

Species, abundance, and time of appearance of fly larvae in white rats (*Rattus norvegicus* Berkenhout 1769) carcass with different burnt times

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ABSTRACT. Insects are still rarely used to determine the post-mortem interval (PMI). The flies during the period of laying eggs and forming larvae can estimate the time of death. This study aims to determine the species abundance, and time of appearance of fly larvae on white rat carcasses that were treated with burning. The experiment was conducted on 12 female white rats of the wistar strain, aged two-three months, healthy, and weighing 150-200 g. The research was experimental which consisted of 4 treatments with 3 repetitions of each treatment with variations in burning time, namely A (10 min), B (20 min), and C (30 min). Observations were made for five days until the carcass underwent complete decomposition. Larvae were taken from each carcass using tweezers, then put into vials containing 70% alcohol. The larvae were then identified morphologically in the laboratory. There were types of fly larvae found in the burnt treated white rat carcasses, namely *Sarcophaga argyrostoma* and *S. haemorrhoidalis*, while the controls were *Chrysomya albiceps*, *C. megacephala*, and *C. bezziana*. *S. argyrostoma* was found with the highest abundance in the treatment (39.9 individuals), while *C. albiceps* was found in the control (346.2 individuals). The first fly larvae that appeared in the burning treatment were *S. argyrostoma* (31 h) and those of the control *C. megacephala* (31 h). The variation of combustion has or does not affect the type, abundance, and time of appearance of fly larvae on the carcass.

Keywords: Burnt time; fly larvae; forensic entomology; *Sarcophaga* sp.; time of death

Article History: Received 18 August 2022; Received in revised form 10 October 2022; Accepted 1 December 2022; Available online 30 December 2022. Ver: Pre-Press

How to Cite This Article: Putra ILI, Rahmadi MIN. 2022. Species, abundance, and time of appearance of fly larvae in white rats (*Rattus norvegicus* Berkenhout 1769) carcass with different burnt times. *Biogenesis: Jurnal Ilmiah Biologi*. vol 10(2): 206–214. doi: <https://doi.org/10.24252/bio.v10i2.31392>.

INTRODUCTION

Unnatural deaths often occur in various places in the world, one of which is death due to fire (Debata *et al.*, 2014). According to data from the World Health Organization (2017), cases of unnatural deaths due to fires around the world ranged from 6.2 million people in 2017. Indonesia, one of which is DKI Jakarta, recorded cases of unnatural deaths due to burning around 53 people in 2020 (Arief, 2020). Cases of unnatural death such as burning are closely related to the determination of the post mortem interval (PMI). PMI is the determination of the estimated time between death and the discovery of a corpse (Samuel & Prainsack, 2019). Several methods have been developed in determining PMI, one of which is forensic entomology (Byrd & Brundage, 2016).

Forensic entomology utilizes the use of insects found in corpses to estimate the time of death (Goyal, 2012). According to Badenhorst and Villet (2018), it is known that insects as forensic indicators in determining PMI that first come to carcasses are flies. Flies have a keen sense of smell like the stench after death. This is because the fly has a sucking-licking type mouth that has a *sponge* that can be used to absorb food. One way to determine the estimated time of death is to look at the life cycle of flies (Umar & Algozi, 2013). According to Widyaningsih (2013), the way to determine the estimated time of death is to look at the growth time of fly larvae, such as humidity, temperature, exposure to corpses, and others. In line with the research of Chen *et. al.* (2011) regarding the parameters and growth time of *Hypopygiopsis violacea* (Family Calliphoridae), stated that if a pupa of this species was found in a carcass, it could be estimated that the age of the carcass was between 7 - 12.5 days, 3 instar larvae (1.5 - 2 days), larvae instar 2 (\pm 1.5 days), larvae instar 1 (\pm 0.5 days), and eggs were estimated to be less than half a day old.

Putra and Yahya (2021) have conducted research on forensic entomology using carcasses of white rats by burning them. Nevertheless, there isn't any research that used corpse dead by burned to determine the time of death. In addition, the determination of PMI on carcasses of white rats by

burning treatment has never been done. Corpse that had been burned need a lot of time to identified the time of death, so this research can help forensic officer to determine the time of death by identifying flies larvae that found in the corpse. This research also important to do because by determining the type, abundance, and time of appearance of fly larvae in white rat carcasses can be used as reference material in determining PMI, especially in cases of death by burning. The purpose of this study was to determine the type of fly larvae found in the carcass of white rats by burning treatment, to determine the abundance of fly larvae species in each variation of the burning time given to white rats, and to know the type of fly larvae that appeared for the first time as well as the timing of their appearance in the carcass of white rats by burning treatment. The benefit of this research is to obtain scientific data regarding the types of fly larvae found in white rat carcasses by burning treatment. In addition, the results of this study can be used as an illustration of determining the PMI in cases of unnatural deaths, especially deaths due to burns.

MATERIALS AND METHODS

The tools used in this study were a cage measuring 38 cm × 32 cm as a place for keeping white rats for 1 week, *stainless* clips measuring 32 cm × 21 cm as clamping white rats when burned, a mercury thermometer to measure the body temperature of the carcass, a stopwatch to count the time. in the treatment of burning white rats, thermohygrometer to measure humidity and air temperature, anemometer (Benetech, GM816) to measure wind speed, lux meter (AS803) to measure light intensity, killing bottle size 12 cm × 26 cm to place anesthesia for white rats, tweezers to take fly larvae, stereo microscope to observe fly larvae, Opti lab to take pictures of fly larvae, slide glass to place fly larvae to be observed, 120 mm diameter petri dish for placing fly larvae when identified, 100 ml plastic bottle for collection fly larvae from carcasses, 300 ml plastic cups for maintenance n fly larvae obtained, MDN brand meter measuring 1.5 meters to measure the distance between one carcass, roll meter measuring 10 m to measuring land area, strimin wire to cover carcasses so that they are not taken by wild animals, cellphone cameras for documentation during research, ATK to record research data and a drinking bottle measuring 60 ml as a place to drink the test rats.

The materials used in this study were 12 female wistar rats aged 2-3 months, healthy, and weighing 150-200 g, coconut shell as an ingredient to produce coals in the burning process of white rats, 70% alcohol, ether 10%, matches, masks, gloves, label paper measuring 17 × 58 cm, cotton, dry tissue and white mouse feed in the form of AD 2.

Procedures (preparation phase). Female white rats aged 2-3 months, healthy, and weighing 150-200 g as many as 12 as test animals. Tools and materials were prepared. The design model used in this study was a completely randomized design (CRD) consisting of 4 treatments and 3 replications with experimental treatment with variations in combustion time and control. White rats were acclimatized for 1 week in cages to uniform the way of life and food in the test animals used. During the acclimatization period, white rats were fed AD 2 which was given 2 times a day with the amount of feed given 10% of the white rat's body weight and given water ad libitum.

Treatment phase. White rats were divided into 4 treatment groups, each group was repeated 3 times. Each group was burned using stainless tweezers on a fire with various variations in burning time, including: Group 1 was burned with a burning time variation of 10 min by first being anesthetized using 10% ether, then immediately dislocating the cervical spine. After confirming that the white rat was killed, it was burned for 10 min using stainless tweezers over a fire; Group 2 was burned with a variation of the burning time of 20 min by first being anesthetized using 10% ether, then immediately dislocating the cervical spine. After confirming that the white rat was killed, it was burned for 20 min using stainless tweezers over a fire; Group 3 was burned with a variation of the burning time of 30 min by first being anesthetized using 10% ether, then immediately dislocating the cervical spine. After confirming that the white rat was killed, it was burned for 30 min using stainless tweezers over a fire; and Group 4 dislocated the cervical spine by first being anesthetized using 10%

ether, then holding the neck of the white rat between the thumb and index finger of the left hand, after that the tail of the white rat was pulled with the right hand.

Carcass placement phase. After all the white rats were treated, then the carcasses of the white rats were placed in an open field covered with strimin wire to cover all parts of the carcass. The distance of each carcass between treatments and repetitions was 2.5 meters.

Observation and fly larvae collection phase. Observation and collection of fly larvae on white rat carcasses was carried out every day in the morning and evening for 5 days. The fly larvae on each carcass were taken using tweezers, then reared first until they reached the 3rd instar in a plastic cup with the carcass meat being fed. After the fly larvae entered instar 3, they were transferred to a vial containing 70% alcohol.

Identification of fly larvae phase. Identification of fly larvae was carried out at the Ecology and Systematics Research Laboratory, Biology Study Program, Universitas Ahmad Dahlan. The fly larvae that have been taken are then grouped based on their morphological similarities. Each group was then identified by looking at the morphology and posterior spiracles of the larvae using a stereo microscope. The characteristics obtained were then matched with the identification key in the Identification of British Insect and the larval identification key journal (Amendt *et al.*, 2004; Tuzun *et al.*, 2010; Ghosh *et al.*, 2017; Mona *et al.*, 2019). The fly larvae that have been obtained are then photographed to identify the species and the number of individuals for each species found is counted.

Data analysis. The data analysis used in this research is descriptive and inferential analysis. Descriptive analysis was used to describe the species of fly larvae found in various combustion treatments, as well as to describe the species of fly larvae that first appeared and the time of their appearance on the carcass of white rats. Inferential analysis was used to calculate the abundance of each fly larvae species in various combustion treatments. The inferential analysis used is the normality test, homogeneity test, and the average difference test. Normality test is used to determine whether the data is normally distributed or not. Normality test using One-Sample Kolmogorov-Smirnov Test. If the data is normally distributed, homogeneity test is carried out to test whether the data distribution is homogeneous or not. Homogeneity test using Levene Test. Furthermore, if the data is not normal, then a non-parametric test is carried out using Kruskal-Wallis to make a decision that the data has no difference or there is a difference in the average abundance of fly larvae by treatment.

RESULTS AND DISCUSSION

Fly larvae species in white rat carcass. Based on the identification of the samples obtained during the research, five species of fly larvae were found in the carcass of white rats. The five species of fly larvae were *Sarcophaga argyrostoma* (Fig. 1), *S. haemorrhoidalis* (Fig. 2) from the Sarcophagidae family; *Chrysomya albiceps* (Fig. 3), *C. megacephala* (Fig. 4), and *C. bezziana* (Fig. 5) from the Family Calliphoridae (Table 1). The fly larvae of *S. argyrostoma* were found in the burn treatment, while *S. haemorrhoidalis* were found in the burn treatment and control. The larvae of flies from the Calliphoridae family (*C. albiceps*, *C. megacephala*, and *C. bezziana*) were only found in the control treatment.

Table 1. Fly larvae species found in white carcass

No	Family	Fly species	Treatment			
			Burnt 10 min	20 min	30 min	Control
1	Sarcophagidae	<i>S. argyrostoma</i>	√	√	-	-
		<i>S. haemorrhoidalis</i>	√	√	-	√
2	Calliphoridae	<i>C. albiceps</i>	-	-	-	√
		<i>C. megacephala</i>	-	-	-	√
		<i>C. bezziana</i>	-	-	-	√

The results obtained in Table 1 show that there is a difference between the burn treatment and the control. The difference in treatment given to white rat carcasses will also get different fly larvae (Putra & Yahya, 2021). The results obtained in the control obtained more fly larvae species than burned. This is because the white rats in the control were killed by cervical dislocation, thus getting more types of fly larvae. According to Switha *et al.* (2019), the carcass of a white rat killed by dislocation will cause the decomposition process to occur naturally. The natural decomposition in question is a change in the condition of the carcass with a long time (Siddiki & Zambare, 2017). These changes include stiffness, bruising, swelling due to gas produced by the anaerobic metabolism of bacteria, emitting a foul odor, and ending with a dry carcass condition (Siddiki & Zambare, 2017). The stages that occur during the natural decomposition will affect the number of species and individual fly larvae in the carcass due to the complete decay process (Archer & Elgar, 2013).

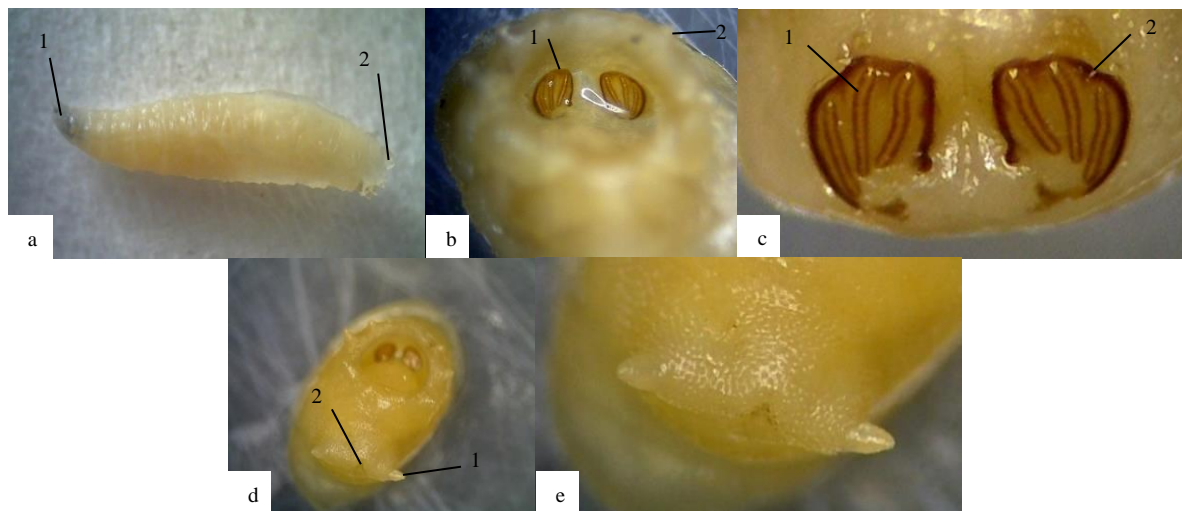


Fig. 1. *Sarcophaga argyrostoma* larvae characteristics: a. 3rd instar larvae: 1. Anterior, 2. Posterior; b. Posterior abdomen of the larvae: 1. Posterior spiracle, 2. Papilla; c. Posterior spiracle: 1. Spiracular slit, 2. peritrem wall; d. Posterior from a far: 1. Anal pad, 2. Anal opening; e. Excretory pore.

In contrast to carcasses that decompose due to certain factors, it will definitely affect the amount of meat contained in the carcass (Ody *et al.*, 2017). This large amount of meat can cause the type and abundance of flies to be found in carcasses with dislocation treatment compared to burning treatment. This is in accordance with the research of Nitiprodjo and Maulana (2018), which found types of fly larvae from the Calliphoridae and Sarcophagidae families in the high abundance dislocation treatment, which indicated that the dislocation treatment was included in natural decomposition, compared to the burning treatment, which was included in decomposition due to certain factors.

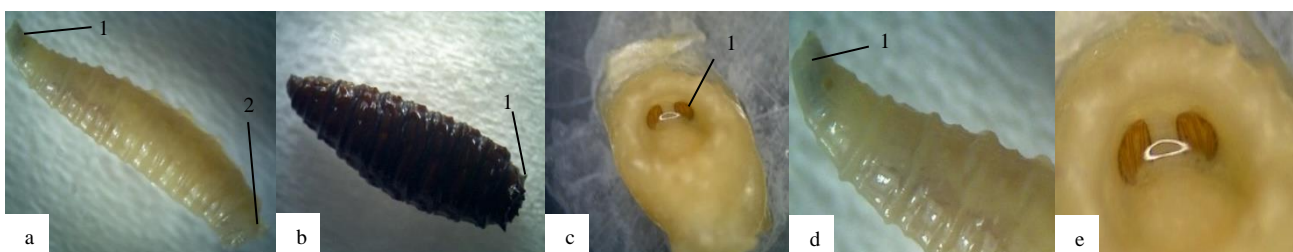


Fig. 2. *Sarcophaga haemorrhoidalis* larvae characteristics: a. 3rd instar larvae: 1. Anterior, 2. Posterior; b. Posterior spiracle with tuberculum: 1. Tuberculum; c. Larvae posterior abdomen: 1. Posterior spiracle; d. Anterior larvae: 1. Head capsule; e. Posterior spiracle

According to Haefner *et al.* (2012), species from the family Sarcophagidae will come under all conditions of exposure to carcasses. In contrast to species from the Calliphoridae family that will come to carcasses under certain conditions, such as carcasses that emit a rancid odor (Behnia &

Desplan, 2015). In addition, the Sarcophagidae family can also carry out its life cycle at a temperature of 25°C and its life cycle will be faster at higher temperatures (Showman & Rutledge-Cornelly, 2012). According to Byrd & Castner (2010), the optimum temperature of the Sarcophagidae family in its development is from 22° - 35°C, so that larvae from this family can be found in burned carcasses (body temperature of the burned carcasses is around 31°C on the first day of treatment, whereas on the second day, the carcasses body temperature that was burned followed the ambient temperature of around 26°C).

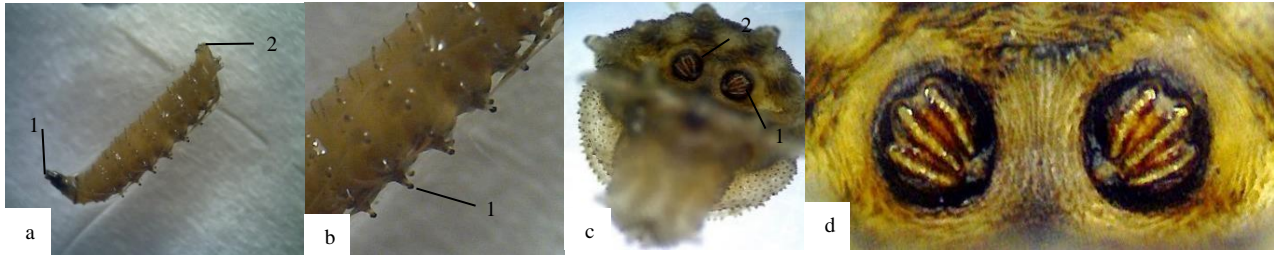


Fig. 3. *Chrysomya albiceps* larvae characteristics: a. 3rd instar larvae: 1. Anterior, 2. Posterior; b. Larvae body: 1. Spina; c. Larvae posterior abdomen: 1. Peritrem wall, 2. Spiracular slit; d. Posterior spiracle

The results of this study are also in line with those of Putra and Yahya (2021), who found fly larvae from the Sarcophagidae family on the carcass of wistar rats that were treated with fire. Fly larvae *S. argyrostoma* in the control treatment because the larvae of this species were secondary invaders of carrion (Tantawi *et al.*, 2018). According to Verves *et al.* (2018), when the carcass contains many fly larvae from other species, *S. argyrostoma* will not come to it. The results of the study did not find fly larvae from the Calliphoridae family in the burned treatment due to a delay in the oviposition of Calliphoridae flies on burned carcasses (Whitaker, 2017). This is because the burned carcasses will experience a dehydration process in the skin which causes the flies from the Calliphoridae family to be unsuitable for oviposition. According to Amendt *et al.* (2004), female flies from the Calliphoridae family will not lay eggs in dehydrated tissue because eggs and larvae from this family require relatively high humidity for development. According to Fisher *et al.* (2011), the relative humidity for female flies from the Calliphoridae family to oviposition is 55 – 90%.

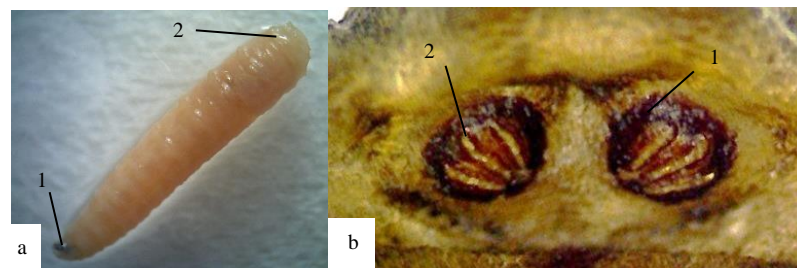


Fig. 4. *Chrysomya megacephala* larvae characteristics: a. 3rd instar larvae: 1. Anterior, 2. Posterior; b. Posterior spiracle: 1. Peritrem wall, 2. Spiracular slit

Burning in this study was divided into three treatments. The treatments were burning with a time of 10 min, 20 min, and 30 min. The results obtained in the 10 min and 20 min burning groups found larval species of *S. argyrostoma* and *S. haemorrhoidalis* from the Sarcophagidae family, while in the 30 min burning group no fly larvae were found. The results of observations on white rats burning for 30 min gave off a scorched odor on their bodies, while those burning for 10 min and 20 min gave off a ripe smell with open flesh. According to Hansbrough and Hansbrough (2012), with variations in the burning time of 30 min or fourth-degree burns (charring injury), the body of a white rat carcass looks black like charcoal, the muscles on the body shrink, all the skin and subcutaneous tissue and bones look charred. This causes the 30 min burning time not found fly larvae. In contrast to the burning time of 20 min and 10 min, the larvae of flies from the Sarcophagidae family were found.

When burning with a range of 30 min, the level of damage to the body will cause extensive fragmentation of the skull, charred, and the absence of remaining flesh on the body. While in the range of 10 – 20 min, the level of damage to the body is still in the moderate range. Burns in the moderate range (degree II) cause the skin to split open so that body tissues become exposed. According to Hansbrough and Hansbrough (2012), the body of a white rat carcass at the time of burning 10-20 min or a second degree burn (partial thickness burn) causes the skin tissue to peel off so that the skin is exposed or the flesh is visible which can attract flies from the Sarcophagidae family to come to it.



Fig. 5. *Chrysomya bezziana* larvae characteristics: a. 3rd instar larvae: 1. Anterior, 2. Posterior; b. Anterior of the larvae: 1. Cephalopharyngeal, 2. Mental spine; c. Posterior abdomen of the larvae: 1. Posterior Spiracle; d. Posterior spirakel: 1. Peritrem wall; 2. Spiracular slit

Fly Abundance in White Rats Carcass. The average abundance of individual fly larvae collected in the burn treatment (10 min, 20 min, and 30 min) and control (dislocation) were then compared. The results of the average abundance of individual fly larvae from each treatment can be seen in Table 2.

Table 2. Fly larvae abundance in each treatment

Treatment	Fly species	Collection in days-					Σ	\bar{X}
		1	2	3	4	5		
A (10 min)	<i>S. argyrostoma</i>	-	37	13	-	-	50	16.7
	<i>S. haemorrhoidalis</i>	-	7	9	5	-	21	7
B (20 min)	<i>S. argyrostoma</i>	21	24	21	4	-	70	23.2
	<i>S. haemorrhoidalis</i>	-	29	11	10	-	50	16.7
C (30 min)	<i>S. argyrostoma</i>	-	-	-	-	-	-	-
	<i>S. haemorrhoidalis</i>	-	-	-	-	-	-	-
D (control)	<i>C. albiceps</i>	-	102	459	343	135	1039	346.2
	<i>C. megacephala</i>	74	208	11	5	-	298	99.2
	<i>C. bezziana</i>	-	-	3	2	2	7	2.2
	<i>S. haemorrhoidalis</i>	-	-	-	29	3	32	16

Based on the results obtained in Table 2, the treatment burned at a burning time variation of 20 min was more abundant with the average number of individual fly larvae of *S. argyrostoma* (23.2 individual) and *S. haemorrhoidalis* (16.7 individual) were compared with variations in burning time of 10 min, even the two flies were not found in variations of 30 min of burning time. This is possible because in the variation of the burning time of 20 min, the carcass of white rats experienced a level of body damage that caused the skin to split open so that it was exposed or visible flesh on the burned body parts. According to Hansbrough and Hansbrough (2012), the body of a white rat carcass at a time of 20 min of burning will cause the skin tissue to peel off so that the flesh is exposed or visible, there are bullae and slight edema of the skin. Thus, attracting *S. argyrostoma* and *S. haemorrhoidalis* from the Sarcophagidae family to visit the carcass at a variation of 20 min of burning time. The presence of exposed skin tissue at 20 min of burning also helps in the development of fly larvae. The ease of getting food will certainly make the two flies lay lots of eggs and their development becomes faster. This is in accordance with research from Mashaly (2016), who found an abundance of larvae

of flies from the Sarcophagidae family in carcasses with exposed skin tissue compared to carcasses without exposed skin tissue.

The average abundance of *S. argyrostoma* (23.2 individual) was higher than *S. haemorrhoidalis* (16.7 Individual) in the 20-min burning treatment. This is possible because *S. argyrostoma* flies appeared first and thrived on white rat carcasses. According to Byrd & Brundage (2016), *S. argyrostoma* flies will first visit the carcasses in a location suitable for growth (optimum growth temperature 22° - 35°C) and will develop rapidly. Unlike the small number of individuals found in *S. haemorrhoidalis*. According to Ren *et.al.* (2018), *S. haemorrhoidalis* will not lay its larvae on a carcasses where there are already many fly larvae from other species on the carcasses. This is because *S. haemorrhoidalis* is known to have a behavior to avoid competition for food, both intra-species and between species (Supriyono *et al.*, 2019). The species of flies that arrive earlier will dominate the carcasses more than the species of flies that arrive later (Rusidi *et al.*, 2019). This is in accordance with the research of Szpila *et. al.* (2015), which states that competition from fly species to get food is usually dominated by fly species that reach the carcasses first.

In contrast to the variation of the burning time of 10 min, white rat carcasses experienced a level of body damage that was only on the surface of the skin, no bullae, slight edema, and no exfoliated skin tissue. This is because the body of the carcass is not suitable for development for *S. argyrostoma* and *S. haemorrhoidalis* flies. The discrepancy from the condition of the carcass at 10 min of burning was that there was no damage or opening in the skin, so that these two species from the Sarcophagidae family had difficulty in getting food. In the 30 min burning time variation treatment, no fly larvae were found on the carcass of white rats. This is because the body of the white rat carcass looks black like charcoal, the muscles in the body shrink, all the skin and subcutaneous tissue and bones look charred.

The average abundance of fly larvae on carcasses with the treatment given was then tested non-parametrically using Kruskal-Wallis. Based on the results of the Kruskal-Wallis test above, it can be concluded that the Asymp.Sig value is 0.714 > 0.05 (no difference or H0 is accepted). This means that there is no difference between the average abundance of fly larvae and the treatment. This was possible because the white rat carcasses were placed in all treatments at the same location and ambient temperature. In addition, the biology of the flies of the Calliphoridae and Sarcophagidae families in their development is in accordance with the location of the carcass, which is ± 26°C.

Fly larva appearance in white rats carcass. Based on observations during the study, it was found that the first fly larvae to appear were *Sarcophaga argyrostoma* with an appearance time of 31 h after placing the carcass in the burned treatment and *Chrysomya megacephala* with an emergence time of 31 h after placing the carcass in the control treatment (Table 4).

Table 3. Time of appearance of fly larvae in white carcass

Fly species	Time of appearance (h)			
	Treatment	10 min	20 min	30 min
<i>S. argyrostoma</i>	-	48	31	-
<i>S. haemorrhoidalis</i>	96	55	48	-
<i>C. albiceps</i>	48	-	-	-
<i>C. megacephala</i>	31	-	-	-
<i>C. bezziana</i>	72	-	-	-

The type of fly larvae that first appeared was *S. argyrostoma* at 31 h after placing the carcass in the burn treatment with a burning time variation of 20 min. The body of the carcass of a white rat that was treated for 20 min was burnt and the body was damaged which caused the skin to split open so that the flesh was visible. This attracts the arrival of *S. argyrostoma* to lay their eggs on the carcass. In contrast to *S. haemorrhoidalis* which appeared for the first time within 48 h after placing the carcass in the burn treatment with a burning time variation of 20 min. This is possible because the carcasses

of white mice previously contained fly larvae from other species so that *S. haemorrhoidalis* avoided competition for food. The results of observations, no larvae of *S. argyrostoma* and *S. haemorrhoidalis* at a 30 min burning time variation because the condition of the level of damage to the body looked black like charcoal, the muscles in the body were shrinking, all the skin and subcutaneous tissue and bones looked charred. This causes the 30 min burning time not to find any fly larvae.

The emergence of *S. argyrostoma* and *S. haemorrhoidalis* fly larvae at 10 min treatment was higher (the emergence time was 48 h for *S. argyrostoma* and 55 h for *S. haemorrhoidalis*). This is because after 10 min of burning, the body of the carcasses experiences a level of damage to the body which is only on the surface of the skin tissue. In addition, *S. argyrostoma* and *S. haemorrhoidalis* flies are not suitable for developing food.

CONCLUSION

The types of fly larvae on the carcasses of white rats that were given burn treatment were *S. argyrostoma* and *S. Haemorrhoidalis*. Variations in burning time of 10 min, the abundance of larvae of *S. argyrostoma* as much as 16.7 and *S. haemorrhoidalis* as many as 7, in burning time of 20 min the number of larvae of *S. argyrostoma* as many as 23.2 and *S. haemorrhoidalis* as many as 16.7, and in 30 min of burning time, no fly larvae were found in the carcass of white rats. The type of fly larvae that first appeared on the carcass of white rats was *S. argyrostoma* at 31 h with a burning time variation of 20 min.

ACKNOWLEDGEMENTS

Authors thank Universitas Ahmad Dahlan for supporting the research process.

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