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Technical note

Patterns of oviposition and development of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) on burned rabbit carcasses

N.A. Mahat [a,*], N.L. Zainol-Abidin [b], N.H. Nordin [a], R. Abdul-Wahab [a], P.T. Jayaprakash [c]

[a] *Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM, Johor Bahru, Johor, Malaysia*
[b] *Department of Chemistry Malaysia, Jalan Sultan, 46661 Petaling Jaya, Selangor, Malaysia*
[c] *School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

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A B S T R A C T

Considering that crimes against animals such as illegal killing and cruelty have been alarmingly increasing and since burning is one of the common ways for disposing cadavers, ability to estimate minimum postmortem interval (PMI) using entomological data merits consideration. *Chrysomya megacephala* and *Chrysomya rufifacies* are common necrophagous species recovered from cadavers in many countries including Malaysia. Specific studies focusing on the oviposition and developmental patterns of both species on cadavers manifesting different levels of burn as described by the Crow–Glassman Scale (CGS) remain scarce. In four replicates, rabbit carcasses were burned to CGS levels #1, #2 and #3 by varying the amount of petrol used and duration of burning. Oviposition by *C. megacephala* and *C. rufifacies* was delayed by one day in the case of carcasses burned to the CGS level #3 ($p < 0.05$) when compared with that of controls. Such delay in oviposition was not observed in the CGS level #1 and #2 carcasses. No significant differences ($p > 0.05$) in the duration of development were found between control and burned carcasses. These findings deserve consideration while estimating minimum PMI since burning as a mean for disposing animal and human cadavers is gaining popularity.

1. Introduction

Forensic entomological data have been demonstrated to be useful for estimating the postmortem interval (PMI), especially for decomposing bodies discovered 72 h or more after death [1]. Similarly, forensic entomology has been utilized for investigating wildlife offences [2] viz. illegal killing and cruelty, trade and possession, as well as poaching [3,4]. Anderson [5] reported that wildlife animals are illegally killed for fur, meat and organs, and in this context, the ability to estimate PMI accurately would be extremely useful in refuting a suspect's alibi. *Chrysomya megacephala* (Fabricius) remains the earliest necrophagous species to oviposit on decomposing corpses and animal models in Malaysia followed by *Chrysomya rufifacies* (Macquart) [6–10]. While climatological factors [8,11] and poisons [8] have been reported to influence oviposition and the development of necrophagous insects, entomological studies on the influence of burned carcasses/bodies appear to have been neglected [12]. Interestingly, Anderson [13] noted that 'killers often try to dispose of a victim by burning the body' and emphasized that 'little research has been conducted on the effects of burning on insect succession'. For facilitating the uniform description of injuries to burned human bodies, Glassman and Crow [14] have described a graded scale (the Crow–Glassman Scale) that is divided into five levels in increasing order of destruction to the body. Unfortunately, a similar gradation for animals remains lacking.

A previous study reported that initial oviposition occurred immediately on both the CGS level #2 burned and control pig carcasses, although the majority of the oviposition occurred the next day [15]. Heo et al. [16] studied partially burned pig carcasses in Malaysia and indicated no significant differences in the sequence of faunal succession or the rate of decomposition between the control and burned carcasses. However, these researchers [16] did not indicate the CGS level of burning, rendering it difficult to make the appropriate comparisons. Recognizing the applications of CGS levels for the estimation of

* Corresponding author. Tel.: +60 7 5534138; fax: +60 7 5566162.
E-mail addresses: naji@kimia.fs.utm.my (N.A. Mahat), liyanamok@gmail.com (N.L. Zainol-Abidin), nurulnordin@gmail.com (N.H. Nordin), roswanira@kimia.fs.utm.my (R. Abdul-Wahab), ptjaya@usm.my (P.T. Jayaprakash).

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PMI, Avila and Goff [15] recommended further investigations on the effects of different degrees of burning on arthropod succession patterns. In this context, it is pertinent to indicate that any factors that could influence initial oviposition or the duration of development of necrophagous insects may subsequently affect the accuracy of the PMI estimate [17]. Hence, the present study explores the patterns of oviposition and the development of *C. megacephala* and *C. rufifacies* across different CGS levels in burned rabbit carcasses and provides baseline data for investigating wildlife offences and human forensics.

2. Methods

2.1. Experimental design

Using rabbits as animal models, this experiment (four replicates) was designed to investigate the possible influences of different levels of burn injury as prescribed in the Crow–Glassman Scale (CGS) [14] on the initial oviposition and completion of the first life cycle of *C. megacephala* and *C. rufifacies*. Male rabbits (*Oryctolagous cuniculus*) (4 for each replicate) (2.0–2.5 kg) sacrificed by slaughtering with the front part of the neck severed partially were purchased as dead carcasses from a rabbit meat seller in Kota Bharu between 7.00 and 7.30 am. The carcasses were transported individually to the decomposition site in separate sealed double plastic bags to prevent exposure to other arthropods prior to placement at the decomposition site. Among the four rabbit carcasses that formed a single replicate, one was used as the control, while the remaining three carcasses were burned to CGS level #1, CGS level #2 and CGS level #3, respectively. Glassman and Crow [14] describe the burn injuries of CGS level #1 as characteristic of typical smoke death (i.e., blistering on the epidermis, singeing of the head and facial hair), with the body remaining recognizable for identification. CGS Level #2 burns occur when the bodies are still recognizable with varying degrees of charring, and the destruction of the body remains limited to the absence of elements of limbs, genitalia and ears [14]. CGS Level #3 occurs when the body undergoes further destruction, leading to the absence of major portions of the limbs with unrecognizable identity, despite the presence of the head [14]. Because extremely extensive burning is required to obtain CGS levels #4 and #5, this study was restricted to CGS level #1, CGS level #2 and CGS level #3 only. For achieving the CGS level #1, CGS level #2 and CGS level #3 burn injuries; the carcasses were burned using 0.5 L of petrol (RON 95) for 1 min, 0.75 L of petrol (RON 95) for 5 min and 1.5 L of petrol (RON 95) for 33 min, respectively (Table 1). As much as possible, the petrol was poured evenly over each carcass. Repeating the above procedure, four replicates (i.e., 16 rabbit carcasses) were included in the present research. The decompositions for all of the four replicate experiments were conducted from the 1st–13th February 2013, 5th–17th February 2013, 9th–21st February 2013 and 27th March–8th April 2013, respectively. While each carcass was separated by a minimum distance of 20 m during every replicate, the decomposition sites for all of the different replicate experiments were further separated by a minimum distance of 50 m to ensure the independence of these replicates.

Table 1
Detail of the four rabbit carcasses that formed one replicate and conditions of burning to achieve the required CGS level.

Rabbits ($n = 4$)	Quantity of RON95 (L)	Duration of burning (min)
Control	Unburned intact carcass	0
CGS level 1	0.5	1
CGS level 2	0.75	5
CGS level 3	1.5	33

2.2. Decomposition site and entomological observation

The experiments (February to April 2013) were conducted in a sunlit habitat located within the Universiti Sains Malaysia Health Campus, Kubang Kerian, Kelantan, an eastern state of Peninsular Malaysia (606"1"N, 102017"5"E) at approximately 4.6 m above the sea level. The soil type at the decomposition site was loam, and human activity was minimal. Each carcass was placed individually with direct contact on bare soil and covered with a slotted plastic basket (basket length: 58 cm; basket width: 45 cm; basket height: 20 cm; slot length: 3 cm; slot width: 1 cm; mould width between slots: 0.5 cm) with two to three bricks on top to prevent scavengers, while allowing for the free access of flies. Entomological observations, as well as data on daily ambient temperature and rainfall, were recorded until the completion of the first developmental cycle of *C. megacephala* and *C. rufifacies* (i.e., evidence of the emergence of tenerals). Following the entomological observation methods prescribed by Mahat et al. [8], field observations, as well as collection, rearing and preservation of entomological specimens, were made. Taxonomic identification of larvae and tenerals was conducted based upon the morphological identification keys provided by previous researchers [18–20].

2.3. Statistical analysis

Data analysis was conducted using IBM SPSS version 20.0 software. The normality of the data used for statistical inference was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Considering the significance level of 0.05, the Kruskal–Wallis *H* with pairwise comparison using Mann–Whitney *U* test was used for comparing the differences in the oviposition and completion of life cycles for *C. megacephala* and *C. rufifacies* among the different groups.

3. Results and discussion

Ambient temperatures remained similar for all four of the carcasses across all four replicates; the ambient temperature data recorded for the control carcasses alone are reported in Table 2. The means for the ambient temperatures and the total daily rainfall recorded during the four replicates ranged between 27.4 and 35.5 °C (Table 2) and 0.0–5.0 mm, respectively. In general, rain was not recorded during the first two days of decomposition in any of the four replicate experiments, except on the second day of decomposition in replicate 1, where drizzling was observed (0.4 mm). Neither incessant nor torrential rains were observed throughout the study.

Results revealed that the times to initial oviposition by *C. megacephala* and *C. rufifacies* in the CGS level #1 and #2 burned carcasses were similar to that of control carcasses ($p > 0.05$, Tables 3 and 4), in concurrence with the findings reported by Avila and Goff [15]. It has been reported that blowflies are attracted by the odour emanating from decomposing corpses/carcasses [13], and burning may lead to the disintegration of charred soft tissues, which may be favourable for the colonization of insects [12]. Adding to the body of current knowledge, we found that initial oviposition by both necrophagous species was delayed by one day in the CGS level #3 burned carcasses ($p < 0.05$, Tables 3 and 4). The CGS Level #3 burned carcasses were relatively drier and had smaller amounts of seeping body fluids when compared with the controls and the CGS level #1 and #2 burned carcasses. Therefore, the delays in oviposition by *C. megacephala* and *C. rufifacies* observed in the CGS level #3 burned carcasses ($p < 0.05$,