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Larval growth of the muscid fly, *Synthesiomyia nudiseta* (Wulp), a fly of forensic importance, in the indoor fluctuating temperatures of Malaysia

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Abstract. Larvae of the *Synthesiomyia nudiseta* (Wulp) were collected from a decomposed human corpse at the Department of Forensic Medicine, Penang Hospital. A colony of this species was established and the eggs were collected for rearing. The developmental times, rearing temperatures, and relative humidity were recorded twice daily from the time the eggs collected until adult emergence. An average of 5 larvae were randomly collected from the rearings twice daily, warm-water killed and preserved in Kahle's solution. The larval instar stages were determined by observing the number of posterior spiracular slits and the length of the preserved larvae were measured. When the larval life cycle was completed, the accumulated developmental times were calculated. A total of 8 replicates were carried out. The temperature of the rearing room was 28.5 ± 1.5°C while the relative humidity was within 67 - 85 %. The total developmental time for *S. nudiseta* was 322 ± 19 hours (13.4 ± 0.8 days).

INTRODUCTION

Muscoids dipterous have always been associated with human and domestic animals due to abundance of food resources found in stables and domestic garbage (Oliveira *et al.*, 2002). Larvae of flies in this group are commonly found in animal and human faeces as well as in decaying vegetable materials, kitchen refuse, decaying cotton seed, and garbage though they have been reported to prefer carrion as the food source of choice (Byrd & Castner, 2001). The fly *Synthesiomyia nudiseta* (Diptera: Muscidae) is one of the forensically important species in many countries such as in Brazil (de Souza *et al.*, 2008; Calderon-Arguedas *et al.*, 2005b), Egypt (Tantawi *et al.*, 1996), southeastern United States (Lord 1990), Thailand (Sukontason *et al.*, 2007) and Malaysia (Omar *et al.*, 1994; Lee *et al.*, 2004; Nazni *et al.*, 2007). In Malaysia, Nazni *et al.* (2007) reported the adults of *S. nudiseta* exhibit eusynanthropic character because it was only found near human premises. According to Byrd & Castner (2001) this species is found throughout the tropical and subtropical regions of the world. The larvae of this species are predacious and are one of the few species that is noted to consume the larvae of *Chrysomya rufifacies* (Byrd & Castner, 2001). The objective of this paper was to report the larval developmental times of *S. nudiseta* in the indoor fluctuating temperatures of Malaysia.

MATERIALS AND METHODS

The larvae of *S. nudiseta* were collected from a decomposed human corpse (active decay stage) at Department of Forensic Medicine, Penang Hospital. The deceased, was a 61 years old lady was found dead on 28th May 2008 at 8.15pm and brought to the Department of Forensic Medicine, Penang

Hospital at 10pm on the same day. She was kept in the body refrigerator (4 ± 3 °C) overnight and the larvae of *S. nudiseta* (third instars, 11mm in length) were collected from the left thigh near the anal region of the deceased on 29th May 2008 at 11am. The cause of death was given as unascertained. According to the investigating police officer, the deceased was found on the bed, doors and windows closed and no foul play suspected. During the autopsy, the larvae were collected using a plastic blunt end forceps. A representative of 7 larvae were preserved (all similar size) while the others (more than 20 larvae) were reared. Rearing was done in a container measuring 11cm (height), 10cm (wide) and 10cm (length) with 2.5 cm thick of sterilized soil. The beef meats (in pieces of 25g each) were given *ad libitum* and wet paper towel was placed on top of it to prevent the meat from drying and acted as skin. The top of the container were covered with paper towel using a rubber band to prevent the infestation of the pest flies. These reared larvae later emerged on 9th June 2008 at 8am and were maintained as a colony in 30 cm (height) x 30 cm (wide) x 30 cm (length) fine wire mesh cage. Beef meat (in pieces of 25g each) provided to the colony once every two days. The rearing was done indoor in a room. The room was 2.3m (height) x 4.2m (length) x 3.2m (wide) with a closed window and an exhaust fan above the room which provides air ventilation. The colony and rearings were exposed to the photoperiod of 9 hours of lights and 15 hours of darkness daily. The daily larval developmental data were recorded and the temperatures and relative humidity were measured using psychrometer (G.H. Zeal Ltd., London) from the time the beef meat placed into the colony, eggs collected and until the emergence. For the eggs collections, a 24-hours decomposed beef meat (in piece) was used, placed into a cup [1.0cm (height) x 4.3cm (length) x 4.3cm (wide)] and introduced to the colony. The eggs of the first 4 replicates were collected on 23rd September 2008 at 10 am (meat was place into the colony at 9am). Then, after one hour, another batch of eggs were collected from the colony and made into 2 more replicates (meat was place into the colony at 10am). On 25th September 2008 at 5pm another batch of eggs were collected and made into 2 more replicates (meat was place into the colony at 2pm). Then, these eggs were reared in rearing containers as described earlier. From each replicate, an average of 5 larvae were randomly collected daily, every morning and evening until the larvae reach pupal stage. These larvae were killed in warm water (52 ± 10ºC) and preserved in Kahle's solution as suggested by Gennard (2007). The larval stages of the preserved larvae were determined by the number of posterior spiracular slits under a stereomicroscope (Olympus, Japan) and the lengths of the larvae were measured using 1.0 mm x 1.0 mm graph paper placed on top of the stereomicroscope stage. For the first and second instars the observation of the posterior spiracles under the stereomicroscope was not feasible. The slide of the posterior spiracles were prepared as suggested by Lee *et al.* (2004) and observed under light microscope (Leica DM2500). When the immature stages completed, the measured larvae lengths for each sampling period averaged and were plotted against time using SPSS software (version 12) at the confidence interval of 95%. A total of 8 replicates (combination of 4 + 2 + 2 replicates) were done to establish the developmental times of this species. The mean ± s.d. (standard deviation) values were determined for each larval stages and their developmental times.

RESULTS AND DISCUSSION

The mean ± s.d. of room temperature throughout the rearing was 28.5 ± 1.5 °C (min.= 27 °C; max.= 30 °C) and the relative humidity of the rearing room fluctuated in between 67-85%. For the first 6 replicates the eggs were collected after one hour the beef meat were placed into the colony. The last 2 replicates, the eggs were collected after 3 hours the beef meat was placed into the colony. We found variation in the times of egg collections with some workers who collected the eggs within one hours (Day &

Wallman, 2006), 2 hours (Greenberg & Tantawi, 1993) and 3 hours (Clark *et al.*, 2006). In this current study, we decided to collect the eggs for the first one hour (two different batches of eggs) and then the eggs of last two replicates were sampled after three hours the beef meat was placed into the colony. We sampled in such a manner as to determine whether there would be any differences on the larval development if the temperature, relative humidity and batches of eggs differs. Though the oviposition periods were one and three hours in differences, the eggs for the first 6 replicates hatched within 22-23 hours after the eggs were collected (oviposition period one hour). However, for the last 2 replicates (oviposition period three hours), we observed the eggs hatched 15 hours after the collection of the eggs. The differences in the eggs incubation periods were probably due to the fluctuating room temperatures. At the time the eggs collected for the first 6 replicates the rearing room temperatures recorded was 29ºC while for the last two replicates the room temperatures was 30ºC at the time the eggs collected. Another main point to be considered is the precocious egg development in the forensic flies in the establishment of the reference data. In an experiment done by Wells & King (2001) they dissected *Calliphora terraenovae* (Macquart), *Calliphora vomitoria* (L.), *Calliphora vicina* (Robineau-Desviody) and *Lucilia sericata* (Meigen) were found to have a single egg held in the oviduct. According to them, when this precociousness occurs, the eggs that were laid will readily hatch while those that not mature eggs will still in the stage of embryonic development and cause variation in the development of the larvae. We do not rule out the possibility of the occurrence of precocious egg development for *S. nudiseta*.

As for the larval stages, the second instar developed around 47.5 ± 8.5 hours (Table 1) after the eggs were collected. The mean ± s.d. developmental hours for each stage of third instar, post feeding, pupation and the emergence was 71.5 ± 22.5, 100.0 ± 4.0, 119.5 ± 8.5 and 322.0 ± 19.0 hours, respectively (Table 1). The mean ± s.d. of immature larvae from eggs until pupal stage was 5.0 ± 0.4 days while mean ± s.d. of the pupation period last about 8.4 ± 0.4. During the early third instar the cuticle of the larvae were noted to be transparent and the crop was visible (3rd – 4th segments towards the posterior end of the larvae). The length of the eggs of this species was within the range of 1.00 – 1.20 mm (Table 2). We noted that the adults of this species prefer to lay eggs by spreading their eggs on the surface area of the decomposing beef meat rather than laying in masses such as blowflies. The third instar was observed at the length of 6.40 mm while the maximum larval length measured was 14.90 mm (Table 2). As the larvae went into the post feeding stage the larvae became opaque and the crop of the larvae was not

Table 1. The mean ± s.d. accumulated developmental time of *Synthesiomyia nudiseta* at 28.5 ± 1.5 ºC and the relative humidity was within 67-85%

Stage	Mean ± s.d. of accumulated developmental time of *S. nudiseta.* (hours)*
Eggs	0.0
First instar	23.5 ± 8.5
Second instar	47.5 ± 8.5
Third instar	71.5 ± 22.5
Post-feeding	100.0 ± 4.0
Pupa	119.5 ± 8.5 (5.0 ± 0.4 days)
Emergence	322.0 ± 19.0 (13.4 ± 0.8 days)

* Data from 8 replicated.

Table 2. The length of *Synthesiomyia nudiseta* according to the larval stages

Stage	Length [min-max (mm.)]*
Eggs	1.00 – 1.20
First instar	1.50 – 2.75
Second instar	2.83 – 6.28
Third instar	6.40 - 14.90
Post feeding	12.25 - 14.67
Puparium	no measurement

* Data from 8 replicated.

visible. In late third instar, the larvae of this species were observed to arrange itself in groups side by side with the anterior portion facing downwards and posterior end facing upwards and encased itself in a cocoon by using the soil around it. Then, the top of posterior ends were observed covered with foamy whitish substance. The adults were noted to emerge through this foamy whitish substance when the pupation period completed. According to Siddons & Roy (1942) the cocoon was formed from a frothy fluid which soon solidifies as a whitish pellicle, in which sand grains are cemented. The puparium formed within this cocoon. The pupation period last for 8.4 ± 0.4 days and occupied about 62% of the total immature stage of this species.

According to Grassberger and Reiter (2001), if the temperature is roughly constant, as is the case with corpses found indoors the use of the growth curves could provide a quick and precise estimate for the post-mortem interval. In such circumstances, the post-mortem estimation of this species can be read directly off the growth curve (Figure 1) on the basis of the length of the individual larvae collected from the scene of death or at autopsy since this species have been reported to infest indoor human corpses only (Omar *et al.*, 1994; Lee *et al.*, 2004; Sukontason *et al.*, 2007). In Malaysia, this species was reported to be forensically important flies (Omar *et al.*, 1994; Lee *et al.*, 2004; Nazni *et al.*, 2007). However, there is no study has been reported on the larval developmental time of this species in Malaysia and there are few published information can be retrieved regarding this species. Rabinovich (1970) reported at 28 ± 1ºC, relative humidity 90%, the durations of the immature stage (egg

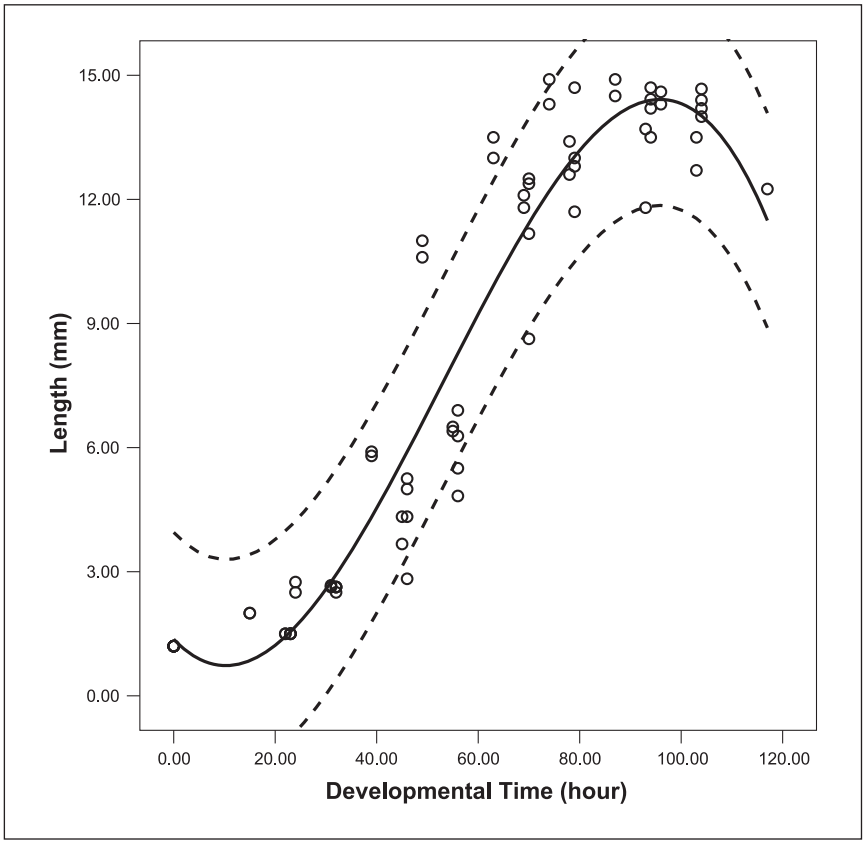

Figure 1. Growth curve of *S. nudistea* larvae (eggs until post feeding stage- 8 replicates) grown at 28.5 ± 1.5 ºC. The fitted cubic function (solid line- R-square 0.923) with 95% confidence limits of the model (dashed lines).

until pupae) was 9.6 ± 1.6 days while the pupation periods was 8.2 ± 0.5 days. His finding of total larvae stage (eggs till emergence) was 17.8 ± 2.1 days while in the current study was 13.4 ± 0.8 days. The mean temperature between his study and current study was same though the relative humidity was much higher in the former study. Meanwhile, Kruger *et al.* (2002) reported the under the constant temperature of 26 ± 1°C and relative humidity above 75%, the eggs incubation period was 21.17 hours, larvae development 25.97; 48.08 and 233.65 hours for the first, second and feeding phase of third instars, respectively. The development period of post feeding larvae, prepupa and pupa development was 322.26 hours (Kruger *et al.*, 2002). In a succession study using rabbit carcasses done by Calderon-Arguedas *et al.* (2005a), they found *S. nudiseta* was commonly occurred in the active decomposition phase. In another separate study, Calderon-Arguedas *et al.* (2005b) have shown that by using rabbit carcass, the collection of *S. nudiseta* larvae began between the 7th and 11th day post-exposition and ended at approximately the 30th day post exposition. In Malaysia, Omar *et al.* (1994) suggested that Malaysian *S. nudiseta* adults might be attracted to corpses that were already putrid. They also reported that in their preliminary studies of arthropod succession on cat carrions placed indoors showed that *S. nudiseta* of late third instar were found on 8th to 10th day and noted the pupation began on day 14 and the first adult emergence was detected on day 20 which conclude the pupation period of 6 days. In the current study, the pupation period was 8.4 ± 0.4 days. The differences in pupation period might probably be due to the differences of the temperature, relative humidity or the medium used. Furthermore, Omar *et al.* (1994) mentioned in their 5 years study, they failed to trap this species using meat and fish baits outside houses and in various outdoor habitats and only able to trap this species when carrion were place indoors. On the contrary, in rural area, D'almeida (1994) found this species only attracted to fish bait in the Tijuca forest in Brazil. While, Oliveira *et al.* (2002) trapped this species using decomposing beef liver around the Rio de Janeiro zoo (urban area) in Brazil. They also reported the population of this species peaked in April and July around the zoo. In Alexandria, Egypt, Tantawi *et al.* (1996) reported this species was a secondary invader of slow decaying carcasses in fall. Other workers, de Souza *et al.* (2008) reported this species occurred on the carcass in large numbers and in specific periods (only in winter) in southern Brazil. In Venezuela, Rabinovich (1970) established colony of this species from larvae collected from 2 dead laboratory rat in the city of Mérida (urban area). Besides the difference in geographic region, the differenent preferences of adults towards the baits were the locations of the study where some workers trapped it in the rural areas (fish bait) while the other workers managed to trap it in the urban area (decomposing beef liver). However, in our opinion, the difference preferences of adults towards the baits and seasonal distribution of this species in Malaysia merit further investigations.

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