BIOLOGICAL PARAMETER AND EFFECT OF ABIOTIC FACTORS ON THE DEVELOPMENTAL STAGES OF *Megaselia scalaris* (LOEW) (DIPTERA: PHORIDAE)

TEO BEE HSIEN

UNIVERSITI SAINS MALAYSIA

2014

BIOLOGICAL PARAMETER AND EFFECT OF ABIOTIC FACTORS ON THE DEVELOPMENTAL STAGES OF *Megaselia scalaris* (LOEW) (DIPTERA: PHORIDAE)

by

TEO BEE HSIEN

Thesis submitted in fulfilment of the requirements
for the Degree of
Master of Science

August 2014

ACKNOWLEDGEMENT

First of all, I would like to express my heartfelt thanks to my supervisor, Dr Hamdan bin Ahmad, for his meticulous and painstaking guidance, advice and enthusiastic encouragements to me in completing this thesis. His willingness to give his time so generously is very much appreciated. I would also like to thank to my co-supervisor, Dr Zary Shariman Yahaya for checking the thesis and giving advice to me.

I would like to acknowledge Department of Veterinary Services of Malaysia for giving me a chance to co-operate with them and run my project. Highly appreciated to the advices and guidance from Dr Kamarudin bin Md Isa (Director of Department of Veterinary Services, Johor), Dr Abu Hassan bin Muhammad Ali (Director of Research Department of Veterinary Services, Malaysia), Mr Chang Kun Wah and Mr Khoo Choon Kiat from Research and Innovation Division, Department of Veterinary Services Malaysia.

My special thanks are extended to all the staff from Institute Veterinary Malaysia (IVM) and Pusat Penyelidikan Walit/ Pembiakan Itik, Kampung Paya Jaras Hilir, Selangor under the Department of Veterinary Service Malaysia for their technical assistance and equipment support. Appreciations also go to Encik Selvaraja, Encik Guna, Kakak Rohani and Encik Hazwan for helping me in laboratory.

I would also like to thank Prof Madya Hamady Dieng, the lecturer of School of Biological Sciences, Universiti Sains Malaysia. His advice and guidance in the biostatistics has been a great help in my data analysis of my research.

Not to forget my dearest friends Eng Hua, Siaw Fang, Yan Fen, Choy Leng, Chun Siang, Wai Heng, and Song Guan, thanks for giving happiness to me.

Finally, I wish to thank my parents and family for their patience, support and love, which kept me moving ahead in my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
˚C	Degree Celcius
cm	Centimetre
D_1	Day 1
DDT	Dichlorodiphenyltrichloroethane
g	Gram
IVM	Institute Veterinary Malaysia
MANOVA	Multivariate Analysis of the Variance
mg	Milligram
mm	Millimetre

LIST OF APPENDICES

PARAMETER BIOLOGI DAN KESAN FAKTOR ABIOTIK TERHADAP PERINGKAT PERKEMBANGAN *Megaselia scalaris* (LOEW) (DIPTERA: PHORIDAE)

ABSTRAK

Parameter biologi dan kesan faktor- faktor abiotik yang berbeza telah dikaji terhadap peringkat larva, pupa dan dewasa *M. scalaris*. Bagi parameter biologi, perbandingan antara jantan dan betina terhadap berat, saiz, dan jangka hayat *M. scalaris* telah diperiksa. Jantan *M. scalaris* mempunyai kadar pertumbuhan yang lebih pendek dan berat badan yang ringan dibandingkan dengan betina semasa peringkat larva dan dewasa. Walau bagaimanapun, pada peringkat pupa, jantan *M. scalaris* mempunyai kadar pertumbuhan yang perlahan berbanding dengan betina. Berat bagi jantan dan betina telah didapati meningkat secara berterusan pada peringkat larva tetapi menurun secara berterusan pada peringkat pupa. Dalam kajian ini, kesan bagi kedua-dua faktor abiotik: suhu dan makanan telah dikaji terhadap kadar pertumbuhan dan kadar kelangsungan hidup bagi larva dan pupa *M. scalaris*. Suhu 27˚C dan 35˚C mencatat kadar pertumbuhan yang lebih pendek dan kadar kelangsungan hidup yang lebih tinggi bagi larva *M. scalaris*. Bagi pupa, suhu 27˚C menunjukkan masa pertumbuhan yang paling singkat dan kadar kelangsungan hidup yang tertinggi. Ia mempunyai perbezaan yang ketara dengan suhu 19˚C dan 35˚C. Kadar pertumbuhan yang pantas dan daya hidup yang tinggi bagi larva *M. scalaris* yang berada dalam makanan ayam. Walau bagaimanapun, makanan tidak menunjukkan sebarang kesan ($P > 0.05$) terhadap masa pertumbuhan dan kadar kelangsungan hidup pupa. Interaksi antara suhu dan makanan memberikan kesan kepada kadar kelangsungan hidup larva, masa pertumbuhan pupa dan kadar kelangsungan hidup pupa bagi *M. scalaris*. Kesan antara lima faktor seperti status

mengawan, jantina, suhu, nisbah cahaya, makanan dan interaksi antara mereka dikaji terhadap jangka hayat dewasa *M. scalaris* dalam kajian ini. Lalat yang tidak mengawan didapati hidup lebih lama jika dibandingkan dengan lalat yang mengawan. Betina *M. scalaris* juga mempunyai jangka hayat lebih lama jika dibandingkan dengan jantan. Suhu 19˚C dan nisbah cahaya kecerahan yang berterusan (LD: 24:0), nisbah cahaya (LD: 16:8) dan nisbah cahaya kegelapan yang berterusan (LD: 0:24) boleh memanjangkan jangkat hayat dewasa. Jangkat hayat yang paling tinggi boleh didapati bagi lalat yang diberikan makanan seperti gula, air dan protein. Bagi kesuburan, bilangan maksimum telur dicatatkan pada suhu 19˚C. Kesimpulannya, kajian tentang parameter biologi dan faktor- faktor abiotik adalah penting kerana ia boleh mempengaruhi perkembangan *M. scalaris* dan ini akan memberikan kesan terhadap kecekapan penghasilan secara besar-besaran.

BIOLOGICAL PARAMETER AND EFFECT OF ABIOTIC FACTORS ON THE DEVELOPMENTAL STAGES OF *Megaselia scalaris* (LOEW) (DIPTERA: PHORIDAE)

ABSTRACT

Biological parameters and the effect of different abiotic factors were tested on larval, pupal and adult stages of *M. scalaris*. For the biological parameters, comparison between males and females on the weight, size and lifespan of *M. scalaris* were examined. Males of the *M. scalaris* had shorter developmental time and lighter in weight and size compared with the females during larval and adult stages. However, during pupal stage, males of *M. scalaris* had longer developmental time compared with the females. Their weights increased continuously during larval stage but decreased incessantly during pupal stage. In this study, the effect of two abiotic factors: temperature and diet were tested on the larval and pupal developmental time and survival of the *M. scalaris*. The temperatures of 27 ˚C and 35 ˚C recorded shorter developmental time and higher survival of the larvae of *M. scalaris*. For the pupae, temperature of 27 ˚C showed the shortest developmental time and the highest survival of *M. scalaris* which was significantly different to those reared at temperatures of 19 ˚C and 35 ˚C. Fast developmental time and high survival of the larvae of *M. scalaris* were also found in the treatment of chicken feed. However, there was no significant difference ($P > 0.05$) of the diets to the pupal developmental time and survival. Interaction between temperature and diet was found on larval survival, pupal developmental time and pupal survival of *M. scalaris*. Effect of five factors such as mating status, sex, temperature, photoperiod, diet and their interactions were evaluated on the adult longevity of *M. scalaris* in present study. Unmated flies were found to live longer than mated flies. Females of *M.*

scalaris also showed that they had longer longevity if compared with them males. Temperature of 19 ˚C and continuous brightness (LD: 24:0), long photoperiod (LD: 16:8) and continuous darkness (LD: 0:24) can prolong the lifespan of the adults. Highest adult longevity was found for those fed with treatment of sugar, water and protein. Results also indicated that there were interactions between several factors. For the fecundity, maximum egg-laying number was recorded at the temperature of 19 ˚C. As a conclusion, the study of biological parameters and abiotic factors was important as it significantly affected the *M. scalaris* development and this can influence the efficiency of mass production.

CHAPTER ONE

GENERAL INTRODUCTION

Insects are a class of invertebrate organisms (Arthropod: Insecta) which have three body part; namely, head, thorax and abdomen. Other distinct features which distinguish insects from other organisms include six jointed legs, a pair of compound eyes and antennae. Insects play different roles in the ecosystem such as pollinators, predators, pests, etc. Besides that, they are also feeds for various insectivores such as fishes, amphibians, reptiles, birds and mammals.

Swiftlet is an aerial insectivore widely found in Indonesia (Nugroho & Whendrato, 1995) and certain places in Peninsular Malaysia (Koon & Cranbrook, 2002). There are high demands towards swiftlet due to their edible nests (also known as bird nests) which generate profitable income for farmers.

In recent years, mass deforestation activities such as shifting cultivation, urbanisation and forest fires have led to major decline in the population of insects as a result of destroyed habitat in the tropical forest (Waugh & Hails, 1983). These ecological changes have reduced the population of insects which serve as a food source for swiftlets. The production of these edible swiflet nests has been deeply affected as a consequence of the decline of the insects which are swiftlets' food sources. Therefore, alternative method such as mass production of insects is essential to fulfill the demand of the swiftlet-farming industry.

Lourie and Tompkin (2000) suggested that insects from the order Diptera are one of the main diets for swiftlet. Nugroho *et al.* (1998) and DVS (2007) also indicated that swiftlets prefer small flying insects. Hence, *Megaselia scalaris* (Loew)

(Diptera: Phoridae), a small and cosmopolitan fly (El- Muniawi & Moustafa, 1965; Manix, 1964; Robinson, 1971) has received substantial attention as preferred feed for swiftlets.

Prior to the mass production of insects, the quality of mass rearing needs to be controlled in order to maximise the insects' populations in laboratory. There are several aspects which need to be monitored routinely in order to ensure good quality of mass production. These aspects include biological values for the immature insects (such as egg viability, density, and yield and survival rate), adult insects (population size and density, sex ratio) and life cycle development for the insects. In addition, proper diet (such as the textures, composition, and microbial loads) and suitable environmental conditions are also monitored to ensure proper mass production (Derrell, 1977). For example, male and female invertebrate organisms have different lifespan. (Stojkovic & Savkovic, 2011). Distinct differences in size between male and female invertebrates are also examined (Head, 1995; Shine, 1979, 1994; Teder & Tammaru, 2005).

It was found that abiotic factor such as temperature affects the lifespan of adult *M. scalaris* (El- Miniawi & Moustafa, 1965; Manzato & Tadei, 2004; Prawirodisastro & Benjamin, 1979). Several studies reported that the amount of eggs by *Drosophila melanogaster* (Rezaei, 2012) and *Drosophila ananassae* (McKenzie, 1975; Parsons, 1978) are significantly reduced at low temperature.

It was also found that photoperiod, an abiotic factor, also affects the insects' lifespan. Diapause will be induced by the photoperiod to insects (Brodeur & McNeil, 1989 & 1990; Pawson & Petersen, 1990; Tisdale & Wagner, 1990; Yonggyun &

Krafsur, 1994; Trimble, 1994). Das *et al.* (2012) showed that photoperiod of 12:12 (L: D) is appropriate for mass production of *Oxya hyla* in acridid farms.

Idris *et al.* (2001) had reported that different diets used to feed *M. scalaris* produce different effect on the development of larvae, pupae and adult emergence. House flies and other insects require adult diets especially sugars and proteins to develop their reproduction systems in order to produce more eggs (Adams & Nelson, 1990; Glaser, 1923).

Nonetheless, information on the mass production of *M. scalaris* is scanty. Up to date, limited information is available on the biology and effect of abiotic factors on different development stages of *M. scalaris*.

Therefore, the objectives of this study were:

i) To determine the biological parameters of males and females *M. scalaris*
ii) To determine the abiotic factors affecting development time and survival rate of larvae and pupae *M. scalaris* in laboratory
iii) To determine the abiotic factors affecting longevity and fecundity of adults *M. scalaris* in laboratory

CHAPTER TWO

LITERATURE REVIEW

2.1 Insect as feed for animals

Insects can be food sources to some animals especially fishes, amphibians, reptiles, birds and mammals (Thompson, 1984). These organisms are known as insectivores. Many fishes and poultry feed on insects. A common example of poultry feeding on insects is chicken. Chickens feed on worms and larvae from the topsoil. Human uses maggots as fish baits for fishing. Fluorescent lights were commonly hanged above fish ponds in Southeast Asia to attract insects which in turn fall into the pond due to the light's reflection and being consumed by fishes (van Huis, 2013).

Insects are used as complementary food source for poultry. Ravindran and Blair (1993) mentioned that poultry were fed with grasshoppers, crickets, cockroaches, termites, lices, stink bugs, cicadas, aphids, scale insects, psyllids, beetles, caterpillars, flies, fleas, bees, wasps and ants. Chickens and guinea fowls in Togo and Burkina Faso were fed with termites (Farina *et al.*, 1991).

Insects are sought as replacement feeds for poultry. The mealworms (*Tenebrio molitor*) were utilized to replace soya meal in poultry. They are reared on low-nutritive waste products and feed to broiler chicken (Ramos Elorduy *et al.*, 2002). The substitute of soya meal in poultry diets with soldier flies (*Hermetia illucens*) that grow on manure and housefly pupae (*Musca domestica*) were discovered by Ravindran and Blair (1993). In Nigeria, maggots used to replace fishmeal with 25 percent of the replacement in the diet produced significant effect on the average weight gain per week and protein efficiency rate of the chickens

(Awonyi *et al.*, 2004). The replacement did not significantly affect the life, dressed and exenterated weights of the chickens along with their lengths, breadths and weights of the pectoral and gastrocnemius muscles.

Chitin from the exoskeleton of insects can be found in lower organisms such as fungi, crabs, lobsters and shrimps. It contained nitrogen and is a well-known component of anti-viral and anti-tumour activities derivative (Lee *et al.*, 2008). It is also believed that chitin can improve the immune system of the chickens. Therefore, chitin served as a substitute to antibiotic in poultry industry against drug-resistant bacterial strains which eventually harm human (van Hall *et al.*, 2011).

2.1.1 Insects as feed for swiftlet

In recent years, swiftlet farming industry has grown exponentially in Indonesia (Nugroho & Whendrato, 1995) and various places in Peninsular Malaysia such as Penang (Langham, 1980), Sitiawan and Taiping in Perak, and in the states of Terengganu, Johor and Malacca (Koon & Cranbrook, 2002). The reasons of the over-exploitation in caves and the rapid development of swiftlet farming industry are due to the high demand and value of its edible nest. Moreover, edible bird nest has suffered from inflation and witnessed increase in its price due to upsurge in demand all over the world owing to its roles as tonic and skin rejuvenation.

Swiftlets are aerial insectivores which feed on flying insects (Charles, 1987; Chantler & Driessens, 2000; Medway, 1962; Nguyen *et al.*, 2002). Kamarudin and Khoo (2011) reported that foraging areas of swiftlets include forests, plantations, and fruit orchards. Manchi and Sankaran (2010) published that swiftlets producing edible nests forage high above the canopy of forested areas.

According to the study by Kamarudin and Anum (2011), insects from the order of Diptera (55.7%) and Hymenoptera (19.9%) were found all over palm oil plantations in Johor where swiftlets nest can be found. Lourie and Tompkin, (2000) showed that the food boluses found in White-nest Swiftlets, *A. fuciphagus*, taken as sample from Gomantong Cave in Sabah which is forest and palm oil canopy consisting of a large quantity of insects from order of Diptera (39.2%) and Hymenoptera (38.6%).

Nonetheless, human activities such as shifting cultivation, urbanization and forest fire caused the declination of insect population in forest canopy (Waugh & Hails, 1983). Marsh and Greer (1992) stated that timber industry in Sabah, Malaysia was the main reason of forest loss and induce the insect habitat destruction.

Hence, culturing insect to feed swiftlets is an astute step to overcome insect shortage in swiftlet farming industry. Nugroho *et al.* (1998) and DVS (2007) mentioned that swiftlets feed on small flying insects only. Kamarudin and Khoo (2011) highlighted that insects from the order of Diptera are the best choice to be cultured for swiftlet feeding. They also listed out some vital criteria such as size, texture, versatility, short life cycle, high feed conversion and high nutritional value of the insect which should be considered for the suitable insect diet for the swiftlets. It was stated that the size of insect should be about two to five millimetres because larger insects would cause difficulty for the swiftlets to swallow them while smaller insects are difficult to be reared in enclosed cage. Hard-bodied insects from the order of Coleoptera such as beetles are knotty to be digested by swiftlets, making these insects not suitable to be fed to the swiftlets. Furthermore, the insects that can be fed to the swiftlets should be easily raised and reproduced in different stages and environment. Insects with short life cycle, i.e., less than three weeks can be a good

candidate for insect culture. In addition, the capability to maximize insect yield using less feed is greatly recommended for insect productions. Moreover, insects with high protein content are favoured so that swiftlets can produce protein rich saliva bird nest. With these criteria, *M. scalaris* has received considerable attention as an ideal feed for swiftlets.

2.2 *Megaselia scalaris*

Megaselia scalaris (Loew) (Diptera: Phoridae), a small and cosmopolitan fly, is also known as "phorid fly". It is being described as "humpbacked fly" due to its large thorax (Peterson, 1992). It is also referred to as "scuttle fly" because of its ability to walk in short bursts with periods of break in between (Miller, 1979). A remarkable trait, brown and yellowish in colour with some dark markings on the abdomen can also be used to identify *M. scalaris*. It is only 2 mm long and it is a holometabous insect which undergoes four distinct stages of growth: egg, larva, pupa and adult.

2.2.1 Eggs

The egg of *M. scalaris* is in the form of boat-shaped. It exhibits a gunwale–like palisade of flat platelets closed to the respiratory plastron with its dotted tubercles when engaged in liquid media (Ferrar, 1987) (Plate 2.1). An exceptional width of plastron of *M. scalaris* and its particularly small egg distinguishes itself from other fly eggs such as *Chrysomya rufifacies*, *Chrysomya megacephala*, *Chrysomya pacifica*, *Chrysomya nigripes*, *Aldrichina graham*, *Lucilia cuprina* and *M. domestica* (Sukontason *et al.*, 2004). Physically, the eggshells of *M. scalaris*

Plate 2.1: Eggs of *M. scalaris.*

are similar to the eggs of *D. melanogaster*. However, the layers of the eggshells of *M. scalaris* are thicker than that of *D. melanogaster*. Such characteristic shows that eggshells of *M. scalaris* are more resistant towards unfavorable factors such as bacteria attack and dryness (Wolf & Liu, 1996). Amoudi *et al.* (1989) reported that the mean egg incubation period was 14.3 ± 1.3 hours and ranged between 12 to 16 hours under laboratory conditions at temperature of 27 ˚C and 75% relative humidity.

2.2.2 Larvae

The larval stage of *M. scalaris* consists of three instars: first-instar, second-instar and third-instar. They are creamy white (Plate 2.2). Boonchu *et al.* (2004) studied the ultrastructure of the first-instar and the second-instar larvae of *M. scalaris*. They described the first-instar larvae of *M. scalaris* as cylindrical, narrowed toward the head and measured about 750 μm in length. Two cephalic lobes are found on cephalic region of the first-instar larvae of *M. scalaris* and six sensory papillae positioned tangentially to the antenna which appear in pair dorsally on the caudal section. Similar morphology is found for the second-instar larva of *M. scalaris*. However, the oral grooves of the second-instar larva are better developed and obvious. The structure of spiracular hairs located at or near the constriction of the slender spiracular plates which contain two straight slits for each plate is observed on the second-instar larva. The third-instar larvae of *M. scalaris* are creamy white and possess the same structure of mouth hooks, oral grooves, and anterior spiracles as the first-instar and the second-instar larvae. Nevertheless, the third-instar larvae have spiracular hairs that appear centrally at the constriction of the slender spiracular plates. Two straight slits are also found on each spiracular plate of the third-instar

Plate 2.2: First-instar of *M. scalaris.*

larvae (Sukontason *et al.*, 2002). The larval phase of the third instar of *M. scalaris* can be subdivided into two stages: feeding stage and post-feeding stage. At temperature of 22 ˚C, the ratio for the feeding stage and post-feeding stage of *M. scalaris* is 1.2:1 and changes to 1.5:1 at 29 ˚C (Greenberg, 1991).

2.2.3 Pupae

Pupa of *M. scalaris* is dorsoventrally compressed. The pair of long and slender pupal respiratory horn with a tip slightly curved outward can be observed on the dorsum at the end of the 5th segment using scanning electron microscope (Plate 2.3). Several spiral papillae are arranged on the surface of the horns. The papillae are oval and domed-shaped with a single longitudinal straight aperture on them. There is an invaginated cephalic segment with a pair of antennae at the ventral part. A pair of round anterior spiracles is found dorsolateral on the prothorax. They bear two straight slits with one end closed while the other end opened. Pointed end spinose setae and short tubercles with spinal apex are located on each body segment. A pair of posterior spiracles can be found on the last segment. These spiracles contain four straight parallel spiracular slits arranged as two opposite groups with large posterior spiracular hairs located centrally between the two groups. An ecdysial scar or button is located near the spiracular hairs (Sukontason *et al.*, 2005). At 25 ˚C, male larvae of *M. scalaris* were found to evolve into pupae two days earlier than the female larvae. It is also noted that the male pupal development period was shorter than that of the females (Benner & Ostermeyer, 1980).

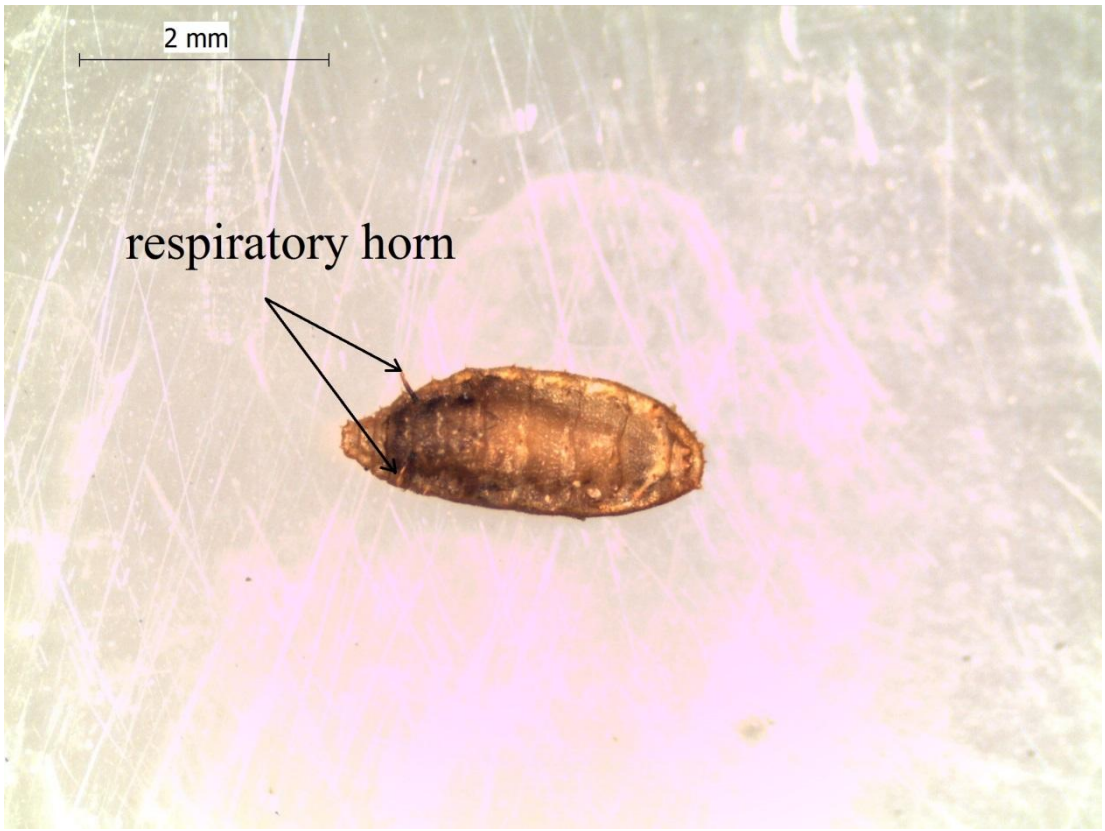

Plate 2.3: Pupa of *M. scalaris*.

2.2.4 Adults

Observing from the head part, the adults have a rather flattened head with some ommatrichia positioned between the facets of their compound eyes. Sometimes, female *M. scalaris* has distended and idiosyncratically shaped of the lower facial margin and clypeus (Peterson, 1992). Both sexes of *M. scalaris* bear sponging mouthparts. Trichoid, conical sensilla and five pairs of sharply pointed teeth are observed in both sexes. However, sexual dimorphism is observed on the surface of labellum for the mouthparts of *M. scalaris*. Female *M. scalaris* has smooth labellum surface whereas its male counterpart has labellum covered fully with microtrichia (Sukontason *et al.*, 2003). Wings are generally large and trimmed with a variety of sizes of setae. It has stout and enlarged legs which are tangentially squeezed behind femur. The third body region which is known as abdomen is well-developed based on different sexes (Peterson, 1992). Female can be recognized by the sclerite of segment six which is extended laterally on the abdomen (Plate 2.4) while the male has distinctive hook-like terminalia (Plate 2.5) (Brown & Oliver, 2007). . Female adult *M. scalaris* is much larger than the male (Harrison & Cooper, 2003) (Plate 2.6). With the earlier emergence of male *M. scalaris*, it may be able to mature its sperm early by feeding on meals before its female counterpart emerges (Disney, 1994). Female *M. scalaris* is receptive to the male about 24 hours after emergence (Ondraschek, 1953). During courtship, the male executes several difficult wing and leg movements while the female will retort with lateral shake its abdomen. The coupling may take place when the male carries the female during conjugal flight (Miller, 1979). The average mating period of *M. scalaris* is about 32 seconds but it may range between 16 seconds to 148 seconds (Benner, 1991).

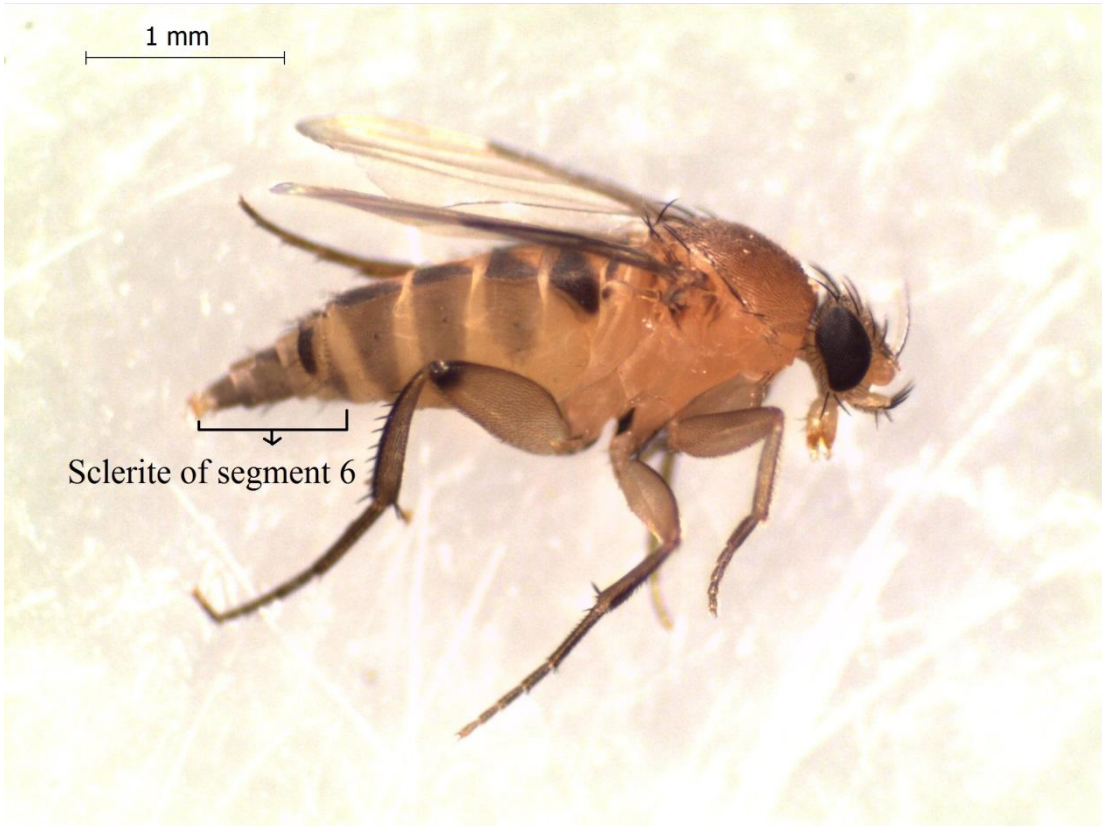

Plate 2.4: Female of *M. scalaris* with distinctive sex recognition.

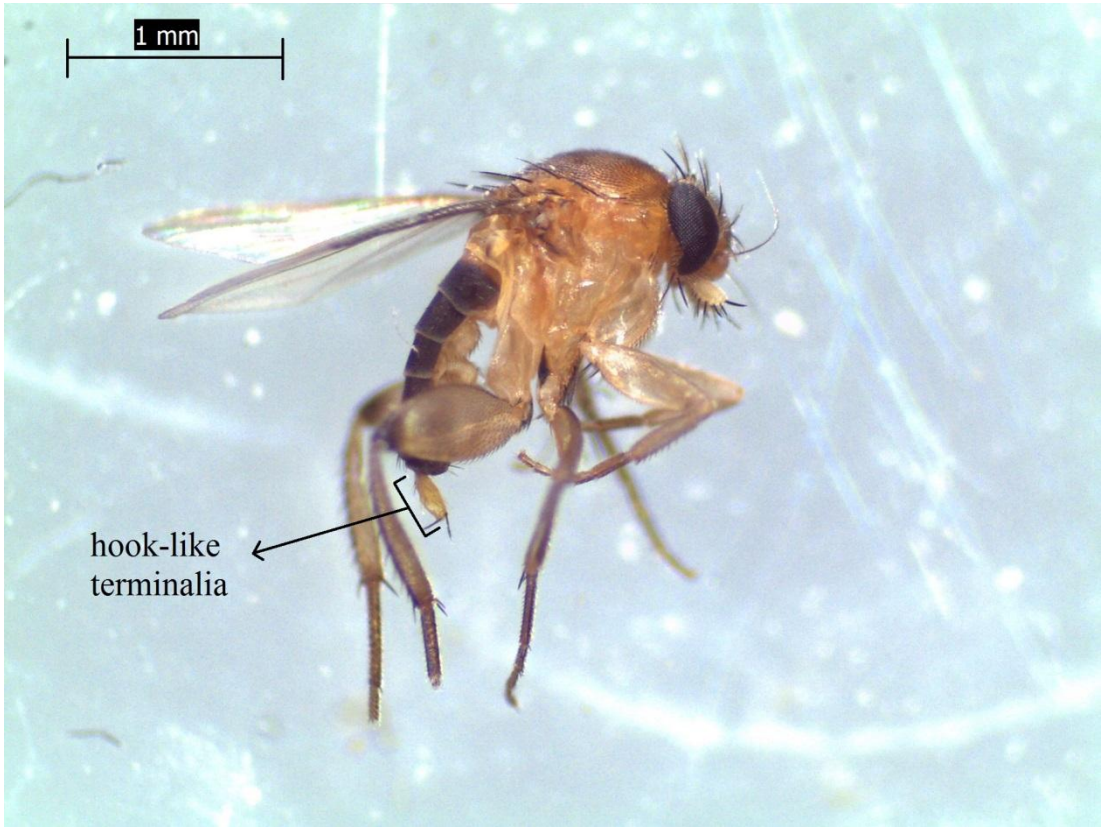

Plate 2.5: Male of *M. scalaris* with distinctive sex recognition.

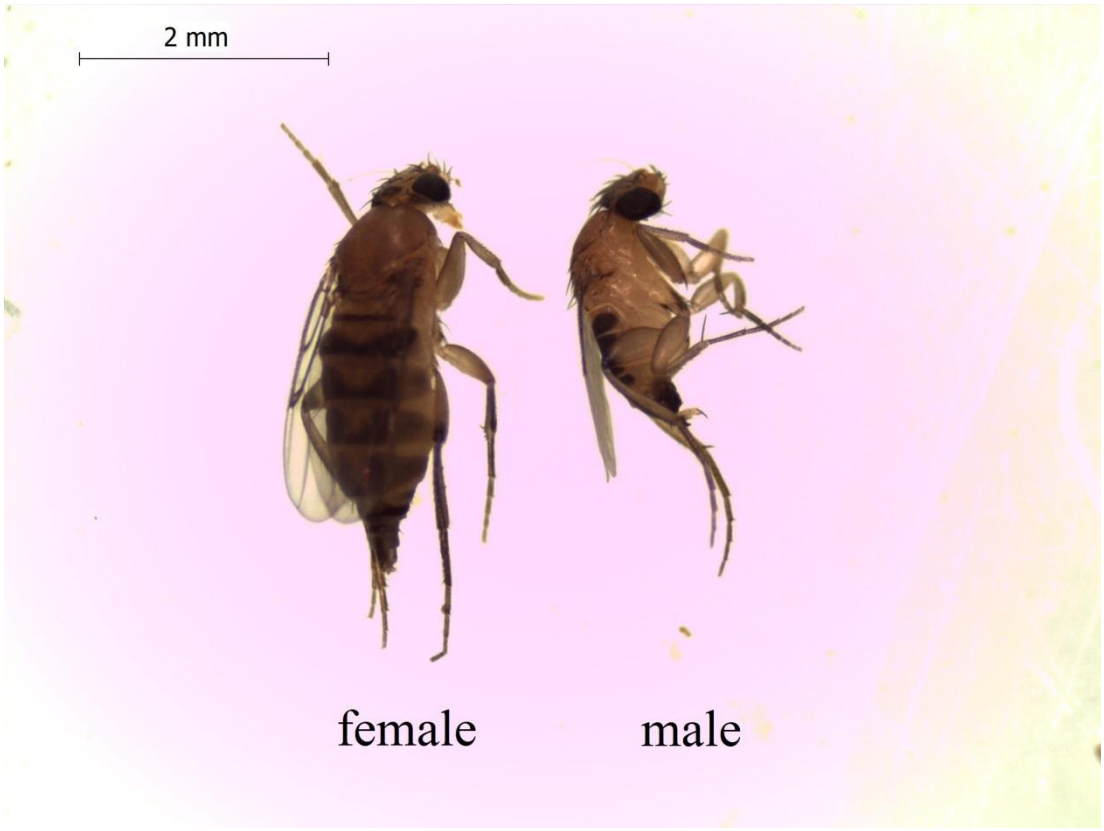

Plate 2.6: Size difference between male and female of *M. scalaris*.

2.2.5 Feeding habit of *M. scalaris*

M. scalaris is an omnivorous insect (Sukontason *et al.,* 2003). As cyclorrhaphan larvae, *M. scalaris* larvae feed on a wide variety of fluid organic materials. Initially, larvae will dissolve the solid organic matter by secreting the digestive enzymes onto the food particles. It will then congregate and pass the food particles backward above the lamellae. This will eliminate the excess fluid as the lamellae are partly obstructing the gutters between the ridges in the floor of the pharynx which act as a sieve (Disney, 1994). Larvae of *M. scalaris* are able to float when immersed into liquid media by swallowing air. This can avoid *M. scalaris* from drowning in its normal habitat (Harrison & Cooper, 2003).

Adults *M. scalaris* can only feed on liquid diets due to their sponging mouthparts. Sugar is the primary food for adults. Females *M. scalaris* require protein food sources to mature their eggs (Disney, 1994). Five pairs of sharp teeth were found in the mouth of adults *M. scalaris*. The teeth aid the digestion and breakdown of nutrients. They are also used to exit their pupal casings by cutting holes through them (Sukontason *et al.,* 2003).

2.2.6 Importance of *M. scalaris*

Commonly, *M. scalaris* is used to estimate the postmortem interval for some forensic cases and it is reared in small numbers for this purpose. However, recently it had been proven that *M. scalaris* can be easily cultured in the laboratory. This is because the larvae are detritivores and feed on various types of food such as decaying meat, moribund insects, plant materials, feaces and others (Robinson, 1971). Hence, it becomes popular to be used as experimental organism to study genetics, developmental biology and biomass (Disney, 2008). However, the production of *M.*

scalaris is restricted to laboratory use. Available information on the mass production of *M. scalaris* is also sparing.

2.3 Mass rearing of insects in laboratory

There are several definitions for mass rearing of insects. One of the definitions of mass rearing is the simplicity of rearing large numbers of insects to the desired numbers to enforce economic control per unit of area or to production effectiveness per unit of attempt and space (Finney & Fisher, 1964; Knipling, 1966; Shorey & Hale, 1965). According to the study by Mackauer (1972), mass production of the entomophagous insects can be defined as the breeding of one million times the mean number of offspring per female per generation cycle. Derrell (1977) defined mass rearing as the number of insects per generation exceeding ten thousand to one million times the mean yield of the native female population and the capability to accomplish program goals with an adequate cost or benefit ratio.

Recently, many insects are domesticated and mass-produced for certain purposes. Some species of the insects are reared in large quantity due to their commercial value, for example: silkworm and bees. Large-scale productions of mealworms and crickets are used as pet food in Europe, North America and many Asian countries (van Huis *et al.*, 2013). In biological control, mass production of predators and parasitoids in agriculture are important to combat pests. *Lucilia sericata* was mass-reared for maggot therapy to clean out the necrotic tissue of wounds in humans and animals (van Huis *et al.*, 2013).

Mass production of insects aims to produce the large quantity of "functional" insects with minimum labour, space and cost. Many insects often have short life cycle, high biotic potential, simple food requirement, and alternative hosts. Process

standardization, programme systematization, well-organized production, quality control maintenance, operative sanitation, microbial control contamination need to be established in the rearing laboratory (Singh, 1982).

During the initial stage of insect production, an origin source of the insect colony needs to be selected and this source is regarded as founder population. The source can affect the eventual genetic and the latent behavioral profiles of the production colony (Mackauer, 1972; 1976). Founder population can be built by sampling and collecting insect samples from sites. Different traps can be used to catch and collect the samples, for example: emergence traps, light traps, pitfall traps, sticky traps, tuna fish baits, etc. Emergence traps introduced by Szadziewska (1977) are used to trap soil dwelling insects from the order Phoridae. An emergence trap is made by using washing up bowl with an exit hole at the bottom which is fitted with tube extended into a collecting pot. Emergence trap for bark-breeding insects can be used to trap Phoridae insects by attaching the trap to a branch or the trunk of a tree (Büchs, 1988). Alternatively, light trap can also be used. Large numbers of *Chonocephalus* are recorded to be attracted to light traps in the tropics (Hardy & Beyer, 1964). Meanwhile, pitfall traps which are widely used by researchers are baited with decaying fungus. This type of traps is highly selective with the result of more than 60% specimens collected from 50 sites in Northern England belonging to the *Megaselia longicostalis* (Brown & Marshall, 1984; Baumann, 1977; Disney *et al.*, 1981). Other baits such as tuna fish baits had successfully attracted fire ants by Feener (1987). Sticky traps with chemical lure are used to trap Diptera insects (Beavers *et al.*, 1972). Generally, Phoridae order insects are strongly attracted to 2, 3-hexadienyl butyrate but the *Megaselia* species, particularly *M. pleuralis* are strongly attracted to traps with 10% 1-phenylethanol bait (Kamm *et al.*, 1987).

Insect production necessitates the establishment of a research colony from founder population. According to the study of Needham (1937), food, protection from enemies, appropriate physical environment and suitable conditions for reproduction are the keys to successful insect rearing. The research colony can also estimate the advance viability of mass production and creates preliminary procedures for insect rearing whether by directly maneuvering or modifying the performance.

During mass production, life history of insects and the environmental conditions (biotic and abiotic factors) are regularly determined to ensure the success of the rearing process as well as the stability in production amenities (Derrell, 1977).

2.3.1 Life history of insects

Insects, which are cold-blooded organisms, undergo different growth and development stage depending on the environment these insects are in. The development of insects is also different for both sexes. Female invertebrate insects such as the *Drosophila* species were found to have a longer life expectancy compared with the males (Tower & Arbeitman, 2009; Stojkovic & Savkovic, 2011). Similar result was found in the study by Amoudi *et al.* (1988) which concluded that female *M. scalaris* adapted in two seasons have longer life expectancy than the males. A plausible explanation is due to the difference in size between the males and the females (Roff, 1980; Faibairn, 1990; Blanckenhorn *et al.*, 2007). In addition to that, a study carried out by Tower and Arbeitman (2009) found that asymmetric inheritance of sex chromosomes gives rise to different lifespan between males and females. Due to the presence of single X chromosome in the male, any recessive mutant phenotype will be expressed by the male compared with the female that present two X chromosomes. These mutations normally will cause the declination of the lifespan.

For example, longevity in *Drosophila*, mice and other species of organisms are decreased owing to the inbreeding that can create the recessive mutations.

Insects can be classified and identified based on their morphology and size. For example, flies are distinguished from other winged insects as flies have only a pair of wings while other winged insects have two pairs of wings. For a specific species of insect, the males and females differ in size and morphology. This is known as sexual dimorphism. For example, the size of female invertebrates and poikilothermic vertebrates are larger than the males (Head, 1995; Shine, 1979, 1994; Teder & Tammaru, 2005). The major factor that causes the size differences between the males and females is fecundity selection. However, it had been reported that the difference in size between the males and females are due to three primary classifications; which are: 1) the individual is large in size from the beginning during hatching/birth period, 2) the individual has higher growth rates, or 3) the individual has a long growth period.

2.3.2 Abiotic factors

Abiotic factor can be defined as non-living chemical or physical factor in the environment that can influence the ecosystem. It can be divided into several groups such as climatic factor, edaphic factor and social factor. Climatic factors are referred to sunlight, humidity, temperature and atmosphere. For the edaphic factors, they are stated to the nature and type of soil, geology of the land while social factors are referred to land use, water resource and others (Hogan, 2010).

Abiotic factors affect an insect's abundance and distribution (Savopoulou-Soultani *et al.*, 2012). Insects will try to adapt themselves to survive in unfavorable

conditions. Failure to adapt would cause the insect's population to decline. Factors such as temperature and humidity also affect an insect's population.

There are several reports emphasizing on manipulating abiotic factors to control pests' populations. For example, Bonsignore (2012) mentioned that buprestid beetle, *Capnodis tenebrionis* which causes a considerate threat to the organic cultivation of various *Prunus* species can be controlled by temperature due to its phenology. Rahmathulla *et al.* (2012) indicated that the interaction between low temperature and high humidity due to increased rainfall is correlated to the increased infestation levels of *Diaphania pulverulentalis* which is a devastating leafroller for mulberry.

Besides that, abiotic factors can assist the biological control of certain insects. A study carried out by Ogah *et al.* (2012) on the African rice gall midge (*Orseolia oryzivora*) and its parasitisms (*Platygaster diplosisae and Aprostocetus procerae*) indicated that the population of African rice gall midge can be controlled efficiently under high temperature and low rainfall. Schirmer *et al.* (2008) stated that abiotic factors such as temperature and light intensity affect the biological and ecological characteristics of aphid parasitoid. An example is the use of *Aphelinus asychis* to control aphid, *Aphis gossypii* in greenhouses.

2.3.2.1 Temperature

Temperature exerts strong effect on arthropod's populations and their interactions (Bentz *et al.*, 1991; Gilbert & Ragworth, 1996; Logan & Bentz, 1999; Logan & Powell, 2001). This is due to the reason that insects are poikilothermic organisms in which their metabolism, growth and development rate and overall behavioral activities are sensitive towards thermal changes (Ward & Stanford, 1982).

For instance, insect pests such as whiteflies, beet armyworms, cabbage loopers and leafminers grow faster at temperatures between 85 ºF to 90 ºF but the growth rate is slower under cool and winter conditions (Palumbo *et al.*, 2010). Typically, there is a linear relationship between most of the range of temperatures to the developmental time of insect (Schirmer *et al.*, 2008). Temperature controls the metabolic rates of the insects by increasing the rates of chemical reactions in the insect's body (Pearl, 1924). Trumble and Pienkowski (1979) have shown an increase in growth rate of larvae *M. scalaris* at increasing temperature. However, diapause can be induced if the insects are exposed to extreme high or cold temperature by prolonging the development period (Chapman, 1998; Tauber *et al.*, 1986). A study by Frouz *et al.* (2002) showed that the temperature followed a bell-shaped curve with a rise at lower temperatures and a wide plateau at higher temperature of the growth rate of *Chironomus crassicaudatus* (Diptera: Chironomidae). According to the study by Palumbo *et al.* (2010), extreme hot temperature (> 48 ºC) or extreme cold condition (< 0 ºC) will also limit growth of the insects which might led to mortality. In a separate case, Peter (1975) indicated exposing *Hemerobius pacificus* to the high temperatures would result in zero survival rates.

Temperature has also known to affect the body size of insect. Bergmann's rule states that there is an increase of the size of organisms based on the weather and many studies had supported this rule (Atkinson, 1994; Hoffmann *et al.*, 2007; Diamond and Kingsolver, 2010). Another related example is larvae mosquitoes will develop into small adults which encounter high mortality when bred at high temperatures and under food restriction in the laboratory (Nayar, 1969; Reisen *et al.*, 1984; *Siddiqui et al.*, 1976).

It is also worth to take note that seasonal temperatures also affect reproduction, feeding and oviposition of insects (Palumbo *et al.*, 2010). For example, temperature was found to have effect on female's fecundity, fertility and the male's mating ability of *Drosophila melanogaster* (Mckenzie, 1975; Parsons, 1978; Schnebel and Grossfield, 1986). Chhgan and Stevens (2007) stated that there is an increase of the oviposition rate of *Heliothrips haemorrhoidalis* with the increasing temperature. However, in the cooler environments, female of *Drosophila pseudoobscura* (Dobzhansky, 1935) and *Drosophila melanogaster* (Tantawy & El Helw, 1970) were found to lay more eggs compared to warmer environments.

2.3.2.2 Photoperiod

Photoperiod is another common environmental factor that affects the developmental rate of insects. Greenberg *et al.* (2005) indicated that female progeny of boll weevil (Coleoptera: Curculionidae) in increased darkness condition (0: 24 and 10: 14 h) developed slower than those in greater brightness condition (24: 0 and 14: 10 h). Insects will develop slowly owing to the induction of diapause when the photoperiod is not suitable to them by prohibiting the emergence gating (Pittendrigh, 1967) of the insect which is referred to as the transition stage coordinated to the environmental cycles occurring in the insect's body (Dallwitz, 1984; Brodeur & McNeil, 1989 & 1990; Pawson & Petersen, 1990; Tisdale & Wagner, 1990; Greenberg, 1991; Yonggyun & Krafsur, 1994; Trimble, 1994; Davies & Ratcliffe, 1994; Byrd & Allen, 2001). For example, nocturnal insects are unable to carry out their mechanism activities during daylight compared with diurnal and crepuscular insects because their motor activities are inhibited under this condition. This observation shows that photoperiod affects the insect's daily activity pattern (Beck &

Hanec, 1960). Besides that, there is a greater changeability in the transition stage of *D. melanogaster* exposed under constant light (Skopik & Pittendrigh, 1967; Pittendrigh & Skopik, 1970). Several studies mentioned that the final larval instar of the European corn borer, *Osterinia nubilalis* confronted photoperiodically induced diapause (Mutchmor & Beckel, 1958 & 1959; Beck & Hanec, 1960). However, a study conducted by Hamdan (2006) on predatory bug, *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) revealed that photoperiod might not have any effect on the development period of an insect.

Photoperiods can influence the insect's life expectancy. The lifespan of *Drosophila* was reported to be influenced by photoperiods (Allemand *et al.,* 1973; Pittendrigh and Minis, 1972). Sahin and Ozkan (2007) stated that the lifespan of *Venturia canescens* was the longest under continuous darkness while the l lifespan decreased in L16: D8. This may due to the low metabolic activity of *V. canescens* under dark conditions which allows it to conserve more body energy compared to those under greater light conditions. Therefore, it can be stated that the insect utilizes its energy effectively to ensure higher life expectancy.

The length of photoperiods creates an effect on the fecundity of insects. For example, Fantinou *et al.* (2004) showed that photoperiods affect the ovipositional activity and ovipositional rate of *Sesamia nonagrioides*. Diapause induced by photoperiods has a relationship to the oviposition (Leather *et al.,* 1993). Deseo & Sarringer (1975) mentioned that the diapause larvae will continuously feed on weeds and winter cereals which cause accumulation of essential nutrients for the insects' reproductions. Thus, diapausing larvae are able to produce more eggs compared to non-diapausing insects. Besides that, diapause larvae can develop into large pupae by