



## Nocturnal Larviposition Behaviour of Necrophilous, Carrion-Associating Flesh Flies (Diptera: Sarcophagidae) in Ungwan-Rimi Area Kaduna, Nigeria

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### ABSTRACT

Insects are known to have been used in the detection of crime for a long time. In order to interpret a crime scene, it is important to know which species of insects are infesting the body and something about their habits and environmental requirements. This study was conducted to investigate specie composition, abundance and nocturnal oviposition/larviposition behaviour by carrion associating flies, 200 grams of fresh beef liver was used as oviposition/larviposition medium and was exposed immediately after sunset at different time intervals (1-hour interval from sunset) in both dry and wet seasons. Exposed baits were taken to forensic laboratory for observation. A total of 1221 insects were collected comprising of 842 (69%) and 379 (31%) in dry and wet seasons respectively. The insects were found to belong into families; Calliphoridae, Muscidae and Sarcophagidae. Muscidae had the highest abundance 546 (64.85%), followed by Calliphoridae 27 (33.14%), Sarcophagidae had the least abundance with 17 (2.01%) in dry season. During the wet season Calliphoridae had the highest abundance with 279 (57.26%), and Muscidae had the least with 162 (42.74%). Sarcophagidae were however not abundant. All six species were abundant in both dry and wet seasons, with the *Musca domestica* being the most dominant. Nocturnal larviposition was observed during dry season only. This occurred at 6.40 to 7.40pm and 8.40 to 9.40pm within a temperature range of 29°C - 35°C. Out of the 23 species of flies that emerged 8 were identified *Sarcophaga inzi* while 15 species as *Sarcophaga* sp, 15(65.22%) were *Sarcophaga inzi* and 8(34.78%) were *Sarcophaga* spp. This study shows that genus *Sarcophaga* has a tendency of nocturnal larviposition, and could be useful material in forensic investigations.

**Keywords:** Forensic, Insects, Kaduna, Larviposition, Nocturnal

### INTRODUCTION

The scientific study of insects has become inevitable because they are the most diversified animals ever known to man. As a group, they have become one single factor that has influenced the entire life system (Omoloye, 2008). One beneficial outcome of insects as the most abundant animals in the world is their ubiquitous presence, including crime scenes (Ekanem and Usua, 2004). From this factor, forensic entomology, the scientific discipline, which aims to interpret information on homicide cases using insects as silent witnesses at the scenes came into being (Ekanem and Usua, 2004). Some insects are referred to as carrion feeders and thus play a prominent role in the decomposition of carrions.

Forensic entomology is the analysis of insect evidence for forensic and legal purposes (Amendt *et al.*, 2007). The most important task in forensic investigation is the estimation of the minimum time since death (Amendt *et al.*, 2011). The majority of cases where entomological evidence has been used are concerned with illegal activities which take place and are discovered within a few time of being committed. Gaudry *et al.* (2004) commented that in France, 70% of cadavers were found outdoors and of this 60 % were less than 1 month old. The applications of forensic entomology are numerous, encompassing any situation which involves an interaction between insects and other arthropods, plus the law.

Insect diversity varies with seasonality, temperature, and geographic location (Erzinclioglu 1983). These factors affect species diversity, but also affect arthropods' lifecycles,

causing a difference in developmental time; physical size, and body mass (Davies and Ratt-Cliffs, 1994), for example, dipterans larvae have an optimum range of temperatures conducive to their development. As temperature increases development rates for dipterans larvae increase. As developmental rates increase metabolic heat generated by the larval mass also increases, thus increasing the temperature within the carrion (Erzinclioglu, 1983).

In Nigeria, few studies relating to forensic entomology are conducted so far (Usua, 2007). Therefore, it has not been applied in the investigation of cases of homicide and mysterious deaths. This necessitates carrying out this study of nocturnal larviposition of Flesh flies in the Kaduna area, using the fresh beef liver as bait, with the aim of investigating nocturnal oviposition/larviposition behaviour of carrion-associating flies their composition and dominance in Ungwan Rimi of Kaduna State, Nigeria.

### MATERIALS AND METHODS

#### Study Site Location

The study was conducted at Bala Crescent, behind ECWA Nursery and Primary School, Federal Low-Cost Housing Estate Ungwar Rimi, Kaduna, Kaduna State Nigeria (Figure 1). The area is located at coordinates 10.5289° N, and 7.4625° E. The study site has residential houses with a cemented floor and about 6 grown trees around. The investigation was conducted at the study site during the dry and wet seasons of 2016. The environment is the urban and experimental site was kept clean of trash and human refuse

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### Trapping of Adult Flies (Control)

Diurnally, trapping of adult flies was done at noon every day using a fly trap (Tesco trap) with the wasted liver bait that had no larviposition after 24 hours. Adult flies collected from the Tesco trap were classified based on their morphological features. All specimens for this study were collected as adults and then taken to the Insect Museum of Institute of Agricultural Research (IAR) Ahmadu Bello University Zaria (A.B.U.) for identification to species level.

### Experimental baited liver in the dry season

Six baits were exposed overnight each day. The exposed baits were taken to the forensic laboratory and observed further for another 24 hours for evidence of oviposition/larviposition.

### Species identification

All specimens for this study were collected as adults and then taken to the Insect Museum of Institute of Agricultural Research (IAR) Ahmadu Bello University Zaria (A.B.U.) for identification to species level using taxonomic keys. In the case of positive nocturnal oviposition/larviposition responses, maggots in the rearing containers were bred through to adulthood before being identified.

### Data Analysis

Results were presented in tables and percentages using Microsoft excel. Shannon Weiner and Simpson's index tests were carried out on the sampled arthropods to determine the diversity; richness and evenness of the species collected. A paired t-test was used to determine if there exist significant differences between rainy and dry season mean total abundance of necrophagous insects.

### RESULTS

A total of 1221 insects belonging to Families Calliphoridae, Muscidae and Sarcophagidae were collected from the control bait exposed diurnally, comprising of 842 and 379 insects in the dry wet seasons respectively (Tables 1 and 2). In dry season, Muscidae had the highest abundance 216(64.85%), followed by Calliphoridae 27(33.14%). Sarcophagidae had the least abundance of 17(2.01%). During wet season, Calliphoridae had the highest abundance 217(57.26%), and Muscidae with the least 162(42.74%). Sarcophagidae were however not abundant in wet season. Six species (*Chrysomyia Chloropyga*, *Chrysomyia albiceps*, *Hemipyrellia fermanica*, *Musca domestica*, *Sarcophaga inzi* curran, *Sarcophaga sp*), were collected. Of these, *Musca domestica* L (64.85%) was the most dominant followed by *Chrysomyia chloropyga* (18.53%), while *Chrysomyia albiceps* recorded (14.01%). Analysis of Shanon (H) and Simpsons index (D<sub>s</sub>) indicates that dry season had a value of (0.726 and 0.470) respectively (Tables 4 and 5), suggesting that species of flies in the dry season had a more diverse and richer population. During the wet season, *Chrysomyia Chloropyga*, *Chrysomyia regalis*, *Isomyia nitida*, *Isomyia dubiosa*, *Musca domestica*, and *Musca sorbens*, were collected. Of these species, *Musca domestica* L (34.83%) was the most dominant followed by *Chrysomyia chloropyga* (28.5%) and *Chrysomyia regalis* (14.8%). Shanon (H) and Simpsons index (D<sub>s</sub>) also indicates species in wet season had a value of (0.683 and 0.491) respectively as seen in Tables 4 and 5. Results shows that there is a significant difference in arthropod abundance between both seasons ( $P < 0.05$ ) with  $t_{stat} < t_{crit}$ .

**Table 1:** Diurnal Abundance of trapped Adult Flies for the control during the dry season

Order	Family	Species	Total	Percentage (%)
Diptera	Calliphoridae	<i>Chrysomyia chloropyga</i>	156	18.53
		<i>Chrysomyia albiceps</i>	118	14.01
		<i>Hemipyrellia fermanica</i>	5	0.59
	Muscidae	<i>Musca domestica</i>	546	64.85
		<i>Sarcophaga inzi. Curran</i>	5	0.59
	Sarcophagidae	<i>Sarcophaga sp</i>	12	1.43
<b>TOTAL</b>			842	100

**Table 2:** Diurnal Abundance of Trapped Adult Flies for the Control During the Wet Season

Order	Family	Species	Total	Percentage (%)
Diptera	Calliphoridae	<i>Chrysomyia chloropyga</i>	108	28.5
		<i>Chrysomyia regalis</i>	56	14.8
		<i>Isomyia nitida</i>	22	5.8
		<i>Isomyia dubiosa</i>	31	8.2
	Muscidae	<i>Musca domestica</i>	132	34.83
		<i>Musca sorbens</i>	30	7.9
<b>TOTAL</b>			379	100

**Nocturnal Larviposition Observed**

Nocturnal larviposition was observed in two plates only during the dry season experiment on the 3<sup>rd</sup> of March, 2016. The larviposition occurred at (6:40 – 7:40pm and 8:40 – 9:40pm) (Table 6) within a temperature range of 29° -35°C. No larviposition was observed in the exposed baits throughout the study during the wet season (Table 7) of the 23 adults that emerged from the exposed liver baits, 8/23 (34.78%) were identified as *Sarcophaga sp* and 15/23 (65.22%) as *Sarcophaga inzi* (curran.).

**Temperature and Humidity of study area in the dry and wet season**

The average temperature of the study site during the dry season was 24.7±4.7 °C while the average humidity was 37.4±16.8%, (Figure 2.). In wet season, the average temperature and humidity in the study site was 27.2±2.4 °C and 81.9±1.3 respectively.

**Table 3:** Adult flies that emerged from nocturnally larviposited eggs on the baited liver during the dry season

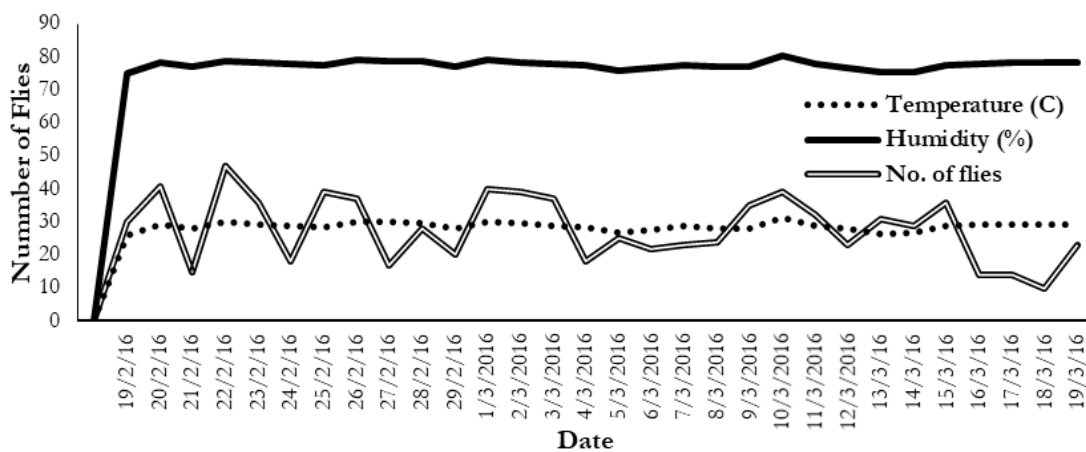
Family	Species	Time	Percentage (%)
Sarcophagidae	<i>Sarcophaga inzi</i> . Curran	8(6.40-7.40)pm	34.78
	<i>Sarcophaga Spp</i>	5(8.40-9.40)pm	65.22
		23	100

**Table 4:** Shanon-Weiner Index for the dry and wet season

Number of Dry	Prop. D	nProp. D	Prop. D*nPropD	Number of Wet	Prop. W	nProp. W	Prop. W*nPropW
279	0.331	1.105	0.366	217	0.573	0.558	0.319
546	0.648	0.433	0.281	162	0.427	0.85	0.363
17	0.02	3.903	0.079	0	0	.	.
842		H	0.726	379		H	0.683

**Table 5:** Simpsons Index for the Dry and Wet Season

	DRY				WET			
	n <sub>i</sub>	n <sub>i</sub> (n <sub>i</sub> -1)	N(N-1)	∑n <sub>i</sub> (n <sub>i</sub> -1)	n <sub>i</sub>	n <sub>i</sub> (n <sub>i</sub> -1)	N(N-1)	∑n <sub>i</sub> (n <sub>i</sub> -1)
Calliphoridae	279	77562	708122	375404	217	46872	143262	72954
Muscidae	546	297570			162	26082		
Sarcophagidae	17	272			0	0		
	842				379			
	D <sub>s</sub> =1-∑n <sub>i</sub> (n <sub>i</sub> -1) / N(N-1)= 0.470				D <sub>s</sub> =1-∑n <sub>i</sub> (n <sub>i</sub> -1) / N(N-1)= 0.491			



**Figure 2:** Temperature, humidity, and number of Adult flies in the study area during the dry season

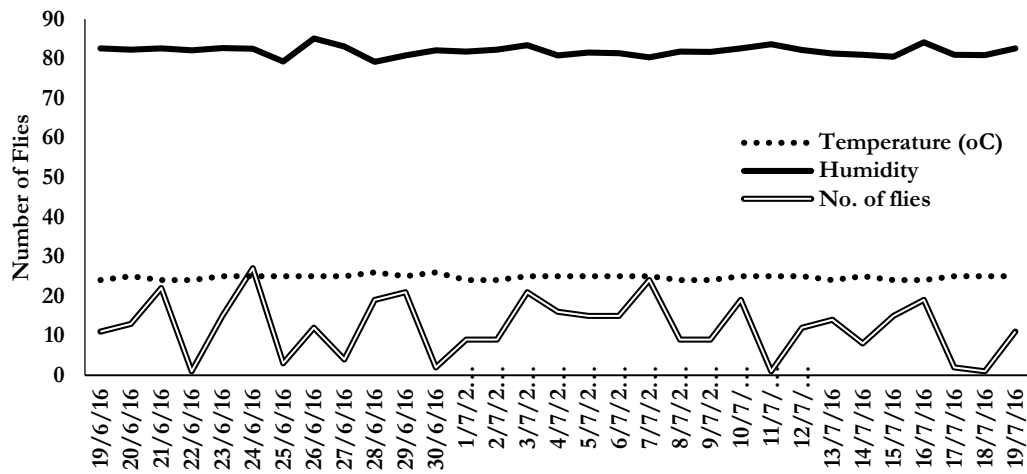


Figure 3: Temperature, humidity, and number of Adult flies in the study area during the wet season

Table 6: Experimental baited liver distribution during the dry season

Date	Sample					
	1	2	3	4	5	6
19/2/16*	-	-	-	-	-	-
20/2/16*	-	-	-	-	-	-
21/2/16*	-	-	-	-	-	-
22/2/16*	-	-	-	-	-	-
23/2/16*	-	-	-	-	-	-
24/2/16**	-	-	-	-	-	-
25/2/16**	-	-	-	-	-	-
26/2/16**	-	-	-	-	-	-
27/2/16**	-	-	-	-	-	-
28/2/16**	-	-	-	-	-	-
1/3/16**	-	-	-	-	-	-
2/3/16**	-	-	-	-	-	-
3/3/16**	+	-	+	-	-	-
4/3/16***	-	-	-	-	-	-
5/3/16***	-	-	-	-	-	-
6/3/16***	-	-	-	-	-	-
7/3/16***	-	-	-	-	-	-
8/3/16***	-	-	-	-	-	-
9/3/16***	-	-	-	-	-	-
10/3/16***	-	-	-	-	-	-
11/3/16***	-	-	-	-	-	-
12/3/16***	-	-	-	-	-	-
13/3/16***	-	-	-	-	-	-
14/3/16***	-	-	-	-	-	-
15/3/16***	-	-	-	-	-	-
16/3/16***	-	-	-	-	-	-
17/3/16***	-	-	-	-	-	-
18/3/16***	-	-	-	-	-	-
19/3/16***	-	-	-	-	-	-
20/3/16***	-	-	-	-	-	-
21/3/16***	-	-	-	-	-	-

KEY: \*- Sunsets at 6.40 pm, \*\*- Sunsets at 6.41 pm, \*\*\*- Sunsets at 6.42 pm; - Larviposition absent, + Larviposition present.

**Table 7:** Experimental baited liver distribution during the wet season

Date	Sample					
	1	2	3	4	5	6
19/6/16***						
20/6/16***	-	-	-	-	-	-
21/6/16***	-	-	-	-	-	-
22/6/16***	-	-	-	-	-	-
23/6/16***	-	-	-	-	-	-
24/6/16**	-	-	-	-	-	-
25/6/16**	-	-	-	-	-	-
26/6/16**	-	-	-	-	-	-
27/6/16**	-	-	-	-	-	-
28/6/16**	-	-	-	-	-	-
29/6/16**	-	-	-	-	-	-
30/6/16**	-	-	-	-	-	-
1/7/16**	-	-	-	-	-	-
2/7/16**	-	-	-	-	-	-
3/7/16**	-	-	-	-	-	-
4/7/16***	-	-	-	-	-	-
5/7/16***	-	-	-	-	-	-
6/7/16***	-	-	-	-	-	-
7/7/16***	-	-	-	-	-	-
8/7/16***	-	-	-	-	-	-
9/7/16***	-	-	-	-	-	-
10/7/16***	-	-	-	-	-	-
11/7/16***	-	-	-	-	-	-
12/7/16***	-	-	-	-	-	-
13/7/16***	-	-	-	-	-	-
14/7/16*	-	-	-	-	-	-
15/7/16*	-	-	-	-	-	-
16/7/16*	-	-	-	-	-	-
17/7/16*	-	-	-	-	-	-
18/7/16*	-	-	-	-	-	-
19/7/16*	-	-	-	-	-	-

**KEY:** \*- Sunsets at 6.40 pm, \*\*- Sunsets at 6.41 pm, \*\*\*- Sunsets at 6.42 pm; - Larviposition absent, + Larviposition present.

## DISCUSSION

The successions of the waves of insects colonizing a body have been studied by a number of researchers, including Carvalho *et al.* (2000) and Centeno *et al.* (2002). As it decomposes, the human corpse is large enough to support colonies of a number of different species of fly. Work by Archer and Elgar (2003) showed that after being exposed outdoors for 24 hours, the preferred colonization sites on a carcass changed from the orifices to skin folds. Such locations included between the legs or under the ear pinnae. Blowflies are amongst the first to colonize a body, the most common species initially present on the body are *Calliphora vicina* and *Calliphora vomitoria*. One of the first clues to their colonization is the presence of eggs on the corpse. The speed of decomposition is influenced by environmental conditions, so dermestids may also be found sooner.

In the dry season of this study, family Calliphoridae, Muscidae and Sarcophagidae were the most numerous families which are in accordance with various surveys performed in different geographical areas of Nigeria (Ekanem and Usua, 2004; Okiwely *et al.*, 2008; Ahmed *et al.*, 2017). The dominance of *Chrysomya Chloropyga* *Chrysomya albiceps* and *Musca domestica* observed in this study supports the report of Ahmed *et al.* (2017). The low prevalence of insects collected in the wet season could be attributed to a lower temperature and excess rainfall that occurred within the study period.

A similar observation was made by Okiwelu *et al.* (2008). Calliphoridae and Muscidae were the major families collected during this study. Shannon & Simpsons index also indicates that species of the wet season were less diversified but more evenly distributed than the species of the dry season (Ekanem and Usua, (2004). The dominance of *Chrysomya Chloropyga*, *Chrysomya regalis*, and *Musca domestica* observed in this study supports the report of Abajue *et al.* (2013)

In this study, both dry and wet seasons were observed for nocturnal larviposition/oviposition. The dry season supports higher relative humidity, and higher temperatures than the wet season, the result of the study revealed a larger number of flies due to the higher relative humidity and higher temperatures, similar report was observed in the work of Saunders (2001) the wet season supports lower relative humidity and lower temperatures, the result revealed a smaller number of necrophagous insect due to lower relative humidity and lower temperature.

It was observed that necrophagous flies oviposited on baits during nocturnal hours. The study showed that the primary fly species larvipositing on baits at the research site were *Sarcophaga* Species. When present and active in a given area, *Sarcophaga* Species is often one of the first necrophagous fly species to arrive on carrion in the fresh stage. This species has been collected from a large list of carrion, including human corpses, pig carcasses, rat carcasses, and processed beef in all seasons of the year and a wide array of habitats, with its development time ranging between 13 and 20 days (Kirkpatrick and Olson, 2001) in Central Texas. Within apparent seasonal or habitat restrictions, *Sarcophaga* Species is a potential key species in estimating post mortem intervals at any time of the year. Yet, little is known morphologically about these necrophagous fly species larvae. It is pertinent that these fly species be studied more thoroughly as to its morphology as well as its reproductive behaviour due to its potential forensic importance because of its nocturnal and year-round oviposition behaviour (Pohjoismaki *et al.*, 2010).

In the wet season of this study, no nocturnal larviposition/oviposition was observed irrespective of the humid conditions. The lack of warm temperature in the wet season of the year which is an environmental factor in nocturnal larviposition by necrophagous flies for this study created conditions in which necrophagous fly activity ceased after sunset. This result is in agreement with reports of Baldrige *et al.* (2006) in central Texas. Heavy rainfall has been reported to restrict the flight of necrophagous flies and consequently unfavorably affect the colonization of remains (Ngoen-Klanet, *et al.*, 2011; George *et al.*, 2013). The low temperature and heavy rainfall that occurred during the study period could be attributed to the absence of larviposition/oviposition on the exposed baited liver.

Several environmental conditions are thought to have a strong influence on the activities of necrophagous carrion-associating blood and flesh flies. Ambient temperature and relative humidity are the major environmental factors considered during this investigation. Because they are

poikilothermic, ambient temperature is the most crucial factor dictating the fly species activity. Generally, activity increases with increasing, and each insect's species has a minimum lure hold temperature for activity. Below this temperature, the energy required to fly cannot be produced (Mellanby, 1939). It is widely accepted that the minimum lures hold temperature is approximately 12°C for blowflies of forensic importance (Erzinioglu, 1996, Amend *et al.*, 2008). On the other hand, extremely high temperatures can be lethal to blowflies and their youngs (Wells and Lomotte 2010).

Relative humidity also affects colonization patterns (Pitts and Wall, 2004). During the period of low humidity, remains can become dehydrated or mummified. There is some evidence to suggest that female blowflies avoid depositing young on desiccated remains because the survival of the young is dependent on moisture and access to soft tissues (Cragg and Ramage, 1945; Campobasso *et al.*, 2009). By contrast, rainfall can adversely affect colonization by restricting the flight activity of blowflies (Greenberg, 1990; Greenberg and Kunich, 2002). Light rain does not appear to inhibit blowfly colonization (Reed Jr., 1958; Mahat *et al.*, 2009; Reibe and Madea, 2010), but colonization can be reduced or halted completely during heavy continuous rain (MacLeod, 1947; Green, 1951; Mann *et al.*, 1990).

There was a higher abundance of flies in the dry season than in the wet season. *Chrysomya chloropyga* and *Musca domestica* was the most prevalent species recovered from the carrion. Temperature and relative humidity affect nocturnal larviposition behaviour of necrophagous carrion-associated with blowflies. Inclusion of forensic entomology is highly recommended to be incorporated into Nigerian legal systems.

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