

RESEARCH NOTE

LARVAL GROWTH OF *LIOSARCOPHAGA DUX* THOMPSON (DIPTERA: SARCOPHAGIDAE) UNDER UNCONTROLLED INDOOR TEMPERATURES IN MALAYSIA

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Abstract. The larval growth of *Liosarcophaga dux* Thompson (Diptera: Sarcophagidae) was studied under varying indoor room temperatures in Malaysia. Five replicates were established. The immature growth of this species from first instar until adult emergence was 307.0±3.0 hours. The mean larval length measured for second instar, third instar, post-feeding stage and puparia were 6.5±0.5 mm ($n=10$), 11.8±3.7 mm ($n=31$), 12.7±0.8 mm ($n=16$), and 9.5±0.5 mm ($n=15$), respectively.

Keywords: *Liosarcophaga dux*, forensic entomology, larval growth, Malaysia

INTRODUCTION

Forensic entomology was established in Malaysia during the 1950s (Reid, 1953). Since then, most of studies conducted in forensic entomology have focused on the larval development of calliphorids (Mohd Iswadi *et al*, 2007, Chen *et al*, 2008, Kumara *et al*, 2010). Only recently has there been a shift towards the species identification of sarcophagids in Malaysia (Kurahashi and Tan, 2009; Tan *et al*, 2010). However, studies on larval growth of these species are still lacking. Sarcophagidae, commonly known as flesh flies, is a large family comprising over 2,000 species, and representatives of this family are found

throughout the world, with most species occurring either in tropical or warm temperate regions (Byrd and Castner, 2010). Sarcophagidae are one of the forensically important families in Malaysia (Kumara *et al*, 2012). In a three-decade review of forensic entomological specimens conducted in Malaysia, 6.25% of the 448 specimens that were sampled from human remains were *Sarcophaga* spp (Lee *et al*, 2004). These species have also been known to cause myiasis (Cheong *et al*, 1973). Meanwhile, others have studied the diurnal and nocturnal distributions of dipterous flies in Malaysia and found that more *Liosarcophaga dux* were caught at daytime compared with nighttime. A succession study conducted by using a pig carcass in a palm oil plantation at Tanjung Sepat, Selangor, Malaysia reported the presence of *Sarcophaga* spp on the second

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day after the carcass was exposed (Heo *et al*, 2007).

To estimate the postmortem interval (PMI), there are two requirements to be fulfilled. First, is the correct identification of the sarcophagids, and second, is to establish the larval development of the respective flesh fly species. Thus, in this paper, the larval growth of *L. dux* was studied.

MATERIALS AND METHODS

Larval rearing

Larvae of *L. dux* were obtained by baiting outside the Department of Forensic Medicine, Penang Hospital (5° 23' N, 100° 21' E), by placing a 24-hour decayed boneless beef steak in a container [5.5 cm (h) x 4.0 cm (w) x 4.0 cm (l)] (As we do not know the species of larvae until the emergence of adults, 9 replicates were done. Five of the replicates were *L. dux*). The top of container was wrapped with pin-holed transparent plastic using a rubber band. From the collected larvae, one to two larvae were preserved in 70% alcohol for larval instar stage determination, while the rest were transferred into the rearing containers measuring 11 cm (h) x 10 cm (w) x 10 cm (l), with a 2.5 cm thick layer of sterilized soil. The boneless meat steak (in pieces of 25 g each) was given *ad libitum*, and a wet paper towel was placed on top to prevent the meat from drying and acted as skin. The top of the container was covered with a paper towel using a rubber band to prevent the infestation of pest flies.

The developmental data, temperature and relative humidity of the rearing room were recorded from the time the larvae were collected until the adult emergence using a RH-520: Humidity

+ Temperature Chart Recorder (©Extech Instruments Nashua, NH). From each replicate, an average of two-to-five larvae were randomly collected, every morning (08:00 AM) and evening (05:00 PM), until the larvae reached the pupal stage. These larvae were killed by placing them in warm water (52±10°C) and preserving in Kahle's solution. The instar stages of the preserved larvae were recorded by determining the number of posterior spiracle slits under the stereomicroscope (©Olympus, Tokyo, 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. After the rearing was completed, the developmental hours were calculated, and the graph of larval length against developmental hours was constructed using JMP 8.0.1 software (©SAS Institute, Cary, NC).

Data analysis

The data analysis was performed on the developmental hours and on the mean larval length of the larvae ($n=79$) to evaluate if there were any significant differences among the replicates. Normality test (Shapiro-Wilk test) was performed before the one-way analysis of variance (One-way ANOVA) test was conducted. If the data was normally distributed, the one-way ANOVA were performed. If the data was not normally distributed, the Levene's test, the Brown-Forsythe test, O'Brien's test and Bartlett's test were performed to determine if the variance between the replicates was unequal. If any of these four tests revealed the variances between the replicates were unequal, the results of the Welch ANOVA for unequal variances was reported instead (Sall *et al*, 2007).

Further, if ANOVA showed there was a significant difference between the mean

Table 1
The developmental hours and mean larval length (mm) of five replicates of *Liosarcophaga dux* at fluctuating temperature of $28.9 \pm 1.2^\circ\text{C}$ and relative humidity $64 \pm 10\%$.

		Developmental stages				
		2 nd instar	3 rd instar	Postfeeding	Pupae	Emergence
Developmental hours	MIN	16.0	26.0	66.0	88.0	304.0
	MAX	22.0	55.0	80.0	94.0	310.0
	MEAN	19.0	40.5	73.0	91.0	307.0
	SE	± 3	± 14.5	± 7	± 3	± 3
Mean larval length ($n=72$) ^a	MIN	6.0	8.0	12.0	9.0	N/M
	MAX	7.0	15.5	13.5	10.0	N/M
	MEAN	6.5	11.8	12.7	9.5	-
	SE	± 0.5	± 3.7	± 0.8	± 0.5	-

MIN, minimum; MAX, maximum; SE, standard error; N/M, no measurement

^aData excluded first instar larvae.

larval length of larvae or developmental times among the replicates, Tukey-Kramer Honestly Significant Difference (HSD) test was used to determine which of the five replicates were different from one another. For the mean larval length, the data analysis was performed until the pupae stage; while for the developmental times, the data analysis was performed until the adult emergence. These data analyzes were described by Linnea (2004). All statistical analysis was performed in JMP 8.0.1 software (©SAS Institute, Cary, NC).

DISCUSSION

The rearing was conducted indoors at the mean temperature of $28.9 \pm 1.2^\circ\text{C}$ and relative humidity $64 \pm 10\%$. The developmental hours of *L. dux* across the five replicates was normally distributed (Shapiro-Wilk test, $p=0.063$). The One-way ANOVA ($F_{4,35} 0.0434$, $p=0.9963$) shows that there was no significant difference in the developmental hours between the five replicates. However, for the mean larval length, the distribution was not normally distributed (Shapiro-Wilk test, $p<0.001$).

Further tests (O'Brien's test, $p=0.9984$; Brown-Forsythe test, $p=0.9932$; Levene's test, $p=0.9903$; Bartlett's test, $p=0.9984$) found the variance was equal among the replicates which allowed for the use of one-way ANOVA. One-way ANOVA ($F_{4,35} 0.0804$; $p=0.9878$) found no significant differences of mean larval length between the five replicates.

The eggs of this genus fly hatch in the female fly's reproductive tract, and therefore it lays first instar larvae (Gunn, 2006). The lengths of the first instar were 2.0 ± 0.1 mm ($n=7$). All the first instar of the five replicates were collected within a duration of 3 hours after the decayed meat was placed outside for baiting. The total larvae collected for each replicate were 43, 14, 32, 52, and 54 larvae, respectively. It has been reported the viviparous females of sarcophagids are less fecund than blowflies and houseflies, and do not deposit all their larvae in the same carrion, rather spreading them evenly among several carcasses (Galante, 2008). The total life history strategy of sarcophagids is geared to produce a few offspring which rapidly use

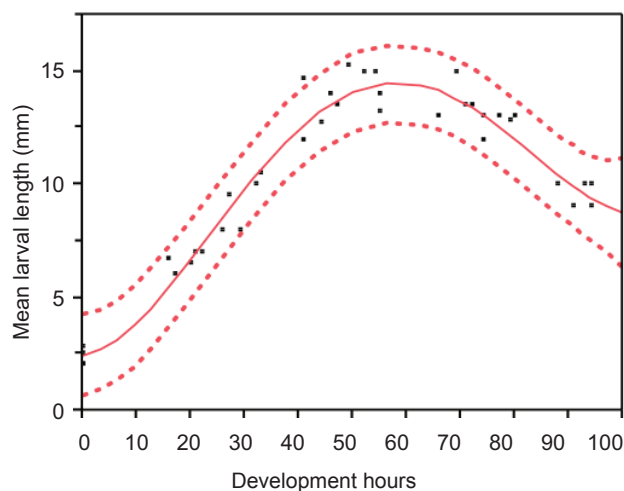


Fig 1—The growth curve of *Liosarcophaga dux* larvae (first instar until pupae stage-five replicates) grown at $28.9 \pm 1.2^\circ\text{C}$. The fit with the quartic polynomial function (solid line- R^2 0.963) has 95% confidence limits of the model (dashed lines).

fresh carrion before exploitation, and often overexploitation, by the opportunistic calliphorids (Denno and Cothran, 1976). The larval stage of *L. dux* from first instar until pupa stage was 91 hours, which was faster when compared with the blowflies *Chrysomya megacephala* (122 ± 12 hours) at $29 \pm 2^\circ\text{C}$ (Kumara *et al*, 2010) and *Chrysomya rufifacies* (121.6 ± 7.9 hours) at $28 \pm 2^\circ\text{C}$ (Chen *et al*, 2008). Thus, in comparing the flesh fly *L. dux* and the blowflies, *C. megacephala* and *C. rufifacies*, the former spend more than 50% of their immature life cycle in the pupation period.

Fig 1 represents the larval growth curve of *L. dux*. The developmental hours for *L. dux* from first instar larvae until adult emergence was 307.0 ± 3.0 hours at the fluctuating temperature of $28.9 \pm 1.2^\circ\text{C}$. In Saudi Arabia, a study conducted by Al-Misned (2004) at a constant temperature of 28°C found the development of *L. dux* from first instar until adults emergence

was 393.9 ± 20.9 hours. In northern Thailand, Sukontason *et al* (2010) studied the developmental rate of this species under natural ambient temperature of $26 \pm 2^\circ\text{C}$ and found the prepupae began at 72 hours, 84 ± 12 hours and 96 hours for summer, rainy season, and winter, respectively. In Riyadh, Saudi Arabia, Amoudi *et al* (1994) studied the developmental rate of *Parasarcophaga (Liopygia) ruficornis* (Diptera: Sarcophagidae), and reported at the constant temperature of 28°C ; the mean development times for feeding larvae, wandering larvae, pupae, and total development were 86.4 ± 8.6 , 76.8 ± 12.2 , 273.6 ± 13.7 , and 436.8 ± 15.4 hours, respectively. In the present study, the post-feeding, pupae, and emergence of *L. dux* was initiated at 73.0 ± 7.0 , 91.0 ± 3.0 and 307.0 ± 3.0 hours at $28.9 \pm 1.2^\circ\text{C}$. The maximum mean larval length measured was 15.5 mm from third instar larvae (Table 1). The pupal length of *L. dux* in current study was 9.5 ± 0.5 mm. Sukontason *et al* (2006) measured the length of puparia of *L. dux* as 9.9 ± 0.3 mm.

From the reviewed literature, there are differences in the development of *L. dux* found by different researchers. These differences occur for various reasons, such as genetic differences, different methods, and data representation by authors (sampling frequency, fluctuating or constant temperature, larval density, and differences in rearing medium used) (Richards and Villet, 2009; Cammack and Nelder, 2010; Gallagher *et al*, 2010). Our focus in this paper was to establish a larval growth data for *L. dux* to use for post-mortem interval estimation of forensic cases in Malaysia.

ACKNOWLEDGEMENTS

The authors thank Universiti Sains

Malaysia for funding provided under the research grant USM RU/1001/PBiology/815009. We would also like to thank Ms Tan Siew Hwa, Universiti Malaya for identifying the sarcophagid species concerned and Mr Phil Withers for proofreading the manuscript.

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