff 1992;

Zehner et al. 2004). These blowflies are the first to arrive at the dead body due to the odour of fresh blood, especially blood from opened wounds and body fluid (Anderson et al. 2000). A study conducted by Lee et al. (2004) noted that among of the large numbers of fly species found on human cadavers, *Chrysomya* sps. were the most dominant of them all. As a result, their larvae are usually collected to be reared to adults and some to be preserved in order to determine the PMI of the victim (Chen et al. 2004; Anderson 2004).

Flies of the species *Chrysomya megacephala* are large with size over 9.5 mm long. The adults are bright metallic green with black margins on the second and third abdominal segments, and have large red eyes almost touching each other. The face below the eyes is usually yellow to orange (Siriwattanarungsee et al. 2005). *C. megacephala* has a life cycle of 4 growth stages, which are egg, larva, pupa and adult. The period from egg to adult usually takes 8 to 9 days. A female fly can lay from 150 to 300 eggs in each batch and the larva and pupa stages each usually last about 4 days (Sukontason et al. 2003).

The succession of arthropods development is mostly affected and influenced by temperature and humidity (Grassberger & Reiter 2001; Ames & Turner 2003). In warmer temperature and high moisture condition, insects have been known to grow faster. The opposite conditions have also been noted to retard insect growth significantly (Anderson et al. 2000). Apart from the effect of surrounding conditions, studies have also noted that heat created by normal putrefaction processes of the body and larva mass has been known to influence the overall rate of insect development (Goff 1991). Based on this, it always has been suggested that when taking larva samples from decomposed body, the history of temperature and humidity should be noted and taken into consideration when determining the estimated time of death (Forbes et al. 2005).

To determine estimated time of death, forensic entomologists usually collect larvae directly from a decomposing body. The larvae then are divided into two groups: one group is preserved to determine larva stage while the second group is grown to determine the species. Only by obtaining these two data can estimated time of death be reliable. Since growing larvae to adults in the laboratory usually take 6 to 8 days, reports on the estimated time of death are usually late. This will cause delays in investigation and reduce the likelihood of solving the crime (Amorim & Ribeiro 2001).

Despite knowing temperature and humidity play a major part in insect development, the use of this knowledge in forensic entomology has been overlooked constantly. For this reason, a study was conducted to determine the optimum temperature and humidity for growing maggots of *C. megacephala* in a laboratory environment.

MATERIALS AND METHODS

Chrysomya megacephala (Diptera: *Calliphoridae*) species was obtained from the insectarium at the Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur.

1. Effect of temperature on the growth period of *C. megacephala* from eggs to adults.

C. megacephala eggs from the room temperature were collected and transferred into rearing containers. All of the larvae were incubated inside a modified incubator (Jeio Tech, Korea) at different constant temperatures (27, 30, 33, 36 and 39°C) and a fixed humidity level of 67.5±7.5%. The hatched larvae were given fish (*Decapterus punctatus*) as staple food. At each level of temperature, eggs, larvae, pupae and adult flies were observed daily. The length of the larvae and pupae were also measured.

2. Effect of humidity on the growth period of *C. megacephala* from eggs to adults.

When the optimum temperature was identified, i.e. 33°C, effect of humidity on the fly cycle development was studied. In this regard, *C. megacephala* eggs from the room temperature were collected and transferred into rearing container. All of the larvae were incubated inside the modified incubator (Jeio Tech, Korea) with a constant temperature of 33°C (optimum temperature with no defect for each stage). The hatched larvae were given fish (*Decapterus punctatus*) as staple food. Humidity inside the modified incubator was calibrated to different levels of relative humidity, i.e. 54.2, 57.6, 76.0 and 67.5%. The humidity inside the incubator was confirmed using a relative humidity meter (Quest Technologies, USA). The length of the larvae and pupae were measured daily.

RESULTS

Table 1 shows the mean period taken for the larvae to reach adulthood at 27°C was 8.5 days. Raising the temperature by 3 degrees reduced this period by 2 days. A further increase of 3 degrees shortens the period by about another 2 days to 5 days. When the temperature was increased to 36 and 39°C, the mean period taken for all the larvae to develop into adults was not significantly different from the period at 33°C. Although some pupae developed into adults as early as day 2 at 39°C, they were not healthy and died.

Fig. 1 shows at a relative humidity of 54.2%, the larvae took the longest time to reach their maximum lengths, i.e. 39 hours. In addition, the mean length reached was the longest among all the humidity levels investigated (Table 2). At a higher

TABLE 1. Total numbers of *Chrysomya megacephala* at different levels of temperature

Temperature (°C)	Stages	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
	Larva	40, 40, 40	40, 40, 40	22, 30, 0						
27	Pupa			0, 10, 40	18, 40, 40	40, 40, 40	40, 40, 40	11, 10, 40	10, 0, 0	
	Adult							29, 30, 0	30, 40, 40	40, 40, 40
	Larva	40, 40, 40								
30	Pupa		40, 40, 40	40, 40, 40	40, 40, 40	1, 40, 40	40, 0, 0			
	Adult					39, 0, 0	0, 40, 40	40, 40, 40		
	Larva	40, 40, 40								
33	Pupa		40, 40, 40	40, 40, 40	0, 40, 12					
	Adult				40, 0, 28	40, 40, 40				
	Larva	40, 40, 40	0, 0, 2							
36	Pupa		40, 40, 38	40, 40, 40	40, 0, 0					
	Adult				0, 40, 40	40, 40, 40				
	Larva	40, 40, 40								
39	Pupa		38, 40, 40	38, 40, 40	38, 30, 0					
	Adult		2, 0, 0	2, 0, 0	2, 10, 40	40, 40, 40				

relative humidity of 57.6%, maximum length was reached the earliest at 21 hours and the mean length was the shortest. At 67.5% and 76.0% relative humidity, the larvae reached maximum lengths at 36 hours. Both have similar lengths.

TABLE 2. Effect of relative humidity on time for maggots to complete growth to adults

Relative humidity (%)	Time (hrs)
54.2	127 ± 3
57.6	102 ± 3
67.5	124 ± 3
76.0	76.5 ± 3.9

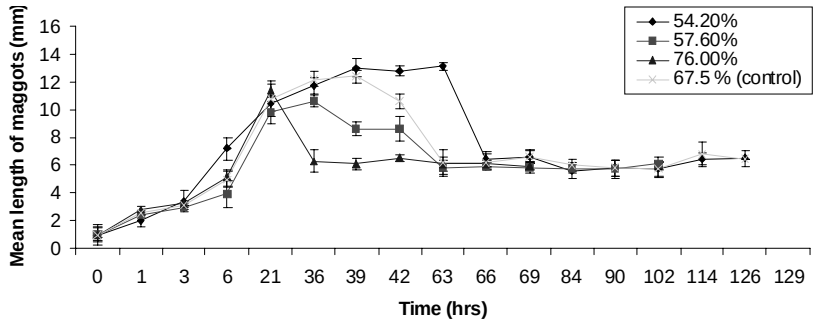

FIGURE 1. Comparison of mean maggot length of *C. megacephala* at different time following larviposition at 4 different levels of humidity. Each vertical bar represents the mean ± 1SD.

DISCUSSION

The results demonstrated that temperature plays a major factor in influencing and controlling the insects' activity, oviposition rate and as well as their overall development as was reported previously (Marinho et al. 2006). In this study the lowest temperature used was 27ºC. At this temperature, development of the insect was the slowest compared to other temperature. Similar result was also noted by Payne et al. (1965) and Smith (1986). On an average, it was found that it took the insects 9.0 days to transform themselves from eggs into adult flies. At 30ºC the maggots' growth has increased greatly and took only 6.3 days to complete a full life cycle. Three degrees higher, i.e. 33ºC, the larvae grew even faster. On

average, all 3 batches of 40 maggots had successfully transformed themselves into adults in 4.67 days. At the two temperatures above 33ºC, i.e. 36ºC and 39ºC, larva growth were accelerated even more and in this regard it only took 4.33 & 4.66 days to transform themselves into adult flies. Despite the accelerated growth, mortality of the larva also increased greatly. For example, at 39ºC, larva growth was found to be retarded or died at some points in their life cycle.

The increase in mortality at 36ºC and 39ºC was believed to be due to the effect of larva mass modifying local temperate. Since the temperature probe measured circulating temperature within the modified incubator, the actual temperature present within the mass of larvae on the fish was likely to be much higher. Past studies have indicated that localized temperature could even reach 5ºC to 20ºC higher than the local air and ground temperature (Goodbrod & Goff 1990; Greenberg 1991). Taking this into consideration, the true temperature within the larva could have reached as high as 59ºC; a temperature unsuitable for protein functions. Since this study took more than four days to complete, prolonged raised temperature likely would have cause protein denaturising, metabolism collapse and eventually death of the growing larvae. Based on the overall result, 33ºC was considered the optimum temperature for larva growth as it hastened larva development but still allowed them to grow to adults.

When optimum temperature was fixed and relative humidity rose, larvae grew much faster. At the lowest relative humidity of 54.2%, the larvae took a long period of 127±3 hours to develop. But when the relative humidity was increased to 57.6%, the larvae grew faster and took 102±3 hours to complete the transformation. Results also showed that the larvae took even shorter time, i.e. 76.5±3.9 hours to transform into adult flies at the highest relative humidity studied (76.0%). At this level of relative humidity, the larvae grew faster because the amount of moisture was considered sufficiently enough for larva growth. It is hypothesized that when larvae received enough moisture, decomposition of the maggots' food (*Decapterus punctatus*) were much faster resulting in a larger supply of simple proteins, fats and carbohydrates. Availability of the simple structural food had then allowed the larvae to consume the food much faster and easier thus allowing a much more rapid growth. Overall there was a significant change in larva growth at the 4 different levels of relative humidity studied.

CONCLUSION

The results of this study showed that the successful development of *C. megacephala* was closely dependent on temperature and humidity of the surroundings. The optimum temperature and humidity for *C. megacephala* growth is 33ºC at 76.0% relative humidity. The conclusion of this study has major implications in forensic science. Firstly by using this data for larva rearing in forensic entomology cases, estimated time of death could be obtained in half the

time compare with current rearing techniques. Secondly, slight variation in temperature and humidity will influence larva growth and indirectly influence estimation of time of death. Thus to ensure a more accurate estimation of time of death, history of surrounding temperature and humidity in the location where a body was found must be taken into consideration.

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