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Life table of forensically important blow fly, *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae)

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Abstract. The life table of the *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) were studied under the laboratory conditions at 28 ± 2.5ºC and 75 ± 10% relative humidity using field strain from Penang, Malaysia. The raw data collected were analysed based on the age-stage, two-sex life table theory in order to consider both sexes and the variable developmental rate among individuals and between sexes. The population parameters; intrinsic rate of natural increase (r) was 0.2361 d^{-1}, the finite rate of increase (λ) was 1.2663 d^{-1}, the net reproduction rate (R_0) was 97.2 and the mean generation time (T) was 19.4. The two-sex life table analysis provides a comprehensive description of the changes in structure stage of *C. rufifacies* which is potentially useful for forensic investigations in the country.

INTRODUCTION

The hairy maggot blow fly, *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) is a forensically important species in many parts of the world (Sukontason *et al.*, 2007). Both larvae and adult of this species have been used to estimate the post-mortem interval (PMI) in human corpses and animal carcasses (Lee *et al.*, 2004; Salleh *et al.*, 2007; Nazni *et al.*, 2011; Kumara *et al.*, 2012). The larvae of this species were found to be the primary carrion feeders, and the adult flies show a preference for fresh remains (Byrd & Castner, 2010). The colonization of adults from the family of *Calliphoridae* on carcasses were observed after 30 minutes of exposure in the environment (Heo *et al.*, 2007). In several studies conducted in Malaysia, the species of *C. rufifacies* were mostly found after *Chrysomya megacephala* (Heo *et al.*, 2007, 2008, 2010). This is due to the predacious activity of this larva which predates other fly larvae (Byrd & Castner, 2010). The occurrence of eggs or larvae of *C. megacephala* were suggested to stimulate oviposition of *C. rufifacies* so that this species could consume on the immature stages of *C. megacephala* (Azwandi & Abu Hassan, 2009). *C. rufifacies* can be found both indoor and outdoor environment either as single or mixed infestation with *C. megacephala* or other species of Calliphoridae (Kumara *et al.*, 2012).

The construction of the life table is an essential component in the understanding of a particular forensically important fly species (Sukontason *et al.*, 2007). In fact, Abou Zied *et al.* (2003) noted that an established life table of a population are very useful since it provides the most understandable description on the growth, survival and fecundity of a particular species of interest. The development rate of blow flies have been reported under natural temperature and photoperiod in Thailand with rapid development occurred in the hot season while slower in the rainy season with potentially giving significance in forensic investigations (Sukontason *et al.*, 2008). Although the development rate of this species have been recorded previously, no life table studies

have been established specifically on *C. rufifacies* in Malaysia, which is one of the primary species found in insect succession pattern of human and animal corpses (Kumara *et al.*, 2012; Syamsa *et al.*, 2017).

To anticipate both sexes and variable development rates among individual, the life table of *C. rufifacies* was constructed based on the age stage, two-sex life table analysis. This will include both the male and female individuals in various age stages in the calculations in order to construct the population parameters of this species. The cohort life table provides a brief description on the survivorship, development and reproduction or the fecundity of a population, which is an essential basic requirements in both the theoretical and applied population ecology (Chi & Yang, 2003). The objective of this study is to establish the life table of *C. rufifacies* with the aim of providing information that will greatly improve the forensic investigations in the country.

MATERIALS AND METHODS

Rearing of the insect colony

The life table study of *C. rufifacies* was conducted in the laboratory at Vector Control Research Unit, University Sains Malaysia (USM), Penang. The temperature and relative humidity in the laboratory were consistent at 28.0 ± 2.5ºC and 75 ± 10% respectively with a photoperiod of 12 hours light and 12 hours dark (12:12). Both temperature and humidity were recorded twice a day using a psychrometer and data logger respectively on a daily basis. A colony of *C. rufifacies* was initially collected from Durian Valley, USM and reared in a (43 cm x 43 cm x 43 cm) cage in the laboratory. The adult flies were fed on 10% sucrose solution and fresh beef liver as a source of protein as well as for oviposition. Approximately 30 gram of fresh beef liver were provided for the colony and after 24 hours, the eggs were obtained from the oviposition medium. Oviposition medium containing the deposited eggs were removed from the colony and 300 eggs were individually transferred from the existing beef liver into 10 gram of fresh beef liver inside the paper cups using a wetted paint brush. This fresh beef liver medium was provided in vented paper cup, and then placed into a plastic container (30 cm diameter x 90 cm height). Three replicates were conducted in this study with 100 individuals per replicate in separate rearing containers. The entire containers were labelled from number 1 to 300. During the post feeding stage, the larvae were provided with sawdust for pupation process. The newly emerged female and male adults were paired and kept in a plastic container contained with fresh beef liver and 10% sucrose in a separate petri dish. Sucrose were checked and added if necessary while beef livers were changed daily. The male and female individual in each of the container were labelled based on their previous numbers. The raw data of the larval emergence from the egg stage, life span of the larval phase (three different larval stage were grouped together as one, due to the complexity in identifying the different stages for the total cohort at a time), the beginning of the pupation, life span of the pupae phase, the adult emergence (based on the sexes), the first female oviposition, daily fecundity; numbers of eggs laid per day and finally the mortality of the adult (based on the sexes) were recorded separately for each individual in a daily basis. The observations and raw data recordings were continued until the last individual of the cohort died.

Life table analysis

The raw data of 300 individuals of *C. rufifacies* were analysed by using the age-stage, two-sex life table approach as developed by Chi & Liu (1985) and the TWOSEX-MSChart computer program (Chi, 2008). Means and standard errors of the population parameters were estimated by using the Bootstrap method (Efron, 1979) which was also included in the previous computer program.

Statistical analysis

The statistical analysis was conducted by using SPSS 20.0. The adult longevity of the female and male were tested for normality by using the Shapiro-Wilk test. Since the data was not normally distributed, a non-

parametric Mann-Whitney U test was conducted to analyse the significant different of the adult longevity between the two sex. A non-parametric Spearman's rank order correlation was performed to test the significant correlation between the female longevity and fecundity.

RESULTS

The developmental time of the immature stages namely egg, larvae and pupae, the longevity of the adult female and male fly, the adult pre-oviposition period (APOP) and total pre-oviposition period (TPOP) of the female, and the fecundity of the female are given in Table 1. Out of 300 eggs, only 254 eggs hatched successfully with egg survival rate of 84.7%. Only 137 adults emerged with the pre-adult survival rate of 45.7%. The mortality rate of the larvae and pupae were 34.0% and 5.0% respectively. The sex ratio of this species was recorded as 1: 1. The mean adult longevity of the female and male were 25.2 ± 1.0 and 22.4 ± 1.1 respectively. The adult longevity were significantly different between the sex with $p < 0.05$ ($z = -2.146$, Mann-Whitney U test). The result showed that the female of *C. rufifacies* lived significantly longer than the male.

The female of *C. rufifacies* had a mean adult pre-oviposition period (APOP) of 5.6 ± 0.1 days, while a total pre-oviposition period (TPOP) was 14.5 ± 1.2 days. The mean fecundity was showed by the number of eggs per female which was 416 ± 19.9. The Spearman rank order correlation analysis, which conducted to disclose the correlation pattern that exists between the female longevity and the fecundity revealed a significant positive correlation between this two life history parameter, $\underline{r}(70) = 0.425$, $p < 0.05$. This suggested that the longer the life-span of the female individual, the higher the fecundity rate of the individual.

The age-stage specific survival rate (s_{xj}) of *C. rufifacies* clearly showed the development and the survival rate was overlaps between different stages. The survival rate explains the probability of the survival of the newly oviposited eggs to the age x and stage j. The survival rate of the egg was 0.85, the larva; 0.51 and the pupa; 0.46. While for the female and male adults were 0.23 and 0.22 respectively. The development of the adult stage of both sexes of *C. rufifacies* occurs on the same day 8 (Figure 1).

The age-specific survival rate (l_x); probability of the new egg survives to age x, ignoring the stage and sex by combining all individuals, age-stage specific fecundity (f_{x4}); mean number of eggs of female (stage, $j = 3$), age-specific fecundity (m_x); the age-specific fecundity of the total population, where data for individuals at age x was pooled, and age-specific maternity ($l_x m_x$); was the product of l_x and m_x of the *C. rufifacies*. A sigmoid curve of the age-specific survival rate (l_x) presented in Figure 2. The age-stage specific fecundity (f_{x4}) of female, age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) showed a maximal peak on day 19, although there were four peaks of

Table 1. Life table data of *C. rufifacies* at 28.0 ± 2.5°C and 75 ± 10% R.H.

Parameter	Stage	(Initial eggs = 300)	
		n	Mean ± SE
Developmental time (days)	Egg	254	1 ± 0
	Larva	152	4.7 ± 0.1
	Pupa	137	3.6 ± 0.8
Adult longevity (days)	Female	70	25.2 ± 1.0
	Male	67	22.4 ± 1.1
Adult pre-oviposition period (APOP) (days)	Female	64	5.6 ± 0.1
Total pre-oviposition period (TPOP) (days)	Female	64	14.5 ± 1.2
Fecundity (eggs/female)	Female	70	416 ± 19.9

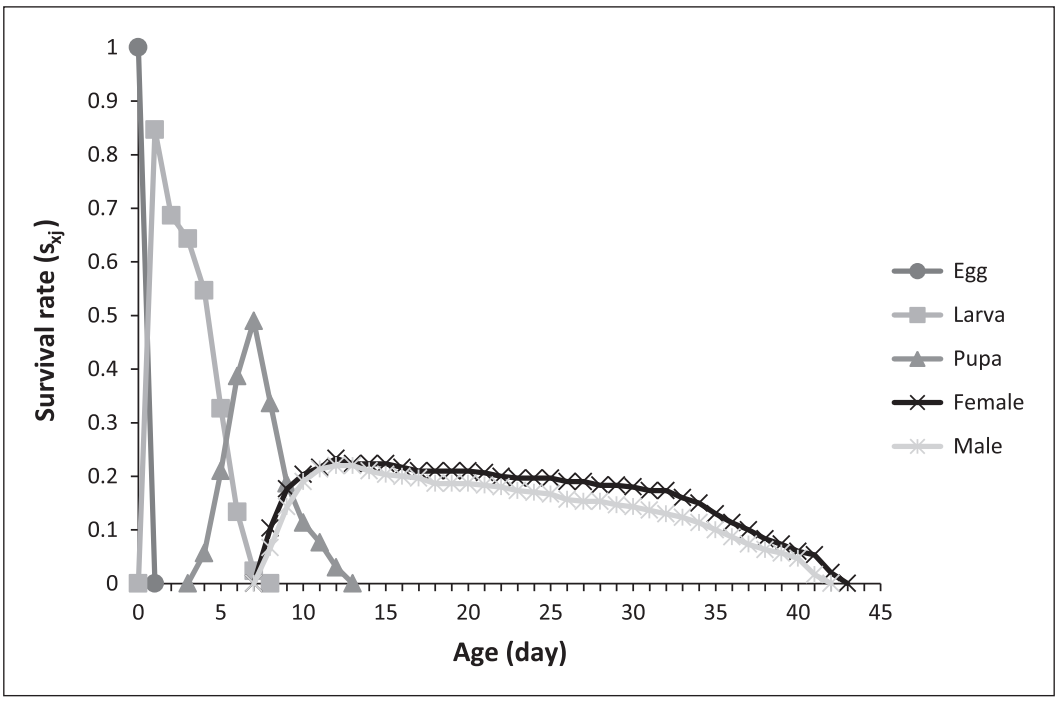

Figure 1. Age-stage specific survival rate (s_{xj}) of *C. rufifacies* at 28.0 ± 2.5 ºC and 75 ± 10% R.H.

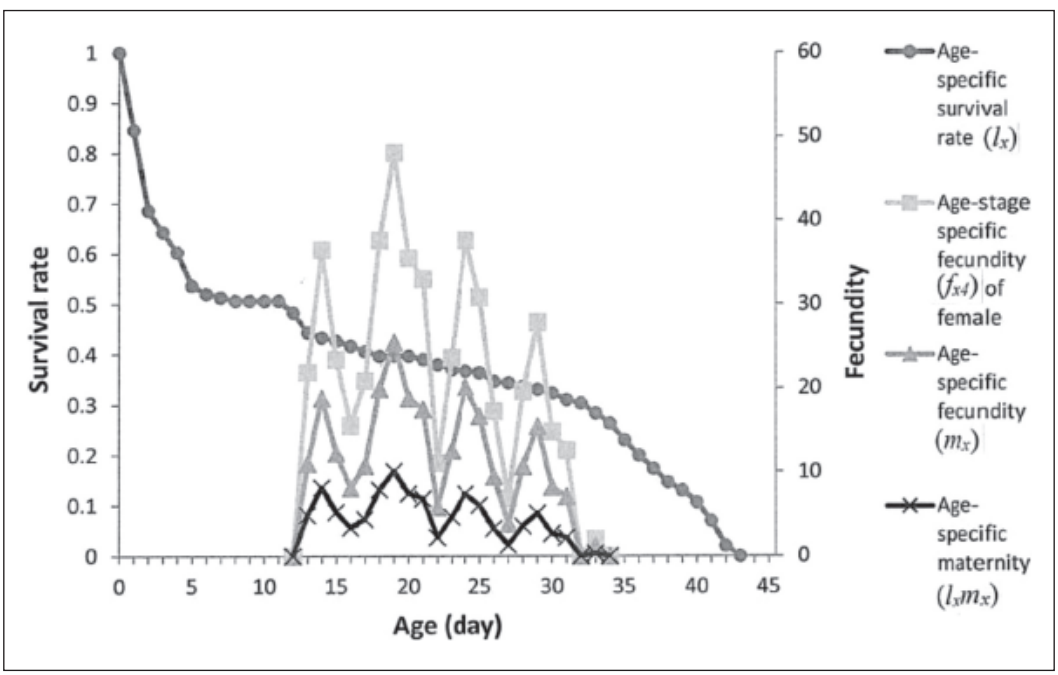

Figure 2. Age-specific survival rate (l_x), age-stage specific fecundity (f_{x4}) of female, age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *C. rufifacies* at 28 ± 2.5ºC and 75 ± 10% R.H.

those parameters along the lifespan of the fly.

The prediction of the future life of *C. rufifacies* population was presented by the age-stage specific life expectancy (e_{xj}) in Figure 3. The age-stage specific life expectancy (e_{xj}) was the time that an individual of age x and stage j was expected to live. The life expectancy of the newly oviposited egg was 17.0. The graph showed that the female life expectancy was higher than the male flies. The peak life expectancy of the female and male were occurred on day 8 of the fly life span which were 26.2 and 23.6 respectively. Based on the sexes, the values of the adult life expectancy were found to decrease gradually with the age.

The age-stage specific reproductive value (v_{xj}) of *C. rufifacies* was illustrated in Figure 4. The age-stage specific reproductive value (v_{xj}) was the expectation of future offspring of an individual at the age x and stage j. The reproductive value for a newly oviposited egg of the *C. rufifacies* was the value of the finite rate of increase (λ), which presented in Table 2. The peak reproductive value of the female individuals occurred during the age of day 18 with the value of 146.4. This value showed that the female during this age contributed the most to the reproductive value of the population.

The population parameters of the intrinsic rate of natural increase (r), the finite rate of increase (λ), the net reproduction rate (R_0) and the mean generation time (T) which were calculated by using the age-stage, two-sex life table theory were presented in Table 2.

DISCUSSIONS

The results of this study showed that the egg survival rate was 84.7% that is similar to those conducted by Abou Zied *et al.* (2003) on the blowfly species of *Lucilia cuprina*. Pre-adult survival rate in this study was 45.7%, also closely resembles to Abou Zied *et al.* (2003). Compared to our finding, life table studies on *C. megacephala* by Gabre *et al.* (2005) reported 38% of pre-adult survival rate. Another study by Sukontason *et al.* (2008)

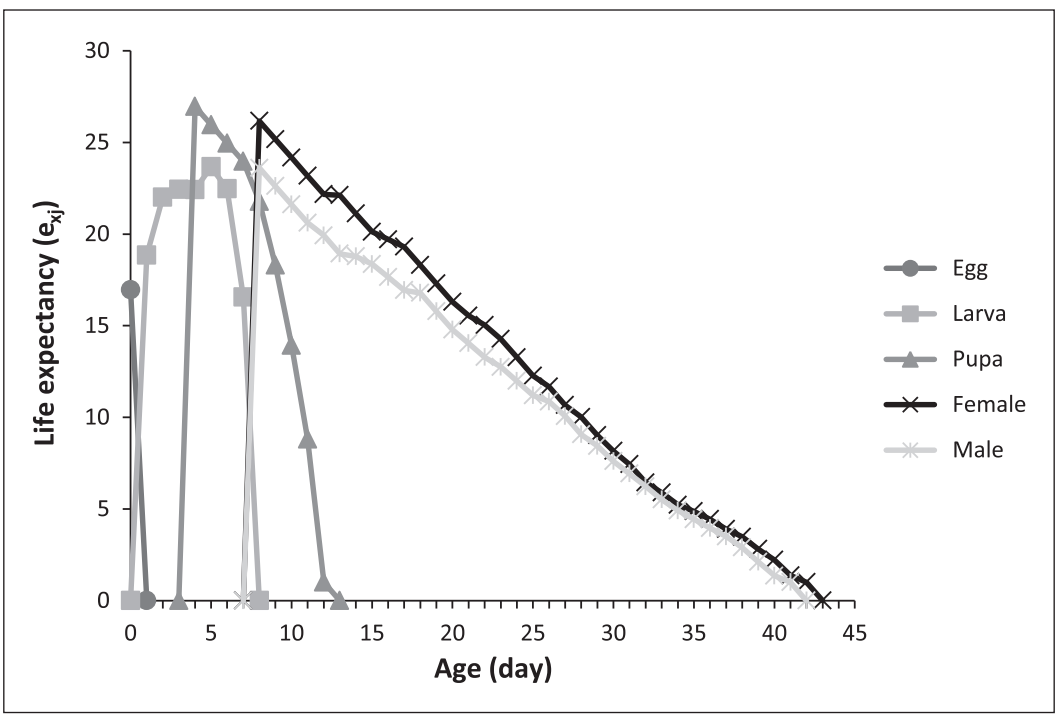

Figure 3. Age-stage specific life expectancy (e_{xj}) of *C. rufifacies* at 28 ± 2.5 ºC and 75 ± 10% R.H.

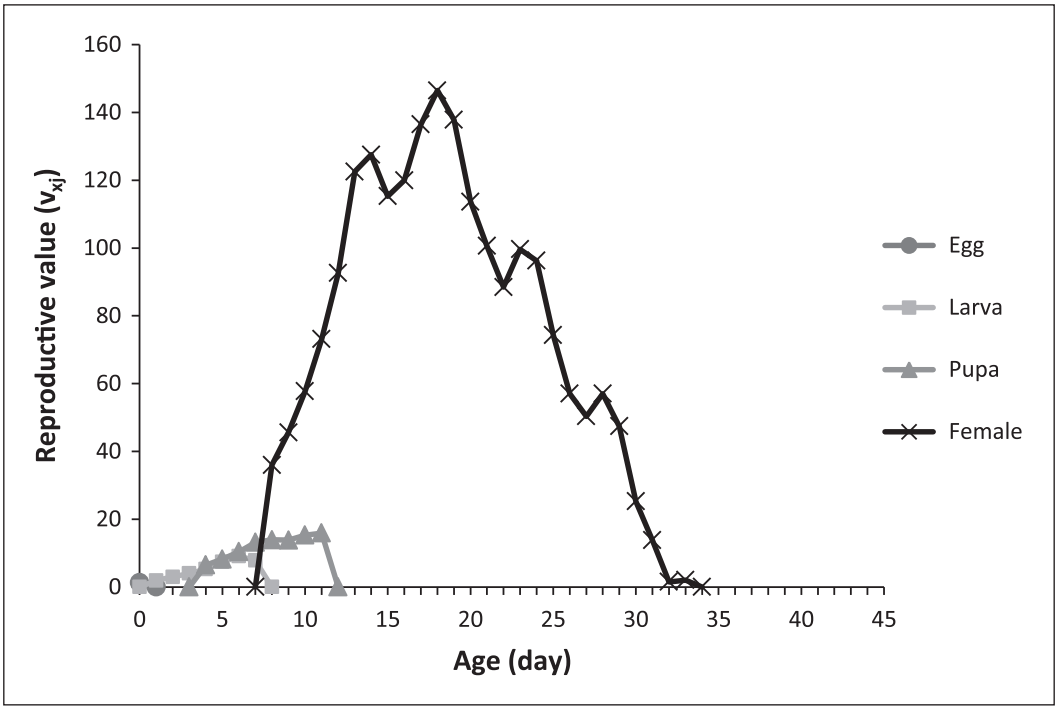

Figure 4. Age-stage specific reproductive value (v_{xj}) of *C. rufifacies* at 28 ± 2.5ºC and 75 ± 10% R.H.

Table 2. Population parameters of *C. rufifacies* at 28 ± 2.5°C and 75 ± 10% R.H.

Population parameters	Mean ± SE*
Intrinsic rate of increase, r (days^{-1})	0.2361 ± 0.0068
Finite rate of increase, λ (days^{-1})	1.2663 ± 0.0086
Net reproductive rate, R_o (offspring/individual)	97.2 ± 10.9
Mean generation time, T (days)	19.4 ± 0.2

* Mean and standard error obtained by using the Bootstrap method (m=10,000)

indicated that the developmental rate from newly hatched larvae of *Chrysomya* sp. until pupation at 28°C to 31°C were between 5 to 7.5 days resemble developmental time recorded in this study which was approximately 8 days at 28.0 ± 2.5°C. Local study by Ahmad Firdaus *et al.* (2009) documented that the development for *C. rufifacies* were 9.92 days at 27°C, 9.13 days at 30°C and 7.44 days at 33°C. Their results clearly showed that the lower temperature lengthen the development of *C. rufifacies*. Differences between previous studies considerably due to temperature and also other various factors such as relative humidity, food sources, geographical origin (Sukontason *et al.*, 2008) and photoperiod (Nabity *et al.*, 2007). Various food sources or culture media were used including beef meat (Gabre *et al.*, 2005), swine liver (Sukontason *et al.*, 2008) and salted cod (Esser, 1991). This variation of food sources largely contributed to the differences in development and survival rate of pre-adult flies.

The study produce a sex ratio of 1:1, which similar to the findings of Esser (1991), who also documented the laboratory study on *C. megacephala*. The mean longevity of male and female adult *C. rufifacies* in this

study were 25.2 and 22.4 days respectively while Gabre *et al.* (2005) also found similar result, with male and female adults recorded mean longevity of 25.3 and 25.8 days respectively. The results suggested that the female longevity was significantly longer than the male. This result was also similar to those of Abou Zied *et al.* (2003) and El-Shazly *et al.* (1995) who studied on blowflies, *Chrysomya albiceps* and *Parasarcophaga argyrostoma*.

The result demonstrated the mean adult pre-oviposition period (APOP) of 5.6 days by the female of *C. rufifacies* (Table 1). This finding was similar to Baumgartner (1993) who indicated that the oviposition of the female of this species could occurred approximately five days after mating. This finding also resemble the result obtained by Gabre *et al.* (2005), with the estimated mean APOP of 6 days. Russo *et al.* (2006) described temperature as the most influential factor which impacts the adult pre-oviposition period. Therefore, similar APOP were documented among the females that reared under the temperature of 28.0 ± 2.5 °C. The age of the first reproduction by females provides a crucial amount of impact on the population growth (Gabre *et al*., 2005). Therefore the estimation of the APOP was an important effect for determination of population growth. The mean total pre-oviposition period (TPOP) obtained in this study was 14.5 days (Table 1) which was considerably similar to the study of Abou Zied *et al.* (2003) conducted at 30 ± 2.5°C. However, the TPOP value recorded by Gabre *et al.* (2005) on *C. megacephala* at 26°C was much longer than recorded on *C. rufifacies* in this study.

The total fecundity in this study was 416 ± 19.9 eggs per female. The previous study conducted by Zumpt (1965) on a blowfly species of *Calliphora vomitoria* discovered that the species could lay 540–720 eggs while Abou Zied *et al.* (2003) reported the total fecundity of 445.69 ± 48.3 eggs per female for *L. cuprina*. However, El-Shazley *et al.* (1993) documented only 229.43 ± 30.33 of the total fecundity of *Chrysomya albiceps*. Thus, clearly showed that there were considerable variations in fecundity between different species of flies. In this study, the fecundity was significantly correlated with the female longevity. Previous studies also documented strong correlation between the fecundity and longevity in various species of flies (Rose, 1984; Partridge, 1986). Muller *et al.* (2001) in a study of Mediterranean fruit flies, *Ceratitis capitate* documented that the fecundity are correlated to longevity of fly individual. Thus, individual fly that are able to gain benefit from the expanded life span by increase the productivity of eggs are indeed tend to live longer.

The population size of *C. rufifacies* was rapidly decline during the immature stages but slowly or gradually decline during the adult stage (Figure 2). There are three general forms of survivorship curves known as type I, II and III. The type III curve mostly display by the insects and typically occurs with higher rate of mortality at early stage of life and slowly reduced the rate of decline with increasing age (Pianka, 2000). The results showed that the survivorship of this species exhibited the criteria of type III curve. The age-specific survival rate (l_x) showed a downward curve, which also indicated the occurrence of the characteristics of the type III curve. The age-stage specific fecundity (f_{x4}) of female, age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) begun from day 13 and retained until day 33 with maximum survival peak on day 19. The age-stage specific life expectancy (e_{xj}) of the adult were found to decrease gradually with the age x. The female life expectancy was higher than that of the male adults. This results explained that the reproductive cost in female was significantly higher than in male where the hormonal and physiological variations might lead to the diversity of the life expectancy in both male and female (Carey *et al.*, 1995).

The age-stage specific reproductive value (v_{xj}) of *C. rufifacies* showed that the maximum value of the reproduction occurred during adult stage of the female, although the immature stages also contributed some significant value. Therefore, it can be concluded that the adult female provided the major input in the growth of the future population. The study indicated that the

blowfly of *C. rufifacies* exhibiting the r-strategy criteria with high intrinsic rate of natural increase (r), high finite rate of increase (λ), high net reproduction rate (R_0), short mean generation time (T), small size of the individual and type III curve of survivorship. In previous study, Abou Zied *et al.* (2003) and Gabre *et al.* (2005) also indicated that *L. cuprina* and *C. megacephala* respectively as the r-strategists. Among all, the intrinsic rate of natural increase (r) being the most useful indicator of the potential population growth (Price *et al.*, 2011).

This present study provides information on the changes of age-stage structure of both sexes in blow fly, *C. rufifacies*. This two-sex life table of *C. rufifacies* is the first recorded in Malaysia and this finding could be crucial in understanding the optimal development of both sexes of this species. Based on this findings, *C. rufifacies* could be used in determination for entomological-based post-mortem when it recovered concurrently with other Calliphoridae species on human or animal corpses. Although this study conducted under laboratory conditions, it is nonetheless provides new insight of blow fly development that reflect the actual population growth in the natural environment. The information on the biology of *C. rufifacies* deserves more attention because it is greatly considered as one of the forensically and medically importance to human and animals.

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