# Insect succession on a decomposing piglet carcass placed in a man-made freshwater pond in Malaysia

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Abstract. This entomological study was conducted in a man-made freshwater pond in a palm oil plantation in Tanjung Sepat, Selangor from 23 July 2007 by using pig (Sus scrofa) as a carcass model. A 1.5 month old piglet (5 kg), which died of asphyxia after being accidentally crushed by its mother, was thrown into a pond. Observation was made for ten days; one visit per day and climatological data were recorded. On the first two days, the piglet carcass sunk to the bottom of the pond. The carcass floated to the surface on the third day but no fly activities were seen. The blow fly, Chrysomya megacephala and Chrysomya rufifacies started to oviposit on the fourth day. Other than adult flies, a spider (Arachnida) was also observed on the carcass. Bubbles accumulated at the mouthpart, and the abdomen was greenish black. A lot of blow fly eggs were seen on the body surface on the fifth day (floating decay), along with first and second instars C. megacephala crawling under the piglet's skin. On the sixth day, adult blow fly, C. megacephala, and C. rufifacies, and muscid flies, Ophyra spinigera and Musca domestica were observed on to the carcass. High numbers of first and second instars of flies were observed wandering around the body surface with C. megacephala larvae being the predominant species. Two prominent maggot masses occurred on seventh and eighth days. Bloated deterioration stage began on day eighth exposing rib bones, humerus bones and intestines. Carcass was partially sinking and the maggot masses were at the water level. On day ninth, the carcass was partially sinking and three maggot masses were observed on the exposed surface. There were very few adult flies, including a scarab beetle was sighted on the carcass at this stage. The carcass along with the maggots sunk on day tenth, leaving an oily layer on the water surface.

## INTRODUCTION

Medicocriminal forensic entomology is defined as the application of the study of insects and other arthropods to violent crimes such as murder, suicide, rape, as well as physical abuse. Human corpses, whether they have been produced naturally or as the result of foul play, are processed by insect decomposers in the same manner as any other carrion (Lord, 1990). A large body of literature has emerged on arthropods colonization of terrestrial carrion (including human) for the past ten years (Catts & Goff, 1992). In spite of this tremendous body of research, the role of freshwater and marine fauna in forensic investigation has received very little attention (Payne & King, 1972; Haskell *et al.*, 1989; Vance *et al.*, 1995; Hobischak, 1997; Nawrocki *et al.*, 1997).

Since corpses are often found in aquatic environment (Haglund *et al.*, 1990; Hobischak & Anderson, 1999), it is important that forensic scientist and police visiting a crime scene have an increased knowledge of the aquatic organisms that could potentially colonize humans and non-human models (Byrd & Castner, 2001).

To date, most empirical evidence examining insect colonization in aquatic systems has concentrated on blow flies (Calliphoridae), and a few other terrestrial species that colonized a corpse after it bloats and rises to the surface. Those species that are restricted to aquatic ecosystems for survival in one or more life stages has been largely ignored (Payne & King, 1972; Nuorteva *et al.*, 1974; Smith, 1986; Goff & Odom, 1987; Mann *et al.*, 1990; Hobischak & Anderson, 1999). In fact, prior submersion in freshwater environment can alter the succeeding terrestrial faunal succession on carrion or corpses and subsequently alter the process of decomposition (Payne & King, 1972; Smith, 1986; Haskell *et al.*, 1989; Rodriques, 1997)

## MATERIALS AND METHODS

The present study was carried out to observe the process of faunal succession and stages of decomposition using a piglet carcass. A 1.5 month old pig (Sus scrofa), weighing  $\cong 5$ kg, from the pig rearing farm in Tanjung Sepat, Selangor ( $\cong 85$  km from Kuala Lumpur) that died of asphyxia was immediately thrown in a man-made freshwater pond inside a palm oil plantation near the pig farm (2.6 N 101.6 E) The pond was irregular in shape (2.0m x 2.8m x 2.8m x 2.5m), with a depth of 1.3 meter. The water was brownish in color, odorless, stagnant, with some floating flora and palm fruits. This pond was made by plantation workers purposely for water storage and was filled with rainwater for a long time. The study was first conducted in the afternoon of 23 July 2007, which was taken as the first day of observation.

Observation was made for 10 days, with one visit per day in the afternoon. Climatological data such as ambient temperature and humidity were recorded for 10 days by using a mercury thermometer and a hygrometer. Water temperature, body surface temperature, and maggot mass temperature were also recorded during each visit. Adult flies were caught around the carcass by using sweep net. Some of the eggs and fly larvae (10-20 larvae) were either collected using forceps for rearing or put into ethyl alcohol 70% for preservation; this collecting procedure is based on that of Haskell & Catts (1990). Adult flies were killed in chloroform, pinned and kept in an insect box. Fly larva were dehydrated in varies concentrations of alcohol and mounted in Canada balsam on a slide. The collected specimens were processed for species identification by using key of Kurahashi *et al.* (1997) for adult flies and key of Ishijima (1967) for fly larvae. Processed specimens were kept as a record in the Department of Parasitology & Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia.

## RESULTS

During the observation periods, the ambient temperature in the palm oil plantation for the ten days period ranged from 27°C to 33°C (mean 28.67, standard deviation  $\pm 1.20^{\circ}$ C), air humidity ranged from 60% to 100% (mean 96, ±3.05%) (Figure 1). Body surface temperature of the carcass ranged from 28°C to 30°C (mean 28.3, ±0.88°C) and maggot mass temperature varied from 28°C to 35°C (mean 31, ±2.08°C) (Figure 2). Rain was recorded three times during the study (at day-6, day-8 and day-9). Five stages of carcass decompositions in aquatic environment was attained based on Byrd & Castner (2001): these were submerge-fresh, early-floating, floating-decay, bloateddeteriorated, and sunk (Table 1).

Body temperatures of the piglet's carcass were measured by placing the mercury thermometer onto the body surface and not in the anus and mouth since those sites were submerged in water. The temperatures were recorded from the early-floating stage until bloated-deteriorated stage only. Maggot mass temperature was taken by inserting the thermometer into the center of larvae mass. Each reading of temperature was recorded after placing the thermometer for three minutes.

The man-made freshwater pond had a few water striders (Gerridae) and frog's eggs. On the first and second days of observation, the carcass submerged in the water (submerged-fresh stage of decomposition). On the third day of death, the carcass floated on the water surface with the head under the water (early-floating stage of decomposition), and there was no adult fly activity observed around the carcass. Fly eggs were seen on the body on the fourth day and the whole body had now floated on the water surface. Some white air bubbles accumulated at the mouthpart of the carcass. This indicating that abdominal gas was pushed out from the mouth. The abdomen appeared greenish black during this stage. Some adult blow flies, *Chrysomya* 

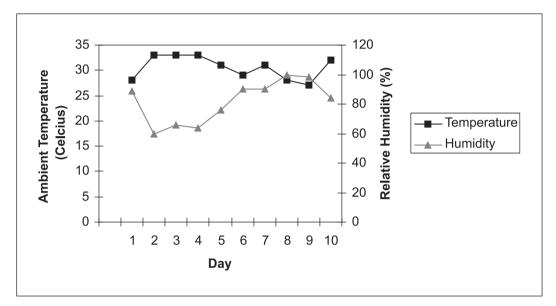


Figure 1. Ambient temperature and air humidity of the study site inside the palm oil plantation in Tanjung Sepat, Selangor, Malaysia (2.6 N 101.6 E) to observe the insect succession of the piglet carcass. The first day of observation began on 23 July 2007.

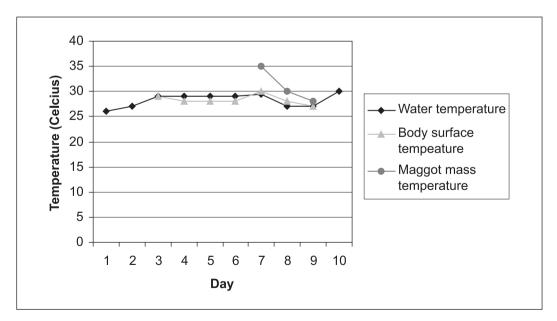


Figure 2. Means of the water temperature of the study site (2.6 N 101.6 E), piglet carcass temperature and maggot mass temperature during observation periods in July-August, 2007.

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Date of collection	Ambient temperature (°C)	Humidity (%)	Water temperature (°C)	Body temperature (°C)	Maggot mass temperature (°C)	Piglet's status
23 July	28	89	26	_	_	Sink
24 July	33	60	27	_	_	Sink
25 July	33	66	29	29	_	Float
26 July	33	64	29	28	_	Float
27 July	31	76	29	28	_	Float
28 July	29	90	29	28	_	Float
29 July	31	90	29.5	30	35	Float
30 July	28	100	27	28	30	Float
31 July	27	98	27	27	28	Float
1 August	32	84	30	_	_	Sink

Table 1. The condition at the studied site to observe the insect succession on piglet carcass at aquatic environment in Tanjung Sepat, Selangor, Malaysia (2.6 N 101.6 E) IN 2007

*megacephala* and *Chrysomya rufifacies* were observed on the body of the carcass. A spider (arachnida) was also seen on the carcass preying on adult flies.

On the fifth day (floating-decay stage of decomposition), 15-28 adult flies were found landing on the floating carcass, and there were a lot of fly eggs seen on the body surface. First and second instars were seen under the skin. At this stage, the fur easily detached from the body. On the sixth day (floating-decay stage of decomposition), various species of adult blow flies, the majority being C. megacephala and C. rufifacies, and a few muscid flies, Ophyra spinigera and Musca domestica were observed at the piglet's skin. Two maggot masses formed on the body surface, and there were many second and third instars C. megacephala crawling all over the exposed body. The skin that was under the water had now began to loosen from the body. On the seventh day, the highest congregations of adult C. megacephala ( $\cong$ 40) were seen along with two prominent maggot masses formed at the left hind legs and abdomen. The highest maggot mass temperature was recorded during this stage (35 °C). No maggot mass was found at the head because this organ was submerged in water.

On the day-eighth (bloated-deterioration stage of decomposition), the carcass was partially sinking, with some of the rib bones, humerus and intestine being visible. Water began reaching the maggot masses. The major species of third instars maggots were C. megacephala followed by C. rufifacies. Very few adult flies were observed on the body perhaps due to the difficulty of landing on the watery surface. On day nine, there were mainly adult C. megacephala, M. domestica and O. spinigera. The carcass was still partially sinking. There were now three maggot masses on the body, and the head part was still intact but no maggot mass was formed there. One scarab beetle (scarabidae) was attracted to the carcass at this stage. On day-tenth, the piglet carcass and the maggots sunk under the pond. There were some bubbles and an oily layer forming on the water surface. There was no adult fly activity observed around the pond by this time.

#### DISCUSSION

The results from these observation revealed that the piglet was submerged on the first and second days of death, and began to float from the third to ninth day (1 week) and sunk on tenth day. Life cycles of flies on decomposing carcass found in aquatic environment could not be completed. The only way to determine the PMI is by looking for immature stages of flies found on the carcass, and adding another four days to the estimated time since the eggs were only found on day-four.

Decomposition of a body submerged in an aquatic environment occurs at a rate roughly half that of decomposition in air, primarily due to the cooler temperature and the inhibition of insect activity (Knight, 1997; Rodrigues, 1997). In our study, we found that the flies will only oviposit eggs on the fourth day, which indicated the process of decomposition will be delayed up to four days. Payne & King (1972) conducted the experiments using immersed pigs (S. scrofa). These authors revised the stages of decomposition to accommodate corpses found in aquatic habitats and divided the process into six stages: submerge fresh, early floating, floating decay, bloated deterioration, floating remains, and sunken remains. In our study, we observed only five stages of aquatic decomposition stages. The floating remains stage, which was defined by Payne & King (1972) as those parts of tissue projecting above the water surface was showed to have little maggot (Calliphoridae) activity, probably due to migration from carcass, death by drowning, or predation from aquatic or other terrestrial insects. However, this stage cannot be determined in our study. Our observation showed that carcass proceeded to sunkenremains stage immediately after bloateddeterioration stage. It may be due to the different climatic condition prevalent in our country.

During the submerged-fresh stage, several investigators found immature stages of aquatic insects, such as hydropsychid caddisflies (Trichoptera: Hydropsychidae), chironomid midges (Diptera: Chironomidae), and heptageniid mayflies (Ephemeroptera: Heptageniidae) (Vance *et al.*, 1995; Hobischak, 1997; Keiper *et al.*, 1997). However, those insects were not found in our experiment. It may be due to different ecology and habitats in our country.

Carrion beetle (Coleoptera: Silphidae) and rove beetles (Coleoptera: Staphylinidae) may feed on larval blow flies or animal flesh. Yellowjacket and bald-faced hornet (Hymenoptera: Vespidae) generally prey on adult and larval blow flies (Payne & King, 1972; Nuorteva et al., 1974; Goff & Odom, 1987; Tomberline & Adler, 1998). On the floating decay stage, intense feeding activity by calliphorid maggots has been observed on pig and rat carcasses floating above the water surface, creating many openings in the exposed skin (Tomberlin & Adler, 1998). Silphid, staphylinid, and histerid (Histeridae) beetles were observed in large numbers during this time, searching for prey and copulating (Payne & King, 1972). During bloated deterioration stage, most of the exposed tissues of pig or rat carcasses floating or projecting above the water surface have disappeared due to the continual feeding activities of blowfly maggots. Haglund & Reay (1993) found that hind limbs became disarticulated, blood and other fluids leaked from carcass orifices, large sections of flesh sloughed off, and severe ulceration were observed on the abdominal wall.

In our study, we found calliphorid fly (C. megacephala and C. rufufacies) and muscid fly (O. spinigera and M. domestica) visiting the floating carcass; with C. megacephala as the dominant species. The conditions of the aquatic decomposition stages were similar to the oversea findings (Payne & King, 1972; Nuorteva et al., 1974; Goff & Odom, 1987; Tomberline & Adler, 1998). No other insects were observed at the vicinity on carcass except a scarab beetle (scarabidae) arriving on day nine. There were several water striders of the family Gerridae on the pond surface prior to the placement of carcass. However, these insects did not come near to the decaying piglet.

In conclusion, this paper has highlighted that there are five stages of decomposition (submerge-fresh, early-floating, floatingdecay, bloated-deterioration and sunkenremains) of the piglet carcass in the aquatic environment, which naturally occurred in Malaysia. The majority of flies observed on the floating carcass was *C. megacephala*, followed by *C. rufifacies*, *O. spinigera* and *M. domestica*. The insects' activities on the floating carcass are not as intense compared to carcass placed on the land (Heo *et al.*, 2007).

More research merits investigation to obtain information of insect succession in other aquatic habitats (e.g. river, lakes, marine situations, mining pool, sewage pond, and wells). So these knowledge can be useful in forensic investigation, in particular the estimation of minimal post-mortem interval.

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