ความเป็นไปได้ในการใช้มวนกรรเชียงจิ๋ว *Micronecta grisea* แทนการใช้เทมีฟอส ในการควบคุมลูกน้ำยุงลาย *Aedes aegypti*

นางสาวจุฑาภรณ์ อัมระปาล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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FEASIBILITY STUDY OF USING PYGMY WATERBOATMAN, *Micronecta grisea*, INSTEAD OF TEMEPHOS LARVICIDES IN CONTROLLING OF *Aedes aegypti* LARVAE

Miss Chutaporn Amrapala

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Thesis Title	FEASIBILITY STUDY OF USING PYGMY WATERBOATMAN, *Micronecta grisea*, INSTEAD OF TEMEPHOS LARVICIDES IN CONTROLLING OF *Aedes aegypti* LARVAE
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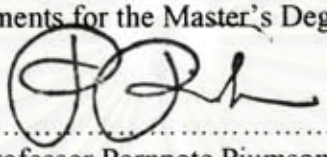
Co-Advisor	Usavadee Thavara, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree



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จุฑาภรณ์ อัมระปาล : ความเป็นไปได้ในการใช้มวนกรรเชียงจิ๋ว *Micronecta grisea* แทนการใช้เทมีฟอสในการควบคุมลูกน้ำยุงลาย *Aedes aegypti.* (FEASIBILITY STUDY OF USING PYGMY WATERBOATMAN, *Micronecta grisea*, INSTEAD OF TEMEPHOS LARVICIDES IN CONTROLLING OF *Aedes aegypti* LARVAE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.ดวงแข สิทธิเจริญชัย, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร.อุษาวดี ถาวระ, 76 หน้า.

ในการศึกษาครั้งนี้ ได้ทำการเก็บตัวอย่างมวนกรรเชียงจิ๋ว (*Micronecta grisea*) จากแหล่งเพาะพันธุ์ธรรมชาติ อำเภอบางบัวทอง จังหวัดนนทบุรี และนำมาเพาะเลี้ยงในห้องปฏิบัติการคณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย เพื่อศึกษาประสิทธิภาพและพฤติกรรมของมวนกรรเชียงจิ๋วแต่ละระยะในการกินลูกน้ำยุงลาย (*Aedes aegypti*) ระยะที่ 3 สำหรับใช้ในการควบคุมโดยชีววิธี จากการวัดความยาวลำตัว ความยาวหัว และความกว้างหัวของมวนกรรเชียงจิ๋วระยะตัวอ่อน 330 ตัว และตัวเต็มวัย 71 ตัว พบว่ามวนกรรเชียงจิ๋วมีระยะตัวอ่อนทั้งหมด 5 ระยะ (N1-N5) และตัวเต็มวัย โดยการจำแนกด้วยความยาวลำตัว ซึ่งมีค่าเท่ากับ 0.54 – 0.65 มม.(N1) 0.69 – 0.84 มม.(N2) 0.9 – 1.11 มม.(N3) 1.29 – 1.56 มม.(N4) 1.74 – 1.98 มม.(N5) และ 2.07 – 2.43 มม. นอกจากนั้นตัวอ่อนของมวนกรรเชียงจิ๋วยังแบ่งออกเป็น 3 กลุ่มตามความยาวลำตัว ได้แก่ ขนาดเล็ก (N1 และ N2) ขนาดกลาง (N3 และ N4) และขนาดใหญ่ (N5) เพื่อทำการทดลองกินลูกน้ำยุงร่วมกับตัวเต็มวัยของมวนกรรเชียงจิ๋ว พบว่าเวลาในการหาเหยื่อและเวลาในการจับกินเหยื่อลดลงเมื่อขนาดของมวนกรรเชียงจิ๋วเพิ่มขึ้น นอกจากนี้ยังพบว่าการกินเหยื่อของมวนกรรเชียงจิ๋วสอดคล้องกับความสัมพันธ์ของผู้ล่ากับเหยื่อแบบที่ 2 จากการเปรียบเทียบการกินลูกน้ำยุงลายของตัวอ่อนระยะสุดท้ายและตัวเต็มวัยของมวนกรรเชียงจิ๋ว และการใช้เทมีฟอสความเข้มข้น 1 ppm พบว่าภายในเวลา 24 ชั่วโมง เทมีฟอสและตัวเต็มวัยของมวนกรรเชียงจิ๋วทำให้ลูกน้ำยุงตายมากที่สุด (100% และ 99% ตามลำดับ) ในขณะที่ตัวอ่อนระยะสุดท้ายทำลายลูกน้ำยุงได้ 64% ระหว่างการทดลองตัวอ่อนระยะสุดท้ายใช้เวลาในการกินเหยื่อมากกว่าตัวเต็มวัยของมวนกรรเชียงจิ๋ว และเมื่อทดลองใช้ตัวเต็มวัยของมวนกรรเชียงจิ๋ว 1 ตัวกินลูกน้ำยุงจำนวนต่างๆ กันในเวลา 72 ชั่วโมง พบว่าเปอร์เซ็นต์การตายของลูกน้ำยุงมีค่าสูงที่สุด ในการทดลองที่เริ่มต้นด้วยจำนวนเหยื่อน้อยที่สุด ซึ่งให้ผลเช่นเดียวกันกับการทดลองในภาชนะขนาด 25 ลิตร และสุดท้ายการทดลองใส่เทมีฟอสในภาชนะที่มีมวนกรรเชียงจิ๋วอยู่ พบว่าเทมีฟอสมีผลให้มวนกรรเชียงจิ๋วตายภายในเวลา 8 ชั่วโมงหลังจากเริ่มการทดลอง จากการที่มวนกรรเชียงจิ๋วเป็นผู้ล่าที่มีประสิทธิภาพสูงในการทำลายลูกน้ำยุงลาย ในขณะที่สารเคมีที่ใช้ควบคุมลูกน้ำยุงในปัจจุบันยังมีความเป็นพิษต่อมวนกรรเชียงจิ๋วในแหล่งน้ำเช่นกัน ดังนั้นวิธีการควบคุมที่เหมาะสมจำเป็นต้องคำนึงถึงผลกระทบต่อสิ่งมีชีวิตอื่นในพื้นที่ และในขณะเดียวกันควรส่งเสริมและให้ความรู้เกี่ยวกับความสำคัญของมวนกรรเชียงจิ๋ว เพื่อประโยชน์สำหรับการควบคุมลูกน้ำยุงลายตามธรรมชาติต่อไป

สาขาวิชาวิทยาศาสตร์สิ่งแวดล้อม ..... ลายมือชื่อนิสิต จุฑาภรณ์ อัมระปาล

ปีการศึกษา 2551 ..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก ดวงแข สิทธิเจริญชัย

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม อุษาวดี ถาวระ

498 90684 20: MAJOR ENVIRONMENTAL SCIENCE
KEYWORDS : *Micronecta grisea* / *Aedes aegypti* / BIOLOGICAL CONTROL/ NYMPHAL INSTARS / TEMEPHOS ZEOLITE GRANULES

CHUTAPORN AMRAPALA : FEASIBILITY STUDY OF USING PYGMY WATERBOATMAN, *Micronecta grisea*, INSTEAD OF TEMEPHOS LARVICIDES IN CONTROLLING OF *Aedes aegypti* LARVAE. ADVISOR : ASST. PROF. DUANGKHAE SITTHICHAROENCHAI, PH.D., CO-ADVISOR : USAVADEE THAVARA, PH.D., 76 pp.

Pygmy waterboatmen, *Micronecta grisea*, were collected and used to establish laboratory cultures in order to study the predation rates and feeding behavior of nymphal instars (N) and adults against third instar larvae (L3) of *Aedes aegypti* to assess their potential for biological control. The body length, head length and head capsule size of 330 nymphs and 71 adults of *M. grisea*, collected from Bang Bua Thong District, Nonthaburi Province, Thailand, were measured using a stereo microscope. Five discrete nymphal instars (N1 – N5) plus adults could be classified by body length; the 1st (N1; 0.54 – 0.65 mm), 2nd (N2; 0.69 – 0.84 mm), 3rd (N3; 0.9 – 1.11 mm), 4th (N4; 1.29 – 1.56 mm) and 5th (N5; 1.74 – 1.98 mm) nymphal instars plus adults (2.07 – 2.43 mm). Nymphs classified as three discrete size categories, small (N1 & N2), medium (N3 & N4) and large (N5), including adults were examined for predation rates and prey handling times when fed L3 *Ae. aegypti* at different predator to prey ratios. Prey searching and handling times decreased with increasing size of *M. grisea* and were consistent with the Type II functional predator-prey response. The 5th nymphal instar and adult of *M. grisea* were tested the ability of *M. grisea* to feed on L3 *Ae. aegypti* to examine their efficiency. Temephos zeolite granules, applied in water at 1 ppm, were also tested against *Ae. aegypti* larvae. The number of live mosquito larvae left was recorded and found that, within 24 hours, temephos and adult showed the highest mortality percentages (100% and 99%, respectively), whereas the fifth instar nymph killed 64% of the mosquito larvae. Feeding behavior of *M. grisea* was observed during the feeding tests. Compared with adult of *M. grisea*, the fifth instar nymph took more time feeding on *Ae. aegypti* larvae. When using one adult *M. grisea*, within 72 h, the highest larval mortality percentages occurred at the lowest prey densities which also showed in further feeding tests in small scale containers, exhibited higher prey mortality (percentages) at the lower prey density. The effects of temephos on *M. grisea* were also tested and results revealed complete mortality of *M. grisea* within 8 hours of exposure. As larvicides applied in the field are toxic to *M. grisea*, but *M. grisea*'s satisfactory results provided excellent control against *Ae. aegypti* larvae, controlling methods should be safe in the areas as well as promoting benefits for conservation of effective predators to control mosquito population in the nature.

Field of Study : Environmental Science Student's Signature Chutaporn Amrapala

Academic Year : 2008 Advisor's Signature Duangkhae Sitthicharoenchai

Co-Advisor's Signature Usavadee Thavara

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CHAPTER I

INTRODUCTION

1.1 Theoretical Background

Nowadays, chemicals are widely used in agricultural activities and for medical insect controls including pesticides for the control of mosquito population which bring about contaminations and accumulations of the chemicals in the environment. Reducing these problems, biological control was developed to be an alternative with benefits to control many kinds of vectors in Thailand.

Aedes aegypti L. (Diptera: Culicidae), the yellow fever or dengue mosquito, is the principal vector for transmission of the dengue flavivirus in humans across the tropics and subtropical regions, including within Thailand. Infection with one of the dengue virus strains can cause dengue fever or the more serious dengue hemorrhagic fever (DHF), and has been estimated to cause an average morbidity in some 50 - 100 million and 500,000 people annually worldwide, respectively, and some 30,000 cases a year in Thailand, although regional epidemics can be large (Duane J. G, 2006). Besides, *Ae. aegypti* is also a vector of Chikungunya fever, a viral illness with joint pain, as well as fever and rash. Epidemics of this fever have been recorded as early as 1824 in India and elsewhere, whereas the virus was first isolated between 1952 to 1953 in the Tanzania. Recently, the widespread occurrence of Chikungunya has been reported in several countries including Thailand (WHO Regional Office for South-East Asia, 2009).

Urban *Ae. aegypti* populations are well adapted to human (their principal blood host) environments and breed in both indoor and outdoor water-storage containers, as well as a diverse array of other suitable temporal water sources of relatively stagnant water, including empty coconut shells, flag pole holders, gutters, plant pot trays, refuse bins, etc. This makes total larval elimination or control unrealistic and restricting these measures to control of the main breeding sites (human water storage containers) and attempted reduction of the minor sites so as to reduce adult mosquito numbers in the proximity of human habitations. To this end, larval control programs in Thailand are

traditionally performed using a mixture of three imperfect approaches, that is physical (water container covers, upturning to drain dustbins, prevention of blockage of gutters and similar drainage channels to reduce pooled rainwater), chemical (e.g. use of the insect growth regulator pyriproxyfen) and biological (e.g. use of bacterial toxins such as *Bacillus thuringensis israelensis* or predators such as *Mesocyclops* copepods) control methods (Vu *et al*., 1998).

However, the choice(s) available are somewhat restricted by whether the water is intended for human consumption or not and the ability to locate, and so treat, all such temporally dynamic potential breeding sites for *Ae. aegypti*. Thus, for example, household water jars may be easily covered (physical) but external natural and man made water sources cannot all be located let alone be covered, whilst chemical control suffers from the same problem of the inability to locate and treat all breeding sites, increasing insecticide and larvicide resistance, and the fact that the larval rearing sites are in close proximity to humans, such as in the house or even drinking water reservoirs, and so is restricted by the risks of long term human exposure in addition to environmental and economic concerns. There is thus an increasing demand for alternative treatments including environmentally acceptable biocontrol based methods for treatment of non-household water container based breeding grounds.

Many biological control methods are, however, not mobile and so again are limited by the problem of locating and treating all the temporally dynamic breeding grounds, with loss of the control agent as a given site dries up or the mosquito moves away as a pharate adult. Thus mobile, prey seeking (or imago phoretic, such as, perhaps, microsporidia) biological agents would potentially offer an alternative weapon, being able to also control neighboring unlocated breeding sites and also new larval rearing sites as they become available.

Micronecta grisea Fieber (Heteroptera: Micronectidae), the pygmy waterboatmen, are true aquatic hemipteran insects with paddle-like legs that have all their developmental stages in water. They are excellent and active swimmers both on the surface and under water, feeding on insect larvae, including mosquito larvae, by capturing the prey in the water and then sucking out the haemolymph from the prey body. Combined with their widespread distribution from India and Sri Lanka to Taiwan and

Indonesia and Java (Nieser & Chen, 1999), their relatively small size and water size requirements, mobility between water sites, their natural habitat being stagnant water or parts of streams with little current flow, broadly the same as *Ae. aegypti* larvae, and that they are potential predators of *Ae. aegypti* larvae (Lekprayoon, 2006), this makes them potential alternative biocontrol agents in mosquito control programs.

A survey on the distribution of *Micronecta* spp. in all regions of Thailand, including inspections of water-storage jars which were breeding sites of *Ae. aegypti*, reported that the presence of *Micronecta* spp. was found to be 100, 89, 62 and 25 % of the outdoor jars in the central and eastern, north-eastern, northern and southern parts of Thailand, respectively (Suphapathom *et al*., 2002).

At the same time, chemicals for mosquito control are still widely used, especially temephos zeolite granules, the newly developed formulation of organophosphate insecticide lacking undesirable odor and water turbidity (Mulla *et al*., 2004). Temephos as an active ingredient at 1 ppm is the concentration currently used in the national control program for *Ae. aegypti* larvae in Thailand which provided highly satisfactory control of *Ae. aegypti* larvae for the period of more than three months (Thavara *et al.*, 2004). Successful mosquito control could be carried out by combining different complementary strategies to improve efficacy, reduce cost and harmful environmental impact. As chemicals are widely used in mosquito breeding sites, integrated control by using combination of larvicides and natural enemies would be improving control results. For chemicals, the study of effects on natural enemies is necessary. In some cases, predators should be studied on the effects of larvicide to guarantee the quality of the integrated control, as for improving the control efficiency not harming non-target species.

From studies related to types of water-storage container used and infested with larval *Aedes*, it was noted that glazed clay jars, especially 200 liters in capacity were the most commonly used water-storage containers (Kittayapong and Strickman, 1993; Jamulitrat *et al*., 1998; Thavara *et al*., 2001). In many areas of Thailand, glazed clay jars are used for drinking as well as daily-use water. Therefore, clay jars are important target containers that should be focused on for treatment when larval control programs against DHF vectors are carried out. Consequently, 1.2 l and 25 l clay jars were treated in this experiment.

As *Micronecta* spp., including *M. grisea*, are common species found throughout the country, this study was carried out to examine some biology of *M. grisea* nymphal instars and adults, together with their feeding ability upon *Ae. aegypti* larvae as a potential biocontrol agent. The study also compared the efficiency of *M. grisea* and temephos zeolite granules in controlling of *Ae. aegypti* larvae in similar water-storage containers with the same water capacity. Additionally, the evaluation of *M. grisea* predation in small scale containers were carried out as well as the observation of the effects of temephos on *M. grisea* to determine the results for future application in the field.

1.2 Objectives

1.2.1 To study some biological characters of *M. grisea*.

1.2.2 To evaluate the efficiency of *M. grisea* for the control of *Ae. aegypti* larvae instead of chemicals.

1.2.3 To compare the efficiency of *M. grisea* and temephos zeolite granules for controlling *Ae. aegypti* larvae.

1.2.4 To investigate the efficiency of *M. grisea* for the control of *Ae. aegypti* larvae in small scale containers.

1.2.5 To determine the effects of temephos zeolite granules on *M. grisea*

1.3 Hypothesis

1.3.1 There will be no difference between the efficiency of *M. grisea* and temephos zeolite granules for controlling *Ae. aegypti* larvae.

1.3.2 The application of temephos will cause *M. grisea* mortality.

1.4 Scopes of study

1.4.1 *M. grisea* were collected from Bang Bua Thong District, Nonthaburi Province, Thailand and were brought to establish laboratory cultures at Department of Biology, Faculty of Science, Chulalongkorn University.

1.4.2 Efficiency of *M. grisea* and temephos zeolite granules on controlling *Ae. aegypti* larvae were evaluated.

1.4.3 Investigation of *M. grisea* predation on *Ae. aegypti* larvae were performed in small scale containers, 25 l jars.

1.4.4 Effects of temephos on *M. grisea* were also determined.

CHAPTER II

LITERATURE REVIEWS

2.1 Mosquitoes

In the world, there are more than 4,300 mosquito species that were officially classified. In that number, more than 600 species exist in Asia with about 60 species being vectors in tropical countries.

In Thailand, there are more than 200 mosquito species including 10 vector species. The vector-borne diseases from mosquito are mainly malaria, dengue fever, Japanese encephalitis (JE) and filariasis which were found in both human and animals (Baimai, 2002). The three main mosquio vectors are *Culex* sp., *Anopheles* sp. and *Aedes* sp.

Culex spp. are vectors of Bancroftian Filariasis and Japanese Encephalitis. Larvae are found in polluted water, rice field as well as pigsty breeding places (Malainual *et al*., 1988; Tawatsin, 2001). *Anopheles* spp., vectors of Malaria, larvae are found in streams especially with plants growing along the shore (Hormchong, 2000). *Aedes* spp. are vectors of the yellow fever, dengue fever and Chikungunya viruses (Sucharit, 1988). Their habitats are mainly household water-storage containers with clean water.

2.2 *Aedes aegypti*

2.2.1 Classification

Kingdom Animalia

Phylum Arthropoda

Class Insecta

Order Diptera

Family Culicidae

Genus *Aedes*

Species *Aedes aegypti* Linnaeus, 1762

Aedes aegypti L. (Diptera: Culicidae), the yellow fever or dengue mosquito, is the principal vector for transmission of the yellow fever, dengue fever and Chikungunya viruses (Sucharit, 1988). It is the principal vector for transmission of the dengue flavivirus in humans across the tropics and subtropical regions, including within Thailand. Infection with one of the dengue virus strains can cause dengue fever or the more serious dengue hemorrhagic fever (DHF), and has been estimated to cause an average morbidity in some 40 million and 2-300,000 people annually worldwide, respectively, and some 30,000 cases a year in Thailand, although regional epidemics can be large.

Urban *Ae. aegypti* populations are well adapted to human environments and breed in both indoor and outdoor water-storage containers, including empty coconut shells, flag pole holders, gutters, plant pot trays, refuse bins, etc. It was also reported from the surveys of *Aedes* mosquito around Samui island in Thailand, from July 1995 to July 1996, that most breeding sites were ceramic jars, bathroom basins, ant traps, automobile tires and natural containers such as coconut shells, mangosteen shells and durian shells (Tawatsin *et al*., 2007).

2.2.2 Life cycle

Ae. aegypti is easily recognized by white spots on the body and head regions and white rings on the legs. It is a holometabolous insect, going through a complete metamorphosis with an egg, larva, pupa and adult stage. The developmental period from eggs to adult range from 9 – 14 days depending on environmental conditions, such as temperature, food supply, densities, etc (Thavara, 2001).

Eggs are laid separately and deposited on damp surfaces within artificial containers like cans, jars or rainwater containers. *Ae. aegypti* eggs are long oval-shaped of one millimeter in length (Sucharit, 1988; Hormchong, 2000; Thavara, 2001). When first laid, eggs appear white but within 12 – 24 hours turn shiny black. Hatching occurs within 1 – 2 days and eggs can survive desiccation for more than a year and hatch once submerged in water. Females preduce 50 – 150 eggs per batch; however, the number of eggs produced depend on the quantity of blood meal. Females can lay 1 – 7 batches of eggs in a lifetime.

After hatching, *Ae. aegypti* larvae develop by molting through four developmental instars, from the first instar larvae of 1 millimeter to the fourth instar larva of 6 – 8 millimeters in length. They spend about 5 – 7 days to pass through four instars. Larvae feed mostly on small aquatic organisms, algae and particles of plant and animal materials. They feed in the bottom and inner surface of the water containers. Larval *Ae. aegypti* breathe oxygen through spiracle and a posteriorly located siphon.

After the last molt, larvae enter the pupal stage with a respiratory tube on the head segment, called trumpets. At this stage, they do not feed and take 1 – 2 days to develop to be adult.

Adult *Ae. aegypti* are 4 – 5 millimeters long. Males develop faster than females with longer life span in females, 1 – 3 months, than male, living 1 – 4 weeks. Mating begins within 24 hours after hatching into adults (Hormchong, 2000). The male's antennae are conspicuous and plumose whilst the female's are pilose. Both females and males feed on nectar, fruit juice, plant juice and sugar water but only females feed on blood in order to be food supply for laying their eggs.

In controlling of DHF vectors, physical, biological and chemical controls are used throughout Thailand. For evaluation of controlling methods in the field, the survey of mosquito population are needed. There are three main targets for the survey consisting of mosquito larvae survey, mosquito adults survey and the survey for the eggs (Chansang, 2001).

2.3 The control of *Ae. aegypti*

Since the time DHF has been reported in Thailand, it has become one of the major public health problems in the country. At present, no effective vaccine is available and therefore, the control of the disease relies mainly on the control of mosquito vectors. The two main approaches used for controlling *Ae. aegypti* in Thailand are adult and larval control programs (Thavara *et al*., 2004). Controlling adult mosquitoes, by space spraying, is usually carried out as an emergency measure for suppressing vector populations during epidemic outbreaks, whereas larval control, such as using natural enemies and applying larvicides in various mosquito breeding sites, are primarily relied upon and used routinely.

The larval controls are performed by three approaches, which are physical, chemical and biological control methods.

2.3.1 Physical control

To reduce the numbers of adult mosquitoes, management of both natural and artificial breeding sites should be performed in all areas. Firstly, all water containers should be covered with clothes, meshes or concrete plates. Secondly, the dustbins should be upturn for draining. Thirdly, the control also requires prevention of blockage of gutters and other drainage channels to reduce pooled rainwater. The final step is to get rid of all the used water containers, such as drinking bottles, cans, broken jars, plastic buckets and old tires (Thavara, 2001).

Oil surfactant was tested against larvae and pupae of vector species, *Ae. aegypti*, *Anopheles dirus* and *Culex quinquefasciatus* (Tawatsin *et al.*, 2001). The strategy was introduced as an alternative physical control of mosquito population by physical wetting action causing hydrophilic in larvae and pupae which can lead to anoxia and mortality. Results revealed that oil surfactant was more effective with the pupae than the larvae. The control showed the best result in *An. dirus* followed by *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. Oil surfactant that should be used in the surface area of 50 cm^3 was 2 µl for the control of *An. dirus* and 5 µl for both *Ae. aegypti* and *Cx. quinquefasciatus*.

2.3.2 Chemical control

The application of chemicals for the control of mosquito is still widely used because of the rapid action and high reliability. A single application may form persistent residue that continue to kill the mosquito for hours, days or even months after application. There are five major groups of chemicals used for mosquito control in Thailand (Thavara, 2001).

(1) Organochlorine compounds (e.g. DDT) These environmentally stable compounds are highly soluable in lipids, making it possible for them to accumulate in the body fat of non-target organisms. Predators, particularly those at the top of a food chain, amass pesticide concentrations many times greater than anywhere else in the environment. Bioaccumulation (or biomagnification) was responsible for high levels of DDT and most organochlorine insecticides were banned during 1970's and 1980's.

(2) Organophosphorus compounds (e.g. malathion, temephos) Most of them are used as to overcome resisitance of organochlorines with higher cost but lower breakdown period (3 – 5 months)

(3) Carbamate compounds (e.g. propoxur, methomyl) Also, Carbamates are sometimes used to avoid resistance of organochlorines for especially air-borne effects on mosquitoes.

(4) Synthetic Pyrethroid compounds (e.g. permethrin, deltamethrin) These compounds are highly effective on controlling of mosquitoes with low toxicity on mammals. They are short residued so should be sprayed weekly.

(5) Natural products Pyrethrum, derived from the dried flower of *Chrysanthemum* sp., is a contact insecticide attacking the nervous system of insects almost immediately. It can knock down the mosquito within 2 – 3 minutes after high absorption causing immobilization, paralysis and death. Pyrethrum has been approved for a wide range of indoor and outdoor uses, including homes, restaurants, broad-scale spraying operations and organic farms. Advantage of this product is the short breakdown period in the environment and that it can be degraded by the combination of sunlight and air.

Thai medicinal plants were also tested for larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* larvae (Chamavit *et al*., n.d.). Eight medicinal plants, *Acorus caramus*, *Annona squamosa*, *Croton tiglium*, *Duranta repens*, *Nerium indicum*, *Sapindus rarak*, *Stemona tuberose* and *Stephania pierrei*, were extracted by maceration with 70% ethanol or water for 3 days. Results showed that, within 24 hours, ethanolic extract of *A. squamosa* performed the highest larvicidal activity with LC_{50} of 34.56 and 4.96 mg/L for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively.

Laboratory studies on volatile oils derived from Lemon grass (*Cymbopogon citratus*), Citronella grass (*Cymbopogon nardus*) and May Chang (*Litsea cubeba*) was evaluated for repellent activity against *Ae. aegypti* and *Cx. quinquefasciatus*. Each oil was prepared as liquid formulation (non-gassed spray) at three different concentrations: 5%, 10% and 15% in ethanol (95%) and tested against mosquitoes at dosage of 0.18 – 0.2 g/m^2. Results showed that repellencies of three volatile oils against *Cx. quinquefasciatus* were significantly higher than those of *Ae. aegypti*. Repellencies of Lemon grass, Citronella grass and May Chang against *Cx. quinquefasciatus* were 28.2 –

68.2%, 33.3 – 80.7% and 48.1 – 76.2%, respectively, indicating equal efficiency as mosquito repellent (Santiwitchaya *et al*., 2007)

For the control of *Ae. aegypti* larvae, chemicals that are mainly used are temephos sand granules (Abate), temephos zeolite granules (AZAI-SS) and Insect Growth Regulators (IGRs) (Thavara, 2001).

(1) Temephos sand granules and temephos zeolite granules

The concentration for applying in water is 1 ppm (10 g temephos/100 l water) with the persistence of 8 – 20 weeks. The resistance was found in *Ae. aegypti* and *Ae. albopictus* of some countries. However, since temephos have been used for 30 years, resistance in *Aedes* mosquitoes of Thailand are also at high risk (Thavara, 2001).

Resistance of *Ae. aegypti* larvae to temephos was investigated in 7 provinces in the north eastern part of Thailand (Sornpeng, n.d.). The tests with 0.02 mg/L of temephos revealed that larvae from Mukdahan Province showed the highest LC_{50} (0.011 mg/L) followed by Sakolnakorn Province (0.007 mg/L) and Sri saket Province and Amnaj Charoen Province, the last two showing the lowest LC_{50} (0.003 mg/L).

(2) Insect Growth Regulators (IGRs)

(a) Juvenile hormone analogues (e.g. methoprene with trade names: Altosid, Apex, Precor, Ovitrol, etc) which caused mortality during the molting period and inhibit larval development with the persistence for 2 – 4 weeks. Pupae already existed in the containers prior to the test were not affected, but, after the test, mortality of the newly emerged larvae and pupae may be found (Thavara, 1979). Methoprene was reported the oral LD_{50} of 34,600 mg/kg.

(b) Chitin synthesis inhibitors (e.g. diflubenzuron with trade names: Dimilin, Difluron, Larvakil, Novaluron, triflumuron, etc) have potential to obstruct the chitin synthesis of the larvae and resulted in mortality within exposure of shorter period of time comparing with juvenile hormone.

The field evaluation of diflubenzuron of two formulations, tablets and granules, reported the high level of residual activity against *Ae. aegypti* larvae. The tests, at 0.05 – 0.1 mg AI/L, in 200 l clay jars, provided long-lasting control for 3 – 4 months (Thavara *et al*., 2007)

Novaluron, an insect growth regulator belonging to the benzoylphenyl urea insecticide, was evaluated against polluted-water mosquitoes, mostly *Cx. quinquefasciatus*, in low-income communities in urban areas of Bangkok, Thailand. The formulation was applied by pressurized spray tank at the rate of 10 mg AI/m^2 and was found to dramatically suppress the immature populations of mosquitoes in the treated areas. Moreover, the population remained at extremely low levels of 3 – 7 weeks after treatment (Tawatsin *et al*., 2007).

The IGRs were with high safety level to all organisms, including mammals, birds, fishes, and plants, as well as the environment. But these compounds are effective when exposed over long period of time, not causing acute toxicity to the larvae. Also, the high cost and toxicity to non-target organisms are concerned.

(3) Others

However, many studies have been carried out on the efficiency of other chemical controls and reported on the efficiency of the methods. For instance, the laboratory study on the effect of the red lime solution on *Ae. aegypti* larvae (Thavara and Phan-Urai, 2001) revealed that red lime solution killed all four larval instar of *Ae. aegypti* with the LC_{50} of 0.063, 0.067, 0.07 and 0.138%, as well as LC_{95} of 0.071, 0.08, 0.11 and 0.26% for the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larvae, respectively.

The new type of insect repellent containing 20% diethyl toluamide and 0.5% permethrin, formulated as a soap, was evaluated the efficacy and results indicated that the formulation provided protection time of 6 hours in laboratory and 5.75 hours in the field (Thavara *et al*., 1990).

Furthermore, the study of the effect of Lysol solution on *Ae. aegypti* larvae showed that LC_{50} and LC_{95} for the 4^{th} larval instars is 0.017 and 0.025%v/v, respectively. The solutions were persistent in water for 11 days after application (Thavara and Phan-Urai, 2001).

The convenience and effectiveness made insecticides and larvicides become standard practice for pest control during the 1960's and 1970's. But overuse, misuse and abuse of these chemicals have led to widespread criticism of chemical control and, in a few cases, resulted in long-term environmental consequences (e.g. soil contamination, water contamination, air pollution, etc). Furthermore, high efficiency can cause serious treat to non-target organisms, including natural enemies, which can have an enduring impact on the ecological balance in the community.

Finally, mosquito may become resistant to insecticides as innate change in behavior may occur to reduce the probability of encountering a toxicant. Some may manage to escape lethal doses of insecticide by avoiding enclosed areas and refusing to land on treated surfaces. Resistance is an inheritable capacity developed in a population of normally susceptible mosquitoes. It derives from the selective effect of exposures that kill or disable a portion of the population. With repeated insecticide applications generation after generation, a population comes to consist mainly of individuals carrying pre-adaptations, by process of Darwinian selection. A population is usually termed "resistant" only when it has reached a level that results in a control failure in the field with the recommended dosage of the insecticide (Watson and Brown, 1977).

After resistance to one insecticide, mosquito may also become less susceptible to other toxicants in the same chemical family, commonly found in organochlorines, organophosphates, carbamates and synthetic pyrethroids.

2.3.3 Biological control

Controlling the mosquito population by biological control refers to natural control strategies that need biological agents, including natural enemies (e.g. predators, bacteria). Biological agents are most effective when they are prey-specific and highly reproductive without side effect on other organisms (Thavara, 2001; Emden, 1989).

(1) Bacteria *Bacillus thuringiensis* H-14 (Bti) is spore-forming bacteria with crystal protein being toxic to mosquito larvae when ingested. The toxicity resulted in mortality within less than 48 hours, depending on the concentration of Bti. There are many formulations of Bti, granules (Bactimos), tablets (Vectobac) and liquid (Teknar). They are not duplicable but degradable in the environment and have low toxic to mammal with oral and dermal LD_{50} more than 30,000 mg/kg (Thavara, 2001).

Several *Bacillus thuringiensis israelensis* (Bti)-formulated products (e.g. water dispersible granules, paperstrips and tablets) were developed in order to improve its potency and efficiency in reducing *Ae. aegypti* larvae. Among all products, a 1 g tablet, with potency of 500 ITU/mg, demonstrated the best performance. Results from efficacy tests revealed that the tablet was highly toxic to *Ae. aegypti*, followed by *Culex* and *Anopheles* larvae (Lerdthusnee *et al*., 2007).

The study of *Bacillus thuringiensis* var. *israelensis* tablet formulation against *Ae. aegypti* larvae in different types of water found that LC_{50} of Bti were 0.2991, 0.4062 and 0.4078 mg/L, whereas LC_{95} were 0.7926, 0.9839 and 1.1834 mg/L for application in rainwater, tap water and groundwater, respectively. In addition, laboratory tests reported the longest persistence occurred in rainwater (18 days) followed by tap water (17 days) and groundwater (12 days), respectively (Thiravirojana, 1994).

From the study on Bactimos Briquets against late 3^{rd} instar larvae of *Ae. aegypti*, LC_{50} was found to be 0.54072 mg/L and the field-simulated experiment provided excellent control, with inhibition of emergence > 90%, at the dosage of ¼ dunk/200 l jar, for at least 8 weeks (Sithiprasasna *et al*., 2007).

The test of efficacy and longevity of Mosquito Dunks were conducted to determine the potency of Bti product against *Aedes* mosquito species. LC_{50} and LC_{95} were 1.02 and 1.86 ppm for *Ae. aegypti* and 0.39 and 0.84 ppm for *Ae. albopictus*. The semi-field evaluation of this product in 200 l clay jars showed satisfactory control of greater than 80% against *Ae. aegypti* larvae at 11 weeks post-treatment (Fansiri *et al*., 2007).

Chui *et al.* (1995) studied on using Bti (granular formulation, Vectobac-G) and teflubenzuron against *Ae. aegypti* larvae. The results showed high efficacy in reducing larval population at LC_{95} of 1.64 mg/L and 4.06 µg/L for Bti and teflubenzuron, respectively. Bioassays showed that teflubenzuron has a higher degree of residual activity than Vectobac.

Bacillus sphaericus (Bs) is also spore-forming bacteria with low toxic to human and animals. Mostly, Bs is used for *Culex* sp. larval control and Bs is duplicable in clean and polluted water (Thavara, 2001).

(2) Larvivorous fish Mosquito controls were successful by using larvivorous fish in many countries (e.g. Spain, Italy, Greece, North Africa, India, China, Malaysia, etc). Many genera of fish were applied to be biological agents and were focused on for the ability of feeding on larvae. Laboratory tests and publications had been reported and published since 1880. Until 1960, numbers of fish were known to be 714 species, mostly being in Suborder Cyprinodontidae.

Poecillia reticulata is the most popular larvivorus fish in Thailand with the application of 3 – 5 individuals per 1 m^2 of the water surface area. It feeds on all preadult stage of mosquito (egg, larval stage and pupal stage). Moreover, *Charias cusus*, *Tilapia mossambicus*, *Gambusia affinis* and *Aplocheilus dayi* are effective control agents of dengue mosquito in other countries (Thavara, 2001).

(3) Invertebrate predators They are natural enemies of mosquito, such as copepods, *Toxorhynchites* and some insect nymphs living in water. Some predators are highly reproductive in short period of time.

Copepods, *Mesocyclops*, are freshwater zooplankton with efficiency in controlling the 1st and 2nd instar larvae of *Ae. aegypti*. Individual copepods can feed on 15 – 20 larvae a day (Thavara, 2001; Vu *et al.*, 1998).

Toxorhynchites splendens larvae feed on larvae of *Anopheles* sp., *Culex* sp. and *Aedes* sp. The study on the efficiency of this predaceous mosquito larvae in controlling of larvae and pupae of *Ae. aegypti* reported that the 3rd instar larvae of predaceous mosquito exhibited higher predation on L3 *Ae. aegypti* than predation on L4 and pupal stage, respectively (Sungsirin, 2004).

So far, no study reported on controlling mosquito larvae by using *Micronecta* spp., pygmy waterboatmen, only the related heteropteran species, *Enithares* sp., were evaluated for the efficiency on mosquito larvae (Chittihunsa, 1980). The laboratory test revealed that the backswimmers showed excellent performance feeding on all four instar larvae of mosquito with higher efficiency on the 1st and 2nd instar larvae than the 3rd and 4th instar larvae. In addition, backswimmers demonstrated the highest mortality in *Ae. aegypti* followed by *Cx. quinquefasciatus* and *An. balabacensis*, respectively

However, biological control obviously takes some time for biological agents to spread from the points of release and result their impact on the prey population. The good point is that it can provide effective results by natural enemies with no side effects. Biological agents do not carry the kind of environmental danger associated with insecticides. Furthermore, they are self-propagating as bio-control agents will persist in time and may spread over large areas from the points of release (Emden, 1989).

2.4 *Micronecta grisea*

2.4.1 Classification

Kingdom Animalia
Phylum Arthropoda
Class Insecta
Order Hemiptera
Suborder Heteroptera
Family Micronectidae
Genus *Micronecta*
Species *Micronecta grisea* Fieber, 1844

Hemipterans, characteristically, have mouthparts adapted for piercing and sucking the fluids from plants or animals (Williams and Feltmate, 1992). Mostly Hemiptera's nymphs differ from adults in such details as numbers of antennal segments and presence of ocelli, as well as wing development. Among the present hemipteran fauna, members of one suborder, the Heteroptera, includes a minority of predatory and ectoparasitic species (Daly, *et al.*, 1978).

Worldwide, there are about 3,200 species of hydrophilic Heteroptera. Typically, life cycle consists of five nymphal instars, after the egg stage, and the adult (Chittihunsa, 1980; Williams and Feltmate, 1992). In common with other hemimetabolous insects (no pupal stage), the nymphs are very similar to the adults in terms of their apperance, habitat and behavior, but are smaller.

Within the Heteroptera, there are several wholly aquatic or semi-aquatic families and many of these are predators. Subsurface predators, such as notonectids, detect prey both visually and through water-borne vibrations. The predator waits at the surface of the water or on some submerged perch (e.g. a plant or stone) and, once prey is detected, chases after it. Prey is caught with the prothoracic legs and once the prey has been subdued, the stylets are inserted through its cuticle and the body fluids are sucked out (Williams and Feltmate, 1992).

Micronecta grisea, pygmy waterboatmen, are aquatic hemipteran insects with paddle-like legs that have all their developmental stages in water. From the diagnosis and distribution of *M. grisea*, Nieser (2002) reported that *M. grisea* is macropterous species of 2.6 – 3.2 mm long and is generally large greyish brown. Corium are shown with two strongly broken longitudinal stripes which may be indistinct. Lateral margins of hemielytra presented with three elongate brown patches of which the middle one is the largest. The lack of a strigil and the peculiar form of the free lobe of left part of the eighth tergite are characteristic.

It is considered to be a widespread species, from India and Sri Lanka through South East Asia to Sumatra, Java, Taiwan and possibly Sulawesi. There are also some specimens from Selangor and Perak. *M. grisea* is only known in the macropterous form. It occurs in both stagnant and running waters and has also been found in light catches. A survey on the distribution of *Micronecta* spp. in all regions of Thailand, including inspections of water-storage jars which were breeding sites of *Ae. aegypti*, reported that adult *Micronecta* spp. were approximately 2 – 3.5 mm long. The presence of *Micronecta* spp. was found to be 100, 89, 62 and 25 % of the outdoor jars in the central and eastern, north-eastern, northern and southern parts of Thailand, respectively (Suphapathom *et al*., 2002). They are excellent and active swimmers both on the surface and under water, feeding on insect larvae, including mosquito larvae, by capturing prey in the water and then sucking out the haemolymph from the prey body. Combined with their widespread distribution from India and Sri Lanka to Taiwan and Indonesia and Java (Nieser & Chen, 1999), their relatively small size and water size requirements, mobility between water sites, their natural habitat being stagnant water or parts of streams with little current flow, broadly the same as *Aedes aegypti* larvae, and that they are potential predators of

Ae. aegypti larvae (Lekprayoon, 2006), this makes them potential alternative biocontrol agents in mosquito control programs.

2.5 Temephos zeolite granules

Temephos is an organophosphate (OP) pesticide registered by EPA in 1965 to control mosquito larvae, and it is the only organophosphate with larvicidal use (United States Environmental Protection Agency, 2001). Trade names for products containing the compound include Abat, Abate, Abathion, Acibate, Biothion, Bithion, Difennthos, Ecopro, Nimitox and Swebate. In addition, temephos is also used to control midge, black fly larvae, fleas on dogs and cats and to control lice on human. Temephos is available up to 50% emulsifiable concentrates, 50% wettable powder and up to 5% granular forms. It is used in areas of standing water, shallow ponds, lakes swamps, marshes, intertidal zones and wetlands (EXTOXNET, 1996).

2.5.1 Toxicological effects

Temephos inhibits the action of enzymes called cholinesterase which control the nervous system, brain and musculoskeletal system including nerve signal transmission. It can overstimulate the nervous system causing nausea, dizziness, loss of muscle coordination, difficult breathing, and at high exposures, respiratory paralysis and death.

Reported oral LD_{50} values of temephos range from 1,226 to 13,000 mg/kg in rats and 460 to 4,700 mg/kg in mice (Gallo and Lawryk, 1991). For effects on aquatic organisms, temephos shows a wide range of toxicity, depending on the formulation. Generally, the technical grade compound (tech) is moderately toxic and the emulsifiable concentrate (ec) and wettable powder (wp) formulations are highly to very highly toxic. The most sensitive species of fish is the rainbow trout with LD_{50} of 0.16 mg/L (ec) and 3.49 mg/L (tech). Other 96-hour LD_{50} values are reported as: coho salmon 0.35 mg/L (ec), largemouth bass 1.44 mg/L (ec), channel catfish 3.23 mg/L (ec) and over 10 mg/L (tech), bluegill sunfish 1.14 mg/L (ec) and 21.8 mg/L (tech) and Atlantic salmon 6.7 mg/L (ec) and 21 mg/L (tech). Freshwater aquatic invertebrates such as amphipods are very highly susceptible to temephos, as are some marine invertebrates such as mysids. The 96-hour LD_{50} of temephos in *Gammarus lacustris* is 0.08 mg/kg and in stoneflies is

0.01 – 0.03 mg/kg. Because the compound is an insecticide and is used effectively to control the aquatic larval stages of mosquitoes, black flies and midges, its high toxicity to these organisms is not surprising (EXTOXNET, 1996).

2.5.2 Environmental breakdown

(1) Breakdown in soil and groundwater Little information is available about the fate and behavior of temephos in the environment. Based on its very low solubility in water, it would probably have a high affinity for soil. Based on this, a half-life of 30 days has been estimated (Wauchope, 1992), indicating a low to moderate persistence.

(2) Breakdown in water Weekly application of temephos at twice the normal application rates in pond water resulted in the rapid disappearance of the compound from the water and from the sediments. Temephos will be photolyzed in water (U.S. Public Health Service, 1995). Pesticide residues were detected in the water 2 hours but not 4 hours after application, indicating a very short persistence in the water.

Temephos zeolite granules, the new formulation of temephos, lacked undesirable odor and water turbidity when applied in water (Mulla *et al*., 2004; Thavara *et al*., 2004). When tested against *Ae. aegypti* larvae under field-simulated conditions, temephos were effective for 3 – 6 months, with the application rate of 1 g/ 10 L water. From the study of efficacy and longevity of temephos zeolite granules tested in village-scale trials against *Ae. aegypti* larvae in water-storage containers, a single application of temephos at 1 ppm provided highly satisfactory control of *Ae. aegypti* larvae for the period of more than three months (Thavara *et al*., 2004). Moreover, the study on the efficacy of temephos zeolite granules in 200-liter water-storage containers found that the temephos resulted in 100% reduction of *Ae. aegypti* larvae within 19 weeks (Tawatsin *et al*., 2007).

CHAPTER III

FEEDING ABILITY OF *Micronecta grisea* NYMPHAL INSTARS AND ADULT ON *Aedes aegypti* LARVAE

3.1 Materials and Methods

3.1.1 *Micronecta grisea*

M. grisea were collected from water-storage clay jars at the Research Station for Mosquito Biology and Control of the Department of Medical Sciences, Ministry of Public Health, Bang Bua Thong District, Nonthaburi Province, Thailand (Fig. 3.1). All samples were brought to, and used to establish laboratory cultures at, the Department of Biology, Faculty of Science, Chulalongkorn University. Laboratory cultures and experiments were performed at 25±1°C and an L: D period of 12:12. Five adult female and male *M. grisea* were kept in a 1.2 l clay jar for stock cultures and fed on *Ae. aegypti* larvae as in *ad libitum*. All clay jars used were covered with nylon meshes (1 mm mesh size) (Fig. 3.2).

Figure 3.1 Research station for mosquito biology and control

Figure 3.2 Clay jars covered with nylon meshes in laboratory

3.1.2 *Aedes aegypti* larvae

Freshly laid *Ae. aegypti* eggs (Fig. 3.3), attached on filter paper and dry, were obtained from the laboratory cultures of the Department of Medical Sciences, Ministry of Public Health, and were reared in 2 l plastic rectangular containers in the laboratory at Chulalongkorn University to obtain late L3 *Ae. aegypti* for use in the experiments. Excess larvae were not reared further but killed and in-house *Ae. aegypti* cultures were not established.

Figure 3.3 *Ae. aegypti* eggs

Figure 3.4 Pedigree

Tap water, for rearing all stadia of *M. grisea* and *Ae. aegypti* larvae, was dechlorinated by leaving to air in containers for 24 h. For *Ae. aegypti*, the dried eggs on filter paper were added to this water whereupon they hatched within 30 minutes and the larvae were fed daily with crushed Pedigree (Fig. 3.4), dog food at 0.1 g of food per 2 l of water containing 200 larvae (Fig. 3.5) and maintained at 25±1°C and an L: D period of 12:12. After 3 – 4 days under these conditions, larvae molted to the L3 stadia, observed under stereo microscope with the size range of 6 – 8 mm long (Fig. 3.6), which were then selected and used in the experiments.

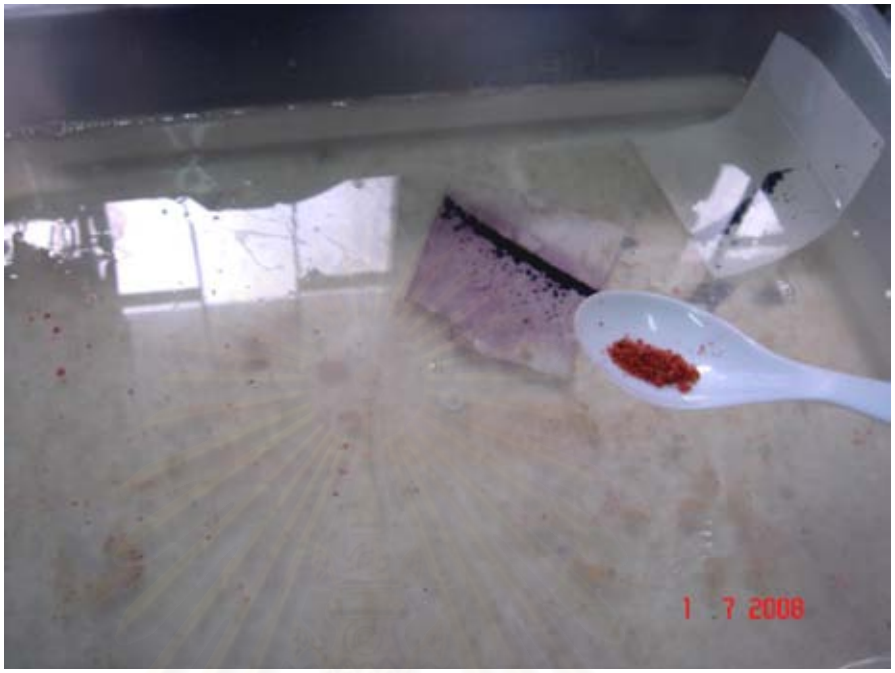


Figure 3.5 Feeding larvae with crushed Pedigree

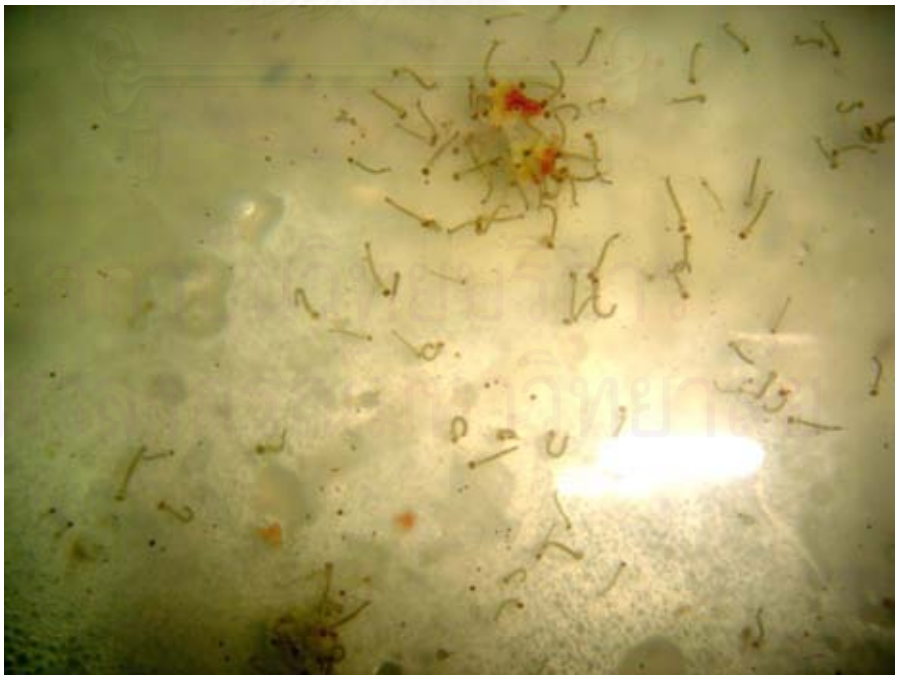


Figure 3.6 Third instar larvae of *Ae. aegypti*

3.1.3 *M. grisea* nymphal instars and adult

Measurements of the body length, head capsule size and head length were made on individual *M. grisea* nymphs and adults using a stereo microscope (32x) (Fig. 3.7). *M. grisea* were put individually on the petri dish by using a dropper. After that, tissue paper was used to dry up the water surrounding the insect body. The three characters were measured once for each individual by stage micrometer attached under the microscope.

Figure 3.7 Stereo microscope

To determine the size range of each of the *M. grisea* nymphal instars, as well as adults, the data for the three measured characters for the nymphal instars and adults were analyzed by scatter graph plots. The individuals were then ascribed to a given nymphal instar or adult category based upon the size distribution scatter plot analysis (see results) and then each category was reanalyzed for size range and variation within and between each defined nymphal instar and adults. The nymphal instars were grouped into three sizes; small (N1 & N2), medium (N3 & N4) and large (N5) for evaluation of their predation and feeding efficiencies and handling time of the different developmental stages of *M. grisea* at different predator (*M. grisea*) and prey (L3 *Ae. aegypti*) densities.

3.1.4 Mosquito larvae consumption

Feeding tests were conducted to determine the ability of the three nymphal size categories, plus adults, of *M. grisea* to feed on L3 *Ae. aegypti* as determined by predation efficiency and handling times.

To broadly standardize the hunger level of *M. grisea* (predator), and thus the potential hunting desire and, when prey is not limiting, the total consumption rate, the three size categories of *M. grisea* nymphs (small, medium and large) and adults were selected randomly from stock cultures and kept separately without food for the same period of time, that is for 24 h prior to experimentation. *M. grisea* were housed at three different densities, *viz.* 5, 10 and 20 nymphs or adults in 1.2 l clay jars filled with 1 l of dechlorinated tap water. After that, third instar *Ae. aegypti* larvae at one of three different densities, *viz.* 10, 20 and 40 larvae, were put into each clay jar containing the *M. grisea* at different densities to start the tests. After 24 h, the number of living larvae and the cadaver remains in the jars was recorded. In all experiments three replicates were performed for each combination.

The mortality numbers were adjusted by Abbott's formula (Abbott, 1925), and then used to calculate the percentage mortality of mosquito larvae in all experiments, and as a measure of predation levels. Plots of prey density against prey attacked were plotted to determine if the predator-prey relationship best fitted a type I or type II functional response.

Abbott's formula:

$$\text{Corrected \%} = 100 \times \left[1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}} \right]$$

When; n = insect population, T = treatment, Co = control

If Co mortality was 1) greater than 20%, tests were discarded.

2) between 5 – 20%, adjust the data by Abbott's formula.

3) less than 5%, use the mortality number in the treatments.

3.1.5 Statistical analyses

Data for the mean sizes of the three measured parameters for nymphal instars and adults were subject to ANOVA and Duncan's multiple means tests with significant differences accepted at the $P \leq 0.05$ level. For evaluation of the predation levels, the data obtained from all experiments were calculated as the mean percentage mortality from all replicates and then the percentage means of *Ae. aegypti* L3 mortality induced by each *M. grisea* category (nymphal instar and adults), as a measure of predation efficiency, was analyzed by *Mann-Whitney U*-test to compare predator efficiency within 24 h.

3.1.6 Feeding behavior of *M. grisea*

During the feeding tests with L3 *Ae. aegypti* mosquito larvae as the prey, searching and handling times of individual *M. grisea* nymphs and adults were recorded separately using a timer for each of the four size categories. Searching time started from the time when the prey (L3 *Ae. aegypti*) were put into containers until the time that the first prey was captured by a predator. The handling time started from the time the prey were captured until the time that predators released their prey or prey remnants, and included capturing, killing, eating and digesting (Holling, 1959).

The feeding time of *M. grisea* nymphs and adults were calculated by summation of both the searching and handling times.

3.2 Results

Measurements of the body length, head capsule size and head length of 401 *M. grisea* specimens of mixed developmental stadia revealed six discrete developmental stages of *M. grisea*, comprised of five nymphal instars (N1 – N5) and the adults, were recognized from the three measured characters when the data was analyzed by scatter graphs in Figs. 3.8A and 3.8B with $r^2 = 0.95$ and 0.81, respectively. No overlap in the body length distribution was found between each nymphal instar of the examined pygmy waterboatmen, whereas some overlap in the head capsule size and head length size distributions was noted between nymphal instars and also adults. Photographs of each stage of *M. grisea* are shown in Fig. 3.9.

Figure 3.8. The (A) body length and head capsule size (mm) and (B) body and head lengths (mm) of *M. grisea*. Data are shown for 401 individuals of mixed developmental stadia.

Figure 3.8A.

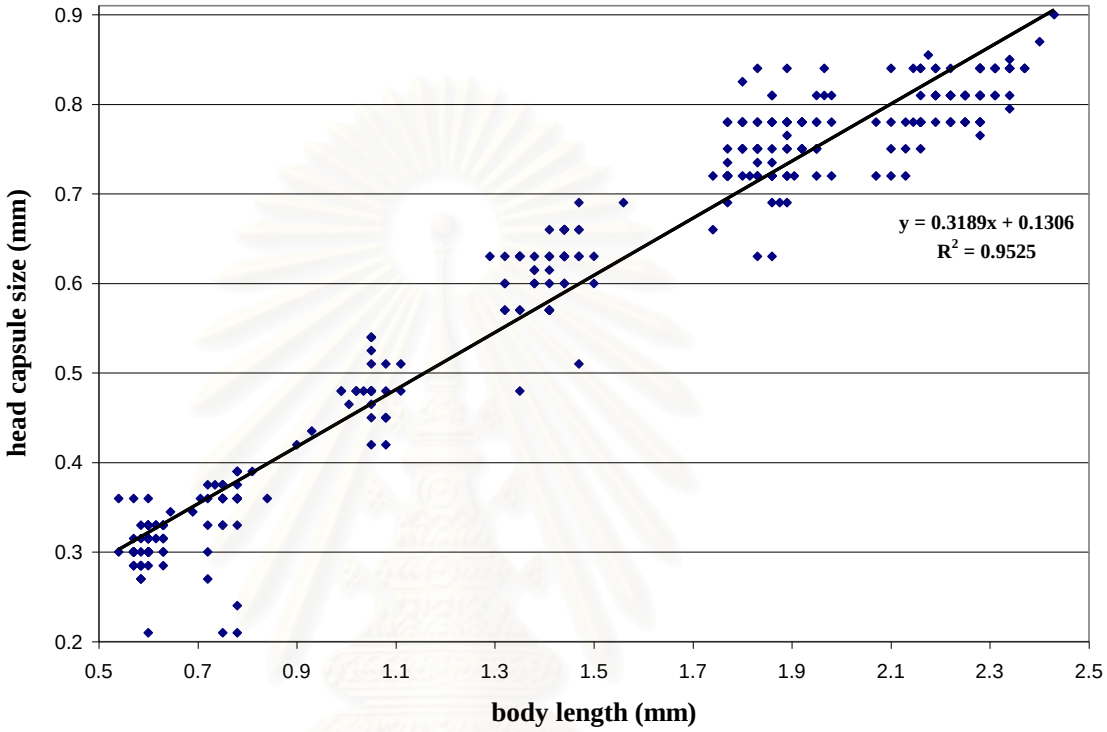


Figure 3.8B.

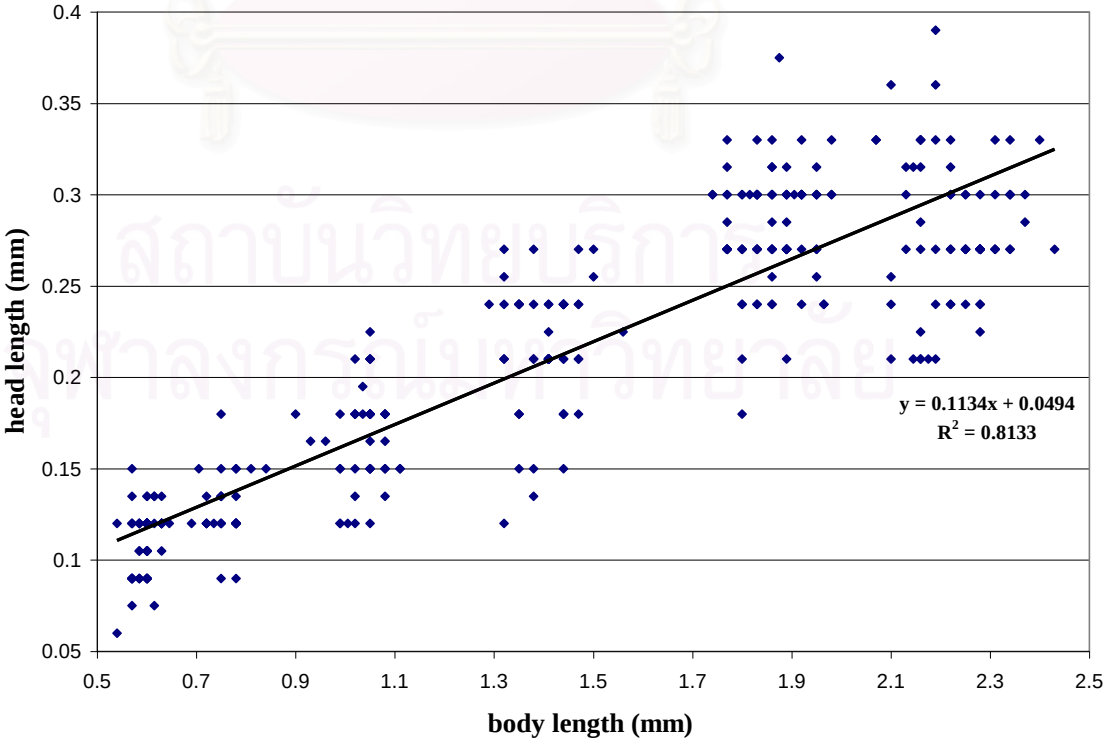
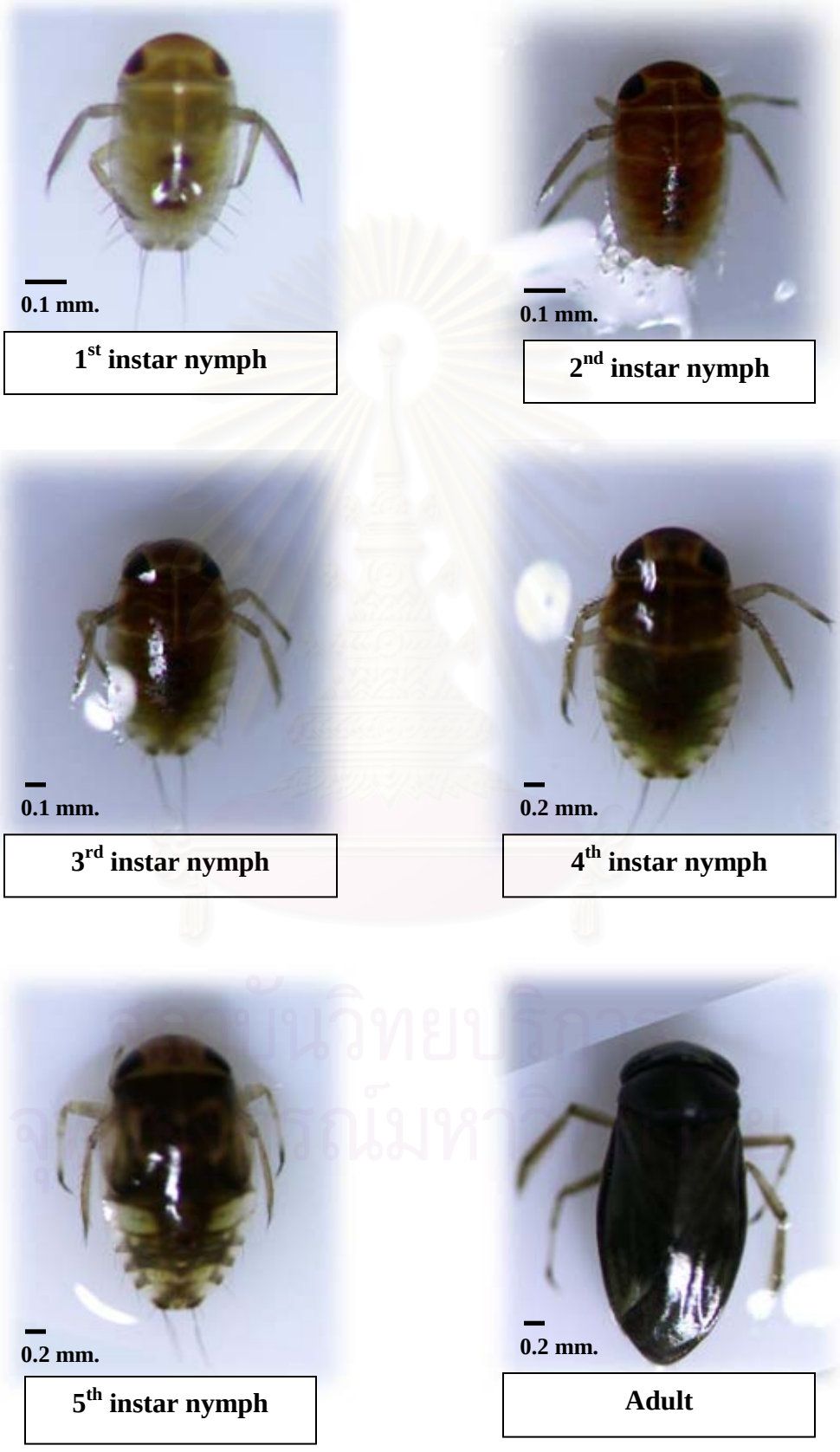


Figure 3.9. Photographs of six stages of *M. grisea* by using a stero microscope (32x)



The normality test and homogeneity test showed that the data were normally distributed and the variances were significantly homogenous at $P \leq 0.05$. A one-way analysis of variance (ANOVA) with Duncan's multiple comparison tests on the data for the three different measurements for each instar of the pygmy waterboatmen showed a significant differences in the mean body length between each instar (df = 5, 395, F = 9383.19, $P = 0.000$), but no significant difference was found within each instar, tested by ANOVA within each instar of pygmy waterboatmen. With discrete non-overlapping distributions between the five apparent nymphal instars plus the adults, the body lengths could be and were subsequently used for classifying these six discrete developmental stages of *M. grisea*, and as such split the 401 specimens into 73, 34, 63, 70, 90 and 71 individual N1, N2, N3, N4, N5 and adults of *M. grisea*, respectively. Analysis of the body length, head capsule size and head length within these six categories (developmental stadia) revealed that each nymphal instar and adults were significantly different ($P \leq 0.05$), except for head lengths between the N5 and adults (Table 3.1).

Using the discrete body size distributions as markers for the different developmental stages of *M. grisea*, the nymphal instars were grouped into small (N1 & N2), medium (N3 & N4) and large (N5) size groups for the predatory feeding tests with L3 *Ae. aegypti*. The feeding tests were performed with four size categories of *M. grisea* at three different densities (5, 10 and 20 per l container), each of which was supplied with live L3 *Ae. aegypti* as prey at three different densities (10, 20 and 40 larvae per l). The mean larval mortality within 24 h, used as the marker for predation rate, was evaluated and the data is summarized in Table 3.2.

Table 3.1. The range and mean (± S.E.) body length, head capsule size, and head length of each developmental stage of *M. grisea* (One-way ANOVA) ($P \leq 0.05$)

Stage	N	Body Length (mm)			Head Capsule (mm)			Head Length (mm)		
		MIN	MAX	MEAN±SE	MIN	MAX	MEAN±SE	MIN	MAX	MEAN±SE
1st nymphal instar	73	0.54	0.65	0.60±0.002a	0.21	0.36	0.31±0.003a	0.06	0.15	0.11±0.002a
2nd nymphal instar	34	0.69	0.84	0.76±0.005b	0.21	0.40	0.35±0.008b	0.09	0.18	0.13±0.003b
3rd nymphal instar	63	0.90	1.11	1.03±0.005c	0.42	0.54	0.48±0.003c	0.12	0.23	0.17±0.003c
4th nymphal instar	70	1.29	1.56	1.40±0.006d	0.48	0.69	0.61±0.005d	0.12	0.27	0.22±0.004d
5th nymphal instar	90	1.74	1.98	1.86±0.007e	0.63	0.84	0.75±0.004e	0.18	0.38	0.28±0.003e
Adult	71	2.07	2.43	2.23±0.010f	0.72	0.90	0.81±0.004f	0.21	0.39	0.28±0.005e

* Means in the same column with the same letters are not significantly different.

Table 3.2. Mean (± S.E.) mortality (%) of third instar *Ae. aegypti* larvae observed within 24 hours using different instars and densities of *M. grisea* (M^*; predator) with different densities of *Ae. aegypti* larvae (A^*; prey) (*Mann-Whitney U*-test) ($P \leq 0.05$)

M^*	S			M			L			ADULT		
A^*	5	10	20	5	10	20	5	10	20	5	10	20
10	$0a^{1*}$	$0a^1$	$0a^1$	$33.3 \pm 8.8a^1$	$40 \pm 5.8a^1$	$46.7 \pm 3.3a^1$	$50 \pm 5.8a^1$	$80 \pm 5.78a^2$	$80 \pm 5.8a^2$	$96.7 \pm 3.3a^1$	$100 \pm 0.0a^1$	$100 \pm 0.0a^1$
20	$0a^1$	$0a^1$	$0a^1$	$31.7 \pm 7.3a^1$	$30 \pm 8.ab^1$	$43.3 \pm 4.4a^1$	$45 \pm 2.9a^1$	$71.7 \pm 4.4a^2$	$80 \pm 5.8a^2$	$95 \pm 5.0a^1$	$100 \pm 0.0a^1$	$100 \pm 0.0a^1$
40	$0a^1$	$0a^1$	$0a^1$	$19.2 \pm 4.2a^1$	$25.8 \pm 1.7b^1$	$35.8 \pm 5.1a^1$	$28.3 \pm 0.8b^1$	$50.8 \pm 0.8b^2$	$69.2 \pm 6.0a^3$	$95.8 \pm 3.0a^1$	$95.8 \pm 2.2a^1$	$100 \pm 0.0a^1$

* Means in the same column with the same letters are not significantly different, whilst those across rows (within the same size of predator) with the same superscript numbers are not significantly different.

Analyses of the predator-prey relationship by plots of prey density against prey attacked were consistent with a Type II functional response (Fig. 3.10), and were tested using the Hollings disc equation. Predator satiation slightly occurred in M and L nymphs' functional response curves, but not existed in adult's curve. The proportion of prey consumed by predators declined with increasing prey densities. Functional response curves revealed that the decreasing rate, as for five medium nymphs, five large nymphs and ten large nymphs, dued to prey saturation of pygmy waterboatmen, while the constant consuming rate showed unsaturated conditions, more prey were still available for predators to consume. The adult, medium and large nymphal categories predated the L3 *Ae. aegypti* in all experiments, whilst in contrast the small nymphs showed no evidence of predation upon L3 *Ae. aegypti* in all tests.

The normality test showed that the data were not normally distributed and the homogeneity test also showed the variances to differ significantly at $P \leq 0.05$. Using *Mann-Whitney U*-test, the results revealed that as the prey numbers (and thus the prey: predator ratio) were increased the predation level (larval mortality) were significantly different when observed for *M. grisea* of the same developmental stage at the same density. Significant differences were shown for 10 of the medium size nymphs as well as 5 and 10 of the large size nymphs.

Comparisons of larval mortality, as a marker for predation levels, within each nymphal stage of *M. grisea* at different predator densities showed that, at the same prey densities, only the large nymphal size category consumption caused a significantly different mortality amongst the different predator densities (Table 3.2). In all prey densities (10, 20 and 40), large size nymphs at density of 5 performed significantly lower mortality than densities of 10 and 20.

Figure 3.10 Type II functional response of the predator (*M. grisea*) – prey (L3 *Ae. aegypti*) relationship at different predator densities (n). Data are shown for the (A) medium nymphs, (B) large nymphs and (C) adults of *M. grisea*

Figure 3.10A.

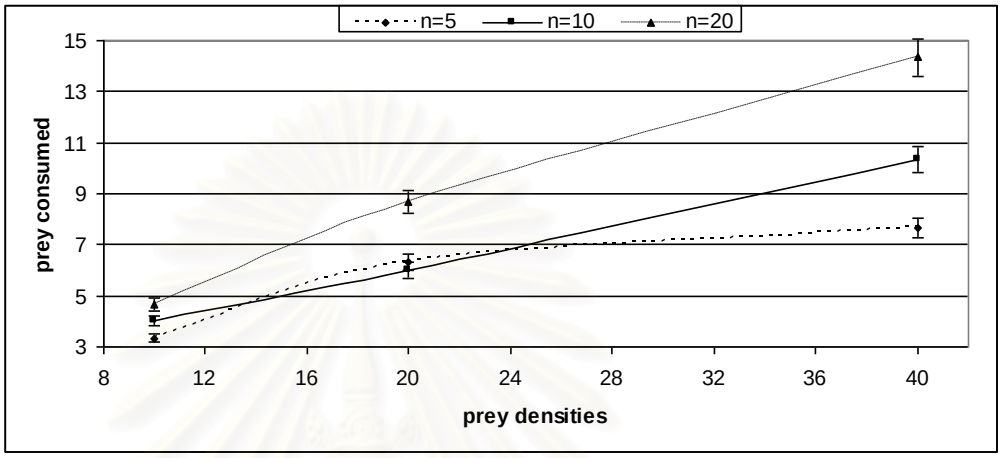


Figure 3.10B.

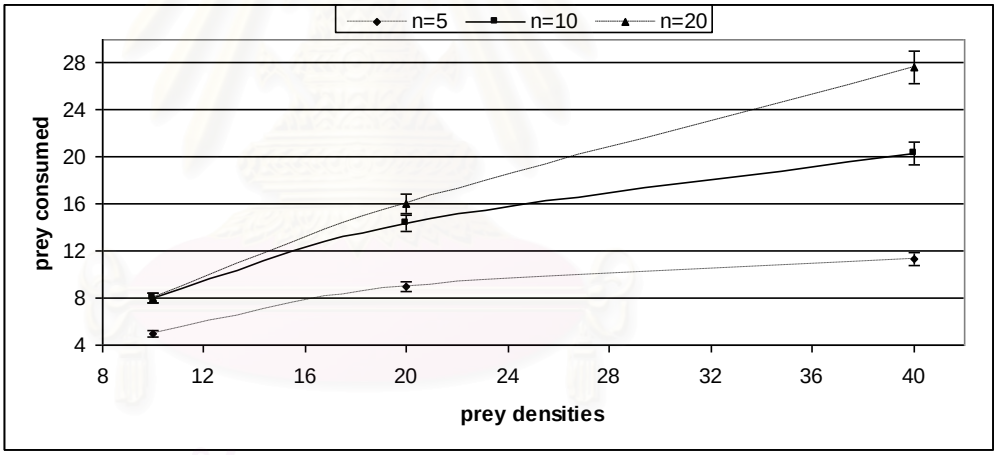
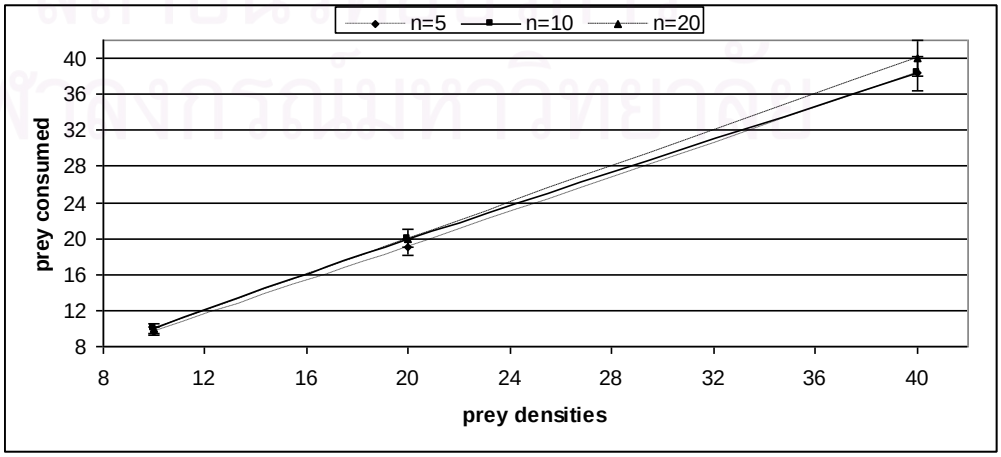


Figure 3.10C.



For a density of five *M. grisea* with 10, 20 and 40 prey (L3 *Ae. aegypti*), the consumption by medium and large nymphs were numerically, but not statistically significantly, different, whereas adults, caused a significantly higher predation level than both medium and large nymphs at all prey densities (Appendix A).

When the *M. grisea* density increased to 10 there were significant differences amongst the predation levels (mortality of L3 *Ae. aegypti*) between each size category of nymphal instars and adults of pygmy waterboatmen, with the predation levels increasing with increasing predator size (developmental stage)

At predator densities of 20, the prey consumption of medium sized nymphs was significantly lower than that of large nymphs, with that of adults being significantly higher still.

The laboratory no-choice feeding tests with *M. grisea* (predator) feeding on L3 *Ae. aegypti* (prey) revealed that, at a predator: prey ratio of 10:20, the searching and handling times of predators were significantly different between the medium and large nymphal stages and the adults (Table 3.3), with adult and medium sized nymphal *M. grisea* having the shortest and longest, respectively, searching and handling times. Therefore, with feeding time being simply the summation of searching and handling times, then the same trend was noted with adults having the shortest feeding time and medium stage nymphs the longest. Note that no data on searching, handling and feeding times is presented for the small *M. grisea* nymphs since no prey were ever caught or killed.

Table 3.3. Mean (± S.E.) searching, handling and feeding times of medium (M) and large (L) size category nymphs and adults of *M. grisea* upon third instar *Ae. aegypti* larvae (One-way ANOVA) ($P \leq 0.05$)

M. grisea stage	No. of *M. grisea* : No. of mosquito larvae	Searching time (s)	Handling time (s)	Feeding time (s)
M-size	10 : 20	93 ± 13a*	1680 ± 312a	1773 ± 314a
L-size	10 : 20	57 ± 10b	867 ± 182b	924 ± 185b
Adult	10 : 20	14 ± 1c	480 ± 42b	494 ± 43b

* Means in the same column with the same letters are not significantly different.

3.3 Discussion

Measurement of the body and head lengths and the head capsule size of different nymphal instars and adults of the pygmy waterboatman, *M. grisea*, revealed that five discrete nymphal instars, plus the adult stage, could be distinguished. Although no allometry was found between the three characters measured, the body length was found to have a discrete and well separated distribution pattern between these six developmental stages, whereas both the head capsule size and head length showed some overlap in their size distribution profiles between related developmental stadia. This notion of five nymphal instars is consistent with studies in the biology of the related waterboatmen (Gerridae) and the backswimmers, *Enithares* sp., which have also been reported to have five preadult nymphal instars in their development (Chittihunsa, 1980; Williams and Feltmate, 1992).

The body lengths of each instar, showing no overlap, were used for classifying each *M. grisea* instar. The non-overlapping discrete body length ranges were 0.54 – 0.65, 0.69 – 0.84, 0.9 – 1.11, 1.29 – 1.56 and 1.74 – 1.98 mm for the 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} (N1 – N5) nymphal instars, respectively, and 2.07 – 2.43 mm for adults. Although the adult size range is lower than that previously reported from the study of *M. grisea* in Singapore and Peninsular Malaysia, such as 2.6 – 3.2 mm (Nieser, 2002), it is in agreement with the results of a recent survey on the distribution of *Micronecta* spp. across all the regions of Thailand which found that adult *Micronecta* spp. were approximately 2 – 3.5 mm long (Suphapathom *et al*., 2002). This may be due to environmental conditions and diet sufficiency in different distribution regions.

In this study differences in the body length, head capsule size and head length of each nymphal and adult stage were noted except for head lengths between the N5 and adult stages which were approximately the same. This may be due to the fact that the anterior part of the head segment in adults was hidden underneath during measurement leading to variable underestimates in their size.

Within the 24 h feeding window, the predation and feeding ability of the pygmy waterboatmen (*M. grisea)* on L3 *Ae. aegypti* differed amongst each size category. Adult waterboatmen showed the highest predation rates (as *Ae. aegypti* L3 mortality) followed by large medium and small nymphs, in that order, with small nymphs revealing no detectable predation of L3 *Ae. aegypti* in all tests. Consistent with this observation is that under laboratory conditions larger sized backswimmers (*Enithares* sp.) caused a higher larval mortality (predation rate) than those of a smaller size (Chittihunsa, 1980). For the small sized nymphs of *M. grisea* in this study, it seems likely that they were too small, compared with the prey size available (L3 *Ae. aegypti*) in these no-choice experiments, to capture their prey whilst the newly hatched instar nymphs would also still have nutrients from the yolk available to them.

M. grisea of the same size categories (small, medium and large nymphal and adults) and at the same densities caused no significant difference in the larval mortality percentages (predation levels). Rather, within each size category *M. grisea* (predators), when at the same predator number (density), they consumed about the same amount of L3 *Ae. aegypti* prey items even when at higher prey densities, and so higher prey: predator ratios, suggesting that prey saturation may influence predator consumption. Of course, as the prey densities are increased for a given number of predators, and the total numbers of prey consumed remain the same (satiation), the evaluated prey mortality percentages would appear to decrease.

Although in general, predators may spend time on three types of activities, searching, handling their prey and then satiation related activities, we observed the former two in these assays. At the same prey density, the searching times of adult, large and medium sized nymphs of *M. grisea* were significantly different. Adults showed the shortest searching time followed by large and finally medium sized nymphs, respectively. The handling time showed a similar relationship, except that the handling time for large nymphs and adults were not significantly different. Consequently, feeding time paralleled the predator handling time.

Furthermore, in early experiments, cannibalism has been described in the backswimmers, *Enithares* sp. It was found that backswimmers kept without food performed higher rate of cannibalism than those provided with mosquito larvae (Chittihunsa, 1980). This indicated that cannibalism occurred in cases of severe stress. Throughout this experiment, as were observed during stock cultures and all feeding tests, *M. grisea* were not seen feeding on other individuals, showing no cannibalism. However, cannibalism may occur when *M. grisea* are in some severe conditions, such as lacking food supply.

Overall, adult *M. grisea* provided the highest L3 *Ae. aegypti* mortality in all tests with the shortest feeding times. This may be due to adults being more active and larger than nymphs, but also may reflect the relatively large prey size used. Thus, whilst it remains important to evaluate the predation efficiency and feeding times of all developmental stages of *M. grisea* upon all larval developmental stages of *Ae. aegypti*, it also remains of interest to evaluate if different developmental stages of *M. grisea* preferentially feed upon different larval developmental stages of *Ae. aegypti*, as well as other prey items, since *Ae. aegypti* development in urban water resources is frequently derived from multiple females and asynchronous.

CHAPTER IV

EFFICIENCY OF *Micronecta grisea* AND TEMEPHOS ON *Aedes aegypti* LARVAE

4.1 Materials and Methods

4.1.1 *Micronecta grisea*

M. grisea were collected from water-storage clay jars at the Research Station for Mosquito Biology and Control of the Department of Medical Sciences, Ministry of Public Health, Bang Bua Thong District, Nonthaburi Province, Thailand. All samples were brought to, and used to establish laboratory cultures at, the Department of Biology, Faculty of Science, Chulalongkorn University. Laboratory cultures and experiments were performed at 25±1°C and an L: D period of 12:12. Five adult female and male *M. grisea* were kept in a 1.2 l clay jar for stock cultures and fed on *Ae. aegypti* larvae as in *ad libitum*. All clay jars used were covered with nylon meshes (1 mm mesh size).

4.1.2 *Ae. aegypti* larvae

Freshly laid *Ae. aegypti* eggs, attached on filter paper and dry, were obtained from the laboratory cultures of the Department of Medical Sciences, Ministry of Public Health, and were reared in 2 l plastic rectangular containers in the laboratory at Chulalongkorn University to obtain late L3 *Ae. aegypti* for use in the experiments. Excess larvae were not reared further but killed and in-house *Ae. aegypti* cultures were not established.

Tap water, for rearing all stadia of *M. grisea* and *Ae. aegypti* larvae, was dechlorinated by leaving to air in containers for 24 h. For *Ae. aegypti*, the dried eggs on filter paper were added to this water whereupon they hatched within 30 minutes and the larvae were fed daily with crushed Pedigree, dog food at 0.1 g of food per 2 l of water containing 200 larvae and maintained at 25±1°C and an L: D period of 12:12. After 3 – 4 days under these conditions, larvae molted to the L3 stadia, observed under stereo microscope with the size range of 6 – 8 mm long, which were then selected and used in the experiments.

4.1.3 Temephos zeolite granules

Temephos zeolite granules, AZAI-SS ZG 1% (Fig. 4.1), containing 1% temephos as an active ingredient, were evaluated against *Ae. aegypti* larvae in water storage jars. They were used at 0.1 g per 1 liter of water, yielding 1 ppm of temephos in each container, a concentration that is currently used in the national control program for *Ae. aegypti* larvae in Thailand (Thavara *et al*., 2004). This formulation was a product of Ikari Trading (Thailand) Company Limited with manufacturing date of 25 September 2007.

Figure 4.1 AZAI-SS ZG 1%

4.1.4 *Ae. aegypti* larvae consumption by the fifth nymphal instar and adult of *M.grisea*

Feeding tests were conducted to determine the ability of two developmental stadia of pygmy waterboatmen, the fifth nymphal instar and adult, to feed on L3 *Ae. aegypti*.

To broadly standardize the hunger level of *M. grisea*, the fifth instar nymphs and adults were selected randomly from stock cultures and kept separately without food for the same period of time, that is for 24 h prior to experimentation. Ten *M. grisea* nymphs or adults were housed in 1.2 l clay jars filled with 1 l of dechlorinated tap water. To conduct the feeding tests, one replicate consisted of one control and two treatments (the fifth instar nymph and adult). After that, third instar *Ae. aegypti* larvae at density of 20 were put into each clay jar containing the *M. grisea* of two different stadia to start the tests. During 24 h, the number of living larvae and the cadaver remains in the jars was recorded at the 1st hour (every 15 min), 2nd hour (every 30 min), 3rd hour, 4th hour, 8th hour, 12th hour, and 24th hour. Each treatment and control (without waterboatmen) were replicated ten times.

The mortality numbers were adjusted by Abbott's formula (Abbott, 1925), and then used to calculate the percentage mortality of mosquito larvae in both experiments (nymphs and adults).

4.1.5 Larval mortality by temephos zeolite granules

Mortality tests were conducted to determine the efficiency of temephos on larval mortality of *Ae. aegypti* in 24 hours.

Ae. aegypti larvae were tested with temephos zeolite granules applied in water at 1 mg/L AI. In this experiment, one replicate consisted of one control and one treatment (tested with 0.1 g temephos). Twenty of L3 *Ae. aegypti* were placed into the 1.2-liter clay jars filled with 1 liter of water. Temephos granules weighed 0.1 g (Fig. 4.2), using 4-digit balance (Fig. 4.3), were added into the jars to start the tests. The number of living larvae and the cadaver remains in the jars was recorded during 24 hours; the 1st hour (every 15 min), 2nd hour (every 30 min), 3rd hour, 4th hour, 8th hour, 12th hour, and 24th hour. Each treatment and control (without temephos) was replicated ten times.

Figure 4.2 Temephos ZG weighing 0.1 g

Figure 4.3 4-digit balance

The mortality numbers were adjusted by Abbott's formula (Abbott, 1925), and then used to calculate the percentage mortality of mosquito larvae. Plots of larval mortality by *M. grisea* (nymphs and adults) and temephos at each period of time were plotted to compare the predation and larvicide efficiency within 24 hours.

4.1.6 *Ae. aegypti* larvae comsumption by using one adult *M. grisea*

Feeding tests were conducted in 1.2 l clay jar to determine the ability of individual *M. grisea* to feed on L3 *Ae. aegypti* as determined by predation efficiency upon L3 *Ae. aegypti* at three different densities.

To broadly standardize the hunger level of predator, the adult *M. grisea* were selected randomly from stock cultures and kept separately without food for 24 h prior to experimentation. *M. grisea* were housed at individually in 1.2 l clay jars filled with 1 l of dechlorinated tap water. To conduct the feeding tests, one replicate consisted of one control and three treatments (tested with 10, 20 and 40 of L3 *Ae. aegypti*). After that, L3 *Ae. aegypti* larvae at one of three different densities, *viz.* 10, 20 and 40 larvae, were put into each clay jar containing one adult *M. grisea* to start the tests. During 72 h, the number of living larvae and the cadaver remains in the jars was recorded at the 1st hour (every 15 min), 2nd hour (every 30 min), 3rd hour, 4th hour, 8th hour, 12th hour, 24th hour, 48th hour and 72nd hour. Each treatment and control (without waterboatmen) was replicated three times.

The mortality numbers were adjusted by Abbott's formula (Abbott, 1925), and then used to calculate the percentage mortality of mosquito larvae in all experiments.

4.1.7 Statistic analyses

For the evaluation of the predation efficiency (by 5th instar nymph and adult) and temephos efficiency, as well as predation by one adult *M. grisea*, the data obtained from all experiments were calculated as the mean percentage mortality from all replicates and then the percentage means of L3 *Ae. aegypti* mortality induced by *M. grisea* and temephos, as a measure of predation efficiency, was analyzed by *Mann-Whitney U* - test to compare efficiency within 72 h.

4.1.8 Feeding behavior of *M. grisea* at different L3 *Ae. aegypti* densities

Feeding behavior was observed through the feeding test conducted by using five adult *M. grisea* with different densities of L3 *Ae. aegypti* (10, 20 and 40).

After the hunger level was standardized by being kept separately without food for 24 h prior to experimentation, five *M. grisea* were housed separately in 1.2 l clay jars. After that, third instar *Ae. aegypti* larvae at one of three different densities, *viz.* 10, 20 and 40 larvae, were put into each clay jar containing five adult *M. grisea* to start the tests.

During the feeding tests with L3 *Ae. aegypti* mosquito larvae as the prey, searching and handling times of individual *M. grisea* were recorded separately using a timer. Searching time started from the time when the prey (L3 *Ae. aegypti*) were put into containers until the time that the first prey was captured by a predator. The handling time started from the time the prey were captured until the time that predators released their prey or prey remnants, and included capturing, killing, eating and digesting (Holling, 1959). The feeding time of *M. grisea* nymphs and adults were calculated by summation of both the searching and handling times.

4.2 Results

Feeding tests were performed with ten of the fifth nymphal instar and ten adults of *M. grisea* along with temephos zeolite granules (0.1 g). Each supplied with twenty live L3 *Ae. aegypti*. The mean larval mortality within 24 h was evaluated and the data is summarized in Table 4.1.

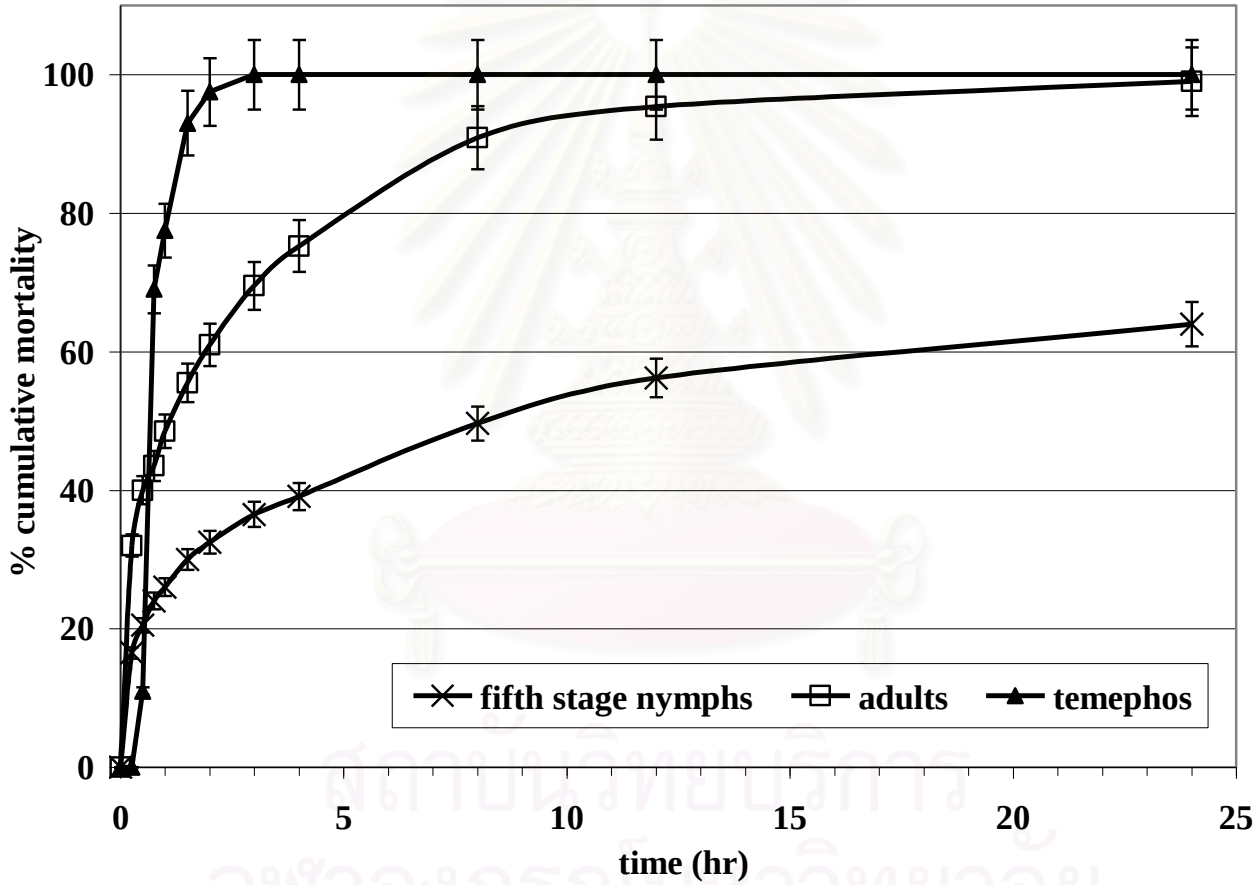
The normality test showed that the data were not normally distributed and the homogeneity test also showed that the variances differed significantly at $P \leq 0.05$. Analyzed by *Mann-Whitney U*-test, from the 1^{st} to 4^{th} hour of the feeding test, temephos showed significantly higher percentage of larval mortality than adult and the fifth nymphal instar of *M. grisea* (Fig. 4.4). Comparisons of larval mortality, by 24 hours, showed that adults (Fig. 4.5) and temephos (Fig. 4.6), with LT_{50} = 0.58 h (Appendix B), performed the highest mortality percentages (99% and 100%, respectively), whereas the fifth instar nymphs killed 64% of the mosquito larvae.

Table 4.1. Mean (± S.E.) mortality (%) of third instar *Ae. aegypti* larvae observed within 24 hours using the fifth nymphal instars and adult of *M. grisea* and temephos zeolite granules (*Mann-Whitney U*-test) ($P \leq 0.05$)

Time(hr)	**0**	**0.25**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**3.00**	**4.00**	**8.00**	**12.00**	**24.00**
5th instar nymph	0a*	16.5 ± 3.6a	20.5 ± 4.5a	24 ± 5.0a	26 ± 5.4a	30 ± 6.4a	32.5 ± 6.4a	36.5 ± 7.2a	39.1 ± 6.9a	49.6 ± 8.2a	56.2 ± 7.7a	64 ± 8.9a
Adult	0a	32 ± 3.7b	40 ± 3.1b	43.5 ± 3.9b	48.5 ± 4.7b	55.5 ± 5.7b	61 ± 6.4b	69.5 ± 6.2b	75.3 ± 5.5b	90.9 ± 2.9b	95.4 ± 2.1b	99 ± 0.7b
Temephos	0a	0 ± 0.0c	11 ± 5.9a	69 ± 4.3c	77.5 ± 6.6c	93 ± 2.6c	97.5 ± 1.5c	100 ± 0.0c	100 ± 0.0c	100 ± 0.0c	100 ± 0.0c	100 ± 0.0b

* Means in the same column with the same letters are not significantly different.

Figure 4.4. Mean mortality of third instar *Ae. aegypti* larvae by the fifth nymphal instar and adult of *M. grisea* and by temephos during a 24-h exposure



* Means in the same period of time with the same letters are not significantly different.

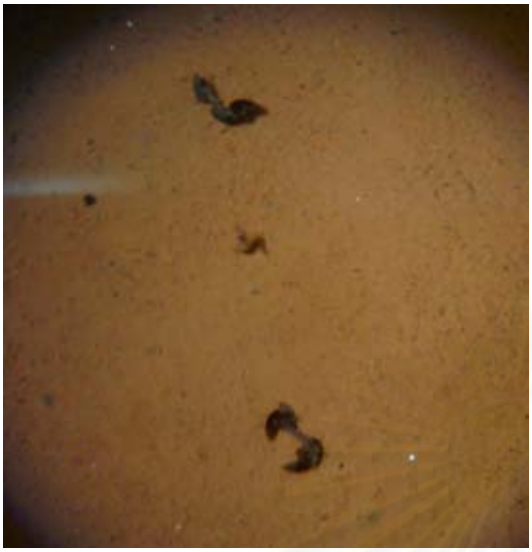


Figure 4.5 Larval mortality by adults

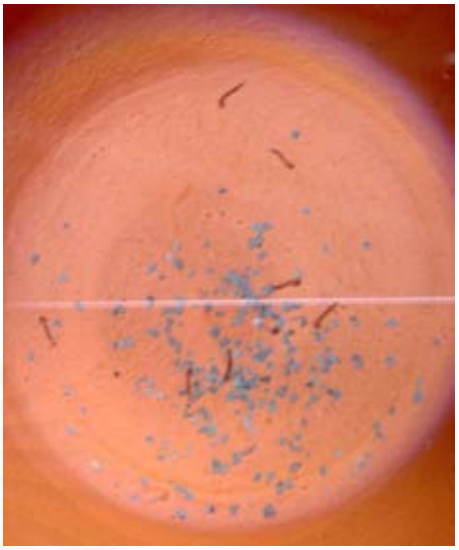


Figure 4.6 Larval mortality by temephos

Feeding tests were also performed with one adult of *M. grisea* and three different densities of L3 *Ae. aegypti* (10, 20 and 40). The mean larval mortality within 72 h was evaluated and the data is summarized in Table 4.2.

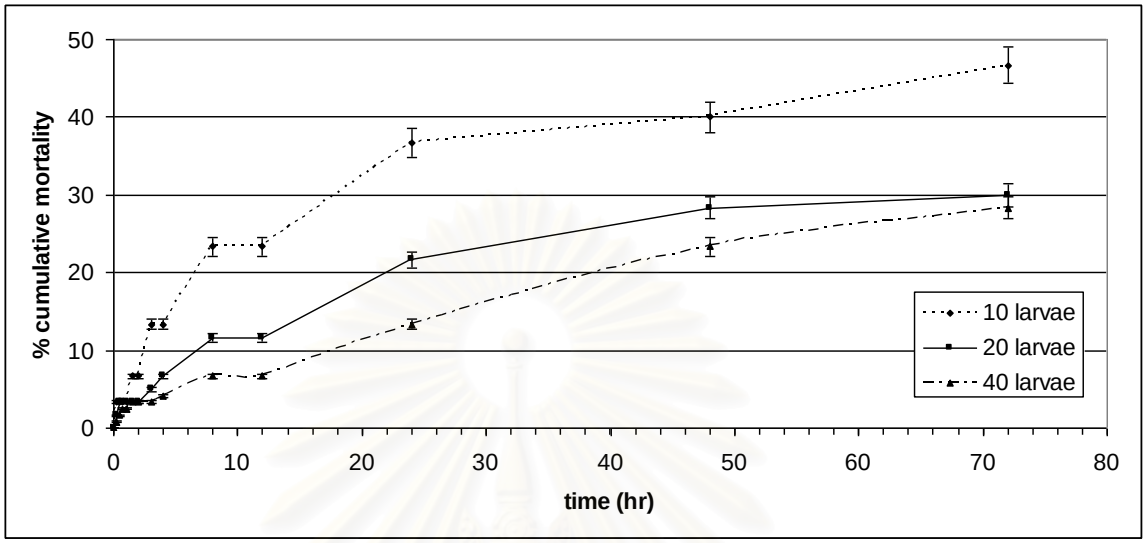
The normality test showed that the data were not normally distributed and the homogeneity test also showed significant difference of the variances at $P \leq 0.05$. Analyzed by *Mann-Whitney U*-test, from the 1st to 4th hour of the feeding test, larval mortality were numerically, but not statistically significantly different among different prey densities. In addition, feeding on prey for longer period, from the 8th hour until the end of the test (72 h), predator showed significantly higher mortality at prey densities of 10 than at prey densities of 40 (Fig. 4.7), whereas L3 *Ae. aegypti* mortality at prey densities of 10 and 20, as well as 20 and 40 were not significantly different. In comparison, within 72 h, one adult *M. grisea* performed the highest larval mortality at prey densities of 10 (46.7%) followed by prey densities of 20 (30%) and prey densities of 40 (28.3%), respectively.

Table 4.2. Mean (± S.E.) mortality (%) of third instar *Ae. aegypti* larvae observed within 72 hours using one adult *M. grisea* with different densities of *Ae. aegypti* larvae (10, 20 and 40) (*Mann-Whitney U*-test) ($P \leq 0.05$)

Time(hr)	0	0.25	0.50	0.75	1.00	1.50	2.00	3.00	4.00	8.00	12.00	24.00	48.00	72.00
No. of larvae														
10	0a*	3.3 ± 3.3a	3.3 ± 3.3a	3.3 ± 3.3a	3.3 ± 3.3a	6.7 ± 3.3a	6.7 ± 3.3a	13.3 ± 6.7a	13.3 ± 6.7a	23.3 ± 8.8a	23.3 ± 8.8a	36.7 ± 8.8a	40.0 ± 5.8a	46.7 ± 6.7a
20	0a	1.7 ± 1.7a	3.3 ± 1.7a	3.3 ± 1.7a	3.3 ± 1.7a	3.3 ± 1.7a	3.3 ± 1.7a	5.0 ± 2.9a	6.7 ± 3.3a	11.7 ± 6.0ab	11.7 ± 6.0ab	21.7 ± 8.8ab	28.3 ± 7.3ab	30.0 ± 7.6ab
40	0a	0.8 ± 0.8a	1.7 ± 0.8a	2.5 ± 0.0a	2.5 ± 0.0a	3.3 ± 0.8a	3.3 ± 0.8a	3.3 ± 0.8a	4.2 ± 0.8a	6.7 ± 0.8b	6.7 ± 0.8b	13.3 ± 2.2b	23.3 ± 0.8b	28.3 ± 4.6b

* Means in the same column with the same letters are not significantly different.

Figure 4.7. Mean mortality of third instar *Ae. aegypti* larvae by one adult *M. grisea* observed within 72 hours using different densities of *Ae. aegypti* larvae (10, 20 and 40)



* Means in the same period of time with the same letters are not significantly different.

Further laboratory feeding tests were conducted with five *M. grisea* (predator) feeding on L3 *Ae. aegypti* (prey) of three different densities (10, 20 and 40). The data revealed that no significant difference in feeding time was found between 5:10 (859.6 seconds) and 5:20 (1318.4 seconds), as well as 5:20 and 5:40 (1476.2 seconds), but feeding times at 5:10 were significantly less than at 5:40 ($P \leq 0.05$) (Table 4.3).

Table 4.3. Mean (± S.E.) feeding time of five adults of *M. grisea* upon third instar *Ae. aegypti* larvae at different densities (10, 20 and 40) (One – way ANOVA) ($P \leq 0.05$)

No. of adult of *M. grisea* : No. of mosquito larvae	**Searching time** (s)	**Handling time** (s)	**Feeding time** (s)
5 : 10	271.6 ± 72.08a*	588 ± 58.17a	859.6 ± 66.16a
5 : 20	34.4 ± 5.63b	1284 ± 219.31b	1318.4 ± 223.45ab
5 : 40	24.2 ± 6.18b	1452 ± 120.6b	1476.2 ± 123.24b

* Means in the same column with the same letters are not significantly different.

When the prey densities increased, there were decreases and increases in searching and handling times, respectively. For instance, searching time at 5:10 (271.6 seconds) was the highest and significantly greater than 5:20 (34.4 seconds) and 5:40 (24.2 seconds), whereas searching times at 5:20 and 5:40 were not significantly different. In contrast, handling time at 5:10 (588 seconds) was the lowest and significantly less than at 5:20 (1284 seconds) and 5:40 (1252 seconds), whereas handling times at 5:20 and 5:40 were not significantly different.

4.3 Discussion

The results revealed that adult *M. grisea* and temephos yielded excellent and equivalent results (99% – 100% larval mortality) for the control of *Ae. aegypti* larvae within 24 hours. Besides, throughout the current feeding tests, adult *M. grisea* showed higher larval mortality than the fifth instar nymphs which is in agreement with laboratory study on the biology and efficiency of *Enithares* sp. for *Ae. aegypti* larvae control (Chittihunsa, 1980), pointing that backswimmers of larger size caused higher larval mortality than those of smaller size. For temephos efficiency on L3 *Ae. aegypti* mortality, in early studies, temephos zeolite granules at 1 ppm, applied in 200 l jars, showed 100% mortality of *Ae. aegypti* larvae in 48 h (Mulla *et al*., 2004). Likewise, in this experiment, 100% larval mortality by temephos began from the 3rd hour until the end of the test (24 h).

Testing with 10 adult *M. grisea* in 1.2 l clay jar may lead to overcrowded condition, as water-storage containers were too small, and prey would be easily encountered. Therefore, experimentation of using individual *M. grisea* in 1.2 l jars was carried out to investigate the feeding ability of a single predator.

Within the extended feeding time, 72 h, the predation of one adult pygmy waterboatmen (*M. grisea*) on L3 *Ae. aegypti* differed amongst each prey densities. In the end of the test, predators showed the highest larval mortality percentages when fed with the lowest prey number (density). The same predator number (density) consumed about the same amount of L3 *Ae. aegypti* prey items even when at higher prey densities, suggesting that prey saturation may influence predator consumption. As the prey

densities are increased and the total numbers of prey consumed remain the same (satiation), the evaluated prey mortality percentages would appear to decrease.

Predators were observed on their searching and handling times during the tests. At different prey densities, adult *M. grisea* showed different abilities in searching for mosquito larvae at different densities. On one hand, predators spent less time searching for prey at higher prey densities. The short searching time suggested that the mosquito larvae could be easily recognized by the predators in the containers, similarly, the higher densities of prey put into the same container causing more prey availability for predators to encounter. On the other hand, predators spent more time handling prey at higher prey densities which implied that, as predator: prey ratio decreases, fewer predators were available to group forage on one prey. So the less prey densities in container led to the more chance for predators to feed on individual prey.

CHAPTER V

SMALL SCALE FIELD TEST OF *Micronecta grisea* PREDATION IN 25-LITER CONTAINERS AND EFFECT OF TEMEPHOS ON *M. grisea*

5.1 Materials and Methods

5.1.1 *Micronecta grisea*

M. grisea were collected from water-storage clay jars at the Research Station for Mosquito Biology and Control of the Department of Medical Sciences, Ministry of Public Health, Bang Bua Thong District, Nonthaburi Province, Thailand. All samples were brought to, and used to establish laboratory cultures at, the Department of Biology, Faculty of Science, Chulalongkorn University. Laboratory cultures and experiments were performed at 25±1°C and an L: D period of 12:12. Five adult female and male *M. grisea* were kept in a 1.2 l clay jar for stock cultures and fed on *Ae. aegypti* larvae as in *ad libitum*. All clay jars used were covered with nylon meshes (1 mm mesh size).

5.1.2 *Ae. aegypti* larvae

Freshly laid *Ae. aegypti* eggs, attached on filter paper and dry, were obtained from the laboratory cultures of the Department of Medical Sciences, Ministry of Public Health, and were reared in 2 l plastic rectangular containers in the laboratory at Chulalongkorn University to obtain late L3 *Ae. aegypti* for use in the experiments. Excess larvae were not reared further but killed and in-house *Ae. aegypti* cultures were not established.

Tap water, for rearing all stadia of *M. grisea* and *Ae. aegypti* larvae, was dechlorinated by leaving to air in containers for 24 h. For *Ae. aegypti*, the dried eggs on filter paper were added to this water whereupon they hatched within 30 minutes and the larvae were fed daily with crushed Pedigree, dog food at 0.1 g of food per 2 l of water containing 200 larvae and maintained at 25±1°C and an L: D period of 12:12. After 3 – 4 days under these conditions, larvae molted to the L3 stadia, observed under stereo microscope with the size range of 6 – 8 mm long, which were then selected and used in the experiments.

5.1.3 Temephos zeolite granules

Temephos zeolite granules, AZAI-SS ZG 1%, containing 1% temephos as an active ingredient, were evaluated against *Ae. aegypti* larvae in water storage jars. They were used at 0.1 g per 1 liter of water, yielding 1 ppm of temephos in each container, a concentration that is currently used in the national control program for *Ae. aegypti* larvae in Thailand (Thavara *et al.*, 2004). This formulation was a product of Ikari Trading (Thailand) Company Limited with manufacturing date of 25 September 2007.

5.1.4 *Ae. aegypti* larvae consumption by adult *M. grisea* in 25 l clay jars

In this experiment, 25-liter clay jar was chosen to be used in the feeding tests to determine the efficiency of adult *M. grisea* in small scale containers. Predators (*M. grisea*) were tested to feed on prey (L3 *Ae. aegypti*) at different combinations.

To broadly standardize the hunger level of *M. grisea* (predator), adults were selected randomly from stock cultures and kept separately without food for 24 h prior to experimentation. *M. grisea* were housed at two different densities, 10 and 30 adults, in 25 l clay jars filled with 20 l of dechlorinated tap water. After that, third instar *Ae. aegypti* larvae at one of two different densities, 100 and 200 larvae, were put into each clay jar containing the *M. grisea* at different densities to start the tests. During the tests, the number of living larvae (or pupae/pupal skins) left in the jars was estimated roughly at 24^{th} and 48^{th} h and counted at the 72^{nd} h. In all experiments five replicates were performed for each combination.

Figure 5.1 Experimentation in 25 l clay jar

The mortality numbers were adjusted by Abbott's formula (Abbott, 1925), and then used to calculate the percentage mortality of mosquito larvae in all experiments. Plots of prey mortality at each predator-prey combinations were plotted to determine the predator efficiency at each test period.

5.1.5 Effect of temephos zeolite granules on adult *M. grisea*

The experiment was conducted to observe effect of temephos zeolite granules on *M. grisea* mortality as determined by mortality percentages.

Adult *M. grisea* were tested with temephos zeolite granules applied in water at 1 mg/L AI. Ten of *M. grisea* were placed into the 1.2-liter clay jars filled with 1 liter of water. Temephos granules weighed 0.1 g were added into the jars containing ten *M. grisea* to start the tests. The number of living *M. grisea* (actively swimming) in the jars was hourly recorded within 24 hours. Three replicates were performed in the experiment.

The mortality numbers were adjusted by Abbott's formula (Abbott, 1925), and then used to calculate the percentage mortality of mosquito larvae. Plots of mortality at each period of time were plotted within 24 hours.

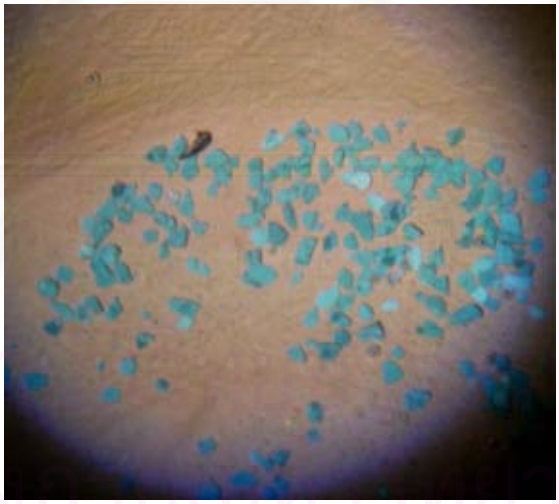


Figure 5.2 *M. grisea* mortality by temephos at the bottom of 1.2 l clay jar

5.1.6 Statistical analyses

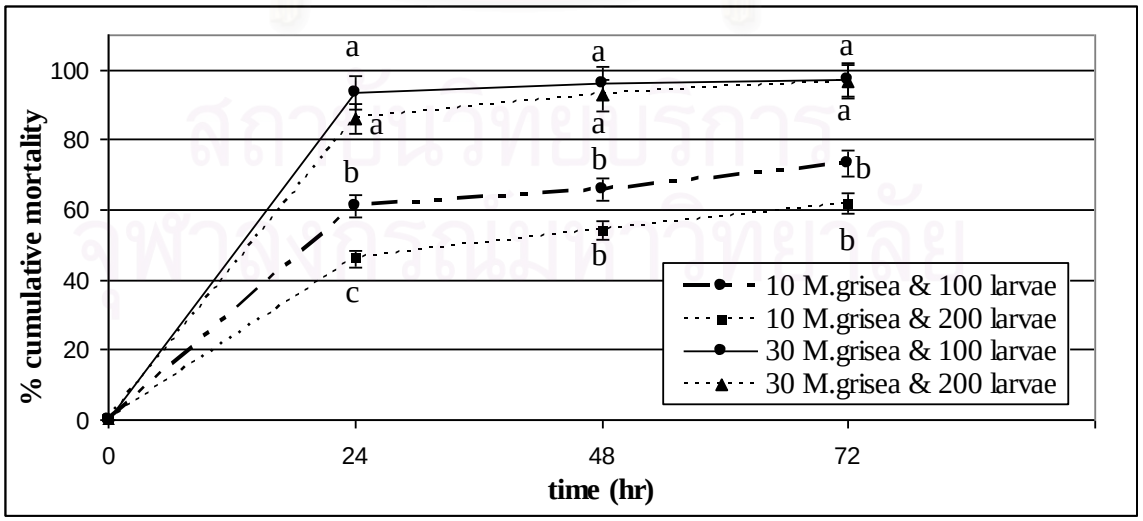
For evaluation of mortality, the data obtained from both experiments were calculated as mean percentage mortality from all replicates and were analyzed by *Mann-Whitney U*-test to compare predator efficiency tested in 25 l containers and compare *M. grisea* mortality by temephos at each hour.

5.2 Results

Feeding tests were demonstrated in 25 l jar, with four combinations of predator: prey which are 10:100, 10:200, 30:100 and 30:200. The mean larval mortality within 72 h was evaluated and the data is summarized (Appendix C).

The normality test showed that the data were not normally distributed and the homogeneity test also showed the variances to differ significantly at $P \leq 0.05$. Using *Mann-Whitney U*-test, the results revealed that, from 24 h to 72 h, the mortality within each combination were numerically, but not statistically significantly, different, whereas the highest predation level, in 72 h, occurred in 30:100 (97.2%), approximately as high as 30:200 (96.7%).

Figure 5.3. Mean mortality of third instar *Ae. aegypti* larvae by adult *M. grisea* at different combinations of predator (*M. grisea*) and prey (L3 *Ae. aegypti*) within 72 hours (*Mann-Whitney U*-test) ($P \leq 0.05$)



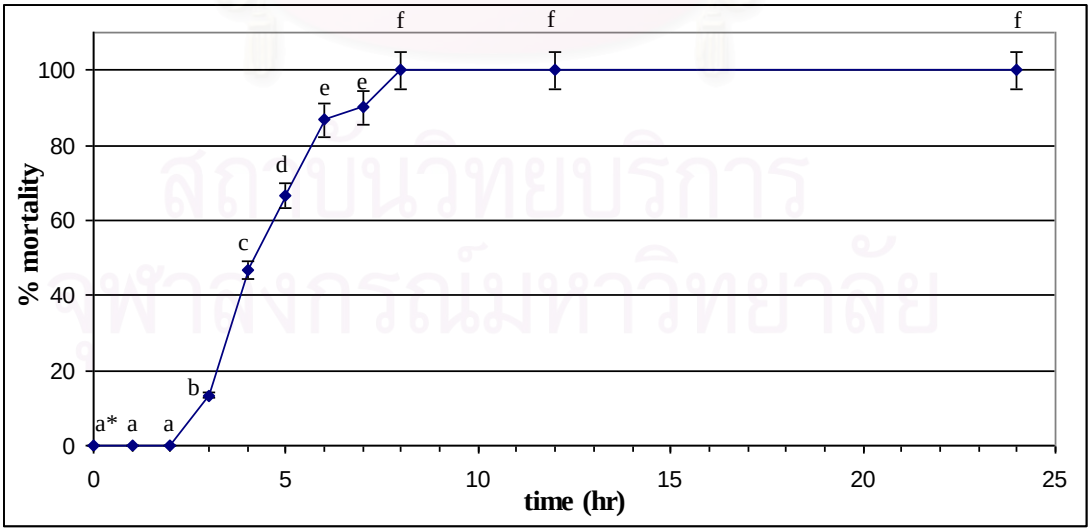
Comparison of different predator-prey combinations, shown in Fig. 5.3, revealed that, at all densities of predators and prey, mortality numbers sharply increased after 24 hours and continued to increase gradually in 48 and 72 hours.

Throughout the feeding test, 30 *M. grisea* exhibited significantly greater larval mortality than 10 *M. grisea* at both prey densities (100 & 200). Besides, testing with same predator numbers, the prey mortality (percentages) slightly increased when predators were fed upon lower prey densities.

From mortality of larvae in percentages, throughout the current feeding tests, higher densities of *M. grisea* resulted in significantly more mosquito larval mortality. Starting with 100 larvae in water containers, at 72 hours, 30 adult waterboatmen (97.2%) caused significantly greater larval mortality than 10 waterboatmen (73.2%). Likewise, when provided with 200 prey, 30 predators (96.7%) also consumed significantly more prey than 10 predators (61.9%) (Appendix D).

In further experiment, *M. grisea* were exposed to temephos at dosage of 1 ppm in 1.2 l clay jar to observe effects within 24 hours. Data from the test showed complete mortality of *M. grisea* within 8 hours of exposure and the mean mortality at each hour were summarized (Appendix E).

Figure 5.4. Mean mortality (%) of adult *M. grisea* by temephos during a 24-h exposure



* Means with the same letters are not significantly different.

The normality test showed that the data were not normally distributed and the homogeneity test also showed significant difference of the variances at $P \leq 0.05$. Analyzed by *Mann-Whitney U*-test, the mortality initiated at the 3rd hour (13.33%) and continued to performed steep increase until the complete mortality at the 8th hour (100%) (Fig. 5.4), with LT_{50} = 4.18 h (Appendix B). It was noted that, from the 3rd hour, there were significant differences amongst mortality of each hour, except at the 6th hour and 7th hour, demonstrating approximately the same mortality, 86.67% and 90%, respectively. Note that no data for mortality after 8 hours is presented since 100% mortality occurred within 8 hours.

5.3 Discussion

In the 72 h feeding test in small scale container, each combination of *M. grisea* (predator) and mosquito larvae (prey) performed different predation ability at different predator densities. Higher predator densities resulted in more prey consumed, supported the higher efficiency in feeding test of more predators. But within the same number of predator, when the prey number were increased to 200, the mortality percentage was numerically, but not statistically significantly, different, while predation at lower prey number caused higher mortality (percentage) than predation at higher prey number. When feeding at the same predator number, they consumed about the same amount of L3 *Ae. aegypti* even when at higher prey densities. The prey saturation may influence predator consumption. As the total numbers of prey consumed remain the same, but the prey densities are increased, the evaluated mortality percentages would decrease.

In small scale field test, mortality at 48 and 72 h slightly increased from 24 h. This revealed that satiation existed for at least 2 days assumed from L3 *Ae. aegypti* mortality percentages at 24, 48 and 72 h. It remains important to conduct the test for longer period as to determine the time when predators caused higher larval mortality again, presenting the duration of satiation. The shorter the satiation period, the higher the potentiality for being an effective biocontrol agent.

M. grisea were initially tested with temephos of 1 ppm and were found to be toxic in causing mortality within short period of time. Therefore, the field application of temephos in water-storage container, mainly being mosquito breeding sites, may cause reduction in population of other non-target aquatic organisms, even predators of *Ae. aegypti* larvae, like *M. grisea*. This indicates that, in those areas where the temephos were used, larvae population will surely decline with serious impacts on *Micronecta* spp. and possibly other sensitive organisms in the same time.

CHAPTER VI

DISCUSSIONS AND CONCLUSIONS

M. grisea, pygmy waterboatman, were found to have five discrete nymphal instars, plus the adult stage, distinguished by the measurement of the body and head lengths and the head capsule size. The body lengths of each instar, showing no overlap, were used for classifying each *M. grisea* instar. The non-overlapping discrete body length ranges were 0.54 – 0.65, 0.69 – 0.84, 0.9 – 1.11, 1.29 – 1.56 and 1.74 – 1.98 mm for the 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} (N1 – N5) nymphal instars, respectively, and 2.07 – 2.43 mm for adults. When each nymphal instars and adults were tested for predation and feeding ability on L3 *Ae. aegypti*, it was found that adult waterboatmen showed the highest predation rates followed by large (N5), medium (N3 & N4) and small (N1 & N2) nymphs, in that order, with small nymphs revealing no detectable predation of L3 *Ae. aegypti* in all tests. For the small sized nymphs of *M. grisea* in this study, it seems likely that they were too small, compared with the prey size available, to capture their prey whilst the newly hatched instar nymphs would also still have nutrients from the yolk available to them.

M. grisea of the same size categories and at the same densities caused no significant difference in the larval mortality percentages. In addition, within each size category *M. grisea*, at the same predator number, they consumed about the same amount of L3 *Ae. aegypti* prey items even when at higher prey densities, and so higher prey: predator ratios, suggesting that prey saturation may influence predator consumption. As the prey densities are increased and the total numbers of prey consumed remain the same, the evaluated prey mortality percentages would appear to decrease. At the same prey density, the searching times of adult, large and medium sized nymphs of *M. grisea* were significantly different. Adults showed the shortest searching time followed by large and finally medium sized nymphs, respectively. The handling time showed a similar relationship, except that the handling time for large nymphs and adults were not significantly different. Consequently, feeding time paralleled the predator handling time.

For the tests of using temephos zeolite granules and *M. grisea* in controlling of L3 *Ae. aegypti,* the results revealed that adult *M. grisea* and temephos yielded excellent and equivalent results (99% – 100% larval mortality) for the control of *Ae. aegypti* larvae within 24 hours. Therefore, the experiments with 10 adult *M. grisea* in 1.2 l clay jar may lead to overcrowded condition, as water-storage containers were too small, and prey would be easily encountered. Further experiments were done by using individual *M. grisea* in 1 l jars to investigate the feeding ability of a single predator. Within the extended feeding time, 72 h, the predation level of one adult pygmy waterboatmen on L3 *Ae. aegypti* differed amongst each prey densities. In the end of the test, predators showed the highest larval mortality percentages when fed with the lowest prey number, resulting from prey saturation that may influence predator consumption as same as the former experiments.

Predators were also observed on their searching and handling times at different prey densities. On one hand, predators spent less time searching for prey at higher prey densities whilst the short searching time suggested that the mosquito larvae could be easily recognized by the predators in the containers. Similarly, the higher densities of prey put into the same container caused more prey availability for predators to encounter. On the other hand, predators spent more time handling prey at higher prey densities which implied that, as predator: prey ratio decreases, fewer predators were available to group forage on one prey. In contrast, the less prey densities in container led to the more chance for predators to feed on individual prey.

When *M. grisea* were tested to control L3 *Ae. aegypti* in small scale containers, in 72 h, each combination of predator and prey performed different predation ability at different predator densities. Higher predator densities resulted in more prey consumed, supported the higher efficiency in feeding test of more predators. But, within the same number of predator, when the prey numbers were increased, the mortality percentage was numerically, but not statistically significantly, different, while predation at lower prey number caused higher mortality. The prey saturation may influence predator consumption, similar to the former experiment.

In small scale field test, mortality at 48 and 72 h slightly increased from 24 h. This revealed that satiation existed for at least 2 days assumed from L3 *Ae. aegypti* mortality percentages at 24, 48 and 72 h. It remains important to conduct the test for longer period as to determine the time when predators caused higher larval mortality again, presenting the duration of satiation. The shorter the satiation period, the higher the potentiality for being an effective biocontrol agent.

Moreover, successful mosquito control could be carried out by combining different complementary strategies to improve efficacy, reduce cost as well as harmful environmental impact. As chemicals are still widely used in mosquito breeding sites, integrated control by using combination of larvicides and natural enemies would be improving control results.

Thus, *M. grisea* were initially tested with temephos of 1 ppm and were found to be toxic in causing mortality within short period of time. Therefore, the field application of temephos in water-storage container, mainly being mosquito breeding sites, may cause reduction in population of other non-target aquatic organisms, even predators of *Ae. aegypti* larvae, like *M. grisea*.

Overall, adult *M. grisea* provided the highest L3 *Ae. aegypti* mortality in all tests with the shortest feeding times. This may be due to adults being more active and larger than nymphs, but also may reflect the relatively large prey size used. Thus, whilst it remains important to evaluate the predation efficiency and feeding times of all developmental stages of *M. grisea* upon all larval developmental stages of *Ae. aegypti*, it also remains of interest to evaluate if different developmental stages of *M. grisea* preferentially feed upon different larval developmental stages of *Ae. aegypti*, as well as other prey items, since *Ae. aegypti* development in urban water resources is frequently derived from multiple females and asynchronous.

As indicated in the results that temephos killed *M. grisea* in short period of time, in those areas where temephos were used, mosquito larvae population will surely decline with serious impacts on *Micronecta* spp. and other sensitive organisms in the same time. It remains important that predators should be studied on the effects of larvicide to guarantee the quality of the integrated control, as for improving the control efficiency not harming non-target species.

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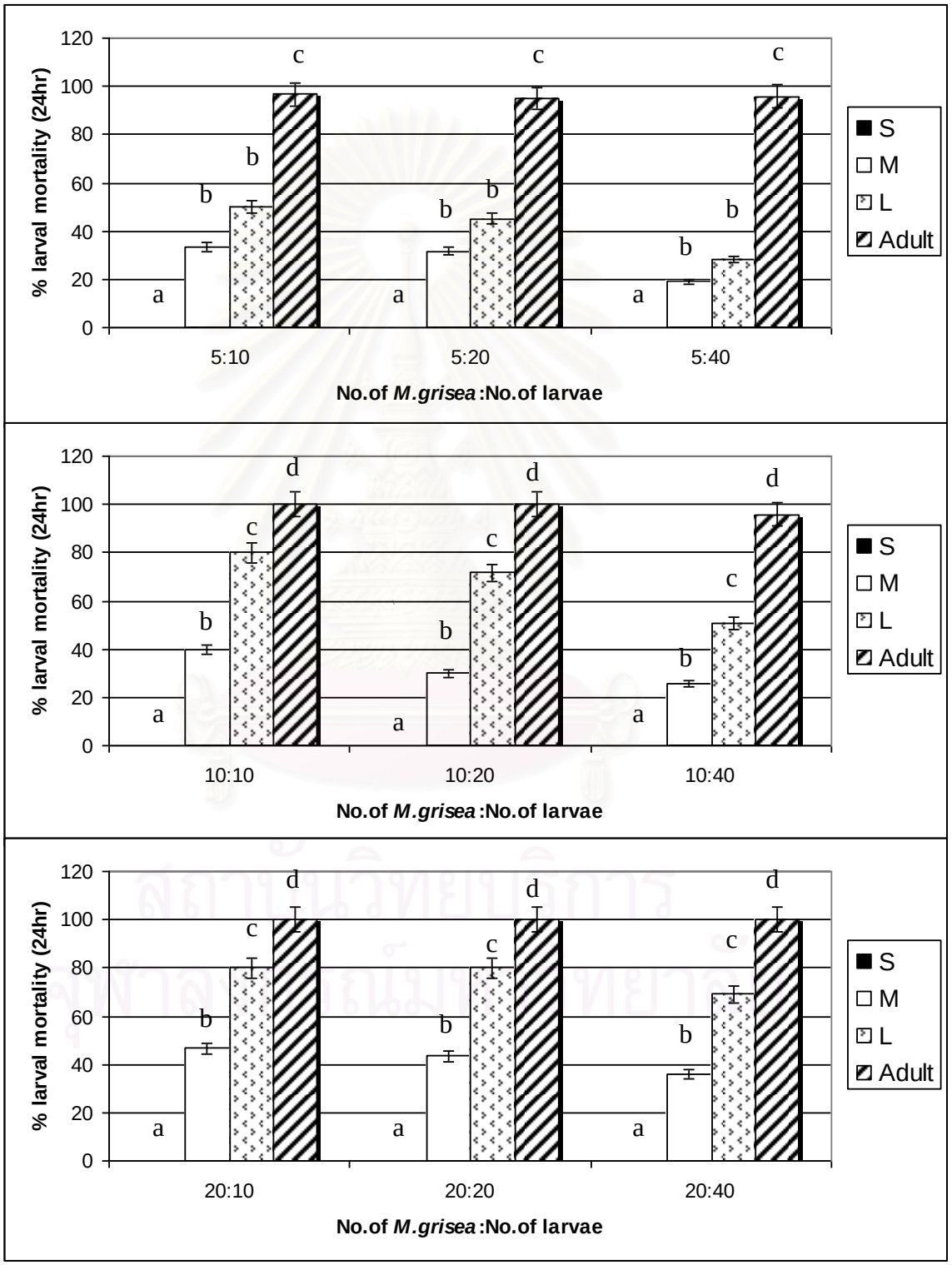
APPENDICES

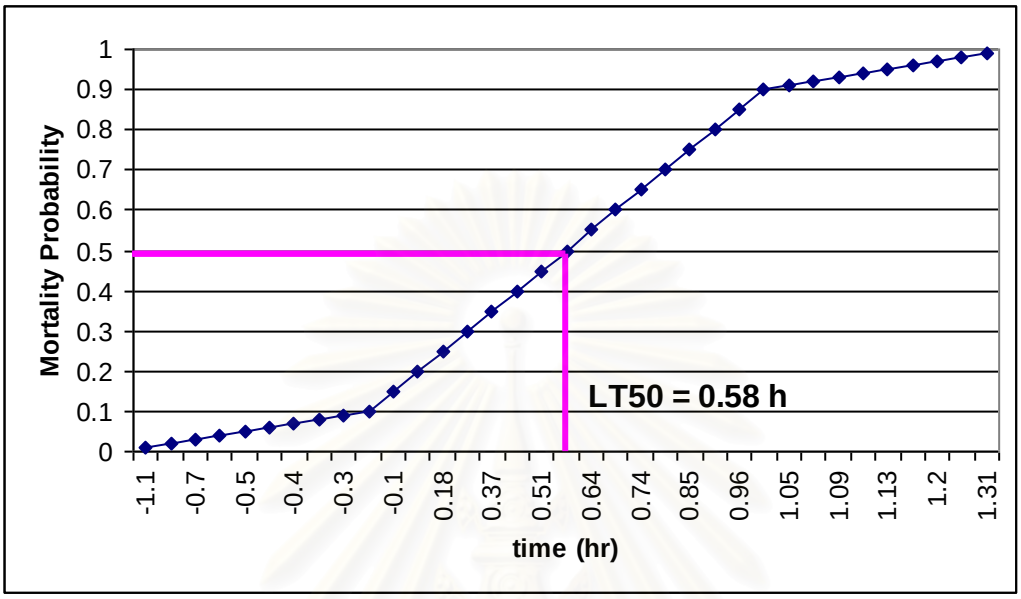
APPENDIX A

Mean mortality (predation) of third instar *Ae. aegypti* larvae by different instars of *M. grisea* at different densities (5, 10 and 20) with different densities of mosquito larvae (10, 20 and 40) (*Mann-Whitney U*-test) ($P \leq 0.05$)

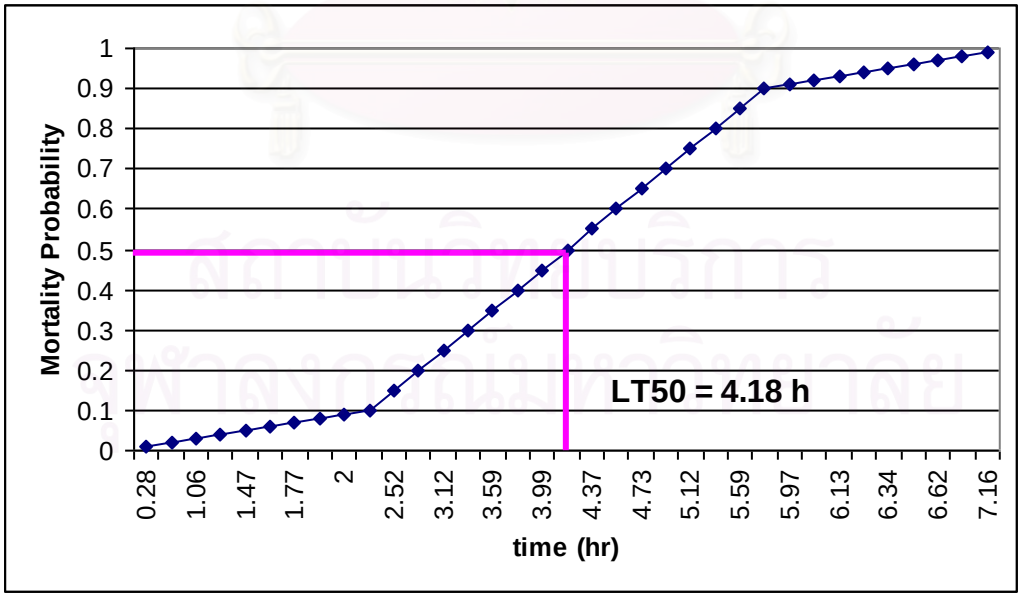


APPENDIX B

Probit analysis of using 20 *Ae. aegypti* larvae and 1 ppm temephos



Probit analysis of using 10 *M. grisea* and 1 ppm temephos



APPENDIX C

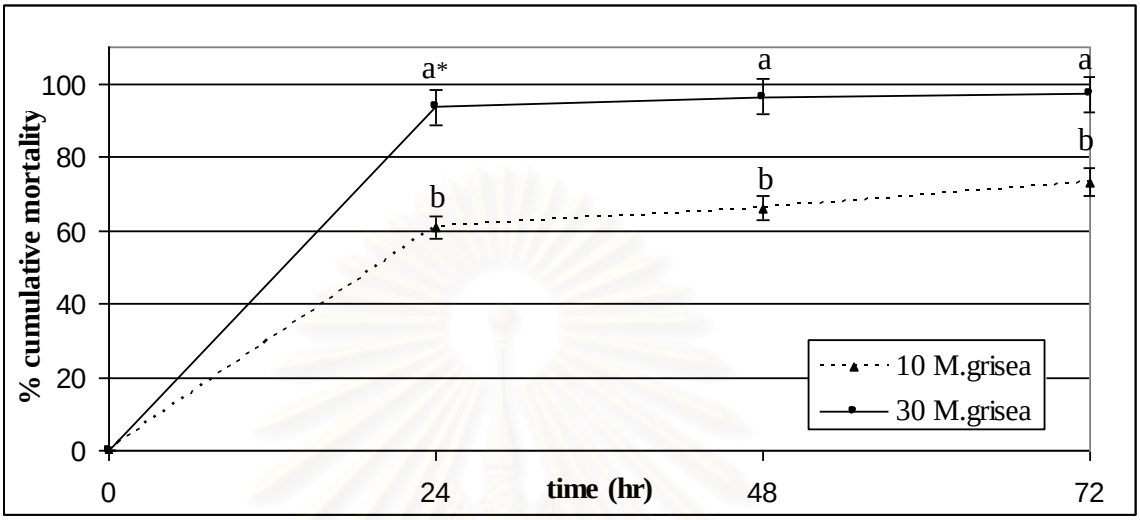
Mean (± S.E.) mortality (%) of third instar *Ae. aegypti* larvae observed within 24, 48 and 72 hours using different densities of adult *M. grisea* (10 & 30) with different densities of *Ae. aegypti* larvae (100 & 200) (*Mann-Whitney U*-test) ($P \leq 0.05$)

No. of mosquito larvae	No. of adult *M. grisea*	Time (hr)			
		0	24	48	72
100	**10**	$0 \pm 0.00a^{1}$	$61.0 \pm 2.4a^{2}$	$66.0 \pm 4.8a^{2}$	$73.2 \pm 6.5a^{2}$
	30	$0 \pm 0.00a^{1}$	$93.6 \pm 3.9b^{2}$	$96.4 \pm 2.9b^{2}$	$97.2 \pm 2.8b^{2}$
200	**10**	$0 \pm 0.00a^{1}$	$46.0 \pm 2.4c^{2}$	$54.0 \pm 6.6a^{2}$	$61.9 \pm 7.1a^{2}$
	30	$0 \pm 0.00a^{1}$	$85.9 \pm 4.8b^{2}$	$92.8 \pm 3.8b^{2}$	$96.7 \pm 2.1b^{2}$

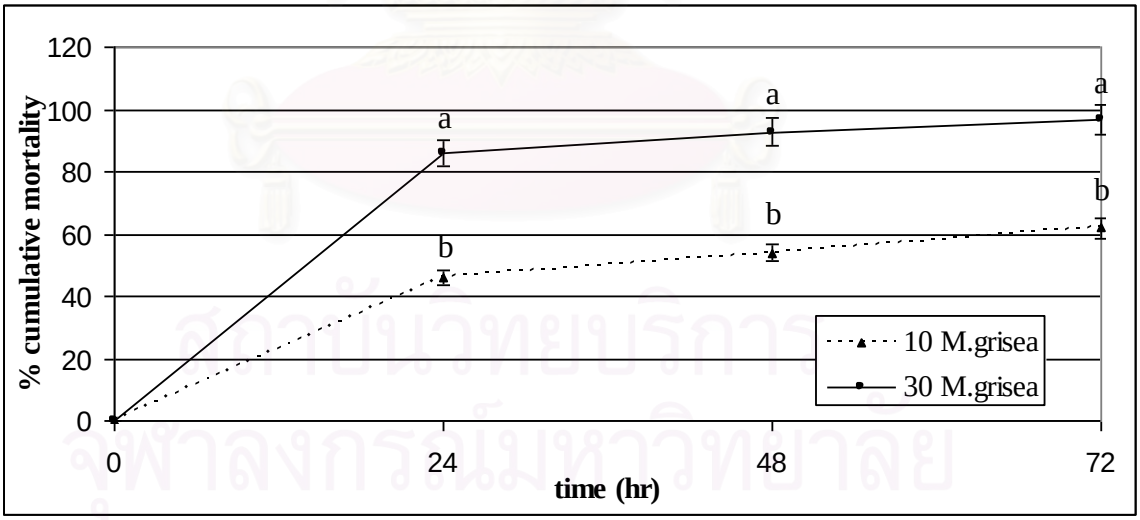
* Means in the same column with the same letters are not significantly different, whilst those across rows with the same superscript numbers are not significantly different.

APPENDIX D

Mean mortality of third instar *Ae. aegypti* larvae by adult *M. grisea* at different densities (10 & 30) with 100 mosquito larvae (*Mann-Whitney U*-test) ($P \leq 0.05$)



Mean mortality of third instar *Ae. aegypti* larvae by adult *M. grisea* at different densities (10 & 30) with 200 mosquito larvae (*Mann-Whitney U*-test) ($P \leq 0.05$)



* Means in the same period of time with the same letters are not significantly different.

APPENDIX E

Mean (± S.E.) mortality (%) of adult *M. grisea* observed within 24 hours by using temephos (*Mann-Whitney U*-test) ($P \leq 0.05$)

Time(hr)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Mortality	0a*	0a	0a	13.33 ± 3.33b	46.67 ± 3.33c	66.67 ± 3.33d	86.67 ± 3.33e	90 ± 0e	100 ± 0f

* Means in the same row with the same letters are not significantly different.

BIOGRAPHY

Chutaporn Amrapala was born on the 12th August, 1984, in Bangkok, Thailand. She received a high school certificate in Math-Science Program from Triamudom Suksa School in 2002. After that, she received a Bachelor degree in Education, majoring in Biology, in 2006, from the Faculty of Education, Chulalongkorn University. As one part of her study in the Faculty of Education, she was a training teacher at Nonsi Wittaya School, teaching Biology and General Science to secondary school students, from May to September 2005. From that, she got an Excellent Teaching Media Award in 2006. Then, she continued her study in the Interdisciplinary Program in Environmental Science for a Master in Environmental Science at Graduate School, Chulalongkorn University in 2006 and completed in 2009.