ARTHROPOD SUCCESSION ON EXPOSED AND SHADED MAMMALIAN CARCASSES IN NSUKKA, NIGERIA

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ABSTRACT

Ten Wistar rats were used as model to study arthropod succession on carcass under shade and exposed conditions. Carcass decomposition took longer periods under shade than on the exposed site. Four decomposition stages (fresh, bloated, decay and dry) were observed. A total of 164 arthropods were collected belonging to three classes: Insecta, Arachnida and Crustacean. Insects formed 95.12 %, arachnids 1.22 % and crustaceans 3.66 %. Large numbers of arthropods were found on the exposed carcass than the shaded carcass. Calliphorids and Sarcophagids were the primary colonizers observed breeding on the carcasses. The most abundant insects include Chrysomya albiceps (13.4 %) and Lucilia serricata (10.37 %). Other dipterans including Muscidae, Phoridae, Sepidae, Fannidae and Anthomyiidae were found during the bloated and decay stages. Families of the coleopterans including; Dermestidae, Silpgidae, Staphylinidae and Histeridae were observed during the bloated to the dry stages and fed on the immature dipterous maggots and carrion remains. Hymenopterans were observed throughout the process of decomposition and played a vital role in carcass decomposition. Other arthropods arrived mostly during the decay stage. Species richness on the carcasses peaked during the decay stage. The rate of decomposition of carcasses is affected by environmental factors such as temperature, relative humidity, precipitation and insect abundance.

Keywords: Post mortem interval, Carcass decomposition, Insect abundance, Forensic entomology

INTRODUCTION

Decomposition is a natural and necessary process responsible for the return of organic material to the environment through the breakdown of dead plant or animal matter. It represents a temporary and changing food source for varied and distinct community of organisms (Putman, 1983). Insects, especially coleopterans and dipterans are the major components of insect community which are primarily involved in the decomposition process (Kočárek, 2003). According to Bornemissza (1957), the decomposer community of a carcass goes through a process of ecological succession. The insects arrive in a determined sequence, producing an addition or substitution of species. The potential of insects as forensic indicators has stimulated detailed research regarding the role of fauna in carcass decomposition in various regions and countries (Tomberlin and Alder, 1998; Centeno et al., 2002; Kočárek, 2003; Okiwelu et al., 2008). The basic reason for using insects in criminal investigations resides in the fact that insects are usually the

first organisms to detect and find a cadaver and are involved in all stages of decomposition. Furthermore, some insect species are specific for certain areas and seasons (Carvalho et al., 2000). Also, it has been observed that oviposition can occur minutes after death (Smith, 1986). The knowledge of this succession pattern as well as their preference for the different stages of decomposition, and the meteorological data (temperature and humidity), have been applied in forensic science to determine the post mortem interval (PMI) of a corpse (Kočárek, 2003; Okiwelu et al., 2008). This field of forensic entomology involves the use of the knowledge of insects in investigation of crimes or even civil disputes. It also includes the application of the study of insects and other arthropods to legal issues, especially in the court of law (Okiwelu et al., 2008). Some examples of criminal cases where forensic entomology was successful in determining the outcome includes, one involving a young prostitute and a thirty-year-old member of the Army (Lord and Rodriguez, 1989). Her family members had informed the police of her vanishing approximately four days before her body was found. She had last been seen in the company of this military man on May 31. A number of adult flies and maggots were noticed and gathered inside and around the wounds of the young girl. A number of these maggots were kept and grown to be adult flies, and others were taken in their present state. Pictures were taken and enlarged showing the flies around the body when it was found. Using this evidence along with associating weather conditions known to be current around the time, an entomologist was able to give a PMI. The evidence showed that the flies had arrived on May 31, which implied a time period of four days since she had died. This information indicated the army man to be the last person to be with the girl. When presented with the evidence the authorities were able to get a confession out of the man. The use of forensic entomology was a strong factor in this case (Lord and Rodriguez, 1989).

Invasion time of the corpse is usually considered important since some insect species are always present, while others disappear and later reappear in the course of decomposition (Wolff et al., 2001). It is important to observe that not all the invertebrates found near a corpse are feeding on it. Based on this, four ecological categories have been recognized for the insect community found around corpses. These include: (i) necrophagous species which are flesh feeding species. They constitute the most important category to establish the time of death. (ii) Predators and parasites of necrophagous species, (iii) omnivorous species and the (iv) adventitious species that use the cadaver like an extension of their ecological niche (Smith, 1986).

The study of the entomofauna associated with cadavers has been an extremely effective tool to clarify numerous cases of homicides, sexual abuses and traffic of organs (Catts and Haskell, 1990). The types of insect present in a decomposing corpse are specific for certain areas and seasons. Invariably, certain groups will colonize first, such as blowflies (Calliphoridae) and flesh flies (Sarcophagidae), but the species involved will usually vary. It is therefore necessary, to study each region for associated arthropods with mammalian carcasses. This is a major factor that has hindered the application of forensic entomology in Nigeria, as only few studies has been carried out in the different geographical location of the country which include works by Okiwelu et al. (2008) in Rivers State, Ekanem and Dike (2010) in Uyo, Ekrakene and Iloba (2009) in Benin City and Abajue et al. (2014) in Okija. Therefore, it is necessary to carry out this study, as this will add to the available data on entomofauna present in mammalian carcasses in Nigeria. The objective of this study was to investigate insects visiting exposed and shaded decomposing rat carcasses to establish relationship between insects and carcasses which may be of forensic importance in Nsukka, Enugu State, Nigeria.

MATERIALS AND METHODS

Site Description: The study was carried out within the University of Nigeria, Nsukka environs. The climate in Nsukka is tropical with monthly temperatures ranging between 24 °C and 29°C (Inyang, 1978). There are two

seasons: the dry and rainy seasons. The dry season is from November to March, while the rainy season is from April to October. Two sites, an open grass area filled with *Pennisetum purpureum* (elephant grass), and abandoned building, 50 m apart opposite the maize farmland of the Faculty of Agriculture, University of Nigeria, Nsukka were used.

Study Design: Ten carcasses of laboratory rats (*Rattus norvegicus*: Berkenhout 1769) each weighing ~ 200 g were used as model. The rats were sacrificed by strangulation and immediately transported to the study area. Five carcasses were placed on each site in a cage (120 mm x 100 mm x 30 mm). The cages were covered with a 5 cm mesh to allow colonization of carcasses by insects, while preventing scavengers attack (Sukontason *et al.*, 2003).

Samples were collected twice a day (9:00 and 13:00) during the first (fresh) and second (bloated) stages of decomposition and thereafter once a day for up to the end of decomposition according to Mabika *et al.* (2014). Flying insects were caught using an insect net, while the immature insects and other arthropods were collected using forceps. The arthropods were killed with ethyl acetate and preserved either dry, or in 70 % alcohol for subsequent taxonomic determination. Samples of larvae were reared and identified (Mabika *et al.*, 2014).

Arthropods were identified to species level using keys of Elzinga (2004), Gennard (2007) and Carvalho and Mello-Patiu (2008). The ambient air temperature was recorded using a mercury column thermometer held inside the cage, 5 cm above ground (Feugang *et al.*, 2012).

Data analysis: Data was analyzed using Microsoft Office Excel® 2013 (Microsoft Incorporation, Redmond, USA). Arthropod relative abundance was computed in percentages as fraction of number of individuals in a given group divided by total number of individuals in groups of interest.

RESULTS

Phases of Carcass Decomposition: During the observation periods, the ambient temperature for both sites ranges from 25 °C to 28 °C (26.8 \pm 0.53 °C) on the exposed site and 25 °C to 29 °C (26.9 \pm 1.05 °C) on the shaded site. Relative humidity ranges from 80 to 100 % (98 \pm 5.05) on both sites, and a total of 5550 mm² of precipitation was recorded.

Decomposition period lasted for 23 days on the exposed carcasses and 25 days on the shaded carcasses. Four carrion decomposition phases, namely fresh, bloated, decay and dry were observed. The fresh stage lasted for less than 24 hours on the exposed carcasses, while on the shaded carcasses it was about 30 hours. Bloating occurred on the second day on both carcasses. It was characterized by the swelling of the abdomen and this lasted for two days on the exposed carcasses while on the shaded carcasses it lasted for three days. The decay stage started on the 5th day, with the breakingup of the skin at the eye and anal region. The abdomen later began to tear-up and the carcasses were relaxing a strong foul odour. Lots of maggots activities were observed on both carcasses, although, the maggots present on the exposed carcasses were greater in number than those of the shaded carcasses. The dry stage began when the maggots were observed moving out from the carcasses and lasted until carrion fauna were no longer found associated with the remains. By the end of the experiment, only dry skin, fur and bones were left of the carcasses.

Visiting Arthropod Species Abundance: A total of 164 arthropods belonging to 3 classes were collected. These include: Insecta, Arachnida and Crustaceans. Insects formed 95.12 % of the total arthropods collected, while the Crustacea contributed 3.66 % and the Arachnida 1.22 % (Table 1). Eight dipteran families (Calliphoridae, Sarcophagidae, Muscidae, Phoridae, Fannidae, Anthomyiidae, Sepsidae and Culicidae) were recovered from the exposed carcasses (Table 2), while six dipteran families (Calliphoridae, Sarcophagidae,

Class	Order	Family	Species	Abundance (%)
Insecta	Diptera	Calliphoridae	Chrysomya albiceps	22(13.4)
			Chrysomya megalocephala	9(5.49)
			Chrysomya chloropyga	7(4.27)
			Lucilia serricata	17(10.37)
			Lucilia illustris	7(4.27)
			Phormia regina	8(4.88)
				70(42.68)
		Muscidae	Musca domestica	10(6.10)
			Muscina stabulans	4(2.44)
				14(8.54)
		Sarcophagidae	Sarcophaga spp.	6(3.66)
		Anthomyiidae	Anthomyia spp.	1(0.61)
		Fannidae	Fannia spp.	5(3.05)
		Sepsidae	<i>Sepsis</i> spp.	15(9.15)
		Phoridae	Megaselia scalaris	3(1.83)
		Culicidae	Culex pipiens	1(0.61)
				115 (70.13)
	Coleoptera	Histeridae	<i>Hister</i> spp.	3(1.83)
			Hypocalus spp.	1(0.61)
		Silphidae	Nicrophoru spp.	3(1.83)
		Staphylinidae	Ocypus spp.	2(1.22)
		Dermestidae	Dermestes lardarius	1(0.61)
				10(6.1)
	Hymenoptera	Formicidae	Messor spp.	10(6.10)
			Camonutus spp.	10(6.10)
			Pheidole spp.	5(3.05)
				25(15.24)
	Orthoptera	Acrididae	Pododula ancisa	4(2.44)
		Gryllidae	Gymnogrylus lucens	2(1.22)
				6 (3.66)
Total				156(95.12)
Arachnida	Araneae	Araneidae	Aranea spp.	2(1.22)
Crustacean	Isopoda	Armadillididae	Armadillidium spp.	6(3.66)
				164(100)

Table 1: Overall arthropod relative abundance on exposed and shaded mammalian carcasses in Nsukka, Nigeria

Table 2: Arthropod relative abundance on exposed mammalian carcasses in Nsukka,Nigeria

Class	Order	Family	Species	Abundance (%)
Insecta	Diptera	tera Calliphoridae Chrysomya albiceps		12(12.37)
			Chrysomya megalocephala	6(6.19)
			Chrysomya chloropyga	5(5.15)
			Lucilia serricata	11(11.34)
			Lucilia illustris	4(4.12)
			Phormia regina	8(8.25)
				46(47.43)
		Muscidae	Musca domestica	7(7.22)
			Muscina stabulans	2(2.06)
				9(9.28)

Arthropod succession on exposed and shaded mammalian carcasses

		Sarcophagidae	Sarcophaga spp.	3(3.09)
		Anthomyiidae	Anthomyia spp.	1(1.03)
		Fannidae	Fannia spp.	3(3.09)
		Sepsidae	<i>Sepsis</i> spp.	9(9.28)
		Phoridae	Megaselia scalaris	2(2.06)
		Culicidae	Culex pipiens	1(1.03)
				19(19.59)
	Coleoptera	Histeridae	<i>Hister</i> spp.	2(2.06)
		Silphidae	Nicrophoru spp.	1(1.03)
		Staphylinidae	<i>Ocypus</i> spp.	1(1.03)
	-	Dermestidae	Dermestes lardarius	1(1.03)
				5(5.15)
	Hymenoptera	Formicidae	Messor spp.	5(5.15)
			Camonutus spp.	5(5.15)
				10(10.31)
	Orthoptera	Acrididae	Pododula ancisa	2(2.06)
		Gryllidae	Gymnogrylus lucens	1(1.03)
				3(3.09)
				92(94.85)
Arachnida	Araneae	Araneidae	Aranea spp.	2(2.06)
Crustacean	Isopoda	Armadillididae	Armadillidium spp.	3(3.09)
Total				97(100)

Muscidae, Phoridae, Fannidae and Sepsidae) were recovered from the shaded carcasses (Table 3).

Arthropod Succession on Carcasses: Primary colonizers on the exposed carcasses during the fresh stage were the Calliphorids and Sarcophagids (Figure 1), while on the shaded carcasses only the Calliphorids were observed from the fresh stage up to the decay stage (Figure 2). Adult Sarcophagids were observed from the fresh stage up to the decay stage on the exposed carcasses, while from the shaded carcasses it was observed from the bloated stage to the decay stage.

The family Muscidae and Sepsidae were observed during the bloated and decay stages on both carcasses. Adult Fannidae, Anthomyiidae and Phoridae were observed during the decay stage on the exposed carcasses. This was similar to those found on the shaded carcasses except for *Anthomyia* species which were absent. The sepsid flies were also observed on both carcasses during the dry stage. The Calliphorids were the highest number of flies from both the exposed and the shaded carcasses (42.68 %), followed by the sepsids (9.15 %) and muscids (8.54 %). Mosquitoes (Culicidae) were observed on the exposed carcasses during the dry stage. Immature of Calliphorids were observed on both carcasses during the decay stage.

During the study, four families of beetles (Coleoptera) were observed. These were Dermestidae, Silphidae, Staphylinidae and Histeridae. *Dermestes lardarius* (Dermestidae) was the first to arrive at the exposed carcasses on the 5th day (decay stage), while on the shaded carcasses, *Nicrophoru* species (Silphidae) was the first to arrive on the 7th day (decay stage). Dermestids were absent on the shaded carcasses while Silphids and Staphylinids were found on both carcasses during the decay and dry stages. Histerids were only found during the dry stage on both carcasses.

Ants (Hymenoptera) were actually the first group of insects to have invaded both carcasses. They were found in less than one hour on the first day of the research. Three species of ants were observed, namely, *Messor* spp., *Camonutus* spp. and *Pheidole* spp. They occurred throughout the process of decomposition.

Other arthropods observed on both carcasses include; *Aranea* sp. (spiders) and terrestrial crustaceans (*Armadillidium* sp.).

Class	Order	Family	Species	Abundance (%)		
Insecta	Diptera	Calliphoridae	Chrysomya albiceps	10(14.93)		
			Chrysomya megalocephala	3(4.48)		
			Chrysomya chloropyga	2(2.99)		
			Lucilia serricata	6(8.96)		
			Lucilia illustris	3(4.48)		
				24(35.82)		
		Muscidae	Musca domestica	3(4.48)		
			Muscina stabulans	2(2.99)		
				5(7.46)		
		Sarcophagidae	Sarcophaga spp.	3(4.48)		
		Fannidae	Fannia spp.	2(2.99)		
		Sepsidae	<i>Sepsis</i> spp.	6(8.96)		
		Phoridae	Megaselia scalaris	1(1.49)		
	Coleoptera	Histeridae	<i>Hister</i> spp.	1(1.49)		
			<i>Hypocalus</i> spp.	1(1.49)		
		Silphidae	Nicrophoru spp.	2(2.99)		
		Staphylinidae	<i>Ocypus</i> spp.	1(1.49)		
				5 (7.46)		
	Hymenoptera	Formicidae	Messor spp.	5(7.46)		
			Camonutus spp.	5(7.46)		
			Pheidole spp.	5(7.46)		
				15(22.39)		
Insecta	Orthoptera	Acrididae	Pododula ancisa	2(2.99)		
		Gryllidae	Gymnogrylus lucens	1(1.49)		
				3(4.48)		
Crustacean	Isopoda	Armadillididae	Armadillidium spp.	3(4.48)		
				67(100)		

Table 3: Insect species relative abundance on shaded mammalian carcasses in Nsukka, Nigeria

The Arachnids were found during the fresh and bloated stages while the crustaceans were observed during the decay up to the dry stages on both carcasses. Orthoptera (grasshoppers and crickets) were also observed on both carcasses.

DISCUSSION

The sequence of the different stages observed during the study (fresh, bloated, decay and dry) was similar to that reported by previous studies (Kočárek, 2003, Ekanem and Dike, 2010; Mabika *et al.*, 2014). However, Okiwelu *et al.* (2008) observed fresh, bloated, wet, dry and skeletonized, while Feugang *et al.* (2012) noted five stages of decomposition; fresh, bloated, active decay, advanced decay and dry. The observations made by the two later studies are different from the present observations only based on nomenclature; their observed stages could be categorized in to the four stages fresh, bloated, decay and dry.

Colonization species of greatest importance in the early stages of decomposition usually are those from the three dipteran families: Calliphoridae, Sarcophagidae and Muscidae (Carvalho and Mello-Patiu, 2008). This was confirmed in the study as the calliphorids and sarcophagids were the first to arrive on both exposed and shaded carcasses. However, the muscids first appeared on the third day (bloated stage).

Generally the sequence and duration of insect succession on the exposed and shaded sites followed the same general pattern previously reported (Centeno *et al.,* 2002; Ekanem and Dike, 2010; Okiwelu *et al.,* 2008;

ARTHROPODA	Stages and Days														
	Fresh Bloated			Decay					Dry						
	1	2	3 - 4		5 - 6	5 - 6 7 - 12			13	-15	16-20		21-30		
Diptera															
Calliphoridae															
Sarcophagidae															
Muscidae															
Fannidae															
Anthomyiidae															
Sepsidae															
Phoridae															
Culicidae															
Coleoptera															
Dermestidae															
Staphtlinidae															
Silphidae															
Histeridae															
Hymenoptera															
Formicidae															
Orthoptera															
Acrididae															
Gryllidae															
Arachnida															
Aranaea															
Crustacea															
Isopoda															

 Key:
 Fresh
 Bloated
 Decay
 Dry

Figure 1: Arthropod succession on exposed mammalian carcasses in Nsukka, Nigeria

ARTHROPODA					St	ages and Days					
		Fresh Bloated			Decay		Dry				
		1-2	2-3	3-4	5 - 8	9 - 12	13-15	16-20	21-30		
Diptera											
Calliphoridae											
Sarcophagidae											
Muscidae											
Fannidae											
Sepsidae											
Phoridae											
Coleoptera											
Staphtlinidae											
Silphidae											
Histeridae											
Hymenoptera											
Formicidae											
Orthoptera											
Acrididae											
Gryllidae											
Crustacea											
Isopoda											
					1 1 1	•					
Key: Fr	resh	E	Bloated		Decay	Dry					

Figure 2: Arthropod succession on shaded mammalian carcasses in Nsukka, Nigeria

Mabika et al., 2014). Exposed carcasses decomposed faster than the shaded carcasses. This was as a result of the large number of insects that visited the exposed carcasses. Maggots were observed on the fifth day of the study, was in contrast with the study of Mabika et al. (2014), where maggots were observed on the fourth day on rabbit carcasses. Feugang et al. (2012) also observed maggots from rat carcasses on the second day of decomposition. These differences could be attributed to the size of the carcasses, vegetation and weather condition of the area (Grassberger and Frank, 2004). Maggot migration from the carcasses was observed on day 9, and this observation was almost similar to that of Mabika et al. (2014) who observed maggot migration on the 8th day.

The earliest coleopterans which were members of Dermestidae, Staphylindae and Silphidae were observed during the decay stage of the study, and corresponded to that of Centeno et al. (2002), but differed from that of Ekanem and Dike (2010), who observed members of the Staphylindae, Scarabidae, Cleridae and Chrysomelidae during the bloated stage. These differences may probably be due to the differences in vegetation of the areas. The beetles (Coleoptera) were observed consuming soft tissues of the decomposing carcasses, as well as preyed on the abundant dipterous larvae present on the carcasses. The late coleopterans arrivals e.g. the Histeridae fed on the dry carcass tissues.

The ants (Formicidae) played a major role in decomposition of the carcasses, since they were present in large numbers throughout the period of the experiment. Their invasion, especially as they came in colonies, may have affected the carcasses decomposition rate, either by predation on immature stages of dipterous flies, or by removal of portions of the carcasses (Ekrakene and Iloba, 2010). Several other arthropods were observed on the carcasses, these include; crickets, grasshoppers, spiders, and terrestrial crustaceans. These formed part of the local entomofauna. For instance, the arachnids in this study were only encountered at the exposed sites. Cobwebs were not observed around the enclosure;

Conclusion: The rate of decomposition of carcasses is affected by environmental factors such as temperature, relative humidity, precipitation and insect abundance. Four distinct stages off carcass decomposition were observed during the study. Succession of arthropods was attracted on the decomposing carcasses, particularly insects. Their breeding biology led to the breakdown and exposure of the carcasses soft tissues. Of the insect species collected during the study, *Chrysomya albiceps* and *Lucilia serricata* could be important for further forensic studies since they were more numerous and observed breeding on the carcasses.

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