ORIGINAL ARTICLE

The Succession Pattern and Development of Predominant Species of Blowflies to Suitcase-enclosed Rabbit Carcasses on Outdoor Versus Indoor Sites

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ABSTRACT

Introduction: Blowfly succession on animal carcasses aids in estimating the minimum post-mortem interval of decomposed bodies. However, colonization differs in cases where the decomposing bodies are enclosed. This study examines blowfly succession on enclosed rabbit carcasses to establish patterns relevant to forensic investigations. **Materials & methods:** Fifteen domestic rabbit (Oryctolagus cuniculus) carcasses were divided into three groups; enclosed indoors and outdoors, and exposed outdoor carcasses. Each carcass was placed in a suitcase as the mode of enclosure. Entomological and environmental data were observed and recorded. **Results:** Enclosement of the carcasses have altered the insect succession pattern during carcasses decomposition. Four species of calliphorid flies were identified on control carcasses, compared to only two species were found on enclosed carcasses. However, both groups of carcasses were colonised by two main blowfly's species; *Chrysomya megacephala* (51.4%) and Chrysomya rufifacies (33.5%). The enclosement has caused delayed in oviposition which took around 49-76 and 50-77 hours for *Ch. megacephala* and *Ch. rufifacies* respectively. During fly development, longer eclosion time was also observed in *Ch. megacephala* (142.5-147.5 hrs) and *Ch. rufifacies* (162.5-169 hrs) compared to exposed carcasses (96-97 hrs). Consequently, the carcasses enclosure disrupted the normal blowfly's oviposition and development, thus affecting the minPMI estimation. **Conclusion:** The enclosement of carcasses significantly altered blowfly succession, delayed oviposition, and prolonged the developmental time. These findings highlight the impact of physical barriers on blowflies succession and colonization of decomposing carcass in forensic investigation, emphasizing the need to consider the effect of enclosure when estimating the minimum post-mortem interval.
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Keywords: *Chrysomya megacephala*, Chrysomya rufifacies, Minimum post-mortem Interval, Succession pattern, Decomposition

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INTRODUCTION

Insects are naturally attracted to specific stages of decomposition and do not appear on a corpse simultaneously. Instead, they arrive in a predictable, chronological order known as insect succession, a concept first introduced by Mŭgnin in 1894 [1,2]. This process has been extensively studied worldwide by observing the consistent and sequential colonization of decomposing remains by various insect species. In cases involving suspicious human or animal deaths, carrion insects play a crucial role in crime scene reconstruction [3]. Necrophagous insects, particularly blowflies (family: Calliphoridae), are commonly used to estimate and determine the postmortem interval (PMI) [4,5].

Forensic entomology primarily relies on the succession patterns and developmental stages of insects [6–7] found on and within a corpse to estimate the time since death. This specialized field plays a vital role in various aspects of criminal investigations, particularly in determining the minimum postmortem interval (minPMI). In addition, minPMI can be inferred by analyzing a range of postmortem changes occurring within the body. The decomposition processes include, physical alterations,

metabolic reactions, autolysis, physicochemical transformations, microbial activity, and the impact of insect colonization [8–10].

After death, the body undergoes a series of processes aimed at returning its components to the environment through the recycling of energy [11]. These processes include cellular oxygen deprivation, carbon dioxide accumulation, and a decrease in pH levels [12]. The disintegration of a body after life ceases follows a predictable pattern, which aids in determining the time since death [13]. Depending on the decomposition stage, degree of exposure, geographic region, and seasonal variations, a variety of insect species, especially flies were applied to estimate the minPMI [14]. Blowflies typically arrive at a corpse within ten minutes after death [15,16]. Within hours, the body emits a strong odor that attracts large numbers of adult blowflies, drawn by the scent produced through bacterial activity on decomposing tissues [17,18]. These stages of decomposition are influenced by environmental factors such as temperature and humidity. In warmer and more humid biogeoclimatic zones, decomposition progresses more rapidly, whereas in colder regions, it slows down due to reduced microbial activity [19].

This study aims to investigate the blowflies colonizing and decomposing enclosed rabbit carcasses to establish a succession pattern of these forensically valuable insects in the event of enclosed dead bodies.

MATERIALS AND METHODS

Animals

Fifteen domestic rabbits (Oryctolagus cuniculus), each weighing approximately 1.2 kg, were used as animal models in accordance with the methods described by [20,21]. The rabbits were divided into three experimental groups, which are outdoor-exposed carcasses, outdoor-enclosed carcasses and indoor-enclosed carcasses, with five rabbits in each group. They were obtained from a local pet shop. The use of rabbit carcasses as human analogues in decomposition studies is widely accepted due to their comparable tissue composition and practical size [20,21]. To obtain the carcasses, each rabbit was humanely euthanized by cervical dislocation [22-23]. The procedure has obtained ethics approval (UNIMAS/AEC/R/F07/050) and was performed by a trained medical laboratory technologist from the Entomology Research Laboratory, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak (UNIMAS).

Study site

The research was conducted at Universiti Malaysia Sarawak campus in Kota Samarahan, Sarawak (1°28'04"N 110°26'50"E, 6 m above sea level). Kota Samarahan, Sarawak has a tropical rainforest climate with a district annual temperature ranged from 22.8 to 30.8°C and 80.71% humidity. The average annual rainfall typically receives about 108.48 mm. The experiments were carried out in five trials of experiment, from May, July, September and November in 2019. Then, the experiment was halted for 9 months due to pandemic COVID-19, and then continued on September of 2020. Entomological, decomposition and environmental data were observed for 30 days or until decomposition was complete, whichever occurred first. A subsequent repeated experiment (trials) were then conducted at least three weeks after the completion of the previous experiment.

The study site was situated in front of an unoccupied faculty building, adjacent to a roadside. The area encompassed of a security guard post with an area of 8 ft (L) x 8 ft (W) x 8ft (H) and small field measuring 630 m2.

Experiment setup

The rabbit carcasses were assigned to three decomposition conditions: outdoor-exposed, outdoor-enclosed, and indoor-enclosed, with one animal placed in each group per trial. The experiment was repeated five times, resulting in a total of five animals used in each group. Enclosed carcasses were placed in a fabric suitcase measuring 60 cm (L) x 40 cm (W) x 18 cm (H) in dimension. The outdoor-exposed carcass was placed on the ground and covered with wire mesh (1 cm x 1 cm) to prevent access from predators while still allowing insect access to corpses. For outdoor-concealed carcass, the carcass was put inside a fabcric suitcase (60 (L) x 40 (W) x 18 (H) cm, which was lined with plastic cover. On the other hand, the indoor-concealed carcass was also put inside a suitcase, and then kept inside the guard's post where all windows were securely closed, no air circulation or light was present, and all small openings were sealed with tape. The guard post was a one storey building, measuring 8 ft (length) × 8 ft (width) Ч 8 ft (height), around 630 square feet. Each of the carcass was placed at least 20 meters from each other to prevent cross-attraction of insects (Fig.1). In this study, the indoor exposed-carcasses was excluded due to space limitations within the indoor facility. Therefore, the comparison of insects succession and decomposition duration was compared between (1) outdoor exposed carcasses and outdoor enclosed-carcasses, and (2) outdoor-enclosed carcasses and indoor-enclosed carcasses. The experiment was repeated five times to ensure the reliability and consistency of the results.

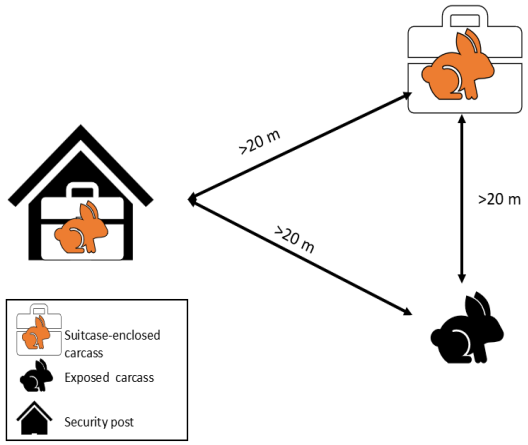

Fig 1: An illustration of the experimental setup and position of suitcase-concealed rabbit carcasses in the study.

Decomposition observation

The decomposition and insect activities on the carcasses were monitored throughout the study period. The stages of decomposition were visually assessed daily by opening the suitcase twice a day, at 8:00 am and 4:00 pm. The stages of decomposition were determined based on physical changes observed on the carcasses and the associated insect activity [24]. For suitcase-enclosed carcasses, observations and sampling were conducted promptly to minimize the entry of unwanted insects and the escape of forensically important species. As a precaution, the suitcase was opened for a maximum of 10 minutes at a time. Criteria used to identify each stage of decomposition was based on the descriptions provided by Goff [24].

Monitoring, insect collection and preservation.

Adult flying insects visiting the carcasses and their larvae were collected using a sweep net and spatula respectively. They were collected twice daily was at 8.00 am and 4.30 pm. Adult insects were placed in a jar and killed using ethyl acetate. Immature stages, primarily fly larvae infesting the carcasses, were collected using spatulas and forceps. Approximately 20–30 larvae were collected from each carcass during every visit. The specimens were then placed in screw-cap containers, appropriately labeled, and transported to the laboratory for further analysis. In the lab, some larvae were killed by immersion in hot water (~80 °C) and then preserved in 70% ethanol before microscopic examination for species identification [25].

Another half of the larvae were reared into adult to facilitate species identification. The larvae were reared by placing them in a glass jar filled with sawdust and covered with a perforated lid. Sufficient beef liver was provided as sustenance and the temperature was kept between 25 oC to 28 oC to ensure their optimal development. Their progression was carefully observed through each stage [25]. All collected insects were brought to the laboratory for species identification. At the field, the environmental parameters were recorded using a data logger equipped with a rain gauge meter (AMTAST, USA). Species identification was carried out based on the key identification and morphology description of the species by Nazni et al. (2011) [26].

Statistical analysis

All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) software, version 24 (USA). In this study, entomological data were compared between the outdoor-exposed and outdoor-enclosed carcass groups, as well as between experimental groups.

Data pertaining to the number of insects between groups were analyzed using t-test whereas the indoor and outdoor environmental data between experiment replicates were analyzed using one-way ANOVA. The results were concluded as statistically significant when the p-value is less than 0.05 ($p < 0.05$). Any significant values were analysed further using Post-hoc Test (Tukey HSD) to determine the siginificantly different group of data. All results are presented as mean ± standard deviation (SD), based on replicates (n = 5 per group).

RESULTS

Meteorological parameters

Generally, both outdoor and indoor ambient temperatures exhibited fluctuations, with outdoor temperatures showing more frequent peaks and drops (Fig. 2). While indoor temperatures also fluctuated, the trend was more stable. Outdoor temperatures ranged from 30.1 °C to 35.9 °C across five experimental trials, whereas indoor temperatures were slightly lower, ranging from 28.1 °C to 32.8 °C. Independent sample t-test was performed to compare the ambient temperatures and relative humidity between outdoor and indoor environment. From the analysis, there is a significant difference of ambient temperatures between outdoor and indoor environment ($p<0.05$) (Fig. 2). Significantly higher ambient temperature was recorded outdoor (33.3 ± 0.5 °C) compared to indoor environment (30.5 ± 0.3 °C). When comparing relative humidity data, there is a significant difference of relative humidity between outdoor and indoor environment ($p <0.05$) (Fig. 2). Significantly higher relative humidity was recorded indoor (82.1±0.8 %) compared to outdoor environment (80.0±0.8 %).

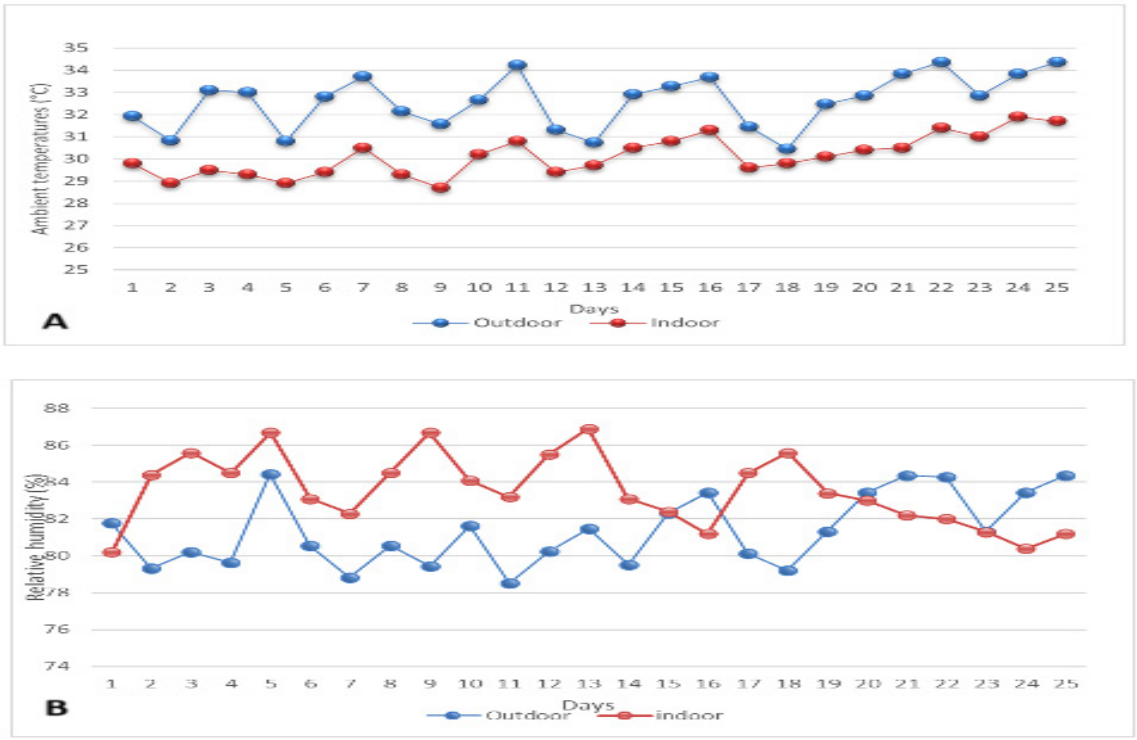

Fig 2: A graph showing (2A) ambient temperatures between outdoor and indoor environment and (2B) relative humidity between outdoor and indoor environment. Outdoor ambient temperatures were significantly ($p < 0.05$) higher than indoor temperatures, meanwhile indoor relative humidity was significantly ($p < 0.05$) higher than outdoor humidity.

Statistical analysis using an independent sample t-test was conducted to compare ambient temperatures and relative humidity between outdoor and indoor environments. The results indicated a significant difference in ambient temperatures ($p<0.001$) and humidity levels ($p=0.004$) between the two sites. Rainfall occurred intermittently throughout the study period, with the highest recorded rainfall of 209.8 mm observed during the third trial, while the lowest accumulated rainfall of 82.8 mm was recorded during the fifth trial (Fig. 3).

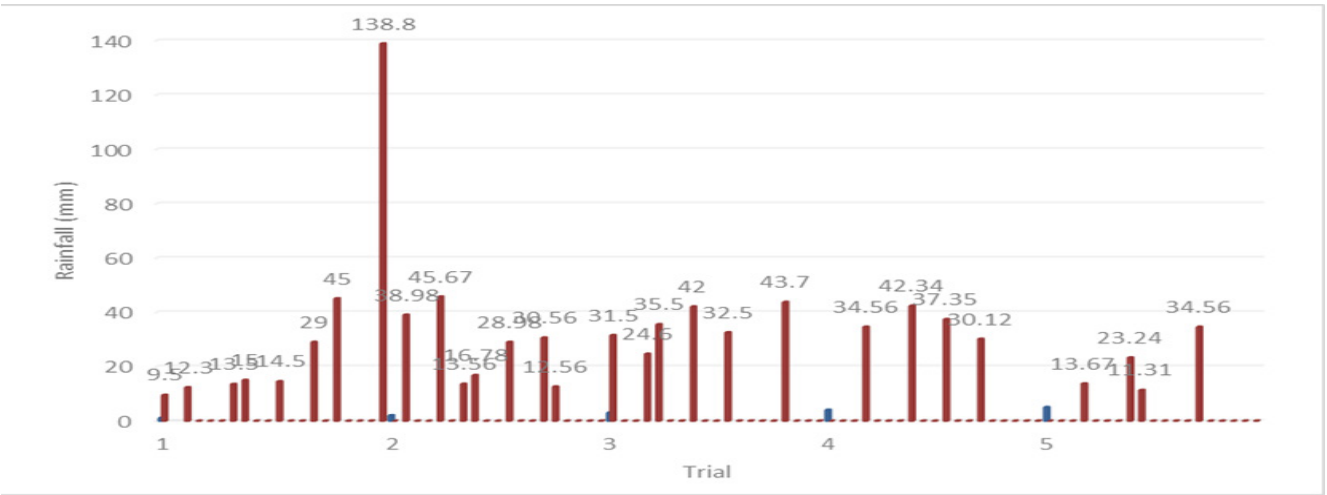

Fig 3: A graph showing rainfall recorded over study period. The highest rainfall recorded was 138.8 mm during the first trial of experiment.

Composition of insects on decomposing rabbit carcasses

Over five experimental trials, a total of 1,799 individual insects were collected from all carcass groups. The highest number was recorded on exposed carcasses in the control group (total =1,133), while fewer insects were found on enclosed carcasses, with totals of 378 and 288 insects, from outdoor and indoor enclosed carcasses, respectively. The insect species collectedon control carcasses consisted of five species of dipteran flies from two families; Calliphoridae and Sarcophagidae. The rest of the insects were unidentified species from the Order Hymenoptera and Coleoptera. As for carcasses enclosed in suitcase, only three species of flies were collected from outdoor carcasses, and two species collected from indoor carcasses (Table I). The predominant flies infesting the carcasses across all groups were similar and consisted of two calliphorid species, *Chrysomya megacephala* and Chrysomya rufifacies. The adult of *Ch. megacephala* can be identified based on its metallic greenish color, distinctive orange-yellow gena, and postgenal area. Meanwhile, *Ch. rufifacies* is characterized by its metallic green color and a whitish or silvery-white postgena. The size of Ch. ruFfifacies is slightly smaller (6.0–9.5 mm) compared to *Ch. megacephala* (8.0–11.0 mm). The larva morphology of *Ch. rufifacies* was easily differentiated from *Ch. megacephala* by its brownish and elongated tubercles on its body segments. *Ch. megacephala* on the other hand, has a cream-yellowish colour, and smooth body segments, with moderate spines present between the first and second of thoracic segments.

Table I: The composition of insects associated with carcasses decomposition in control and suitcase-enclosed group.

Order/Family	Species	Control (exposed outdoor)	Suitcase-enclosed group			Total insect	Relative abundance (%)
			Outdoor	Indoor	Total		
Diptera	*Chrysomya megacephala*	542	213	169	382	924	51.4
Calliphoridae	*Chrysomya rufifacies*	343	141	119	260	603	33.5
	Hemipyrella ligguriens	15	0	0	0	15	0.8
	Hypopygiopsis violacea	10	0	0	0	10	0.6
Sarcophagidae	*Sarcophaga sp.*	10	24	0	24	34	1.9
Hymenoptera Formicidae	*Unidentified*	210	0	0	0	210	11.7
Coleoptera	Unidentified	3	0	0	0	3	0.2
	Total	**1133**	378	288	**666**	**1799**	**100.0**

The relative abundance of *Ch. megacephala* and *Ch. rufifacies* accounted for 51.4% and 33.5%, respectively, of the total insect population collected from all groups of carcasses. Other flies' species found on carcasses were Hemipyrellia ligurriens (Diptera: Calliphoridae) (0.8%), Hypopygiopsis violacea (Diptera: Calliphoridae) (0.6%) and Sarcophaga sp. (Diptera: Sarcophagidae) (1.9%). These flies were only found on the exposed carcasses (control group), except for sarcophagid fly which were also found on enclosed carcasses placed outdoor. Besides flies, other insects such as ants (Hymenoptera: Formicidae) (11.7%) and coleopteran beetles (0.2%) were also collected from carcasses in control group (Table I).

One-way ANOVA test had shown that the number of individual blowflies collected was not significantly different in between five trials of experiments. Meanwhile, comparison on the number of blowflies collected on outdoor-enclosed carcasses was shown to significantly declined compared to control. Adding to that, the number of blowflies collected from outdoor-enclosed carcasses was significantly higher ($p < 0.001$) than those collected from the indoor- enclosed carcasses.

Succession patterns of insects associated with decomposing rabbit carcasses

The decomposition of rabbit carcasses in this study consisted of five stages: fresh, bloated, active decay, advanced decay, and dry remains (Table II). However, the duration of each stages and insects involved during the succession differ between the control and the enclosed groups. For the control group, the fresh stage was between 1 to 2 days before the carcasses began to bloat starting from day 2 to 3. The carcasses entered active decay as early as day three and proceed to advanced decay whuch ended on day 7. By the 9th day, the carcass already entered dry remains stage. However, for the enclosed carcasses placed outdoors and indoors, the fresh stage was longer and abdominal inflation was observed only after three and four days, respectively. Active decay started on day 6th for the outdoor enclosed carcasses and 7th day on the indoor enclosed carcasses. Advanced decay stage occurred on day-11 for outdoor enclosed carcasses and day-13 on the indoor enclosed carcasses. The outdoor and indoor enclosed carcasses enter the dry remains stage on day 16th and 19th, respectively.

Table II: The insect succession pattern during each decomposition stages of carcasses in control and enclosed group.

Group	Order	Family	Genus/ Species	Decomposition stages/day-n				
				Fresh	Bloated	Active decay	Advanced decay	Dry remains
				1-2	2-3	3-5	5-7	7-9
Control	Diptera	Calliphoridae	*Ch. megacephala*	√	√	√	√	√
			Ch. rufifacies	√	√	√	√	√
			H. ligurriens		√	√	√	√
			Hy. violacea			√		
		Sarcophagidae	*Sarcophaga* sp.			√		
	Hymenoptera	Formicidae	Unidentified			√	√	√
	Coleoptera	Unidentified	Unidentified			√	√	√
				1-3	4-5	6-10	11-15	16-21
Carcass enclosed (outdoor)	Diptera	Calliphoridae	*Ch. megacephala*		√	√	√	√
			Ch. rufifacies		√	√	√	√
		Sarcophagidae	*Sarcophaga* sp.		√	√		
				1-4	5-6	7-12	13-18	19-24
Carcass enclosed (indoor)	Diptera	Calliphoridae	*Ch. megacephala*		√	√	√	√
			Ch. rufifacies		√	√	√	√

The succession of insects on the control carcasses which are exposed outdoors started when eggs was first observed on the first day of carcass placement. During fresh stage, *Ch. megacephala* and *Ch. rufifacies* were recorded. During the fresh stage, blowfly eggs were only observed on the exterior of the suitcase but none inside the suitcase for both indoor and outdoor locations. Calliphorids and Sarcophagid started to lay eggs on the outdoor suitcase starting on the fourth day, while only calliphorids lay eggs on the indoor suitcase starting from the fifth day of the suitcase-enclosed carcasses placement. The larvae were first observed on the external area of suitcase and eventually on the carcass on the sixth day for the outdoor suitcase-enclosed carcasses and on seventh day for the indoor suitcase-enclosed carcasses. The larvae that hatched from the external area of the suitcase make way into the suitcase through the suitcase zippers. The larvae actively infesting carcasses throughout the active decay stage which continued for five days for outdoor-enclosed carcasses and six days for indoor-enclosed carcasses. Three species of larvae found inside the outdoor suitcase were *Ch. megacephala*, *Ch. rufifacies* and Sarcophaga sp. while only *Ch. megacephala*, *Ch. rufifacies* were found in the indoor suitcase. During this stage, control carcasses also recorded *H. ligurriens* and *Hy.violacea* on the carcasses.

The advanced decay stage begins on the fifth day for control carcasses, where the third instar larvae left the carcass to pupate. During this stage, the decomposed fluid started to dried up and the putrid odour was not as strong. The carcasses became less attractive to flies, as the number of flies visiting decreased. Similar carcass condition was observed on enclosed carcasses, although the progress was much slower than the control group. For both outdoor and indoor enclosed carcasses, most of the carcass's tissues were gone during advanced decay stage, and the decomposed fluid dried up and less larvae were seen on carcasses as the larvae were leaving the carcass to pupate. The duration of advanced decay stage took five and six days for carcasses placed outdoor and indoor respectively. Then, dry stage begun when all the carcass tissues have gone, the carcasses dried up and left with bones and fur. The observation for decomposing enclosed carcasses were continued for six days until the carcasses were completely dried. In total, the decomposition process for enclosed carcasses took 21 days for outdoor carcasses and 24 days for indoor carcasses while the control carcasses required only nine days to complete the decomposition.

Development duration of predominant blowflies.

Ch. megacephala eggs were observed at 48-50 hours and 75-77 hours on outdoor and indoor enclosed carcasses, respectively (Table III). The oviposition period was significantly delayed compared to the control carcasses, where *Ch. megacephala* laid eggs within 2-4 hours after carcass placement. The time required for egg hatching was similar between the control and enclosed carcass groups. In the control group, first-instar larvae were observed 24-27 hours after oviposition, whereas in the outdoor and indoor enclosed carcasses, newly hatched larvae appeared at approximately 23-28 hours and 27-28 hours, respectively. Larval development from the first until the third instar stage was faster in the control carcasses than in the enclosed ones. For instance, first-instar larvae in the control group developed into second-instar larvae within 7-9 hours. In contrast, larvae in outdoor enclosed carcasses required 23-25 hours to reach the second instar, while those in indoor enclosed carcasses took 19-22 hours. The transition from the second instar until post-feeding stage took approximately 24 hours for each developmental stage in control carcasses. However, in enclosed carcasses, both outdoors and indoors, larval development was delayed by about 7-8 hours before reaching the post-feeding stage. A similar delay was

observed in pupation. Additionally, adult emergence from *Ch. megacephala* pupae was delayed in enclosed carcasses, occurring at 141-144 hours outdoors and 146-149 hours indoors, compared to 96-97 hours in the control group. Overall, the mean development time to adulthood for *Ch. megacephala* from exposed carcasses (control) was 195.0±1.8 hours, which was significantly faster ($p< 0.001$) than the developmental duration of *Ch. megacephala* from enclosed carcasses, which was approximately 310.0±2.1 and 342.0±4.5 hours for outdoor and indoor locations, respectively. Furthermore, development was significantly delayed in indoor enclosed carcasses compared to both outdoor enclosed and exposed carcasses.

Table III: The development stages and duration of *Chrysomya megacephala* in control and enclosed carcass groups.

Group	***Ch. megacephala* development stages and duration (Day/hours)**							**Average development duration of *Ch. megacephala* (Mean±S.D)**
	Eggs	1st instar larvae	2nd instar larvae	3rd instar larvae	Post-feeding larvae	Pupae	Adult	
Control	**Day 1**	**Day 2**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 9**	195±1.8
	2-4	24-27	7-9	16-17	23-25	23-25	96-97	
Enclosed outdoor	**Day 2**	**Day 3**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 13**	310±2.1
	48-50	23-24	23-25	23-26	30-32	19-20	141-144	
Enclosed indoor	**Day 3**	**Day 4**	**Day 4**	**Day 5**	**Day 6**	**Day 10**	**Day 15**	342±4.5
	75-77	27-29	19-22	24-26	23-25	22-23	146-149	

Ch. rufifacies took 3-5 hours to oviposit on exposed carcasses, whereas in the outdoor and indoor enclosed carcass groups, eggs were first observed at 49-51 hours and 76-78 hours, respectively, after carcass placement (Table IV). In all carcass groups, 1st instar larvae were observed the following day, approximately 21-27 hours after the eggs were first recorded. Larval development from 1st instar to 3rd instar larvae was fastest for the exposed control groupand the larvae reached post-feeding stage by day four. While for the outdoor enclosed carcasses, the larval reached post-feeding stage on day five. The indoor enclosed carcasses require the longest time of larval development and reached the post-feeding larva stage only by day six. During the pupal stage, development to adult emergence was longest in the enclosed carcasses, lasting approximately 161-164 hours in outdoor conditions and 168-170 hours in condition, whereas in the control group, adult emergence occurred within just 96 hours. The total developmental duration of *Ch. rufifacies* in the control group was significantly faster (205.2 ± 1.6 hours) compared to its development in enclosed carcasses, which required 337.6±1.5 hours in the outdoor and 369.6 ± 1.1 hours in the indoor environment (Table IV). Similar to *Ch. megacephala*, the development of *Ch. rufifacies* was significantly delayed in indoor enclosed carcasses compared to both outdoor enclosed carcasses and exposed carcasses ($p< 0.001$).

Table IV: The development stages and duration of *Chrysomya rufifacies* in control and enclosed carcass groups.

Group	*Ch. rufifacies* **development stages and duration (hours)**							**Average development duration of *Ch. rufifacies* (Mean±S.D) (hr)**
	Eggs	1st instar larvae	2nd instar larvae	3rd instar larvae	Post-feeding larvae	Pupae	Adult	
Control	**Day 1**	**Day 2**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 9**	205.2±1.6
	3-5	25-26	7-9	16-18	27-29	26-28	96	
Enclosed outdoor	**Day 2**	**Day 3**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 13**	337.6±1.5
	49-51	21-25	26-28	23-24	27-30	21-24	161-164	
Enclosed indoor	**Day 3**	**Day 4**	**Day 4**	**Day 5**	**Day 6**	**Day 10**	**Day 15**	369.6±1.1
	76-78	27-29	22-26	22-26	23-26	22-26	168-170	

DISCUSSION

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Human corpses found in enclosed or indoor environments present significant challenges for forensic investigators, particularly in estimating the post-mortem interval (PMI). Indoor conditions often delay insect colonization and reduce species diversity compared to outdoor cases. Studies have reported restricted insect activity and delayed colonization in indoor and vehicular settings due to physical barriers that limit insect access [27,28]. Closed indoor environments tend to be colonized by specific sarcosaprophagous species, such as Lucilia sericata, which are better adapted to these conditions [29].

In a unique case, a corpse discovered inside a suitcase exhibited altered insect development stages, showing how confinement modifies decomposition and succession patterns [30]. This underscores the importance of understanding the environmental variables that impact insect behavior. Furthermore, accurate PMI estimation in indoor cases requires detailed entomological data tailored to specific scenarios and environments [31]. Forensic entomologists must consider species ecology, local climate, and barriers to colonization in order to draw reliable conclusions.

In forensic entomology, the estimation of post mortem interval was determined by decomposition stages, pattern of succession and the age of the immatures developing on the body [32]. The primary colonizers usually consisted of blowflies and followed by flesh flies and muscid flies. However, the pattern of succession may differ in certain conditions of the body when there is physical barrier such as confinement and burial, which limit the insect access and thus change the order of insect arrival [33]. In this study, although the indoor setting and the suitcase were properly sealed, the insects could somehow access and colonize the concealed carcasses. It was believed that the insects entered during opening and closing the guard post's door.

In terms of insect's diversity and abundance, in current study, the insects were more varied in control and enclosed carcasses. More fly species were observed on exposed carcasses (control) compared to the enclosed carcasses. Similar observation was also reported in previous study in Australia where nine insect taxa were identified during decomposition of the control pig carcasses, meanwhile the pig carcasses in suitcases reported only two to six species of insects [34]. The flies usually will visit and oviposit on carcasses within hours of exposure. The odours released from exposed carcasses attracts flies, especially blowflies which leads to oviposition on the natural orifices such as ears, eyes, nose and genital [35]. In this study, four species of blowflies were identified on exposed carcasses, while only two species were found on enclosed carcasses as a result of limited insect access. The two species found on enclosed carcasses were *Ch. megacephala* and *Ch. rufifacies*, which also the predominant flies infesting the exposed carcasses in control group and among the most important blowflies in estimation of PMI. The insect pattern recorded on exposed carcasses represent the typical pattern of carrion decomposition in this area of Sarawak as it has some similarity with previous studies [22,36]. Goff [37] has found a significant difference in insect colonization at outdoor versus indoor carrion, which prove that flies would easily discover outdoor carrion [33]. In previous study, muscid flies which usually arrive during active and advanced decay stage may be the first to arrive and become the primary colonizers of the carrion that were subjected to a restricted environment [30]. For example, Muscina stabulans and Muscina prolapsa (Diptera: Muscidae) become a primary colonizer on buried remains as access for other flies was limited [38]. However, in this study, the pattern of insect arrival and colonization was similar in both control and enclosed carcasses, although there is a significant difference in species diversity between both groups. In control carcasses, other blowflies namely, Hemipyrellia ligurriens and Hypopygiosis violacea were present on carcasses, whereas for concealed carcasses, these blowflies' species were absent. Similar observation was also reported in previous studies where Calliphora albifrontalis, *Ch. rufifacies* and Fannia canicularis were found both in control and suitcase-enclosed pig carcasses. Whereas, Megaselia scalaris was only found in enclosed carcasses and become the indicator for enclosed decomposition in Australia [34]. In peninsular Malaysia, a study on enclosed rabbit carcasses in sealed plastic waste bin has reported various species of flies present at exterior and interior part of the waste bin. The earliest flies found inside the waste bin was Megaselia scalaris followed by Chrysomya chani, Sarcophaga sp. and Hermetia illucens. In 40 days of observation, six species of scuttle flies were found, namely, Dahliphora sigmoides, Gymnoptera simplex, Megaselia scalaris (Loew), Puliciphora borinquenensis, Puliciphora obtecta Meijere and Spiniphora sp. [40]. Due to their smaller size, it was easier for these species to enter and colonize the enclosed carcasses [40]. However, in present study conducted in Borneo Malaysia, the enclosed carcasses in both indoor and outdoor decomposition were colonized by the same species of blowflies' and there were no flies from Family Phoridae and Family Muscidae observed.

Another important tool for estimating the post-mortem interval is determining the developmental stages and age of the primary colonizers [35]. In current study, the development duration was observed by monitoring the primary colonizers development from oviposition until adult emergence. Based on the finding, the average development duration of both *Ch. megacephala* and *Ch. rufifacies* were delayed compared to the development duration of the same species in control group. The prolonged duration of fly development in enclosed carcasses was due to the delayed in oviposition

and longer eclosion time (from pupation to adult eclosion) (Table III and IV). For example, in this study, oviposition of *Ch. megacephala* on enclosed carcasses in the outdoor environment was delayed by 46 hours compared to exposed carcasses in the control group. Meanwhile, for indoor-enclosed carcasses, the indoor environment caused a further delay of approximately 27 hours compared to outdoor-enclosed carcasses. A similar trend was observed for *Ch. rufifacies*. These findings align with a case study report by Goff [37], which reported a 60-hour delay in the development of predominant fly species in wrapped corpse and thus the PMI estimation was adjusted accordingly.

In another study, a comparison of insect arrivals on decomposing pork, which served as surrogate body parts, was carried out. The pork was enclosed and wrapped in either a zipped plastic bag (to simulate a suitcase), a black plastic bag, or a plastic sack. The finding shows all of the treatment delayed the initial contact of primary colonizer and oviposition by 30 hours in 48 hours of observation. In their study, the arrivals of blowflies on exposed pork meat were in average of eight minutes after the experiments started [40]. This shows that the act of concealing, wrappings or encasement can interfere with the insect's arrival and delayed the time for the flies to oviposit.

Aside from the delay in oviposition, the duration for adult eclosion was also prolonged in the current study. When larvae reach the pre-puparial stage, they typically migrate away from the decomposing remains to find a suitable and drier substrate to pupate. The behaviour is known as post-feeding dispersal which help them to avoid moisture which can detriment on pupal development [41]. In an enclosed environment, with non-absorbent lining and poorly ventilated condition like in the suitcases, the decomposition fluid can accumulate and thus prevent larvae from dispersing properly. The wet environment can interfere with pupae development [42]. A study has been carried out to determine the survival rate of pupae submerged in water (salt water, tap water and river water). The study found that the survival rate of Calliphora vomitoria pupae were significantly decreased in the three types of water (survival rate: 6-15%): when compared to unsubmerged control group (survival rate: 81%). In addition, the eclosion time the survived pupae was longer in treated group, where it took around 14-15 days compared to control pupae which took around 12 days [42]. As a result of delayed oviposition and a longer eclosion time, a prolonged decomposition process was observed.

In addition, the enclosed decomposition can be affected by key physiological factors such as; moisture rentention, oxygen availability and microbial activity. Although these factors were not measured in current study, the effects are well-documented. In an enclosed conditions, the moisture usually retained and the oxygen was limited. These can influenced the microbial activities and insect behaviour [43]. Futhermore, the limited space may restrict post-feeding larval dispersal, which affecting the larval growth and survival. These factors were likely contributed to the findings in current study and should be investigated further in future studies using appropriate instruments.

In estimating the post-mortem interval, ambient temperature and humidity are crucial factors to consider, as they directly influence the rate of decomposition, insect arrival, and development [10]. It has been established that ambient temperature, putrefactive bacterial activities, and exposure to certain chemicals prior to death may alter the rate of decomposition. For example, a body exposed to direct sunlight or immersed in warm water demonstrates faster decomposition. The phenomenon was seen in the victims of the 2004 Tsunami due to high temperature, humidity, and insufficient refrigeration [1]. Over the study period, although indoor temperatures were significantly lower than outdoor temperatures, they remained within the optimal range for insect development. The optimal temperature for *Ch. megacephala* and *Ch. rufifacies* development is 25-35°C [44] and 34-35.1°C [45], respectively. Therefore, in this study, the ambient temperatures in both the outdoor and indoor environments did not adversely affect blowfly development. In addition to temperature and humidity, rainfall also played a significant role during the experimental period. As illustrated in Figure 3, rainfall varied considerably across the five trials, with Trial 2 showing a notable spike of 138.8 mm. Rainfall can significantly influence insect activity by affecting both access to remains and the survivability of early developmental stages. Heavy or prolonged rainfall may hinder blowfly colonization by impeding oviposition, washing away eggs or larvae, or altering microclimatic conditions around the body [46]. Periods of low or no rainfall may favour continuous insect activity and faster decomposition. Although rainfall fluctuated, blowfly colonization occurred in all trials, indicating that rain may delay but not prevent insect activity. This highlights the adaptability of necrophagous flies in variable tropical climates. Thus, rainfall should be considered a modifying factor in PMI estimations, especially in outdoor cases [47].

Seasonal variation likely influenced the results of this study, as it spanned over 17 months (May 2019–September 2020), encompassing multiple seasonal cycles. Malaysia is a non-seasonal country with a tropical climate, experiencing hot and rainy weather throughout the year, encircling the Southeast Monsoon and Northwest Monsoon [48]. Environmental factors such as ambient temperature, humidity, and rainfall which are known to vary seasonally showed significant fluctuations, particularly outdoors. For instance, temperature and humidity levels were notably higher during certain trials, likely aligning with warmer, wetter months,

which can accelerate decomposition and insect activity. Conversely, lower temperatures and reduced rainfall during other trials may have slowed these processes. Such variability could have affected insect succession patterns, oviposition timing, and developmental rates, thereby introducing inconsistencies across trials and previous studies also confirm that seasonal differences affect blowfly activity and development rates [46]. However, based on environmental data collected in this study, the onset and retreat of these monsoons are not sharply defined in this location of the study. This primarily due to the location of Sarawak which lies near the equator.

CONCLUSION

The concealment of carcasses significantly altered blowfly succession, leading to delayed oviposition and prolonged development time. *Ch. megacephala* and *Ch. rufifacies* remained the primary colonizers in both exposed and concealed carcasses. The average development duration of *Ch. megacephala* and *Ch. rufifacies* in outdoor-enclosed was delayed compared to the exposed group of carcasses. Similarly, prolonged development duration of both blowflies was also observed in outdoor-enclosed carcasses when compared with indoor-enclosed carcasses. These findings highlight the impact of physical barriers in forensic entomology investigation, demonstrating that enclosement can disrupt normal insect succession patterns and influence minimum post-mortem interval (PMI) estimations. Future forensic investigations should account for the effects of enclosement when interpreting insect evidence to improve minPMI accuracy.

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