EFFECTS OF SOME ENVIRONMENTAL FACTORS ON PREDATION OF MOSQUITO LARVAE (*AEDES AEGYPTI* LINNAEUS) BY GUPPIES (*POECILIA RETICULATA* PETERS)

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ผลของสภาพแวดล้อมบางประการต่อการล่าลูกน้ำยุงลาย (*AEDES AEGYPTI* LINNAEUS) โดยปลาหางนกยูง (*POECILIA RETICULATA* PETERS)

นางสาววณิชยา จรูญพงษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree

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ผลของสภาพแวดล้อมบางประการต่อการล่าลูกน้ำยุงลายโดยปลาหางนกยูง ผลการศึกษาพบว่า อุณหภูมิ ช่วงของแสง ความชื้นสัมพัทธ์ และความเป็นกรดด่าง น้ำเสียเข้า และน้ำเสียออกจากบ่อบำบัดน้ำเสีย ไม่มีผลทำให้ประสิทธิภาพการล่าลูกน้ำยุงลายแตกต่างกัน แต่พบว่าความเค็มมีผลทำให้ประสิทธิภาพการล่าลูกน้ำยุงลายของปลาหางนกยูงเพศผู้ 1 ตัวกับปลาหางนกยูงเพศเมีย 1 ตัว มีความแตกต่างกันในแต่ละระดับความเค็ม ที่ระดับนัยสำคัญ 0.05 ประสิทธิภาพการล่าลูกน้ำยุงลายแตกต่างกันระหว่างปลาหางนกยูงเพศเมีย 2 ตัวและปลาหางนกยูงเพศผู้ 1 ตัว กับปลาหางนกยูงเพศเมีย 1 ตัว ที่ความเค็ม 3 และ 5 ppt นอกจากนั้นพบว่า ประสิทธิภาพการล่าลูกน้ำยุงลายแตกต่างกันระหว่างปลาหางนกยูงเพศเมีย 2 ตัว และปลาหางนกยูงเพศผู้ 2 ตัว ที่ความชื้นสัมพัทธ์ 60 และ 80% และ ปลาหางนกยูงเพศผู้ 2 ตัว และปลาหางนกยูงเพศผู้ 1 ตัวกับปลาหางนกยูงเพศเมีย 1 ตัว ที่ความชื้นสัมพัทธ์ 60 และ 80% ที่ระดับนัยสำคัญ 0.05 ดังนั้นผลของสภาพแวดล้อมบางประการควรถูกพิจารณาสำหรับการเลี้ยงปลาหางนกยูง เพื่อการควบคุมลูกน้ำยุงลาย

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WANICHAYA CHAROONPHONG : EFFECTS OF SOME ENVIRONMENTAL FACTORS ON PREDATION OF MOSQUITO LARVAE (*AEDES AEGYPTI* LINNAEUS) BY GUPPIES (*POECILIA RETICULATA* PETERS). THESIS ADVISOR : DR. NATHAWUT THANEE, Ph.D. 86 PP. ISBN 974-533-326-3

ENVIRONMENTAL FACTORS/PREDATION/MOSQUITO LARVAE/GUPPIES

This reseach studied effect of some environmental factors on predation of guppy. The results showed that temperature, photoperiod, relative humidity, pH, influent and effluent wastewater from wastewater treatment plants did not demonstrate the difference of effectiveness of guppies as predators of larvae. However, salinity showed the difference of effectiveness of guppies as predator of larvae. One male guppy with one female guppy has showed the difference in effectiveness of guppies predation on larvae at different level of salinity ($p \leq 0.05$). The means of effectiveness on predation larvae between two male guppies and one male guppy with one female guppy were different at salinity of 3 and 5 ppt. Additionally, the means of effectiveness on predation larvae between two female guppies and two male guppies were different at 60 and 80% relative humidity, two male guppies and one male with one female guppy were different at 60 and 80% relative humidity ($p \leq 0.05$). Therefore, effects of some environmental factors should be taken into consideration in guppies breeding to serve a purpose of mosquito larvae control.

School of Biology Student's Signature……..……...……..

Academic Year 2003 Advisor's Signature…….…….

Co-advisor's Signature..……...

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Wanichaya Charoonphong

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LIST OF ABBREVIATIONS

A. aegypti L.	*Aedes aegypti* Linnaeus
∝	alpha
BOD	Biochemical Oxygen Demand
cm^3	cubic centrimeter
$inch^3$	cubic inch
m^3	cubic meter
D	dark
°C	degree Celsius
DO	Dissolved Oxygen
Hz	Hertz
L	light
LSD	Least-Significant Different
mg/L	microgram per litter
mL	mililiter
ppm	part per million
ppt	part per thousand
%	percent
SD	Standard Deviation
µL	microliter

CHAPTER I

INTRODUCTION

Mosquitoes are insects belonging to the order of Diptera, found in many different environments. Mosquito population increases rapidly and distributes widely around the world. The most harmful vector species belong to the genera *Anopheles*, *Cules*, and *Aedes* (Service, 1996). The mosquito is a vector of serious diseases including protozoan diseases, such as malaria, and viral diseases, such as dengue haemorrhagic fever (DHF), encephalitis, and yellow fever (Service, 1993, 1996). Most of these parasites and pathogens can be transmitted to human and their pets or livestock because the adult female mosquito in most species is blood feeder and needs protein from human or animal blood to produce mature eggs. In addition, mosquito bites can also cause skin irritation through an allergic reaction (Eldridge and Edman, 2000).

Mosquitoes are the cause of enormous medical and economic problems. Vast amount of human effort has been invested to control this insect where it has become a major target of chemical control campaign in the tropical countries. Nowadays, chemical insecticides are widely used as means of insect control and cause environmental pollution due to the toxic contents. In recent years, it has been found that mosquitoes become resistant to insecticides, for example dieldrin, and pollutants alter the phototactic behavior in *Aedes aegypti* and build a resistant mechanism to the insecticides. The adoption of an Integrated Mosquito Management (IMM) or

Integrated Pest Management (IPM) reported by Wongsiri (1982) helps to achieve successful result in mosquito control. The concepts integrate multidisciplinary methodologies with pest management strategies and create effective practices in public health and environmental care as well as improve life quality. At the present time, biological control of mosquito larvae is being taken into serious consideration as a choice of mosquito control. There are 2 approaches to get rid of mosquito: adulticides approach and larvicides approach. Larvicides are proving to be the more successful and practical approach in mosquito control. Mosquito life cycle consists of four stages - egg, larva (also called "wriggler"), pupa (also called "tumbler") and adult. Mosquito larvae and pupae need water for life development; therefore it is easier to control these two stages of life cycle than in adult. One of the best methods nowadays in biological control is the use of larvivorous fishes, for example mosquitofish (*Gambusia affinis*) and guppy (*Poecilia reticulata*). The mosquitofish is a freshwater fish, which has been widely used since early 1900s in biological control of mosquito larvae (Linden and Cech, 1990). Similarly the common guppy has received considerable attention in Thailand in this regard (Sasa, 1965). The use of guppy has proved to be very effective against mosquitoes breeding in temporary standing water or man-made breeding sites. The guppy has now been used in many areas because of its ecological acceptability, rapid maturation, high fecundity, tolerance to polluted water and feeding preference for mosquito larvae (Wongsiri and Andre, 1984).

This research investigated the use of *Poecilia reticulata* to control and destroy mosquito larvae in laboratory. The goal of research is to reduce the mosquito population, especially larvae of *Aedes aegypti*, which is a fatal principal vector of

dengue and yellow fever viruses (Guzmán and Kouri, 2001; Monath, 2001). *A. aeypti* has associated relationship with the outbreaks in Thailand and several Southeast Asian countries (Pant, Jatanasen and Yasuno, 1973). It has successfully adapted its biology and habits to fit the changes in urban environment, and has now become dominant household mosquito, called domestic mosquito (Robinson, 1996; Gubler and Clark, 1996). The objectives of this research were 1) to study effectiveness of guppy on predation of *A. aegypti* larvae, 2) to study environmental factors affecting effectiveness of guppy on predation of *A. aegypti* larvae and 3) to study effects of wastewater on effectiveness of guppy on predation of *A. aegypti* larvae.

The environmental factors studied in this research were temperature, pH, photoperiod, salinity, and relative humidity. Influent and effluent of wastewater from Suranaree University of Technology's wastewater treatment plant were also taken to analysis. The numbers of 800 mosquito larvae in the second instar were used per day in the experimental unit of a 2-liter beaker containing 1 liter of dechlorinated tap water. Each experimental unit had male and female wild guppies with standard length of approximately 1 inch. The number of mosquito larvae consumed by guppies was recorded after 24 hours. *A. aegypti* larvae were obtained from the Infective Disease Insect Control Office in Saraburi province and the Department of Communicable Disease Control, Ministry of Public Health. These *A. aegypti* larvae were especially bred and raised for the experiment.

The results of this research provide a better understanding of the effectiveness of guppy as predator of *A. aegypti* larvae in both general and extreme environmental conditions, and information on the effectiveness of guppy on predation of *A. aegypti* larvae in optimum conditions.

CHAPTER II

LITERATURE REVIEW

2.1 History and characteristics of guppy

The scientific name of guppy is *Poecilia reticulata* and its family name is Poeciliidae. Guppy is also commonly known as "livebearer." The family consists of 30 genera, with about 293 species (Nelson, 1994). Poeciliidae family represents the group of members consisting of freshwater live-bearing fish distributed in southern USA, Central America, and northern Argentina. Nowadays, Poeciliidae family is found also outside the native range, in warmer regions to which they were introduced (Petrovicky, 1998).

Guppy is a live-bearing native fish of Trinidad and northeastern coast of South America (Bruce and White, 1995). The history of guppy began in 1859 when a German ichthyologist, Wilhelm C. H. Peters found a specimen in his collection and brought it to Germany from Venezuela. He was the first scientist to describe guppy and called his specimen *Poecilia reticulata*. The word "reticulata" refers to overlapping scales that form a lace-like pattern on guppy's body. Then in 1866 an English botanist, Robert John Lechmere Guppy brought some of this fish to the British Museum from Trinidad in British West Indies for identification. Albert K. Gunther, the museum director at that time, named the species *Girardinus guppyi* in believing that Mr. Guppy had discovered a new species. The popular name of "guppy" has been used ever since, in honor of Robert J. L. Guppy.

The other common names of guppy are millions fish and rainbow fish (Axelrod and Whitern,1965).

Classification of guppy

A recent taxonomic status for common guppy *Poecilia reticulata* belongs to (Nelson, 1994):

Kingdom Animalia

Phylum Chordata

Class Actinopterygii

Order Cyprinodontiformes

Family Poeciliidae

Genus *Poecilia*

Species *Poecilia reticulata*

The fish in this family share one unique characteristic, which are the mark differences that distinguish fish between the sexes. Male is normally smaller than female and has a developed external mating organ that aids male to project sperm into female's oviducts (Petrovicky, 1998).

Guppy is classified in subfamily Poecilinae of which consists of 20 genera. The popular aquarium fish include guppy and molly of the genus *Poecilia*, swordtail and platy or platy fish of the genus *Xiphophorus*. The mosquitofishes, *Gambusia affinis* and *G. holbrooki*, were introduced to many parts of the world for mosquito control (Nelson, 1994)

The other interesting characteristics of guppy are its sexual dimorphic and size. Besides the highly variable coloration, male guppy is usually smaller and brighter in

color than female. Female guppy is usually in light brown or silver grey. Mature male grows up to approximately 1.2 inches long, while the measurement female is 2.4 inches long. Male guppy has a gonopodium. At birth the anal fin of male guppy appears much alike that of female. But by growth the fin becomes gradually modified and eventually appears like a tube, which is narrow and longer than that of female. Individual male guppy is easily identified by its different color markings as shown in Figure 2.1.

a) male guppy b) female guppy

Figure 2.1 Guppies: a) male guppy and b) female guppy

(McBride, Online, 2004)

Guppy is a popular exported beautiful fish that generates high profit to exporters, especially in Singapore where guppy counts 38% of overall exported beautiful fish (กรมประมง, 2541). In Thailand, Ratchaburi province is the biggest breeding source where farmlands earn a living on beautiful fish. Guppy has become very popular not only for its attractive colors, but also because it is easy to take care and resistant to changing environment. Moreover, guppy breeds rapidly so it becomes widely

available and in affordable price to all aquarium lovers. The wild Guppy is found in natural water resource. There was an attempt to make wild guppy an attractive aquarium fish but, unfortunately, this fish did not pass the species selection process. However, fancy guppy has been improved from wild guppy to get beautiful colors and attractive appearances. The good appearances of guppy can be described as: small size, long flat body, attractive flipper and tail color, enjoying to stay in community and ability to eat both plants and animals.

2.2 Biology of guppy

Guppy is a small poeciliid fish originated from the Northeastern of South America. It lives in tropical forests, in clear streams with clean gravel or sand bottoms, and occasional patches of leaf litter (Endler, 1987). Guppy is tropical fish with wide range of habitats and salinity. The fish feeds on zooplankton, small insects and detritus.

Guppy is live-bearing fish (ovoviviparous). The young guppies develop fully within female and get born alive. The parents may chase and eat newborn fish immediately after spawning. Male guppy is typically smaller than female and extremely polymorphic in its bright secondary sexual coloration. Female guppy is uniformly grey-brown with no bright coloration. The morphological dimorphism characterizes the entire family, in which the anal fin of the male is developed into a gonopodium and enabling internal fertilization of the female as shown in Figure 2.2 (Farr, 1974).

Fertilization is the most interesting phenomenon created by guppy. Sperms enter the ovary where they wait for ripening of the first batch of eggs. After birth has taken

place, the new eggs develop rapidly and are fertilized by waiting sperms. Sperms can wait dormantly in female's body for eight months and still be able to fertilize with the gestation period of 22-24 days. Broods are produced under optimum condition every 27-30 days. The 5-6 days difference is accounted for the time necessary for eggs development into a fertilizable condition.

At the age of 30-35 days, guppy sexes can be distinguished by the development of larger body of female and her gravid spot, of which male does not show. Male guppy begins to show the developing gonopodium in this stage as shown in Figure 2.2.

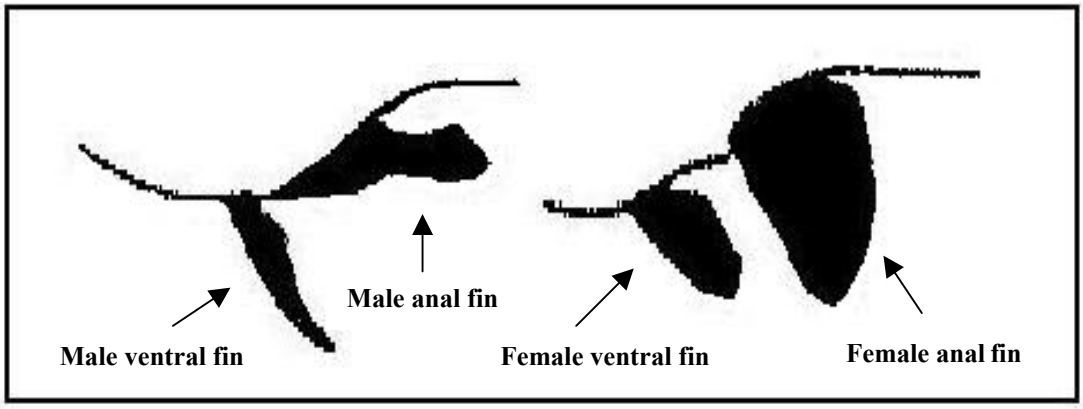

Figure 2.2 Comparison of male and female ventral and anal fins

(Whitney, Online, 2003)

2.3 Characteristics of mosquito and transmission of diseases

In 300 B.C., Aristotle referred to mosquito as "empis" in his "Historia Animalum" where he documented its life cycle and metamorphic ability (Floore, Online, 2002). Mosquito is an insect belonging to the order Diptera that characterizes two wings. An adult mosquito is small and fragile, ranging in body size from 3 to 6

mm. It can be distinguished from other flies by small scales on its wings and body (Robinson, 1996). The mouthparts of female mosquito form a long piercing-sucking, which is proboscis. The antennae of female have whorls of short hairs called “pilose”. Male mosquito differs from female with its feathery antennae called “plumose” and male’s mouthparts are not suitable for piercing skin. Thus, males of all species are incapable of feeding on blood (Service, 1996).

There are about 3,450 species and subspecies of mosquitoes belonging to 38 genera; all mosquitoes are classified in the family Culicidae, including the genera *Anopheles*, *Culex* and *Aedes.* Mosquito is among the most primitive flies that have adapted to human environment. Mosquito has distributed almost worldwide, being found throughout the tropics and temperate regions and even well beyond the Arctic Circle. It is absent only from Antarctica and a few islands (Service, 1993). Mosquito is found in many diverse aquatic habitats because its immature stages are entirely aquatic and larvae feed by filtering microorganisms from the water (Robinson, 1996). Both male and female adults feed on nectar or honeydew to provide energy for flight. Females, however, also feed on blood, which is needed to synthesize yolk and developed eggs (Klowden, 1995).

The relation of mosquito to disease transmission is well known. Parasites and pathogens, such as protozoa and virus, are transmitted to other organisms by mosquito biting (Eldridge and Edman, 2000). Furthermore, mosquito is a nuisance, annoying and interfering outdoor work and other activities of human. Mosquito also brings diseases to human, for example, dengue, which is one of the hazardous causes of morbidity and mortality occurred earlier in several areas in Southeast Asia, Western Pacific, and the Americas. The dengue virus is transmitted to human when *Aedes*

aegypti salivates into bloodstream, as well as by another species vector namely *Aedes albopictus* (Guzmán and Kouri, 2002). Both species breed in natural and manmade containers in the habitats such as water pots and tires (Service, 1996). Dengue is caused by an RNA flavivirus exhibiting 1 to 4 serotypes: dengue-1, dengue-2, dengue-3, and dengue-4. The disease's symptoms vary according to the serotype (Eldridge, Scott, Day and Tabachnich, 2000). *Aedes aegypti* is normally recognized as yellow fever mosquito (Service, 1996). The report said that virus could transfer to mosquito through eggs (transovarian). The virus originated in Africa where earliest description of yellow fever was found in a Mayan manuscript in 1648. Yellow fever occurs in South America and tropical Africa, but with an absence in Asia. Yellow fever is caused by flavivirus and characterizes a single serotype. The symptoms of yellow fever are described: pansystemic viral sepsis with viraemia, fever, prostration, hepatic, renal and myocardial injury, haemorrhage, shock, and high lethality (Monath, 2001). Monath (2001) reported that yellow fever virus has caused many urban epidemics a high mortality rates of up to 5,000 cases in Africa and 300 cases in South America annually. Between 1990-1999, the number of 11,297 cases was reported with 2,648 deaths in Africa.

In Thailand, hemorrhagic fever has been found and spread in Bangkok and Thonburi province since 1949. The impact became very severe in 1958 and spread to other provinces, such as Chiangmai, Nakhon Sawan, Saraburi, Lopburi and Nakhon Ratchasima. Most patients were found in only big cities. Nowadays, the hemorrhagic fever is found in every province and usually spreads in rainy season. According to the statistic in 1994, the number of hemorrhagic fever patients increased in May and reached the highest peak in June. The statistic leaded to a conclusion that, in order to

eradicate *Aedes*, the operation should be conducted from February to April when high temperature and humidity most affect the death rate of mosquitoes; and as a result, the operation will successfully stops the circulation of virus in mosquito's body (สุทารัตน์ พรจรรยา, 2545).

2.4 Life cycle of *Aedes aegypti*

Aedes is a very large genus composing of 962 species, for example, *Aedes aegypti* that cause serious medical problems. Even though a large numbers of *Aedes* species are nocturnal biters, the disease vectors also include many of those that bite during the day or in early evening. *Aedes* adults have conspicuous patterns on the thorax and arrangements formed by black, white or silvery scales. The adults also have conspicuously banded appearance on legs and abdomen. *A. aegypti* adults have dorsal surface of the thorax showing typical 'lyre'-shaped silver markings as shown in Figure 2.3 (Service, 1996).

Figure 2.3 *Aedes aegypti* with typical 'lyre'-shaped marking (สีวิกา, Online, 2003)

Classification of *Aedes aegypti*

Kingdom Animalia

Phylum Arthropoda

Class Insecta

Order Diptera

Family Culicidae

Sub Family Culicinae

Genus *Aedes*

Species *Aedes aegypti*

The arthropods have an external skeleton that does not grow, and must be replaced several times to allow size increase in size and physiological change; this change is called metamorphosis. There exist 2 principal types of metamorphosis in insect: simple and complete (Robinson, 1996). Mosquito has 4 distinct life stages and has complete life cycle called “complete metamorphosis”. The life cycle consists of egg stage, larval stage, pupal stage and adult stage (shown in Figure 2.4). The young insects with complete metamorphosis are usually quite different from the adults and often live in different habitats.

A. aegypti breeds in pots and water-storage jars being placed either inside or outside living places. Larvae exist mainly in the containers with clean water intended for drinking. In some areas, *A. aegypti* also breeds in rock-pool and tree-holes (Service, 1996). Under the most favorable climatic and environmental conditions, the entire immature or aquatic cycle can complete in as short as 10 days. The life cycle of *A. aegypti* is shown in Figure 2.4.

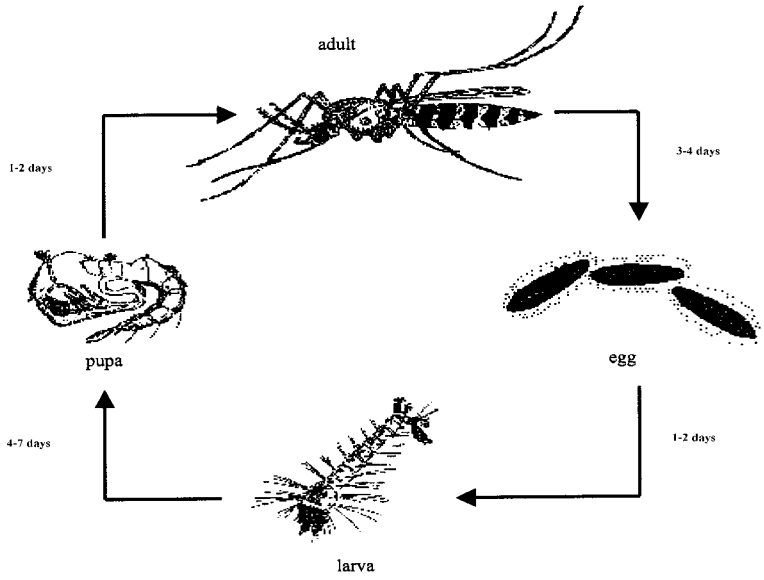

Figure 2.4 Life cycle of *Aedes aegypti* (สุทารัตน์ พรจรรยา, 2545)

2.4.1 Egg

Eggs of *A. aegypti* are blackish and cigar-shaped. Eggs are kept at a relative humidity of 70 % or more in which eggs remain viability for as long as 15.5 months. Exposure to high humidity for 2-3 days is necessary for larvae in order to hatch from eggs. However, if eggs dry out before developmental period, they will collapse and then embryos will die. When *Aedes* eggs are flooded, the hatching can occur within a few minutes while the other genera may require prolonged immersion in water. *For A. aegypti*, a staggered hatch over many days occurs after a single soaking, larvae appear in installments up to 45 days for *A. aegypti* (Service, 1993).

2.4.2 Larva

Ther 4 larval stages take 7-10 days for development. Larva molts (shed their skin) four times and grows larger after each molt; the stage between molts is called “instar”. Larval development rate depends on food supply, water temperature and species. Larva lives in water and comes to the surface to breathe. Swimming to the surface in frequent intervals is necessary for obtaining oxygen through a breathing tube called “siphon”. For breathing, larva has siphon tubes that hangs upside down from the water surface. Larva usually has short barrel-shaped siphon and only one pair of siphonal subventral tufts. The additional characters are: the existing of at least three pairs of setae in the ventral brush, the antennae are not greatly flattened and no enormous setae on the thorax. These characters should separate *Aedes* larvae from most of the culicine genera (Service, 1993). Larva changes into pupal stage during the fourth molt.

2.4.3 Pupa

In this stage, pupa is unable to feed or rest. Pupa takes oxygen through breathing tubes called “trumpets". When disturbed, it dives in a jerking, tumbling motion toward protection and then floats back to the surface. Pupation occurs at the water surface. The larval integument splits along the middorsal line allowing pupal thorax to emerge and, very soon after, the trumpets spring up and come into contact with water surface. The whole process takes about 3-5 minutes. At first the pupal abdomen is straight; it takes a little longer for it to curve underneath the cephalotorax and for the pupa to assume its characteristic comma shape. Transformation from the pupal stage to the adult stage generally takes 2-3 days (Service, 1993).

2.4.4 Adult

The newly emerged adult rests on surface of water for a short time to allow t to dry and all parts of its body to harden. Blood feeding and mating do not occur for a couple of days after the adult emerges. Female mosquito can mate before the first feeding or feed before its first mating (Klowden, 1995).

2.4.4.1 Mating and swarming

Mating behavior is stimulated by the release of juvenile hormone by the female's corpora allata glands (Klowden, 1995). Mating usually occurs in flight. The tone of female's wing-beat attracts males whose antennae perform as sound receptors aiding in mate location. The tone of female's wing-beat varies normally around 250-320 Hz. The tone of male's wing beat is around 100-200 Hz or higher. Male stays below female and holds onto her as the two fly around "face to face". Copulation normally lasts 5-60 seconds. Once a female has mated, it can store sperms for the mating in later reproductive cycles. Male swarm most commonly occurs during sunset.

2.4.4.2 Sugar feeding

Sugar is the basic food of adult mosquitoes. Energy for flight is supplied by glycogen that is rapidly assimilated from a sugar meal; female can also derive energy for flying from blood meals. *A. aegypti* usually flies only 25-100 meter (Service, 1993). Foster (1995) described the sugar feeding appears in both male and female mosquitoes. Both sexes take sugar within hours of emergence and almost every 1-2 day. Most female mosquitoes take sugar before taking blood. The direct effect of sugar feeding and energy reserves on male's reproduction has not been

investigated. For female, however, numerous studies have examined direct effects on the reproductive system such as blood meal size, egg size and delayed oviposition.

2.4.4.3 Blood feeding

Female *A. aegypti* also comes to feed on a host. Feeding blood volume controls the expression of host-seeking behavior in *A. aegypti.* The adult bites mainly during the day or early evening and the biting peaks are clearly defined just after sunrise and just before sunset (Service, 1993). Klowden (1995) reported that mosquito could ingest a blood volume of approximately 5 μl from a single sitting. After a blood meal, mosquito's abdomen is dilated and turns bright red, then becomes darker red some hours later. The gonotrophic cycle of a female mosquito starts from unfed adult, passing through a blood-fed, half-gravid and finally gravid condition. After a full meal, mosquito flies away and no longer attracted by host stimuli (Service, 1996).

2.4.4 Host seeking behavior

The research found that *A. aegypti* likes to stay around human (endophagic) for obtaining carbon dioxide and lactic acid that evaporate from human skin (บุญล้วน พันธุมจินดา, 2515). *A. aegypti* increases in large number during rainy season. It breeds in a narrow place called "stenogamy" where male *A. aegypti*, attracted by female's flying sound, flies to female *A. aegypti*. *A. aegypti* in both sexes consume syrup from pollen and honey as food. After female *A. aegypti* sloughs off, it will suck blood within 24 hours. Mosquito prefers human's blood than that of animals (anthropophic) and suck the blood in arms and legs rather than in face. The blood consummation of 1.5-2 attacks is equal to 4.2 cm^3. For taking this blood quantity,

mosquito has to make space in its stomach by driving water through bottom for 5-15 minutes to remove 1.5 cm^3 (2-3 drops) (สุทารัตน์ พรจรรยา, 2545).

2.4.4.5 Oviposition

A. aegypti in nature discretes oviposition peaks within two hours after sunrise and before sunset, taking place in dark-colored water containing organic material such as decaying leaves, or in water container which are wide openings; The containers located in the shade are preferred. Female mosquito lays eggs singly by the side of container at the water line in batches of 30-50 (Service, 1993). The egg laying appears to be mainly under a control of a circadian rhythm and occurs three days after blood feeding.

The main factor affecting eggs laying nature of mosquito is its breed source; *A. aegypti* always lays eggs on surface of the containers. The eggs absorb water, chemical and organic substances in water containing phosphate, carbonate and chlorideiron, then adjust pH equal to 6-12. *A. aegypti* normally lives in clean water and inside the households, however the mosquito larva can also be found in wastewater drains.

After the oviposition, the host-seeking behavior of female mosquito is again activated. The activation is also according to visual cues, heat, and emanations such as carbon dioxide, lactic acid and volatile fatty acids; it is typical of those organisms containing blood. Reproductive cycle is initiated when mosquito locates another host and ingests the blood. Blood ingestion and oviposition defining the gonotrophic cycle is shown in Figure 2.5. These gonotrophic cycles can occur

every two or three days for a month or so during the lifetime of adult mosquito (Klowden, 1995).

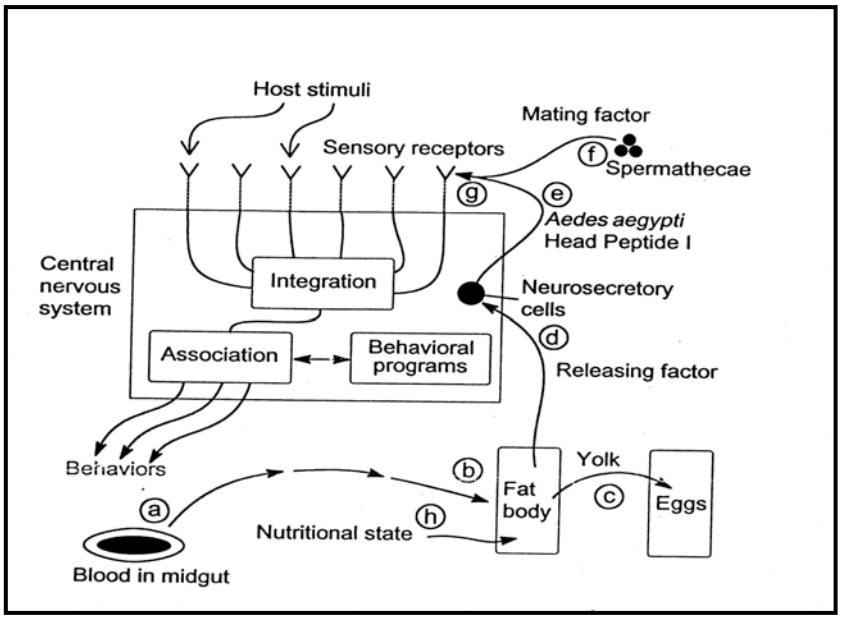

Figure 2.5 The expression of host-seeking behavior in *Aedes aegypti* mosquitoes (Klowden, 1995)

Once the source of protein is located by host-seeking behavior, the hormones released in the insect midgut after blood ingestion activate the fat body to produce yolk for egg development and produce a releasing factor. Neurosecretory cells in the central nervous system are activated to release *Aedes aegypti* Head Peptide I and, together with factors transferred from the male at mating, reduce the sensitivity of sensory receptors. Nutritional state may also affect the metabolic priorities of the fat body so that neither eggs nor *Aedes aegypti* Head Peptide I is produced when female is suboptimally nourished.

2.5 Mosquito management

The conference of Ministry of Public Health in 1994 in regards to control of disease vectors had reported the following development and success in vector control.

2.5.1. Chemical control

Starting from the old times, chemical control methods varied from gasoline, paris green, and a chemical synthesis named DDT which was believed to abolish malaria from the world. In Thailand, there was a successful use of DDT to control adult vector mosquitoes in Sarapi district, Chiangmai province in 1949. Then the use of DDT had expanded to other areas but without high success because the vector mosquitoes became resistant to the chemicals. In 1975 World Health Organization (WHO) reported the 42 species of *Anopheles* became resistant to the chemical substances. Another report by WHO in 1981 indicated that the number of chemical resistant *Anopheles* had increased to 47 species and become 50 species in 1986. Besides, another side effect caused by DDT is environmental pollution. As a result, nowadays, many countries prohibit the use of DDT and adopt other groups of environmental-friendly chemicals such as pyrythroids, rotenoids and nicotinoids as well as the plant extracts such as neem, citronella grass etc.

2.5.2. Physical control

Physical control is a very effective method of controlling mosquitoes because it eliminates their breeding sites. The source reduction refers to any method that physically alters mosquito-breeding site to render its unsatisfactory for completing mosquito life cycle. Source reduction projects will vary in size and cost depending on the type, but typically are the most effective and economical long-term method of mosquito control.

1. Environmental adjustment is an alternative way to successfully control vectors. The method depends on mosquito breed source. The easy and effective ways are removing water from containers to withdraw breed source and inverting the containers that could be breed source.

2. Environmental conversion to make the places unsuitable for mosquito breeding brings a short-term good result. The example methods are using washing powder, and vinegar etc.

3. Reduction of contact between human and mosquito vector and pathogen is another practical alternative. The easiest way is to protect oneself from mosquito by using mosquito protection chemicals or making mosquito wide screen.

2.5.3 Biological control

Biological control is the use of biological organisms to control pests; in this case are the insect pests. The theory of biological control is popular due to its nature of being host-specific, creating no impact on the non-target group.

2.5.3.1 Advantages of biological control

There are some obvious advantages to biological control are:

1. Minimal pollution of the environment
2. Little or no effect on beneficial and nontarget organisms
3. Slow development or nondetectable levels of host resistance
4. Possible recycling or establishment of biologicals to permanently reduce pest populations

2.5.3.2 Disadvantages of biological control

Certain disadvantages of biological control:

1. Each biological is host-specific, that is, effective against only

one or a few species

2. Mass production of biologicals is difficult
3. Generally they are more expensive initially than conventional methods
4. They require highly trained personnel to as the circumstances under which they can be used effectively
5. Scientific experiments in assessing their biological control potential may be greater than one-year duration
6. They are generally more difficult to use than conventional pesticides

2.5.3.3 Type of biological control

Biological control methods fall into six groups (Schrieber and Jones,Online, 2004).

1. Invertebrate predators (insects, flatworms)
2. Pathogens (bacteria, protozoa, fungi, viruses)
3. Parasites (nematodes)
4. Autocidal (genetic)
5. Botanicals (plants)
6. Vertebrate predators (fish, birds)

2.5.3.4 Larvivorous fish

Adopting fish to control mosquito larva is an interesting alternative biological. There have been many researches related to the subject since 1930 in using *Gambusia* fish to control mosquito in United States and other places in the world. At that time, there were not any of such biological practices related to vector mediated

illness control. The research and discovery by WHO in 1974 concerning the use of 12 different types of fish had leaded to the beginning of vector mosquito control. In the research, *Gambusia* is most used, followed by black fish and *Poecilia* respectively. In Maldives Island, *Poecilia* was used to control *An. tessellatus and An. subpictus* successfully. In India, there were many studies demonstrating the effectiveness of many local fish species in hunting mosquito larva of *An. stepensi* and *An. subpictus*, in both laboratory and field scales. Among all fish, *Poecilia* gave most successful result in mosquito larva control.

There are various kinds of fish that eat mosquito larva, but only a few types are being used in mosquito population control. Garcia and Legner (1999) described the well-known fish in mosquito larva control: *Gambusia* or mosquito fish in *Gambusia* parentage; it was originated in South America, Mexico and Caribbean. In United States, *Gambusia* was used to control mosquito in North Carolina for the first time, then used in New Jersey in 1905. The use of *Gambusia* in control mosquito larva, especially the malaria mosquito *Anopheles,* is under the purpose to decrease malaria vectors. *Gambusia* fish was first imported to Thailand in 1944 to control mosquito larva (Wongsiri and Andre, 1984). However, there were direct and indirect effects caused by its primary origin, for example, it was found that 30 fish types in the water source were affected by this import.

2.6 Use of guppy in mosquito control

A research by Wongsiri (1982) discovered the natural enemies of *Aedes aegypti and Culex quinquefasciatus* are not insects. The other researchers in Thailand also found that 20 mosquito enemies are non-insect predators, while there are 14 species of

insect predators being reported to eat mosquito in a period of larva. The important 3 species of fish are *Tilapia niclotica*, *Trichopos vittatus* and *Dermogenys pusillus*. It is also found that guppy is able to eat mosquito larva and *A. aegypti* pupa in critical environment.

เลาจนา ธีรภัทรสกุล (2522) studied the ability of *A. aegypti* mosquito larva in developing to adult stage in drains and garbage water (representing wastewater) containing Biochemical Oxygen Demand (BOD) of 9 and 14 ppm respectively; the research results in wastewater were compared with the results obtained by using tap water and distilled water (representing clean water). It was found that *A. aegypti* larva had nearly in all water types had nearly the same survival percentage and generated indifferent statistical result. Guppy can be used to control *A. aegypti* mosquito larva in polluted water. The strong characters of guppy are its resistance and endurance in high water temperature and water containing organic substance; for this reason, guppy can be used to control mosquito larva in wastewater. However, due to the fact that guppy is an attractive kind of fish; it is normally bred for pleasure purpose rather than for mosquito larva control.

มานิตย์ นาคสุวรรณ (2542) studied the effects of neem extract and neem oil on *Aedes aegypti* and guppy. The test of toxin with guppy showed that neem extract with 10 and 100 ppm did not affect guppy to die. When using neem extract with 1,000 ppm concentration, guppy's death rate reached 23.33% within 48 hours. After changing to neem oil, guppy's death rate was 90% within 48 hours and reached 100% when using 1,000 ppm concentration. The experimental was concluded that neem extract had no or little effect on guppy while neem oil caused mortal effect on guppy. It was explained that neem oil contains scum that blocks guppy's ability to breathe at

the water surface and also reduced level of oxygen in water to lower than vital standard, as a result, all guppies died.

There were many studies in comparing the effectiveness of guppy in eating other types of mosquito larva and *Aedes aegypti* mosquito larva during the first to fourth instar (ฐิติธัช สายยาโน, 2542). In each instar, the experiment was conducted in 5 plastic containers. A guppy aged 30 days was placed in each container together with 25 *A. aegypti* /day/container. The study time was 12 hours per day for 2 days between 6.00 am to 18.00 pm. The result showed that guppy ate all *A. aegypti* in first and second instar before 12 hours period, while *A aegypti* in third and fourth instar were not eaten. The study on the difference between an average number of *A. aegypti* in each instar being eaten by guppy during 1 hour showed that the difference in effectiveness of guppy on predation of *A. aegypti* in each instar by 95%.

The study of ability of guppy in eating *Anopheles minimus* by considering guppy's gender and time, showed that two female guppies ate 131 *An. minimus* between 6.00 am to 18.00 pm and ate 61 *An. minimus* between 18.00 pm to 6.00 am; this made an accumulated number of 192 *An. minimus* eaten by two female guppies. One female guppy and one male guppy ate 120 *An. minimus* between 6.00 am to 18.00 pm and 60 *An. minimus* between 18.00 pm to 6.00 am; made a total number of 180 *An. Minimus* eaten by one female guppy and one male guppy (วีระพล โพธิจิติ, 2540, quoted in ฐิติธัช สายยาโน, 2542).

ประภากร พ่วงฟัก (2542) conducted a research to study an ability of guppy in

eating *Anopheles maculatus* in first to fourth instars. In each period, the experiment was made in 5 plastic containers. A guppy aged 30 days was put in each container together with 25 *An. maculatus*/day/container. The study time was 12 hours per day for 2 days between 6.00 am to 18.00 pm. The experiment showed that an average number of 244 *An. maculatus* in first instar were eaten during 12 hours, 195.2 were eaten in second instar, 53.6 were eaten in third instar and 34.5 were eaten in fourth instar. The study on difference between an average number of *An. maculatus* in each instar being eaten by guppy during 1 hour showed that the different in effectiveness of guppy on predation of *An. maculatus* in each instar at 95%.

สมชาย แพทย์สันเทียะ (2542) conducted a research on ability of guppy in eating *Anopheles dirus* in first to fourth instar. In each instar, the experiment was done in 5 plastic containers. A guppy aged 30 days was put in each container together with 25 *An. dirus*/day/container. The study time was 12 hours per day for 2 days between 6.00 am to 18.00 pm. The experiment showed that an average number of 197 *An. dirus* in first instar were eaten during 12 hours, 72 in second instar, 41 in third instar and 18 in fourth instar. The study on the difference between average number of *An. dirus* in each instar being eaten by guppy during 1 hour found that the different in effectiveness of guppy on predation of *An. dirus* in each instar at 95%.

บุษกร บำรุงธรรม (2543) explained that the size of guppy's mouth is related to guppy's length, although the biggest prey that guppy can eat is as large as its mouth size when spreading out in 90 degree. The factor-affecting guppy's eating behavior is whether it is hungry or full. The standard food size of guppy is equal to its mouth size when spreading out in 45 degree. Guppy size has direct relation with food size in guppy's stomach. It clearly means that mouth size controls the food size. The study

also found that guppy grew up when being fed with live food and artificial food during 15-20 days. The same experiment after 45 days found that the difference in size of artificial food did not affect guppy growth. When guppy grows up to 20 days old (the width of mouth is 0.75 mm and total length is around 8.09 mm), it can be fed with food in any size but not too big.

CHAPTER III

MATERIALS AND METHODOLOGY

3.1 Instrumentation and reagents

3.1.1 Mosquito rearing

1. Guinea pig
2. Earthenware cup diameters 10 cm^3
3. Mosquitoes cage 0.5×0.5×1 m^3 and 11.5×12.5×12.5 $inch^3$
4. Guinea cage
5. Dog biscuit
6. Vitamin syrup
7. Cotton

3.1.2 Guppy rearing

1. Fish tank size 18.5×48×18.5 $inch^3$
2. Air pump
3. Fish food
4. Fish net

3.1.3 Important instruments

1. Environmental growth chamber	Hotpack
2. Conductivity/TDS meter	Jenway
3. pH meter	Denver
4. Stereo microscope	Olympus

5. BOD bottle	Kimble
6. Beaker	Pyrex
7. Heater	VELP Scientifica
8. Thermometer	
9. Dropper	
10. Ruler	
11. Tray	
12. Equipment for titration	
(flasks, pipettes, burettes and measuring cylinders)	

3.1.4 Important reagents

Reagents can prepared follow appendix B

1. Manganous sulfate solution	Carlo erba reagenti
2. Alkaline iodide-sodium azide solution	Carlo erba reagenti
3. Sulfuric acid (concentration)	Carlo erba reagenti
4. 0.025 N sodium thiosulfate solution	Carlo erba reagenti
5. Starch solution	Carlo erba reagenti
6. 0.0021M standard potassium bi-iodate solution	Carlo erba reagenti
7. Phosphate buffer solution	Carlo erba reagenti
8. Magnesium sulfate solution	Carlo erba reagenti
9. Calcium chloride solution	Carlo erba reagenti
10. Ferric chloride solution	Carlo erba reagenti
11. Sodium chloride	Carlo erba reagenti
12. Sodium carbonate	Carlo erba reagenti
13. Sodium phosphate	Carlo erba reagenti

3.2 Research procedure

3.2.1 Mosquito culture

The colonies of *Aedes aegypti*, the batches of laid eggs on filter pater or cover paper, were obtained from Infective Disease Insect Control Office in Saraburi province and the Department of Communicable Disease Control, Ministry of Public Health. *A. aegypti* eggs were hatched in water tray containing 4 liter of water at temperature of 25-30°C and kept for approximately 1-1.30 hours. Hatching took place within 10-12 hours.

After hatching, ground dog food was put into a tray for mosquito larvae. A new tray was replaced every one hour in order to obtain new batch of mosquito larvae at the same age and size. In case of incomplete hatching, the cover paper would be air-dried at room temperature to pressure eggs for the next hatch.

At the age of 4-5 hours, mosquito larvae could swim and, after 2-3 days, grew into second instar lavae, which was used in this study. Water in the tray and dog food must be changed every two days to prevent water pollution. At this stage, mosquito larvae grew up rapidly and molted 2-3 times, then pupated on the water surface. Pupae were transferred into beaker containing water; the beaker was then place in the wire cage for adult emergence. The emergence took 2-3 days.

A small container containing syrup and cotton was provided for adult mosquitoes. A young guinea pig was confined in small cage as blood source for female mosquitoes. A small earthenware cup containing water and filter paper was put in a cage for being used as an egg-laying site. After egg laying, filter paper with eggs was removed from the cage and left dried at room temperature, then kept at 15-20°C for next experiments.

3.2.2 Guppy rearing

Guppies were purchased from a fish shop and reared in a fish tank containing dechlorinated tap water. In order to acclimatize guppies to the new tank, guppies remained in the bag filled with water from the fish shop while the bag was placed into the tank where guppies were soaked slowly for 20 minutes. Then, guppies were released carefully into the tank when water temperatures in the bag and the tank were equalized (Mills, 1984). Guppies were fed with fish food 1-2 times a day.

3.2.3 Experimental plans

The planning of this research was completely randomized design with the use of 3 experimental units; first unit contained two female guppies (FF), second unit contained two male guppies (MM) and third unit contained one male guppy with one female guppy (MF). Guppies of both genders had same standard length of approximately 1 inch, as shown in Figure 3.1. This figure demonstrated standard length from tip of snout to caudal peduncle of fish model. Each experiment unit had 3 replications and divided into 6 experiments.

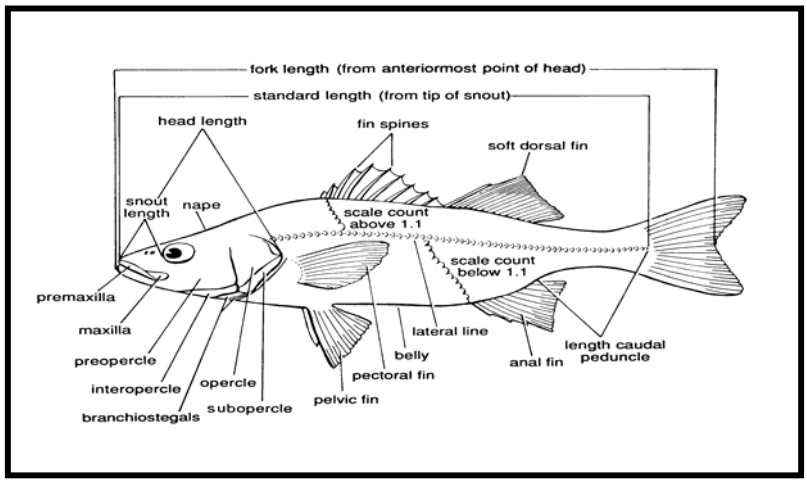

Figure 3.1 External features of fish model (Strauss and Bond, 1990)

The control experiment, where eight hundred mosquito larvae in second instar were used daily, was set up to control every experiments. The mosquito larvae were put into 1 liter of decholrinated tap water in a 2-liter beaker. The water quality related to temperature, DO, pH and air temperature was recorded.

Physical and chemical factors on guppy predation of mosquito second instar larvae

3.2.3.1 Effect of temperature on guppy predation of mosquito larvae

This experiment consisted of 4 treatment sets. Temperatures were set at 10, 20, 30 and 40 °C. The water temperature was controlled through environmental growth chamber. Eight hundred mosquito larvae in second instar were put into 1 liter of dechlorinated tap water in a 2-liter beaker. A group of two female guppies or two male guppies or a group of one male and one female guppy was put into the experimental set separately. Numbers of larvae being eaten by guppies were recorded for 24 hours.

3.2.3.2 Effect of photoperiod on predation of mosquito larvae

This experiment consisted of 4 treatment sets. The photoperiods were set at 9L:15D, 10L:14D, 12L:12D and 14L:10D. L represents the hours of light and D was represents the hours of nonlight (dark). The light was controlled through environmental growth chamber. Eight hundred mosquito larvae in second instar were put into 1 liter of dechlorinated tap water in a 2-liter beaker. A group of two female guppies or two male guppies or a group of one male and one female guppy was put into the experimental set separately. Numbers of larvae being eaten by guppies were recorded for 24 hours.

3.2.3.3 Effect of relative humidity on guppy predation of mosquito larvae

The experiment consisted of 4 treatment sets. Relative humidity was set at 50, 60, 70 and 80%. The relative humidity was controlled by environmental growth chamber. Eight hundred mosquito larvae in second instar were put into 1 liter of dechlorinated tap water in a 2-liter beaker. A group of two female guppies or two male guppies or a group of one male and one female guppy was put into the experimental set separately. Numbers of larvae being eaten by guppies were recorded for 24 hours.

3.2.3.4 Effect of pH on guppy predation of mosquito larvae

This experiment consisted of 4 treatment sets. The pH values were set at 5, 6, 7 and 8. The pH values were adjusted by using sodiumbicabonate and bisodiumphosphate. Eight hundred mosquito larvae in second instar were put into 1 liter of dechlorinated tap water in a 2-liter beaker. A group of two female guppies or two male guppies or a group of one male and one female guppy was put into the experimental set separately. Numbers of larvae being eaten by guppies were recorded for 24 hours.

3.2.3.5 Effect of salinity on guppy predation of mosquito larvae

The experiment consisted of 4 treatment sets. Salinity was set at 3, 4, 5 and 6 ppt. The salinity was adjusted by using NaCl. Eight hundred mosquito larvae in second instar were put into 1 liter of dechlorinated tap water in a 2-liter beaker. A group of two female guppies or two male guppies or a group of one male and one female guppy was put into the experimental set separately. Numbers of larvae being eaten by guppies were recorded for 24 hours.

3.2.3.6 Effect of wastewater on guppy predation of mosquito larvae

The experiment consisted of 2 wastewater samples. Wastewater consisted of influent and effluent obtained from Suranaree University of Technology's wastewater treatment plant. The water quality related to water temperature, DO, BOD, pH and air temperature was recorded. Eight hundred mosquito larvae in second instar were put into 1 liter of dechlorinated tap water in a 2-liter beaker. A group of two female guppies or two male guppies or a group of one male and one female guppy was put into the experimental set separately. Numbers of larvae being eaten by guppies were recorded for 24 hours.

3.2.4 Water analysis

3.2.4.1 Physical factors analysis

1. Water temperature was measured by a pH meter
2. Water pH was measured by a pH meter

3.2.4.2 Chemical factors analysis

1. Salinity was measured by Conductivity/TDS meter
2. Dissolved oxygen (DO) was measured by Azide Modification of the Winkler method (Sawyer, McCarty and Parkin, 1994)
3. Biochemical oxygen demand (BOD) was measured by 5 Days Incubation and Azide Modification of the Winkler method (Sawyer, McCarty and Parkin, 1994)

3.2.4.3 Dissolved oxygen (DO) measurement

Most volumetric methods of determining dissolved oxygen depend upon reactions that release an amount of iodine equivalent to the amount of oxygen

originally present, with subsequent measurement of the amount of iodine released by means of a standard solution of a reducing agent. Sodium thiosulfate is the reducing agent normally used, and starch solution is used to determine the end point. All reactions in the determination of oxygen involve oxidation and reduction. Starch is used as the end-point indicator, and forms a starch-iodine complex with iodine from dilute solutions to produce a brilliant blue color, and returns to a colorless form when the iodine is all reduced to iodide ion (Sawyer, McCarty and Parkin, 1994).

Procedure

1. Completely fill a 300 mL BOD bottle with the sample to be analyzed by siphoning the sample slowly into the bottle and allowing it to overflow for a period to displace the volume of the bottle two or three times.
2. By holding the tip of the pipet below the suface of the liquid, add 2 mL maganous sulfate solution and 2 mL alkaline iodide-sodium axide solution.
3. Replace stopper, avoiding trapping air bubbles, and mix well with gentle inversion. Repeat mixing after floc has settled halfway. Allow floc to settle again.
4. Remove stopper and add 2 mL of concentrated sulfuric acid. Hold pipet above the surfaced of the liquid. Mix until no floc is visible.
5. Withdraw 203 mL of the solution into the Erlenmeyer flask and titrate with sodium thiosulfate solution until the yellow color almost disappears.
6. Add 1 mL starch solution and continue titration until the blue color

disappears.

7. Record the volume of the thiosulfate used. Disregard any return of the blue color**.**

Calculations

The dissolved oxygen presence is expressed in mg/L and is equal to the number of volume of sodium thiosulfate used in the titration.

3.2.4.4 Biochemical oxygen demand (BOD) measurement

The BOD test determines the relative amount of oxygen necessary for biological oxidation of wastewates, effluents, and polluted water. It is the only test available to determine the amount of oxygen required by bacteria while stabilizing decomposble organic matter. Complete stabilization requires too long and incubation period for practical purposes; the 5 day period has been accepted as a standard. Samples are incubated in the dark at 20 ± 1 °C. Dissolved oxygen levels are measured initially and at the end of the 5 day period. The 5 day incubated sample must deplete more than 2 mg/l dissolved oxygen and have more than 0.5 mg/L dissolved oxygen left.

Procedure

1. Preparation of dilution water: Place desired volume of water in a suitable bottle and add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$, solutions/L of water.
2. Fill four 300 mL BOD bottles about half full with dilution water. Then use a large-tipped pipet to measure the predetermimed amount of sample into the four 300 mL BOD bottles. Fill each bottle with dilution water and insert stoppers. See that all air bubbles are excluded

3. Fill two bottles with straight dilution water and insert stoppers the same way (blank).
4. Incubate one dilution water bottle with three diluted sample bottles at 20°C.
5. Run a dissolved oxygen determination on both remaining bottles and record the initial DO content.
6. Run a dissolved oxygen determination on the first sample bottle after 5 days. The dilution water bottle should not have a change in DO concentration of more than ±0.2 mg/L in the 5 day period.

Calculations

$$\frac{\text{Initial DO of diluted sample - DO of sample after 5 days}}{\text{Percent of sample added}} \times 100 = \quad BOD_5 \text{ (mg/L)}$$

3.3 Data analysis

The results from each treatment were calculated to find average amount of *Aedes aegypti* larvae that had been eaten by guppies. The experiments 2, 3 and 5 were analyzed by using Analysis of Variance (ANOVA) method. The experiments 1, 4 and 6 were analyzed by using Student *t*-test method. All experiments between experimental units were analyzed by pair sample *t*-test. All experimental results of this study were analyzed by using SPSS program 11^{th} version.

An average amount of *A. aegypti* larvae eaten by guppies must be adjusted by abbott's formula equation.

$$\% \text{ Corrected Mortality} = \frac{\text{test mortality - control mortality}}{\text{test mortality}} \times 100$$

3.4 Location of research

The research was conducted at the Center for Scientific and Technological Equipment Building 2, Suranaree University of Technology, Nakhon Ratchasima province.

3.5 Study period

The research was conducted for 1 year.

CHAPTER IV

RESULTS AND DISCUSSION

The study of some environmental factors on predation of *Aedes aegypt* lavae by guppies is conducted under different environmental factors, which are temperature, photoperiod, relative humidity, pH and salinity. The study of effects created by wastewater on effectiveness of guppies on predation mosquito larvae is using pre-treatment wastewater (influent) and post-treatment wastewater (effluent) from wastewater treatment plants in Suranaree University of Technology. The experimental results from 3 experimental units composing of: 1) two female guppies (FF), 2) two male guppies (MM) and 3) one male and one female guppy (MF). Number of *A. aegypti* larvae eaten by guppies must be adjusted to mean of % corrected mortality. The experimental results are as follows:

4.1 The effect of temperature on predation of *A. aegypti* larvae by guppies

The effect of temperature did not show difference in effectiveness of guppies as predators of *A. aegypti* larvae in different temperature treatments at 20°C and 30°C. However, in the temperature treatments at 10°C and 40°C during 24 hours, guppies could not survive and then died before the end of experiment. The mean of effectiveness of guppies as predators of *A. aegypti* larvae at 20°C temperature was

lower than that of 30°C temperature in FF and MF. The temperatures suitable for the living of guppies are between 25-29°C (อมรรัตน์ เสริมวัฒนากุล, 2542). In conclusion, the mean of effectiveness of guppies in mosquito larvae predation was insignificantly different amongst the experimental units at each temperature

Table 4.1 The means of effectiveness on predation of mosquito larvae by guppies at different temperatures

Experimental units	Mean±SD	
	20°C	**30°C**
FF	99.20±0.74	99.67±0.36
MM	99.22±0.53	98.52±2.03
MF	98.44±0.47	98.96±0.62

Student *t*-test method was used to analyze the difference between two means at desired temperature and was shown in Table 4.1. The mean of effectiveness on predation of mosquito larvae by guppies at temperature of 20 °C was equal to the mean of effectiveness on predation of mosquito larvae by guppies at temperature of 30°C.

Table 4.2 The means of effectiveness on predation of mosquito larvae between experimental units at each temperature

Pair of experimental units	Paired differences of mean	
	20°C	**30°C**
FF and MM	**-0.02**	**1.15**
FF and MF	**0.76**	**0.71**
MM and MF	**0.78**	**-0.44**

The means of effectiveness on predation mosquito larvae between experimental units were not difference both of 20°C and 30°C as shown in Table 4.2.

Water temperature is an important factor to all aquatic life. In essence, when water temperature rises, life actitivities will increase (body metabolism). Likewise, when water temperature falls, life activities will decrease. Water temperature normally changes relatively to air temperature, which depends on season, altitude, and geographic location. In natural water sources, temperature changes slowly in a way that does not affect aquatic organisms, especially poikilotherms like fish, which is unable to maintain the level of its body temperature like homeotherms do. Therefore, body temperature of aquatic organisms will change relatively to water temperature and environment of the habitat. Additionally, water temperature also affect physical environment of the water sources, for example, the dissolved oxygen concentration will decrease when water temperature rises. The highly increased water temperature results in oxygen insufficiency in the water sources.

4.2 The effect of photoperiod on predation of *A. aegypti* larvae by guppies

The experiment on photoperiod affecting the effectiveness of guppies as predators of *A. aegypti* larvae did not find difference amongst the mean of effectiveness resulted from different photoperiod in 3 experimental units. The experimental results could be explained that there was only a few hours difference between each photoperiod. The study by สมชาย แพทย์สันเที๊ยะ (2542) on effectiveness of guppies as predators of second instar *Anopheles dirus* larvae during 12 hours experimental period, found effectiveness reducing from first hour where the mean was 77.2% to 21.6% in twelfth hour. ประภากร พ่วงฟัก (2542) conducted a similar experi

ment with *Anopheles maculates* larvae and also found effectiveness reducing from first hour where the mean was 71.2% to 60.8% in twelfth hour. It was noted that an increase in photoperiod did not affect the mean of effectiveness, and that the mean of effectiveness was indifferent in overall picture of the experiment. However, this experiment has also found the mean of effectiveness of guppies as predators of *A. aegypti* larvae at 14L:10D photoperiod, was higher than that of 9L:15D photoperiod in every experimental unit. In conclusion, the mean of effectiveness of guppies in mosquito larvae predation amongst experimental units at each photoperiod was indifferent.

Table 4.3 The means of effectiveness on predation of mosquito larvae by guppies at different photoperiod

Treatment	**Mean ± SD**		
	FF	**MM**	**MF**
9L:15D	72.79±22.30	89.80±10.22	98.41±1.51
10L:14D	84.08±19.32	99.51±0.11	99.73±0.20
12L:12D	99.50±0.43	75.59±27.86	99.55±0.33
14L:10D	86.87±22.08	99.81±0.06	99.78±0.65

One-way ANOVA analytical method was used in the experiment to find statistical difference in effectiveness of guppies on predation mosquito larvae in each photoperiod as shown in Table 4.3

Table 4.4 The means of effectiveness on predation of mosquito larvae between experimental units at each photoperiod

Pair of experimental units	Paired differences of mean			
	9L:15D	**10L:14D**	**12L:12D**	**14L:10D**
FF and MM	-17.00	-15.43	23.91	-12.94
FF and MM	25.62	-15.65	0.62	-12.91
MM and MF	8.61	-0.22	-23.29	0.27

The means of effectiveness on predation of mosquito larvae between experimental units were not differences at 9L:15D, 10L:14D, 12L:12D and 14L:10D as shown in Table 4.4.

4.3 The effect of relative humidity on predation of *A. aegypti* larvae by guppies

The experiment on relative humidity affecting the effectiveness of guppies as predators of *A. aegypti* larvae did not find difference in the mean of effectiveness at different relative humidity. The mean of effectiveness at 80% relative humidity in all experimental units was lower than that of other relative humidity. At 80% relative humidity, the mean showed predation effectiveness of 98.47% in FF, 99.46% in MM, and 99.17%. The comparison of the mean in pairs of FF and MM, MM and MF at 60% and 80% relative humidity, showed the difference at 0.05 level of significance. Both 60% and 80% relative humidity treatments on MM generated higher mean of effectiveness than in other experimental units, because MM composted of two male guppies that spent more time on foraging, because the intensity of guppy predators is low, as well as an increase in male-male competition for foods and females (Endler,

1995). In addition regarding temperature and relative humidity, evaporation rate will decrease when relative humidity rises. For this reason, temperature difference between water and air should not exceed 5°C to keep evaporation at a minimum (Christensen, Online, 2004).

Table 4.5 The means of effectiveness on predation of mosquito larvae by guppies at each level of relative humidity

Treatment	Mean ± SD		
	FF	**MM**	**MF**
50%	99.55±0.19	99.60±0.25	99.72±0.06
60%	99.34±0.49	99.78±0.12	99.32±0.21
70%	99.87±0.21	99.62±0.23	99.61±0.20
80%	98.47±1.69	99.46±0.63	99.17±1.01

One-way ANOVA analytical method was used in the experiment to find statistical difference in effectiveness of guppies on predation of mosquito larvae in each relative humidity as shown in Table 4.5.

Table 4.6 The means of effectiveness on predation of mosquito larvae between experimental units at each relative humidity

Pair of experimental units	Paired differences of mean			
	50%	**60%**	**70%**	**80%**
FF and MM	-0.53	-0.45*	0.26	-0.99*
FF and MF	-0.17	0.03	0.26	-0.70
MM and MF	-0.12	0.48*	0.00	0.30*

*The mean difference is significant at the 0.05 level.

The means of effectiveness on predation mosquito larvae between experimental units were different with level of significance 0.05 as shown in Table 4.5.

4.4 The effect of pH on predation of *A. aegypti* larvae by guppies

The experiment on pH affecting the effectiveness of guppies as predators of *A. aegypti* larvae and using bisodiumphosphate as an agent to decrease pH in water, found that bisodiumphosphate could only reduce pH by little and adjust pH to medium value. In this experiment, water had pH 8 in normal condition, and pH 7 after adjustment. The experiment was carried out on pH 7 and 8. The experiment did not generate different mean of guppies' effectiveness at pH 7 and 8 in all experimental units. It was also found that, MM and MF at pH 7 contained a higher mean of effectiveness than at pH 8. The suitable pH for the living of guppies is between 6.5-7.5 (อมรรัตน์ เสริมวัฒนากุล, 2542). In conclusion, the mean of effectiveness of guppies in mosquito larvae predation amongst the experimental units at each pH value was indifferent.

Table 4.7 The means of effectiveness on predation of mosquito larvae by guppies at different pH

Experimental units	Mean±SD	
	pH7	pH8
FF	99.41±0.57	99.50±0.45
MM	99.30±0.69	98.36±0.17
MF	99.49±0.43	96.83±4.40

Student *t*-test method was used to analyze the difference between two means at pH level and shown in Table 4.7. The means of effectiveness on predation of mosquito larvae by guppies at pH 7 are equal to the means of effectiveness on predation mosquito larvae by guppies at pH 8.

Table 4.8 The means of effectiveness on predation of mosquito larvae between experimental units at each pH

Pair of experimental units	Paired differences of mean	
	pH7	**pH8**
FF and MM	**-0.83**	**2.67**
FF and MF	**0.11**	**0.14**
MM and MF	**0.19**	**-2.53**

The means of effectiveness on predation mosquito larvae between experimental units were not differences both pH 7 and pH 8 as shown in Table 4.8.

Temperature and pH are related to ammonia which derives from a process to remove amino radical from amino acid or other amino compound (Deaminization) as well as the breakdown of organicmaterials through the use of water (hydrolysis), besides its natural derivation from nitrate reduction in the oxygenless environment. When water contains high pH and temperature, ammonia will transform into un-ionized form (NH_3), which is harmful to the aquatic animals because ammonia poison will destroy the sensitive tissue of fish gills and results in malfunction of the dissolved oxygen diffusion into gill cells. The poisonous property of ammonia increases in the water containing low level of dissolved oxygen.

4.5 The effect of salinity on predation of *A. aegypti* larvae by guppies

The experiment on salinity affecting the effectiveness of guppies as predators of *A. aegypti* larvae in MF, found difference between the mean of effectiveness at different salinity: which were, 3 and 5 ppt, 3 and 6 ppt, 4 and 5 ppt, 4 and 6 ppt, at 0.05 level of significance. The suitable salinity for guppies in early stage should be

between 0-3 ppt (กรมประมง, 2541). In MF at 0.05 level of significance, the mean of effectiveness at 3 ppt salinity was 98.68% and became 99.79% at 4ppt salinity, which were higher than the mean at 5 and 6 ppt salinity, which were carrying 94.72% and 94.23% respectively. Additionally, in FF, 3 ppt salinity created the highest mean of effectiveness.

The mean of effectiveness of guppies in mosquito larvae predation amongst MM and MF at 3ppt salinity was different. Male courtship behaviour occurred in MF, which represented a balance between natural selection (through predation) and sexual selection. High degree of courtship behavior increases male mating success. When low predation exists in environment, male has more display courtship behavior (Braun and Harper, 1993). In this study, the MF had low effectiveness because male spent times displaying courtship behavior.

Table 4.9 The means of effectiveness on predation of mosquito larvae by guppies at each level of salinity

Treatment	**Mean ± SD**		
	FF	**MM**	**MF**
3 ppt	99.89±8.09	97.14±2.13	98.68±1.34
4 ppt	98.48±1.53	91.77±11.14	99.79±0.37
5 ppt	85.67±12.32	99.64±0.10	94.72±1.49
6 ppt	82.46±30.38	86.90±22.22	94.23±0.26

One-way ANOVA analytical method was used in the experiment to find statistical difference in effectiveness of guppies on predation of mosquito larvae in each level of salinity as shown in Table 4.9.

There were differences in effectiveness of guppies on predation mosquito larvae in different level of salinity at least 2 points at the level of significance 0.05. Therefore used Post Hoc Test by LSD method to find the differences between 2 variable pairs in 4 different levels of salinity. Given the level of significance at 0.05 as shown in Table 4.10.

Table 4.10 The statistical analysis in comparison of each variable pair in 4 different levels of salinity by LSD method

Treatment	Mean Difference		
	FF	**MM**	**MF**
3 ppt and 4 ppt	-6.60	5.37	-1.10
3 ppt and 5 ppt	6.21	-2.50	3.96*
3 ppt and 6 ppt	9.42	10.23	4.45*
4 ppt and 5 ppt	12.81	-7.87	5.06*
4 ppt and 6 ppt	16.02	4.86	5.56*
5 ppt and 6 ppt	3.21	12.73	0.49

*The mean difference is significant at the 0.05 level.

Table 4.11 The difference of means of effectiveness on predation mosquito larvae between experimental units at each salinity

Pair of experimental units	Paired differences of mean			
	3 ppt	**4 ppt**	**5 ppt**	**6 ppt**
FF and MM	-5.25	6.71	-13.97	-4.44
FF and MF	-6.80	-1.31	-9.05	-11.77
MM and MF	-1.54*	-8.02	4.91*	-7.32

* The mean difference is significant at the 0.05 level.

The means of effectiveness on predation of mosquito larvae between MM and MF were different at 3 and 5 ppt at level of significance 0.05 as shown in Table 4.11.

4.6 The effect of wastewater on predation of *A. aegypti* larvae by guppies

The experiment on wastewater affecting the effectiveness of guppies as predators of *A. aegypti* larvae did not find difference in the mean of effectiveness amongst all experimental units containing wastewater from different sources in a wastewater treatment plant. It was noticed that, in FF and MM, the mean of effectiveness in influent wastewater was lower than that of effluent wastewater. This could be explained that influent wastewater contains higher BOD than effluent wastewater. It was also found that guppies could survive even in influent wastewater containing BOD of not more than 13 ppm and DO of not more than 5 ppm. Generally, fish will die when oxygen concentration becomes lower than 1 ppm, while fish's growth and breeding rate will slowly reduce if continually living in the water containing oxygen concentration between 1-5 ppm (Alablaster and Lloyd, 1980; Conroy and Herman, 1970; Boyd, 1982 quoted in ศรีสมร สิทธิกาญจนกุล, 2545). In conclusion, the mean of effectiveness of guppies in mosquito larvae predation amongst the experimental units containing different kinds of wastewater was indifferent.

Table 4.12 The means of effectiveness on predation of mosquito larvae by guppies in wastewater treatment plants

Experimental units	Mean±SD	
	influent	effluent
FF	99.82±2.24	98.18±1.52
MM	96.08±2.24	98.18±1.52
MF	59.21±28.05	53.00±35.64

Student *t*-test method was used to analyze the difference between two means at different wastewater and shown in Table 4.12. The means of effectiveness on predation of mosquito larvae by guppies in influent were equal to the means of effectiveness in predation mosquito larvae by guppies in effluent.

Table 4.13 The means of effectiveness on predation of mosquito larvae between experimental units at each wastewater

Pair of experimental units	Paired differences of mean	
	influent	**effluent**
FF and MM	**3.75**	**1.69**
FF and MF	**40.61**	**46.87**
MM and MF	**36.86**	**45.18**

The means of effectiveness on predation of mosquito larvae between experimental units were not differences both of influent and effluent as shown in Table 4.13.

CHAPTER V

CONCLUSION

This study, in regards to the effects of some environmental factors on effectiveness of guppies as predators of *A. aegypti* larvae, can be concluded that guppies in each experimental unit have level of effectiveness as predators of *A. aegypti* higher than 50%. Besides the environmental factors given in this experiment that affect effectiveness of guppies, there were also internal factors of guppies, which were uncontrollable variables such as genetics variation, and biology of guppy itself.

The results of this experiment have shown the environmental factors which were temperature, pH and wastewater did not affect the effectiveness of guppies as predators of mosquito larvae, for the reasons that treatments relating to each environmental factors in this experiment stayed within the range where guppies could live on; except the temperature treatments at 10 and 40°C where guppies could not live in. This experiment carried out study on pH 7 and pH 8 by using bisodiumphosphate as an adjustment agent. The future experiments should be conducted on lower value than pH 7 by using other types of adjustment agents that are not harmful to fish, in order to compare the effectiveness of guppies as predators of mosquito larvae.

In regards to other envionmental factors, it was found that photoperoid and relative humidity treatments given at different levels did not cause the effectiveness of guppies in mosquito larvae predation to be different, amongst all experimental units.

However, in regards to salinity, it was found that the effectiveness of mosquito predation in MF experimental unit differed from one treatment to the others, while the effectiveness was indifferent amongst other experimental units at all levels of treatments. It could be concluded that suitable salinity for the living of guppies is between 0-3 ppt due to the findings in this experiment, showing the effectiveness of guppies in MF experimental units containing 3 ppt and 4 ppt salinity was higer than that of 5 ppt and 6 ppt salinity. It should also be noted that internal factors of guppies, for example, courtship behaviour, could decrease the predation effectiveness.

The comparison of predation effectiveness of guppies in experimental units based on environmental factors: which were, temperature, photoperiod, pH and wastewater, did not find difference in predation effectiveness amongst experimental units; except when studying on other environmental factors: salinity and relative humidity.

The experiment on salinity found difference in predation effectiveness between MM and MF units at 3 ppt and 5 ppt salinity as already explained in one part of this report concerning the effect of salinity on effectiveness of guppies in mosquito larvae predation. This part of the report concerns comparison of predation effectiveness between the experimental units of each pair containing different salinity. It was found that MF unit contained higher predation effectiveness than MM unit at 3 ppt salinity, while MM unit carried higher predation effectiveness than MF unit at 5 ppt salinity, at level of significance.

The results could be explained in a way that each salinity treatment affected guppies, as well as the internal factors of guppies that also affect the effectiveness in mosquito larvae predation. In MM unit, the predation effectiveness increased due to

the foraging competition behaviour amongst guppies of same sexes which is a normal instict of animals.

The experiment on relative humidity found difference in predation effectiveness between MM and MF unit at 60% and 80% relative humidity. At 60% relative humidity, MM maintained the predation effectiveness higher than MF and, at 80% relative humidity, MM also generated higher predation effectiveness than MF, at level of significance. It was also found relative humidity at 80% in all experimental units created low predation effectiveness. This can be explained in a way that the lower the relative humidity, the higher the water evaporation rates. It is also due to the fact related to vast temperature difference between water and air, whereas in normal condition, water temperature is lower than air temperature by a little. Therefore, the change in temperature and increased evaporation rate provoked guppies to react and resulted in increased predation effectiveness. The knowledge is crucial for fish breeding, for example, in fish aquarium where room temperature and water temperature must be defined in order to prevent water evaporation from aquarium.

According to the environmental factors studied in this experiment, the conclusions are temperature should be between 20-30°C, pH should be around 7-8, salinity should be taken into consideration in guppies breeding to serve a purpose of mosquito larvae control. Levels of photoperiod and relative humidity do not result in any difference in effectiveness of guppies in mosquito larvae predation.

In addition, guppies should be separated by sex, not mixing both sexes in same place. It is due to the fact that foraging competition occurs amongst guppies of same sex and it helps increasing effectiveness in mosquito larvae predation. Contrary, courtship behavior occurs when male and female guppies stay in same place, it results

in decreased predation effectiveness. The knowledge can be adapted to serve the business purposes in guppy-breeding farms, in order to increase efficiency in guppy production.

Suggestion

1. The further study should separate male and female including study each of guppies in order to know the effectiveness of guppies predation on *A. aegypti* in the gender form.
2. The fancy guppies should be selected in another experiment.
3. Because guppies have genetic variation, they should be cultured in the laboratory and should be controlled the involved factor on guppies.

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APPENDICES

APPENDIX A

Data of experiments

Table A.1 The study of temperature on predation of *Aedes aegypti* at 20°C

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No. larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
20°C	1	female and female	8.27	34	29.2	5.7	5.8	800	0	5.3	4	99.5
	2	female and female	8.28	30	28.7	6	6.4	799	1	6	2	99.75
	3	female and female	8.28	30	28.7	6	6.4	305	495	6.4	5	98.36
			8.28	31.33	28.87	5.90	6.20	634.67	165.33	5.90	3.67	99.20
	1	male and male	8.27	27	24.7	6.5	6.4	297	503	7.2	4	98.65
	2	male and male	8.25	28	25.9	6.8	7	299	501	7.2	2	99.33
	3	male and male	8.25	28	25.9	6.8	7	650	150	6	2	99.69
			8.26	27.67	25.50	6.70	6.80	415.33	384.67	6.80	2.67	99.22
	1	male and female	8.47	28	25.5	6.5	6.2	391	409	7	4	98.98
	2	male and female	8.47	28	25.5	6.5	6.3	225	575	7	4	98.22
	3	male and female	8.42	28.5	26.5	6.5	6.4	534	266	6.5	10	98.13
			8.45	28.17	25.83	6.50	6.30	383.33	416.67	6.83	6.00	98.44

Remark: temperatures at 10 and 40°C were not conducted.

Table A.2 The study of temperature on predation of *Aedes aegypti* at 30°C

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No. larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
30°C	1	female and female	8.34	28	27.8	6.2	5.5	800	0	6.3	6	99.25
	2	female and female	8.39	27.5	26.6	6.2	5.6	800	0	6.15	1	99.88
	3	female and female	8.45	31	28.3	6	5.7	800	0	5.75	1	99.88
			8.39	28.83	27.57	6.13	5.60	800.00	0.00	6.07	2.67	99.67
	1	male and male	8.4	29	27.3	6.8	5.2	679	121	6.6	26	96.17
	2	male and male	8.33	31.5	28.8	5.33	6.8	799	1	6.8	2	99.75
	3	male and male	7.22	28	29.6	5.53	5.65	800	0	6.4	3	99.63
			7.98	29.50	28.57	5.89	5.88	759.33	40.67	6.60	10.33	98.52
	1	male and female	8.19	28	29	5.5	5.6	795	5	7.7	3	99.62
	2	male and female	8.28	27	26.5	6.13	4.7	798	2	7	9	98.87
	3	male and female	8.26	28	25.2	6.23	6	787	13	7	0	98.38
			8.24	27.67	26.90	5.95	5.43	793.33	6.67	7.23	4.00	98.96

Table A.3 The study of photoperiod on predation of *Aedes aegypti* at 9L:15D

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
9L:15D	1	female and female	8.34	31	30.5	5.97	5.93	670	130	6.03	0	83.75
	2	female and female	8.42	30	30.1	6	5.7	700	100	6	0	87.5
	3	female and female	8.24	32	31.4	5.8	6	377	423	6	0	47.13
			8.33	31.00	30.67	5.92	5.88	582.33	217.67	6.01	0.00	72.79
	1	male and male	8.3	33	31.7	5.57	6.2	734	66	6	0	91.75
	2	male and male	8.13	33	32.1	5.7	6.4	630	170	6	0	78.75
	3	male and male	8.24	32	31.4	6	6.8	182	618	6.2	2	98.9
			8.22	32.67	31.73	5.76	6.47	515.33	284.67	6.07	0.67	89.80
	1	male and female	7.89		31.7	5.96	6.45	428	372	6.35	14	96.73
	2	male and female	8.44	33	31.8	6.07	5.25	800	0	4.8	9	98.88
	3	male and female	8.22	33	31.8	5.67	5.45	800	0	5.2	3	99.63
			8.18	33.00	31.77	5.90	5.72	676.00	124.00	5.45	8.67	98.41

Table A.4 The study of photoperiod on predation of *Aedes aegypti* at 10L:14D

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
10L:14D	1	female and female	8.06	30	28.5	6.07	5.8	500	300	6.1	0	62.5
	2	female and female	8.06	30	28.5	6.07	5.8	720	80	6.1	0	90
	3	female and female	8.06	30	28.5	6.07	5.8	798	2	6.1	0	99.75
			8.06	30.00	28.50	6.07	5.80	672.67	127.33	6.10	0.00	84.08
	1	male and male	8.35	33	31	5.83	6	488	312	6	3	99.39
	2	male and male	8.35	33	31	5.83	6	770	30	6	3	99.61
	3	male and male	8.35	33	31	5.83	6	647	153	6	3	99.54
			8.35	33.00	31.00	5.83	6.00	635.00	165.00	6.00	3.00	99.51
	1	male and female	8.24	31	30.3	6.17	5.95	695	105	6.1	2	99.71
	2	male and female	8.24	31	30.3	6.17	5.95	800	0	6.1	2	99.75
	3	male and female	8.24	31	30.3	6.17	5.95	730	70	6.1	2	99.73
			8.24	31.00	30.30	6.17	5.95	741.67	58.33	6.10	2.00	99.73

Table A.5 The study of photoperiod on predation of *Aedes aegypti* at 12L:12D

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
12L:12D	1	female and female	8.34	32	30.7	6.5	5.35	800	139	6.25	0	100
	2	female and female	8.55	32	30	6.5	5.35	800	0	5.85	6	99.25
	3	female and female	8.55	32	30	6.5	5	800	0	5.85	6	99.25
			8.48	32.00	30.23	6.50	5.23	800.00	46.33	5.98	4.00	99.50
	1	male and male	8.34	32	30.7	6.5	6.05	359	441	6.25	0	44.88
	2	male and male	8.34	32	30.7	6.5	6.15	661	0	6.25	0	82.63
	3	male and male	8.55	32	30	6.5	5.85	800	0	5.85	6	99.25
			8.41	32.00	30.47	6.50	6.02	606.67	147.00	6.12	2.00	75.59
	1	male and female	8.55	32	30	6.5	4.05	800	0	5.85	6	99.25
	2	male and female	8.42	33	31.6	5.63	4.7	796	4	6.2	12	98.49
	3	male and female	8.2	32	30.2	6.33	5.35	731	69	6.1	8	98.91
			8.39	32.33	30.60	6.15	4.70	775.67	24.33	6.05	8.67	98.88

Table A.6 The study of photoperiod on predation of *Aedes aegypti* at 14L:10D

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
14L:10D	1	female and female	8.44	31	29.8	6.63	6	730	70	6.57	2	99.73
	2	female and female	8.48	31	29.5	6.63	6.57	200	600	6.27	1	99.5
	3	female and female	8.48	31	29.5	6.63	6.3	491	309	6.27	0	61.38
			8.47	31.00	29.60	6.63	6.29	473.67	326.33	6.37	1.00	86.87
	1	male and male	8.44	31	29.8	6.63	6.6	745	55	6.6	1	99.87
	2	male and male	8.48	31	29.8	6.63	6.2	524	276	6.27	1	99.81
	3	male and male	8.25	32	29.5	6.3	5.9	798	2	6.27	0	99.75
			8.39	31.33	29.70	6.52	6.23	689.00	111.00	6.38	0.67	99.81
	1	male and female	8.44	31	29.8	6.63	6.67	458	342	6.6	1	99.78
	2	male and female	8.48	31	29.5	6.63	6.13	670	130	6.27	1	99.85
	3	male and female	8.38	32	30.3	6.57	6.3	702	98	6.4	2	99.72
			8.43	31.33	29.87	6.61	6.37	610.00	190.00	6.42	1.33	99.78

Table A.7 The study of relative humidity on predation of *Aedes aegypti* at 50%

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
50%	1	female and female	8.42	29	28	6.47	6	772	28	6.1	5	99.35
	2	female and female	8.42	28.5	28	6.47	5.5	478	322	6	2	99.58
	3	female and female	8.42	28.5	28	6.47	5.3	712	88	6	2	99.72
			8.42	28.67	28.00	6.47	5.60	654.00	146.00	6.03	3.00	99.55
	1	male and male	8.5	30	29.1	6.5	6.7	693	107	6.47	3	99.57
	2	male and male	8.5	30	29.1	6.5	6.77	476	324	6.47	3	99.37
	3	male and male	8.51	30.5	28.9	6.5	6.53	795	5	7	1	99.87
			8.50	30.17	29.03	6.50	6.67	654.67	145.33	6.65	2.33	99.60
	1	male and female	8.51	30.5	28.9	6.17	5.4	461	339	7	1	99.78
	2	male and female	8.51	30.5	28.9	6.17	6.37	298	502	7	1	99.66
	3	male and female	8.51	30.5	28.9	6.17	6.67	375	425	7	1	99.73
			8.51	30.50	28.90	6.17	6.15	378.00	422.00	7.00	1.00	99.72

Table A.8 The study of relative humidity on predation of *Aedes aegypti* at 60%

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
60%	1	female and female	8.38	29	28.3	6.47	5.9	800	0	6.9	5	99.38
	2	female and female	8.38	29	28.3	6.47	6.2	702	92	6.9	5	99.29
	3	female and female	8.38	29	28.3	6.47	6.53	797	3	6.9	5	99.37
			8.38	29.00	28.30	6.47	6.21	766.33	31.67	6.90	5.00	99.35
	1	male and male	8.48	31	29.5	6.63	5.93	773	27	6.33	1	99.87
	2	male and male	8.48	31	29.5	6.63	6.23	770	30	6.33	1	99.65
	3	male and male	8.48	31	29.5	6.63	6.27	760	40	6.33	1	99.87
			8.48	31.00	29.50	6.63	6.14	767.67	32.33	6.33	1	99.80
	1	male and female	8.44	31	29.8	6.63	5.9	790	10	6.83	4	99.49
	2	male and female	8.44	31	29.8	6.63	6.2	433	367	6.83	4	99.08
	3	male and female	8.44	31	29.8	6.63	6.53	649	151	6.83	4	99.38
			8.44	31.00	29.80	6.63	6.21	624.00	176.00	6.83	4.00	99.32

Table A.9 The study of relative humidity on predation of *Aedes aegypti* at 70%

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
70%	1	female and female	8.49	31	29.3	6.73	6.3	791	0	6.6	3	99.63
	2	female and female	8.45	31	29.3	6.77	6.4	791	0	6	0	100
	3	female and female	8.45	31	29.3	6.77	6.47	800	0	6	0	100
			8.46	31.00	29.30	6.76	6.39	794.00	0.00	6.20	1.00	99.88
	1	male and male	8.51	29.5	28.9	6.57	5.6	800	0	6.63	3	99.63
	2	male and male	8.51	29.5	28.9	6.57	5.47	799	0	6.63	3	99.63
	3	male and male	8.51	29.5	28.9	6.57	6.5	725	70	6.63	3	99.59
			8.51	29.50	28.90	6.57	5.86	774.67	23.33	6.63	3.00	99.62
	1	male and female	8.51	29.5	28.9	6.57	5.93	796	1	6.63	3	99.62
	2	male and female	8.51	29.5	28.9	6.57	6.63	795	0	6.63	3	99.63
	3	male and female	8.51	29.5	28.9	6.57	6.43	733	60	6.63	3	99.59
			8.51	29.50	28.90	6.57	6.33	774.67	20.33	6.63	3.00	99.61

Table A.10 The study of relative humidity on predation of *Aedes aegypti* at 80%

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No.larvae were prey	No.larvae were remained	DO Control ppm	No.larvae were died	% corrected mortality
80%	1	female and female	8.42	29	28	6.5	7.07	115	685	6.5	4	96.52
	2	female and female	8.42	28.5	28	6.5	6.87	652	148	6.5	4	99.39
	3	female and female	8.42	28.5	28	6.5	7	799	1	6.5	4	99.5
			8.42	28.67	28.00	6.50	6.98	522.00	278.00	6.50	4.00	98.47
	1	male and male	8.62	30	29.3	6.7	5.87	652	148	6.3	4	99.39
	2	male and male	8.62	30	29.3	6.7	6.8	794	6	6.27	4	99.5
	3	male and male	8.54	29	28.1	6.73	6.4	800	0	6.27	4	99.5
			8.59	29.67	28.90	6.71	6.36	748.67	51.33	6.28	4.00	99.46
	1	male and female	8.52	28	27.9	6.5	6	416	384	6.6	1	99.76
	2	male and female	8.52	28	27.9	6.5	6.5	396	404	6.27	1	99.74
	3	male and female	8.54	29	28.1	6.7	6.27	500	300	6.4	10	98
			8.53	28.33	27.97	6.57	6.26	437.33	362.67	6.42	4	99.17

Table A.11 The study of pH level on predation of *Aedes aegypti* at pH 7

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No.larvae were remained	DO Control ppm	No. larvae were died	% corrected mortality
pH 7	1	female and female	7.26	27	26	6.17	6.5	797	3	6.4	2	99.75
	2	female and female	7.11	26.5	26	6.4	6.4	760	40	6.8	2	99.73
	3	female and female	7.2	27	26.2	6.57	6.5	790	10	6.2	0	98.75
			7.19	26.83	26.07	6.38	6.47	782.33	17.67	6.47	1.33	99.41
	1	male and male	7.26	27	26	6.17	6	747	53	6.4	2	99.73
	2	male and male	7.11	26.5	26	6.4	6.3	786	14	6.8	2	99.75
	3	male and male	7.2	27	26.2	6.57	6.2	792	8	6.2	0	99
			7.19	26.83	26.07	6.38	6.17	775.00	25.00	6.47	1.33	99.49
	1	male and female	7.26	27	26	6.17	6.13	650	150	6.4	2	99.69
	2	male and female	7.11	26.5	26	6.4	6.3	695	105	6.8	2	99.71
	3	male and female	7.2	27	26.2	6.57	6.5	788	12	6.2	0	98.5
			7.19	26.83	26.07	6.38	6.31	711.00	89.00	6.47	1.33	99.30

Remark: pH5 and pH6 were not conducted.

Table A.12 The study of pH level on predation of *Aedes aegypti* at pH 8

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No.larvae were remained	DO Control ppm	No. larvae were died	% corrected mortality
pH 8	1	female and female	8.1	25	24.2	7	6.76	793	7	7.2	0	99.13
	2	female and female	8.1	25	24.2	7	6.86	795	5	7.2	0	99.38
	3	female and female	8.1	25	24.2	7	6.63	800	0	7.2	0	100
			8.1	25	24.2	7	6.75	796	4	7.2	0	99.50
	1	male and male	8.13	27	26.4	7.2	7.3	769	31	7.3	0	99.5
	2	male and male	8.13	27	26.4	7.2	7.3	733	66	7.3	0	91.75
	3	male and male	8.13	27	26.4	7.2	7.3	793	6	7.3	0	99.25
			8.13	27.00	26.40	7.20	7.30	765.00	34.33	7.30	0.00	96.83
	1	male and female	8.05	24	22	6.5	7.5	797	3	7.5	5	99.37
	2	male and female	8.05	24	22	6.5	7.5	756	42	7.5	5	99.34
	3	male and female	8.05	24	22	6.5	7.5	797	3	7.5	5	99.37
			8.05	24.00	22.00	6.50	7.50	783.33	16.00	7.50	5.00	99.36

Table A.13 The study of salinity on predation of *Aedes aegypti* at 3 ppt

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No. larvae were remained	Conductivity (ms)	DO Control ppm	No. lavae were died	% corrected mortality
3 ppt	1	female and female	8.48	28	26.9	6.3	6.67	545	255	6.95	6.83	31	94.31
	2	female and female	8.48	28	26.9	6.6	6.43	70	730	6.93	6.43	12	82.86
	3	female and female	8.38	28	26.5	6.6	6.4	795	5	6.89	6.43	12	98.49
			8.45	28.00	26.77	6.50	6.50	470.00	330.00	6.92	6.56	18.33	91.89
	1	male and male	8.48	28	26.9	6.6	5.7	678	122	6.95	6.43	12	98.23
	2	male and male	8.48	28	26.9	6.6	5.7	226	574	6.95	6.43	12	94.69
	3	male and male	8.2	29	26.9	5.53	5.8	800	0	7.56	6.43	12	98.5
			8.39	28.33	26.90	6.24	5.73	568.00	232.00	7.15	6.43	12.00	97.14
	1	male and female	8.39	27	28.1	6.7	5.8	798	2	6.71	7	4	99.5
	2	male and female	8.39	27	28.1	6.7	6.9	140	660	6.87	7	4	97.14
	3	male and female	8.39	27	28.1	6.7	6.6	676	124	6.81	7	4	99.41
			8.4	27.0	28.1	6.7	6.4	538.0	262.0	6.8	7.0	4.0	98.7

Table A.14 The study of salinity on predation of *Aedes aegypti* at 4 ppt

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No. larvae were remained	Conductivity (ms)	DO Control ppm	No. lavae were died	% corrected mortality
4 ppt	1	female and female	8.08	28	26.7	6.9	6.27	594	196	9.15	6.53	20	96.74
	2	female and female	7.93	28	27.3	6.8	6	147	471	9.06	6.77	3	99.09
	3	female and female	7.93	28	27.3	6.8	6.53	746	53	9.15	6.77	3	99.6
			8.0	28.0	27.1	6.8	6.3	495.7	240.0	9.1	6.7	8.7	98.5
	1	male and male	8.56	30	28.6	6.73	6.3	97	700	9.07	6.27	21	79
	2	male and male	8.56	30	28.6	6.73	5.77	609	180	9.12	6.27	21	96.77
	3	male and male	7.93	28	27.3	6.8	5.9	631	167	9.34	6.77	3	99.53
			8.4	29.3	28.2	6.8	6.0	445.7	349.0	9.2	6.4	15.0	91.8
	1	male and female	8.2	29	27	6.67	6.77	620	179	9.13	5.95	4	99.36
	2	male and female	8.2	29	27	6.67	6.5	798	0	9.27	6.77	0	100
	3	male and female	8.24	28	27.1	5.53	5.7	794	0	9.04	6.77	0	100
			8.21	28.67	27.03	6.29	6.32	737.33	59.67	9.15	6.50	1.33	99.79

Table A.15 The study of salinity on predation of *Aedes aegypti* at 5 ppt

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No. larvae were remained	Conductivity (ms)	DO Control ppm	No. lavae were died	% corrected mortality
5 ppt	1	female and female	8.3	29	26.9	6.3	6.37	798	2	11.35	6.7	0	99.75
	2	female and female	8.3	29	26.9	6.3	6.53	615	185	11.1	6.7	0	76.88
	3	female and female	8.3	29	26.9	6.3	6.53	643	157	11.08	6.7	0	80.38
			8.30	29.00	26.90	6.30	6.48	685.33	114.67	11.18	6.70	0.00	85.67
	1	male and male	8.06	30.5	27.7	5.47	5.8	790	10	11.44	6.2	2	99.75
	2	male and male	8.06	30.5	27.7	5.47	5.73	503	297	11.35	6.2	2	99.6
	3	male and male	8.06	30.5	27.7	5.47	6.13	451	349	11.23	6.2	2	99.56
			8.06	30.50	27.70	5.47	5.89	581.33	218.67	11.34	6.20	2.00	99.64
	1	male and female	8.6	29	27.3	6.43	5.27	786	14	11.32	6.03	35	95.55
	2	male and female	8.6	29	27.3	6.43	5.53	799	1	11.21	6.03	35	95.62
	3	male and female	8.6	29	27.3	6.43	5.27	500	300	11	6.03	35	93
			8.60	29.00	27.30	6.43	5.36	695.00	105.00	11.18	6.03	35.00	94.72

Table A.16 The study of salinity on predation of *Aedes aegypti* at 6 ppt

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	DO (after) ppm	No. larvae were prey	No. larvae were remained	Conductivity (ms)	DO Control ppm	No. lavae were died	% corrected mortality
6 ppt	1	female and female	8.13	27	26.4	7.5	6.55	800	0	13.13	7	0	100
	2	female and female	8.13	27	26.4	7.5	6.27	800	0	13.33	7	0	100
	3	female and female	8.13	27	26.4	7.5	6.53	379	421	13.32	7	0	47.38
			8.13	27.00	26.40	7.50	6.45	659.67	140.33	13.26	7.00	0.00	82.46
	1	male and male	8.24	31	28.8	6.73	6.17	800	0	13.27	6.5	1	99.88
	2	male and male	8.24	31	28.8	6.73	6.37	237	563	13.2	6.5	1	99.58
	3	male and male	8.24	31	28.8	6.73	6.03	490	310	13.23	6.77	0	61.25
			8.24	31.00	28.80	6.73	6.19	509.00	291.00	13.23	6.59	0.67	86.90
	1	male and female	8.29	29	26.8	6.7	6.63	793	7	13.04	7.04	46	94.2
	2	male and female	8.29	29	26.8	6.7	6.83	800	0	13.26	7.04	46	94.25
	3	male and female	8.29	29	26.8	6.7	6.67	799	1	13.35	7.04	46	94.24
			8.29	29.00	26.80	6.70	6.71	797.33	2.67	13.22	7.04	46.00	94.23

Table A.17 The study of wastewater on predation of *Aedes aegypti* at influent wastewater

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	BOD ppm	No. larvae were prey	No. larvae were remained	DO Control ppm	No. lavae were died	% corrected mortality
influent	1	female and female	7.61	23	23.7	3.2	12	799	1	5	1	99.87
	2	female and female	7.61	23	23.7	3.2	12	671	129	5	1	99.85
	3	female and female	7.61	23	23.7	3.2	12	398	402	5	1	99.75
			7.61	23.00	23.70	3.20	12.00	622.67	177.33	5.00	1.00	99.82
	1	male and male	7.61	25	24.6	3	13	247	553	5.2	16	93.52
	2	male and male	7.61	25	24.6	3	13	690	110	5.2	16	97.68
	3	male and male	7.61	25	24.6	3	13	540	260	5.2	16	97.03
			7.61	25.00	24.60	3.00	13.00	492.33	307.67	5.20	16.00	96.08
	1	male and female	7.7	28	27.9	1.3	11.3	661	139	3.3	0	82.63
	2	male and female	7.7	28	27.9	1.3	11.3	535	265	3.3	0	66.88
	3	male and female	7.7	28	27.9	1.3	11.3	225	575	3.3	0	28.13
			7.7	28.0	27.9	1.3	11.3	473.7	326.3	3.3	0.0	59.2

Table A.18 The study of wastewater on predation of *Aedes aegypti* at effluent wastewater

Treatment	Replication	Experimental units	pH	Air temp °C	Water temp °C	DO (before) ppm	BOD ppm	No. larvae were prey	No. larvae were remained	DO Control ppm	No. lavae were died	% corrected mortality
effluent	1	female and female	8.32	23	23	7.1	2.03	794	6	6.2	1	99.87
	2	female and female	8.32	23	23	7.1	2.03	732	68	6.2	1	99.86
	3	female and female	8.32	23	23	7.1	2.03	755	45	6.2	1	99.88
			8.32	23.00	23.00	7.10	2.03	760.33	39.67	6.20	1.00	99.87
	1	male and male	8.21	26	24.5	7.1	2.17	400	400	7.2	4	99
	2	male and male	8.21	26	24.5	7.1	2.17	349	450	7.2	4	99.11
	3	male and male	8.21	26	24.5	7.1	2.17	112	688	7.2	4	96.43
			8.21	26.00	24.50	7.10	2.17	287.00	512.67	7.20	4.00	98.18
	1	male and female	8.5	22.5	23.6	6.2	1.73	103	697	7.2	0	12.88
	2	male and female	8.5	22.5	23.6	6.2	1.73	648	350	7.2	0	81
	3	male and female	8.5	22.5	23.6	6.2	1.73	521	279	7.2	0	65.13
			8.50	22.50	23.60	6.20	1.73	424.00	442.00	7.20	0.00	53.00

APPENDIX B

Reagent preparation protocol

1. Dissolved oxygen (DO)

Reagents

a) Manganous sulfate solution: Dissolve 480 g $MnSO_4.4H_2O$, 400 $MnSO_4.2H_2O$, or 364 g $MnSO_4.H_2O$ in distilled water, filter, and dilute to 1 L. The $MnSO_4$ solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

b) Alkali-iodide-axide reagent: Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L. Add 10 g NaN_3 dissolved in 40 mL distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

c) Sulfuric acid, H_2SO_4, conc: One mL is equivalent to about 3 mL alkali-iodide-azide reagent.

d) Starch: Use either and aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

e) Standard sodium thiosulfate titrant: Dissolve 6.205 g $Na_2S_2O_3.5H_2O$ in distilled water. Add 1.5 mL 6N NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

f) Standard potassium bi-iodate solution, 0.0021M: Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1000 mL.

Standardization

Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6N H_2SO_4 or a few drops of conc H_2SO_4 and 20 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solutions are of equal strength, 20 mL 0.025M $Na_2S_2O_3$ should be required.

2. Biochemical oxygen demand (BOD)

Reagents

a) Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7H_2O$, and 1.7 g NH_4Cl in about 500 mL distilled water and dilute to 1 L.

b) Magnesium sulfate solution: Dissolve 22.5 g $MgSO_4.7H_2O$ in distilled water and dilute to 1 L.

c) Calcium chloride solution: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.

d) Ferric chloride solution: Dissolve 0.25 g $FeCl_3.6H_2O$ in distilled water and dilute to 1 L.

APPENDIX C

Statistical analysis

1. Independent samples *t*-test

Student *t*-test method was used to analyze the difference between means of treatment

Statistical Hypothesis

H_o: $\mu_1 = \mu_2$

H_1: $\mu_1 \neq \mu_2$ (i≠j)

Given $\propto = 0.05$ It is found that level of significance from the result is higher than the given $\propto$ at 0.05. So, it is concluded that

Accept null hypothesis H_o: $\mu_1 = \mu_2$

2. One-way ANOVA

One-way ANOVA analytical method was used to analyze the difference of means from treatment.

Statistical Hypothesis

H_o: $\mu_1 = \mu_2 = \mu_3 = \mu_4$

H_1: $\mu i \neq \mu j$ (i≠j)

Given $\propto = 0.05$ It is found that level of significance from the result is higher than the given $\propto$ at 0.05. So, it is concluded that

Accept null hypothesis H_o: $\mu_1 = \mu_2 = \mu_3 = \mu_4$

3. Paired sample *t*-test

Student *t*-test method was used to analyze the difference between two means in same treatment.

Statistical Hypothesis

H_o: $\mu_1 - \mu_2 = d_0$

H_1: $\mu_1 - \mu_2 \neq d_0$

$d_0 = 0$

Given $\propto = 0.05$ It is found that level of significance from the result is higher than the given $\propto$ at 0.05. So, it is concluded that

Accept null hypothesis H_o: $\mu_1 - \mu_2 = d_0$

CURRICULUM VITAE

Miss Wanichaya Charoonphong was born on April 5th, 1978, in Udon Thani province and finished high school from Satri Rachinuthit School. She graduated bachelor's degree in Environmental Science from Khon Kaen University in 1998. She continued her graduated study for a master's degree in Environmental Biology in School of Biology at Suranaree University of Technology in 2000.