

EFFECT OF TEMPERATURES ON THE DEVELOPMENTAL RATES OF BLOW  
FLY, *CHRYSOMYA RUFIFACIES* (MACQUART)

SURASUK YANMANEE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE MASTER DEGREE OF SCIENCE  
IN BIOLOGICAL SCIENCE  
FACULTY OF SCIENCE  
BURAPHA UNIVERSITY

JUNE 2016

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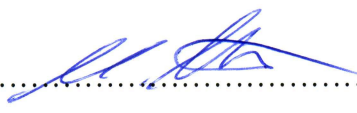
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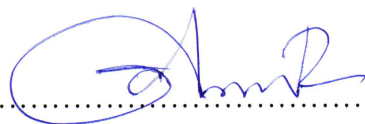
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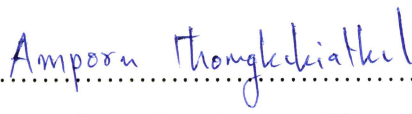
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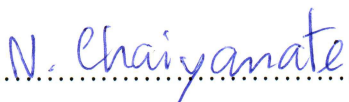
  
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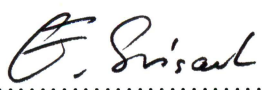
  
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KEYWORDS: *CHRYSOMYA RUFIFACIES*/ ISOMEGALEN  
DIAGRAM/ ISOMORPHEN DIAGRAM/ PMI.

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The developmental times from oviposition to eclosion of the forensically important blow fly, *Chrysomya rufifacies* were investigated at seven constant temperature regimes: 15, 18, 27, 30, 33, 36 and 39 °C. They were significantly positively influenced by rearing ( $F_6= 1572.22$ ,  $p<0.0001$ ). Times (mean± SD) were 618.62± 33.84, 547.52± 27.59, 216.6± 10.64, 187.09± 10.33, 168.03± 4.69, 172.63± 9.31 hours for 15, 18, 27, 30, 33 and 36 °C, respectively. No pupae emerged at 39 °C. Furthermore, larval body size was significantly influenced rearing temperatures. Using linear regression showed that temperature (x) explains 99% of variance in body size (y),  $y= 0.0007x - 0.0077$ ,  $R^2= 0.9906$ . The threshold temperature estimated from the linear regression was 11 °C for oviposition to pupariation,  $y= 0.0007x - 0.0077$ ,  $R^2= 0.9906$ , while it was 9.5 °C for oviposition to eclosion,  $y= 0.0002x - 0.0019$ ,  $R^2= 0.9589$ . The thermal constant (K) was 526.32. The ADD at 15, 18, 27, 30, 33 and 36 °C were 128.88, 182.51, 153.68, 155.91, 161.03 and 187.02 ADD respectively. However, it was 65.19 at 39 °C from egg to pupariation. No emergence was found at 39 °C. Additionally, the isomegalen and isomorphen diagrams were generated from these studies to provide more precise PMI estimates. Our results therefore provide new developmental information on *Ch. rufifacies* for forensic applications that include estimating PMI.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Statements and significance of the problems

When a suspicious death occurs “How long has person been death?” is the first immediately question in crime scene investigation. If the time in question is not known or unclear, the method for estimation of post-mortem interval (PMI) will be used by a number of techniques, especially the medical and scientific techniques (Adams & Hall, 2003). The investigator who judges the PMI by mean of decomposition could refer to the stage of decomposition since many types of changes to the dead body occur after death, including rigor mortis, livor mortis, dehydration, algor mortis, autolysis, autodigestion and putrefaction (Campobasso, Di Vella, & Introna, 2001). However, some of these are limited to the first 72 hours after death and can only be used in short PMI estimates (Henssge, Knight, Krompecher, Madea, & Nokels, 1995; Al-Alousi, 2002; Bourel, Callet, Hédouin, & Gosset, 2003). The estimation of PMI by mean of forensic entomology is the time that has elapsed since the oviposition of first colonized insects. The identification of the age of insects present on the dead body can be calculated to determine the PMI. After a person died, bacteria located in the large intestine begins decomposition results producing large among of foul-smelling gas attracted insects to colonized and feed on the carrion. The first group of insects colonization of corpse are usually blow flies, they can be attracted within minutes after death (Gennard, 2007). In particular, the hairy maggot blow fly, *Chrysomya rufifacies* (Diptera: Calliphoridae), is a forensically important fly often detected on human corpse (Zumt, 1965; Guimaraes, Papavero, & Prado, 1983). This fly species is distributed widely, covering the southeastern, central and southwestern portion of the United States, Japan, India, and the remainder of the Old World including Thailand. The adults usually arrive within the first ten minutes after death as long as atmospheric conditions are favorable for activity (Catts & Goff, 1992). Their life stages method seems to give good results only when the larvae of interest have been exposed to temperatures similar to those used in generating the reference value applied in the PMI calculation (Anderson, 2000). Moreover, the

temperature range in which the development rate is actually linear that is not wide enough to cover all temperatures. Furthermore, neither developmental times nor base temperatures for development have been calculated for *Ch. rufifacies* originate existing in Thailand. In addition, it is necessary to know the development rates for species involved in decomposition for the same condition at the possible crime scene (Introna, Altamura, Dell Erba, & Dattoli, 1989; Grassberger & Reiter, 2001). Developmental data are simply data pertaining to the duration of development of immature stages recorded at different constant temperatures and then could be summarized and generated in four developmental models, namely curvilinear regression (Ullyet, 1950; Higley & Haskell, 2001), isomorphen diagram (Grassberger & Reiter, 2001), isomegalen diagram (Reiter, 1984) and thermal summation models (Réaumur, 1735; Higley & Haskell, 2001), for the purpose of estimating PMI. In Thailand there is has no published study reports of any specific insect species isomorphen, isomegalen diagrams and minimum developmental threshold. For all those reasons I mentioned previously I aimed to investigate developmental data of, *Ch. rufifacies* under seven constant temperatures of 15, 18, 27, 30, 33, 36 and 39 °C to constructed isomorphen, isomegalen diagrams and minimum thermal threshold for specific species and provide a precise developmental time, development rates, ADD and ADH for *Ch. rufifacies*.

## 1.2 Objectives

**The main objectives of this research are as follows:**

1. To determine developmental times and the developmental rate of *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C.
2. To generate isomorphen diagram for *Ch. rufifacies* at seven different constant temperatures of 15, 18, 27, 30, 33, 36 and 39 °C.
3. To generate isomegalen diagram for *Ch. rufifacies* at seven different constant temperatures of 15, 18, 27, 30, 33, 36 and 39 °C.
4. To calculate the developmental minimum threshold temperature of *Ch. rufifacies*.
5. To calculate the ADD and ADH of and *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C.

### 1.3 Hypotheses

To accomplish the objectives of this research the following hypotheses were investigated.

#### 1.3.1 Hypothesis I:

$H_0$ : The developmental times of *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C will not differ.

$H_1$ : The developmental times of *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C will differ.

#### 1.3.2 Hypothesis II:

$H_0$ : The ADD and ADH of *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C will not differ.

$H_1$ : The ADD and ADH of *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C will differ.

#### 1.3.3 Hypothesis III:

$H_0$ : The larvae size of *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C will not differ.

$H_1$ : The larvae size of *Ch. rufifacies* at 15, 18, 27, 30, 33, 36 and 39 °C will differ.

### 1.4 Anticipate benefit from thesis

The information of the effect different seven constant temperatures on the developmental times and development rates of *Ch. rufifacies* at different constant temperatures have been provided background data for future forensic entomological casework in Thailand. Additionally, the thermal constant (K), the minimal developmental threshold ( $T_L$ ), accumulated degree hour and accumulated degree day for this species were provided valuable information have been generated. In addition, the isomorphen diagram of *Ch. rufifacies* at different seven constant temperatures for the purpose of estimating postmortem intervals have been constructed and contributed important information to forensic entomology investigations.

## 1.5 Boundary of study

Eggs and larvae of *Ch. rufifacies* were collected from the chicken baits around Burapha University. They were reared in the laboratory colonies maintained at the Department of Biology, Faculty of Science, Burapha University, Thailand. They were identified using the morphological characteristics described by Well and Kurahashi (1994). A hundred adults consist of 50:50 male and female of each *Ch. rufifacies* were caged (13 x 18 x 6 cm<sup>3</sup>) at 15, 18, 27, 30, 33, 36 and 39 °C, humidity: 40-80% relative humidity (RH) and a photoperiod of 12:12 (L:D). Each cage was supplied with water, 50% sucrose and granular sugar. The first day, adult blow fly was starved, the next 2 days was fed with blended pig liver to allow their ovary development, and at days fourth they were provided with beef muscle meat, ad libitum in a Petri dish. Eggs batches were collected within 30 minutes of oviposition and transferred to fresh piece of beef placed in the plastic container (7 x 9.5 x 5 cm<sup>3</sup>). The 100 hatching larva were transferred to a 20 g fresh piece of beef placed in plastic container (7 x 9.5 x 5 cm<sup>3</sup>). The time was recorded when 1<sup>st</sup> instar larvae (1<sup>st</sup> ecdysis) become to 2<sup>nd</sup> instar larvae and 2<sup>nd</sup> instar larvae changed to 3<sup>rd</sup> instar larvae (2<sup>nd</sup> ecdysis). The 3<sup>rd</sup> instar larvae were stopped feeding and become to post feeding stage, and then move them into the 200 g. sterile soil sawdust mixture 50% (w/w) in the plastic container (12 x 25 x 6 cm<sup>3</sup>). Time of each emergent adult was recorded and they were identified using the same method as described above. Data were used to generated the linear developmental rate model described by Bourel et al. (2003), calculated the accumulated degree hour, accumulated degree day of minimum threshold temperature each species.

## CHAPTER 2

### LITERATURE REVIEWS

#### **2.1 Biology of blow flies in the genus *Chrysomya***

##### **2.1.1 Taxonomy of *Chrysomya rufifacies***

Blow flies are insects belonging to family Calliphoridae, commonly known as bottle flies or calliphorids. They are characterized typically as being shiny with metallic coloring often with blue, green, purple and black thoraces and abdomens, and noisy in flight with an average size of 8-10 mm (Robinson, 2005). They have frontal sutures and also present branched RS 2 veins and developed calypters. Adult blow flies feed on various types of foods, but their maggots are scavengers. Over 1,500 species of blow flies are distributed over all continents worldwide (Carvalho & Mello-Patiu, 2008). The most common areas to find blow flies are Asia, central American and North America, with 228 species in the Neotropics (Hall, 1995). In Thailand, there are 93 blow fly species belonging to 9 subfamilies including Subfamily Ameniinae, Calliphoridae, Luciliinae, Phumosiinae, Polleniinae, Bengaliinae, Auchmeromyiinae, Chrysomyinae and Rhiniinae (Kurahashi & Bunchu, 2011)

Blow flies of the genus *Chrysomya* are considered to be of profound forensic importance for determination of the PMI, due to their rapid colonization of corpses, extensive oviposition and abundance in many habitats (Byrd & Butler, 1997). Representatives from this genus constitute 44% of the blow fly species present in murder or suspicious death cases investigated in New South Wales, Australia (Levot, 2003). *Chrysomya* species can be distinguished from other calliphorids by the combination of a ciliated stem vein, a convex occiput and a thoracic squama with hairs in the center of the upper surface. Adults of *Chrysomya* are typically metallic colored with thick setae on the meron and plumose arista. (Wells & Kurahashi, 1996), while members of this genus are often present as primary colonizers, they are particularly stimulated to oviposit by distinctive chemical cues produced by maggots of other genera (e.g. *Calliphora*), and as such may also be present as secondary colonizers (Fuller, 1934; Norris, 1965; Byrd & Castner, 2001a). This behavior is due

to the predatory nature of *Chrysomya* larvae particularly *Ch. rufifacies* (Macquart), which feed on, repel and often totally displace the primary larvae due to competition (Faria & Godoy, 2001; Bharti & Singh, 2002, 2003). The heavily sclerotized spines and processes on third- instar ‘hairy maggots’ of *Ch. rufifacies* may enhance their ability to capture and restrain their prey (Baumgartner, 1993). The Scientific classification of *Ch. rufifacies* is as followed.

Kingdom	Metazoa
Phylum	Arthropoda
Class	Hexapoda
Order	Diptera
Family	Calliphoridae
Genus	<i>Chrysomya</i>
Species	<i>Ch. rufifacies</i> Macquart, 1842

### 2.1.2 Morphology and distribution

*Ch. rufifacies* is one of special interest that has established itself throughout the central United States (Figarola & Skoda, 1998; Shahid, Hall, Haskell, & Merrit, 2000). They are usually known as “hairy maggot” blow fly, their body segment possesses a median row of fleshy tubercles giving a slightly hairy appearance which was first noted in the Gulf Coast region (Martin, Carlton, & Meek, 1996). They have shiny, blue green color, body size are ranging from 6-12 mm length. The fully mature third instar of *Ch. rufifacies* is robust and muscoid shaped, with a length of about 14 mm. The anterior spiracle had nine to twelve marginal branches. The third instar of *Ch. rufifacies* appeared hairy and was consequently known as the “hairy maggot”, which had distinct, stout, elongated tubercles encircling the body segments. Each tubercle had large base, with a gradually tapering tip having about 3 circular rows of spines apically. The structure and distribution of the dorsal cuticular spines between the prothorax and mesothorax are arranged singly and close together. Each spine is heavily pigmented at the tip and had one to three dark pointed tips. On close examination, the surface integument was covered with several net like patches. Each patch comprises many denticles with a single, the much larger one being more or less located. When viewed at the caudal segment, six peripheral tubercles (inner dorsal, median dorsal, outer dorsal, outer ventral, median ventral and

inner ventral tubercles) are extremely developed. The outer dorsal tubercles appear to be the largest one. Moreover, a pair of the posterior spiracles is clearly seen at the caudal end (figure 2.1). The peritreme is incomplete and heavily pigmented as a dark structure encircling the three relatively straight spiracular openings (slits), with the middle slits appearing slightly bent inwards (Sukontasan, Piangjai, Siriwattananarungsee, & Sukontason, 2008).

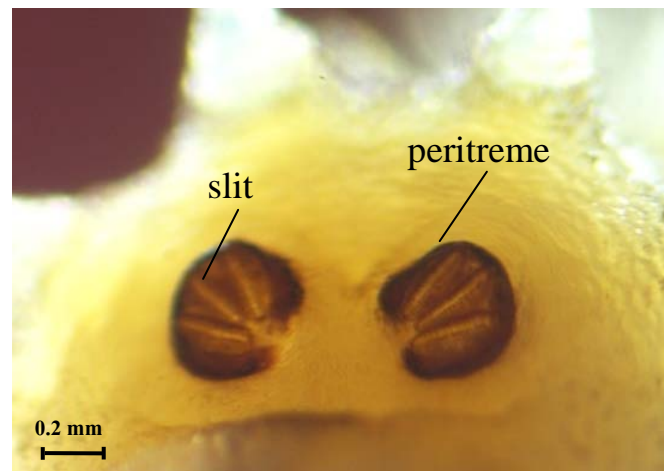


Figure 2.1 Stereo micrograph of post feeding larva of *Ch. rufifacies* showing complete peritreme and heavily pigment.

The female of *Ch. rufifacies* exhibit monogenic reproduction, they produce either male or female off springs depending on their genetic constitution (Kirchhoff & Schroeren, 1986). This phenomenon is rare for Diptera (Roy & Siddons, 1939; Subramanian & Mohan, 1980) and is determined by maternal genotype (Kirchhoff & Schroeren, 1986). Adults mate between 2 - 10 days after eclosion (Schmidt & Kunz, 1985; Baumgartner, 1993). Development of mature ovaries depends on the ingestion of a protein meal. Females lay between 210 - 368 eggs per clutch (Schmidt & Kunz, 1985). Developmental time of this species is temperature dependent and perhaps depends on latitude (Schmidt & Harris, 1989; Byrd & Butler, 1997). Eggs had a lower temperature threshold of 9 °C (Baumgartner, 1993). Larvae passed through three instar and take about 600 and 200 hours at 5 and 32 °C, respectively to develop until eclosion (Byrd & Butler, 1997). Adult flight activity has a minimum threshold of

13 °C (Baumgartner, 1993), and live approximately 23-30 days (Schmidt & Kunz, 1985). This species was thought to be active during warm weather (Baumgartner, 1993), however, researches recently collected adults during November in South Carolina with the temperature ranging from 8.2- 12.8 °C (Cammack & Nelder, 2010).

The bulk of *Ch. rufifacies* interactions with vertebrate animals are on carrion, however, the first maggots were discovered in the U.S. in 1981 on a dead cheetah (Baumgartner, 1985). The first incidence of *Ch. rufifacies* myiasis reported in the United States was on a dog in 1983, and several cases on animals and humans in Brazil had been documented as well (Baumgartner, 1985). *Ch. rufifacies* was able to cause sheep strike and is an important parasite of new born calves in wet areas of Hawaii (Wells & Greenberg, 1992).

#### 2.1.2.1 External morphology of eggs

The eggs of *Ch. rufifacies* were elliptical in shape, creamy-white in color, and the anterior pole was somewhat tapered, consisting of micropyle extremity. The median area expanded slightly at the anterior pole, appearing as Y-shaped and extending along the egg at the midline. The median area and hatching pleats are narrow in *Ch. rufifacies* (figure 2.2).

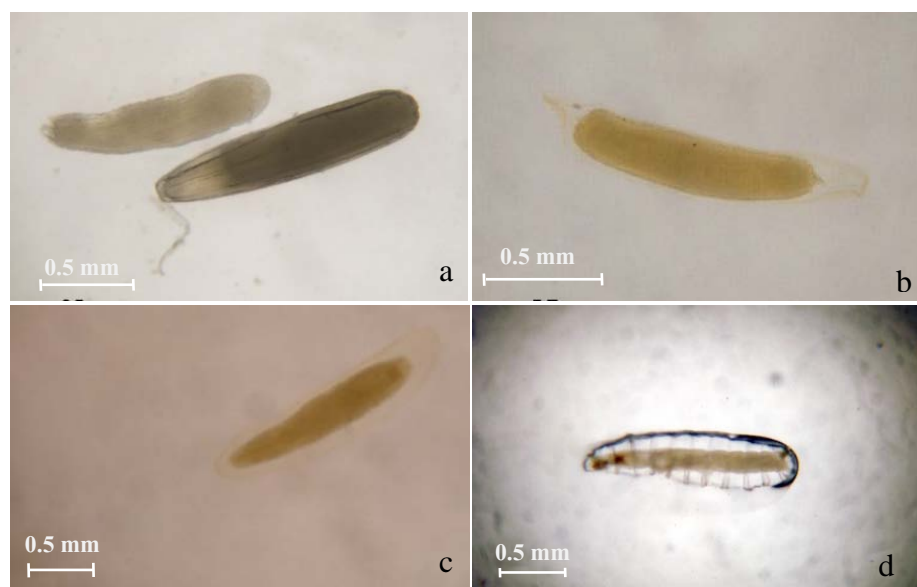


Figure 2.2 Light micrographs of *Ch. rufifacies* eggs (a) at developmental time of 0 hour (b) 3 hours (c) 9 hours and (d) first hatching at 30 °C.

### 2.1.2.2 External morphology of larvae

The first instar larvae of *Ch. rufifacies* have white color (figure 2.3a). The second instar larvae are very easily identified with sharp fleshy tubercles running down their bodies (figure 2.3b), the third instar larvae were about 14 mm in length with a dirty yellowish color hence the common name, hairy maggot blow fly. The peritreme of the posterior spiracle is very wide with a narrow gap that contains forked edges; the slits are short and wide, almost filling the plate (figure 2.3 c, d).

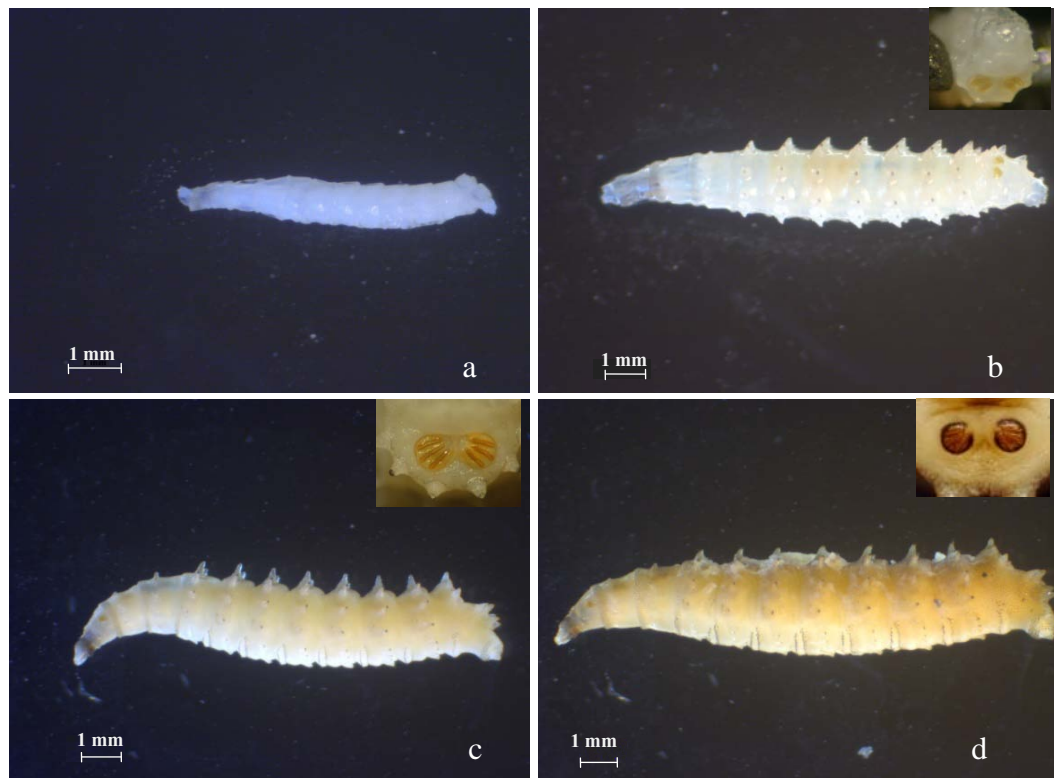


Figure 2.3 Stereo micrograph of (a) 1<sup>st</sup> instar larva (b) 2<sup>nd</sup> instar larva (c) 3<sup>rd</sup> instar larva (d) post-feeding larva.

### 2.1.2.3 External morphology of pupae

Pupae of *Ch. rufifacies* have tubercles along the dorsal and lateral segments. The tubercles contain many sharp spines (figure 2.4). The 9-12 numbers papillae on the anterior spiracle are present in *Ch. rufifacies*.

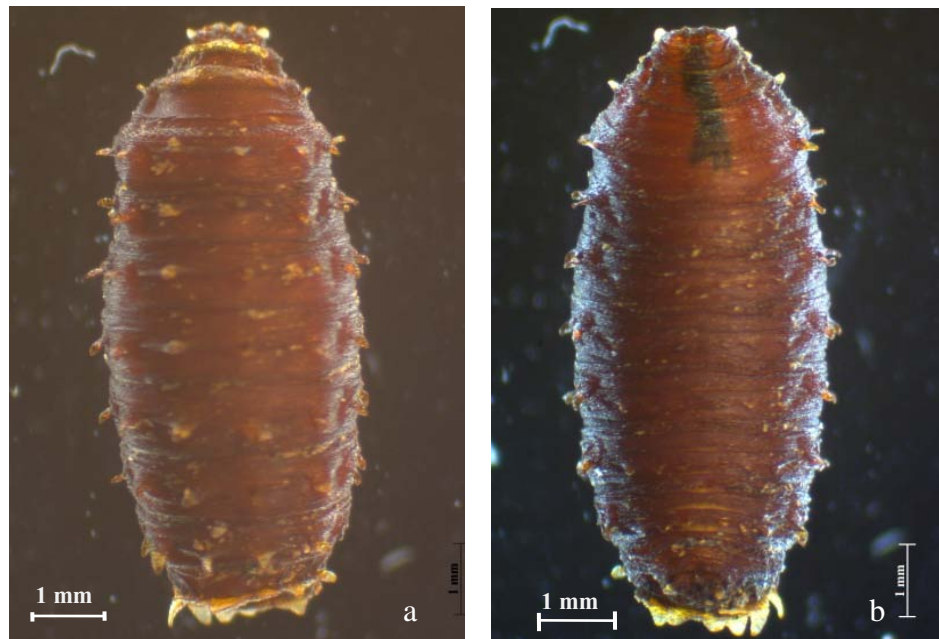


Figure 2.4 Stereo micrograph of *Ch. rufifacies* pupa (a) tubercles along the dorsal and lateral segments (b) ventral without tubercles.

#### 2.1.2.4 External morphology of adult

The hairy maggot blow fly can be readily identified by examining for a shiny metallic blue-green color, a pale genal dilation, and a vestiture of the anterior thoracic spiracle that is pale in color. The mature adult is about 6 - 12 mm in length (Whitworth, 2006).

The use of a microscope allows the investigator to identify setae on the fly's meron, a greater ampulla with stiff erect setae, black first and second abdominal tergites, and a black posterior margin of the third and fourth abdominal tergites. These features are characteristic of the genus *Chrysomya*. The differentiation between *Ch. megacephala* and *Ch. rufifacies* is accomplished by observing the anterior thoracic spiracle color. *Ch. rufifacies* has a pale or white anterior thoracic spiracle (figure 2.5 a, b). Gena present orange-brown to black with pale dusting and silvery hairs.

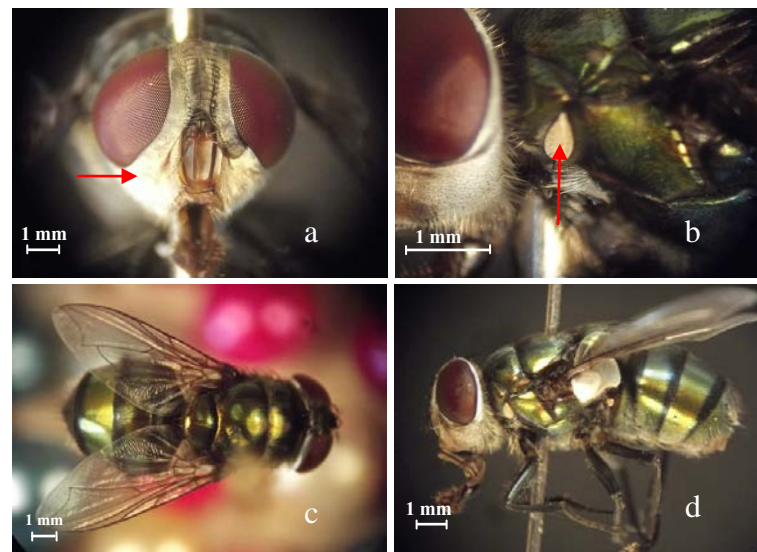


Figure 2.5 Stereo micrographs of *Ch. rufifacies* adult. (a) gena showing orange brown to black with pale dusting and silvery hairs. (b) a pale or white anterior thoracic spiracle (c) the dorsal of body in top view (d) a greater ampulla lateral site.

### 2.1.3 Life cycle of blow fly

The lifecycle of blow flies are described as holometabolous, or complete metamorphosis, consisting of four life stages in their lifecycle includes the egg, larva, pupa and adult (Tao, 1927). The cycle starts when the adult female lays eggs (Gennard, 2007). The eggs are approximately 1 mm long and are laid in a loose mass of 50 - 200 eggs (figure 2.6). Group oviposition by several females results in masses of thousands of eggs that may completely cover a decomposing carcass (Catts & Goff, 1992)

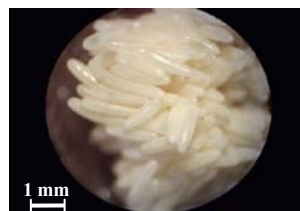


Figure 2.6 Batches of 50-200 eggs of *Ch. rufifacies* on beef meat after 30 minutes oviposition.

The eggs hatch into the first larval stage with 24 hours or as ten hours at 30 °C (depending on temperature) and the larvae feed on carrion and molt into the next stage and molt until they reach maturity. Upon maturity, they migrate away from the carrion searching of a pupariation site. Pupariation occurs within the first inch of top soil or under leaf litter/rock to metamorphose to be adults. During this time, the larval skin shrinks and hardens to form the puparium which is dark brown. This stage may last for 7-12 days depends on the temperature. The adults can live up to six weeks (figure 2.7) (Baumgartner, 1986; Byrd & Butler, 1996).

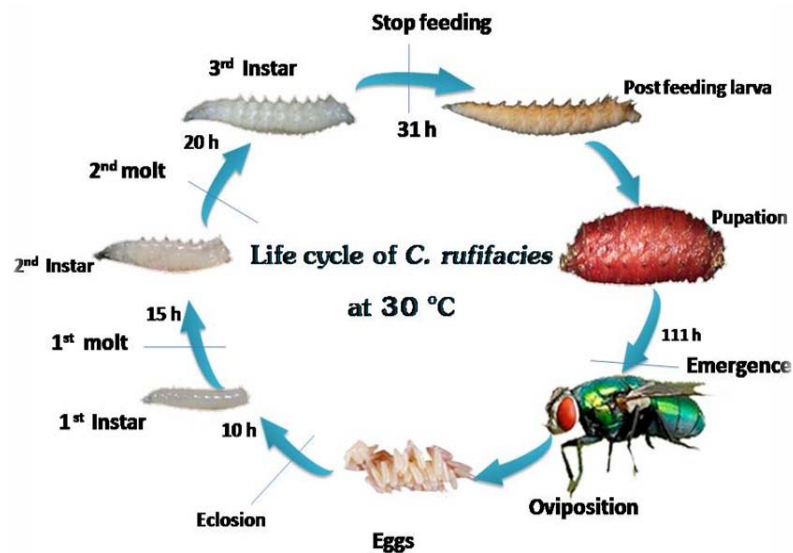


Figure 2.7 Life cycle of *Ch. rufifacies* at  $30 \pm 2$  °C, it take 197 hours to complete the cycle (modified from Goff, 2000).

#### 2.1.4 Identification of blow fly

The important morphological characteristics of the blow fly for species identification shows in figure 2.8. The Blow flies of family Calliphoridae (subfamilies Chrysomyinae, Calliphorinae and Lucillinae) can be distinguished from other metallic or partly metallic calyptrate Diptera by the row of bristles on the meron and the absence of a prominent subscutellum. The plumose (hairy) arista and sharply bent wing vein M are also characteristic of blow flies (Marshall, Whitworth, & Roscoe, 2011).

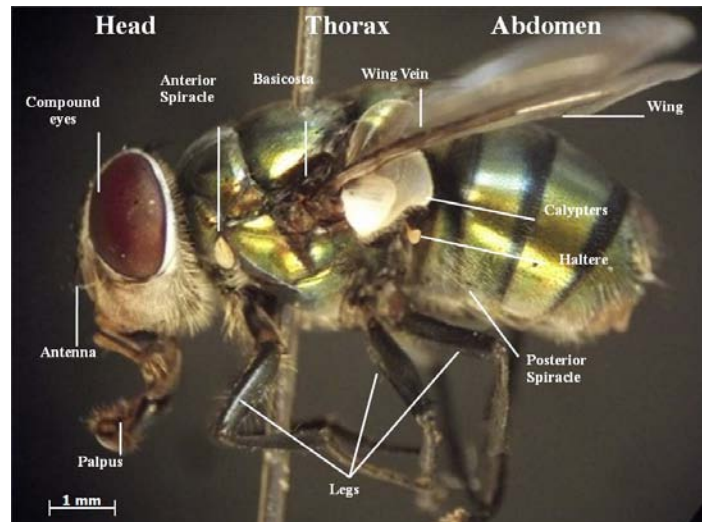


Figure 2.8 Important morphological characteristics of the blow fly species identification.

### 2.1.5 Identification of blow fly sex

The adult head is composed of two large compound eyes. Those of female are more widely spaced than those of male. In male, the whole head is smaller, and the compound eyes are more obliquely placed compare to those of female. The forehead of female is broad and trapezoidal, but in male it is narrow and triangular (figure 2.9).



Figure 2.9 The adult of *Ch. rufifacies*, with an average body size 6-12 mm, the compound eyes of (top) female and male (bottom).

## 2.2 The importance of blue bottle fly

Blow flies are important in forensic analyses in cases of homicide, murders and crime of human death, because their life developmental stages can provide clue of the times and conditions of death. A major focus of forensic entomology is to calculate an accurate and precise estimate of the time between death and discovery of a corpse known as the minimum post mortem interval (Greenberg, 1991; Catts & Goff, 1992). However, the definition of PMI base on forensic entomology is the time between insect colonization and a dead body discovery, because the time of the colonization of insect is not always concurrent with the time of death since blow flies are often arrive a fresh corpse firstly and lay eggs almost immediately, the focus of these estimates is on them (Catts & Goff, 1992). The most common method have been used to calculate minimum PMI is the thermal summation model using a linear regression model based on the temperature-dependent rate of development of insects. It works on the assumption of a linear relationship between developmental rate and the temperatures experienced by the growing insects (Sharpe & DeMichele, 1977; Byrd & Allen, 2001; Higley & Haskell, 2001; Greenberg & Kunish, 2002). The age of an immature insect can be evaluated from developmental times and temperature-specific development rates, and this age is used to estimate the PMI (Turner & Howard, 1992), they are ideal candidates for estimating minimum PMI. The precision of the estimate of age from temperature is affected by the number of constant temperatures used in the regression analysis (Ikemoto & Takai, 2000). The good regression models are based on at least six points along the linear section of the relationship (Ikemoto & Takai, 2000; Sokal & Rohlf, 2005). However, the relationship between developmental rate and temperature is generally sigmoidal, more points are needed in practice to identify the limits of its (approximately) linear mid-section, that mean, at least eight constant temperatures are needed to characterize the model comprehensively. Many researchers reported different in number and ranges of temperatures created the models which were ten different constant temperatures ranging from 15°C-34°C by Grassberger and Reiter (2001), different constant eight temperatures by O' Flynn (1983) and by Hanski (1976), Reiter (1984), different seven temperatures by Byrd and Allen (2001) and different six temperatures by Williams and Richardson (1984). One possible solution to this problem is to pool data from

separate studies for data quality and the interpretation of forensic entomological evidence.

In Thailand, the most common species initially present on the death bodies are *Ch. megacephala* and *Ch. rufifacies* (Sukontason et al., 2008). *Ch. rufifacies* is one of the most important blow fly because its extremely predictable developmental times, minimum difference of larval length. Female can lay average 210 eggs on corpse during daylight within few minutes after body discovery at suitable temperature (Baumgartner, 1993). Sometime oviposition is primarily a daylight activity, and as such, may be delayed for several hours if death occurs at night (Haskell, Hall, Cervenka, & Clark, 1997). Eggs are roughly 2 mm long and are initially deposited around naturally moist open areas or orifices such as eyes, nose, ears and mouth. The first instar larvae emerge from the eggs, typically in 12-24 hours, upon environmental temperature (Gennard, 2007). The maximal preferential temperature for the larvae of this species is about 35 °C (Baumgartner, 1993). The first instar larvae aggregate and sometimes feed as a maggot mass on the decomposing remain. They will pass through three sequential larval instar during that time (Byrd & Castner, 2001b). The third instar larvae and post feeding instar have a feeding and non-feeding larval stage. During the migratory stage, larvae will leave food resource and search for a pupariation site and typically pupate away from the remains, the length of post-feeding larvae decreases during this period (Gennard, 2007). During pupariation period the exoskeleton of the prepupae become darkens and hardens to form a shell or puparial case. Pupariation usually occurs near the soil surface or near the decaying corpse. It has been reported that when bodies are located outdoor, migrating larvae are known to burrow into loose soils and substrates at a distance from the remains (Arnott & Turner, 2008). Puparia of blow flies are football shaped and resemble mouse or rat dropping. The adult will develop inside the puparium and eventually emerge (Lord & Rodriguez, 1989).

In addition *Ch. rufifacies* has also relevance to forensics medical entomology and public health in Africa, Asia, Australia and South America (Prins, 1982; Pont, 1985; Wells, 1991; Wells & Kurahashi, 1994; Von Zuben, Von Zuben, & Godoy, 2001). Developmental time of *Ch. rufifacies* at 28 °C was  $16.2 \pm 0.78$  days from egg to eclosion. Each stage occurred in succession from first instar larvae  $1.1 \pm 0.25$  days,

second instar larvae developed  $2.3 \pm 0.25$  days later, and the third instar larvae developed  $5.7 \pm 0.41$  days later. The larvae pupated  $10.0 \pm 0.57$  days after oviposition (Swinger, Hogsette, & Butler, 2014a). Byrd in 1997, found that *Ch. rufifacies* (Byrd & Butler, 1997) under mean cyclic temperatures of 15.6, 21.1, 26.7, and 35.0 °C and a constant temperature of 25.0 °C. Growth curves for development from egg to adult at 15.6, 21.1, 26.7, and 35.0 °C and a constant temperature of 25.0 °C ranged from 190 to 598 hours. A constant temperature of 25 °C produced a range of pupariation times from 134 to 162 hours, with adult emergence ranging from 237 to 289 hours. Therefore, the developmental times of the species at 25, 30 and 35 °C were 10.9, 9.0, and 8.1 days, respectively (Yisseff, 2007).

### **2.3 Effect of temperature on blow fly development**

The developmental rates of blow flies are usually related to the environmental temperature they exposed during development, since they are ectothermics. The development slows at extreme cold and increases at warm temperature. Some blow flies develop faster than others in the same environmental temperature (Greenberg, 1991). Environmental temperatures influence the survival which is different in different insect species. Each species requires a specific temperature, if it is too high or too low, the development stops. The heat they required for growth and development can be measured as a thermal units or degree days (DD). The minimum threshold temperature for growth (base temperature) varies with the species (Gennard, 2007). The overall energy budget to achieve life stages can be specifically calculated. The thermal units measured based on 24 hours periods of time are called accumulated degree days. If the period is shorter and the length of time being discussed is in hours then thermal units will be measured as accumulated degree hours. It is possible to predict how long it takes for an insect to develop from egg to adult by measuring temperature over time and calculating species specific accumulated thermal units. Such estimation of time since death is based on the speed of insect growth (figure 2.10) (Gennard, 2007).

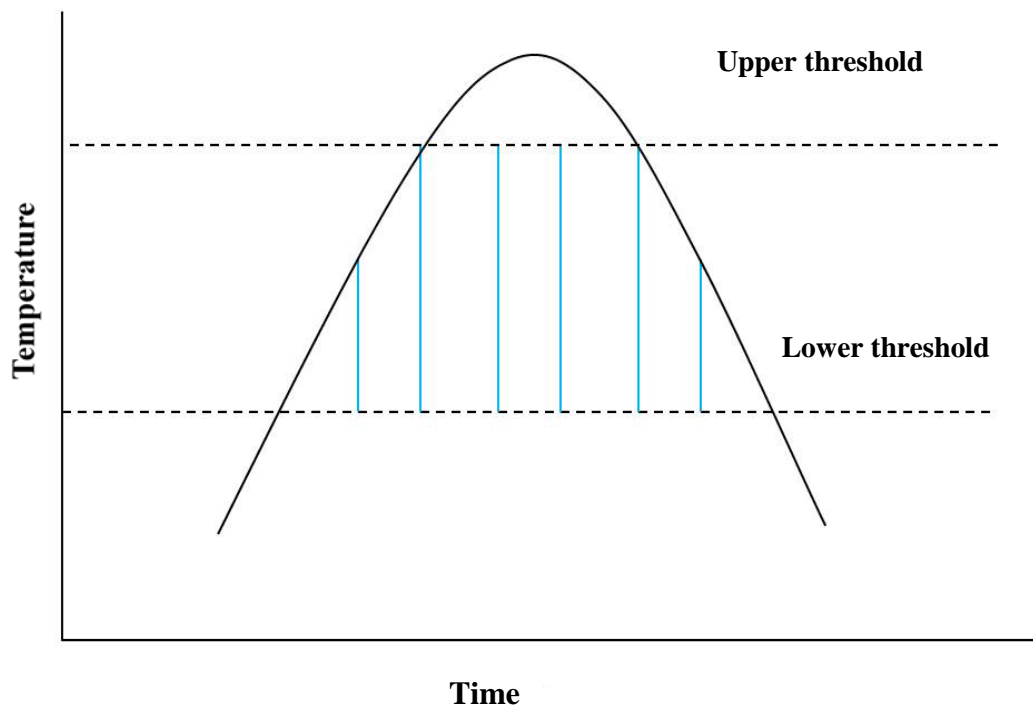


Figure 2.10 Insect growth in relation to upper and lower temperature thresholds (Gennard, 2007).

## 2.4 Lower and upper threshold temperature

Lower threshold temperature or base temperature or baseline is the minimum temperature at which insects first start to develop. If the temperature is lower than baseline the growth and development stop. The maximum temperature at which insects stop developments is called *upper threshold temperature* or *cutoff*. The specific base temperature for particular species is worked out in the laboratory from the insect's growth rate at set experimental temperatures. The base temperature is calculated by plotting temperature against  $1 \div \text{total days for development}$ , i.e. the time between the larvae initially emerging from eggs adults, using a range of temperatures (figure 2.11). Threshold temperatures differ for different species and should be determined by raising insects at the extremely low temperatures (Ames & Turner, 2003). Forensic entomologists often use a baseline ranging from 6 and 10 °C when they determined ADD. *Phormia regina* have base temperature ranging from 10 °C to 14 °C (Deonier, 1942; Haskell, 1993; Nabity, Higley, & Heng-Moss, 2006). Bourel and his colleagues studied developmentaleggs of blow fly, *Lucilia sericata* at

various constant temperatures. The developmental minimum threshold for eggs development was estimated by the x-intercept method. The threshold temperature estimated from this regression was 9.4 °C (figure 2.11) (Bourel et al., 2003). The temperature threshold of *Cynomya mortuorum* (Diptera: Calliphoridae) was calculated from the developmental rates at different constant temperature 14, 16, 18, 20 and 22 °C it was 4.06 °C (Bourguignon, Braet, Vanpoucke, Drome, & Hubrecht, 2014). The lower temperature threshold for *Protophormia terraenovae* was approximately 9 °C (Grassberger & Reiter, 2001; Grassberger & Reiter, 2002), whereas it was 7.8 °C in Russian strain (Marchenko, 2001). Warren showed that egg eclosion could not occur at a temperature as low as 9.8 °C (Warren, 2006). The differences of thresholds in many species also were found in many reports. Davies and Ratcliffe demonstrated a threshold of 3.5 °C for *Calliphora vicina* in north of England (Davies & Ratcliffe, 1994), whereas Marchenko recorded a base temperature of 2 °C for the same species (Marchenko, 2001). Donovan, Hall, Turner, and Moncrieff in 2006 explored *Ch. vicina* growth in London, at temperature between 4 °C and 30 °C and found the base temperature there to be 1 °C for *Ch. vicina* (Donovan et al., 2006). This may simply be due to regional behavioral differences (Wells & LaMotte, 2001). The difference threshold also found in different stages of the same species such as for egg stage of *Ch. rufifacies* eggs had a lower temperature threshold of 9 °C, while in adult fly was 13 °C (Baumgartner, 1993), which depended on its distribution, this fly has been noted to oviposit on fresh and uninfested carcass remains (O'Flynn & Moorehouse, 1979; Shishido & Hardy, 1969; Wells & LaMotte, 2010), but in other locations does not colonize remains until other larvae are first present (Bohart & Gressitt, 1951; Wells & Greenberg, 1994; Wells & LaMotte, 2010; Zumpt, 1965). There is a few research of lower threshold temperature for this species, the application of the development rates of this species from other geographic regions should be avoided as the species development can vary regionally.

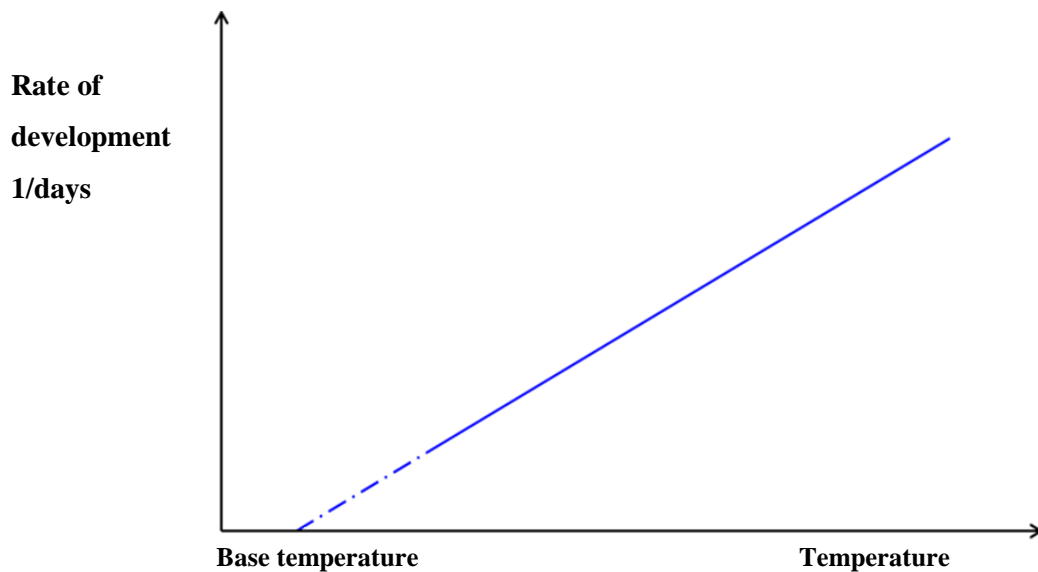


Figure 2.11 Base temperature determination using the linear approximation method.

The point at which the graph extends to the x axis is the approximation of the base temperature (Gennard, 2007).

## 2.5 Estimating the post mortem interval (PMI)

The PMI estimated are usually made using thermal summation models (some time we called degree-days method), isomorphen and isomegalen diagrams (Grassberger & Reiter, 2001; Higley & Haskell, 2001; Greenberg & Kunich, 2002). In forensic case work, the PMI can be in two ways, the first is using isomorphen or isomegalen diagrams by the age of maggots presented on a corpse, mostly of the family Calliphoridae, they are the most accurate forensic indicators of death timing (Schoenly, Griest, & Rhine, 1991; Van Laerhoven & Anderson, 1996; El-Kady, 1999). They recognized as the first insects of the faunal succession on a human dead bodies (Grassberger & Reiter, 2001). It is based on the application of existing developmental data for the species of blow fly present together with available environmental temperature measurements (Sukontason et al., 2008). The initial PMI estimate can then be amended to address scene-specific characteristics, such as insect accessibility to the body (Byrd & Castner, 2010). The developmental data in the relation to the temperature as the size and developmental stage of the blow fly larvae collected in a case provide important indicators for more precise PMI estimates.

### 2.5.1 Isomegalen diagram

The combination between the length of larvae and the means temperatures in a single diagram can be generated by using time from egg hatching to post feeding of species plot against different temperature, each line representing identical larval length at various temperatures are nearly constant or not undergoing fluctuations. Previous studies on isomegalen diagram of blow flies in the genus *Chrysomya* have been carried out by Grassberger and Reiter who generated for *L. sericata* under 10 different temperature regimes (Grassberger & Rieter, 2001). However, data obtained from these researches were not always accurate for the species found in different geographic region (Reiter, 1984). The data studied from specific region should be more precise estimates PMI (figure 2.12).

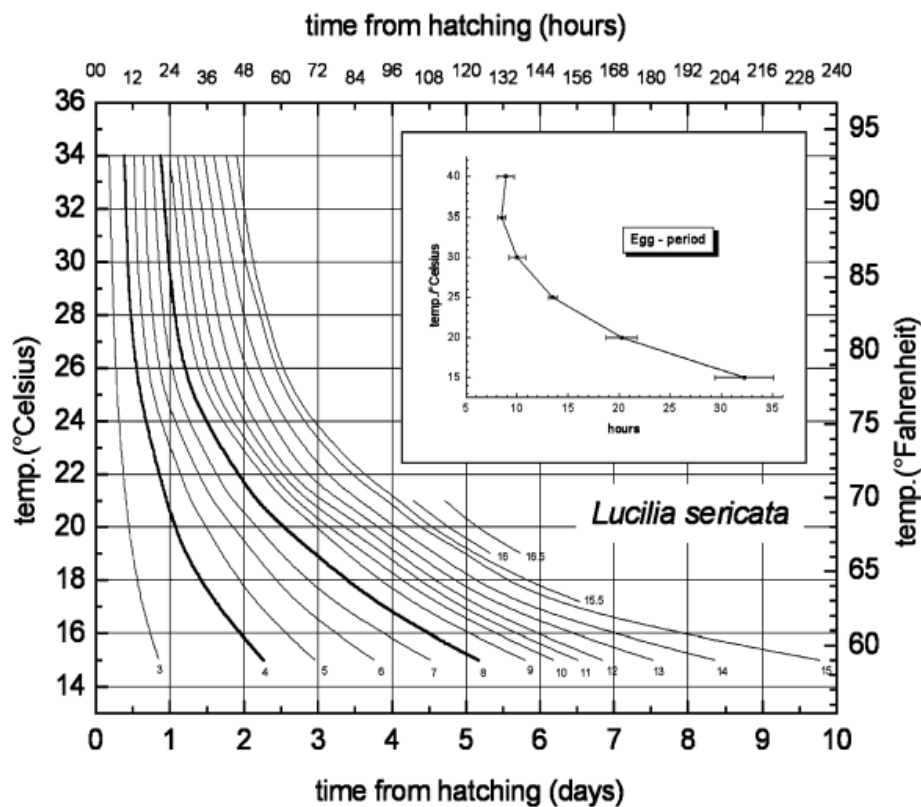


Figure 2.12 Isomegalen diagram for *Lucilia sericata* larvae from hatching to peak-feeding. Time is plotted against temperature, each line representing identical larval length (mm). The small graph shows the analogous egg-periods between 15 and 40 °C (Grassberger & Rieter, 2001).

### 2.5.2 Isomorphen diagram

Developmental Times of all stages from oviposition to eclosion is plotted against different constant temperatures, each line represent morphological changes, areas between lines represent identical stage of the blow fly species (Grassberger & Rieter, 2001). Previous report showed the isomorphen diagram of *L. sericata* generated by five different constant temperatures (15, 20, 25, 30 and 35 °C) between times from hatching to eclosion (figure 2.13) (Grassberger & Rieter, 2001).

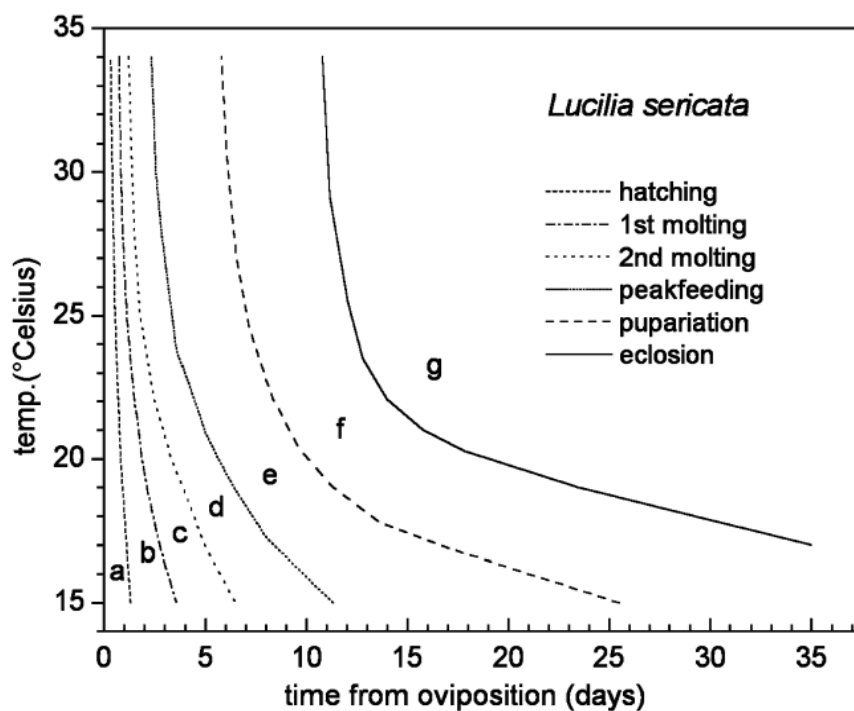


Figure 2.13 Isomorphen diagram of *L. sericata*, showing all stages from oviposition to eclosion (15-34 °C). Area between lines represent identical morphological stages at various temperatures. Where is a= egg; b=1<sup>st</sup> instar; c= 2<sup>nd</sup> instar; d= 3<sup>rd</sup> instar; e= postfeeding larvae; f =pupa; g = imago. Each line represents identical morphological changes (Grassberger & Rieter, 2001).

### 2.5.3 Degree days (DD)

Development of many organisms which cannot internally regulate their own temperature is dependent on temperatures they exposed in the environment. An organism needs amount of heat to develop is known as physiological time which is

often expressed in units called degree-days (figure 2.14). Developmental are influenced by temperature. Degree-day models, based on the relationship between the rate of insect development and its temperature above certain base temperature which is linear and is commonly used for calculating the temperature dependent development of insects for prediction of events in their life cycle (Lindblad & Sigvald, 1996).

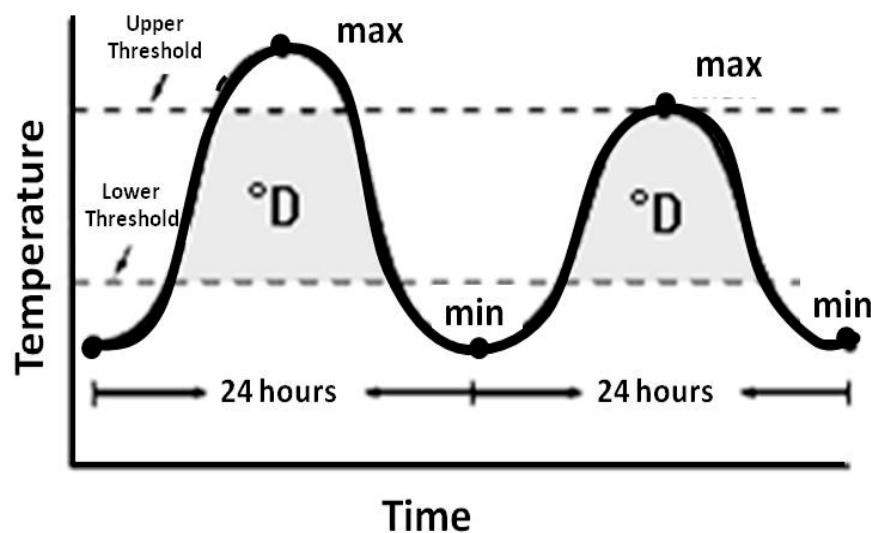


Figure 2.14 Thresholds and accumulated degree-days. The area in gray under the curve represents the number of degree days that fall between a lower and upper threshold, for each 24-hour period (Baskerville & Emin, 1969).

#### 2.5.4 Accumulated degree day (ADD) and Degree hours (ADH)

Living organisms including insects require a certain amount of heat for their development in their life cycles (Uvarov, 1931; Andrewartha & Birch, 1955). The amount of heat required for complete development of a certain species is always the same. The accumulated heat for the development can be measured by the physical time combines with the temperature, the physical time is often approximated in unit called degree days ( $^{\circ}\text{D}$ ) (Baskerville & Emin, 1969; Wilson & Barnett, 1983)

*A degree day (DD)* is a measurement of thermal unit over time of development calculated from daily maximum and minimum ambient temperatures or at the constant temperature in the laboratory (Baskerville & Emin, 1969).

*Accumulated degree day* are a measurement of thermal or heat units required for growth and development of insects for a day. Each developmental stage of an organism has its own total heat requirement (Marchenko, 1988; Highley & Haskell, 2001).

*Accumulated degree hours* measure the same heat requirements in hours instead of day (Marchenko, 1988; Highley & Haskell, 2001).

The calculation used for ADD was based upon a standard technique called the rectangular method (Arnold, 1960). The heat accumulation is the difference between the average temperature and the lower threshold temperature (base temperature) the days taken to develop (Wilson & Barnett, 1983). Determining thermal required for completing each stage of development from egg to adults are expressed in temperature time units. The formula for calculating ADH and ADD are given by

$$\text{ADH} = (\text{temperature} - \text{base temperature}) \times \text{Time}_{(\text{hours})}$$

$$\text{ADD} = \frac{(\text{temperature} - \text{base temperature}) \times \text{Time}_{(\text{hours})}}{24}$$

24

Base temperature is specific value which is x-intercept of the linear equation of insect development (Arnold, 1959; 1960). The ADH value for complete developmental in each species stays constant within temperature threshold (Reibe, Doetinchem, & Madea, 2010). The published report showed the developmental duration to complete developmental stage of *L. sericata* was 14 days at 25 °C resulted in 238 ADD using a base temperature of 8 °C, however, a developmental duration of 19 days at 21 °C resulted in 231 ADD, both ADD-values were in the same range (231-238) (Grassberger & Reiter, 2001), new effect for the younger and also shorter developmental phases the ADH values were nearly constant over the complete range of temperatures, but for the post-feeding and the pupal stages showed that the ADH values are strongly temperature dependent (Reibe et al., 2010). The developmental time of *Cynomya mortuorum* (Diptera: Calliphoridae) from eggs to adults ranged from 29.67 days ( $\pm 2.38$ ) at 14.45 °C, to 16.82 days ( $\pm 0.40$ ) at 22.11 °C. The accumulated degree days (ADD) of total development was 305.75 ( $\pm 15.67$ ) DD. The accumulated degree days (ADD) at 22.25 °C needed to complete total life cycle was 300.87 ( $\pm 14.71$ ) (Bourguignon et al., 2014).

The ADH and ADD of larval growth rate of a forensically important fly species, *Ch. vicina* at temperatures between 4 °C and 30 °C were 4700 and 195.83, respectively (Donovan et al., 2006). Thermal constant (K) for *P. terraenovae* were studied in the laboratory at five different constant temperature regimes (15, 20, 25, 30 and 35 °C) overall it was  $240.2 \pm 9.3$  ADD (Grassberger & Reiter, 2002).

## **CHAPTER 3**

### **MATERIALS AND METHODS**

#### **3.1 Instruments**

- Analytic balance (Sartorius analytic, Germany)
- Camera (Fuji, Japan)
- Hot air oven (Clayson, Austraria)
- Incubator (Taksin N&D Medical LTD., Thailand))
- Light microscope (Olympus, Japan)
- Stereoscopic microscope (Olympus, Japan)

#### **3.2 Inventory supplies**

- Aluminum foil (Diamond, USA)
- Analog soil moisture, pH and light meter (CMT-1288, China)
- Beakers 5, 25, 80, 125, 500 and 1,000 ml (Pyrex, Germany)
- Collection vials
- Light tough forceps (Mira, Germany)
- Hand lens
- Hygrometer
- Insect nets
- Needles
- Muslin cloth
- Paintbrush
- Petri Dish (Pyrex, Germany)
- Plastic container (7 x 9.5 x 5 cm<sup>3</sup>)
- Rearing cages for adult (6 x 18 x 13 cm<sup>3</sup>)
- Ruler (Koyo, Japan)
- Thermometer (Sangi, Thailand)

### 3.3 Chemicals

- Absolute ethyl alcohol (Merck, Germany)
- Distilled water
- Sodium chloride (Ajax Finechem, Australia)
- Sucrose (Ajax Finechem, Australia)

### 3.4 Specimens and sample sizes

- Adults *Ch. rufifacies* (800 adults per treatment), seven treatment
- Eggs (18 batches of eggs per treatment), 400-500 eggs per batch
- First instar larvae, second instar larvae and third instar larvae and
- Third instar larvae (4,374 larvae)
- Pupae (900 pupae per treatment)

### 3.5 Study site

Specimens were collected from the chicken baits around Burapha University (13° 17' 9.22" N, 100° 55' 29.11" E) (figure 3.1). Studies effect of constant temperatures on developmental rate were carried out at the laboratory, Department of Biology, Faculty of Science, Burapha University, Thailand.



Figure 3.1 The known time of death chicken baits in a metal mesh wooden cage for blow flies collecting.

### 3.6 Identification of blow flies from carcass

#### 3.6.1 Identification of adults

A hundred of adults and larvae of blow fly were collected from the chicken baits around Burapha University. They were identified using the morphological characteristics described by Marshall et al. (2011).

#### 3.6.2 Identification of third instar larvae

A hundred larvae of blow fly were collected and kept at 30 °C until become third instar larvae and then they were identified using the morphological characteristics described by Smith (1986).

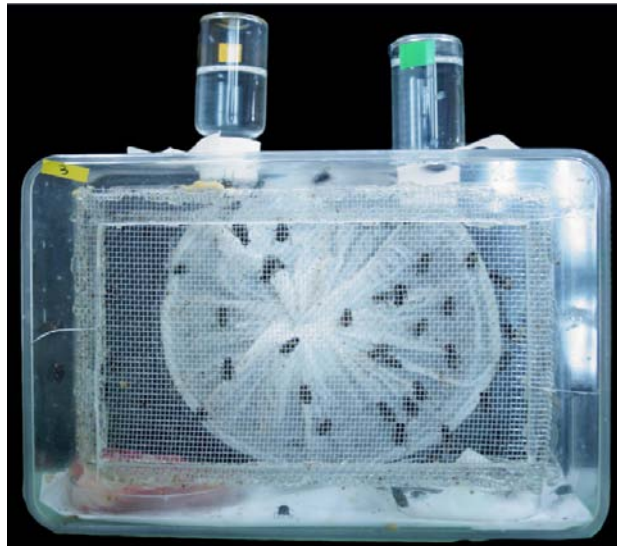


Figure 3.2 Adults male and female of *Ch. rufifacies*, sex ratio of 50:50 rearing in plastic cages (6 x 18 x 13 cm<sup>3</sup>), provided with 50% sucrose solution and water.

#### 3.6.3 Species confirmation

A hundred adults consist of 50:50 male and female of *Ch. rufifacies* were caged in plastic container (6 x 18 x 13 cm<sup>3</sup>) under different constant temperature at 30 °C, humidity: 40-80% relative humidity (RH) and a photoperiod of 12:12 (L:D). They were supplied with water, 50% sucrose and granular sugar for three days (figure 3.2). The next 2 days were fed with blended pig liver to allow their ovary

development, and at days fourth were provided with beef meat. The beef were provided ad libitum in a Petri dish. Eggs batches were collected within 30 minutes of oviposition and transferred to fresh piece of beef placed in the plastic container (7 x 9.5 x 5 cm<sup>3</sup>). The 100 hatching larva were transferred to a 20 g fresh piece of beef placed in plastic container (7 x 9.5 x 5 cm<sup>3</sup>). When 1<sup>st</sup> instar larva become to 3<sup>rd</sup> instar larva (2<sup>nd</sup> ecdysis), they were collected and identified the species. Another cage were set up and were kept until they emerged to be adults and then they were identified as the same methods as described above.

### **3.7 Eggs period under seven different constant temperature regimes**

Adults of *Ch. rufifacies*, with a 1:1 sex ratio were reared in plastic cages (6 x 18 x 13 cm<sup>3</sup>) with 100/cage. They were reared by the same method as above at different constant temperature of 15, 18, 27, 30, 33, 36 and 39 °C until oviposition. Eggs batches were collected within 30 minutes after oviposition and transferred to 20 g fresh beef meat placed in the plastic container (7 x 9.5 x 5 cm<sup>3</sup>). Time of hatching of 100 larvae were recorded and then transferred and spread on 20 g fresh beef meat. Three replicates were done at one of seven temperature regimes.

### **3.8 Effect of different constant temperature on developmental time of larvae**

A hundred first instar larvae were transferred into a 20 g beef meat placed on aluminum foil boat (4 x 5 x 1 cm<sup>3</sup>), provided in the plastic container (7 x 9.5 x 5 cm<sup>3</sup>). Ten larvae were collected and killed by placing in hot water (80 °C) for 30 seconds to prevent shrinkage (e.g. ten larvae of each stage were collected until all ten larvae were molt to next stage, sampling time were followed by preliminary studies), then the stage of changing were identified using the posterior spiracle under stereo microscope, the times of the first molting (changing stage from 1<sup>st</sup> instar larvae to be the 2<sup>nd</sup> instar larvae) and the second molting (from second instar larvae to be 3<sup>rd</sup> instar larvae) were recorded. Ten molted larvae were recorded. A 20 g fresh piece of beef was changed daily for each cage (figure 3.3a, b, c, d).

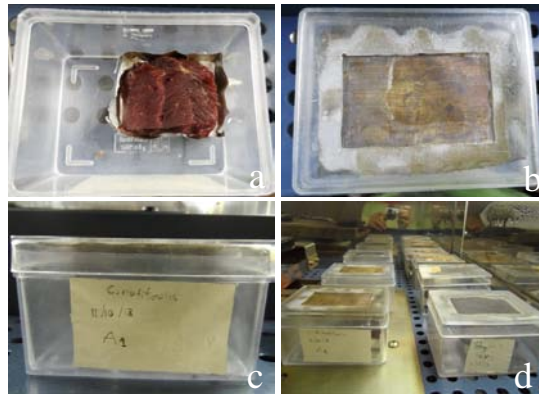


Figure 3.3 Blow fly rearing containers and cages (a) A 20 g beef meat placed on aluminum foil boat ( $4 \times 5 \times 1 \text{ cm}^3$ ) in the plastic container ( $7 \times 9.5 \times 5 \text{ cm}^3$ ), (b and c) Plastic rearing cage covered with a nylon, (d) Cages containing larvae kept in the incubator at constant temperature.

### 3.9 Effect of different constant temperatures on developmental time of pupae and adults

When the third instar larvae stopped feeding (post feeding larvae), wandering behavior were observed and then they were moved on to 200 g sterile moistened soil-sawdust mixture in the plastic container ( $13 \times 18 \times 6 \text{ cm}^3$ ) (figure 3.4a). Time when each of which individual migrated and buried were recorded (figure 3.4b). Time of each pupae to reach adult stage (eclosion) were recorded, all emergences were recorded for each temperature.



Figure 3.4 (a) A 200 g of moistened sterile soil-sawdust mixture in the plastic container ( $13 \times 18 \times 6 \text{ cm}^3$ ), (b) Post feeding larvae migrated from the remain and buried for pupariation.

### **3.10 Effect of seven different constant temperatures on size of larvae**

Four of the largest maggots of each larval stage were removed from the plastic container every 3 hours until 50% of maggots stop feeding. Specimens were killed in hot water 80 °C for 30 seconds and then the measurement was followed immediately under stereo microscope using standard ruler, and larvae were preserved in 75% ethanol.

Larval length were measured at hours 0, 3, 6, 9, 12 and so on after molting until they become post feeding larvae. Three replicates were done for each treatment (1044 larvae at 15 °C, 756 larvae at 18 °C, 81 larvae at 27 °C, 81 larvae at 30 °C, 288 larvae at 33 °C, 252 larvae at 36 °C and 192 larvae at 30 °C).

### **3.11 Developmental threshold and thermal constant**

Base temperature or lower threshold temperature (B) for the species development were calculated by generating the linear regression of developmental rate ( $y = 1/\text{developmental time}$ ) model described by Bourel et al. (2003) on seven different constant temperature (x). X-intercept was estimated as a baseline or lower threshold temperature (Broufas & Koveos, 2000). The thermal constant was calculated from the equation  $DD = \text{developmental time (rearing one of seven different temperatures)} - \text{lower threshold temperature gotten from the equation, } ^\circ\text{C}$ . The thermal constant or DD was generated for each of seven constant temperature to obtain the overall ( $X \pm SD$ ) for total development from egg to pupae), and for six different constant temperature, (except 39 °C) was calculated for total immature development. The reciprocal of slope of the regression line represent the sum of degree hour (accumulated degree hour).

### **3.12 Growth curves from seven different constant temperature regimes**

Means of larval lengths of all rearing were plotted against times for each of seven temperatures. Means of developmental times ( $\pm SD$ ) from eggs to eclosion at each of six constant temperatures (except 39 °C) were calculated and from eggs to pupariation of seven different temperature regimes were also calculated.

### **3.13 Isomorphen diagram**

Data from Developmental times of eggs, larva and pupa of blow fly species *Ch. rufifacies* were used to generate isomorphen diagram by Microsoft office Excel 2007. In this diagram: Time of all stages from oviposition to eclosion were plotted against temperatures 15, 18, 27, 30, 33, 36 and 39 °C. Areas between lines represent identical morphological stages of *Ch. rufifacies*.

### **3.14 Isomegalen diagram**

Lengths of larvae from hatching to post feeding were used to construct isomegalen diagrams for *Ch. rufifacies*. In this diagram time from hatching to post feeding is plotted against temperature each line representing identical larval length (mm) at various temperatures (15, 18, 27, 30, 33, 36 and 39 °C).

## CHAPTER 4

### RESULTS

#### 4.1 Effect of Seven Different Constant Temperature Regimes on Developmental time and Developmental Rates of *Chrysomya rufifacies*

##### 4.1.1 Developmental times of *Ch. rufifacies* at 15 °C

The developmental times (mean± SD) of *Ch. rufifacies* from egg to eclosion at 15 °C were 27.14± 1.46, 60.14± 4.20, 73.15± 2.19, 144.15± 10.48, 314.04± 15.51 hours (table 4.1), respectively. The accumulation degree hours and accumulation degree days for *Ch. rufifacies* were 3093.10 ADH and 128.88 ADD, respectively. The developmental rate was 0.00162 1/hours.

Table 4.1 Developmental times, DH, ADH, DD and ADD (B<sub>10</sub>) of *Ch. rufifacies* at 15 °C

Stages	Time (hours) (mean± SD)	DH-B <sub>10</sub>	ADH-B <sub>10</sub>	DD-B <sub>10</sub>	ADD-B <sub>10</sub>
egg	27.14± 1.46	135.70	135.70	5.65	5.65
1 <sup>st</sup> instar	60.14± 4.20	300.70	436.40	12.53	18.18
2 <sup>nd</sup> instar	73.15± 2.19	365.75	802.15	15.24	33.42
3 <sup>rd</sup> instar	144.15± 10.48	720.75	1522.90	30.03	63.45
pupariation	314.04± 15.51	1570.20	3093.10	65.43	128.88
total	<b>618.62± 33.84</b>	<b>3093.10</b>		<b>128.88</b>	

##### 4.1.2 Effect of temperature on larval size

Means (± SD) of the maximal measured lengths of first instar larvae to post feeding larvae of *Ch. rufifacies* were plotted against time (figure 4.1). The length showed significantly different ( $F_{87}= 272.88$ ,  $p < 0.0001$ ). It was ranged from 1.80± 0.20 - 13.80± 0.80 mm. The larval sizes during period of 0 - 12 hours were not significant different, the average size was about 2.00 mm (figure 4.2a), but at 54 hours after hatching the length was 4.0 mm (figure 4.2b), the third instar larva spend development time 159 hours to reach their body length to 8.0 mm (figure 4.6c). The average longest larval sizes were found in 240 hours duration from hatching, it was 13.8± 0.63 mm (figure 4.2d).

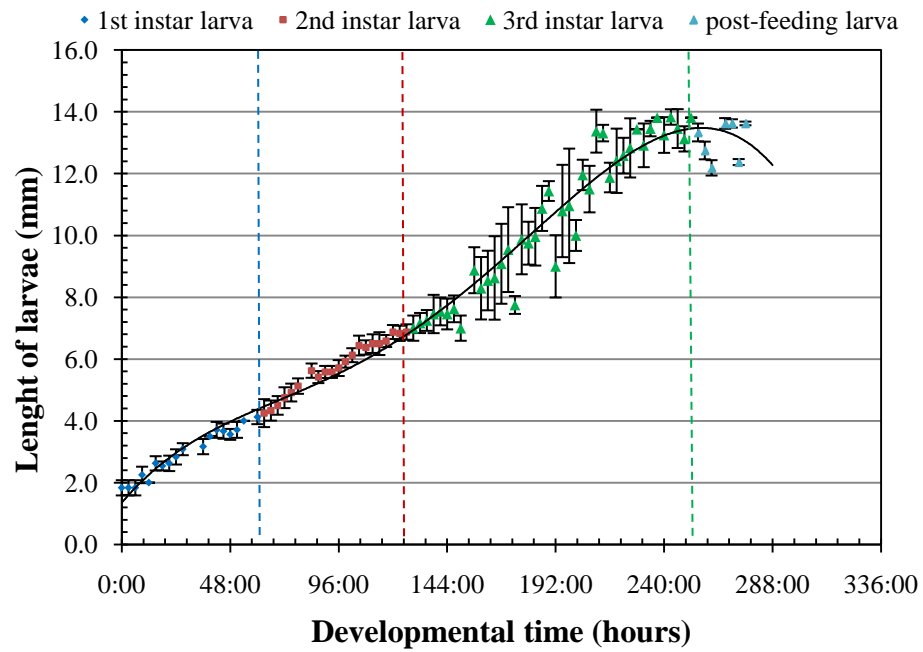


Figure 4.1 Larval growth of *Ch. rufifacies* at 15 °C (mean $\pm$  SD) ( $F_{87}= 272.88$ ,  $p < 0.0001$ ,  $n = 877$ ).

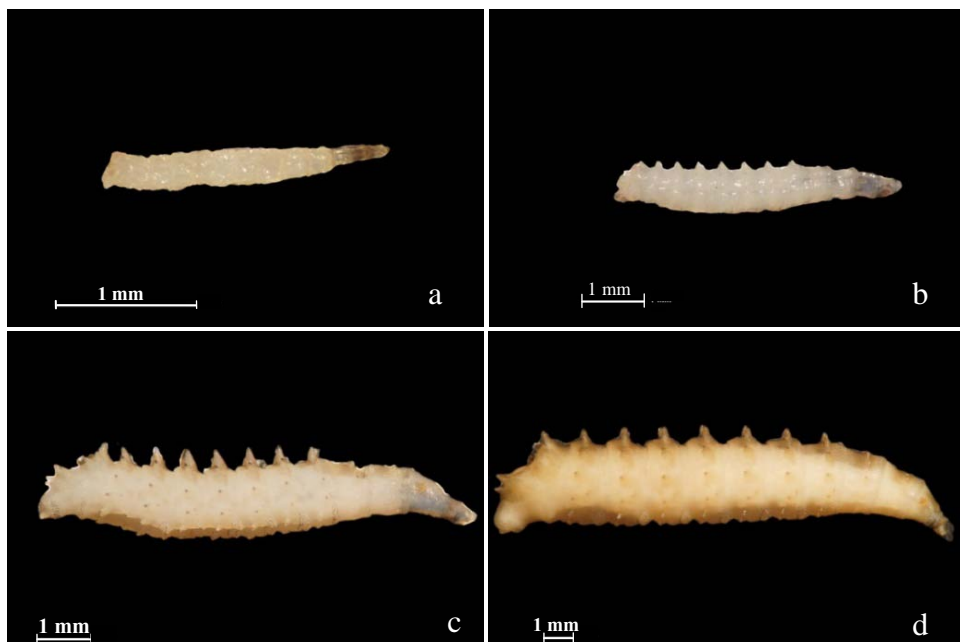


Figure 4.2 Larvae of *Ch. rufifacies* at 15 °C (a) 2.0 mm long 1<sup>st</sup> instar larva at 12 hours (b) 4.0 mm long 2<sup>nd</sup> instar larva at 54 hours (c) 8.0 mm long 3<sup>rd</sup> instar larva at 159 hours (d) 14.0 mm peak feeding larva at 240 hours.

#### 4.1.3 Developmental time of *Ch. rufifacies* at 18 °C

The developmental time (mean± SD) of *Ch. rufifacies* under constant at 18 °C from egg to adult were 26.49± 2.48, 50.37± 2.17, 52.40± 10.52, 129.18± 5.46 and 289.08± 6.16 hours (mean± SD), respectively. ADD and ADH were shown in table 4.2.

Table 4.2 Developmental times, DH, ADH, DD and ADD (B<sub>10</sub>) of *Ch. rufifacies* at 18 °C

Stages	Time (h)(mean ±SD)	DH-B <sub>10</sub>	ADH-B <sub>10</sub>	DD-B <sub>10</sub>	ADD-B <sub>10</sub>
Eggs	26.49± 2.48	211.92	211.92	8.83	8.83
1 <sup>st</sup> Instar	50.37± 2.17	402.96	614.88	16.79	25.62
2 <sup>nd</sup> Instar	52.40± 10.52	419.20	1034.08	17.47	43.09
3 <sup>rd</sup> Instar	129.18± 5.46	1033.44	2067.52	43.06	86.15
Pupariation	289.08± 6.16	2312.64	4380.16	96.36	182.51
<b>Total</b>	<b>547.52± 27.59</b>	<b>4380.16</b>		<b>182.51</b>	

#### 4.1.4 Effect of temperature (18 °C) on larval size

The means (± SD) of the maximal lengths of *Ch. rufifacies* from larvae hatching to peak-feeding were plotted against time (figure 4.3). Body length showed significantly different and ranging from 1.90± 0.32 - 15.62± 0.48 mm ( $F_{66}= 340.27$ ,  $p<0.0001$ ). The average size of hatching first instar larvae was 1.90± 0.32 mm (figure 4.4a), the length of second instar larva started 5.5± 0.67 mm long at 51 hours from hatching (figure 4.4b), the third instar larva started at 105 hours from hatching its average size was about 9.5± 0.8 mm (figure. 4.4c). The peak-feeding larva found in 177 to 192 hours of developmental time, the average largest size was 15.6± 0.9 mm (figure 4.4d).

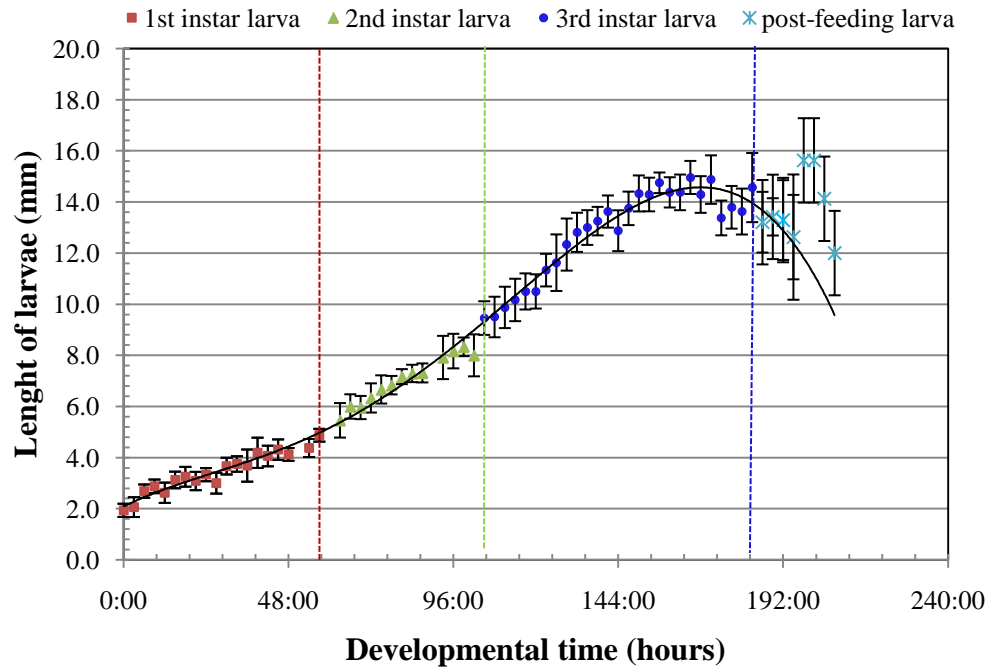


Figure 4.3 Laval growth of *Ch. rufifacies* at 18 °C ( $F_{66} = 340.27$ ,  $p < 0.0001$ ,  $n = 604$ ).

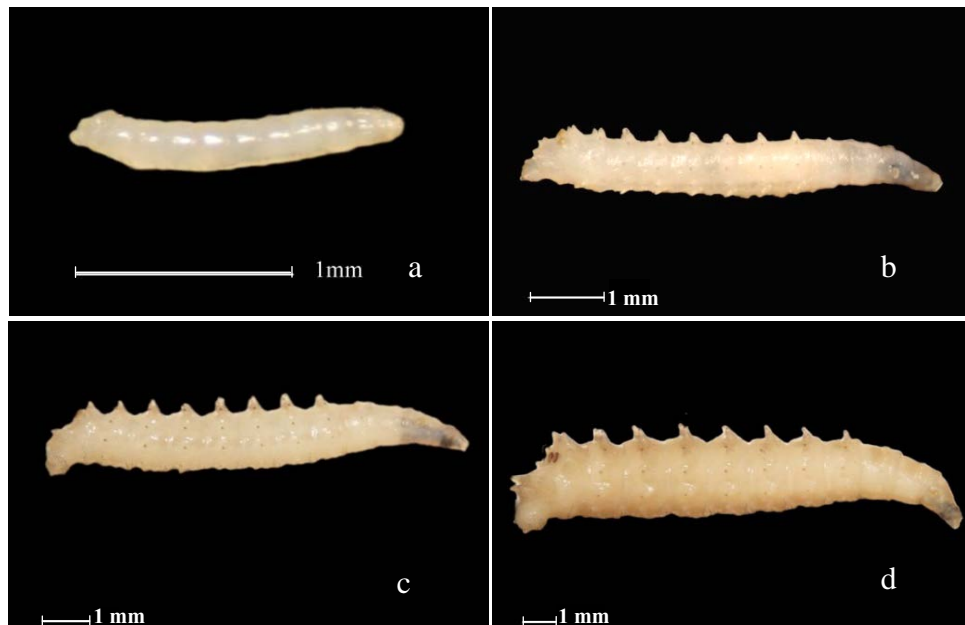


Figure 4.4 Larvae of *Ch. rufifacies* rearing at 18 °C (a) 2.0 mm long 1<sup>st</sup> instar larva at 0 hour (b) 4.0 mm long 2<sup>nd</sup> larva 51 hours (c) 9.0 mm long 3<sup>rd</sup> instar larva at 102 hours (d) 15.0 mm long peak feeding larva at 177 hours.

#### 4.1.5 Developmental time of *Ch. rufifacies* at 27 °C

The developmental time (mean± SD) of *Ch. rufifacies* under constant temperature at 27 °C of eggs, 1<sup>st</sup> instar larva, 2<sup>nd</sup> instar larva, 3<sup>rd</sup> instar larva and pupariation were 10.29± 1.39, 19.23± 0.45, 26.18± 10.46, 43.13± 4.15, 118.13± 4.19 hours respectively. The ADD and ADH were also calculated (table 4.3).

Table 4.3 The developmental times, DH, ADH, DD, and ADD (B<sub>10</sub>) of developmental stage from egg to eclosion of *Ch. rufifacies* at 27 °C

Stages	Time (h) (mean ±S D)	DH-B <sub>10</sub>	ADH-B <sub>10</sub>	DD-B <sub>10</sub>	ADD-B <sub>10</sub>
eggs	10.29± 1.39	174.93	174.93	7.29	7.29
1 <sup>st</sup> instar	19.23± 0.45	326.91	501.84	13.62	20.91
2 <sup>nd</sup> instar	26.18± 0.46	445.06	946.90	18.54	39.45
3 <sup>rd</sup> instar	43.13± 4.15	733.21	1680.11	30.55	70.00
pupariation	118.13± 4.19	2008.21	3688.32	83.68	153.68
<b>total</b>	<b>216.96± 10.64</b>	<b>3688.32</b>		<b>153.68</b>	

#### 4.1.6 Effect of temperature on the larval size rearing at 27 °C

The means (± SD) of the maximal lengths of *Ch. rufifacies* larvae from hatching to peak-feeding of rearing at 27 °C were plotted against time (figure 4.5). The body size were significantly different ( $F_8= 269.25$ ,  $p < 0.0001$ ), ranging from 1.83± 0.12-13.20± 0.50 mm. The length was 3.5 mm at 16 hours (figure 4.6a), 7.0 mm at 34 hours (figure 4.6b) and 8.5 mm at 42 hours after hatching (figure 4.6c). The highest length of larvae was 13.2± 0.5 at 75 hours after hatching (figure 4.6d).

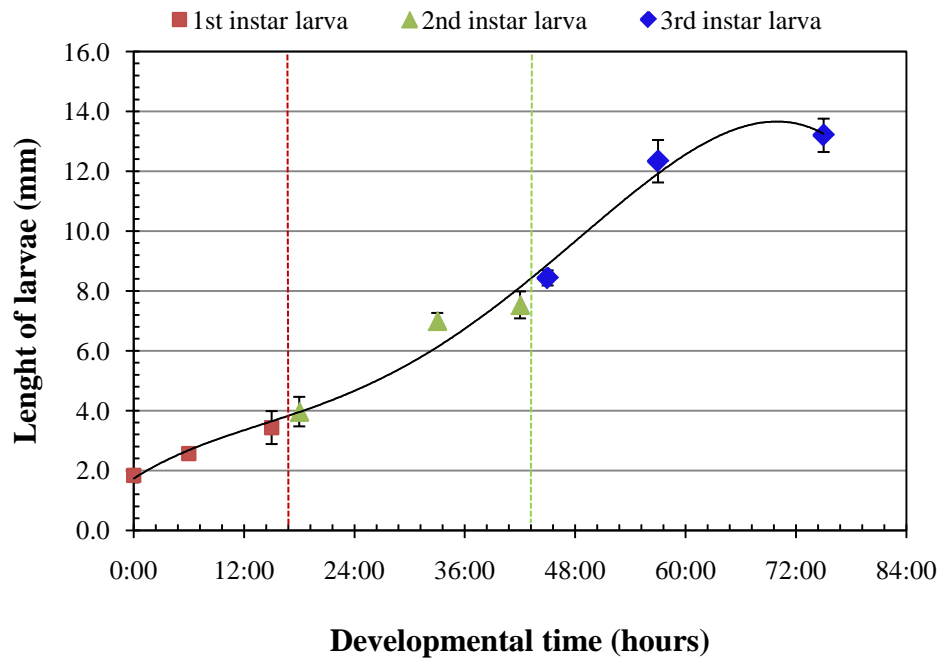


Figure 4.5 Larval growth of *Ch. rufifacies* rearing at 27 °C ( $F_8 = 269.25$ ,  $p < 0.0001$ ,  $n = 27$ ).

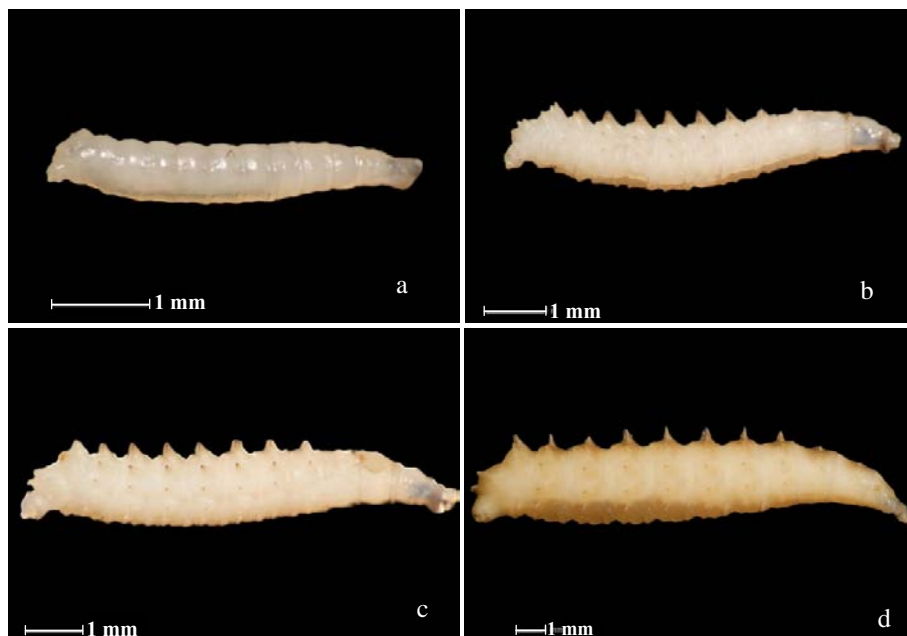


Figure 4.6 Larvae of *Ch. rufifacies* rearing at 27 °C (a) 3.5 mm long 1<sup>st</sup> instar larva at 16 hours (b) 7.0 mm long 2<sup>nd</sup> instar larva at 34 hours (c) 8.5 mm 3<sup>rd</sup> instar larva at 42 hours (d) 13.0 mm post feeding larva at 75 hours.

#### 4.1.7 Developmental time of *Ch. rufifacies* at 30 °C

The developmental time (means $\pm$  SD) of eggs, 1<sup>st</sup> instar larva, 2<sup>nd</sup> instar larva, 3<sup>rd</sup> instar larva and pupariation of *Ch. rufifacies* were 9.25 $\pm$  1.70, 15.56 $\pm$  0.60, 20.01 $\pm$  1.23, 31.16 $\pm$  0.58, 111.11 $\pm$  6.22 hours (table 4.4), respectively. The ADH and ADD were 3741.80 and 155.91, respectively.

Table 4.4 Developmental times, DH, ADH, DD and ADD (B<sub>10</sub>) of *Ch. rufifacies* rearing at 30 °C

Stages	Time (h) (mean $\pm$ SD)	DH-B <sub>10</sub>	ADH-B <sub>10</sub>	DD-B <sub>10</sub>	ADD-B <sub>10</sub>
eggs	9.25 $\pm$ 1.70	185.00	185.00	7.71	7.71
1 <sup>st</sup> instar	15.56 $\pm$ 0.60	311.20	496.20	12.97	20.68
2 <sup>nd</sup> instar	20.01 $\pm$ 1.23	400.20	896.40	16.68	37.35
3 <sup>rd</sup> instar	31.16 $\pm$ 0.58	623.20	1519.60	25.97	63.32
pupariation	111.11 $\pm$ 6.22	2222.20	3741.80	92.59	155.91
total	<b>187.09<math>\pm</math> 10.33</b>	<b>3741.80</b>		<b>155.91</b>	

#### 4.1.8 Effect of constant temperature (30 °C) on larval length

The means ( $\pm$  SD) of the maximal measured lengths of *Ch. rufifacies* larvae from hatching to post-feeding of rearing at 30 °C were plotted against time (figure 4.7). The measured ranges of body length were significant different ranging from 1.96 $\pm$  0.06 -14.83 $\pm$  1.40 mm ( $F_8= 151.63$ ,  $p < 0.0001$ ). The size started from 2.0 mm after hatching (figure 4.8a), the length reached 6.0 mm at 25 hours (figure 4.8b). The larvae become to third instar at 33 hours which averaged size 8.0 $\pm$  0.26 mm long (figure 4.8c) and reached the largest size 55 hours, it was 15.1 $\pm$  1.44 mm (figure 4.8d).

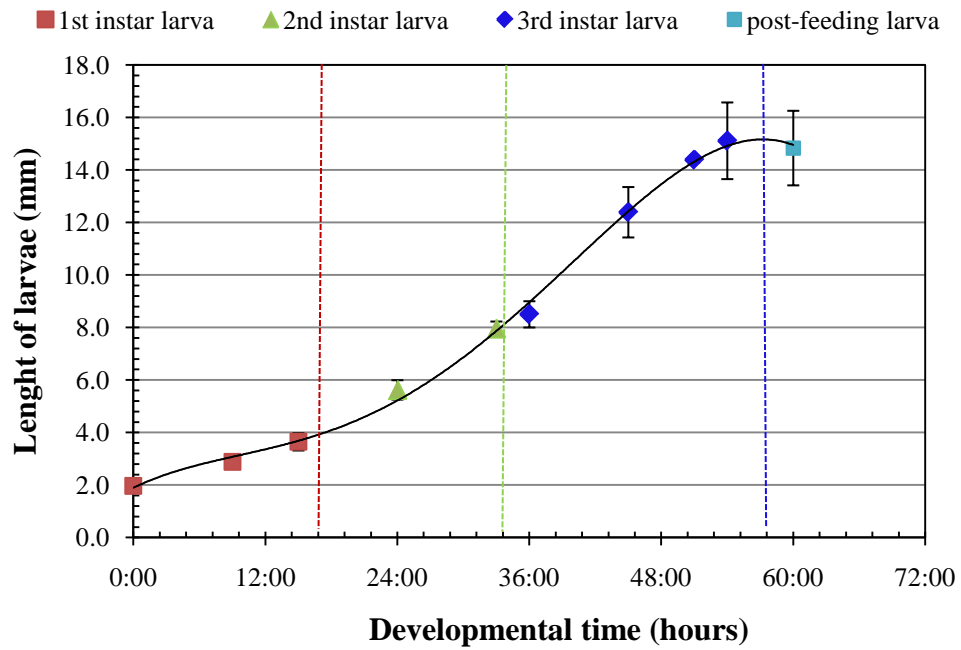


Figure 4.7 Larval growth of *Ch. rufifacies* rearing at 30 °C ( $F_8 = 151.63$ ,  $p < 0.0001$ ,  $n = 27$ ).

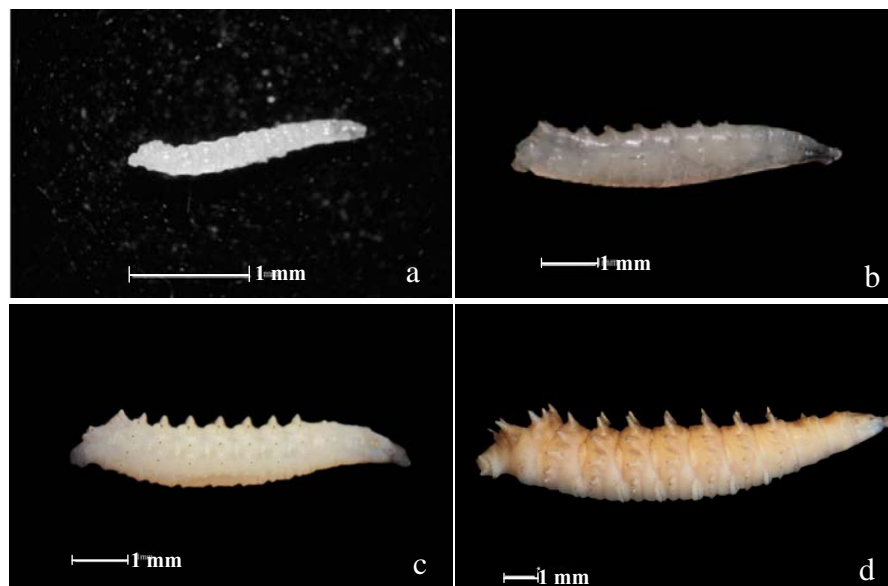


Figure 4.8 Larvae of *Ch. rufifacies* rearing at 30 °C (a) 2.0 mm long 1<sup>st</sup> instar larva at 0 hour (b) 6.0 mm long 2<sup>nd</sup> instar larva at 25 hours (c) 8.0 mm long 3<sup>rd</sup> instar larva at 33 hours (d) 15.0 mm post feeding larva at 55 hours.

#### 4.1.9 Developmental time of *Ch. rufifacies* at 33 °C

The developmental time (means $\pm$  SD) of eggs, 1<sup>st</sup> instar larva, 2<sup>nd</sup> instar larva, 3<sup>rd</sup> instar larva and pupariation of *Ch. rufifacies* under constant temperature at 33 °C were 8.27 $\pm$  1.8, 12.26 $\pm$  0.19, 14.29 $\pm$  0.23, 30.15 $\pm$  1.56, 103.06 $\pm$  2.80 hours, respectively. The ADH and ADD were 3864.69 and 161.03, respectively.

Table 4.5 The developmental times, DH, ADH, DD, and ADD (B<sub>10</sub>) of *Ch. rufifacies* rearing at 33 °C.

Stages	Time (h) (mean $\pm$ SD)	DH-B <sub>10</sub>	ADH-B <sub>10</sub>	DD-B <sub>10</sub>	ADD-B <sub>10</sub>
eggs	8.27 $\pm$ 0.18	190.21	190.21	7.93	7.93
1 <sup>st</sup> instar	12.26 $\pm$ 0.19	281.98	472.19	11.75	19.67
2 <sup>nd</sup> instar	14.29 $\pm$ 0.23	328.67	800.86	13.69	33.37
3 <sup>rd</sup> instar	30.15 $\pm$ 1.56	693.45	1494.31	28.89	62.26
pupation	103.06 $\pm$ 2.80	2370.38	3864.69	98.77	161.03
total		3864.69		161.03	

#### 4.1.10 Effect of temperature (33 °C) on larval length

The means ( $\pm$  SD) of the measured larval lengths from hatching to post-feeding of at 33 °C were plotted against time (figure 4.9) The average measured body lengths were significantly different and ranging from 1.79 $\pm$  0.26 - 15.79 $\pm$  0.96 mm ( $F_{23} = 553.98$ ,  $p < 0.0001$ ). The larval length was 2.92 $\pm$  0.20 at 6 hours after hatching (figure. 4.10a), the length of the second instar larvae reached 6.3 $\pm$  0.59 mm at 21 hours (figure. 4.10b), the third instar larva spend development time of 33 hours to reach their body length to 10.0 mm (figure. 4.10c), the highest length of larvae were 15.79 $\pm$  0.96 mm long found in 54 hours during development (figure 4.10d).

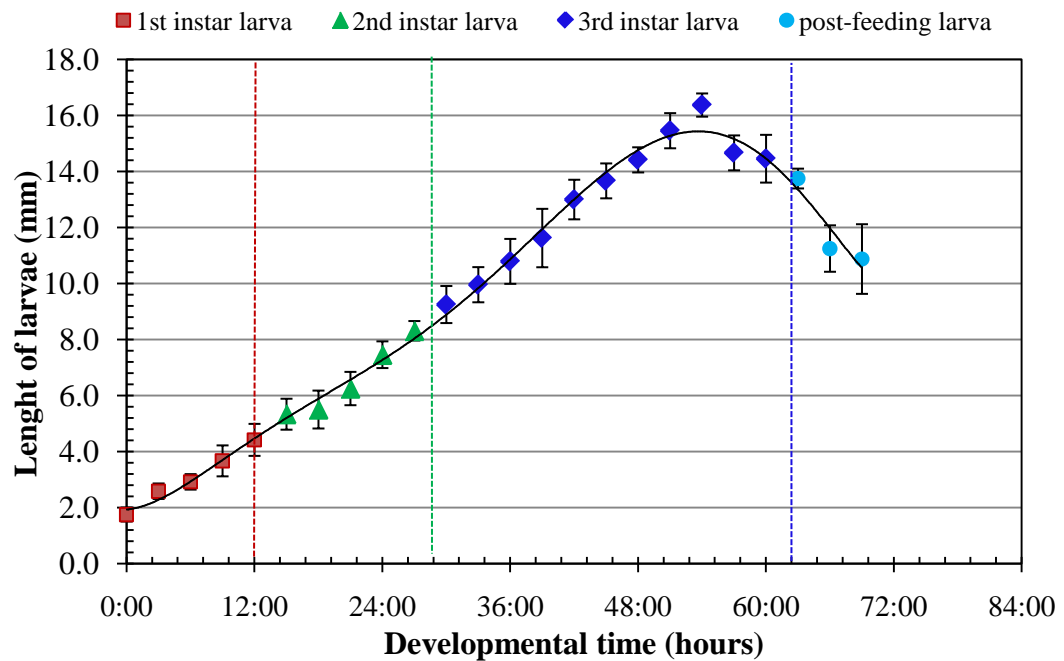


Figure 4.9 Larval growth of *Ch. rufifacies* rearing at 33 °C ( $F_{23} = 553.98$ ,  $p < 0.0001$ ,  $n = 252$ ).

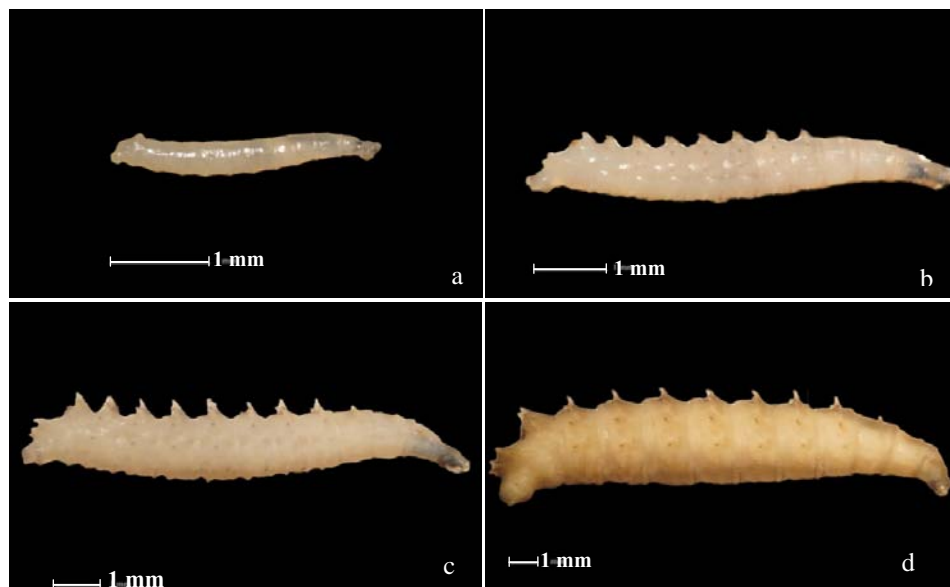


Figure 4.10 Larvae of *Ch. rufifacies* rearing at 33 °C (a) 3.0 mm long 1<sup>st</sup> instar larva at 6 hours (b) 6.5 mm long 2<sup>nd</sup> instar larva at 21 hours (c) 10.0 mm long 3<sup>rd</sup> instar larva at 33 hour (c) 15.0 mm long peak-feeding larva at 51 hour.

#### 4.1.11 Effect of Temperature (36 °C) on the Developmental Time

##### of *Ch. rufifacies*

The developmental time (means $\pm$  SD) of *Ch. rufifacies* under constant temperature at 33 °C was presented in Table 4.6. The developmental time of eggs, 1<sup>st</sup> instar larva, 2<sup>nd</sup> instar larva, 3<sup>rd</sup> instar larva and pupariation were 7.10 $\pm$  0.39, 10.39 $\pm$  0.20, 13.52 $\pm$  1.22, 26.51 $\pm$  3.50, and 115.11 $\pm$  4.00 hours, respectively. The ADH and ADD were 4488.38 and 187.02, respectively.

Table 4.6 The developmental times, DH, ADH, DD and ADD (B<sub>10</sub>) of *Ch. rufifacies* rearing at 36 °C

Stages	Time (h) (mean $\pm$ SD)	DH-B <sub>10</sub>	ADH-B <sub>10</sub>	DD-B <sub>10</sub>	ADD-B <sub>10</sub>
egg	7.10 $\pm$ 0.39	184.60	184.60	7.69	7.69
1 <sup>st</sup> instar	10.39 $\pm$ 0.20	270.14	454.74	11.26	18.95
2 <sup>nd</sup> instar	13.52 $\pm$ 1.22	351.52	806.26	14.65	33.59
3 <sup>rd</sup> instar	26.51 $\pm$ 3.50	689.26	1495.52	28.72	62.31
pupariation	115.11 $\pm$ 4.00	2992.86	4488.38	124.70	187.02
total	<b>172.63<math>\pm</math> 9.31</b>	<b>4488.38</b>		<b>187.02</b>	

#### 4.1.12 Effect of temperature (36 °C) on larval lengths

The means ( $\pm$  SD) of the measured lengths of *Ch. rufifacies* larvae from hatching to post-feeding of rearing at 36 °C were plotted against time (figure 4.11). The larval lengths were statistically significant different ( $F_{20} = 512.15$ ,  $p < 0.0001$ ), ranging from 1.96 $\pm$  0.26 - 15.42 $\pm$  0.79 mm. The average length of the first instar larvae have their size 3.4 $\pm$  0.38 mm at 9 hours (figure. 4.12a), for the second instar larvae was 8.0 $\pm$  1.30 mm at 24 hours (figure. 4.12b), third instar larvae was 11.2 $\pm$  0.84 mm long at 33 hours (figure. 4.12c), the largest size was 15.42 $\pm$  0.79 mm long at 48 hours (figure 4.12d).

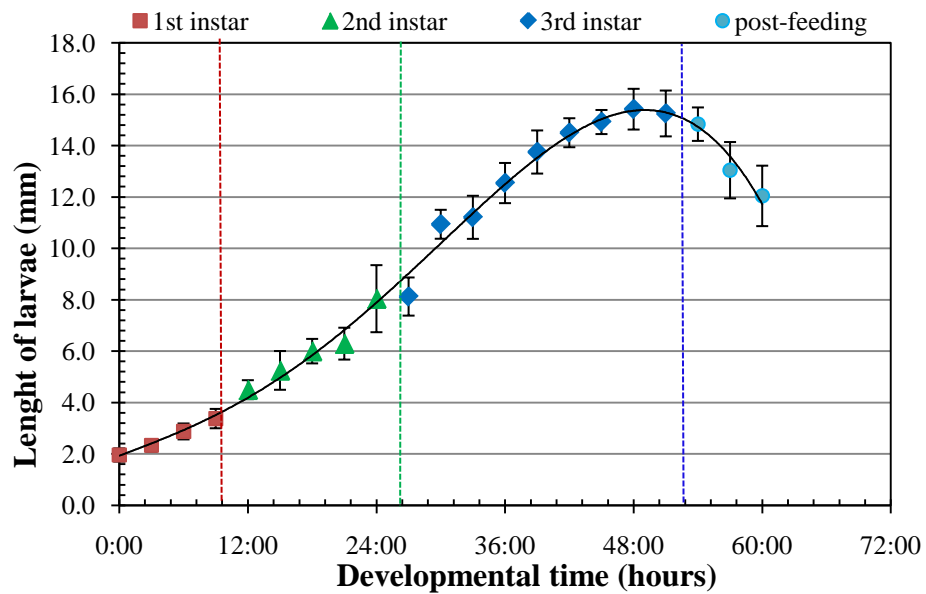


Figure 4.11 Larval growth of *Ch. rufifacies* rearing at 36 °C ( $F_{20} = 512.15$ ,  $p < 0.0001$ ,  $n = 252$ )

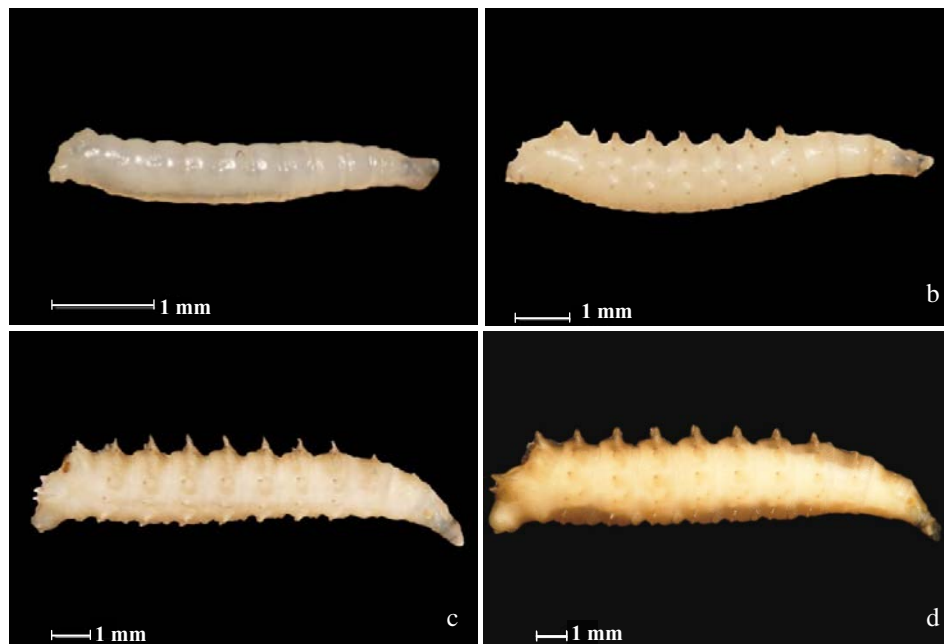


Figure 4.12 Larvae of *Ch. rufifacies* rearing at 36 °C (a) 3.5 mm long 1<sup>st</sup> instar larva at 9 hours (b) 8.0 mm long 2<sup>nd</sup> instar larva at 24 hours (c) 11.5 mm long 3<sup>rd</sup> instar larva at 33 hours (d) 15.5 mm long peak feeding larva at 48 hours.

#### 4.1.13 Developmental time of *Ch. rufifacies* at 39 °C

The developmental time (means $\pm$  SD) of *Ch. rufifacies* under constant temperature at 39 °C from eggs to pupariation; eggs, 1<sup>st</sup> instar larva, 2<sup>nd</sup> instar larva and 3<sup>rd</sup> instar larva were 6.14 $\pm$  0.40, 11.37 $\pm$  0.38, 12.07 $\pm$  0.10, 24.37 $\pm$  0.10 hours, respectively (table 4.7). No emergences were found for this condition. The ADH and ADD were 1564.55 and 65.19, respectively.

Table 4.7 The developmental times, DH, ADH, DD and ADD (B<sub>10</sub>) *Ch. rufifacies* rearing at 39 °C

Stages	Time (h) (mean $\pm$ SD)	DH-B <sub>10</sub>	ADH-B <sub>10</sub>	DD-B <sub>10</sub>	ADD-B <sub>10</sub>
egg	6.14 $\pm$ 0.40	178.06	178.06	7.42	7.42
1 <sup>st</sup> instar	11.37 $\pm$ 0.38	329.73	507.79	13.74	21.16
2 <sup>nd</sup> instar	12.07 $\pm$ 0.10	350.03	857.82	14.58	35.74
3 <sup>rd</sup> instar	24.37 $\pm$ 0.10	706.73	1564.55	29.45	65.19
pupariation	–	–	–	–	–
<b>total</b>		<b>1564.55</b>		<b>65.19</b>	

#### 4.1.14 Effect of temperature (39 °C) on the larval length

The means ( $\pm$  SD) of the larval measured lengths of larvae from hatching to post-feeding of rearing at 39 °C were plotted against time (figure 4.13). The body length ranged from 1.87 $\pm$  0.22 - 15.57 $\pm$  0.94 mm ( $F_{15} = 756.72$ ,  $p < .0001$ ). The length of the first instar larvae was 4.5 $\pm$  0.45 mm at 12 hours (figure 4.14a), the second instar larvae increased body size to 6.16 $\pm$  0.24 mm at 18 hours (figure 4.14b), the third instar larvae reach the length of 11.87 $\pm$  0.93 mm at 30 hours (figure 4.14c), the longest length of larvae were found in 39 hours of developmental time which was 15.57 $\pm$  0.94 mm (figure 4.14d).

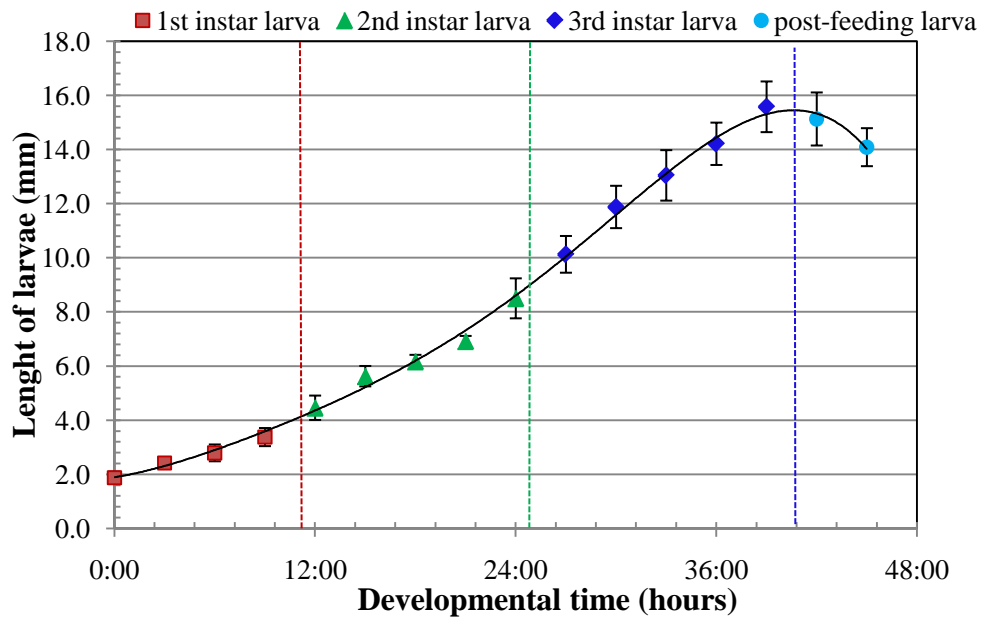


Figure 4.13 Larval growth of *Ch. rufifacies* rearing at 39 °C ( $F_{15} = 756.72$ ,  $p < .0001$ ,  $n = 192$ )

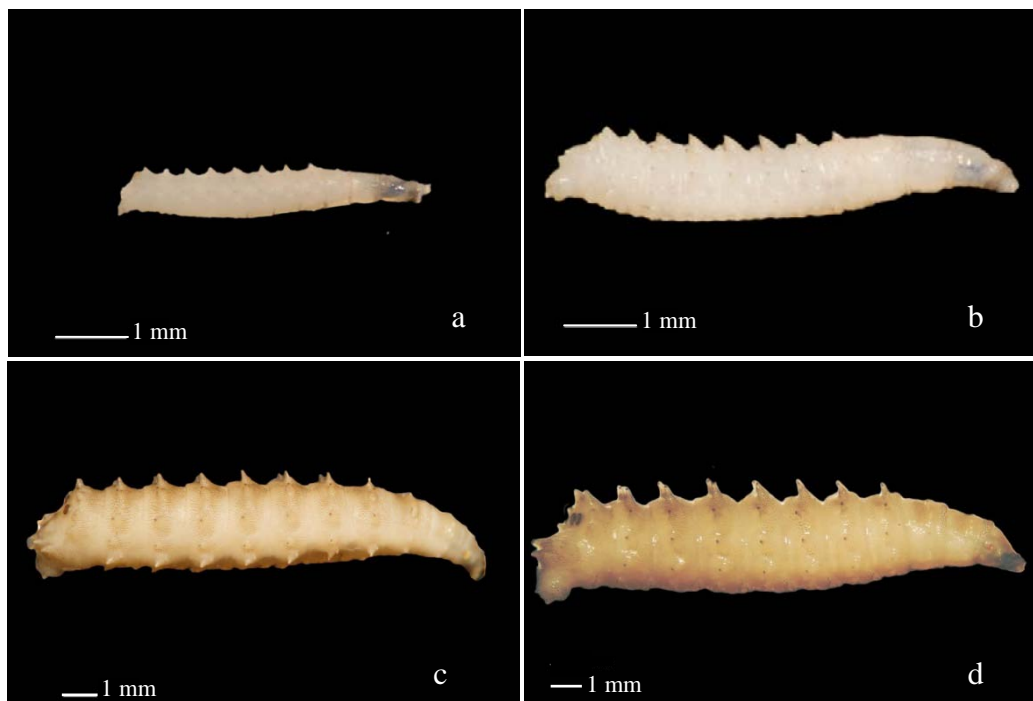


Figure 4.14 Larvae of *Ch. rufifacies* kept at 39 °C (a) 4.5 mm long 1<sup>st</sup> instar larva at 12 hours (b) 6.0 mm 2<sup>nd</sup> instar larva at 18 hours (c) 12.0 mm 3<sup>rd</sup> instar larva at 30 hours (c) 15.5 mm peak feeding larva at 39 hours.

## 4.2 Development curves from constant temperature regimes

The means  $\pm$  SD of the maximal measured body lengths of all instar larvae were plotted against time for each temperature regimes (from the hatching until the end of third instar). The body length of larvae show significant different between temperatures ( $F_6 = 2288.20$ ,  $p < 0.0001$ ), they were  $1.80 \pm 0.20$  -  $13.80 \pm 0.80$ ,  $1.90 \pm 0.32$  -  $14.60 \pm 0.42$ ,  $1.83 \pm 0.12$  -  $13.20 \pm 0.50$ ,  $1.96 \pm 0.06$  -  $14.83 \pm 1.40$ ,  $1.79 \pm 0.26$  -  $15.79 \pm 0.96$ ,  $1.96 \pm 0.26$  -  $15.41 \pm 0.79$ ,  $1.86 \pm 0.22$  -  $15.57 \pm 0.94$  mm for rearing at 15, 18, 27, 30, 33, 36 and 39 °C, respectively (figure 4.15). The lengths of larvae on development time of 42 hours at seven different temperature regimes were showed in figure 4.17. Growth curves of all regimes for this species displayed similar in shapes with progressively longer periods as temperatures decreased, and the larval lengths reached the highest points at peak-feeding stages. The development time of all developmental stages (from egg to pupariation) were shortest at 39 °C, while they were longest at 15 °C. The developmental time of pupariation was shortest at 33 °C, ( $103.06 \pm 2.80$  hours), but it was longest at 15 °C ( $314.04 \pm 15.51$  hours). No emergence was found at 39 °C.

Table 4.8 Developmental time (mean  $\pm$ SD) of *Ch. rufifacies* life stages at seven constant temperature regimes.

Stage	Time (mean $\pm$ SD hours) of <i>Ch. rufifacies</i> at seven constant temperature regimes						
	15 °C	18 °C	27 °C	30 °C	33°C	36 °C	39 °C
eggs	27.14 $\pm$ 1.46 <sup>a</sup>	26.49 $\pm$ 2.48 <sup>a</sup>	10.29 $\pm$ 1.39 <sup>b</sup>	9.25 $\pm$ 1.14 <sup>bc</sup>	8.27 $\pm$ 0.18 <sup>bcd</sup>	7.10 $\pm$ 0.39 <sup>cd</sup>	6.14 $\pm$ 0.40 <sup>d</sup>
1 <sup>st</sup> instar	60.14 $\pm$ 4.20 <sup>a</sup>	50.37 $\pm$ 2.17 <sup>b</sup>	19.23 $\pm$ 0.45 <sup>c</sup>	15.56 $\pm$ 0.60 <sup>d</sup>	12.26 $\pm$ 0.19 <sup>e</sup>	10.39 $\pm$ 0.20 <sup>e</sup>	11.37 $\pm$ 0.38 <sup>e</sup>
2 <sup>nd</sup> instar	73.15 $\pm$ 2.19 <sup>a</sup>	52.40 $\pm$ 10.52 <sup>b</sup>	26.18 $\pm$ 0.46 <sup>c</sup>	20.01 $\pm$ 1.23 <sup>cd</sup>	14.29 $\pm$ 0.23 <sup>d</sup>	13.52 $\pm$ 1.22 <sup>d</sup>	12.07 $\pm$ 0.14 <sup>d</sup>
3 <sup>rd</sup> instar	144.15 $\pm$ 10.48 <sup>a</sup>	129.18 $\pm$ 5.46 <sup>b</sup>	43.13 $\pm$ 4.15 <sup>c</sup>	31.16 $\pm$ 0.58 <sup>d</sup>	30.15 $\pm$ 1.56 <sup>d</sup>	26.51 $\pm$ 3.50 <sup>d</sup>	24.37 $\pm$ 0.10 <sup>d</sup>
pupariation	314.04 $\pm$ 15.51 <sup>a</sup>	289.08 $\pm$ 6.16 <sup>b</sup>	118.13 $\pm$ 4.19 <sup>c</sup>	111.11 $\pm$ 6.22 <sup>cd</sup>	103.06 $\pm$ 2.80 <sup>d</sup>	115.11 $\pm$ 4.00 <sup>cd</sup>	*
total	<b>618.6 <math>\pm</math> 33.84<sup>a</sup></b>	<b>547.5 <math>\pm</math> 27.59<sup>b</sup></b>	<b>216.96 <math>\pm</math> 10.64<sup>c</sup></b>	<b>187.09 <math>\pm</math> 10.33<sup>d</sup></b>	<b>168.03 <math>\pm</math> 4.96<sup>f</sup></b>	<b>172.63 <math>\pm</math> 9.31<sup>e</sup></b>	-

\* No emergence of adults.

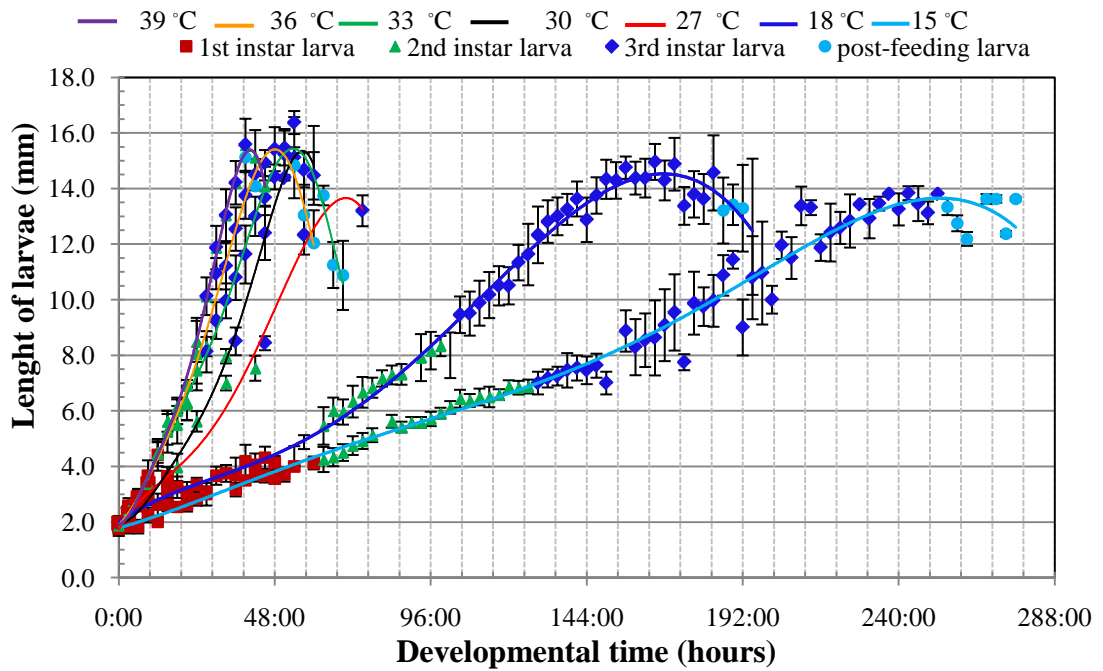


Figure 4.15 Larval development of *Ch. rufifacies* from hatching to pupariation at seven constant temperatures ( $F_6= 2288.20$ ,  $p<0.0001$ ,  $n= 1765$ ).

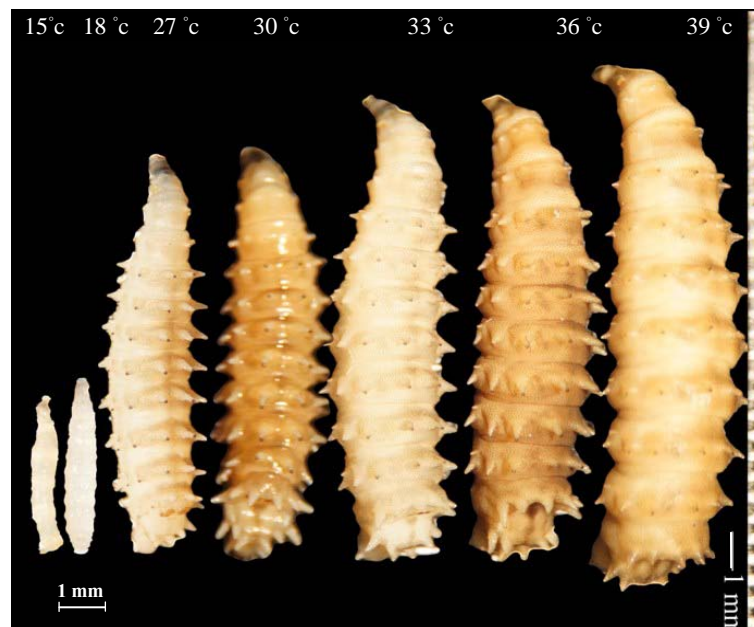


Figure 4.16 The 42 hours aged larvae of *Ch. rufifacies* kept at seven constant temperature regimes showing a significant lengths at a various exposed temperatures.

The various larvae lengths on developmental time of 15-18, 19-24, 33-36 and 45 hours at those seven temperature regimes were found and shown in figure 4.17. At various time of exposure of 15-18 hours were  $2.5 \pm 0.14$ ,  $3.1 \pm 0.33$ ,  $3.4 \pm 0.55$ ,  $3.6 \pm 0.33$ ,  $5.3 \pm 0.55$ ,  $5.3 \pm 0.75$  and  $5.6 \pm 0.38$  mm (figure 4.76a), at 19-24 hours were  $2.8 \pm 0.25$ ,  $3.1 \pm 0.36$ ,  $4.0 \pm 0.49$ ,  $5.6 \pm 0.37$ ,  $7.5 \pm 0.47$ ,  $8.0 \pm 0.130$  and  $8.5 \pm 0.74$  mm (figure 4.17b), 33-36 hours were  $3.2 \pm 0.25$ ,  $3.7 \pm 0.32$ ,  $7.0 \pm 0.26$ ,  $8.0 \pm 0.26$ ,  $10.0 \pm 0.63$ ,  $11.2 \pm 0.84$  and  $13.0 \pm 0.93$  mm (figure 4.17c), and at 45 hours were  $3.7 \pm 0.25$ ,  $4.3 \pm 0.39$ ,  $8.4 \pm 0.25$ ,  $12.4 \pm 0.96$ ,  $13.7 \pm 0.62$ ,  $14.09 \pm 0.47$  and  $14.1 \pm 0.7$  mm (figure 4.17d).

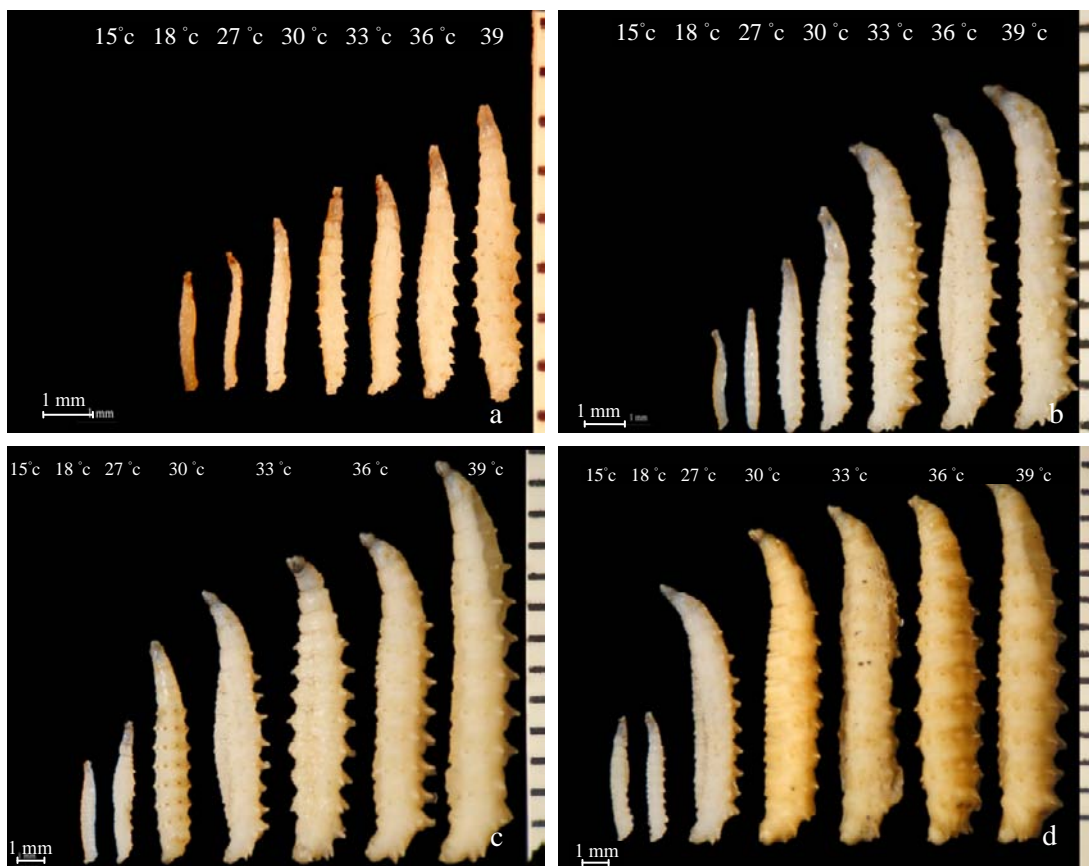


Figure 4.17 Larvae of *Ch. rufifacies* on developmental time of at seven different temperature regimes showing different lengths and they increase when increasing of temperature of rearing (a) 15-18 hours (b) 19-24 hours (c) 33-36 hours (d) 45 hours.

### 4.3 Isomegalen-diagram

Isomegalen-diagram was constructed from data of time from hatching to peak-feeding was plotted against temperature, each line represent identical larval length at those of temperature. It can be used to estimate the post mortem interval in forensic case works (figure 4.18).

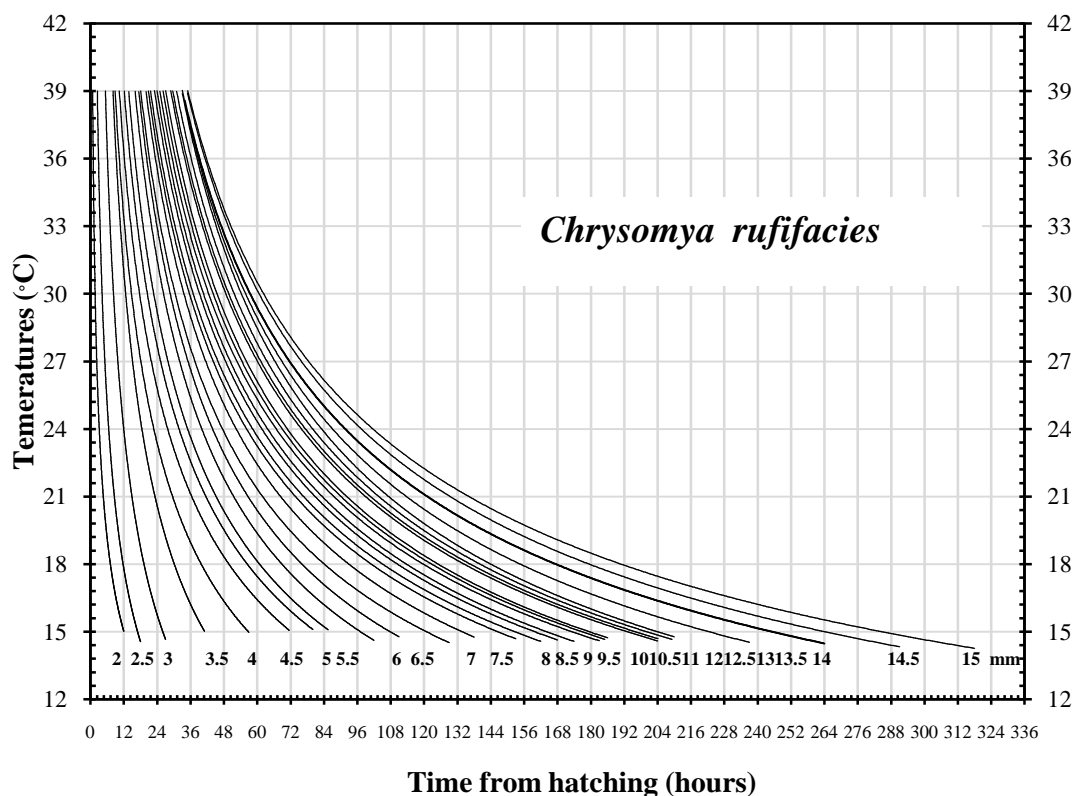


Figure 4.18 Isomegalen-diagram for *Ch. rufifacies* larvae from hatching to peak-feeding larvae. Time (x-axis) are plotted against temperature (y-axis), each line represent identical larval length (mm) ranged from 2-15.5 mm.

### 4.4 Isomorphen- diagram

Related to the antecedently published isomorphen-diagram (Grassberger & Reiter, 2001), time (x-axis) of all stages from oviposition to eclosion of *Ch. rufifacies* from this study were plotted against seven different constant temperatures (y-axis) where each line represents morphological changes. Areas between lines define identical stages (figure 4.19).

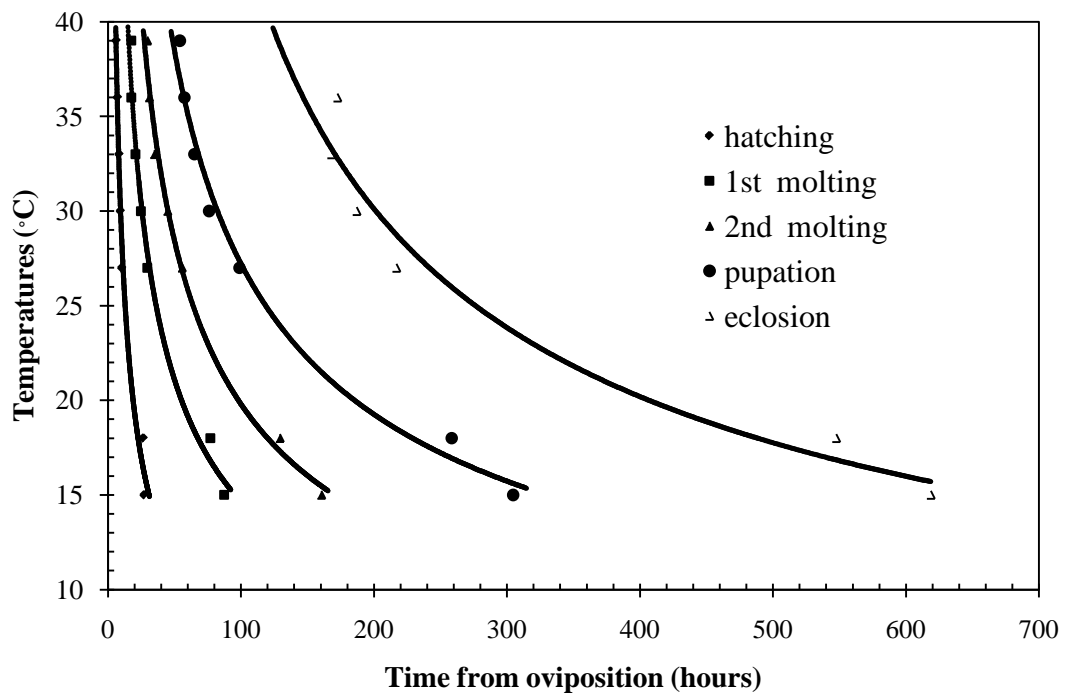


Figure 4.19 Isomorphen-diagram for *Ch. rufifacies*, presenting the egg, larval and pupal and mature stages among the temperature conditions.

#### 4.5 Developmental rate and thermal constant

The developmental rates of *Ch. rufifacies* kept at seven different constant temperature regimes from oviposition to pupariation were 0.00328, 0.00387, 0.01012, 0.01316, 0.01539, 0.01739 and 0.01856 1/hours at 15, 18, 27, 30, 33, 36 and 39 °C, respectively and for oviposition to eclosion were 0.00162, 0.00183, 0.00461, 0.00535, 0.00595 and 0.00579 1/hours at 15, 18, 27, 30, 33 and 36 °C, respectively. From the linear regression,  $y = 0.0007x - 0.0077$  of developmental rates from oviposition to pupariation showed that the minimum development threshold (B) was 11°C, however it was 9.5°C for total immature development (figure 4.20). The thermal constant (K) for this species from this study was 526.32 Degree days. The ADH for total immature development at seven different temperature regimes of 15, 18, 27, 30, 33 and 36 °C were 3093.10, 4380.16, 3688.32, 3741.80, 3864.69 and 4488.38, respectively. However, no emergence was found at 39 °C, its ADH was 1564.55 for the development from oviposition to pupariation.

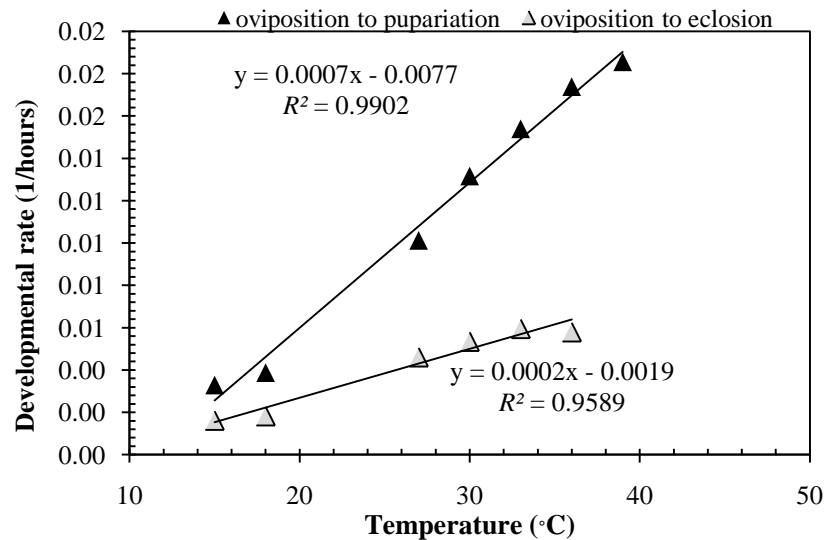


Figure 4.20 Linear regression of rearing temperature regimes and development rates from oviposition to pupariation;  $y = 0.0007x - 0.0077$ ,  $R^2 = 0.9902$  and from oviposition to adult;  $y = 0.0002x - 0.0019$ ,  $R^2 = 0.9589$  of *Ch. rufifacies*.

#### 4.6 Linear and non-linear model

A linear and a non-linear model were generated. The developmental minimum threshold for oviposition to pupariation development was estimated by x-intercept method (Greenberg, 1990). The linear equation of the development rates to temperatures was  $y = 0.0007x - 0.0077$ ,  $R^2 = 0.9906$ . The threshold temperature estimated from this regression was 11 °C (figure 4.21). For non-linear model, developmental time from oviposition to pupariation was plotted against temperature was  $y = 68820x^{-1.978}$ ,  $R^2 = 0.987$ .

Similar as above the linear and non-linear models were calculated from the relationship between the developmental rate of oviposition to eclosion and the rearing temperatures. We determined consequently the value of the intersection of the regression line x-axis which indicates the threshold temperature which was 9.5 °C (figure 4.22). The linear equation of the development rate was  $y = 0.0002x - 0.0019$ ,  $R^2 = 0.9589$ , while the non-linear for developmental time was  $y = 60725x^{-1.676}$ ,  $R^2 = 0.966$ .

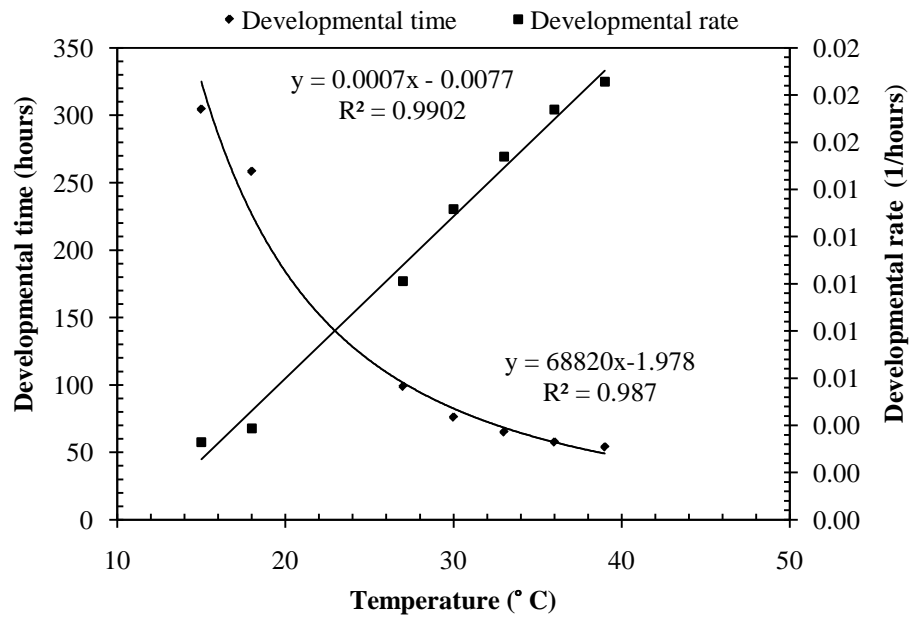


Figure 4.21 Linear and non-linear models of development (ADH) from oviposition to pupariation: development rates:  $y = 0.0007x - 0.0077$ ,  $R^2 = 0.9902$ ; developmental time:  $y = 68820x^{-1.978}$ ,  $R^2 = 0.987$

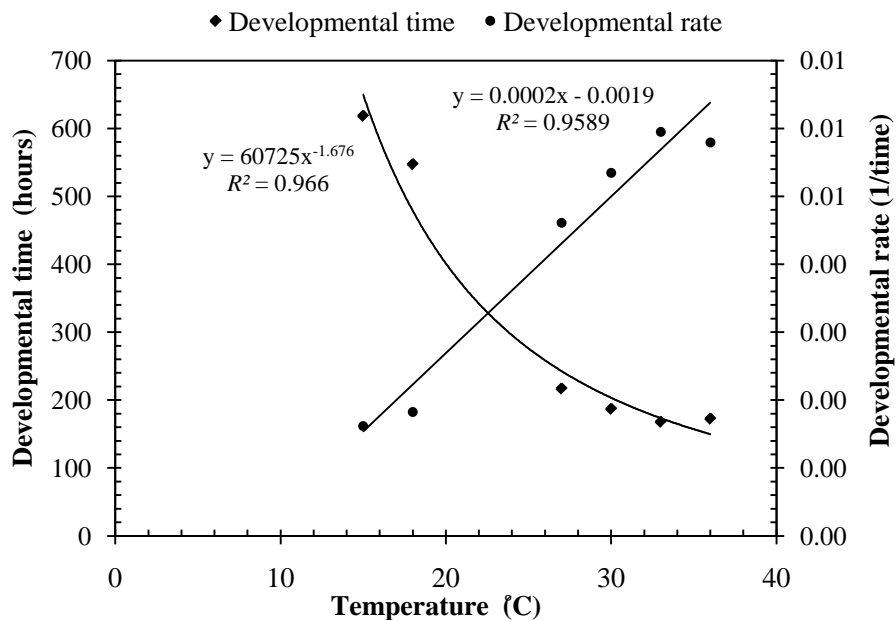


Figure 4.22 Linear and non linear models of development (ADH) from oviposition to eclosion: developmental rate:  $y = 0.0002x - 0.0019$ ,  $R^2 = 0.9589$ ; developmental time:  $y = 60725x^{-1.676}$ ,  $R^2 = 0.966$ .

## CHAPTER 5

### DISCUSSION AND CONCLUSION

#### 5.1 Effect of temperature on developmental time

The developmental rate of *Chrysomya rufifacies* was significantly and positively associated with temperature. The longest developmental times of eggs, 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> instar and pupae were measured at the lowest rearing temperature 15 °C, while the shortest developmental times were at the highest rearing temperature of 39 °C. Further, there was no pupal survival at this highest temperature, indicating that the upper threshold temperature for *Ch. rufifacies* is near this temperature. The upper threshold temperature reported here is in the range of previous thresholds documented for this species at 43 °C. (Johnson & Wallman, 2014). These findings are also in agreement with studies on heat shock protein increases near these temperatures (Rivers, Ciarlo, Spelman, & Brogan, 2010). The development time from egg to eclosion of *Ch. rufifacies* at 27 °C was similar to the study by Byrd (1997). These results showed accelerated developmental time compared to findings by Swiger et al. (2014b) (Table 5.1).

However, there may be genetic variation and environmental factors in different geographic regions for individual species (Conner & Hartl, 2004), thus, populations from different regions should be studied independently to better understand intra- and inter-species variation in development temperature thresholds (Table 5.2). Table 5.2 summaries several studies, including work by Greenberg and Kunich (2002) who studied *Calliphora vomitoria* at 12.5 and 23 °C and *Protophormia terraenovae* at identical constant temperatures and showed differences between species and each constant temperature. In addition, Kamal (1958) investigated the lifecycles of six fly species developing at 26.7 °C (*Ch. vomitoria*, *Ch. vicina*, *Lucilia sericata*, *Phormia regina*, *P. terraenovae* and *Cynomyopsis cadaverina*). There have been many other studies on blow fly development (Anderson, 2000; Gressberger & Reiter, 2001; Byrd & Allen, 2001; Russo et al., 2006; Byrd, 1995). The study by Byrd and Butler (1997), they reported development from egg to adult ranged from 190 to 598 hours under mean cyclic temperatures of 15.6, 21.1, 26.7 and 35.0 °C and a

constant temperature of 25.0 °C. A constant temperature of 25 °C produced a range of pupation times from 134 to 162 hours, with adult emergence ranging from 237 to 289 hours. The maximal preferential temperature of 35.1 °C was determined for larvae using a gradient system. When considering effect of the main factor (temperature). The validation of these gave reasonable that temperature is a well-known abiotic factor that affects blow fly development. Higher temperatures accelerated development while lower temperatures had an inverse impact (Byrd & Allen, 2001; Byrd & Butler, 1996; Byrd & Butler, 1997; Greenberg, 1991). Additionally, blow flies can influence their rate of development with temperatures increasing or decreasing development times, depending on the species (Byrd & Allen, 2001; Byrd & Butler, 1996; Byrd & Butler, 1997; Greenberg, 1991; Niederegger, Pastuschek, & Mall, 2010).

Table 5.1 Developmental hours for each life stage from Byrd (1997) and Swinger et al. (2014b), compared to data of the present study (27 °C) (average minimum duration of each stage).

Source	Temp. °C	Egg +1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	pupa	Total	ADH-B <sub>10</sub>
Byrd (1997)	26.7	32.0	24.0	78.0	82.0	216.0	3607.2
Present study	27.0	29.5	26.2	43.1	118.1	217.0	3682.0
Swinger (2014)	28.0	26.4	28.8	81.6	103.2	240.0	4320.0

Table 5.2 Average minimum life cycle durations of a selected blow flies species at fixed temperatures.

Species	Temp. (°C)	Egg (hours)	L1 (hours)	L2 (hours)	L3 (hours)	Pupa (hours)	Source
<i>Calliphra vomitoria</i> L.	12.5	64.8	55.2	60.0	434.4	717.6	Greenberg and Kunich (2002)
	23.0	21.6	25.2	19.1	210.4	247.2	
	26.7	26.0	24.0	48.0	420.0	260.0	Kamal (1958)

Table 5.2 (Cont.)

Species	Temp. (°C)	Egg (hours)	L1 (hours)	L2 (hours)	L3 (hours)	Pupa (hours)	Source
<i>Calliphora vicina</i> R-D	16.1	41.4	83.0	128.0	522.0	719.7	Anderson (2000)
	20.6	22.5	57.0	84.0	368.5	514.8	
	26.7	24.0	24.0	20.0	176.0	288.0	Kamal (1958)
<i>Lucilia sericata</i> Meigen	17.0	28.0	39.0	54.0	279.0	442.0	Gressberger and Reiter (2001)
	20.0	22.0	24.0	35.0	161.0	209.0	
	26.7	18.0	20.0	12.0	130.0	168.0	Kamal (1958)
<i>Lucilia illustris</i> Meigen	15.0	70.3	75.0	135.0	573.0	458.0	Byrd and Allen (2001)
	25.0	14.0	16.0	19.0	123.0	125.0	Anderson (2000)
<i>Phormia regina</i> Meigen	20.0	21.2	30.0	55.0	274.0	244.0	Byrd and Allen (2001)
	25.0	18.9	25.0	44.0	251.0	209.0	
	26.7	16.0	18.0	11.0	36.0	228.0	Kamal (1958)
<i>Protophormia terraenovae</i> R-D	12.5	91.2	290.4	240.0	832.8	722.4	Greenberg and Kunich (2002)
	23.0	16.8	26.4	27.6	118.8	144.0	
	26.7	25.0	28.0	22.0	188.0	288.0	Kamal (1958)
<i>Piophilina casei</i> Linnaeus	15.0	177.6	211.6	156.0	408.0	417.6	Russo et al. (2006)
	25.0	33.6	91.2	98.4	156.0	165.6	
<i>Cynomyopsis cadaverina</i>	26.7	19.0	20.0	16.0	168.0	216.0	Kamal (1958)
<i>Chrysomya rufifacies</i>	15.0	27.14	60.14	73.15	144.15	314.04	present study*
	18.0	26.49	50.37	52.4	129.18	289.08	present study
	26.7	14.0	18.0	24.0	78.0	82.0	Byrd (1995)
	27.0	10.29	19.23	26.18	43.13	118.13	present study*
	30.0	9.25	15.56	20.01	31.16	111.11	present study*
	33.0	8.27	12.26	14.29	30.15	103.06	present study*
	36.0	7.1	10.39	13.52	26.51	115.11	present study*
39.0	6.14	11.37	12.07	24.37	–	present study*	

## 5.2 Accumulated degree hours and accumulated degree days

Calculating accumulated degree hours and accumulated degree days is one of methods for the estimating the minimum PMI when using insect development. Accumulated degrees are considered the thermal units required for insect growth and ADH values represent a certain number of “energy hours” that are necessary for the development of insects larvae, whereas the ADD represents the number of “energy

days”. Both assume that the developmental rate is proportional to the temperature within a certain species-specific temperature range (Table 5.3) (Higley & Haskell, 2009). Greenberg and Kunich (2002) studied *Ch. vomitoria* development at 12.5 °C and 23.0 °C and Kamal (1958) evaluated it at 26.7 °C and found the ADH and ADD for this species was 3330, 6806 and 12993 ADH and 138.8, 283.6 and 541.4 ADD, respectively. *Lucilia sericata* has also been extensively investigated among several temperatures and reported variable ADH and ADD depending on temperature (Gressberger & Reiter, 2001; Kamal, 1958) Furthermore, the developmental variation in *L. illustris* (Byrd & Allen 2001; Anderson, 2000) and *Ch. rufifacies* has been extensively studied (Baumgartner, 1993). From these studies, it is clear that each species shows species-specific values for a minimum developmental threshold temperature or base temperature and this variation is important for calculating ADH and ADD. This is especially important in Thailand, where there have been few published development data sets on blow flies.

Table 5.3 Accumulated degree hours and accumulated degree days from several blow fly species from multiple studies.

Species	Temp. (°C)	ADH-B <sub>10</sub>	ADD-B <sub>10</sub>	Source
<i>Calliphora vomitoria</i> L.	12.5	3330.0	138.8	Greenberg and Kunich (2002)
	23.0	6805.5	283.6	
	26.7	12992.6	541.4	Kamal (1958)
<i>Calliphora vicina</i> R-D	16.1	9114.0	379.8	Anderson (2000)
	20.6	11096.1	462.3	
	26.7	8884.4	370.2	Kamal (1958)
<i>Lucilia sericata</i> Meigen	15.0	3060.0	127.5	Gressberger and Reiter (2001)
	17.0	5894.0	245.6	
	19.0	5076.0	211.5	
	20.0	4510.0	187.9	
	21.0	4169.0	173.7	

Table 5.3 (Cont.)

Species	Temp. (°C)	ADH-B <sub>10</sub>	ADD-B <sub>10</sub>	Source
<i>Lucilia sericata</i> Meigen	22.0	4068.0	169.5	Gressberger and Reiter (2001)
	25.0	4455.0	185.6	
	26.7	5811.6	242.2	Kamal (1958)
	28.0	4950.0	206.3	Gressberger and Reiter (2001)
	30.0	5360.0	223.3	
	34.0	6216.0	259.0	
<i>Lucilia illustris</i> Meigen	15.0	6556.5	273.2	Byrd and Allen (2001)
	25.0	4455.0	185.6	Anderson (2000)
<i>Phormia regina</i> Meigen	20.0	6242.0	260.1	Byrd and Allen (2001)
	25.0	8218.5	342.4	
	26.7	5160.3	215.0	Kamal (1958)
<i>Protophormia terraenovae</i> R-D	12.5	5442.0	226.8	Greenberg and Kunich (2002)
	23.0	4336.8	180.7	Greenberg and Kunich (2002)
	26.7	9201.7	383.4	Kamal (1958)
<i>Piophilha casei</i> Linneaus	15.0	6854.0	285.6	Russo et al. (2006)
	25.0	8172.0	340.5	
<i>Cynomyopsis cadaverina</i>	26.7	7331.3	305.5	Kamal (1958)
<i>Chrysomya rufifacies</i>	15.0	6242.0	260.1	present study
	18.0	8218.5	342.4	present study
	26.7	5160.3	215.0	Byrd (1995)
	27.0	5442.0	226.8	present study
	30.0	4336.8	180.7	present study
	33.0	9201.7	383.4	present study
	36.0	6840.0	285.0	present study
39.0*	8316.6	346.5	present study*	

A lower threshold used for calculated accumulated degree hours usually 10 °C (Higley & Haskell, 2001 cited in Byrd & Castner, 2001, pp. 287-302). However, a alternate value for the lower developmental threshold, which has been widely adopted, is to use the value obtained by extrapolating the linear portion of the relationship between developmental rate and temperature back to intercept the x-axis.

The specific lower threshold temperature for *Ch. rufifacies* from presented studied were 11.0 and 9.5 °C for oviposition to pupation and oviposition to eclosion, respectively. The ADH calculated from three different values lower threshold temperature (B<sub>10</sub> table 5.4, B<sub>9.5</sub> table 5.5 and B<sub>11</sub> table 5.6) were significantly different both ADH from oviposition to pupation ( $F_2=53.04$ ,  $p<0.0001$ ) and oviposition to eclosion ( $F_2=90.36$ ,  $p<0.0001$ ). From our studied and observation we recommened to use 11.0 °C as a base temperature for calculated ADH from oviposition to pupation, it seem to be more precise than 9.5 °C. On the other hand, the base temperature 9.5 °C more precise when use for complete cycle.

Table 5.4 Accumulated degree hours calculated from B<sub>10</sub>.

Stage	ADH of <i>Ch. rufifacies</i> at different temperature (B <sub>10</sub> )						
	15 °C	18 °C	27 °C	30 °C	33°C	36 °C	39 °C
Eggs	135.7	211.92	174.93	185	190.21	184.6	178.06
1 <sup>st</sup> instar	436.4	614.88	501.84	496.2	472.19	454.74	507.79
2 <sup>nd</sup> instar	802.15	1034.08	946.9	896.4	800.86	806.26	857.82
3 <sup>rd</sup> instar	1522.9	2067.52	1680.11	1519.6	1494.31	1495.52	1564.55
Pupa	3093.1	4380.16	3688.32	3741.8	3864.69	4488.38	

Table 5.5 Accumulated degree hours calculated from B<sub>9.5</sub>.

Stage	ADH of <i>Ch. rufifacies</i> at different temperature (B <sub>9.5</sub> )						
	15 °C	18 °C	27 °C	30 °C	33°C	36 °C	39 °C
Eggs	149.27	225.165	180.08	189.63	194.35	188.15	181.13
1 <sup>st</sup> instar	480.04	653.31	516.60	508.61	482.46	463.49	516.55
2 <sup>nd</sup> instar	882.365	1098.71	974.75	918.81	818.27	821.77	872.61
3 <sup>rd</sup> instar	1675.19	2196.74	1729.53	1557.59	1526.80	1524.28	1591.53
Pupa	3402.41	4653.92	3796.80	3835.35	3948.71	4574.70	

Table 5.6 Accumulated degree hours calculated from B<sub>11</sub>.

Stage	ADH of <i>Ch. rufifacies</i> at different temperature (B <sub>11</sub> )						
	15 °C	18 °C	27 °C	30 °C	33°C	36 °C	39 °C
Eggs	108.56	185.43	164.64	175.75	181.94	177.50	171.92
1 <sup>st</sup> instar	349.12	538.02	472.32	471.39	451.66	437.25	490.28
2 <sup>nd</sup> instar	641.72	904.82	891.20	851.58	766.04	775.25	828.24
3 <sup>rd</sup> instar	1218.32	1809.08	1581.28	1443.62	1429.34	1438.00	1510.60
Pupa	2474.48	3832.64	3471.36	3554.71	3696.66	4315.75	

### 5.3 Lower developmental threshold

The minimum temperatures for growth and development vary among species. The temperature threshold below which growth and development will not take place is called the base temperature. The base temperature varies among species and geographic locations. Davies and Ratcliffe (1994) demonstrated a minimum baseline temperature threshold of 3.5 °C for *Ch. vicina* in north of England, whereas Marchenko (2001), working in Russia, recorded a base temperature of 2 °C for the same species. Donovan et al. (2006) explored *Ch. vicina* growth in London, England at temperatures between 4 - 30 °C and found the base temperature was 1°C. In this study, we observed base temperatures of *Ch. rufifacies* by plotting temperatures (15, 18, 27, 30, 33, 36 and 39± 2 °C) against developmental rate (1/time) and used the x-intercept to estimate a base temperature of 9.5 °C for total immature development while it was 11 °C from oviposition to pupation. These results suggest that the specific base temperature for a particular species depends on the geographic location of the population and reinforces the need to ensure that the most appropriate base temperature is chosen with respect to crime scene location (table 5.7).

Table 5.7 Lower developmental thresholds (base temperatures) for several blow flies species by Marchenko (2001) at a high latitude compared to the present study carried on in Thailand near the equator.

Fly species	Base temperature (°C )	Source
<i>Calliphora vicina</i>	2.0	Marchenko (2001)
<i>Calliphora vomitoria</i>	3.0	
<i>Protophormia terraenovae</i>	7.8	
<i>Lucilia sericata</i> <sup>a</sup>	9.0	
<i>Chrysomya albiceps</i>	10.2	
<i>Phormia regina</i>	11.4	
<i>Muscina stabulans</i>	7.2	
<i>Muscina assimilis</i>	7.9	
<i>Boettcherisca septentrionalis</i>	7.8	
<i>Piophilila foveolata</i>	6.4	
<i>Chrysomya rufifacies</i>	9.5	present study*

<sup>a</sup> According to Kozhanchikov (1961).

#### 5.4 Use of the isomorphen and isomegalen-diagrams

Entomological evidence found on and around the corpse should be collected and preserved according to standard medico-legal procedures that have been described elsewhere (Haskell et al., 1997). On site microclimatic temperatures that affect larval development in the immediate environment are often established and correlated retrospectively with nearby air temperature records (e.g., weather station). Assuming an average constant temperature, as is the case with corpses found indoors larvae or pupae recovered from the scene should be stored at a constant temperature, until they pupate or the first adults emerge. Their age can then be determined retrospectively, often using a isomorphen-diagram like the one presented in this study. Where temperature is variable, an age range can be estimated between the points where the observed morphological change (pupation or eclosion) divides the graph at the maximum and minimum temperatures. Length of larvae found on crime scene should be collected and use isomegalen-diagram to determined age of larvae where the temperature was exactly the same with crime scene.

## 5.5 Conclusion

5.5.1 Developmental time from egg to adult was significantly different between the temperatures ( $F_6 = 1572.22$ ,  $p < 0.0001$ ), with development times of  $618.62 \pm 33.84$ ,  $547.52 \pm 27.59$ ,  $216.6 \pm 10.64$ ,  $187.09 \pm 10.33$ ,  $168.03 \pm 4.69$ ,  $172.63 \pm 9.31$  hours for 15, 18, 27, 30, 33 and 36 °C, respectively.

5.5.2 At 39 °C pupae did not survive and development was not completed.

5.5.3 Larval body length was significantly different among the seven different temperatures ( $F_6 = 2288.20$ ,  $p < 0.0001$ ). Body length was  $1.80 \pm 0.20$  -  $13.80 \pm 0.80$ ,  $1.90 \pm 0.32$  -  $14.60 \pm 0.42$ ,  $1.83 \pm 0.12$  -  $13.20 \pm 0.50$ ,  $1.96 \pm 0.06$  -  $14.83 \pm 1.40$ ,  $1.79 \pm 0.26$  -  $15.79 \pm 0.96$ ,  $1.96 \pm 0.26$  -  $15.41 \pm 0.79$ ,  $1.86 \pm 0.22$  -  $15.57 \pm 0.94$  mm at temperatures of 15, 18, 27, 30, 33, 36 and 39 °C, respectively

5.5.4 Linear regression of development rates from oviposition to pupation resulted in a minimum development threshold of 11 °C, while for total immature development was 9.5 °C.

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## **APPENDIX**

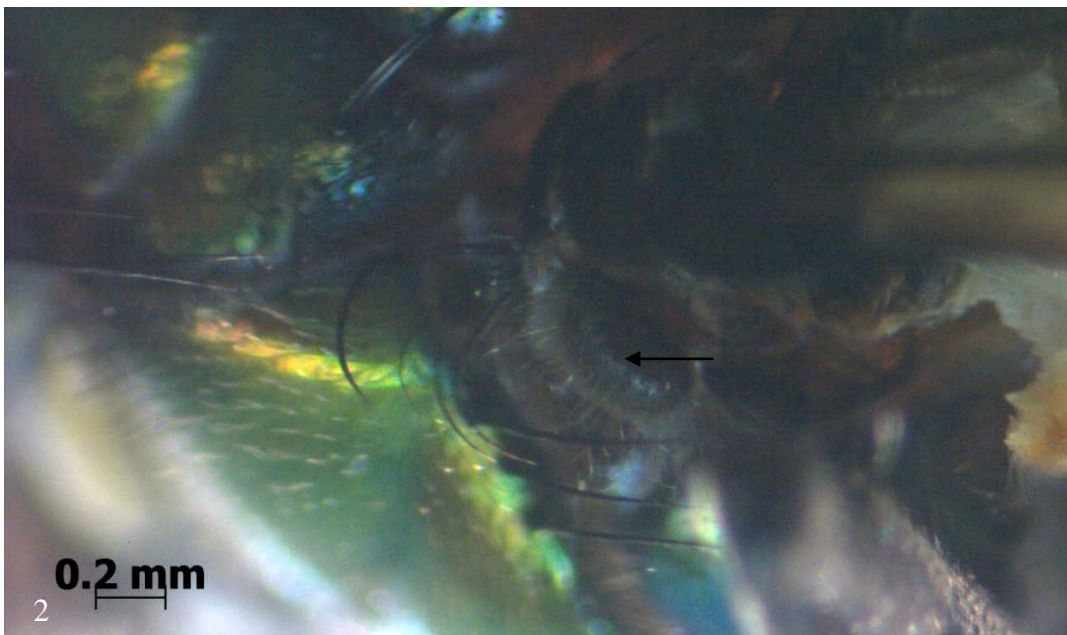
## **APPENDIX 1**

### **Characteristics Key of Blow fly *Ch. rufifacies***

**Key to adult of *Chrysomya rufifacies***  
**(by Marshall, Whitworth, and Roscoe)**



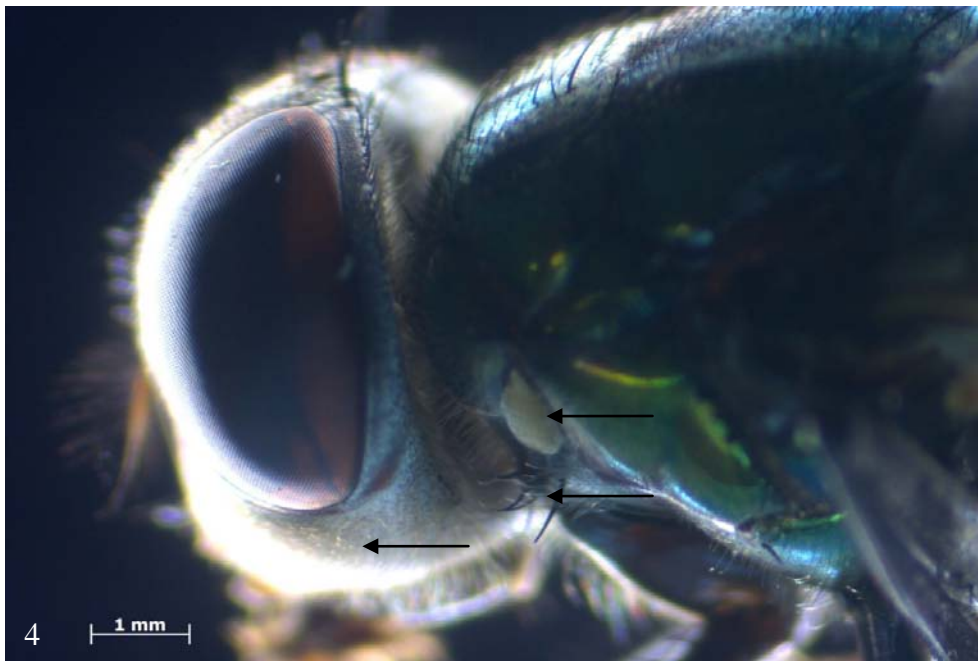
1. stem vein hairs present.(figure 1).....2



2. greater ampulla with stiff, erect setae..(figure 2).....  
.....Chrysomyinae (go to 3)



3. first abdominal tergite and posterior margins of second and third tergites black.....  
 (figure 3).....*Chrysomya*..go to 4



4. gena white, orange-brown to black with pale dusting and silvery hairs; a pale or  
 white anterior thoracic spiracle with proepisternal seta present (figure 4) .....  
 .....*Chrysomya rufifacies*



separated compound eyes (figure 5).....female

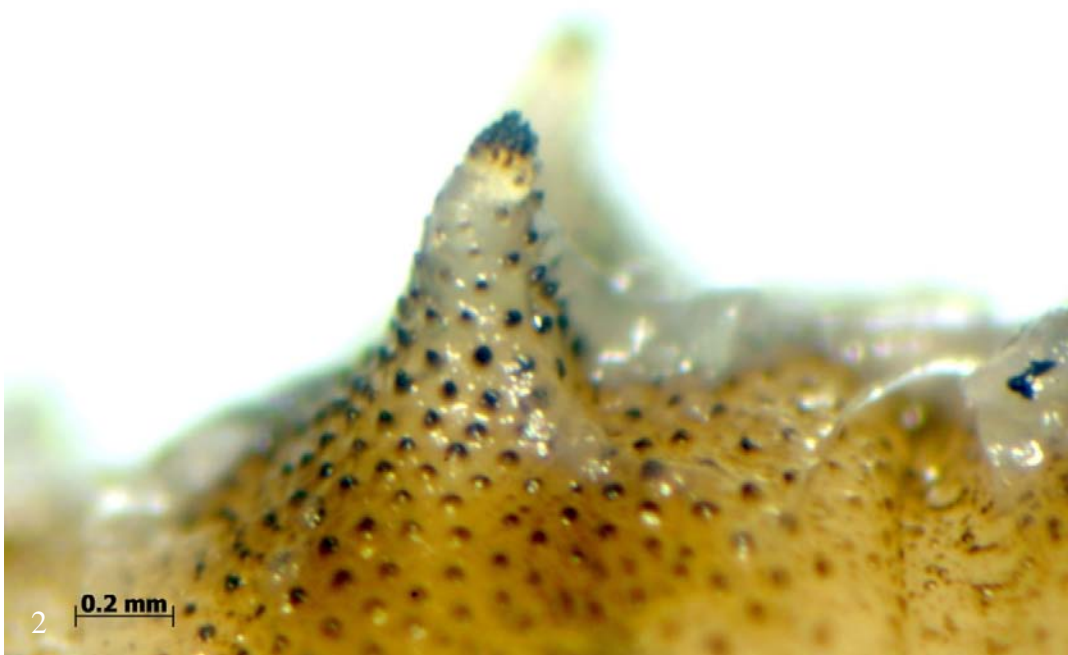


closed compound eyes (figure 6).....male

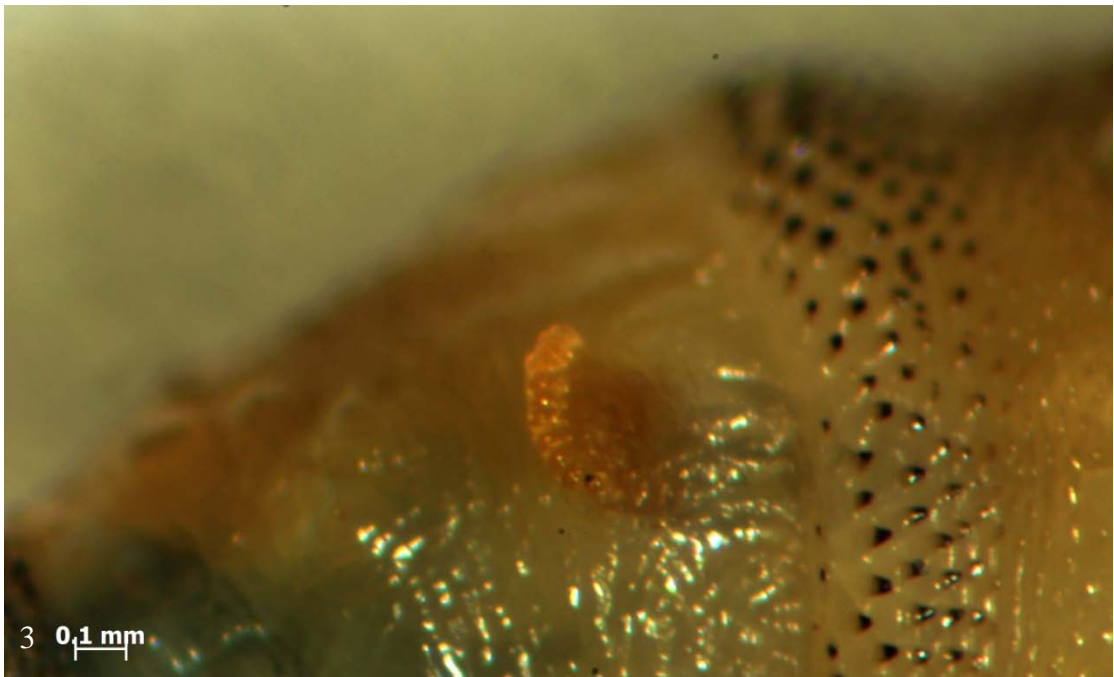
**Key to third instar larvae of *Ch. rufifacies* (by Smith)**



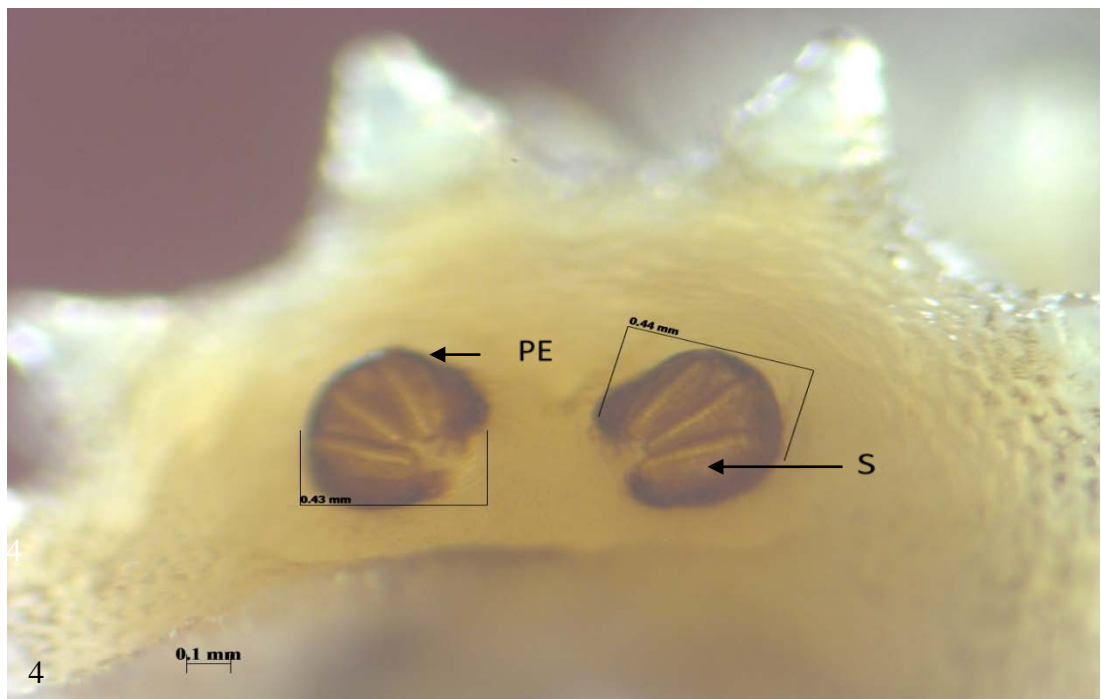
1. Tubercle along the body (figure 1).....go to 2



2. tubercle tips bearing numerous small spines (figure 2).....go to 3



3. anterior spiracles consist of 10 papillae (figure 3).....go to 4



4. posterior spiracle with incomplete, heavily pigmented peritreme (PE) encircling three relatively straight spiracular openings (slits, S) (figure 4).....*Ch. rufifacies*



Higher magnification of posterior spiracle of *Ch. rufifacies* (figure 5).....

**APPENDIX 2**  
**Data of Developmental Times**

Table 6-1 Developmental times of blow fly, *Ch. rufifacies* at 15 °C

Stages	Developmental time at 15 °C (hour:min:sec)			
	Rep.1	Rep.2	Rep. 3	average
<b>Eggs</b>	25:10:45	28:06:00	28:24:00	27:13:35
<b>1<sup>st</sup> Instar</b>	55:17:00	63:27:00	61:57:00	60:13:40
<b>2<sup>nd</sup> Instar</b>	75:12:00	73:53:00	70:40:00	73:15:00
<b>3<sup>rd</sup> Instar + prepupa</b>	152:41:00	148:00:00	132:03:30	144:14:50
<b>Pupation</b>	329:13:00	297:35:00	315:23:30	314:03:50
<b>Total</b>	637:33:45	611:01:00	608:28:00	619:00:55

Table 6-2 Developmental times of blow fly, *Ch. rufifacies* at 18 °C

Stages	Developmental time at 18 °C (hour:min:sec)			
	Rep.1	Rep.2	Rep.3	average
<b>Eggs</b>	25:01:00	25:24:00	30:03:00	26:49:20
<b>1<sup>st</sup> Instar</b>	48:48:30	53:03:30	52:26:00	51:26:00
<b>2<sup>nd</sup> Instar</b>	52:32:00	63:37:00	41:52:00	52:40:20
<b>3<sup>rd</sup> Instar + prepupa</b>	123:48:00	128:48:00	135:19:00	129:18:20
<b>Pupation</b>	294:07:00	282:05:00	291:13:40	289:08:33
<b>Total</b>	544:16:30	552:57:30	550:53:40	549:22:33

Table 6-3 Developmental times of blow fly, *Ch. rufifacies* at 27 °C

Stages	Developmental time at 27 °C (hour:min:sec)			
	Rep.1	Rep.2	Rep.3	average
Eggs	11:48:00	11:03:00	8:38:00	10:29:40
1 <sup>st</sup> Instar	19:08:00	18:47:00	20:14:00	19:23:00
2 <sup>nd</sup> Instar	25:51:00	25:51:00	27:12:00	26:18:00
3 <sup>rd</sup> Instar + prepupa	39:37:00	42:08:00	47:56:00	43:13:40
Pupation	119:38:00	121:42:00	113:23:00	118:14:20
Total	216:02:00	219:31:00	217:23:00	217:38:40

Table 6-4 Developmental times of blow fly, *Ch. rufifacies* at 30 °C

Stages	Developmental time at 30 °C (hour:min:sec)			
	Rep.1	Rep.2	Rep.3	average
Eggs	10:08:00	10:00:00	8:07:00	9:25:00
1 <sup>st</sup> Instar	15:57:00	16:02:00	15:49:00	15:56:00
2 <sup>nd</sup> Instar	21:20:00	20:12:00	18:33:00	20:01:40
3 <sup>rd</sup> Instar + prepupa	30:11:00	31:32:00	32:05:00	31:16:00
Pupation	116:48:00	104:16:00	112:29:00	111:11:00
Total	194:24:00	182:02:00	187:03:00	187:49:40

Table 6-5 Developmental times of blow fly, *Ch. rufifacies* at 33 °C

Stages	Developmental time at 33 °C (hour:min:sec)			
	Rep.1	Rep.2	Rep.3	average
<b>Eggs</b>	8:06:00	8:34:00	8:41:00	8:27:00
<b>1<sup>st</sup> Instar</b>	12:43:00	12:31:00	12:05:00	12:26:20
<b>2<sup>nd</sup> Instar</b>	14:07:00	14:27:00	14:53:00	14:29:00
<b>3<sup>rd</sup> Instar + prepupa</b>	31:53:00	30:46:00	28:06:00	30:15:00
<b>Pupation</b>	101:54:00	105:34:00	101:49:00	103:05:40
<b>Total</b>				168:43:00

Table 6-6 Developmental times of blow fly, *Ch. rufifacies* at 36 °C

Stages	Developmental time at 36 °C (hour:min:sec)			
	Rep.1	Rep.2	Rep.3	average
<b>Eggs</b>	6:29:00	6:50:00	7:45:00	7:01:20
<b>1<sup>st</sup> Instar</b>	10:57:00	10:17:00	10:43:00	10:39:00
<b>2<sup>nd</sup> Instar</b>	13:37:00	15:21:00	12:38:00	13:52:00
<b>3<sup>rd</sup> Instar + prepupa</b>	30:17:00	27:35:00	22:42:00	26:51:20
<b>Pupation</b>	118:56:00	115:40:00	110:58:00	115:11:20
<b>Total</b>	180:16:00	175:43:00	164:46:00	173:35:00

Table 6-7 Developmental times of blow fly, *Ch. rufifacies* at 39 °C

<b>Stages</b>	<b>Developmental time at 39 °C (hour:min:sec)</b>			<b>average</b>
	<b>Rep.1</b>	<b>Rep.2</b>	<b>Rep.3</b>	
<b>Eggs</b>	6:12	6:12	6:19	6:14
<b>1<sup>st</sup> Instar</b>	11:15	11:15	12:22	11:37
<b>2<sup>nd</sup> Instar</b>	12:06	12:06	12:09	12:07
<b>3<sup>rd</sup> Instar + prepupa</b>	24:38:00	24:38:00	24:35:00	24:37:00
<b>Pupation</b>	*	*	*	*
<b>Total</b>				

*Note:*\* No adults emerge all replication.

**APPENDIX 3**  
**Data of Developmental Rates**

Table 7-1 Developmental rates of blow fly, *Ch. rufifacies* at 15 °C

Stages	Developmental rate at 15 °C (1/time)			average
	Rap.1	Rap.2	Rap.3	
<b>Eggs</b>	0.0398	0.0356	0.0354	0.0370
<b>1<sup>st</sup> Instars</b>	0.0181	0.0158	0.0162	0.0167
<b>2<sup>nd</sup> Instars</b>	0.0133	0.0136	0.0142	0.0137
<b>3<sup>rd</sup> Instars</b>	0.0066	0.0068	0.0076	0.0070
<b>Pupariation</b>	0.0030	0.0034	0.0032	0.0032
<b>Total</b>	0.0016	0.0016	0.0016	0.0016

Table 7-2 Developmental rates of blow fly, *Ch. rufifacies* at 18 °C

Stages	Developmental rate at 18 °C			average
	Rap.1	Rap.2	Rap.3	
<b>Eggs</b>	0.0400	0.0396	0.0333	0.0376
<b>1<sup>st</sup> Instars</b>	0.0206	0.0189	0.0191	0.0195
<b>2<sup>nd</sup> Instars</b>	0.0188	0.0158	0.0241	0.0195
<b>3<sup>rd</sup> Instars</b>	0.0081	0.0078	0.0074	0.0078
<b>Pupariation</b>	0.0034	0.0035	0.0034	0.0035
<b>Total</b>	0.0018	0.0018	0.0018	0.0018

Table 7-3 Developmental rates of blow fly, *Ch. rufifacies* at 27 °C

Stages	Developmental rate at 27 °C (1/time)			average
	Rap.1	Rap.2	Rap.3	
<b>Eggs</b>	0.0871	0.0907	0.1193	0.0990
<b>1<sup>st</sup> Instars</b>	0.0524	0.0541	0.0497	0.0521
<b>2<sup>nd</sup> Instars</b>	0.0392	0.0392	0.0369	0.0384
<b>3<sup>rd</sup> Instars</b>	0.0254	0.0238	0.0210	0.0234
<b>Pupariation</b>	0.0084	0.0082	0.0046	0.0071
<b>Total</b>	0.0046	0.0046	0.0046	0.0046

Table 7-4 Developmental rates of blow fly, *Ch. rufifacies* at 30 °C

Stages	Developmental rate at 30 °C (1/time)			average
	Rap.1	Rap.2	Rap.3	
<b>Eggs</b>	0.0992	0.1000	0.1239	0.1077
<b>1<sup>st</sup> Instar</b>	0.0642	0.0624	0.0646	0.0637
<b>2<sup>nd</sup> Instar</b>	0.0472	0.0497	0.0546	0.0505
<b>3<sup>rd</sup> Instar</b>	0.0332	0.0319	0.0089	0.0247
<b>Pupariation</b>	0.0086	0.0096	0.0089	0.0090
<b>Total</b>	0.0051	0.0055	0.0053	0.0053

Table 7-5 Developmental rates of blow fly, *Ch. rufifacies* at 33 °C

Stages	Developmental rate at 33 °C (1/time)			average
	Rap.1	Rap.2	Rap.3	
<b>Eggs</b>	0.1241	0.1199	0.1189	0.1210
<b>1<sup>st</sup> Instar</b>	0.0805	0.0812	0.0830	0.0816
<b>2<sup>nd</sup> Instar</b>	0.0711	0.0701	0.0688	0.0700
<b>3<sup>rd</sup> Instar</b>	0.0317	0.0328	0.0356	0.0334
<b>Pupariation</b>	0.0098	0.0095	0.0099	0.0097
<b>Total</b>	0.0059	0.0058	0.0060	0.0059

Table 7-6 Developmental rates of blow fly, *Ch. rufifacies* at 36 °C

Stages	Developmental rate at 36 °C (1/time)			average
	Rap.1	Rap.2	Rap.3	
<b>Eggs</b>	0.1590	0.1538	0.1342	0.1490
<b>1<sup>st</sup> Instar</b>	0.0946	0.0983	0.0959	0.0963
<b>2<sup>nd</sup> Instar</b>	0.0748	0.0657	0.0808	0.0738
<b>3<sup>rd</sup> Instar</b>	0.0331	0.0366	0.0446	0.0381
<b>Pupariation</b>	0.0084	0.0087	0.0090	0.0087
<b>Total</b>	0.0056	0.0057	0.0061	0.0058

Table 7-7 Developmental rates of blow fly, *Ch. rufifacies* at 39 °C

<b>Stages</b>	<b>Developmental rate at 39 °C (1/Time)</b>			<b>average</b>
	<b>Rap.1</b>	<b>Rap.2</b>	<b>Rap.3</b>	
<b>Eggs</b>	0.1634	0.1634	0.1616	0.1628
<b>1<sup>st</sup> Instar</b>	0.0897	0.0897	0.0818	0.0871
<b>2<sup>nd</sup> Instar</b>	0.0829	0.0829	0.0827	0.0829
<b>3<sup>rd</sup> Instar</b>	0.0410	0.0410	0.0411	0.0410
<b>Pupariation</b>				
<b>Total</b>	0.0185	0.0185	0.0181	0.0184

**APPENDIX 4**  
**Data of Larval Sizes**

Table 8-1 The first replication lengths of blow fly larvae rearing at 15 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	1.5	1.5	1.5	1.5
3.0	1.5	1.5	1.5	1.5
6.0	1.5	1.5	1.5	1.5
9.0	-	-	-	-
12.0	2.0	2.0	2.0	2.0
15.0	2.5	2.5	2.5	2.5
18.0	2.5	2.5	2.5	2.5
21.0	2.5	2.5	3.0	2.5
24.0	2.5	2.5	2.5	2.5
27.0	3.0	3.0	3.0	3.0
30.0	-	-	-	-
33.0	-	-	-	-
36.0	3.0	3.0	3.0	3.0
39.0	3.5	3.5	3.5	3.5
42.0	4.0	4.0	4.0	4.0
45.0	4.0	4.0	4.0	4.0
48.0	-	-	-	-
51.0	4.0	4.0	4.0	3.5
54.0	4.0	4.0	4.0	4.0
57.0	-	-	-	-
60.0	4.5	4.5	4.0	4.0
63.0	4.5	4.0	4.0	4.0
66.0	4.5	4.5	4.0	4.0
69.0	4.5	4.5	4.0	4.0
72.0	5.0	5.0	5.0	5.5
75.0	5.0	5.0	5.0	5.0
78.0	5.0	5.0	5.0	5.5
81.0	-	-	-	-
84.0	-	-	-	-
87.0	5.5	5.5	5.5	5.0
90.0	6.0	6.0	5.5	5.5
93.0	5.5	5.5	5.5	5.5
96.0	5.5	5.5	6.0	6.0
99.0	6.0	6.0	6.0	6.0
102.0	6.0	6.0	6.0	6.0
105.0	-	-	-	-
108.0	7.0	6.5	6.0	6.0

Table 8-1 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
111.0	6.5	6.5	6.0	6.0
114.0	6.5	6.5	6.0	6.0
117.0	6.5	6.5	6.5	6.5
120.0	7.0	7.0	7.0	6.5
123.0	7.0	6.5	6.5	6.5
126.0	7.0	7.0	7.0	6.5
129.0	7.0	7.0	6.5	7.5
132.0	7.0	7.0	7.0	7.0
135.0	7.0	7.0	7.0	7.0
138.0	7.0	7.0	7.0	7.0
141.0	7.0	7.5	7.0	7.0
144.0	7.0	7.0	7.0	7.0
147.0	7.5	7.5	7.5	7.0
150.0	-	-	-	-
153.0	-	-	-	-
156.0	7.5	7.0	7.0	6.5
159.0	7.5	7.0	7.0	7.0
162.0	7.5	7.5	7.5	7.5
165.0	7.5	7.0	7.0	7.0
168.0	7.5	7.5	7.5	7.5
171.0	8.0	7.5	7.5	8.5
174.0	7.5	7.5	8.0	8.0
177.0	7.5	7.5	8.0	8.0
180.0	-	-	-	-
183.0	9.0	8.5	7.5	9.5
186.0	-	-	-	-
189.0	-	-	-	-
192.0	9.0	8.5	9.5	9.0
195.0	9.0	8.5	8.5	8.0
198.0	8.0	9.0	9.0	8.5
201.0	10.5	10.0	9.5	10.0
204.0	11.0	11.0	12.0	12.0
207.0	9.0	10.0	11.5	9.5
210.0	-	-	-	-
213.0	-	-	-	-
216.0	12.0	12.0	12.0	11.5
219.0	11.5	11.5	11.0	12.0

Table 8-1 (Cont.)

Developmental times (hours)	Larvae sizes (mm)			
	L1	L2	L3	L4
222.0	14.0	12.5	10.5	11.0
225.0	11.0	12.0	13.0	12.0
228.0	-	-	-	-
231.0	12.5	12.5	12.0	12.5
234.0	13.0	13.0	13.5	13.0
237.0	14.5	13.0	12.0	13.5
240.0	-	-	-	-
243.0	13.5	13.5	13.0	13.0
246.0	13.0	12.5	14.0	12.0
249.0	12.0	12.5	12.5	13.0
252.0	-	-	-	-
255.0	13.0	14.0	14.0	14.0
258.0	14.0	14.5	14.5	14.0
261.0	-	-	-	-
264.0	-	-	-	-
267.0	14.0	13.5	13.5	13.5
270.0	14.0	13.0	13.5	14.0
273.0	12.5	12.0	12.0	13.0
276.0	13.0	13.5	14.0	14.0

Table 8-2 The second replication lengths of blow fly larvae rearing at 15 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	2.0	2.0
3.0	2.0	2.0	2.0	2.0
6.0	2.0	2.0	2.0	2.0
9.0	2.5	2.5	2.5	2.5
12.0	-	-	-	-
15.0	2.5	2.5	3.0	3.0
18.0	2.5	2.5	2.5	2.5
21.0	-	-	-	-
24.0	3.0	3.0	3.0	3.0
27.0	3.5	3.5	3.0	3.0
30.0	-	-	-	-
33.0	-	-	-	-

Table 8-2 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
36.0	3.5	3.5	3.0	3.0
39.0	3.5	3.5	3.5	3.5
42.0	3.5	3.5	3.5	3.5
45.0	3.5	3.5	3.5	3.5
48.0	3.5	3.5	3.5	3.5
51.0	3.5	3.5	3.5	4.0
54.0	-	-	-	-
57.0	-	-	-	-
60.0	4.5	4.5	4.0	4.0
63.0	5.5	4.0	4.0	4.5
66.0	4.5	4.5	4.5	4.0
69.0	4.5	4.5	5.0	5.0
72.0	4.5	4.5	4.5	4.5
75.0	5.5	4.5	5.0	5.0
78.0	-	-	-	-
81.0	-	-	-	-
84.0	5.5	5.5	5.5	5.5
87.0	5.5	5.5	5.5	5.5
90.0	5.5	5.5	5.5	5.5
93.0	6.0	6.0	5.5	5.5
96.0	6.0	6.0	6.0	5.5
99.0	6.0	6.0	6.0	6.0
102.0	6.5	6.0	6.0	6.0
105.0	7.0	6.5	6.5	6.5
108.0	6.5	6.5	6.5	6.5
111.0	7.0	7.0	6.5	6.5
114.0	6.5	6.5	7.0	7.0
117.0	7.0	6.5	7.0	6.5
120.0	7.0	7.0	7.0	7.0
123.0	7.0	7.0	7.0	7.0
126.0	-	-	-	-
129.0	-	-	-	-
132.0	7.5	6.5	7.0	7.5
135.0	7.5	7.5	7.0	7.0
138.0	8.5	8.0	8.0	8.5
141.0	8.5	8.0	7.5	8.0
144.0	7.5	8.0	7.5	7.5

Table 8-2 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
147.0	8.0	7.0	8.5	8.0
150.0	-	-	-	-
153.0	-	-	-	-
156.0	10.0	9.0	9.0	8.5
159.0	8.5	8.5	10.0	9.0
162.0	8.0	8.5	9.0	10.0
165.0	10.0	10.0	11.0	9.5
168.0	10.0	10.0	10.5	9.5
171.0	11.0	11.0	11.5	10.0
174.0	-	-	-	-
177.0	13.0	11.5	11.0	11.5
180.0	10.5	10.5	10.0	9.0
183.0	10.0	11.5	11.5	11.0
186.0	12.0	12.0	11.0	11.0
189.0	11.5	11.5	10.5	11.5
192.0	-	-	-	-
195.0	12.5	11.0	12.5	13.0
198.0	12.5	12.5	12.0	12.0
201.0	-	-	-	-
204.0	12.0	12.5	13.0	11.5
207.0	13.0	12.5	12.0	11.0
210.0	14.0	14.0	12.5	14.0
213.0	14.5	14.0	14.0	13.0
216.0	-	-	-	-
219.0	13.0	13.0	12.5	12.0
222.0	13.0	12.5	12.0	13.0
225.0	13.0	12.0	13.0	13.0
228.0	14.0	13.0	13.0	13.0
231.0	13.5	13.0	13.0	13.0
234.0	14.0	13.5	12.5	13.5
237.0	14.0	14.0	14.0	14.0
240.0	-	-	-	-
243.0	15.5	14.5	14.5	13.0
246.0	13.5	13.5	13.0	14.0
249.0	14.5	13.0	12.0	11.5
252.0	14.0	14.0	14.0	14.5
255.0	13.0	12.0	14.0	14.0

Table 8-2 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
258.0	11.0	12.5	12.0	11.5
261.0	12.5	12.0	12.0	12.0
264.0	14.5	13.0	14.0	12.0
267.0	-	-	-	-
270.0	13.0	13.0	13.0	12.0
273.0	15.0	14.0	13.0	13.0
276.0	13.0	13.0	12.5	11.0

Table 8-3 The third replication lengths of blow fly larvae rearing at 15 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	2.0	2.0
3.0	2.0	2.0	2.0	2.0
6.0	2.0	2.0	2.0	2.0
9.0	2.0	2.0	2.0	2.0
12.0	-	-	-	-
15.0	2.5	2.5	2.5	2.5
18.0	2.5	2.5	2.5	3.0
21.0	-	-	-	-
24.0	3.0	3.0	3.0	3.0
27.0	3.0	3.0	3.0	3.0
30.0	-	-	-	-
33.0	-	-	-	-
36.0	3.5	3.5	3.0	3.0
39.0	3.5	3.5	3.5	3.5
42.0	3.5	3.5	3.5	4.0
45.0	3.5	3.5	3.5	3.5
48.0	3.5	3.5	3.5	4.0
51.0	3.5	4.0	3.5	3.5
54.0	-	-	-	-
57.0	-	-	-	-
60.0	4.0	4.0	4.0	4.0
63.0	4.0	4.5	4.0	4.0
66.0	4.0	4.0	5.0	4.5
69.0	4.5	4.5	4.5	4.5

Table 8-3 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
72.0	4.5	4.5	4.5	5.0
75.0	5.0	5.0	4.5	4.5
78.0	-	-	-	-
81.0	-	-	-	-
84.0	5.5	6.0	6.0	5.5
87.0	5.5	5.5	5.5	5.0
90.0	5.5	5.5	5.5	5.5
93.0	5.5	5.5	5.5	5.5
96.0	5.5	5.5	5.5	5.5
99.0	6.0	6.0	5.5	5.5
102.0	6.5	6.5	6.0	6.0
105.0	6.5	6.0	6.5	6.0
108.0	6.5	6.5	6.0	6.0
111.0	6.5	6.5	6.5	6.5
114.0	6.5	7.0	6.5	6.0
117.0	6.5	6.5	6.5	6.5
120.0	7.0	7.0	6.5	6.5
123.0	7.0	7.0	7.0	6.5
126.0	-	-	-	-
129.0	-	-	-	-
132.0	7.5	7.5	7.0	7.5
135.0	8.0	7.5	7.5	7.0
138.0	7.5	7.0	7.0	7.0
141.0	7.5	7.5	7.5	7.5
144.0	8.5	7.0	8.0	7.5
147.0	7.5	7.5	8.0	7.5
150.0	-	-	-	-
153.0	-	-	-	-
156.0	9.0	8.5	7.5	9.5
159.0	9.5	8.0	9.0	8.5
162.0	10.0	8.5	9.0	9.5
165.0	9.0	9.0	8.0	8.5
168.0	9.0	10.0	9.0	11.0
171.0	9.5	10.0	10.0	10.0
174.0	-	-	-	-
177.0	10.5	10.5	10.0	9.5

Table 8-3 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
180.0	9.5	10.0	9.0	9.5
183.0	10.5	10.0	10.5	10.0
186.0	10.5	10.5	10.5	9.5
189.0	12.0	12.0	11.5	11.0
192.0	-	-	-	-
195.0	11.5	11.5	11.5	12.0
198.0	12.5	12.5	11.5	11.5
201.0	-	-	-	-
204.0	13.0	12.5	12.0	11.0
207.0	12.5	11.0	13.0	13.0
210.0	13.0	14.0	12.5	13.0
213.0	13.0	12.5	12.5	13.0
216.0	-	-	-	-
219.0	12.0	14.0	13.5	13.0
222.0	13.5	13.0	13.0	13.0
225.0	14.5	13.0	13.5	14.0
228.0	14.5	13.5	14.0	12.5
231.0	13.0	14.0	13.0	13.0
234.0	14.5	13.5	14.0	13.5
237.0	14.5	13.5	14.0	12.5
240.0	-	-	-	-
243.0	14.5	13.0	14.0	14.0
246.0	15.0	13.0	14.0	14.0
249.0	14.5	14.5	13.5	14.0
252.0	13.0	13.5	13.5	14.0
255.0	13.0	13.5	12.5	13.0
258.0	13.5	11.5	12.0	12.0
261.0	13.0	11.5	12.5	12.0
264.0	13.0	14.0	11.0	12.5
267.0	-	-	-	-
270.0	13.0	14.5	13.5	14.0
273.0	13.0	13.0	10.0	12.5
276.0	12.5	12.0	12.5	13.0

Table 8-4 The first replication lengths of blow fly larvae rearing at 18 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	2.0	2.5
3.0	2.0	2.0	2.0	2.0
6.0	2.5	2.5	2.5	3.0
9.0	-	-	-	-
12.0	3.0	2.5	2.5	2.5
15.0	3.5	3.0	3.0	3.5
18.0	3.5	3.0	3.0	3.5
21.0	3.5	3.0	3.0	3.0
24.0	3.5	3.5	3.5	3.5
27.0	-	-	-	-
30.0	4.0	4.0	4.0	4.0
33.0	4.0	4.0	4.0	3.5
36.0	4.0	4.0	4.0	3.5
39.0	4.5	5.0	5.0	5.0
63.0	6.0	6.5	6.0	5.5
66.0	7.0	6.5	6.5	6.0
69.0	7.0	6.5	6.0	6.0
72.0	6.5	6.5	6.5	6.5
75.0	7.5	7.5	7.5	6.5
78.0	7.0	7.0	6.5	7.5
81.0	7.5	7.5	7.5	7.5
84.0	7.5	7.5	7.5	7.5
87.0	7.5	7.5	7.5	7.0
90.0	-	-	-	-
93.0	8.0	7.5	7.5	8.0
96.0	7.5	9.0	9.0	7.5
99.0	8.5	9.0	8.5	8.0
102.0	-	-	-	-
105.0	10.0	10.0	10.5	9.0
108.0	10.0	9.0	8.5	9.0
111.0	8.5	10.0	10.0	11.0
114.0	10.5	10.5	10.0	10.0

Table 8-4 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
117.0	12.0	10.0	10.0	10.0
120.0	11.0	11.0	10.0	10.0
123.0	11.5	11.5	11.5	11.0
126.0	-	-	-	-
129.0	13.0	13.5	13.0	13.0
132.0	14.0	12.0	12.5	13.5
135.0	13.5	14.0	12.5	13.5
138.0	14.0	13.0	14.0	13.5
141.0	13.0	14.0	13.5	13.5
144.0	-	-	-	-
147.0	-	-	-	-
150.0	15.0	14.5	15.0	15.5
153.0	15.5	14.5	14.0	15.0
156.0	14.5	15.0	15.0	15.0
159.0	14.5	14.0	15.0	14.0
162.0	14.0	13.5	14.0	14.0
165.0	16.0	15.0	15.0	14.5
168.0	15.0	14.0	15.0	14.0
171.0	14.5	15.0	14.5	14.5
174.0	-	-	-	-
177.0	13.0	13.5	13.0	15.0
180.0	12.0	14.5	14.0	14.0
183.0	15.0	14.0	14.5	15.0
186.0	13.0	14.5	15.5	14.5
189.0	14.0	14.0	14.0	12.0
192.0	13.5	13.5	13.0	14.0
195.0	8.0	13.5	11.5	11.0
198.0	15.5	15.0	17.0	15.0
201.0	16.0	15.5	15.0	16.0
204.0	14.0	14.5	13.5	14.5
207.0	10.0	14.0	11.0	13.0

Table 8-5 The second replication lengths of blow fly larvae rearing at 18 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	1.5	1.5	2.0	2.0
3.0	2.5	2.0	2.0	2.0
6.0	3.0	3.0	2.5	2.5
9.0	3.0	3.0	3.0	2.5
12.0	-	-	-	-
15.0	2.5	3.5	3.0	3.0
18.0	-	-	-	-
21.0	4.0	3.5	3.0	3.0
24.0	3.5	3.5	3.5	3.5
27.0	-	-	-	-
30.0	4.0	3.5	3.5	3.5
33.0	4.0	4.0	3.5	3.5
36.0	-	-	-	-
39.0	4.0	3.5	3.5	3.0
42.0	4.5	4.5	4.0	4.0
45.0	5.0	5.0	4.5	4.0
48.0	-	-	-	-
51.0	-	-	-	-
54.0	-	-	-	-
57.0	-	-	-	-
60.0	-	-	-	-
63.0	6.0	6.0	5.5	5.5
66.0	6.0	6.0	6.0	6.0
69.0	6.0	6.0	5.5	6.0
72.0	6.5	7.0	6.5	6.5
75.0	7.5	6.5	6.0	6.0
78.0	7.5	7.0	6.5	6.5
81.0	7.0	7.0	7.0	7.0
84.0	7.0	6.5	7.5	7.5
87.0	7.5	7.5	7.5	6.5
90.0	-	-	-	-
93.0	9.0	9.5	8.5	6.5
96.0	10.0	9.0	7.5	7.0
99.0	8.0	9.0	8.5	8.5
102.0	-	-	-	-
105.0	10.0	9.5	9.5	9.5
108.0	9.0	9.5	10.0	11.0

Table 8-5 (Cont.)

Developmental times (hours)	Larvae sizes (mm)			
	L1	L2	L3	L4
111.0	10.0	9.0	10.5	10.0
114.0	11.5	11.0	11.0	10.5
117.0	10.5	11.0	11.5	11.5
120.0	10.0	10.5	11.5	10.5
123.0	11.0	12.0	11.5	11.0
126.0	-	-	-	-
129.0	12.5	14.0	12.5	12.5
132.0	12.5	12.0	12.0	14.0
135.0	13.5	13.5	13.0	12.5
138.0	13.5	14.0	13.5	12.5
141.0	14.0	13.5	14.0	13.5
144.0	-	-	-	-
147.0	-	-	-	-
150.0	14.0	13.5	14.5	14.0
153.0	13.5	13.5	14.5	15.0
156.0	14.0	15.0	15.0	14.5
159.0	14.0	14.5	14.5	14.5
162.0	16.0	14.0	15.0	14.5
165.0	15.5	15.0	14.5	15.0
168.0	15.0	15.0	14.0	14.5
171.0	16.0	15.5	15.0	15.5
174.0	-	-	-	-
177.0	14.0	14.0	14.0	14.0
180.0	14.0	14.5	14.0	12.0
183.0	15.0	14.5	14.0	14.5
186.0	13.5	13.0	13.5	15.0
189.0	13.0	13.0	13.5	15.0
192.0	12.5	14.0	12.0	13.0
195.0	14.0	13.0	15.0	15.0
198.0	15.5	15.0	17.0	15.0
201.0	16.0	15.5	15.0	16.0
204.0	14.0	14.5	13.5	14.5
207.0	10.0	14.0	11.0	13.0

Table 8-6 The third replication lengths of blow fly larvae rearing at 18 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	1.5	1.5	2.0	2.0
3.0	2.5	2.0	2.0	2.0
6.0	3.0	3.0	2.5	2.5
9.0	3.0	3.0	3.0	2.5
12.0	-	-	-	-
15.0	2.5	3.5	3.0	3.0
18.0	-	-	-	-
21.0	3.0	3.0	2.5	2.5
24.0	3.0	3.0	3.0	3.0
27.0	3.0	3.0	3.0	3.0
30.0	3.5	3.5	3.5	3.0
33.0	4.0	4.0	3.5	3.0
36.0	3.5	3.5	3.5	3.5
39.0	-	-	-	-
42.0	4.0	3.5	4.0	4.0
45.0	4.0	4.0	4.0	4.0
48.0	4.0	4.5	4.0	4.0
51.0	-	-	-	-
54.0	4.5	4.5	4.5	4.0
57.0	5.0	5.0	4.5	5.0
60.0	-	-	-	-
63.0	5.0	5.0	4.5	4.0
66.0	5.5	5.5	6.0	5.0
69.0	6.0	5.5	5.5	5.5
72.0	5.5	6.0	6.0	6.0
75.0	6.0	6.0	6.0	7.0
78.0	6.5	6.5	6.5	7.0
81.0	7.0	7.0	7.0	7.0
84.0	7.5	7.5	7.0	7.0
87.0	-	-	-	-
90.0	-	-	-	-
93.0	8.0	8.0	7.5	7.0
96.0	8.0	8.0	7.5	8.0
99.0	8.0	8.0	8.0	8.0
102.0	8.0	8.0	8.0	8.0
105.0	9.0	8.5	9.0	9.0
108.0	-	-	-	-

Table 8-6 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
111.0	-	-	-	-
114.0	9.5	8.5	9.5	9.5
117.0	9.5	10.5	9.5	10.0
120.0	11.0	11.0	10.0	9.5
123.0	10.5	11.5	12.0	11.0
126.0	13.0	12.0	10.5	11.0
129.0	11.0	11.0	10.5	11.5
132.0	-	-	-	-
135.0	13.5	11.5	12.5	12.5
138.0	13.0	13.5	12.0	12.5
141.0	14.0	14.0	13.5	13.0
144.0	13.5	13.5	13.0	11.5
147.0	13.5	14.0	14.0	13.5
150.0	14.0	13.0	14.5	14.5
153.0	14.0	15.0	14.0	13.0
162.0	-	-	-	-
165.0	14.0	15.0	14.5	15.5
168.0	13.5	14.0	14.0	13.5
171.0	15.0	15.0	15.0	13.0
174.0	14.0	13.0	13.0	13.5
177.0	13.0	13.0	15.0	14.0
180.0	-	-	-	-
183.0	-	-	-	-
186.0	11.0	11.0	12.0	12.0
189.0	13.0	12.5	14.0	13.0
192.0	14.0	14.0	13.0	13.0

Table 8-6 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
195.0	-	-	-	-
198.0	15.5	15.0	17.0	15.0
201.0	16.0	15.5	15.0	16.0
204.0	14.0	14.5	13.5	14.5
207.0	10.0	14.0	11.0	13.0

Table 8-7 The first replication lengths of blow fly larvae rearing at 27 °C

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
0.0	2.0	1.8	1.9
3.0	-	-	-
6.0	2.7	2.8	2.1
9.0	-	-	-
12.0	-	-	-
15.0	4.3	3.7	3.4
18.0	3.3	3.9	2.9
21.0	-	-	-
24.0	-	-	-
27.0	-	-	-
30.0	-	-	-
33.0	7.2	7.3	7.5
36.0	-	-	-
39.0	-	-	-
42.0	8.3	7.6	8.2
45.0	8.5	7.2	9.5
48.0	-	-	-
51.0	-	-	-
54.0	-	-	-
57.0	12.0	12.7	12.0
60.0	-	-	-
63.0	-	-	-
66.0	-	-	-
69.0	-	-	-
72.0	-	-	-
75.0	13.5	13.2	14.5

Table 8-8 The second replication lengths of blow fly larvae rearing at 27 °C

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
0.0	2.0	1.8	1.9
3.0	-	-	-
6.0	2.9	2.4	2.5
9.0	-	-	-
12.0	-	-	-
15.0	3.0	2.9	2.6
15.0	3.0	2.9	2.6
18.0	4.3	4.2	4.2
21.0	-	-	-
24.0	-	-	-
27.0	-	-	-
30.0	-	-	-
33.0	7.0	7.1	6.5
36.0	-	-	-
39.0	-	-	-
42.0	7.5	7.2	7.8
45.0	8.0	8.7	8.0
48.0	-	-	-
51.0	-	-	-
54.0	-	-	-
57.0	13.0	12.8	13.6
60.0	-	-	-
63.0	-	-	-
66.0	-	-	-
69.0	-	-	-
72.0	-	-	-
75.0	12.2	13.0	12.5

Table 8-9 The third replication lengths of blow fly larvae rearing at 27 °C

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
0.0	1.9	1.8	1.5
3.0	-	-	-
6.0	2.9	2.2	2.6
9.0	-	-	-
12.0	-	-	-
15.0	3.7	3.8	3.5
18.0	4.4	4.2	4.3
21.0	-	-	-
24.0	-	-	-
27.0	-	-	-
30.0	-	-	-
33.0	7.5	6.0	6.9
36.0	-	-	-
39.0	-	-	-
42.0	6.3	7.8	7.2
45.0	9.0	8.1	9.0
48.0	-	-	-
51.0	-	-	-
54.0	-	-	-
57.0	11.5	12.6	11.0
60.0	-	-	-
63.0	-	-	-
66.0	-	-	-
69.0	-	-	-
72.0	-	-	-
75.0	13.2	13.5	13.3

Table 8-10 The first replication lengths of blow fly larvae rearing at 30 °C

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
0.0	2.0	2.1	2.0
3.0	-	-	-
6.0	-	-	-
9.0	3.0	2.9	2.8
12.0	-	-	-
15.0	3.1	3.5	3.2
18.0	-	-	-
21.0	-	-	-
24.0	5.0	5.5	5.1
27.0	-	-	-
30.0	-	-	-
33.0	8.0	8.1	8.1
36.0	8.5	7.5	8.0
39.0	-	-	-
42.0	-	-	-
45.0	11.5	11.5	12.5
48.0	-	-	-
51.0	14.5	15.0	13.5
54.0	16.0	16.0	14.5
57.0	-	-	-
60.0	15.0	14.0	15.0

Table 8-11 The second replication lengths of blow fly larvae rearing at 30 °C

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
0.0	2.0	2.0	1.9
3.0	-	-	-
6.0	-	-	-
9.0	3.0	2.9	3.0

Table 8-11 (Cont.)

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
12.0	-	-	-
15.0	4.0	3.5	4.0
18.0	-	-	-
21.0	-	-	-
24.0	6.0	6.0	5.5
27.0	-	-	-
30.0	-	-	-
33.0	7.5	7.5	8.0
36.0	9.0	8.0	8.5
39.0	-	-	-
42.0	-	-	-
45.0	13.5	11.0	11.0
48.0	-	-	-
51.0	14.5	16.0	13.0
54.0	14.0	13.5	13.0
57.0	-	-	-
60.0	14.0	13.5	13.0

Table 8-12 The third replication lengths of blow fly larvae rearing at 30 °C

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
0.0	1.8	1.9	2.0
3.0	-	-	-
6.0	-	-	-
9.0	2.5	2.9	2.9
12.0	-	-	-
15.0	4.0	3.5	4.0
18.0	-	-	-

Table 8-12 (Cont.)

Developmental times (hours)	Larval sizes (mm)		
	L1	L2	L3
21.0	-	-	-
24.0	5.5	6.0	6.0
27.0	-	-	-
30.0	-	-	-
33.0	8.5	8.0	8.0
36.0	9.0	9.5	8.5
39.0	-	-	-
42.0	-	-	-
45.0	12.5	14.0	14.0
48.0	-	-	-
51.0	15.0	14.0	14.0
54.0	16.5	16.5	16.0
57.0	-	-	-
60.0	16.5	16.5	16.0

Table 8-13 The first replication lengths of blow fly larvae rearing at 33 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	1.5	1.5	2.0
3.0	3.0	2.5	2.5	2.0
6.0	3.5	3.0	3.0	2.5
9.0	3.5	3.0	3.0	3.0
12.0	4.0	4.0	4.0	3.5
15.0	5.0	4.5	4.5	5.0
18.0	5.0	4.5	4.5	4.5
21.0	6.0	6.0	5.5	5.0
24.0	7.0	7.5	7.0	7.5
27.0	-	-	-	-

Table 8-13 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
30.0	-	-	-	-
33.0	9.5	9.0	10.0	10.0
36.0	11.0	10.5	11.0	10.5
39.0	11.0	12.0	11.5	10.0
42.0	14.0	13.0	14.0	14.0
45.0	14.0	14.0	14.0	13.5
48.0	14.5	15.0	15.0	15.0
51.0	16.0	17.0	15.5	16.0
54.0	17.0	16.5	16.5	16.0
57.0	15.0	14.5	15.0	14.0
60.0	14.0	13.5	13.5	13.0
63.0	13.5	14.0	14.0	14.0
66.0	12.0	11.0	10.0	12.0
69.0	12.5	11.0	9.0	11.0

Table 8-14 The second replication lengths of blow fly larvae rearing at 33 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	1.5	1.5	1.5	2.0
3.0	2.5	2.5	2.5	3.0
6.0	2.5	2.5	3.0	3.0
9.0	4.5	4.0	4.5	4.5
12.0	5.5	5.0	5.0	5.0
15.0	6.0	6.0	6.0	6.0

Table 8-14 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
18.0	6.0	6.0	5.5	6.0
21.0	6.0	7.0	6.5	6.5
24.0	7.0	7.0	7.0	7.5
27.0	8.0	8.0	8.5	8.0
30.0	9.0	9.5	9.0	9.0
33.0	9.5	9.0	10.0	10.0
36.0	10.0	11.0	9.5	9.5
39.0	11.0	10.5	12.0	11.0
42.0	12.0	12.0	12.5	12.5
45.0	13.5	13.0	12.5	13.0
48.0	14.0	14.5	14.0	14.0
51.0	16.0	15.0	15.0	15.0
54.0	17.0	16.5	16.5	16.0
57.0	15.5	15.5	15.0	15.5
60.0	16.0	14.5	15.0	15.0
63.0	14.0	13.5	14.0	13.0
66.0	12.0	11.0	10.0	12.0
69.0	12.5	11.0	9.0	11.0

Table 8-15 The third replication lengths of blow fly larvae rearing at 33 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	2.0	1.5
3.0	2.5	2.5	3.0	2.5
6.0	3.0	3.0	3.0	3.0
9.0	3.5	3.5	3.5	3.5

Table 8-15 (Cont.)

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
12.0	4.0	4.5	4.0	4.5
15.0	5.5	5.0	5.0	5.5
18.0	6.0	6.0	5.5	6.5
21.0	7.0	7.0	6.5	6.0
24.0	8.0	8.5	7.5	8.0
27.0	8.0	9.0	8.5	8.5
30.0	10.5	9.5	8.0	9.5
33.0	10.0	11.0	11.0	10.5
36.0	11.5	12.0	12.0	11.0
39.0	13.5	13.0	11.0	13.0
42.0	12.5	13.0	13.5	13.0
45.0	14.0	15.0	13.5	14.0
48.0	14.0	15.0	14.0	14.0
51.0	15.0	15.0	15.0	15.0
54.0	16.5	15.5	16.0	16.5
57.0	14.0	14.0	14.0	14.0
60.0	14.0	15.5	15.0	14.5
63.0	13.5	14.0	14.0	14.0
66.0	12.0	11.0	10.0	12.0
69.0	12.5	11.0	9.0	11.0

Table 8-16 The first replication lengths of blow fly larvae rearing at 36 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	2.0	2.0
3.0	2.5	2.5	2.5	2.5
6.0	3.0	3.0	3.5	3.5
9.0	4.0	3.5	3.5	3.5
12.0	4.5	5.0	5.0	5.0
15.0	5.5	5.0	5.5	4.5
18.0	6.0	6.0	5.5	5.5
21.0	7.5	6.0	6.0	6.5
24.0	10.0	9.0	9.0	8.5
27.0	9.0	9.0	8.5	9.0
30.0	10.5	10.5	11.0	10.5
33.0	11.5	13.0	11.0	10.5
36.0	13.0	14.0	13.0	13.5
39.0	14.5	14.5	15.0	14.0
42.0	14.0	15.5	15.0	14.5
45.0	15.0	15.0	14.5	15.0
48.0	16.0	17.0	16.0	15.0
51.0	16.0	17.0	16.0	15.0
54.0	16.0	15.0	15.0	16.0
57.0	13.0	14.5	12.0	12.5
60.0	13.0	13.5	12.0	13.0

Table 8-17 The second replication lengths of blow fly larvae rearing at 36 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	2.0	2.0
3.0	2.0	2.0	2.0	2.5
6.0	3.0	3.0	3.0	2.5
9.0	3.5	3.5	3.0	3.0
12.0	4.5	4.5	4.0	4.0
15.0	4.5	4.5	4.5	4.5
18.0	6.0	5.0	6.0	6.0
21.0	5.5	5.5	6.0	6.0
24.0	6.5	6.5	6.0	6.5
27.0	7.0	7.5	7.5	7.5
30.0	-	-	-	-
33.0	10.5	10.5	11.0	10.0
36.0	11.5	12.5	12.0	12.0
39.0	15.0	13.0	13.5	13.0
42.0	14.0	15.0	14.0	14.0
45.0	16.0	15.0	15.0	15.0
48.0	15.0	15.0	16.0	15.0
51.0	15.0	14.0	14.5	15.5
54.0	14.5	15.0	14.5	14.5
57.0	13.0	14.0	13.5	14.5
60.0	10.5	13.0	12.5	12.0

Table 8-18 The third replication lengths of blow fly larvae rearing at 36 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	1.5	1.5	2.5
3.0	2.5	2.5	2.5	2.0
6.0	2.5	2.5	2.5	2.5
9.0	3.5	3.5	3.0	3.0
12.0	4.5	4.5	4.5	4.0
15.0	6.0	6.0	6.5	6.0
18.0	6.5	6.5	6.5	6.5
21.0	7.0	7.0	6.0	6.5
24.0	9.0	8.5	8.5	8.5
27.0	9.0	7.5	8.0	8.0
30.0	12.0	11.0	10.5	11.5
33.0	11.5	11.0	12.0	12.0
36.0	13.0	12.0	11.5	12.5
39.0	13.5	12.5	13.0	13.5
42.0	14.0	14.0	15.0	15.0
45.0	15.0	14.0	14.5	15.0
48.0	14.0	16.0	15.0	15.0
51.0	16.0	15.0	15.0	14.0
54.0	14.5	15.0	14.0	14.0
57.0	12.0	14.0	11.0	12.5
60.0	11.5	10.0	10.5	13.0

Table 8-19 The first replication lengths of blow fly larvae rearing at 39 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	1.5	2.0	2.0	2.0
3.0	2.5	2.0	2.0	2.5
6.0	3.0	2.5	2.5	2.0
9.0	3.5	3.5	3.0	3.0
12.0	4.0	4.0	4.0	4.5
15.0	6.0	6.0	5.5	5.5
18.0	6.0	6.0	6.0	6.0
21.0	7.0	7.0	6.5	7.0
24.0	9.0	9.5	9.0	8.0
27.0	10.5	10.5	11.0	11.0
30.0	12.0	13.0	12.0	12.0
33.0	14.0	13.0	14.0	13.0
36.0	14.5	14.5	15.5	15.5
39.0	17.0	16.0	17.0	16.0
42.0	16.0	15.5	16.0	16.0
45.0	14.5	15.0	15.0	15.0

Table 8-20 The second replication lengths of blow fly larvae rearing at 39 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	1.5	2.0
3.0	2.5	2.5	2.5	2.5
6.0	3.0	3.0	3.0	3.0
9.0	3.5	3.5	3.5	3.5
12.0	5.0	5.0	5.0	5.0
15.0	6.0	5.5	6.0	6.0
18.0	6.5	6.5	6.0	6.0
21.0	7.0	7.0	7.0	7.0

Table 8-20 (Cont.)

Developmental times(hours)	Larval sizes (mm)			
	L1	L2	L3	L4
24.0	9.0	9.0	9.0	8.5
27.0	10.5	10.0	9.0	9.0
30.0	11.0	11.0	10.5	11.0
33.0	11.5	12.0	13.0	13.0
36.0	14.5	13.5	13.0	13.5
39.0	15.0	15.0	14.0	15.5
42.0	16.0	14.5	16.0	14.5
45.0	13.0	13.0	13.5	14.0

Table 8-21 The third replication lengths of blow fly larvae rearing at 39 °C

Developmental times (hours)	Larval sizes (mm)			
	L1	L2	L3	L4
0.0	2.0	2.0	1.5	2.0
3.0	2.5	2.5	2.5	2.5
6.0	3.0	3.0	3.0	2.5
9.0	3.5	3.0	4.0	3.0
12.0	4.5	4.5	4.0	4.0
15.0	5.5	5.5	5.0	5.0
18.0	6.5	6.5	6.0	6.0
21.0	6.5	7.0	7.0	7.0
24.0	7.5	7.0	8.0	8.5
27.0	9.5	10.0	10.0	10.5
30.0	13.0	12.0	13.5	11.5
33.0	14.0	13.0	13.5	12.5
36.0	14.5	14.0	13.5	14.0
39.0	14.4	16.0	16.0	15.0
42.0	14.0	15.0	15.0	13.0
45.0	14.0	14.0	14.0	14.0

## **APPENDIX 5**

### **Picture of Larvae at Seven Temperatures**

Table 9-1 Blow fly, (*Ch. rufifacies*) larvae rearing at 15 °C with the age of 0- 276 hours


















Time (hours)	Larval number			
	1	2	3	4
0				
9				
12				
15				
18				
21				
24				
27				

Table 9-1 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
36				
39				
42				
45				
48				
51				
60				
63				

Table 9-1 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
66				
69				
72				
75				
78				
84				
87				
90				

Table 9-1 (Cont.)









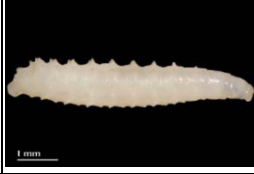























Time (hours)	Larval number			
	1	2	3	4
93				
96				
99				
102				
105				
108				
111				
114				

Table 9-1 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
117				
120				
123				
126				
129				
132				
135				
138				

Table 9-1 (Cont.)














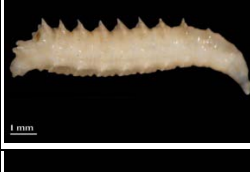





Time (hours)	Larval number			
	1	2	3	4
141	 1 mm	 1 mm	 1 mm	 1 mm
144	 1 mm	 1 mm	 1 mm	 1 mm
147	 1 mm	 1 mm	 1 mm	 1 mm
156	 1 mm	 1 mm	 1 mm	 1 mm
159	 1 mm	 1 mm	 1 mm	 1 mm
162	 1 mm	 1 mm	 1 mm	 1 mm
165	 1 mm	 1 mm	 1 mm	 1 mm
168	 1 mm	 1 mm	 1 mm	 1 mm

Table 9-1 (Cont.)
























Time (hours)	Larval number			
	1	2	3	4
171				
174				
177				
180				
183				
186				
189				
192				

Table 9-1 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
195				
198				
201				
204				
207				
210				
213				
216				

Table 9-1 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
219				
222				
225				
228				
231				
234				
237				
243				

Table 9-1 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
246				
249				
252				
255				
258				
261				
264				
270				

Table 9-1 (Cont.)









Time (hours)	Larval number			
	1	2	3	4
273				
276				

Table 9-2 Blow fly, (*Ch. rufifacies*) larvae rearing at 18 °C with the age of 0- 195 hours





















Time (hours)	Larval number			
	1	2	3	4
0				
3				
6				
12				
15				

Table 9-2 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
18				
21				
24				
30				
33				
36				
39				
63				

Table 9-2 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
66				
69				
72				
75				
78				
81				
84				
87				

Table 9-2 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
93				
96				
99				
105				
108				
111				
114				
117				

Table 9-2 (Cont.)



















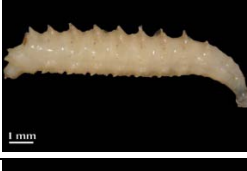
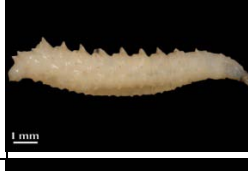












Time (hours)	Larval number			
	1	2	3	4
120				
123				
129				
132				
135				
138				
141				
150				

Table 9-2 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
153				
156				
159				
162				
165				
168				
171				
177				

Table 9-2 (Cont.)




















Time (hours)	Larval number			
	1	2	3	4
180	 1 mm	 1 mm	 1 mm	 1 mm
183	 1 mm	 1 mm	 1 mm	 1 mm
186	 1 mm	 1 mm	 1 mm	 1 mm
189	 1 mm	 1 mm	 1 mm	 1 mm
192	 1 mm	 1 mm	 1 mm	 1 mm
195	 1 mm	 1 mm	 1 mm	 1 mm

Table 9-3 Blow fly, (*Ch. rufifacies*) larvae rearing at 27 °C with the age of 0- 75 hours












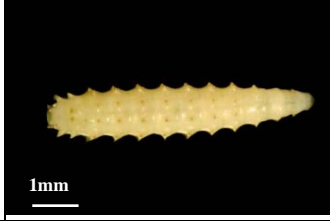






Time (hours)	Larval number		
	1	2	3
0			
6			
15			
18			
33			
42			

Table 9-3 (Cont.)










Time (hours)	Larval number		
	1	2	3
45			
57			
75			

Table 9-4 Blow fly, (*Ch. rufifacies*) larvae rearing at 30 °C with the age of 0- 60 hours







Time (hours)	Larval number		
	1	2	3
0			
9			

Table 9-4 (Cont.)



















Time (hours)	Larval number		
	1	2	3
15	 1 mm	 1 mm	 1 mm
24	 1 mm	 1 mm	 1 mm
33	 1 mm	 1 mm	 1 mm
36	 1 mm	 1 mm	 1 mm
45	 1 mm	 1 mm	 1 mm
51	 1 mm	 1 mm	 1 mm

Table 9-4 (Cont.)







Time (hours)	Larval number		
	1	2	3
54			
60			

Table 9-5 Blow fly, (*Ch. rufifacies*) larvae rearing at 33 °C with the age of 0- 72 hours

















Time (hours)	Larval number			
	1	2	3	4
0				
3				
6				
9				

Table 9-5 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
12				
15				
18				
21				
24				
27				
30				
33				

Table 9-5 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
36				
39				
42				
45				
48				
51				
54				
57				

Table 9-5 (Cont.)





















Time (hours)	Larval number			
	1	2	3	4
60				
63				
66				
69				
72				

Table 9-6 Blow fly, (*Ch. rufifacies*) larvae rearing at 36 °C with the age of 0- 60 hours









Time (hours)	Larval number			
	1	2	3	4
0				
3				

Table 9-6 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
6				
9				
12				
15				
18				
21				
24				
27				

Table 9-6 (Cont.)

































Time (hours)	Larval number			
	1	2	3	4
27				
30				
33				
36				
39				
42				
45				
48				

Table 9-6 (Cont.)

















Time (hours)	Larval number			
	1	2	3	4
51				
54				
57				
60				

Table 9-7 Blow fly, (*Ch. rufifacies*) larvae rearing at 39 °C with the age of 0- 45 hours









Time (hours)	Larval number			
	1	2	3	4
0				
3				

Table 9-7 (Cont.)





















































Time (hours)	Larval number			
	1	2	3	4
6				
9				
12				
15				
18				
21				
24				
27				

Table 9-7 (Cont.)

Time (hours)	Larval number			
	1	2	3	4
30				
33				
36				
39				
42				
45	