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RESEARCH ARTICLE

New developmental data of *Chrysomya megacephala* (Diptera: Calliphoridae) in tropical temperatures and its implications in forensic entomology

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ABSTRACT

The estimation of the postmortem interval (PMI) is an essential information in death investigations. It is necessary to know the developmental data of the most important necrophagous insect species in every geographical area. *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) is one of the most common species associated with human body decomposition, especially in the tropics, so a precise knowledge of its life cycle is crucial. However, despite its ubiquity, developmental data in a range of tropical temperatures is scarce. For this reason, the aim of this study is to provide the developmental data of the blowfly, *C. megacephala,* in a range of tropical temperatures and to apply these data in forensic entomology. Four temperatures were examined (i.e., 27.0°C 29.5°C, 32.0°C and 34.5°C) and the time of developments from egg to adult were recorded. To build the growth curves, five larvae per day were measured with a digital caliper. Accumulated degree-days were calculated and the isomorphen diagram for this species was plotted. As we predicted, the results showed that the life cycle of this species was affected by the increasing temperature. The implications of these new data for determining the minimum PMI in forensic entomology were discussed.

Keywords: Forensic entomology; growth curves; accumulated degree-days; isomorphen diagram; *Chrysomya megacephala*.

INTRODUCTION

Arthropods associated with corpses are studied by forensic entomologists to determine the time since death or also known as postmortem interval (PMI). The necrophagous Diptera species are important tools in the medico-legal field of forensic entomology, they are being used in death investigations since they allow estimating the precise colonization time of the corpse (Greenberg, 1991; Catts & Goff, 1992). Diptera are presence in a wide variety of habitats and are the predominant group of insects associated with the early stages of decomposition of organic matter of carrion origin (Catts & Goff, 1992), and "forensic indicators" are those whose larvae feed and develop exclusively on the carcass. As such, the most relevant families are Calliphoridae, Sarcophagidae, Muscidae, Fanniidae, Phoridae, and Piophilidae (Byrd & Castner, 2001). The fly species distribution may differ depending on the type of habitat, the trophic source, the environmental conditions, and the time of year, among other factors (Goff, 2000). In addition, cosmopolitan fly species may present with variations in their biology, such as different behaviours depending on their biogeographic origin, or differences in their developmental duration due to climatic conditions (Martínez-Sánchez *et al*., 2007). However, necrophagous fly developmental data is mostly limited to conditions of the Palearctic region, making necessary data from other climatic regions, i.e., tropical to improve the calculation of the time of the death.

The estimation of the PMI in death investigations consists of the maximum and minimum probable times from death to the discovery of the corpse (Catts, 1992). There are two fundamental models for its calculation based on entomological evidence and depending on the elapsed time; both methods can be used together or separately. The first method is the analysis of the succession waves of insects found on a body, it is useful when the body is in advanced stages of decomposition, and although it depends on many factors, one of the most important parameters is the biogeographic area and the exact location where the body is found (Byrd & Castner, 2001; Gennard, 2007). For estimating the time of the death in the early decay stage, a second method is used based on the preimaginal development of the species found on the corpse. In this method, it is important that both correct identification of the species, as well as the relationship between the stages of development and the temperature in the death scene where the samples have been collected (Marchenko, 2001). The larval stage is the most useful evidence for PMI estimation and the most applied method to predict the age of an insect is the accumulated degree-days or ADD (Catts & Haskell, 1990). This method is derived from growth curves obtained under similar climatic and geographical conditions and is used until

the larvae reach the prepupal stage (Greenberg, 1991). Recently, other technique to analyse the PMI was improved: the isomorphen diagram that is useful in fly pupae and prepupae stage (Grassberger & Reiter, 2001, 2002).

Despite having all these methods, previous works have been concentrated in applied research to facilitate the estimation of PMI with a common forensic indicator in the tropics such as *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) (Badenhorst & Villet, 2018). The blowfly *C. megacephala*, commonly named as oriental latrine fly (Zumpt, 1965), is a species of widespread distribution in tropical and temperate climates (Badenhorst & Villet, 2018). Its life cycle has been well studied in America (Aguirre-Gil *et al*., 2015; Arias-Di Donato & Liria, 2016), in Africa (Prins, 1982; Gabre *et al*., 2005; Kamel *et al*., 2016) and in Asia (Wells & Kurahashi, 1994; Bharti *et al*., 2007; Ahmad Firdaus *et al*., 2009; Hu *et al.*, 2010). It has medical importance because it is one of the most dominant Diptera species representing forensic entomofauna in Malaysia (Nazni *et al*., 2015). Moreover, this blowfly appears in the early stages of decomposition; and for this reason, it has been used to estimate the postmortem interval in forensic cases around the world (Goff, 1992; Lee *et al*., 2004; Oliveira-Costa & de Mello-Patiu, 2004; Sukontason *et al*., 2007).

Chrysomya megacephala is a dominant species of insect community of corpses in Malaysia (Nazni *et al*., 2015); however, information of its developmental duration at a range of common tropical temperatures is lacking (Ismail *et al*., 2007; Ahmad Firdaus *et al*., 2009). The objective of this study is to describe the life cycle for this blowfly species at different temperatures under a tropical condition. To achieve this aim, the development cycle of this species from Malaysia under four different temperatures was studied; and accumulated degree-days, isomorphen diagram, and larvae growth curves were generated. Although the life cycle of this species is well known in other parts of the world, this work represents a new forensic data for *C. megacephala* from Malaysia in a range of tropical temperature. The implications of these results were also discussed.

MATERIALS AND METHODS

A permanent colony of *C. megacephala* was established using pupae from other *C. megacephala* colony at the parasitology laboratory of the Institute for Medical Molecular Biotechnology (IMMB), Universiti Teknologi MARA, Malaysia. The colony was maintained in a colony cage of 40×30×40cm with a controlled environment [23°–25°C, 60–70% RH, 12:12 (L:D)]. Water and a mixture of sugar and milk powder in a proportion of 2:1 was offered *ad libitum*. For the fly oviposition and for rearing the larvae, beef liver was used.

To study the life cycle, the eggs obtained from the colony were introduced in an incubator chamber at the designated temperatures. Four temperatures were chosen for this study (i.e., 27.0°C 29.5°C, 32.0°C and 34.5°C) with 60–70% RH and a photoperiod of 12:12 (L:D) and a minimum of three replicates were done per each temperature. When the larvae hatched (±12 h), a group of 100 first instar larvae were transferred into a 100 g of fresh beef liver in a container covered with a mesh to avoid the larvae from escaping. Five largest larvae from each replicate were collected daily, boiled in hot water at 80–90°C for 5 minutes and preserved in 70% ethanol. After three days, the mesh was removed, and the container was relocated into a bigger container with sawdust at the bottom to facilitate the pupation of the larvae. The first twenty pupae (n=20) of each replicate were isolated in Eppendorf tube until the adult emergence, after that, the emerged adults were sexed to determine the sex ratio. The ambient temperature and humidity were recorded with a data logger during the whole experiment period.

For practical use in forensic investigations, growth curves and isomorphen diagram were plotted and accumulated degrees-days (ADD) were calculated for each development stage. To build larval growth curves, their lengths were measured using a digital vernier (0.01 mm) and the change of the instar were differentiated by examining the posterior spiracles under a stereo microscope (Olympus SZX7, Japan). The mean of the maximum length was plotted against the time for each temperature regime. The date of each stage of development was recorded and the minimal developmental time for larval and pupal stages was determined when the first five individuals in each replicate had changed to the next stage. All plots were recorded as the average of developmental durations. To calculate accumulated degree-days (ADD) for larval and total preimaginal development, the following formula was used: ADD = y ($D-D_0$), where y is the development time in days, D is the temperature of rearing and D_0 is the minimum development threshold temperature. Lastly, the isomorphen diagram was constructed using the developmental time from oviposition to adult emergence against the temperature, where identical morphological stages were indicated by the areas between the lines and the lines represent morphological changes.

The statistical analysis was carried out using the SPSS software version 27. Normality was checked using the Kolmogorov–Smirnov test and homogeneity of variance of data was also tested. The parametric data were analysed using the one-way analysis of variance (ANOVA, F) and the t-test (t), followed by a post hoc test (Holm-Sidak method). For the non-parametric independent samples, the Kruskal-Wallis (H) test was carried out to compare development between temperatures. In the cases where significant differences were found, post hoc pairwise comparison procedures were employed using Dunn's test or the Tukey test (U).

RESULTS

Duration and survival of developmental stages

The duration of each developmental stage under four temperature regimes is presented herein (Table 1, Figure 1 – isomorphen diagram). The increase of temperature influenced the duration of developmental stages, which decreased slowly, especially in larval stage; however, the duration for pupal stage decreased faster than larval stage. The total development (from egg to adult) ranged from 8.51±0.12 days at 27°C to 7.52±0.13 days at 32.0°C. There were significant differences in larval duration ($H=10.968$, $df=3$, $p=0.012$), pupal duration ($H=165.811$, $df= 3$, $p=0.000$) and total development ($H= 154.485$, $df=3$, $p=0.000$). The survival rate varied between 72.73% at 34.5°C to 85% at 27°C, being significant difference at 29.5°C with 48.89% of survival rate ($p<0.05$).

Postmortem interval estimation methods

Different methods to estimate the PMI have been developed namely growth curves, ADD and isomorphen diagram. First, the growth curves of *C. megacephala* larvae were plotted in Figure 2 and the larval growth at all temperatures showed sigmoidal curves. The maximum larval length was reached comparatively faster at 34.5°C on 3rd day; meanwhile, at 27°C and 29.5°C, it was attained on 4th day. In other words, the growth rate of the larvae was faster when temperatures increased, appearing as the longest larva the day before the prepupa stage. After this moment, their lengths decreased slightly when larvae entered the post-feeding stage and abandoned the food to pupate. The maximum larval length reached by the larvae showed significant differences between all the days at different temperatures ($p<0.05$). The largest larvae were obtained at 29.5°C (16.47±0.86 mm) on day 4, and the largest individual of *C. megacephala* larvae was 18.00 mm at the same temperature (Figure 2).

Regarding the ADD, the rate of larval development varied between 61.93 and 92.24 DD for larval stage and between 65.46 and 73.67 DD for pupal stage. For the total development, the degree-days ranged from 132.73 to 174.75 DD (Table 1). These data were estimated using 11.57°C (for larvae), 10.31°C (for pupae) and 11.41°C (for total) following the developmental threshold temperature (D_0)

Table 1. Duration of each developmental stages (average ± SD), and accumulated degree-days for each temperature regime of *Chrysomya megacephala*

Stage	Minimum duration (days)			
	27.0°C	29.5°C	32.0°C	34.5°C
Egg	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
Larva	4.01 ± 0.12[a]	4.14 ± 0.36[b]	4.00 ± 0.00[a]	4.02 ± 0.26[a]
Pupa	4.00 ± 0.00[a]	3.61 ± 0.57[a]	3.02 ± 0.13[b]	3.05 ± 0.30[b]
Egg to adult	8.51 ± 0.12[a]	8.25 ± 0.52[a]	7.52 ± 0.13[b]	7.57 ± 0.25[b]
Survival rate[+]	85.00%[a]	48.89%[b]	74.43%[a]	72.73%[a]
ADD larvae[*]	61.93	74.28	81.72	92.24
ADD pupae[*]	66.76	69.22	65.46	73.67
ADD total[*]	132.73	149.24	154.79	174.75

[abc] Different letters between temperature (within rows) indicate significant differences $p<0.05$.
[*] For the minimum temperature threshold (D_0), we used 11.57°C (larvae), 10.31°C (pupae) and 11.41°C (total) as calculated by Zhang *et al.* (2018).
[+] Emerged adults out of 20 isolated pupae.

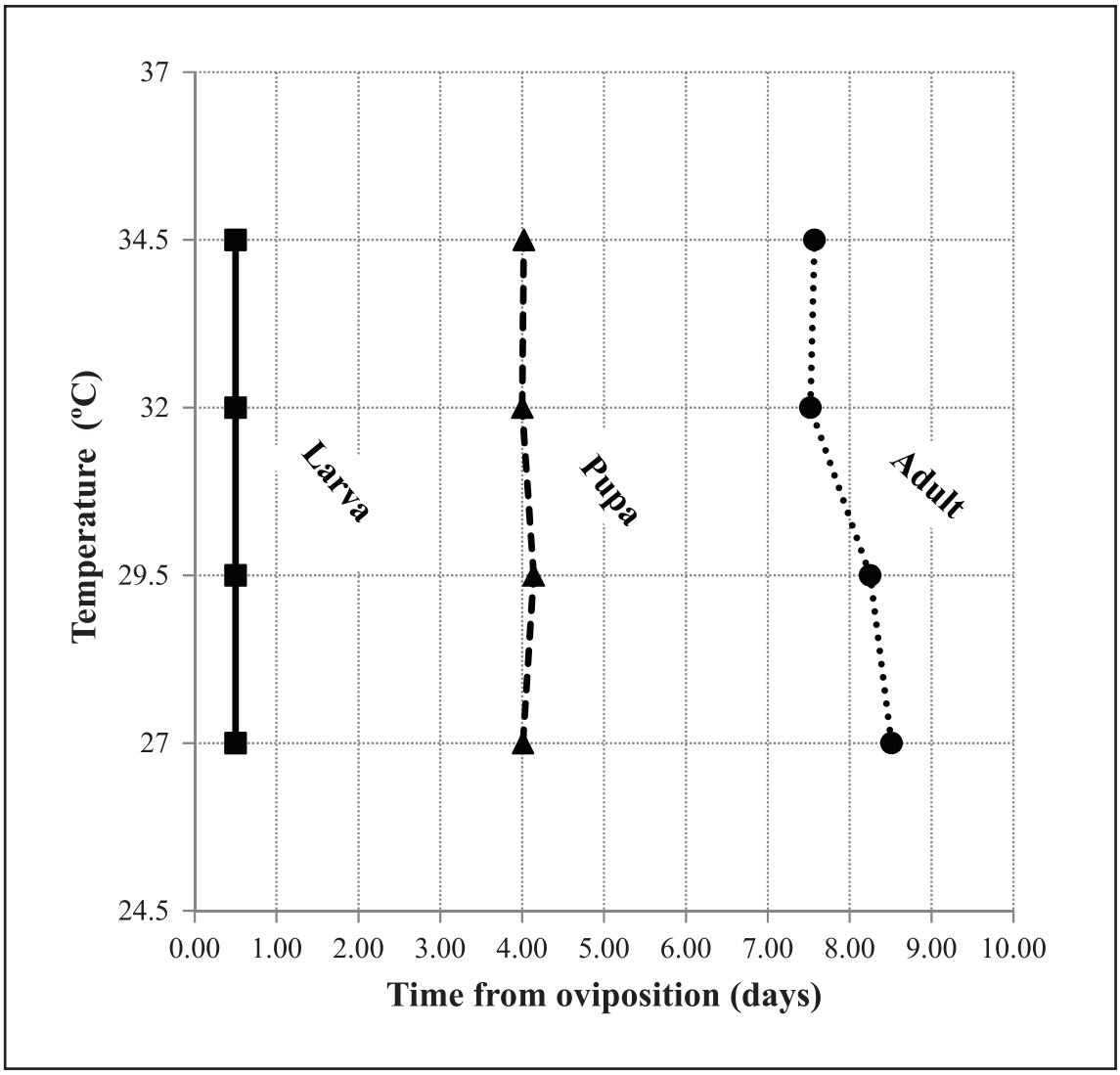

Figure 1. Isomorphen-diagram for *Chrysomya megacephala* showing the duration of developmental stages from oviposition to adult eclosion, at 27.0, 29.5, 32.0 and 34.5°C. ■, egg hatching; ▲, pupation; ●, adult emergence.

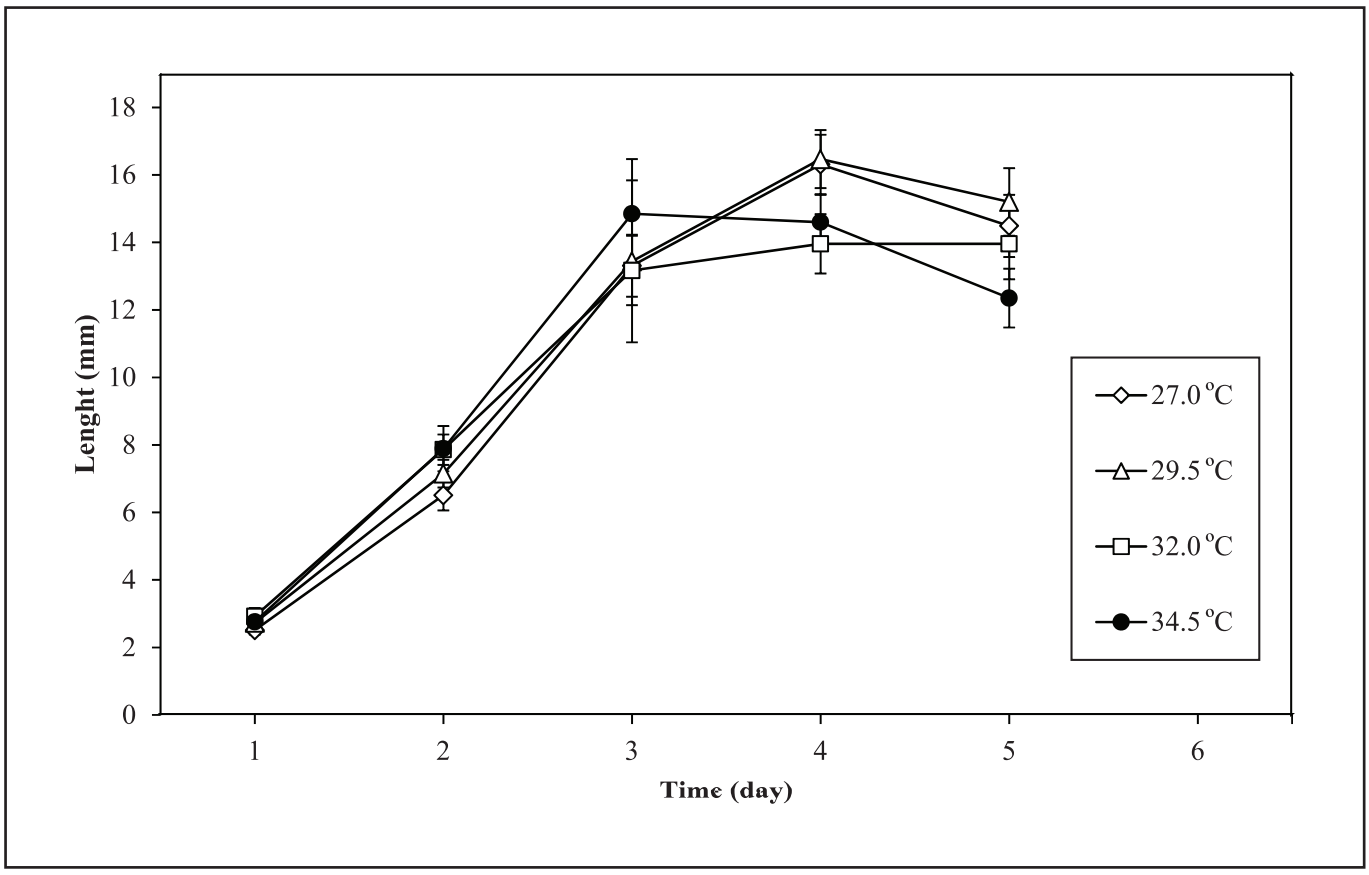

Figure 2. The mean (±SD) length of *Chrysomya megacephala* larvae, measured at 24-h intervals for each temperature regime (n=20).

calculated by Zhang *et al*. (2018). In the present study, we did not calculate the D_0 due to the impossibility to complete the regression analysis of our data.

DISCUSSION

The life cycle of *C. megacephala* is well-known in different regions of the world such as America (Aguirre-Gil *et al*., 2015; Arias-Di Donato & Liria, 2016), Africa (Prins, 1982; Gabre *et al*., 2005; Kamel *et al*., 2016) and in Asia (Wells & Kurahashi, 1994; Bharti *et al*., 2007; Ahmad Firdaus *et al*., 2009; Hu *et al*., 2010). However, despite being one of the most common forensic indicator species, there is a lack of life cycle studies in Malaysia especially for the use in postmortem interval estimation (Ismail *et al*., 2007; Ahmad Firdaus *et al*., 2009; Kumara *et al*., 2010). The overall aim of this study was to determine its life cycle at four different constant temperatures (i.e., 27°C, 29.5°C, 32°C, 34.5°C) and its application to estimate the PMI through three different methods (i.e., growth curves, ADD and isomorphen diagram).

Kumara *et al*. (2010) published a case report where they found *C. megacephala* larvae in Penang, Malaysia; and they studied its life cycle at 29°C±2°C to estimate the PMI after the corpse being placed in a morgue cooler for 12 days. The complete life cycle in Kumara *et al*. (2010) was 9.0±0.5 days, which was a little longer than in our results at the 29.5°C (8.25±0.52 days); however, their study was under a fluctuating indoor temperature (±2°C) while our study was conducted in an incubator with less variations (±0.5°C). On the other hand, the study by Ismail *et al*. (2007), the life cycle of *C. megacephala* at 27°C in their study was 8.5 days, it was the same as in our results at 27.0°C (8.51±0.12 days), but shorter than the results reported by Ahmad Firdaus *et al*. (2009) (9.15 days at 27°C). However, in the case of higher temperatures, Ismail *et al*. (2007) demonstrated a faster developmental duration (6.3 days at 30°C and 4.57 days at 33°C) than ours (8.25±0.52 at 29.5°C and 7.52±0.13 days at 32°C), and the same tendence was found by Ahmad Firdaus *et al*. (2009) (6.76 days at 33°C). Comparing our results with other countries, our findings were slower than those found in India at 30°C (6.3 days) (Bharti *et al*., 2007), but faster than in Brazil at 27°C (13.1–13.6 days) (Mendonça *et al*., 2012), in India at 27°C (9.75 days) (Wells & Kurahashi, 1994) and at 30°C (19 days) and 35°C (8 days) (Bansode *et al*., 2016), and in Egypt at 30°C (11.42 days) (Kamel *et al*., 2016). Nevertheless, our results were similar as in Bharti *et al*. (2007) at 28°C (8.5 days) in India. Differences between life cycle studies can be due to different methodologies used during experimentation (e.g., type of diet, number of larvae, temperature); additionally, some species reveal different growth patterns in different geographical areas (Greenberg, 1991; Grassberger & Reiter, 2001; Donovan *et al*., 2006; Martínez-Sánchez *et al*., 2007), therefore, it is imperative to produce development data for local fly populations.

Regarding the larval survival rate, our findings showed the range of survival rate between 72.73%–85%, except at 29.5°C where the mortality was significantly higher (48.89%; $p<0.05$). The differences in the larval mortality between the temperatures may be due to the manipulation of the larvae when they were counted. Gabre *et al*. (2005) reported a 38% pre-adult survival rate, similar results were obtained by Arias-Di Donato and Liria (2016) with a 36.5%–37.5% of survival with sardine and beef liver diets, respectively (63.5% for sardine and 62.5% for beef liver of mortality). On the other hand, a 44% of total pre-adult mortality (56% of survival) was reported by Goodbrod and Goff (1990), and Kamel *et al*. (2016) who observed 43.42%±2.30%, 65.75%±1.8% and 55.23%±4.20% of survival rate at 20°C, 25°C and 30°C, respectively; however, our survival rates were higher than those reported in the previous studies. According to Zumpt (1965), the length of the third instar of *C. megacephala* can reach up to 16.0 mm, however, it was smaller compared to our findings where 18.0 mm was the highest length obtained in one of

the individual larvae. In comparison to Kumara *et al*. (2010), the maximum length of larvae at 29°C±2°C was less than 15.0 mm, it was considerably lower than our values at 29.5°C, but it is possible that the variation of the studied temperature (±2°C) can affect the length of the larvae. The maximum larval length obtained in Ismail *et al*. (2007) study was less than 13.0 mm, but they plotted the growth curves comparing different levels of humidity, which is one of the factors that can affect the results. Previous published growth curves of *C. megacephala* from China reported a maximum larval length was less than 17 mm at 16°C (Yang *et al*., 2016) and a range of the mean maximum larval length from 16.2 mm to 17.0 mm at 16°C to 34°C (Zhang *et al*., 2018), it was similar to our results, but our study still recorded a higher maximum larval length. Nevertheless, a maximum larval length higher than 20.0 mm was obtained by Wells and Kurahashi (1994).

Considering the PMI estimation method with this fly species in Malaysia is still limited (Kumara *et al*., 2010), this is the first time that the ADD and the isomorphen diagram were published from Malaysian *C. megacephala* population. For ADD analysis in our study, the minimum threshold temperature (D_0) calculated in China by Zhang *et al*. (2018) was used in the ADD calculation due to its geographical closeness to Malaysia. Isomorphen diagrams have been published before in other studies in China using seven different temperatures (Yang *et al*., 2016; Zhang *et al*., 2018). Although our study is preliminary, studying local populations is essential to estimate the PMI accurately in forensic entomology, especially when dealing with an important forensic species such as *C. megacephala*; thus, more studies are needed by increasing the range of temperature regimes to improve the applicability of this blowfly species in forensic cases in Malaysia.

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Conflicts of Interest

The author declares that they have no conflict of interests.

REFERENCES

Aguirre-Gil, O.J., Valente, F.I., dos Santos, L.S., Viana, D.L. & Busoli, A.C. (2015). Desarrollo larval de *Chrysomya megacephala* (Diptera: Calliphoridae) en diferentes dietas y densidades larvales. *Revista Colombiana de Entomologia* **41**: 48-57.

Ahmad Firdaus, M.S., Anita, T., Mohamed, A.M., Noor Hayati, M.I., Syamsa, R.A., Zuha, R.M. & Omar, B. (2009). Pengaruh suhu ke atas perkembangan larva lalat *Chrysomya megacephala* (Fabricius) dan *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae): aplikasi dalam sains forensik. *Jurnal Sains Kesihatan Malaysia* **7**: 89-96.

Arias-Di Donato, L. & Liria, J. (2016). Vital statistics of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) under different diets from Venezuela. *Journal of Entomology and Zoology Studies* **4**: 247-251.

Badenhorst, R. & Villet, M.H. (2018). The uses of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) in Forensic Entomology. *Forensic Sciences Research* **3**: 2-15. https://doi.org/10.1080/20961790.2018.1426136

Bansode, S.A., More, V.R., Zambare, S.P. & Abd Algalil, F.M. (2016). Effect of constant temperature (20°C, 25°C, 30°C, 35°C, 40°C) on the development of the Calliphorid fly of forensic importance, *Chrysomya megacephala* (Fabricus, 1794). *Journal of Entomology and Zoology Studies* **4**: 193-197.

Bharti, M., Singh, D. & Sharma, Y.P. (2007). Effect of temperature on the development of forensically important blowfly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). *Entomon* **32**: 149-151.

Byrd, J.H. & Castner, J.L. (2001). Forensic Entomology: The utility of arthropods in legal investigations, Byrd, J.H. & Castner, J.L. (editors) 1st edition. Boca Raton: CRC Press.

Catts, E.P. (1992). Problems in estimating the postmortem interval in death investigations. *Journal of Agricultural Entomology* **9**: 245-255.

Catts, E.P. & Goff, M.L. (1992). Forensic entomology in criminal investigations. *Annual Review of Entomology* **37**: 253-272. https://doi.org/10.1146/annurev.en.37.010192.001345

Catts, E.P. & Haskell, N.H. (1990). Entomology & Death: A Procedural Guide, Catts, E.P. & Haskell, N.H. (editors). Joyce's Print Shop.

Donovan, S.E., Hall, M.J., Turner, B.D. & Moncrieff, C.B. (2006). Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures. *Medical and Veterinary Entomology* **20**: 106-114. https://doi.org/10.1111/j.1365-2915.2006.00600.x

Gabre, R.M., Adham, F.K. & Chi, H. (2005). Life table of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). *Acta Oecologica* **27**: 179-183. https://doi.org/10.1016/j.actao.2004.12.002

Gennard, D. (2007). Forensic Entomology: An Introduction. West Sussex: John Wiley and Sons.

Goff, M.L. (1992). Problems in estimation of postmortem interval resulting from wrapping of the corpse: a case study from Hawaii. *Journal of Agricultural Entomology* **9**: 237-243.

Goff, M.L. (2000). A fly for the prosecution. how insect evidence helps solve crimes. Massachusetts: Harvard University Press.

Goodbrod, J.R. & Goff, M.L. (1990). Effects of larval population density on rates of development and interactions between two species of Chrysomya (Diptera: Calliphoridae) in laboratory culture. *Journal of Medical Entomology* **27**: 338-343. https://doi.org/10.1093/jmedent/27.3.338

Grassberger, M. & Reiter, C. (2001). Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram. *Forensic Science International* **120**: 32-36. https://doi.org/10.1016/S0379-0738(01)00413-3

Grassberger, M. & Reiter, C. (2002). Effect of temperature on development of the forensically important holarctic blow fly *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae). *Forensic Science International* **128**: 177-182. https://doi.org/10.1016/S0379-0738(02)00199-8

Greenberg, B. (1991). Flies as forensic indicators. *Journal of Medical Entomology* **28**: 565-577. https://doi.org/10.1093/jmedent/28.5.565

Hu, Y., Yuan, X., Zhu, F. & Lei, C. (2010). Development time and size-related traits in the oriental blowfly, *Chrysomya megacephala* along a latitudinal gradient from China. *Journal of Thermal Biology* **35**: 366-371. https://doi.org/10.1016/j.jtherbio.2010.07.006

Ismail, M.I., Osman, K., King, O.H., Hassan, N., Elias, E., Ambia, K.M., Ghazali, A.R., Mohamed, J. & Omar, B. (2007). Accelerating *Chrysomya megacephala* maggot growth for forensic entomology cases. *Jurnal Sains Kesihatan Malaysia* **5**: 17-26.

Kamel, A.S., Helmy, N.M., Marwan, D.A. & Ibrahim, A.A. (2016). Influence of temperature and larval food oncertain biological aspects of *Chrysomya megacephala* (Diptera/ : Calliphoridae). *IOSR Journal of Pharmacy and Biological Sciences* **11**: 5-11. https://doi.org/10.9790/3008-1103030511

Kumara, T., Ahmad, A.H., Che, C.S. & Singh, B. (2010). Growth of *Chrysomya megacephala* (Fabricius) maggots in a morgue cooler. *Journal of Forensic Sciences* **55**: 1656-1658. https://doi.org/10.1111/j.1556-4029.2010.01485.x

Lee, H.L., Krishnasamy, M., Abdullah, A.G. & Jeffery, J. (2004). Review of forensically important entomological specimens in the period of 1972-2002. *Tropical Biomedicine* **21**: 69-75.

Marchenko, M.I. (2001). Medicolegal relevance of cadaver entomofauna for the determination of the time of death. *Forensic Science International* **120**: 89-109. https://doi.org/10.1016/S0379-0738(01)00416-9

Martínez-Sánchez, A., Smith, K.E., Rojo, S., Marcos-García, M.A. & Wall, R. (2007). Geographic origin affects larval competitive ability in european populations of the blow fly, *Lucilia sericata*. *Entomologia Experimentalis et Applicata* **122**: 93-98. https://doi.org/10.1111/j.1570-7458.2006.00497.x

Mendonça, P.M., Dos Santos-Mallet, J.R. & De Carvalho Queiroz, M.M. (2012). Ultrastructure of larvae and puparia of the blowfly *Chrysomya megacephala* (Diptera: Calliphoridae). *Microscopy Research and Technique* **75**: 935-939. https://doi.org/10.1002/jemt.22017

Nazni, W.A., Lee, H.L., Chew, W.K., Roziah, A., Jeffery, J. & Heo, C.C. (2015). Malaysia. In: Forensic Entomology International Dimensions and Frontiers, Tomberlin, J.K. & Benbow, M.E. CRC Press, pp. 19-35.

Oliveira-Costa, J. & de Mello-Patiu, C.A. (2004). Application of forensic entomology to estimate of the postmortem interval (PMI) in homicide investigations by the Rio de Janeiro police department in Brazil. *Aggrawal´s Internet Journal of Forensic Medicine and Toxicology* **5**: 40-44.

Prins, A.J. (1982). Morphological and biological notes on six South African blowflies (Diptera, Calliphoridae) and their immature stages. *Annals of the South African Museum* **90**: 201-217.

Sukontason, K., Narongchai, P., Kanchai, C., Vichairat, K., Sribanditmongkol, P., Bhoopat, T., Kurahashi, H., Chockjamsai, M., Piangjai, S., Bunchu, N. *et al*. (2007). Forensic entomology cases in Thailand: a review of cases from 2000 to 2006. *Parasitology Research* **101**: 1417-1423. https://doi.org/10.1007/s00436-007-0659-8

Wells, J.D. & Kurahashi, H. (1994). *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) development: rate, variation and the implications for forensic entomology. *Japanese Journal of Sanitary Zoology* **45**: 303-309.

Yang, Y-Q., Li, X-B., Shao, R-Y., Lyu, Z., Li, H-W., Li, G-P., Xu, L-Z. & Wan, L-H. (2016). Developmental times of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) at constant temperatures and applications in forensic entomology. *Journal Forensic Science* **61**: 1278-1284. https://doi.org/10.1111/1556-4029.13159

Zhang, Y., Wang, Y., Yang, L., Tao, L. & Wang, J. (2018). Development of *Chrysomya megacephala* at constant temperatures within its colony range in Yangtze River Delta Region of China. *Forensic Sciences Research* **3**: 74-82. https://doi.org/10.1080/20961790.2017.1403007

Zumpt, F. (1965). Myiasis in man and animals in the old world: A textbook for physicians, veterinarians, and zoologists. London: Butterworths.