EFFECTS OF LARVAL DENSITY AND FEED FORMULATION ON DEVELOPMENT AND NUTRITIONAL VALUE OF *Megaselia scalaris* (LOEW) (DIPTERA: PHORIDAE)

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by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
°C	Degree Celcius
CF	Chicken Feed
cm	Centimetre
FP	Fish Pellet
g	Gram
kcal	Kilocalorie
kg	Kilogram
kJ	Kilojoule
mg	Milligram
ml	Millilitre
mm	Millimetre
MP	Mouse Pellet
v	Volume
w	Weight
W_x	x % Water Content

KESAN DENSITI LARVA DAN FORMULASI MAKANAN TERHADAP PERKEMBANGAN DAN NILAI NUTRISI *Megaselia scalaris* (LOEW) (DIPTERA: PHORIDAE)

ABSTRAK

Oleh sebab nilai economi sarang burung yang tinggi, burung walit diternak secara ekstensif dalam rumah burung walit buatan manusia untuk meningkatkan penghasilan sarang burung. *Megaselia scalaris* (Diptera) merupakan satu makanan yang berpotensi kepada burung walit dan dapat dihasilka secar besar-besaran. Walau bagaimananpun, untuk menghasilkan serangga secara besar-besaran, kawalan kualiti dan faktor yang memberi kesan kepada pertumbuhan serangga tersebut perlu dikaji. Dalam penyelidikan ini, kesan densiti larva dan formulasi makanan terhadap perkembangan larva, pupa dan dewasa *M. scalaris* telah dikaji dengan mengolah kandungan air dalam makanan, jenis makanan dan densiti larva yang berbeza. Kadar pertumbuhan larva, kadar kelangungan hidup pupa, keberatan pupa, kadar kemunculan dewasa dan saiz dewasa diperolehi dan dianalisis. Sampel larva, pupa dan dewasa yang dipelihara dalam tiga jenis makanan dianalisis untuk memperolehi kandungan protein mentah, lemak mentah, tenaga dan mineral sampel. Kandungan air dalam makanan mempengaruhi kadar pertumbuhan larva ($F= 41.44$; $df= 5, 64$; $p< 0.05$), kadar kelangsungan hidup pupa ($F= 10.89$; $df= 5, 64$; $p< 0.05$), berat pupa ($F= 1.08$; $df= 5, 64$; $p< 0.05$) dan kemunculan *M. scalaris* dewasa ($F= 12.62$; $df= 5, 64$; $p< 0.05$) dengan signifikan. Kadar pertumbuhan larva yang tinggi, kadar kelangsungan hidup pupa yang tinggi, saiz jantan dewasa yang besar diperhatikan semasa larva dipelihara dalam makanan yang mempunyai 60% kandungan air. Pupa yang berat dan saiz betina yang besar diperolehi daripada larva yang diberi makanan yang mempunyai 90% kandungan air. Antara tiga jenis makanan yang dikaji, pelet ikan memaparkan tempoh masa pertumbuhan larva yang singkat, kadar kelangsungan hidup pupa dan kadar kemunculan dewasa yang tinggi dan saiz lalat dewasa yang besar berbanding dengan pelet ayam dan tikus. Interaksi antara kandungan air dan jenis makanan adalah signifikan. Densiti larva yang kurang daripada 100 larva, dipelihara dalam 5g makanan merupakan ratio yang terbaik. Keputusan ini menunjukkan tempoh masa pertumbuhan larva yang paling singkat (4.02 ± 0.054 hari), berat pupa yang tertinggi (3.57 ± 0.090 mg) dan saiz lalat dewasa jantan dan betina yang terbesar (0.698 ± 0.004 mm dan 0.830 ± 0.003 mm masing-masing). Dewasa *M. scalaris* mengumpul nutrisi lebih baik apabila kandungan protein mentah yang tertinggi direkodkan daripada pelet makanan ayam, pelet makanan ikan dan pelet makanan tikus (66.10 ± 1.21 %, 63.99 ± 1.23 % dan 69.93 ± 1.00 % masing-masing) berbanding dengan larva dan pupa. Kandungan lemak mentah (31.94 ± 0.47 %, 32.85 ± 1.29 % dan 21.59 ± 2.95 % masing-masing) dan tenaga kasar (24.00 ± 0.18 kJ/g, 23.97 ± 0.36 kJ/g dan 22.32 ± 0.47 kJ/g masing-masing) adalah tertinggi dalam peringkat larva.

EFFECTS OF LARVAL DENSITY AND FEED FORMULATION ON DEVELOPMENT AND NUTRITIONAL VALUE OF *Megaselia scalaris* (LOEW) (DIPTERA: PHORIDAE)

ABSTRACT

Due to the high economic value of edible bird nest, swiftlets are being reared extensively in man-made houses to increase the production of edible bird nest. *Megaselia scalaris* (Diptera) has become a potential diet for swiftlet and could be mass produced. However, quality control and factors that affect the development of insect for mass production should be studied. In this research, the influence of larval density and feed formulation on the larval, pupal and adult development of *M. scalaris* was tested by introducing different water content in the feed, type of feed and larval density as treatments. Larval developmental time, pupal recovery, pupal weight, adult emergence and adult size were analysed. Sample of larvae, pupae and adults reared from different feeds were analysed to obtain crude protein, crude fat, energy and mineral of the samples. Water content of diet significantly affected the larval developmental time ($F = 41.44$; $df = 5, 64$; $p < 0.05$), pupal recovery ($F = 10.89$; $df = 5, 64$; $p < 0.05$), pupal weight ($F = 1.08$; $df = 5, 64$; $p < 0.05$) and adult emergence ($F = 12.62$; $df = 5, 64$; $p < 0.05$). Shorter larval developmental time, higher pupal recovery and bigger male adult size were observed when the larvae were reared in feed with 60% water content. Highest pupal weight and female adult size were recorded from the larvae that reared on feed containing 90% water. Shorter larval developmental time, higher pupal recovery, pupal weight, adult emergence and adult size were recorded on fish pellet compared to chicken and mouse pellets. Interaction between the water content and types of feed was significant. Larval density less than 100 larvae was reared in 5g feed was the best feeding ration. It showed shortest larval developmental time (4.02 ± 0.054 days), produced heaviest pupae (3.57 ± 0.090 mg) and the largest male and female adults (0.698 ± 0.004 mm and 0.830 ± 0.003 mm respectively). Studies on nutritional values between larvae, pupae and adults of *M. scalaris* showed highest crude protein content was found in adults from chicken feed, fish pellet and mouse pellet (66.10 ± 1.21 %, 63.99 ± 1.23 % and 69.93 ± 1.00 % respectively) compared to larvae and pupae. Crude fat content (31.94 ± 0.47 %, 32.85 ± 1.29 % and 21.59 ± 2.95 % respectively) and also gross energy (24.00 ± 0.18 kJ/g, 23.97 ± 0.36 kJ/g and 22.32 ± 0.47 kJ/g respectively) were the highest in larval stage from chicken, fish and mouse pellet.

CHAPTER ONE

GENERAL INTRODUCTION

Edible bird nest production has been growing since last few decades. In the past, humans harvested bird nests from the caves for human consumption. Nowadays, swiftlet houses were established and modified the interior condition into cave-like environment to attract the swiftlets to build their nests inside. With human's innovations and modernisation, captive breeding and domestication of swiftlet has been introduced to increase the swiftlet population and edible bird nests production. Recently, there have been efforts in building houses that simulate the environment of caves in to increase swiftlet population. Some farmers domesticate swiftlets through translocation of swiftlet eggs from cave, hand-raising them and return them to the cave. However, a large quantity of insects is needed to feed the captive chicks and release for the wild swiflets. Thus, mass production of *M. scalaris* may be a suitable approach since this species is found to be a main diet for swiftlets (Kamarudin & Khoo, 2011).

Insects are mass produced for some purposes. For instance, sterile insects are mass-reared in large scale upon releasing to natural population to control the population of the insect (Parker, 2005). Anderson & Leppa (1992) and Ochieng'-Odero (1994) had reared a few insect species for integrated pest management (IPM). Black dump fly (*Hydrotaea anescens*) had been mass reared to control house fly population in swine and poultry farms (Turner *et al.*, 1992; Hogsette & Washington, 1995). Hence, they can be mass produced but this would require a quality control to monitor the performance of mass reared insects. The quality control needs routine check on the development of insect such as pupal weight, adult emergence and size (Resilva *et al*., 2007).

Megaselia scalaris (Loew) (Diptera: Phoridae) is a small-bodied Diptera that is commonly found in the tropical and subtropical areas. It looks similar to *Drosophila melanogaster* but it has distinctive characteristics that differentiate itself from *D. melanogaster*. *Megaselia scalaris* is also known as 'scuttle fly' because of its locomotive behaviour. The adult moves in a fast burst with short rests in between (Douglas, 2003). It is a very small insect and capable of penetrating through or from tightly closed containers (Disney, 2008). Douglas (2003) mentioned that *M. scalaris* fed on a large variety of food sources. As they are omnivores, they usually feed on nectar to obtain energy (Sukontason *et al.*, 2003). They can also feed on cornmeal-based diet, plants, animal carcasses and faeces (Douglas, 2003; Idris, 2001).

The life cyle of *M. scalaris* is short and varies with different temperatures (Disney, 2008). The mean developmental time at 27°C and 75% relative humidity, for egg, larva, pupa and adult of *M. scalaris* are 14 hours, four to six days, nine days and two to three days respectively. *Megaselia scalaris* requires approximately 14-16 days to complete one life cycle at 27°C. At 22°C, the life cycle completes in 22 days, whereas 11.1 days at 29°C (Greenberg & Wells, 1998). From these review, *M. scalaris* can be said easy to culture in laboratory. It can also be a potential candidate to be served as one of the swiftlet's food source, as swiftlets might have encountered insufficient food supply due to deforestation and unrestrained pesticide usage in forest areas.

There are many factors affecting the development of *M. scalaris* such as larval crowding within a quantity of diet, types of diets and water content of the diet (David *et al.*, 1995; Sarah, 2005; Sukarsih *et al.*, 1988). Therefore, experiments should be carried out to investigate the effects of those factors on the development of insect, which may be necessary for a successful mass production. Proximate analysis of different stages of this insect would determine their nutritional value. The results generated from these experiments could be useful information for mass production of this insect.

Therefore, the objectives of this study are：

i. to investigate the effects of feed formulation and larval crowding on the development of *M. scalaris* in different feeds.

ii. to investigate the nutritional value of *M. scalaris* produced from different feeds.

CHAPTER TWO

LITERATURE REVIEW

2.1 Edible bird nest industry

Edible bird nest was found to be an important medication cuisine in China since the 16^{th} century. Over the 18^{th} and 19^{th} century, bird nest was imported in a great volume into China each year. Because of high demanding, this industry was thus becoming a business invest with minimal capital but can generates great income in future (Ibrahim, 2009).

2.1.1 Nutritional values of edible bird nest

The major contents of edible bird nest are glycoprotein and carbohydrate (Kathan & Week, 1969). The other nutrient components found are lipid, ash and some trace elements such as calcium, sodium, magnesium, zinc, manganese and iron (Marcone, 2005). Kathan and Week (1969) reported that high content of crude protein is found in the bird nest, i.e., 32.3%. Marcone (2005) reported 62-63% of crude protein in both white and red "blood" nests. Carbohydrate in edible bird nests constitutes of sialic acid and glucosamine (Tung *et al.*, 2008). The function of sialic acid is to enhance the brain function of infants (Colombo *et al.*, 2003). From several studies, glucosamine was found posing an effect on hip osteoarthritis (Bruyere *et al.*, 2008; Rozendaal *et al.*, 2008). Moreover, non-essential (aspartic acid, glutamic acid, and proline) and essential amino acids (threonine and valine) were found in edible bird nest by Kathan and Weeks (1969). These authors then mentioned that these amino acids help in repairing and providing immunity.

2.1.2 Issues of edible bird nest industry

Edible bird nest industry is a high-income industry because the nest was once sold at USD 1600 per kilogram. Koon and Cranbrook (2002) reported that the white nests and "red-blood" were retailed for USD 2000 to USD 10000 per kilogram. Until the most recent, the unprocessed nests can be sold at RM8000 to RM12000 per kilogram whereas, the cleaned nests were sold at RM12000 to RM24000 in international market. The price was so expensive because of the nests harvesting method. During breeding seasons, the collectors will have to climb up on the cave walls where the nests are built hundred feet on top. The collectors require a scaffolding which is made of bamboo or ironwood. It is often considered as a painstaking and dangerous job. After the nests are harvested, they need to be cleaned and this process needs 8 hours (Koon & Cranbrook, 2002). Undoubtedly, the most expensive ingredient in the bird nest is the salivary nest cement. This leads to the case of adulteration of 'white' bird nests with cheaper materials in few years ago. Adulterants such as karaya gum, red seaweed, and Tremella fungus were identified in the nests served to increase the net weight of the nests (Goh *et al.*, 2001). These compounds have the similar colour, taste and texture to the genuine bird nest salivary cement and thus become the perfect adulterants and are difficult to be detected. There are maximum of 10% of overall net weight of bird nests (Marcone, 2005). In fact, the adulterants added resulted in a reduction of overall crude protein content in the genuine edible bird nest as much as 1.1% - 6.2% which decrease the nutritional value of the nests (Marcone, 2005). Another health issue is being concerned recently, that is, the ban on Malaysian bird nests in July 2011 by China due to the nitrite content found in the bird nests. Nitrite, a food preservative, could be carcinogenic if consumed beyond safety level. China prohibited any bird nests with nitrite contamination but our country hardly produced zero-nitrite bird nests. However, related parties had been carrying out policies and guidelines to control the content of nitrite level of edible bird nests from Malaysia (Noriah *et al.*, 2012). Mismanagement of the nesting caves posed threats on the wild swiftlet populations. Nests are over-exploited and this reduced the production of edible bird nests. This was because during the discriminatory harvesting process, many nests and eggs were destroyed. Wild swiftlets feed on wild aerial insects in the forests or vegetation areas. However, the forests were cleared for converting the pristine forest vegetation into monoculture commercial crops led to the reduction of wild insects. Many lands were used to build new oil palm estates (Koon & Cranbrook, 2002).

2.1.3 Potential diet for swiftlet

Swiftlets feed on ample supply of wild insects in the forests. However, deforestation and pesticides used in agriculture to control insects had reduced the insect populations. Urbanization and forest fire are also the causes of insect populations decline which subsequently reduced the populations of swiftlets (Lourie & Tompkins, 2000). To solve the issue of swiftlet poplation decline, many entrepreneurs start to do captive breed swiftlets. Large aviaries are built to allow the birds to fly around and insects are released to feed them. In this case, supply of insects have to be sufficient to support the birds' diet. The knowledge of their diet is important to manage the breeding of swiftlets in captivity since bird nest houses farming is practised in many countries today. Knowing the diet is one of the key to maintain the colonies of swiftlets in the farms. A study by Lourie and Tompkins (2000) using the food boluses to investigate the diet of swiftlets indicated that swiftlets were found to feed on insect group of Hymenoptera and Diptera. Between these two groups, hymenoptera was the most abundant prey items of swiftlets, followed by Diptera. However, Diptera is the prey that Glossy swiftlets tend to feed on in the urban area compared to forest and rural habitats. Study from Kamarudin and Khoo (2011) reported that swiftlets mostly foraged the dipterans (55.7%) followed by Hymenoptera (19.9%). The authors also suggested that *Megaselia scalaris* was a potential candidate as a main diet component for swiftlets (Kamarudin & Khoo, 2011).

2.1.4 Insect mass production

Mass production of insects is not a new issue today. Many industrial farmers have started mass-rearing for integrated pest management strategies and biological control (Pritam, 1982; Jesper *et al.*, 2012). In fact, mass-rearing and marketing the insects can be a prosperous business (Irwin & Kampmeier, 2002). However, a desirable insect mass production is to produce quality insects in large-scale with minimum labour, space and cost. It needs a lot of understanding in the biology of insect and processes. Thus, laboratory rearing usually starts before approaching mass production (Pritam, 1982).

M. scalaris could serve as a potential feed or supplement for the insectivorous swiftlets. Irwin & Kampmeier (2002) shared the information that *Drosophila melanogaster* is a species that has its commercial value. It is used as a basic tool for research in genetics and developmental biology. Insect has to be produced in a large quantity without neglecting its quality and effectiveness.

2.2 Diptera

Order Diptera belongs to the Insecta class. Any member from order Diptera is called a true fly. In this world, there is approximately 120000 species of insect in this order and it has formed the largest group followed by Coleoptera. More species have been discovered which include mosquitoes, house flies, fruit flies, midges and gnats (Wiegmann & Yeates, 2007). These insects perform a wide variety of feeding strategies as they are found to be predators, detritivores, parasites, scavengers and pollinators (Wiegmann & Yeates, 2005). Diptera can adapt very well and the larvae develop successfully in a wide range of media such as soil, rotting wood, plants and animal tissues (Smith, 1989). The order Diptera is classified into three main suborders, ie., the Nematocera, Cyclorrhapha and Brachycera.

2.2.1 Suborder Nematocera

Members in suborder Nematocera are generally primitive flies, all with filamentous antenna of 6-14 segment. Usually their bodies and legs are elongated, with a relatively long abdomen. Larvae are mostly aquatic. Crane Flies and mosquitoes are classified in order Diptera, which mean two wings. The insects in this order have only one pair of membranous flying wings. The second pair of wings are reduced to small knobs, called halteres, for the purpose of balancing. Their body is relatively soft and hairy. They have a pair of large compound eyes, a pair of very short antennae and a sucking mouth (Peterson, 1956). Crane Flies and mosquitoes develop by complete metamorphosis with four stages, i.e., eggs, larva, pupa and adult. Their larvae are known as maggots. Usually there are four larval instars. The mosquito larvae are mainly aquatic

(Oliver & Bode, 1985).

2.2.2 Suborder Brahycera

It is a major suborder consisting of around 120 families. Their most distinguishing characteristic is reduced antenna segmentation. The maxillary palp (an elongated appendage near the mouth) has two segments or fewer. The back portions of the larval head capsule extend into the prothorax (the anterior part of the thorax, which bears the first pair of legs). Two distinct parts make up of the larval mandible (lower jaw). Brachyceran flies can also be distinguished through behavior. Many of the species are predators or scavengers. The brachycera are generally 'well-built' flies and include the family Stratiomyidae, or soldier flies, somewhat flattened insects, often with bright metallic colours, of which there are 50 British species. Soldier flies like to take it easy, often sunning themselves on the ground on fine days. The only harmful flies in the Brachycera are in the group known as the Rhagionidae, which includes horse flies. These are stoutly built flies and have bright, bulging eyes. Female horse flies can give painful bites to horses and man (Yeates 2002).

2.2.3 Suborder Cyclorrhapha

These flies pupate in barrel shaped puparia and escape from them through circular openings. The larvae of many species are known as maggots. They are headless, and taper towards the front (Hackman & Väisänen, 1982). The sub-order Cyclorrhapha is further classified into the Aschiza and the Schizophora, depending on whether they have a little tool called a ptilinum to help them escape from their puparia. The Aschiza are the ones that don't have a ptilium. Examples from the Aschiza group of the

Cyclorrhapha include the scuttle flies (Phoridae) of which there are about 280 species in Britain alone. The front edge of their wings is spiny and they are humpbacked. They scuttle across vegetation and sometimes swarm on window panes (Griffiths, 1972).

2.3 *Megaselia scalaris*

2.3.1 Biology of *Megaselia scalaris*

Megaselia scalaris (Loew) (Phoridae: Diptera) is a small, warm climate fly species. It is cosmopolitan and synanthropic which is widely distributed in the tropics and subtropical areas around human environment (Disney, 2008). It has various common names such as scuttle fly, humpbacked fly, manure fly, coffin fly and phorid.

Being small size (2-4 mm), *M. scalaris* is able to penetrate to well close containers. It was found that it can infest container that was covered by double layer of organdy cloth (Garris, 1983). A study reported that this species can be found in a six feet deep coffin and this rendered its name coffin fly (Triplehorn *et al.*, 2005). The name of humpbacked fly came from the appearance of itself which has a humpbacked structure on its thorax. Major bristles on its thorax are the unique characteristic of this species (Peterson, 1992). *Megaselia scalaris* is known as scuttle fly because of its locomotion behaviour. This insect moves in a rapid and jerky pace with short pauses in between (Triplehorn *et al.*, 2005). It is called a phorid due to the fact that it belongs to the family Phoridae. The biology of Phoridae is very much diverse on the planet (Disney, 1990a). The small size and difficulties in identifying the species make the study of Phoridae an impediment.

2.3.2 Morphology of *Megaselia scalaris*

Scuttle fly eggs are normally different from those of other flies as they are elongated, symmetrical, oval shape with few obvious morphological features (Disney, 2004). The egg of *M. scalaris* is yellowish white with a boat-like shape and its surface is covered by scattered tubercles. The eggs are laid in several batches throughout its life cycle (Disney, 2007).

The larva consists of three different stages, that is, first instar, second instar and third instar. Generally, the morphology of first instar and second instar larvae is similar. The body of the larva is cylindrical with its head tapered and the length is about 0.75mm (Plate 2.1). The third instar larva is approximately 4mm in length. Fewer changes are observed in the development from second instar to the third instar. Little change can be seen throughout the transformation during larval stage (Noppawan *et al.*, 2004; Kabkaew *et al.*, 2002).

The pupa of *M. scalaris* is flattened dorsoventrally. The fifth segment of its dorsum bears a pair of long pupal striking respiratory horn (Kabkaew *et al.*, 2006) (Plate 2.2). Female pupae are larger than male pupae (Amoudi *et al.*, 1989).

The 'humpbacked' feature described on *M. scalaris* is found in the adult. The adult is a tiny fly with flattened head. It is blackish, brownish and yellowish. The legs and wings are attached on the thoracic region. The abdomen is found after the head and thorax, which is developed with six tergites and seven spiracles (Peterson, 1992). The morphology of male and female adult *M. scalaris* is different. There is a distinctive male terminalia at the end of its abdomen of a male adult (Plate 2.3) whereas, the female adult is recognizable by the laterally extended sclerite of segment six on the abdomen (Brown & Oliver, 2007) (Plate 2.4).

2.3.3 Life cycle

The development of *M. scalaris* is a complete metamorphism. The life cycle starts from egg, followed by larva, pupa and adult. It has short life cycle, but varies at different temperatures (Disney, 2008). The mean egg incubation period at 27°C and 75% relative humidity is 14.3 hours. The mean developmental time of larvae of the same condition is 4.6 - 6.3 days, whereas that of the pupae is 9.8 days. Benner and Ostermeyer (1980) reported that the male larvae reached the pupal stage of *M. scalaris* two days earlier than the female at 25°C. Disney (2008) suggested that the male adults emerged earlier to feed and reached their sex maturity before the female. Another finding reported that the adult longevity of *M. scalaris* was 2.8 days. Overall, it takes approximately 18.5 days to complete one generation (Mikky *et al.*, 1988). Species of the same order as *M. scalaris*, for instance *Ae. cantans*, increasing the larval density of also leads to extension of developmental time (Renshaw *et al.*, 1993). Apart from temperature and larval density, diet is also one of the factors that influence insect developmental time. The developmental time is affected by the type of diet, whether the diets are solid or liquid. The diets which are moistened also lead to variation of developmental time (Arong *et al.*, 2011). Idris *et al.* (2001) discovered that the developmental time of *M. scalaris* larvae varied when the larvae were fed with different diets.

Plate 2.1: Larva of *Megaselia scalaris* (Loew).

Plate 2.2: Pupa of *Megaselia scalaris* (Leow).

Plate 2.3: Male *Megaselia scalaris* (Loew).

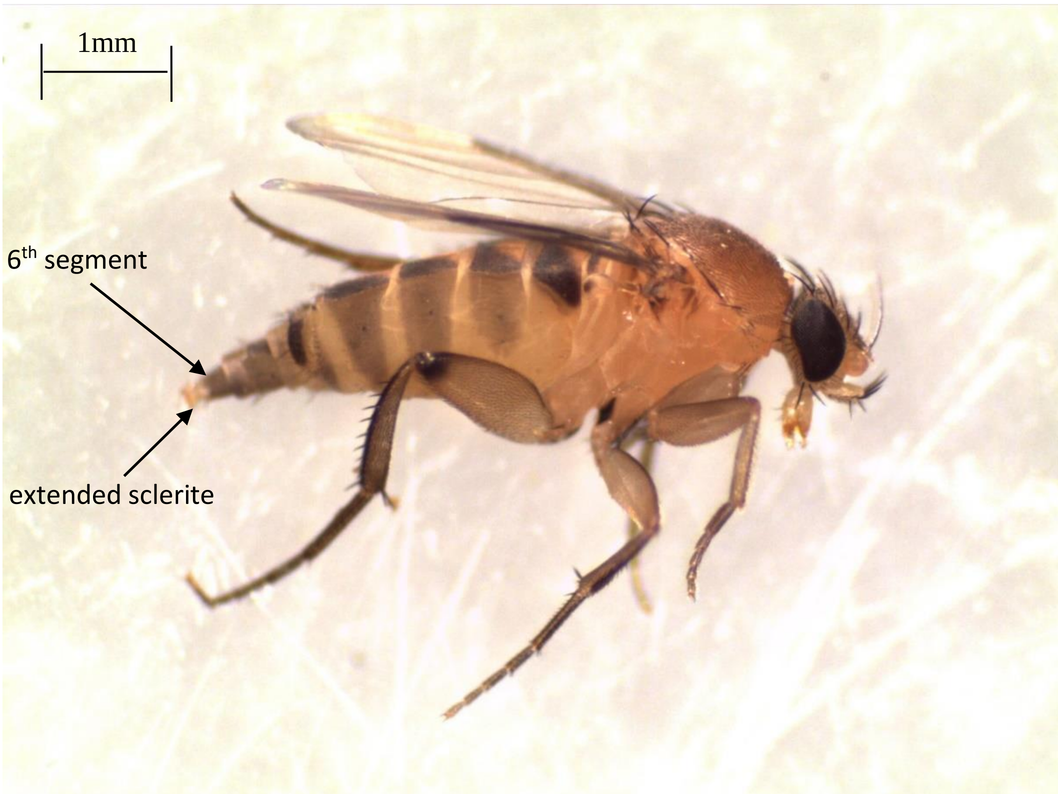

Plate 2.4: Female *Megaselia scalaris* (Loew).

2.4 Rearing of *Megaselia scalaris*

Megaselia scalaris has been very important to medical entomology and forensic entomology studies. This species is favored to become experimental animal for the research on insect developmental, bioassay and genetics studies, because it is easy to culture (Disney, 2008). Recently, there is a demand of insects in edible bird nest industry where insects are needed as a food source for swiftlets. Unfortunately, due to deforestation, forest fire, urbanization and usage of pesticides, wild insect population has declined and this leads to insufficient supply of food source for swiftlets (Lourie & Tompkins, 2000). However, *M. scalaris* is suggested to serve as a potential candidate to be the main diet component for swiftlets (Kamarudin & Khoo, 2011). Hence, a success rearing of insects either in laboratory or mass-rearing requires the manipulation of environmental conditions for insects.

At high temperature, larvae develop rapidly and may result in less active adults, whereas low temperature will destroy the sperm-producing capability of male adults. Low relative humidity results in the growth of fungi while high relative humidity can cause asphyxiation of adults (Singh, 1982). Thus, the author suggested that a temperature about 26°C and 55%-65% relative humidity should be satisfactory for many insects. While temperature and relative humidity are under controlled, there are still many other conditions that could influence the quality of insects produced such as larval crowding, types of insect feed and feed formulation. Developmental rate, weight, emergence and body size are the parameters of quality of insects that largely depends on the conditions of mass-rearing (Ekesi & Samira, 2011).

2.4.1 Larval crowding

Larval crowding will affect the survival rate, developmental time of larvae, pupal weight and also the adult size of insects (Kneidel, 1985; Peters & Barbosa, 1977; Kazimírová, 1996). Overcrowding in immature stage could be observed if there is a limited food resource where larvae will compete for the nutrients (Agnew *et al.*, 2000). Restriction of diet impacts directly to the mass gain and thus results in reduced body size and delayed developmental time (Brent, 2010).

Mazyad & Soliman (2006) reported that various larval densities was researched in the laboratory and these relate to mean life stage rate of development and survival. In the study of Prawirodisastro & Benjamin (2014), adverse effect of larval crowding of *M. scalaris* reflected at 100 larvae / 10g diet medium, where larval and pupal periods increased. Larval survival declined at 200 larvae / 10g diet. There is a few other orders of insect that face the similar situation (Disney, 2008). A study on dipteran such as Mediterranean fruit fly larvae showed that the larval developmental time increased from 24 to 59 days as a result of density increment. A high density of this fly also caused high mortality (Peters & Pedro, 1977). They also stated that the effect of increase in density outweighed the decrease in temperature, suggesting that the effect of larval crowding could be neglected. Larval developmental time of *Drosophila melanogaster* with incubation of 480 eggs in a medium extended 8 days if compared to 30 eggs. Likewise, for mosquitoes, Maciá (2009) reported that the developmental time of *Aedes aegypti* increases from average of 5-6 day (16 larvae/ container) to 8-12 days (64 larvae/ container). Another good evidence is that *Anopheles quadrimaculatus* pupated at day 8 under low density population but 2-13 days later under overpopulated condition (Peters & Barbosa, 1977).

Overcrowded culture of *Calliphora vomitoria* (Calliphoridae) encounters slow development as they were reared in a limited food resource. The larvae will have to attain a critical minimum weight before they can undergo pupation successfully. If the food had completely consumed by the larvae before they could reach to third instar, the development halts (Turner & Sarah, 2006). Some larvae will either die or continue to consume more if the food is not limiting in order to reach the critical weight (Borash *et al.*, 1998; Joshi, 2001). Therefore, the pupal recovery depends on the quantity of food source provided. Pupal recovery refers to the percentage of larva that successfully pupates. Larval nutrition plays an important role in influencing the size of adult blowfly besides the genetic factor (Stoffolano *et al.*, 2000). The larvae might have acquired enhanced competitive ability after many generations by becoming susceptible to high density of larvae. They will act fast in consuming food (Joshi, 2001).

High population densities of house fly negatively affect the weight of pupae. The weight of house fly pupae reduced from 4.15 ± 0.071 mg at 40 larvae per unit medium to 0.69 ± 0.021 mg at 2560 larvae per unit medium (Sullivan & Sokal, 1963). Some studies indicated that the pupal weight of *Dacus oleae* decreases at density of 20 eggs/g diet and above (Cirio & Gherardini, 1981) and a similar trend was observed for Olive fruit flies, *Bactrocera oleae* (Burrack *et al.*, 2009). Inhibition of pupation and higher mortality also could be observed if the larvae fail to undergo larval-pupal ecdysis in a crowding medium (Woolever & Pipa, 1970). The survival of pupa may depend on its location.

Pupa could be crushed by larvae or got disturbed and drowned in the food under crowded condition (Joshi & Mueller, 1993).

The larval stage is of utmost importance as the eclosion of adult is very much depending on this stage. The low percentage of adult emergence is likely due to the larval death before they can reach pupal stage (Turner & Sarah, 2006). Some authors reported the effect of crowding in adult size of other dipterans. The adult size of *Drosophila melanogaster* reduced 20% at the density 480 per vial as compared to density at 3 per vial (Lints & Lints, 1969). Working on *Calliphora vicina* and *Calliphora victoria*, studies showed that there is a reduction in adult size when larval density is increased per culture (David & Alix, 1995; Turner & Sarah, 2006). Turner (2006) also drew a conclusion that the effect of larval density towards the mortality and adult size is more pronounced than the effects of different types of feed. However, adult size changes significantly in different degrees of larval density on each type of feed. This highlights that the effect of food type cannot be neglected if mass-rearing were to be conducted.

In general, besides the retardation of larval developmental time, crowding may cause mortality where larvae fail to undergo process of pupation (Tschinkel, 1971; Woolever & Pipa, 1970). Tschinkel & Willson (1971) suggested that excessive contact of *Zophobas rigipes* (Coleoptera: Tenebrionidae) in overcrowded condition inhibits the pupation. Some species exhibit changes in developmental rate and physiology due to group effect that is, grouping of insect. The group effect was defined as a phenomenon where the effect occurred when the individuals were close to each other in small population. Development rate and survival may be resulted from the small population of