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First study on the larval growth parameter and growth rate of a forensically important blow fly, *Hypopygiopsis violacea* (Macquart, 1835) (Diptera: Calliphoridae)

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Abstract. Blowfly larvae play an important role on ecological function in decomposition of animal remains. Most commonly, the blowfly larvae are used to estimate the minimum time since death, which is the minimum post-mortem interval (PMI), using growth parameter and larval length as a 'biological clock'. This study examined the larval growth of forensically important fly, *Hypopygiopsis violacea* (Macquart, 1835) (Diptera: Calliphoridae) at a temperatures of 28 ± 2 ºC, relative humidity at 70 ± 5% and photo period at 12 dark : 12 light. Detailed observations were made on rate of development of *Hypopygiopsis violacea* by measuring the length of larvae. The larval growth rates were determined. The growth parameter of egg, first, second and third larval instar, total larval period, pupal period and egg to adult period were also studied and determined. This is the first report on the growth characteristics of *Hypopygiopsis violacea*.

Keywords: forensic entomology, blowfly, *Hypopygiopsis violacea*, post-mortem interval, Malaysia

1. Introduction

Blow flies are among the first insects to discover and colonise human remains. The larvae of blow flies are also used extensively in forensic entomology, predominantly to establish the minimum time elapsed since death, or minimum post-mortem interval (PMI), using the larval length as a 'biological clock'.

Estimate of PMI on forensic analysis of the age of blowfly larvae on a corpse, are generated from standardised larval development rates. Many studies on the growth parameter and development rates have been conducted on the forensically important flies around the world, such as *Calliphora vicina* (Robineau-Desvoidy) [1,3], *Phormia regina* (Meigen) [2,3], *Lucilia sericata* (Meigen) [4], *Protophormia terraenovae* (Robineau-Desvoidy) [5], *Eucalliphora latifrons* (Hough) [3] and *Chrysomya rufifacies* (Macquart) [6]. Nevertheless, these works were mostly conducted in Europe where conditions are vastly different from the tropics such as Malaysia.

In experimental studies, calliphorid flies have been recorded arriving at carcasses within minutes of their exposure. Chen *et al*. [7] reported that within an hour, *Hypopygiopsis* (Townsend) sp. (Diptera: Calliphoridae) was observed as the first visitor on the monkey carcass placed outdoor and maggot masses were found on the same day. Until now, no detail studies on growth parameter and development rate of *Hypopygiopsis* sp. has been documented in the world.

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This study was conducted to study the growth parameters and growth rates of *Hypopygiopsis violacea* (Macquart, 1835) (Diptera: Calliphoridae) recovered from animal remains in Malaysia for more accurate determination of PMI.

2. Materials and Methods

2.1. Source of larvae

Adult flies of *Hypopygiopsis violacea* were used for this study. There were collected from a monkey (*Macaca fascicularis*, Raffles) carcass located at a forested area in the Wildlife Research Centre, University of Malaya, 16 Miles, Jalan Gombak, Selangor, Malaysia. Adult flies of *Hypopygiopsis violacea* were caught by using sweep net. The flies obtained from field were bought back to laboratory of Medical Entomology Unit, Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. A total of 20 grams beef was provided as oviposition medium immediately. Adults were kept at temperature of 28 ± 2˚C, relative humidity at 70 ± 5% and photo period at 12 dark : 12 light. Flies were allowed access to water and granulated sugar at all times. Observations were made hourly until eggs were oviposited on the beef. Once the eggs have been laid, the beef was removed and replaced with fresh beef.

2.2. Observation and measurement of larval length

Eggs on the beef were observed every 30 minutes, so that newly hatched larvae could be counted and removed. Once the newly hatched larvae were observed, a total of 3 larvae were randomly collected every 4 hours. For measurement purpose, the larvae were collected, killed and preserved in 70% ethyl ethanol. The length of the preserved eggs, larvae and pupae were measured to the nearest 0.01 mm using an electronic digital calliper. Samplings continued until first pupa was observed. The parameters for the time of pupation were determined by failure of the larvae to elongate and move in response to being disturbed [8]. Observations on pupae were made every 4 hours until the first adult has emerged. Only 2 experimental replications were conducted in this study, owing to the difficulties of colonising this forested blow fly species.

2.3. Data analysis

The length of eggs, larvae, pupae and adults were measured by using a calliper and pooled to obtain the mean number (± SE). The duration of egg, first, second and third larval instar, total larval period, pupal period and egg to adult period were calculated. All statistical analysis was performed by using SPSS v10 software.

3. Results and Discussion

All The larval growth of *Hypopygiopsis violacea* showed a pattern of rapid increase in length, followed by shrinking prior to pupation (Figure 1). Table 1 shows the duration of egg, first, second and third larval instar, total larval period, pupal period and egg to adult period of *Hypopygiopsis violacea*. In our study, it was shown that the *Hypopygiopsis violacea* was able to lay 120 – 150 eggs per female. Once the eggs were oviposited, the period for eggs maturation and hatching took about 6 hours to reach the first instar larvae. In the feeding phase, the larvae of this species gone through first, second and early third instar stages with mean duration of 12, 22 and 16 hours, respectively. After about 50 hours (2 days 2 hours) of feeding phase, the third instar larvae transformed into post-feeding larvae (late third instar) with a mean duration of 114 hours (4 days 18 hours) before pupation. The pupae took about 138 hours (5 days 18 hours) to develop and emerge as adults. Thus, the total period from eggs to larvae, pupae and emerged as adults took about 308 hours (12 days 20 hours).

Growth parameter and growth rate for the immature stages of *Hypopygiopsis violacea* were presented in Table 2. Once the eggs (mean length = 1.69 ± 0.05 mm) hatched, the first instar feeding on the beef and grew rapidly to second instar and early third instar. These was significantly increase of length of larvae from 1.71 ± 0.01 mm to 6.05 ± 0.28 mm, followed by 14.75 ± 0.19 mm in first, second and early third instar larvae, respectively ($p < 0.05$). However, the length of third instar larvae was significantly decreased once they

entered the post-feeding phase ($p < 0.05$). The duration for all stages of larvae was also significantly different ($p < 0.05$).

The growth rate of *Hypopygiopsis violacea* increased significantly in feeding phase, with 0.14 ± 0.00, 0.28 ± 0.04 and 0.98 ± 0.23 mm/hour in first, second and third instar ($p < 0.05$). However, significant decrease in length was observed on the third instar post-feeding larvae by 9 folds ($p < 0.05$).

The size of larva, usually represented by its length, is related to its age as a function of time and temperature, and so theoretically should be able to provide the minimum age for the oldest (longest) larvae and therefore the minimum PMI. In terms of estimating larval age, there are potential problems in confusing the two phase (feeding and post-feeding) in estimation of larval length [1]. However, it is possible to discriminate between them. If the larvae are alive, those in the post-feeding phase can be identified through their behaviour of actively moving away from the food source, stop feeding and by the way they contract into barrel-shape when touched. If they are already dead, the crop of the post-feeding larva (visible in dorsal view or by dissection) is emptying or empty [9]. Once fully grown, the post-feeding larvae usually migrate away from the body to pupate, although some species will pupate on or in the immediate vicinity of the body [10].

This study indicated that the larvae of *Hypopygiopsis violacea* grew rapidly on the fresh carcass (≤ 48 hours). When the carcass was bloating (> 48 hours), the larvae in post-feeding stage were actively moved away from the carcass. The general absence of *Hypopygiopsis violacea* in monkey carcass may be due to their rapid growth and subsequent migration out of the carcass.

This study emphasizes on generate of local data when applying growth parameter and growth rate to estimate larval age in specific forensic cases. This is the first report on the growth characteristics of *Hypopygiopsis violacea* conducted in this region.

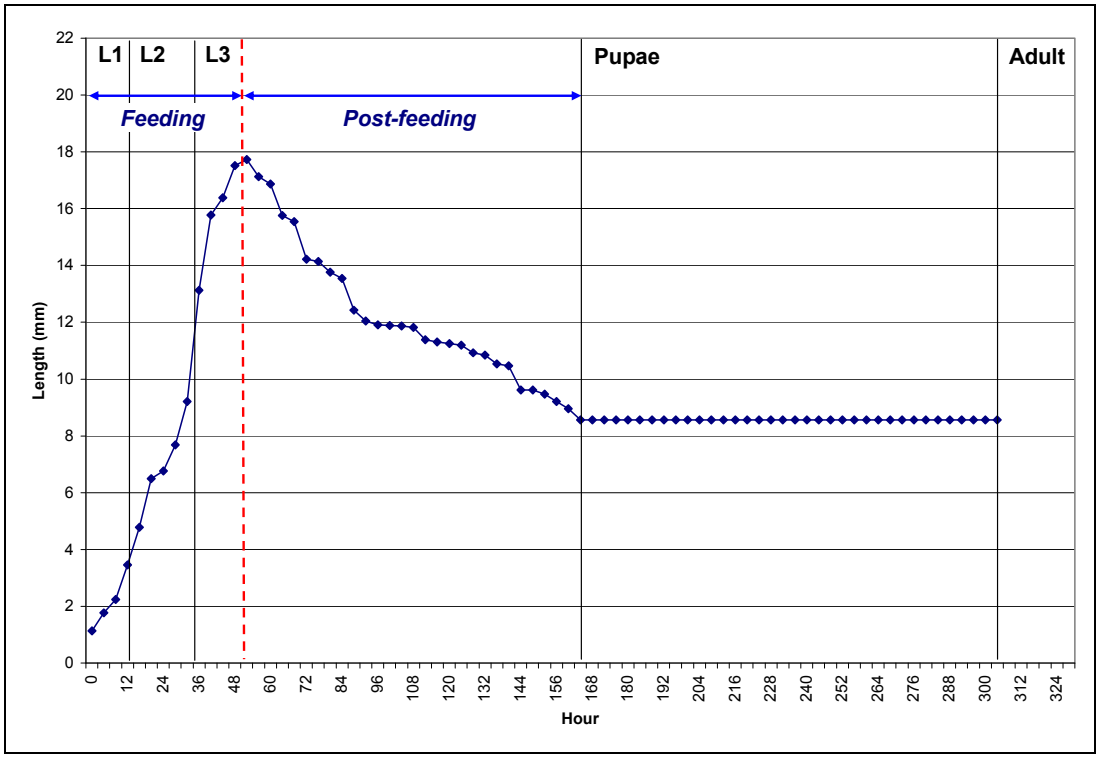

Fig. 1: Summary of body length over time for *Hypopygiopsis violacea* larvae grown at temperature 28.0 ± 2.0˚C, relative humidity at 70 ± 5% and photo period at 12 dark: 12 light.

Table 1. The duration of egg, first, second and third larval instar, total larval period, pupal period and egg to adult period of *Hypopygiopsis violacea*.

Stage	Duration	
	Hour (Mean ± SE)	Day and Hour
• Egg	6.25 ± 2.25	≈ 6 hours
• First instar, L1	12.00 ± 0.00	12 hours
• Second instar, L2	22.00 ± 2.00	22 hours
• Third instar, L3		
o Early L3 (Feeding phase)	16.00 ± 4.00	16 hours
o Late L3 (Post-feeding phase)	114.00 ± 2.00	4 days 18 hours
• Total feeding phase (L1 – early L3)	50.00 ± 2.00	2 days 2 hours
• Total larval period (L1 – L3)	164.00 ± 0.00	6 days 20 hours
• Pupal period	138.00 ± 6.00	5 days 18 hours
• Egg to adult period	308.25 ± 8.25	≈ 12 days 20 hours

Table 2. Growth parameter and growth rate for the immature stages of *Hypopygiopsis violacea.*

Phase	Stages	Mean ± SE		
		Length (mm)	Duration (hour)	Growth rate (mm/hour)
Pre-feeding	Egg	1.69 ± 0.05	6.25 ± 2.25	-
Feeding	L1	1.71 ± 0.01[a]	12.00 ± 0.00[b]	0.14 ± 0.00[c]
	L2	6.05 ± 0.28[a]	22.00 ± 2.00[b]	0.28 ± 0.04[c]
	L3	14.75 ± 0.19[a]	16.00 ± 4.00[b]	0.98 ± 0.23[c]
Post-feeding	L3	12.43 ± 0.05[a]	114.00 ± 2.00[b]	0.11 ± 0.00[c]
	Pupa	8.56 ± 0.29	138.00 ± 6.00	-

[a] = significantly different (F = 1211.42, P = 0.000)
[b] = significantly different (F = 379.56, P = 0.000)
[c] = significantly different (F = 12.22, P = 0.018)

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