



A STUDY ON THE STAGES OF DECOMPOSITION AND FORENSIC INSECTS ASSOCIATED WITH CHICK AND RABBIT CARCASSES IN WATER

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ABSTRACT

This study investigates the decomposition and insect colonization on chicken and rabbit carcasses in water. There were five decomposition stages: submerged fresh, early floating, floating decay, advanced floating decay and sunken remains. *Chrysomya megacephala*, *C. rufifacies*, *Sarcophaga sp.*, *Hydrotaea sp.*, *Saprinus sp.*, *Staphylinus sp.*, *Onthophaga sp.* colonized the carcasses during four seasons. *C. megacephala* and *C. rufifacies* were the first colonizers and *Onthophaga sp.* was last. The seasonal variation in the species composition was prominently noticed due to difference in temperature and relative humidity. *C. megacephala* was the dominant species and its third instar larva could not enter into pupation.

KEYWORDS: Decomposition, Insect Succession, Aquatic Conditions, Forensic Entomology

INTRODUCTION

Forensic entomology is a branch of science that utilizes insects in medico legal investigations. It aids in estimating the time since death or Post-mortem Interval (PMI), specially focusing on the period between death and the recovery of the body (Amendt *et al.*, 2007). Insects play a crucial role in colonizing carcasses, forming one of the most exact relationships between necrophagous insects and dead remains (Amendt *et al.*, 2004). This substantial connection is important for determining PMI and can give authoritative cues to predict the precise time of death of an individual. The fauna engaged in decomposition process, encompasses a variety of organisms, including necrophagous insects, predators and parasitic insects, each utilizing the remains as an integral part of their living environment, through their interactions, feeding habits and contribute invaluable clues for forensic entomologists to determining Postmortem Interval (PMI) (Greenberg, 1991; Merritt *et al.*, 2001; Campobasso *et al.*, 2001; Byrd *et al.*, 2009). Insect species of several taxa are employed to study the decomposition patterns of carcasses in both terrestrial and aquatic environments. The maggots of blow flies (Diptera: Calliphoridae), being the first colonizers of dead bodies, exploit them for their development during the first week of decomposition, however the time taken for their development differs from species to species (Erzinclioglu, 1996). Nazni *et al.*, (2011) have shown the time taken by the blow flies to visit the dead body of a monkey varied from indoor and outdoor conditions, it takes 1.5 hours indoor and 6 hours outdoor. Although there has been an extensive research on insect colonization and decomposition of carcass by insects on land, the taxa associating with the carcasses on the ground and the buried ones, (Simmons *et al.*, 2010) a notable gap exists in research focusing on decomposition, particularly regarding insect colonization of carcasses in aquatic bodies (Catts and Goff, 1992). It is challenging to predict the Postmortem

Submersion Interval (PMSI) of the carcasses found in the aquatic bodies because the terrestrial flies may colonise once the carcass has started to float (Merritt and Wallace, 2010).

We have a little knowledge about the species of insects that visit the carcasses in aquatic bodies since their distribution and biology show a remarkable difference across habitats and environments. Due to difference in the temperature on land and in the aquatic bodies, the metamorphosis of the forensic insects certainly different from that of the insects found in the carcasses in terrestrial environment. Also, there are a smaller number of species accessing dead bodies in urban areas in comparison with that of periurban areas (Baz *et al.*, 2015) and species composition varies in urban and rural sites (Simpson and Strongman, 2002). Many studies have been made in natural water bodies like rivers, lakes and ponds in rural areas but a very rare literature is found on forensic insects in artificial water bodies located in urban environments. The time between instars and pupation of such insects may also be different from that of the terrestrial environment. It is very critical to predict the cause and time of death in case dead bodies retrieved from overhead tanks (Sudhir, 2024), wells (Ambade *et al.*, 2012) due to absence of natural aquatic insect fauna in these water bodies. The carcasses placed in tanks will attract terrestrial blow flies, rove beetles and scarab beetles and they may thoroughly be investigated for estimation of PMSI. With this background, the present investigation is designed to study the stages of decomposition, insect colonizers on chick and rabbit carcasses in an artificial water container located in an urban area.

MATERIALS AND METHODS

Experimental Setup

The study was conducted in each of four seasons (Winter: December – February; Summer: March- May; Monsoon: June –

August; Autumn: September – November) during 2021-2022 in an urban area in Puducherry, India (11° 57' 49" N; 79° 48' 41" E). To mimic natural decomposition, chicken (*Gallus gallus*) (Chakraborty *et al.*, 2015) and rabbit (*Oryctolagus cuniculus*) (Dalal *et al.*, 2020) carcasses were procured from market, weighing 1.15 kg and 1.45 kg respectively and carefully placed in plastic container measuring 71.5cm×57.5cm×45cm. Two third of the container was filled with fresh water and covered with mesh to protect the carcasses from scavengers and left them undisturbed for observation (Fig. 1A and 1B). Animal ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC), Pondicherry University, Pondicherry, India for this study (PU/CAHF/25th IAEC/2020/16).

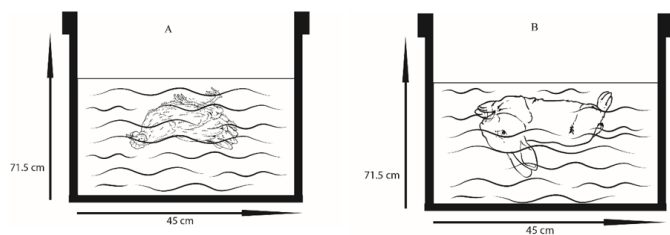


Fig. 1. Experimental set up: A. Chick Carcass B. Rabbit Carcass

Collection and Preservation of Necrophagous Insects:

Adult insects were carefully collected from floating carcasses using sweeping nets and forceps, while maggots, egg masses were collected with fine brushes, spoons and forceps. This careful approach to specimen collection aimed to capture every aspect of insect activity associated with carcass decomposition.

The collected specimens were preserved in 70% ethanol (Sharif and Qamar, 2021) and transferred to Kahle's fluid when needed to store the specimens for prolonged periods.

Observation:

The carcasses were monitored and examined every three hours daily, the temperature and relative humidity were recorded (Fig. 2) during the study period.

RESULTS

Stages of decomposition in aquatic condition

There were five different stages of decaying viz submerged fresh, early floating, floating decay, advance floating decay and sunken remains. Table 1 shows the time taken for different stages of decomposition in chick and rabbit carcasses through four seasons.

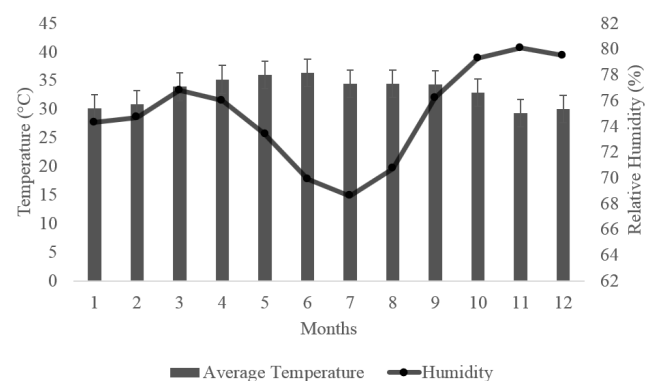


Fig. 2. Average Temperature and humidity during 2021 and 2022

Sl. No	Stage of Decomposition	Time Taken (Hours)							
		Winter		Summer		Monsoon		Autumn	
		Chick	Rabbit	Chick	Rabbit	Chick	Rabbit	Chick	Rabbit
1.	Submerged stage	0- 48	0- 24	0-40	0-20	0-43	0-23	0-45	0-22
2.	Early floating stage	48-74	24-49	40-70	20-46	43-72	23-47	45-72	22-48
3.	Floating decay stage	74-124	49-102	70-121	46-96	72-120	47-97	72-122	48-98
4.	Advance decay stage	124-168	102- 144	121-166	96-145	120-164	97-150	122-170	98-146
5.	Sunken remains stage	168- 217	144-188	166-213	145-193	164-212	150-190	170-216	146-190

Table 1: Time taken for decomposition in water by the chick and rabbit carcasses

Submerged fresh stage: This stage (Figure 3A and 3B) was characterized by the early phases of decomposition occurring underwater before the carcasses began to bloat. There were no external changes of carcasses and insects were yet to visit. Submerged stage lasted 44 ± 3.3 and 22.25 ± 1.4 hours in chick and rabbit respectively.

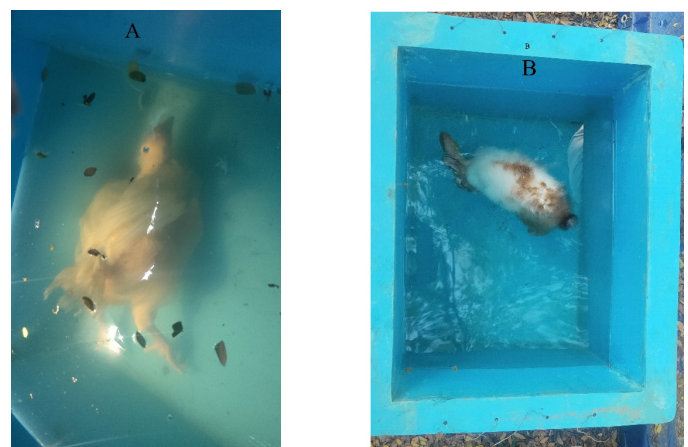


Fig.3. Submerged Fresh Stage of A. Chick Carcass B. Rabbit Carcass

Early floating Stage: During the early floating stage (Figure 4A and 4B), gases produced by anaerobic bacterial respiration caused the carcasses to float to the surface, pushing the abdomen above the surface of water. The putrefaction of proteins was apparent, carcasses attracted adult dipterans especially species of *Chrysomya* from the family Calliphoridae, followed by *Sarcophaga* and *Hydrotaea*. *Chrysomya* sp. dominated over the other two flies and it lasted 69 ± 3.9 and 48.25 ± 2.2 hours in chick and rabbit respectively. Within an hour after their visit, they laid eggs in the orifices of the carcasses. The eggs hatched within 24 hours and larvae started appearing all over the body. Due to eggs laid subsequently by the adult flies, all the three instar maggots were found at this stage.



Fig.4. Early Floating Stage of A. Chick Carcass B. Rabbit Carcass

Floating decay stage: Carcasses (Figure 5A and 5B) floating above the water surface exhibited vigorous activity of the maggots of *Chrysomya*, creating numerous openings on the exposed skin. The carcasses were completely disfigured and every part of the carcass was occupied by these maggots. The maggots of *Hydrotaea* and *Sarcophaga* could rarely be noticed. The adults of *Saprinus* sp., *Staphylinus* sp. and *Onthophaga* sp. were found in this stage, actively preying on the maggots and engaging in copulation. This stage lasted 121.75 ± 1.7 and 98.25 ± 2.6 hours respectively.



Fig.5. Early Floating Stage of A. Chick Carcass showing Egg mass B. Rabbit Carcass

Advance floating stage: During this stage (Figure 6A and 6B) the majority of exposed tissues of carcasses floating above

the water surface have disappeared due to the continuous feeding activities of maggots. The maggots of *Chrysomya* sp. have removed almost every flesh part of the carcasses and some maggots exhibited migration from the carcasses, often resulting in drowning into the tank and some moved out of the tank through its walls. Pupation could not be accomplished and therefore life cycle ended here. We could not notice any *Staphylinus* sp. at this stage; *Saprinus* sp. and *Onthophaga* sp. were very few in number. The time taken by this stage was 167 ± 2.7 and 146.25 ± 2.6 hours respectively.



Fig.6. Advance Floating Stage of A. Chick Carcass B. Rabbit Carcass

Sunken remains stage: The remnants (Figure 7) of the carcasses consisted of bones and insignificant amount of tissues. The adult stages of all flies disappeared, all the maggots died and found floating on the surface of water, a few *Onthophagus* sp. were moving among the dead maggots. At the end of 214.5 ± 2.3 and 190.25 ± 2.0 hours, the observation was brought to termination.



Fig.7 Sunken stage of carcass

Succession insects associated with carcasses in water

During the submerged stage, there was no activity of insects. In the early floating stage, blow flies especially *Chrysomya megacephala* and *Chrysomya rufifacies*, visited and began to lay their eggs on the exposed parts of the floating carcasses and followed by *Sarcophaga* sp. and *Hydrotaea* sp. After the floating decay stage, the egg masses hatched into maggots which fully colonized the floating carcasses. The arrival of *Staphylinus* sp and *Saprinus* sp. was also noticed during the floating decay stage, as these beetles prey on the maggots

present on the floating carcasses. In the advanced decay stage, most of the maggots reached the third instar and began to move away from the carcasses, often drowning in the water while crawling around the corners of the container in search of soil for pupation and the arrival of *Onthophaga* sp. was observed in this stage. Maggots got drowned into the water and a few maggots moved away from the tank. Season wise composition of species appeared during decomposition of carcasses is given in Table 3.

Name of Insect	Appearance of necrophagous insect									
	Submerged		Early Floating		Floating Decay		Advance Floating		Sunken	
	Chick	Rabbit	Chick	Rabbit	Chick	Rabbit	Chick	Rabbit	Chick	Rabbit
<i>C. megacephala</i>	-	-	+	+	+&++	+&++	++	++	-	-
<i>C. rufifacies</i>	-	-	+	+	+&++	+&++	++	++	-	-
<i>Sarcophaga</i> sp.	-	-	+	+	++	++	++	++	-	-
<i>Hydrotaea</i> sp.	-	-	-	-	-	-	+	+	-	-
<i>Saprinus</i> sp.	-	-	-	-	+	+	+	+	-	-
<i>Staphylinus</i> sp.	-	-	-	-	+	+	-	-	-	-
<i>Onthophaga</i> sp.	-	-	-	-	-	-	+	+	+	+

+ indicates the appearance of adult insects; ++ indicates the appearance of maggots; +&++ indicates appearance of both adults and maggots; - indicates the absence

Table 2. Association of terrestrial insects in aquatic decomposition

Name of the insect	Winter		Summer		Monsoon		Autumn	
	Chick	Rabbit	Chick	Rabbit	Chick	Rabbit	Chick	Rabbit
<i>C. megacephala</i>	+	+	+	+	+	+	+	+
<i>C. rufifacies</i>	+	+	+	+	+	+	+	+
<i>Sarcophaga</i> sp.	+	+	+	+	+	+	+	+
<i>Hydrotaea</i> sp.	-	-	+	+	+	+	-	-
<i>Saprinus</i> sp.	+	+	+	+	+	+	+	+
<i>Staphylinus</i> sp.	-	-	-	-	+	+	-	-
<i>Onthophaga</i> sp.	-	-	+	+	-	-	+	+

+ indicates the appearance of adult insects; - indicates the absence

Table 3. Association of adult insects with chick and rabbit carcasses through four seasons

DISCUSSION

We observed five stages of decomposition: submerged, early floating, floating decay, advanced decay, and sunken remains, similar to the study conducted on pig carrion (Payne.,1991) and rabbit carrion (Dalal *et al.*,2020). Many other researchers have suggested six stages of decomposition, including the bloated deterioration stage (Hobischak,1997; Keiper *et al.*,2001; Merrit *et al.*,2001). During the initial stage of decomposition, the carcasses were immersed in water, resulting from the absence of adult insect activity. The submerged stage lasted for 48 hours in chicken, 24 hours in rabbit, and the difference may be due to the sinking ability of the carcasses. The submerged stage lasted 24 hours in human cadaver (Sukontason *et al.*, 2005) which is very similar with that of rabbit carcass whereas in case of piglet spent 4 days in submerged state in a study made by Heo *et al.*, (2008) in Malaysia. Therefore, the time taken for submerged stage may vary among different carcasses. After 48 hours and 30

minutes, blow flies started to visit the floating chicken carcass, in case of rabbit carcass after 24 hours and 15 minutes. In both carcasses, *Chrysomya megacephala* and *Chrysomya rufifacies* were the first insects to visit and colonize during floating stage. *Sarcophaga* sp. *Hydrotaea* sp. visited during advanced floating decay stages and a similar observation was also made by Payne *et al.*,1972. Due to The odour from the decaying carcasses is responsible for attraction of the blow flies. The first and second instar larval periods of *C. megacephala* lasted for 24 hours each. *C. rufifacies* larvae were observed minimally in each season, may be due to difference in the population size in this locality. Third instar larva and pre-pupa were observed moving away from the carcass and gathering in the corner of the water tank in search of soil for the pupation. However, due to the unavailability of soil, some maggots escaped from the tank, and most of them drowned in the water (Payne *et al.*,1972; Aylers *et al.*,2010; Heo, et al., 2008). Adults of *Saprinus* sp. made their

appearance once the maggots of *Chrysomya* sp., appeared and lasted till the active decaying stage. *Saprinus* sp. predated on the maggots of flies. *Staphylinus* sp. appeared floating decay stage whereas *Onthophaga* sp. was found in the last stages of decay. Once the carcass reached sunken stage, almost all insects disappeared. Estimation of PMSI is a challenging task in case of carcasses found in water but if one has a complete profile of insects associated with decomposing dead bodies, it is very much possible to estimate the time of death. This pioneer attempt in South India will definitely help the entomologists of other regions to make detailed studies on the forensic insects associated with the carcasses in water.

CONCLUSION

An attempt has been made to study the association of necrophagous insects on chick and rabbit carcasses in water. There were five stages of decomposition and a particular group of insects was associated with each stage of decomposition. The temperature and relative humidity played vital role in the appearance of necrophagous insects on carcasses. The flies of family Calliphoridae were the first visitors, there was a succession of other flies and coleopterans through various stages of decomposition of the carcasses. The life stages of these insects may be used to estimate the PMSI of the cadavers in legal investigations but it needs further conformation with more studies in this direction.

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