ความชุกชุมและพฤติกรรมเกี้ยวพาราสีของหิ่งห้อย *Luciola* sp. HK 1
ที่ศูนย์ศึกษาการพัฒนาห้วยฮ่องไคร้อันเนื่องมาจากพระราชดำริ
อำเภอดอยสะเก็ด จังหวัดเชียงใหม่

นางสาวณัฏฐา พรฐิติฉันท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ABUNDANCE AND COURTSHIP BEHAVIOR OF A FIREFLY *Luciola* sp. HK 1 AT THE HUAI HONGKHRAI ROYAL DEVELOPMENT STUDY CENTER, DOI SAKET DISTRICT, CHIANG MAI PROVINCE

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ณัฏฐา พรฐิติฉันท์ : ความชุกชุมและพฤติกรรมเกี้ยวพาราสีของหิ่งห้อย *Luciola* sp. HK 1 ที่ศูนย์ศึกษาการพัฒนาห้วยฮ่องไคร้อันเนื่องมาจากพระราชดำริ อำเภอดอยสะเก็ด จังหวัดเชียงใหม่ (ABUNDANCE AND COURTSHIP BEHAVIOR OF A FIREFLY *Luciola* sp. HK 1 AT THE HUAI HONGKHRAI ROYAL DEVELOPMENT STUDY CENTER, DOI SAKET DISTRICT, CHIANG MAI PROVINCE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. ดวงแข สิทธิเจริญชัย, หน้า.

การศึกษาความชุกชุมและพฤติกรรมเกี้ยวพาราสีของหิ่งห้อย *Luciola* sp. HK 1 ที่ศูนย์ศึกษาการพัฒนาห้วยฮ่องไคร้อันเนื่องมากจากพระราชดำริ อำเภอดอยสะเก็ด จังหวัดเชียงใหม่ ตั้งแต่เดือน กรกฎาคม 2557 จนถึงเดือนมิถุนายน 2558 บริเวณแนวถนนริมบึงด้านหลังสำนักงานศึกษาและพัฒนาอาชีพเพาะเลี้ยงกบ เส้นทางศึกษายาว 120 เมตร โดยการนับจำนวนแสงของหิ่งห้อยโดยวิธีการนับโดยตรง จากนั้นทำการโฉบหิ่งห้อยทุกตัวที่อยู่ในระยะศึกษา เพื่อนำมาหาอัตราส่วนจำนวนตัวเต็มวัยของหิ่งห้อย *Luciola* sp. HK 1 จากหิ่งห้อยทั้งหมดในหน่วย ตัว/ช่วง 40 เมตรของเส้นทางศึกษา พบว่าจำนวนโดนประมาณจากการคำนวณของหิ่งห้อย *Luciola* sp. HK 1 ในเดือนกรกฎาคมเท่ากับ 2.57±0.76, เดือนสิงหาคมเท่ากับ 2.77±0.64, เดือนกันยายนเท่ากับ 2.64±0.84, เดือนตุลาคมเท่ากับ 3.68±0.83, ไม่พบหิ่งห้อยชนิดนี้ในบริเวณศึกษาตั้งแต่เดือนพฤศจิกายน 2557 ถึงเดือนมีนาคม 2558 มาพบหิ่งห้อยอีกครั้งในเดือนเมษายนเท่ากับ 4.90±0.99 เดือนพฤษภาคมเท่ากับ 4.40±0.80 และเดือนมิถุนายนเท่ากับ 4.30±0.86 โดยพบว่าหิ่งห้อยกระพริบแสงสูงสุดในช่วงเวลา 55 นาทีหลังกระพริบแสงแรก ช่วงที่แนะนำให้ท่องเที่ยวชมหิ่งห้อยในบริเวณนี้คือ 19:00-20:00 นาฬิกา การศึกษาพฤติกรรมเกี้ยวพาราสีของหิ่งห้อย *Luciola* sp. HK 1 ซึ่งนำตัวอย่างหิ่งห้อยตัวเต็มวัยมาทดลองในห้องปฏิบัติการ โดยนำหิ่งห้อยเพศผู้และเพศเมียอย่างละ 1 ตัว ใส่ในกล่องพลาสติกใสสี่เหลี่ยมมีฝาปิด จากนั้นทำการบันทึกภาพวิดีโอการเคลื่อนที่และการกระพริบแสงของหิ่งห้อย โดยแบ่งออกเป็น 3 ช่วงด้วยกันคือ ก่อนที่เพศผู้และเพศเมียจะมองเห็นกัน ขณะที่เพศผู้และเพศเมียมองเห็นกัน และขณะที่เพศผู้และเพศเมียเข้าผสมพันธุ์กัน พบว่าหิ่งห้อยมีการกระพริบแสง 3 รูปแบบ คือ แสงกระพริบของเพศผู้ แสงกระพริบตอบของเพศเมีย และแสงกระพริบเพื่อผสมพันธุ์

ภาควิชา	ชีววิทยา	ลายมือชื่อนิสิต	....
สาขาวิชา	สัตววิทยา	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
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NUTTA PORNTHITICHANT: ABUNDANCE AND COURTSHIP BEHAVIOR OF A FIREFLY *Luciola* sp. HK 1 AT THE HUAI HONGKHRAI ROYAL DEVELOPMENT STUDY CENTER, DOI SAKET DISTRICT, CHIANG MAI PROVINCE. ADVISOR: ASST. PROF. DUANGKHAE SITTHICHAROENCHAI, Ph.D., pp.

Abundance and courtship behavior of *Luciola* sp. HK 1 at the Huai Hongkhrai Royal Development Study Center, Doi Saket District, Chiang mai Province were investigated from July 2014 to June 2015. Adults of the *Luciola* sp. HK 1 firefly were sampled along a 120-meter transect line nearby a creek. The numbers of adult firefly flashed were recorded by direct counting method using a counter. Then, an aerial net was used to catch all adult fireflies in each transect line section. The numbers of all adult fireflies caught and the numbers of adults *Luciola* sp. HK 1 caught were recorded and the indirect estimated numbers of individual of *Luciola* sp. HK 1 were calculated in the unit of individuals/ 40-meter section of transect line. The indirect estimated numbers of individual of *Luciola* sp. HK 1 were 2.57±0.76 in July, 2.77±0.64 in August, 2.64±0.84 in September, 3.68±0.83 in October, 4.90±0.99 in April, 4.40±0.80 in May, 4.30±0.86 in June; and adult *Luciola* sp. HK 1 was not found in November 2015 to March 2015. Firefly flash display peaks at about 55 minutes after first flash, so the recommended time for ecotourism is around 7:00-8:00 PM. To study of courtship behavior of a firefly *Luciola* sp., the study was conducted with adult fireflies in the laboratory during 6:00-8:00 PM by keeping a couple specimen of one adult male and one adult female fireflies in a clear square box with lid, then, started recording the moving and flashing of the couple specimen. The video was recorded in 3 phases; before the couple saw each other, during the period that the couple saw each other and the mating period between the couple specimen. It was found that there were 3 flashing patterns; male flash, female respond and mating flash patterns.

Department:	Biology	Student's Signature	....
Field of Study:	Zoology	Advisor's Signature	....
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CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

Firefly is a soft-bodied beetle belonging to order Coleoptera, family Lampyridae. There are about 2,000 firefly species worldwide (Lewis and Cratsley, 2008; McDermott, 1966; Ohba, 2004). The firefly taxonomist, Dr. Angoon Lewvanich, suggested that there were 15 genera of fireflies in Thailand, namely, *Abscondita, Asymmetricata, Colophotia, Curtos, Diaphanes, Inflata, Lamprigera, Luciola, Pteroptyx, Pygoluciola, Pyrocoelia, Selerotia, Sternocladius, Triangulara* and *Vesta* (Personal communication). Eight genera, 55 species are recorded from central and eastern Thailand, and 7 genera, 21 species are recorded from the northeastern part. The most common firefly species found in Thailand belong to genus *Luciola* (Chunram and Lewvanich, 2000).

Firefly life cycle consists of 4 stages: eggs, larvae, pupae and adults. The pupal stage is the longest stage comparing to other stages in the life cycle (Chunram and Lewvanich, 2000).

Fireflies have an important role in ecosystem as an abundance indicator (Ballantyne and Buck, 1979). As a natural predator, fireflies play the role as natural enemies against medical and veterinary pests. Firefly larvae prey on small fresh water shellfishes, which some of them are carriers of diseases such as fasciolopsiasis and eosinophilic meningoencephalitis, to human and animal (Longcore and Rich, 2006). Firefly larvae also prey on golden applesnails; known as *Pomacea canaliculata, which is an* introduced species causing a lot of ecological and economic damages as rice field and horticultural pests (Sherley, 2000). Firefly larvae have a potential to control the parasite spreading, for example, *Opisthorchis viverrini* that is the major risk factor for cholangiocarcinoma in endemic countries throughout Southeast Asia, due to their preying behavior that prey on the parasitic carrier, freshwater snails (Sherley, 2000).

Therefore, fireflies should be considered as an important natural enemy for medical and agricultural pest control.

Furthermore, in the term of ecotourism, one of the most popular activities is the firefly-watching tour which seasonally routine activity in many Asian countries and United States of America (Ho and Chu, 2002; Nurancha et al., 2013; Ohba and Wong, 2004). Since 2000, Amphawa has become the popular place for both domestic and international tourists for firefly watching activities in Thailand. (Lertsuchatavanich, 2005; Utarasakul, 2012). In Kuala Lumpur, the synchronous *Pteroptyx tener* firefly is found along the Selangor River; so, firefly watching is the major component of ecotourism activity. However, the negative impacts from the tourists have been ignored and continued to contribute to the degradation in many natural areas; for example, a lot of firefly habitats in Malaysia have been destroyed. So, this is a powerful incentive for conservation in many parts of the world. An appropriate environmental management system is necessary for the local environment conservation.

The firefly watching business is at present effective for the conservation of the site. The financial benefit is recognized well, this business generated broadly revenue such as local guides, local restaurants, handicraft shop and home stay businesses. The local people who understand the natural environment needs to be protected and used in sustainable development (Abe, 2002). Nowadays, some information of firefly watching has been providing to the tourists, for example, the light and noise pollution are disturbing behavior of fireflies (Pongjun, 2013). In the present time, fireflies are considered as rare insect species, as their proper habitats have been destroying by urban growth boundary (Yuma, 2007).

Firefly is a self-bioluminescent insect, with the illuminating organs or light organs located in its 5^{th} and 6^{th} abdominal segments in male and 5^{th} abdominal segment in female (Demary et al., 2006; Hanson, 1962). The light organs compose of 7,000-8,000 big cells called photocytes. The light generating ability is the result of bioluminescent reaction in the cells, producing non-heated light. This reaction is caused by luciferase enzyme stimulating luciferin to change its composition (McElroy et al., 1969). Fireflies use bioluminescent signals to communicate as courtship system, warning displays and predation (Lloyd, 1983). Mating system affects directly on the number of firefly populations, studying this courtship pattern would lead to firefly conservation planning, as well as adapting ecosystem to match firefly habitats.

The flashing displays in fireflies are the behavior that seldom be found in any other insects. The luminescence is reported by biologists as "cold light" become the process to produce the bioluminescence does not produce heat. This behavior is crucial to adult fireflies; they attract their mates using this light. As well, firefly larvae can also illuminate, but for the pray-luring purpose (Longcore and Rich, 2006).

In addition, only a few studies of firefly abundance, flashing displays and courtship behavior of adult fireflies are published. In Thailand, also none of the study or report about fireflies has ever been conducted in The Huai Hong Khrai Royal Development Study Center, Doi Saket District, Chiang Mai Province.

Therefore, the objectives of this research are to study of the moving and flashing patterns in courtship behavior of *Luciola* sp. HK 1 (unidentified species); and focus especially on the abundance and peak flashing period of the adults *Luciola* sp. HK 1 all year round at the Huai Hongkhrai Royal Development Study Center, Doi Saket District, Chiang Mai Province.

CHAPTER II

LITERATURE REVIEWS

2.1 Classification and distribution

Firefly is classified into the order Coleoptera, family Lampyridae (Barrows et al., 2008). They are small to medium-sized beetles, normally not longer than 3 cm. They are distributed worldwide, with highly diverse, especially in warm and humid environments (McDermott, 1964). There are several documents reported about the unusual habitats such as intertidal spray zone, ant nests, marshes, running water, seepage environments, hot springs and decaying tree trunks (Jeng et al., 2007). Nowadays, over 2,000 firefly species are classified in more than 100 genera (McDermott, 1966). They were discovered around the world, including Asia, Americas, Australia and Europe (Stanger-Hall et al., 2007). The largest subfamily of Lampyrids is Luciolinae, with more than 300 species (McDermott, 1964). In Thailand, there are over 100 species recorded, the largest genus is *Luciola* with 90 species (Chunram and Lewvanich, 1996). *Luciola* spp. are highly diverse in morphological character (Jeng, 2007). The systematics of fireflies is continuing flux, as new species are kept being discovered. Moreover, the family-group compositions and boundaries have been still often changing. There are three major classifications; Olivier (1907), McDermott (1964) and Crowson (1972), to group firefly genera (Figure 1). Currently, they are using a mix of McDermott's and Crowson's system to classification of Lampyridae (Jeng et al., 2007).

Modern Family-group Classifications of Lampyridae

Olivier, 1907 (9 subfamilies, 48 genera)	McDermott, 1966 (7 subfamilies, 85 genera)	Crowson, 1972 [a] (8 subfamilies)
Subfamily Lampyrinae Latreille (9)[b]	Subfamily Lampyrinae Latreille (53)	Subfamily Lampyrinae Latreille
Subfamily Lamprocerinae Olivier (14)	Tribe Lampyrini Latreille (11)	Subfamily Amydetinae Olivier
Subfamily Photininae LeConte (6)[c]	Tribe Pleotomini Green (5)	Subfamily Photurinae Lacordaire
Subfamily Lucidotinae LeConte (7)	Tribe Lamprocerini Olivier (5)	Subfamily Luciolinae Lacordaire
Subfamily Dadophorinae Olivier (1)	Tribe Cratomorphini Green (5)	Subfamily Ototretinae McDermott
Subfamily Megalophthalminae Olivier (2)	Tribe Photinini LeConte (27)	Subfamily Pterotinae LeConte
	Subtribe Photinina LeConte (13)	Subfamily Cyphonocerinae Crowson
Subfamily Amydetinae Olivier (1)	Subtribe Luciodotina Lacordaire (10)	Subfamily Ototretadrilinae Crowson
Subfamily Photurinae Lacordaire (1)	Subtribe Dadophorina Olivier (1)	
Subfamily Luciolinae Lacordaire (7)	Subtribe Phosphaeina McDermott (2)	
	Subtribe Lamprigerina McDermott (1)	
	Subfamily Amydetinae Olivier (12)	
	Tribe Amydetini Olivier (2)	
	Tribe Vestini McDermott (5)	
	Tribe Psilocladini McDermott (5)	
	Subfamily Photurinae Lacordaire (4)	
	Subfamily Luciolinae Lacordaire (9)	
	Tribe Luciolini Lacordaire (6)	
	Tribe Curtosini McDermott (1)	
	Tribe Ototretini McDermott (2)	
	Subfamily Matheteinae LeConte (2)	
	Subfamily Pterotinae LeConte (1)	
	Subfamily Rhagophthalminae Olivier (4)	

[a] The more recent classifications of Nakane (1991) and Branham and Wenzel (2001) recognized the same eight subfamilies although with modified generic compositions for Lampyrinae, Amydetinae, and Cyphonocerinae (the synonymy of Cyphonocerinae by Jeng et al. [1998a] was based on Nakane's [1991] redefinition of the subfamily). Branham and Wenzel's cladistic study indicated that Lampyrinae, Amydetinae, and Ototretinae were polyphyletic; that Luciolinae and perhaps Photurinae were monophyletic; that Ototretinae and Pterotinae should be considered incertae sedis in Elateroidea; while the status of two subfamilies could not be commented upon (Ototretadrilinae was excluded and Cyphonocerinae was represented by a single genus). Kazantsev (2006) created Cheguevarini and tentatively placed the tribe as incertae sedis in Lampyridae.

[b] Value in parentheses indicates the number of explicitly included genera.

[c] A name homonymous with the subfamilial name Photininae Giglio-Tosi, in the Mantidae (Mantodea).

Figure 1 The three major classifications of Lampyridae (Jeng et al., 2007).

There are three types of firefly larvae defined by their habitats; aquatic, semi-aquatic and terrestrial. Aquatic fireflies such as *L. cruciata*, *L. owadai* etc. live under water and adapt the body for aquatic life, lateral abdominal gills (Figure 2). Semi-aquatic fireflies; such as *Pristolycus lucifera*, *Pteroptyx valida* Olivier, *P. malaccae* Gorham, live in coastal environments (Figure 3). They normally get into water to search for their prey. This group of firefly larvae has no gills.

Terrestrial fireflies larvae live on damp ground, while, sometimes, some larvae are found on trees; including *Photuris* and *Photinus* (Figure 4), they lack any specialized respiratory for an aquatic life like gills (Fu et al., 2012). They are usually found under stones in ground depression or under leaf litter.

Figure 2 The aquatic firefly larvae of *Luciola cruciata* (photo by Yoshihito Furukawa).

Figure 3 The semi-aquatic firefly larvae of *Pteroptyx malaccae* Gorham (photo by Sutisa Loomboot).

Figure 4 The terrestrial firefly larva of *Photinus* sp. at Fontenelle Forest, Nebraska, USA (Padelford and Padelford, 2012).

2.2 Morphology and description of an adult firefly

The study that conducted on adult firefly samples, most of them are males, by examining the appearances such as pronotum, wings, antennae, males' aedeagus and light organs. Identification of fireflies in immature stages is hardly conducted as the samples must be raised in the laboratory to confirm their types by comparing them with other identified adult fireflies, then describing their characteristics. However, there are few reports of larval characteristics.

Most fireflies have the characteristics like other insects in order Coleoptera (Figure 5). Adult fireflies' body size varies from 5-25 mm. The firefly body is divided into three parts; head, thorax and abdomen. The body is tube-shaped where the head is covered by a hard carapace named "pronotum". Males' characteristics mostly are big compound eyed, full grown wings, 2 segments of luminescent organs. On the opposite, females are found with differ appearances. Females of *Photinus extensus* Gorham have short wings, while *Lampyris noctiluca* Linnaeus look like wingless worms, which are also known as “glowworms”. Some female firefly species have similar appearances to males; they have full grown wings, a slightly bigger body, smaller eyes and only 1 luminescent segment (Thancharoen, 2009).

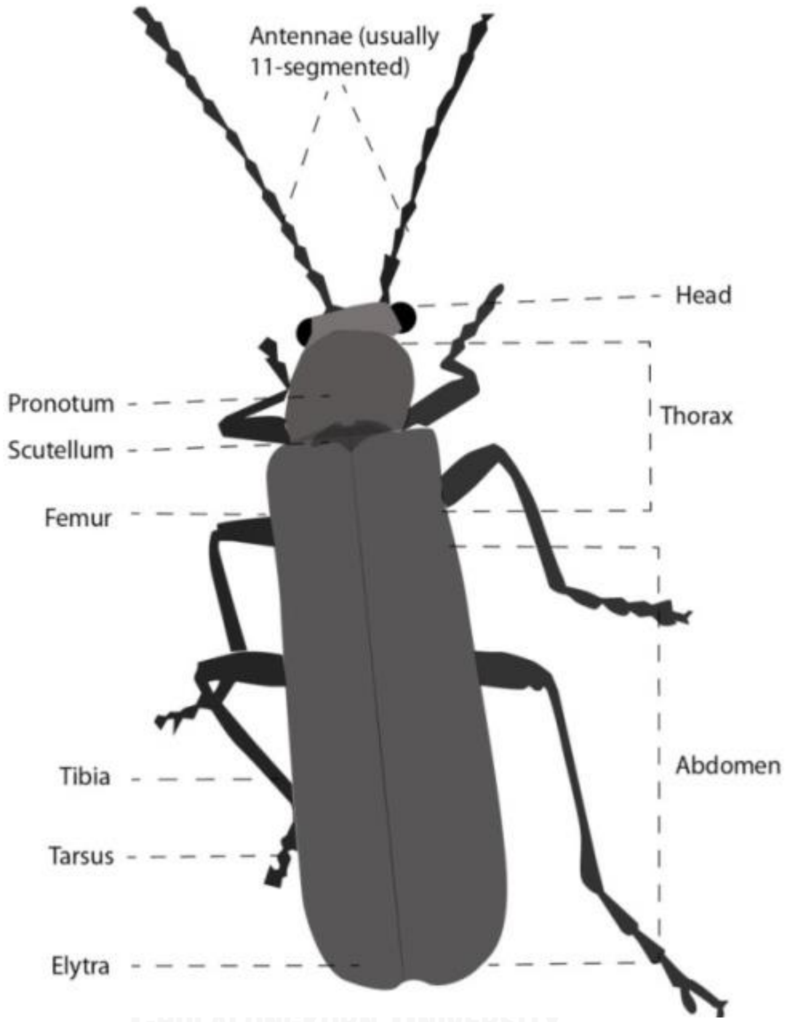


Figure 5 The basic beetle anatomy (Tejon, 2015).

2.3 Life cycle in general

The life cycle of *Luciola* sp. HK 1 has not been studied yet. Therefore, there is no basic information about its life cycle and biology. Here, life cycle of the fireflies closely related to this particular *Luciola* sp. HK 1 is explained.

Luciola's life cycle is complete metamorphosis, composed of 4 stages: egg, larva, pupa and adult (Figure 6).

Eggs

A gravid female *Luciola aquatilis* was reported to lay white eggs within two days after copulation. These eggs were attached under aquatic plant leaves. Each female laid and attached around 40 eggs per leaf and took around 1-5 days for laying eggs at night. The eggs took approximately 10-12 days to hatch and become larvae. Eggs of some fireflies species could luminate (Buschman, 1984). In *L. ficta*, eggs were about 0.57±0.02 mm in diameter, with milky yellow color and lasted approximately 19.1±1.5 days before hatching (Ho et al., 2010).

Larvae

There are three types of larvae: aquatic, semi-aquatic and terrestrial larvae. They are predaceous and their diets are various depending on the types of the larvae, such as snails, slugs, earthworms and other insect larvae. Firefly Larvae are able to emit light but they cannot control the flash pattern as in the adult fireflies. *Luciola* sp. HK 1 is aquatic firefly, inhabits in freshwater, such as marshes, ditches and ponds. In 2007, *L. aquatilis* larvae were reported to have six instars classified by size and morphological characters. The larval stage was the longest period in firefly's life cycle, varied from 57-99 days (Thancharoen, 2007). In *L. ficta*, the larval body length ranged from 1.80±0.12 to 14.55±0.46 mm depending on the larval stage. They were black, soft bodies with clear dorsal line. A larva had eight abdominal segments, each segment had a pair of lateral tracheal gills. The larval stages lasted about 328.9±33.2 days under water and 10.9±7.8 days on terrestrial (Ho et al., 2010).

Pupae

Each species has different papal stage durations. Gender was reported as a factor affecting pupal duration; male pupae took longer time to develop than female pupae (Fu et al., 2005). The body weight of a female *L. ficta* pupa was 0.5424±0.1397 mg and 0.403±0.0586 mg in male. A pupa was milky white color with head, antennae, wings and visible legs. A female was about 9.06±0.63 mm in length and 3.43±1.36 mm in width, and male was 8.56±0.40 mm in length and 2.56±0.18 mm in width. The pupal duration was 14.7±5.3 days (Ho et al., 2010).

Adults

Most of male firefly species have developed their wings, while females are found dissimilar kinds of wings: short-winged or wingless. The lighting organ is located at the 5^{th}-6^{th} abdominal segments in males, and only in 6^{th} abdominal segment in female fireflies. The adult duration of *L. ficta* was reported at about 15.7±5.2 days (Ho et al., 2010).

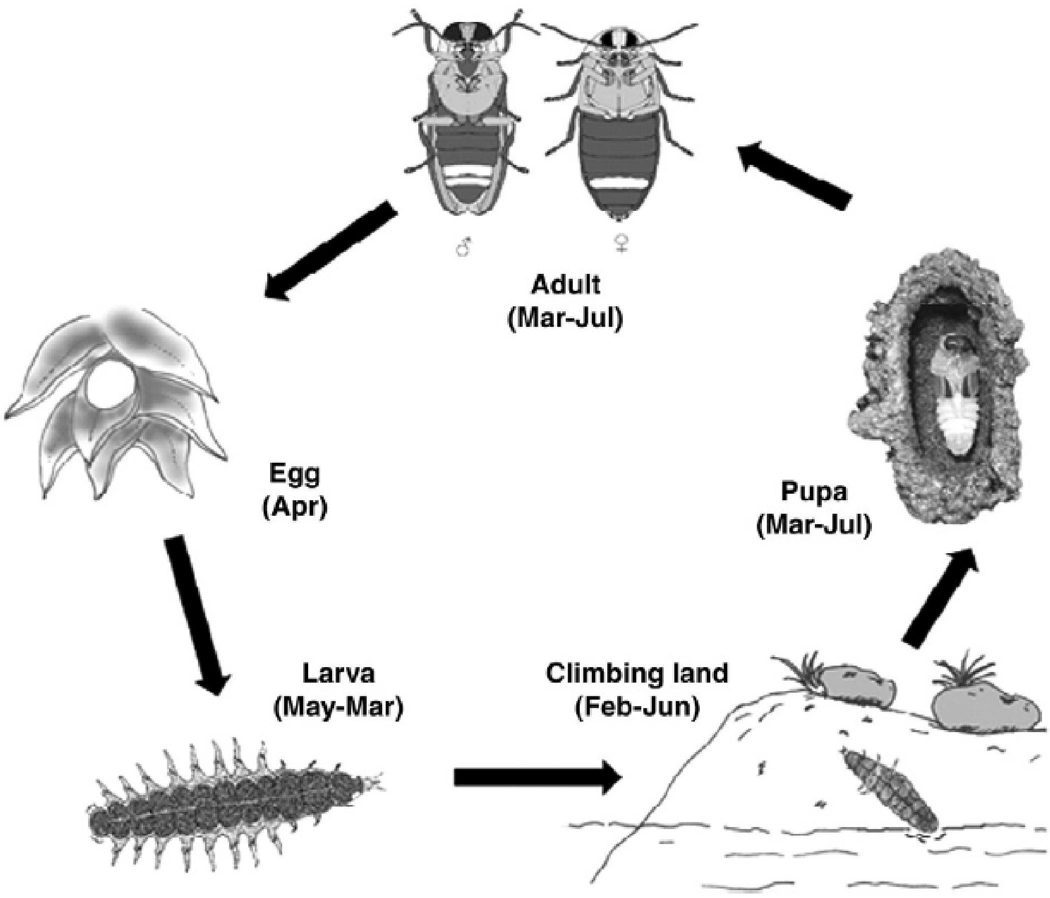


Figure 6 Life cycle of *Luciola ficta* in Jiji, Nantou country of central Taiwan (Ho et al., 2010).

2.4 Flash signal of adult fireflies

2.4.1 Bioluminescent signals and mechanism of flash production

Fireflies use bioluminescent signals to communicate among individuals in the same species as courtship system, warning displays and predation (Lloyd, 1983). Flash patterns are different in each species, and they can produce from green to red light. The primary structure is determined by color differences, which in turn affects the active-site environment around the emitter (Wood, 1995). Previous studies showed that the light (bioluminescent) signals were transmitted faster than pheromones (De Cock and Matthysen, 2005). This signal produced for the male that already developed wings that could fly to search for a female (Fister et al., 2013). In term of evolution, larvae were first produced and emitted the photic signals to function as an aposematic warning display. After that, it was gained in adults to use as a sexual signal (Branham and Wenzel, 2003). The bioluminescent flashing is used for the reproduction as light communication between males and females. The process is begun with a male flash to searching for a female, then the female flash to response to the male (Lewis and Cratsley, 2008). This response based on the female's reaction to the flash length and speed of the male's mating signal. These flashes are specific to each firefly species (Carlson and Copeland, 1985). In 1966, Lloyd classified the mating system into 2 types: signal system I and II. Signal system I, the wingless females emitted bright continuous light without flashing, for example as in *Lamprigera* spp. and *Lampyris* spp. Signal system II, both male and female displayed the flashes when flying to search for a mate, for example as in *Luciola* spp., *Photinus* spp. and *Aspisoma* spp. (Carlson et al., 1976).

Fireflies have the light organs located at the 5th abdominal in female and 5th and 6th abdominal segments in male that contain the cells called photocytes (Smith, 1963). There are three layers of firefly light organ structures. The first layer is clear-mirror-like transparent cuticle. The second layer is photogenic layer, a layer with bioluminescent chemical with plenty of photocyte surrounding the air tubes. There is mitrocondria as the source of cellular energy. These cells have a lot of tracheoles and nerves supplying on. The third layer is reflector layer. This layer accumulates of many uric crystals for reflecting the light from inside the body to outside, like the mirror coated with mercury (Thancharoen, 2009)(Figure 7).

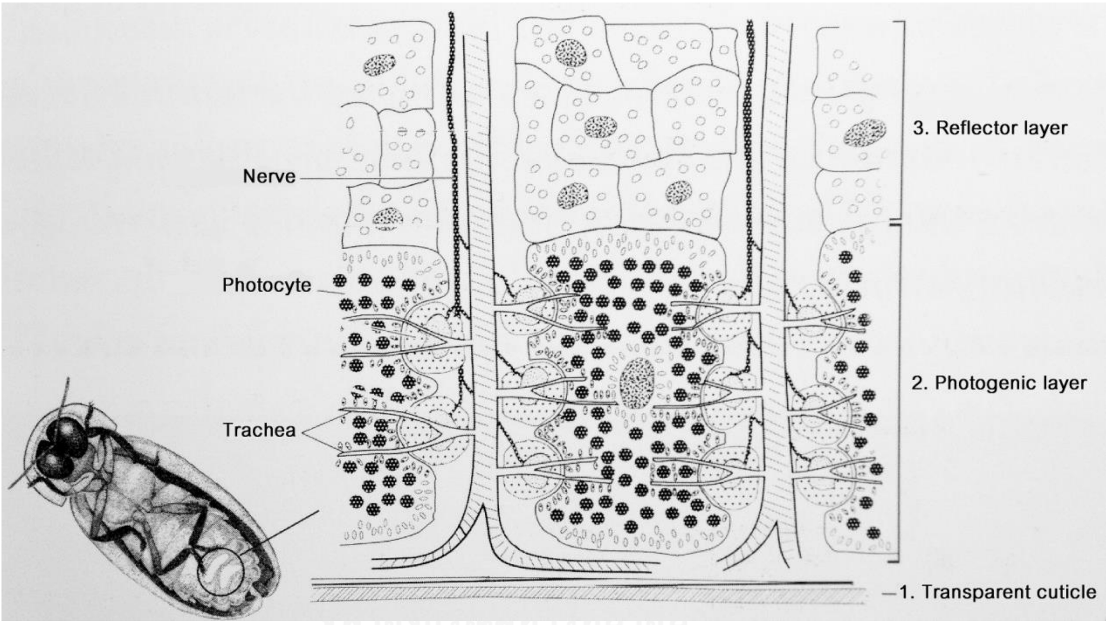


Figure 7 The three layers of firefly light organ structures, modified from Thancharoen, 2009.

A photocyte is a cell that specializes in catalyzing enzymes to produce light (bioluminescence). There are luciferin and luciferase in each photocyte (Neuwirth, 1981). The firefly flash light is the product of bioluminescence reaction which the luciferase acts as a catalyst. The reactions start with luciferrin, luciferase (catalyst), Mg^{++} and adenosine triphosphate (ATP) to form luciferyl adenylate-luciferase and pyrophosphate (McElroy and DeLuca, 1978). Then, luciferyl adenylate-luciferase is oxidized to form an oxidized complex and emission of light (Seliger and McElroy, 1960).

The flash rhythm is controlled by the neural pattern generators called octopamine (Bagnoli et al., 1976). This light is reported as cold light that gives off less than 10% of the energy as body heat and no infrared or ultraviolet frequencies. Fireflies also emit the green-yellow to red-orange visible wavelength lights of the spectrum from 510 to 670 nm (Erez and Huppert, 2010).

Firefly has sensitive eyes with ommatidia constructed to sum light (Horridge, 1969). In 1984, Case stated that the luminescent lampyrid eyes and facets were larger than in non-luminescent lampyrids. They detected the flash light with wide visual angel (Case, 1984). Flash timing parameters might be important in flash codes; but color, intensity and movement were less important (Lloyd, 1966).

There are bioluminescent insects besides the firefly. Of 140 animal genera in the three phyla (Annelida, Mollusca and Arthropoda) were reported that could make bioluminescence (Hastings and Morin, 1991; Oba et al., 2011). Three luminescence orders confirmed in insects have been Coleoptera, Diptera and Collembola. Five species of luminous true fly in genus *Keroplatus* have been reported to produce faint light in the larval and pupal stages. The larval luminescence was depended on fat body cells around the digestive tract, but still lacked of biochemical studies (Baccetti et al., 1987; Kato, 1953). In Collembola, bioluminescence depended on oxygen (Harvey, 1952). The light organs located at the Malpighian tubules in *Arachnocampa* species (Williams, 1915) for emitting luminescence to attract prey, then they caught the prey with sticky threads excreted by the larvae (Fulton, 1941; Meyer-Rochow, 2007).

2.4.2 Pheromones

Animals use pheromones, the semichemicals, for communication such as sex pheromones, alarm pheromones, aggregation pheromones or trail pheromones (Wyatt, 2003). Pheromones are main sexual signals in many insects active in diurnal or well-developed antennae species (Branham and Wenzel, 2001).

The adult fireflies of some species are not luminous at all; instead, they use pheromones to locate their mate. The use of pheromones as sexual signals appears to be the primitive condition in fireflies along with the use of luminous sexual signals, which is more recent development such as *Phosphaenus hemipterus*, the diurnal firefly species use pheromones for sexual communication (De Cock and Matthysen, 2005). Diurnal male fireflies usually had larger antennae, part in olfaction, than nocturnal male fireflies (McDermott, 1964). It is suggested that the diurnal fireflies use pheromones instead of flashing signal. In 2003, pheromones were reported to use in conjunction, then, replaced by photic signals in some lampyrid lineages (Branham and Wenzel, 2003). There are some species that employ both pheromones and luminous components in their mating systems. These species appear to be evolutionarily intermediate between the pheromone-only fireflies and flash-only fireflies. Some female fireflies attract males using a combination of pheromones and bioluminescent signals (Branham and Wenzel, 2003). From the phylogenetic analysis, the primative lampyrids were mostly diurnal that used the pheromones to communicate (Stanger-Hall et al., 2007). The advantages of uses pheromone were its persistent, long-range and energetically efficient signal (Zuk and Kolluru, 1998). Pheromone signals are also gain by the ground-dwelling fireflies that live in the sheltered habitat where in contrast to visual signals. On the other hand, pheromone signals are difficult to locate when compared to the flash signals (Lloyd, 1983). A few fireflies were reported using pheromones in combination with bioluminescent signals for mate location (Lloyd, 1971).

2.5 Mating behavior of fireflies

Mating behavior of fireflies can be classified by many characters such as behavioral patterns in mating behavior, flash behavior and sex-attractant pheromone (Thancharoen, 2007).

There were five basic stages of *Luciola obsolete* (Olivier) mating behavior (Lloyd, 1972). At first, the sedentary signaling stage was its sedentariness-only; standing or walking along the leaf edges. Males emitted single flash, duration was 0.06-0.13 sec and flicked with often shortly and erratically. There were variations of flash periods or flash intervals. Female emitted various intensities of modulated and unmodulated glows. The second stage was chasing stage which the flickering males chased flying and glowing females. Mostly, a single female was pursued by one or two males and ended within 30 feet from where they started. After that, the males that successful in pursuing landed with females. The next step was walking-luminescing stage which observed after a male and female landed together. Males emitted single flash and flicked as same as in the sedentary signaling stage. Female flash patterns were also glows and beady glows as in the sedentary signaling stage, but the flash patterns had more variations such as bright flashes and glows with stepped modulations. After that, they moved to the mounting stage. In this stage, male mounted on female. Males usually flashed while mounting, but in some cases males flashed a few seconds before mounting. At the same time, the male antennated the pronotum of the female and locked the female's body with all legs. The last stage was copulation that the male-female interaction was in a tail-to-tail position. Male flashed flickeringly and singly while female flashed continuously. After copulation, males separated immediately, but female still stood on the perch and flashed for about 5 minutes (Lloyd, 1972).

Rooney and Lewis (2000) reported the mating behavior of diurnal firefly, *Ellychnia corrusca*. Despite it closely related to the ge nus *Photinus*, nocturnal firefly, *Ellychnia* lack of adult light organ. In stage I, a male mounted a female dorsally and brushed its antennae across the female pronotum. Besides, the male used maxillary palps to contact the junction between the female's pronotum and elytra. This stage took place for 2 to 30 minutes. In stage II, the male rotated its body around for several hours, and the copulating couples often moved for a distances (Rooney and Lewis, 2000).

Thancharoen (2007) has founded that *Luciola aquatilis* displays 4 stages of mating sequences, while fireflies in European and North America have only one stage. The sequences started with, preening behavior, when a male and a female firefly used their legs to preen their antennae, eyes, mouthparts and abdomens. The males flashed rapidly. The females extended and retracted their ovipositors. Then they proceeded to the next stage, looking for mating partner, when there was more frequent in the flashing to attract female. As soon as the female responses, the courtship phase began with the male getting closer to the female, then getting on the female's back; the flashing frequency then reduced. So the last stage, the mating, started as the male and the female attached their bottoms to together. The luminescence in this stage was brightest, with long dark periods in between. This is believed to be an alarm or warning light as firefly stops flashing during the mating process, however, the flashing resumed when they were disturbed by the environment, or when there was another male around. This flashing has implied a similar mean to alert other creature not to get closer.

Additional to that, Thancharoen (2007) also has mentioned that there are 4 patterns in the flashing pattern of male *Luciola aquatilis*.

Flash Type 1: Beginning flashes - these flashes make males active after dusk. This lasts continuously without a consistent interpulse interval.

Flash Type 2: Advertising flashes - these flashes display when males walking to find a mate. These are with a high fast rate. Some males flash this flash type when received flash responses from female.

Flash Type 3: Courtship flashes – these flashes occur when a male receives a female response, then the male flashes again when walking to the responding female. The flash rate is lower than the flash type 2.

Flash Type 4: Warning flashes – the flashes are the light that both males and females flash when disturbed during the copulation. This light is symmetrical and brightest among the four types.

CHAPTER III

STUDY AREA

3.1 Study area

The research was conducted in the area of the Huai Hongkhrai Royal Development Study Center, Doi Saket district, Chiang Mai province (Figure 8 and 9).

Figure 8 Map of Doi Saket district, Chiang Mai province in the north of Thailand.

Figure 9 The study area (circle) at the Huai Hongkhrai Royal Development Study Center, Doi Saket district, Chiang Mai province in the northern of Thailand (Google, 2016).

The Huai Hongkhrai Royal Development Study Center was established from His Majesty the King's will on 11th December 1982 to be the center of study in the northern provinces as the role model for people to get a chance to learn and adapt the knowledge to their daily life. The forest developing purposes were for fruits, charcoal, for soil and water conservation, and also for improve the moisturizer to the ground. The studies were begun with the upstream forest geology, leading to the fishery in the reservoirs downstream, along with the industrial agricultural study in the field of farming, keeping cattle, and dairy milking. All of these have made this center to be the complete study center for local people, practice, and adapt to their daily routine, according to His Majesty the King's speech "let this center be the living museum." - to conduct, conclude, and simplify the study for people to learn and practice. This place is also the ecotourism site for visitors.

The data were sampled from a creek along the Standard Frog Culture Facility of the Huai Hongkhrai Royal Development Study Center with the total length of 120 meters

(Figure 10). The study period was 12 months from July 2014 to June 2015. Because of the moonlight affected to the fireflies flashing display, fireflies peak flashing perform in the moonless and least in the full moon; so, the study was conducted during the waning moon period of each month (Abe, 2002)(Figure 11). This area was chosen to be the study site due to the benefits to promote this area to be the ecotourism site, also, there is no research or report on fireflies conducted in this area before and there are at least 10 species of fireflies recorded from this area (Figure 12). Moreover, this area has a lot of visitors such as local people, students and tourists.

Figure 10 The study line transect behind the Standard Frog Culture Facility of the Huai Hongkhrai Royal Development Study Center with the total length of 120 meters.

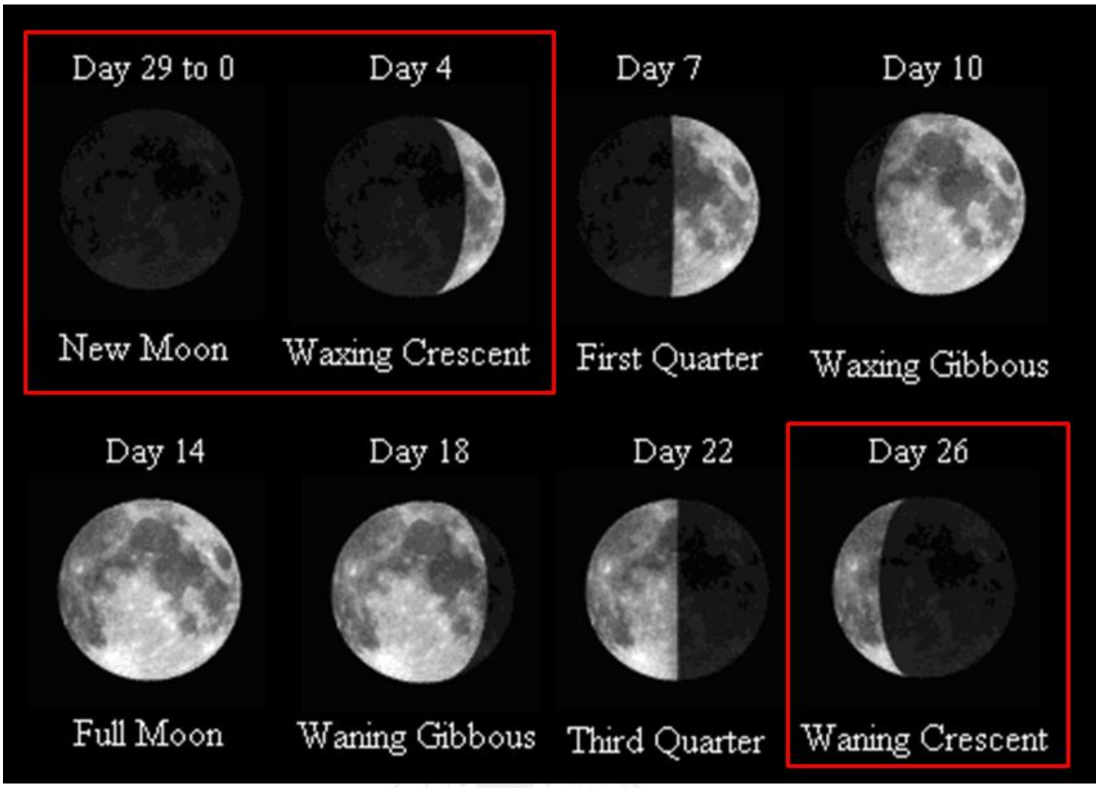


Figure 11 The waning moon period

This area also has a high variation of microhabitat; so, the diversity of firefly species should be high. From the preliminary study also found that the numbers of firefly species are diversely different month by month (Figure 12 and Table 1).

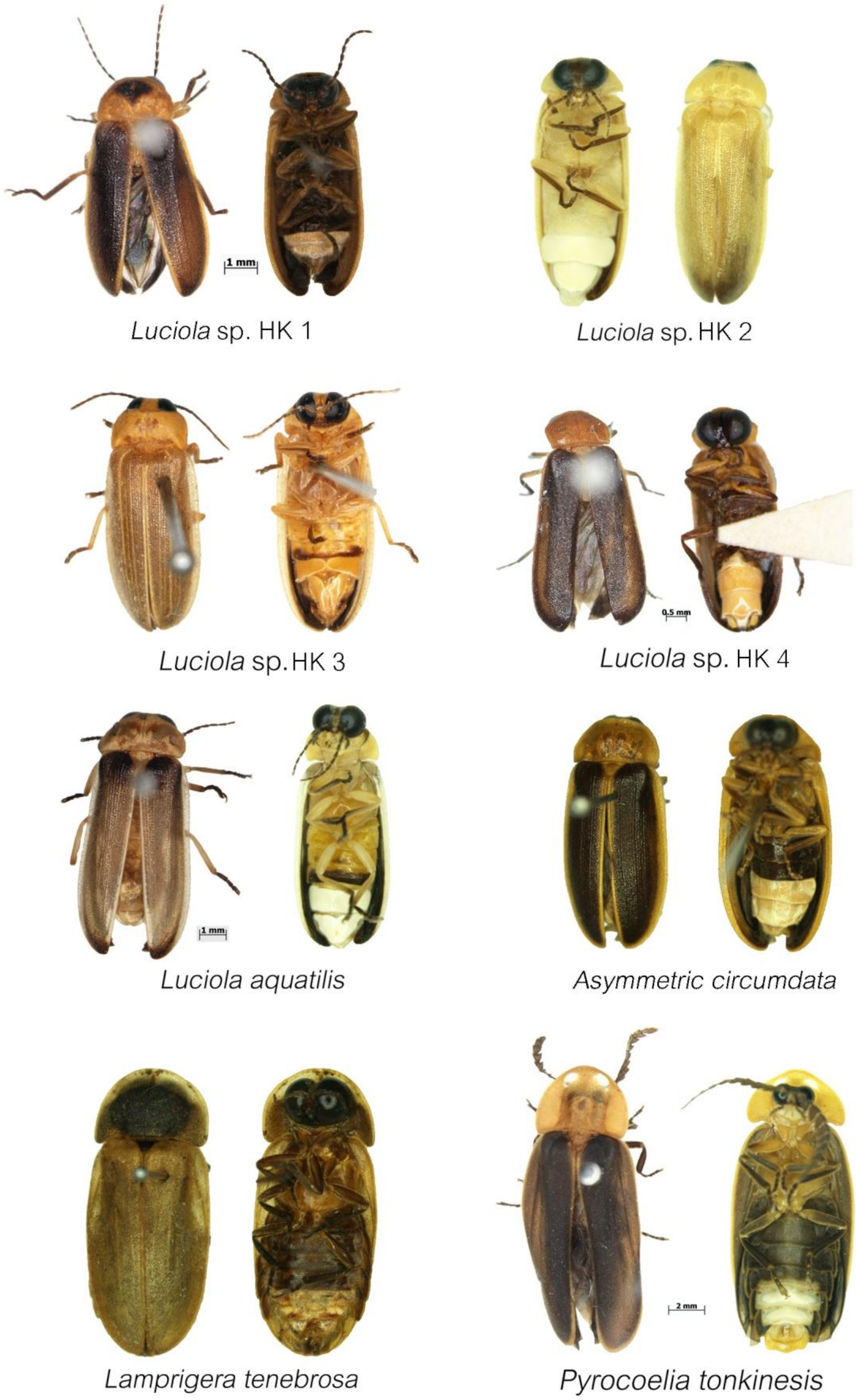


Figure 12 The firefly species that were found from July 2014 to June 2015 at the creek along the Standard Frog Culture Facility of the Huai Hongkhrai Royal Development Study Center.

Table 1 The firefly species that were found from July 2014 to June 2015.

	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN
Lamprigera tenebrosa		✓										
Asymmetric circumdata											✓	
Pyloceolia tonkinesis	✓	✓	✓						✓	✓	✓	✓
Luciola sp. HK 4	✓	✓	✓								✓	✓
Luciola sp. HK 3	✓	✓	✓	✓						✓	✓	✓
Luciola sp. HK 2	✓	✓	✓	✓					✓	✓	✓	✓
Luciola aquatilis	✓	✓	✓	✓					✓	✓	✓	✓
Luciola sp. HK 1	✓	✓	✓	✓						✓	✓	✓

3.2 Morphology and description of an adult *Luciola* sp. HK 1

Luciola sp. HK 1 was chosen to be the study model because this firefly is dominant species of this area and also found in almost every month of the year.

3.2.1 Males

The adult *Luciola* sp. HK 1 males are about 7 mm long and 3 mm wide. The distinctive characteristic is a single darker marking on the pronotum. They are dark brown with elongated and soft body (Figure 13 and 14). Head is totally covered by pronotum with two compound eyes. Antennae are dark brown filiform with 11 segments, all flagellomeres are slender and elongate (Figure 15). Forewings and hindwings are fully developed, forewings are dark brown elytra and hind-wings are membranous. They are able to flash with well-developed photogenic organs. The lanterns are located on 5th and 6th abdominal segments. Legs have femora and tibias, simple and straight (Figure 16).

Figure 13 General characteristics of adult male *Luciola* sp. HK 1, dorsal (left) and ventral (right) aspects.

Figure 14 General characteristics of male *Luciola* sp. HK 1, dorsal (left) and lateral (right) aspects.

Figure 15 *Luciola* sp. HK 1, male, head and thorax, ventral aspect.

3.2.2 Females

The adult *Luciola* sp. HK 1 females are similar to the males, with darker marking on pronotum. Females are larger than males, about 8 mm long and 3 mm wide. Head is located under pronotum with two compound eyes. There are 11 segments of dark brown antenna, all antennomeres are slender and elongate. Forewings and hind-wings are fully developed, forewings are elytra and the hind-wings are membranous which coloration and shape similar to the male. Antennae are dark brown filiform. Females are able to flash with the lantern on the fifth abdominal segment.

Figure 16 The lanterns of male (left) and female (right) *Luciola* sp. HK 1

CHAPTER IV

ABUNDANCE AND FLASHING DISPLAYS OF ADULT *Luciola* sp. HK 1 FIREFLY AT THE HUAI HONGKHRAI ROYAL DEVELOPMENT STUDY CENTER, DOI SAKET DISTRICT, CHIANG MAI PROVINCE

4.1 Introduction

The abundance and population of fireflies are affected from various physical factors such as temperature and relative humidity, especially the temperature which is the main factor to induce egg-hatching, death and resistance of newly hatched larvae. These factors also impact the survivability of the firefly pupal stage. When the relative humidity is low or dry, firefly eggs may not be able to survive, hatch and develop to larval and pupal stages. Most of firefly larvae are vulnerable to the dry weather. This leads to the number of the firefly population in different seasons; high abundance in the rainy season as the redundant of food sources and low abundance in the winter season (Thancharoen, 2000). The objective of this study is to study the firefly, *Luciola* sp. HK 1, abundance in the one-year round from July 2014 to June 2015. The obtained data can be used as referring database to improve the firefly preserving management and the sustainable ecotourism planning.

4.2 Materials and Methods

4.2.1 Abundance of *Luciola* sp. HK 1 fireflies

The data were collected along a 120-meter line transect near a creek. The line transect was split into three sections, 40-meter section each. Three study points were set at each of the three sections (Figure 17). A study point was located at the center of each 40-meter section. The data was collected monthly, 3-day period (one day was spent for one section).

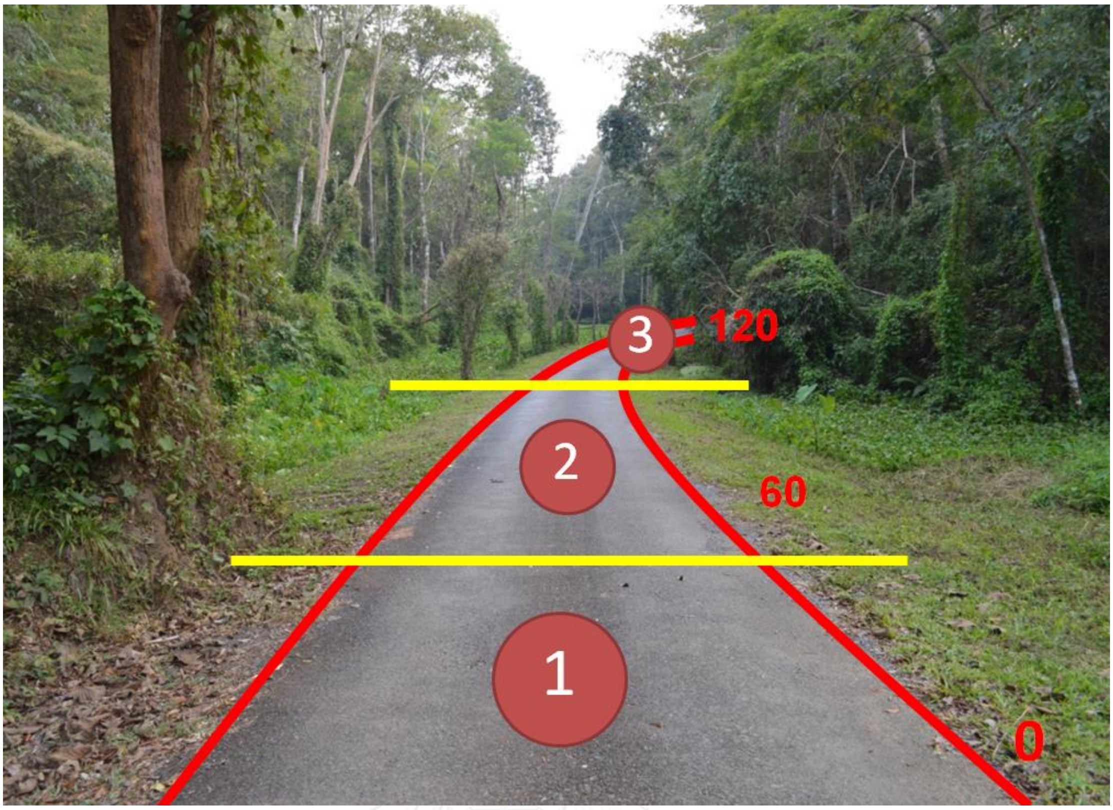


Figure 17 The 120-meter transect line was split into three sections, 40-meter section each, collected the data by one day was spent for one section.

The experiment was done by counting and catching (two people worked together, one for counting and another one for catching) the adult fireflies from 7:00 PM to 8:30 PM every 5 minutes in each night. Numbers of all adult firefly flashes were counted by direct counting method using a counter. In each study point, the flashes were counted at 360° around the point. After that, an aerial net was used to catch every adult fireflies in each 40-meter section along the transect line (Figure 18). The aerial net was with extendable 140-centimeter handle, 30 centimeters in diameter and a bag depth of 80-centimeter.

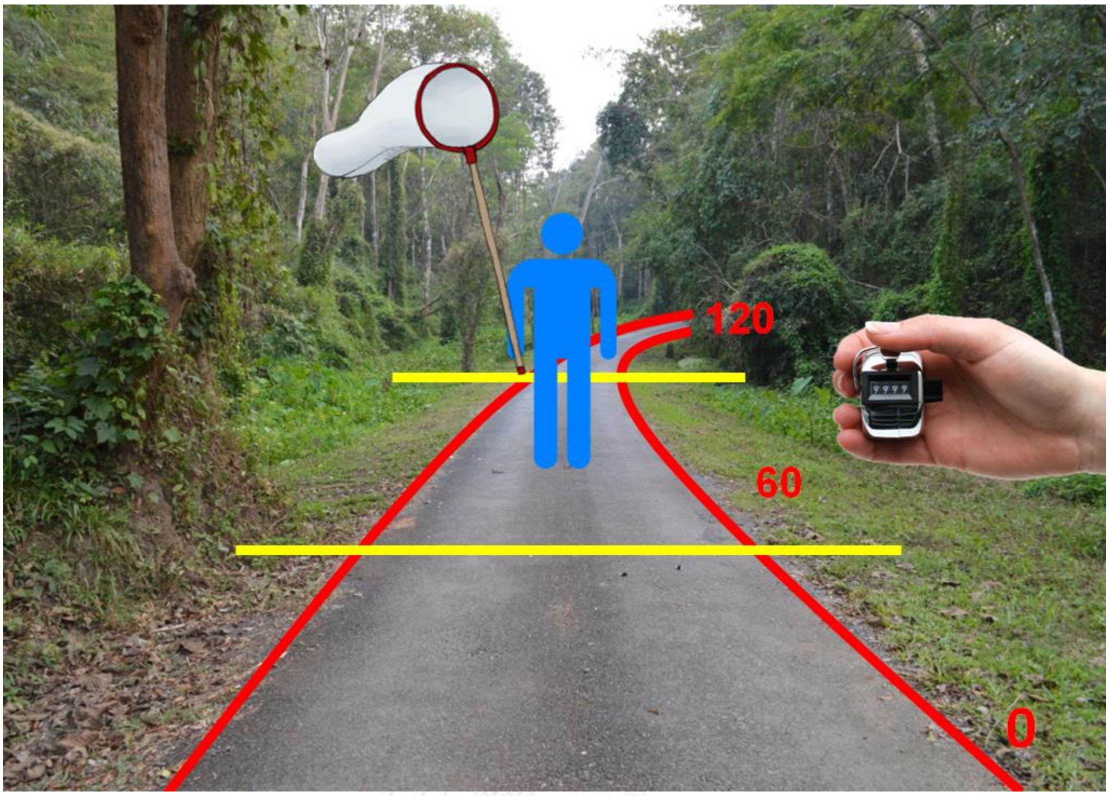


Figure 18 The counting and catching method, the flashes were counted at 360° around the point and used an aerial net to catch all adult fireflies in each 40-meter section along the transect line.

In each section, the numbers of all adult fireflies caught and the numbers of adult *Luciola* sp. HK 1 caught were recorded. All of the fireflies caught were then released after finishing the experiment each night. The data obtained were calculated and averaged for the indirect estimated number of individual of *Luciola* sp. HK 1 (individuals/ 40-meter section of transect line) in each month using the equation;

$$\text{Indirect estimated number of individual} = \frac{\textit{Luciola}\text{ sp. HK 1}}{\text{Fireflies caught}} \times \text{Direct count of firefly individuals by flashes}$$

Physical factors such as temperature and humidity were recorded by thermometer and hygrometer, respectively. The start flashing time of *Luciola* sp. HK 1 and the sunset time in each day were recorded.

Data analysis

The flow diagram in Figure 19 gives a simple decision tree to choose the right test (Pirk et al., 2013). The data were checked as non-parametric data by checking normality and homogeneity. Mann-Whitney U-test statistical method in SPSS program was used to compare the data among the study months.

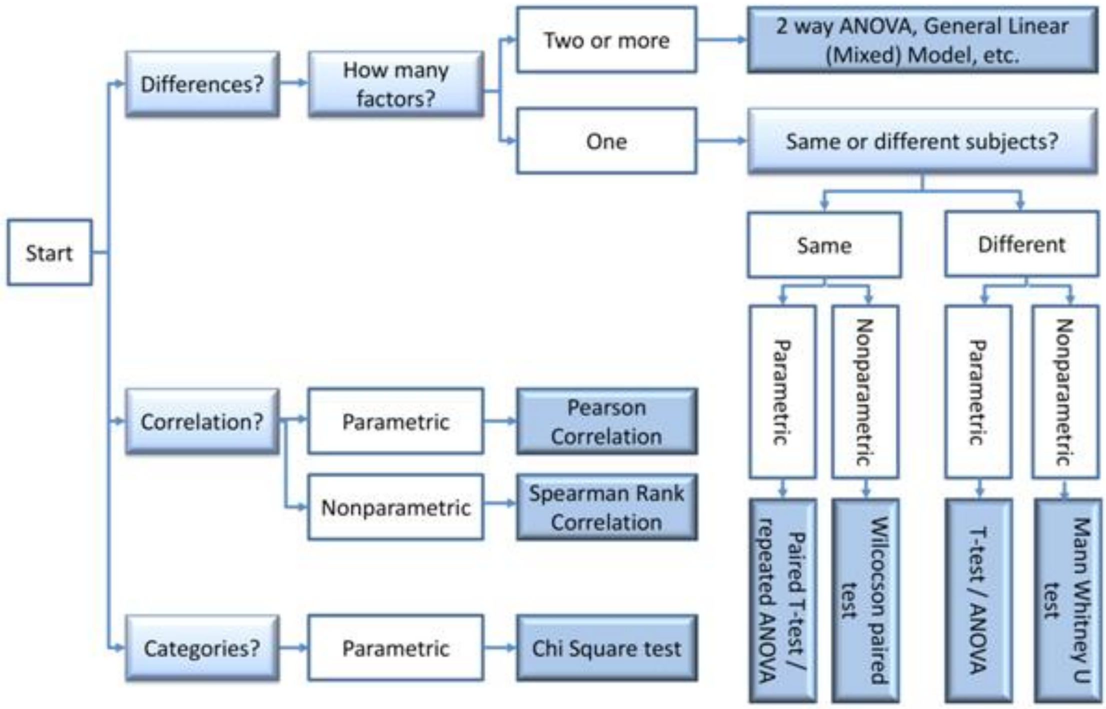


Figure 19 Guideline to statistical analyses in abundance and peak flash period of *Luciola* sp. HK 1 (Pirk et al., 2013).

4.2.2 Peak flashing periods of *Luciola* sp. HK 1 firefly

The data were collected along a 120-meter line transect near a creek. The line transect was split into three sections, 40-meter section each. Three study points were set at each of the three sections. A study point was located at the center of each 40-meter section. The data was collected monthly, 3-day period (one day was spent for one section). The experiment was done by counting and catching the adult fireflies from 6:00 PM to 5:00 AM, totally 11 hours in each night. Numbers of all adult firefly flashes were counted by direct counting method using a counter. Each study point, the flashes were counted at 360° around the point. After that, an aerial net was used to catch all adult fireflies in each 40-meter section along the transect line. In each section, the numbers of all adult fireflies caught and the numbers of adult *Luciola* sp. HK 1 caught were recorded. The data obtained were calculated and averaged for the indirect estimated number of individual of *Luciola* sp. HK 1 (individuals/ 40-meter section of transect line) in each month using the equation;

$$\text{Indirect estimated number of individual} = \frac{\textit{Luciola}\text{ sp. HK 1}}{\text{Fireflies caught}} \times \text{Direct count of firefly individuals by flashes}$$

All of the fireflies caught were released after finishing the experiment in each study period (Table 2).

Table 2 The counting and catching method periods.

Hour	Counting and catching
1^{st} - 2^{nd}	every 5 minutes
3^{th} - 4^{th}	every 15 minutes
5^{th} - 6^{th}	every 30 minutes
7^{th} - 8^{th}	every 1 hour
9^{th} - 11^{th}	every 2 hours

Physical factors such as temperature, humidity and sunset time were recorded.

Data analysis

The flow diagram in Figure 19 gives a simple decision tree to choose the right test (Pirk et al., 2013). The data were checked as non-parametric data by checking normality and homogeneity. Wilcoxon signed-rank test statistical method in SPSS program was used to compare the peak flashing times among the study months.

4.3 Results and Discussion

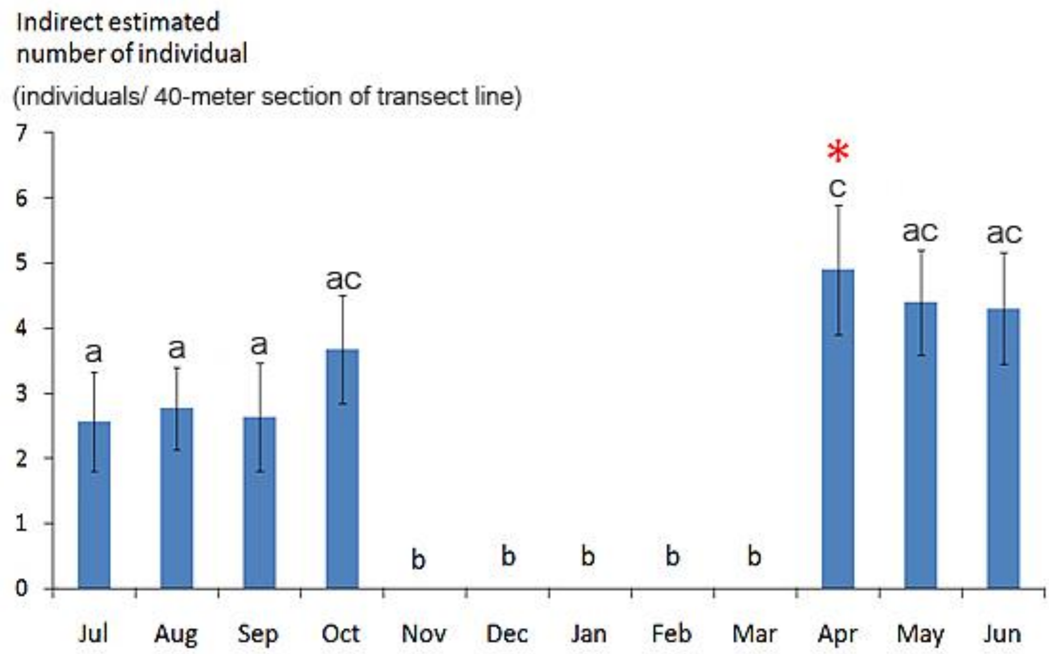
4.3.1 Abundance of *Luciola* sp. HK 1 fireflies

From the study, the indirect estimated number of individual in 12 months (Table 3) indicated that the firefly *Luciola* sp. HK 1 was highest abundance in April 2015 (Mann-Whitney U-test, $p<0.05$) and there was no adult firefly found from November 2014 to March 2015 (Figure 20).

Table 3 The averages of indirect estimated number of individual (individuals/ 40-meter section of transect line; Mean±SE) recorded 12 months from July 2014 to June 2015.

Month	Indirect estimated number of individual
July 2014	2.30 ± 0.76^{a}
August 2014	2.14 ± 0.64^{a}
September 2014	2.91 ± 0.84^{a}
October 2014	2.98 ± 0.83^{ac}
November 2014	0.00 ± 0.00^{b}
December 2014	0.00 ± 0.00^{b}
January 2015	0.00 ± 0.00^{b}
February 2015	0.00 ± 0.00^{b}
March 2015	0.00 ± 0.00^{b}
April 2015	4.92 ± 0.99^{c}
May 2015	4.39 ± 0.80^{ac}
June 2015	4.26 ± 0.86^{ac}

*Different letters in superscript following values indicated statistical significance



*Different letters in superscript following values indicated statistical significance

Figure 20 The averages of indirect estimated number of individual (mean±SE) from July 2014 to June 2015 (The adult firefly was not found from November 2014 to March 2015).

The recommended months for firefly-watching ecotourism are April, May and June due to the high abundance of fireflies and the low possibility of rain (Table 4 and Figure 21). Tourists would easily access the area for observing fireflies at the site. July-October period is not a suitable duration for watching fireflies because the firefly abundance is low, as well as there is a high possibility of rain. Because of no firefly found during November-March, so it is recommended not to visit the firefly site during this period.

Table 4 Total and average rainfall from July 2014 to June 2015.

Month	Total rainfall (mm/month)	Average rainfall (mm/day)	Number of day
JUL	175.20	6.04±13.28	15
AUG	231.30	7.46±13.71	21
SEP	177.50	5.92±10.76	18
OCT	129.30	4.17±11.27	8
NOV	16.00	0.55±1.90	4
DEC	0.00	0.00±0.00	0
JAN	78.90	2.55±9.82	2
FEB	0.00	0.00±0.00	0
MAR	27.50	0.89±3.99	4
APR	53.80	1.86±6.05	8
MAY	76.50	2.47±5.41	9
JUN	15.20	0.54±1.18	11

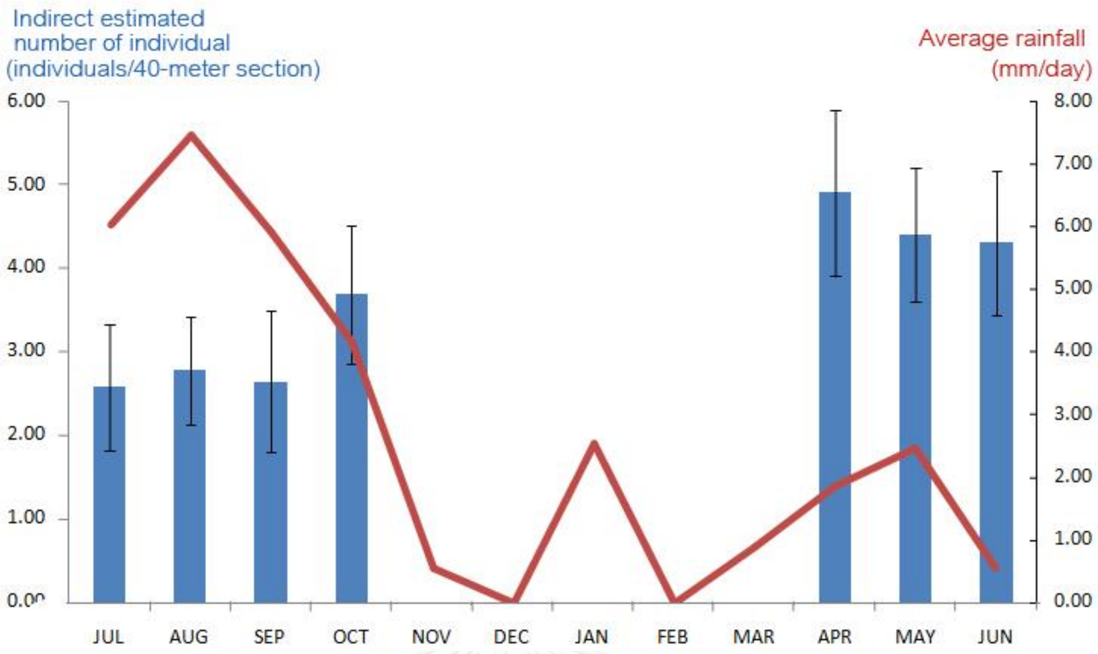


Figure 21 The averages of indirect estimated number of individual (individuals/ 40-meter section of transect line) and average rainfall (mm/day) from July 2014 to June 2015.

The temperature and relative humidity are shown in Table 5 and Figure 22. Temperature were significantly correlated with the indirect estimated number of individual (Spearman's correlation, p value=0.000). The result could be drawn that the raising temperature had influence to increase the abundance of firefly population in the area. Moreover, the relative humidity was significantly correlated with indirect estimated number of individual (Spearman's correlation, p value=0.028), while the rainfall was not significantly correlated with the indirect estimated number of individual (Spearman's correlation, p value=0.117). However, the indirect estimated numbers of individual were high correlated with temperature, relative humidity and rainfall when using Multiple Regression Analysis (R=0.926, R^2=0.857).

Table 5 The average monthly temperature and humidity of study sites from July 2014 to June 2015.

Month	Temperature (^{o}C)	Relative humidity (%)
July 2014	24.40±0.31	94
August 2014	24.07±0.07	96
September 2014	24.60±0.21	94
October 2014	23.47±0.15	94
November 2014	21.90±0.06	96
December 2014	17.10±0.26	89
January 2015	17.87±0.46	93
February 2015	18.77±0.50	78
March 2015	23.23±0.14	64
April 2015	28.40±0.15	96
May 2015	27.23±0.15	96
June 2015	27.50±0.17	96

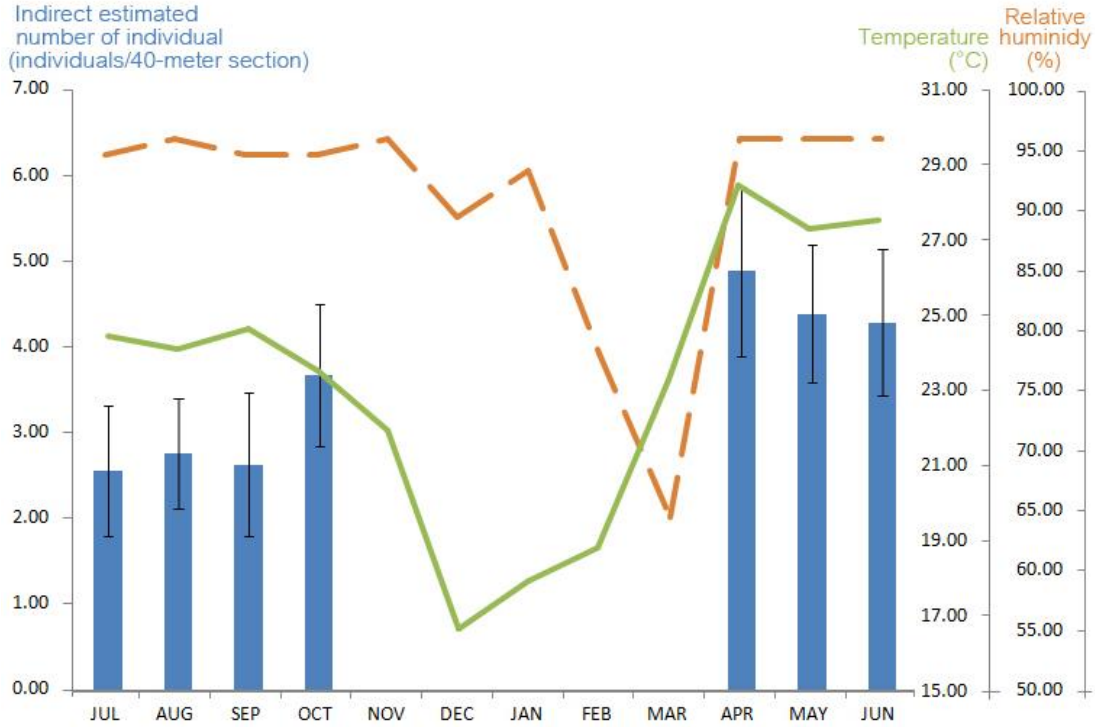
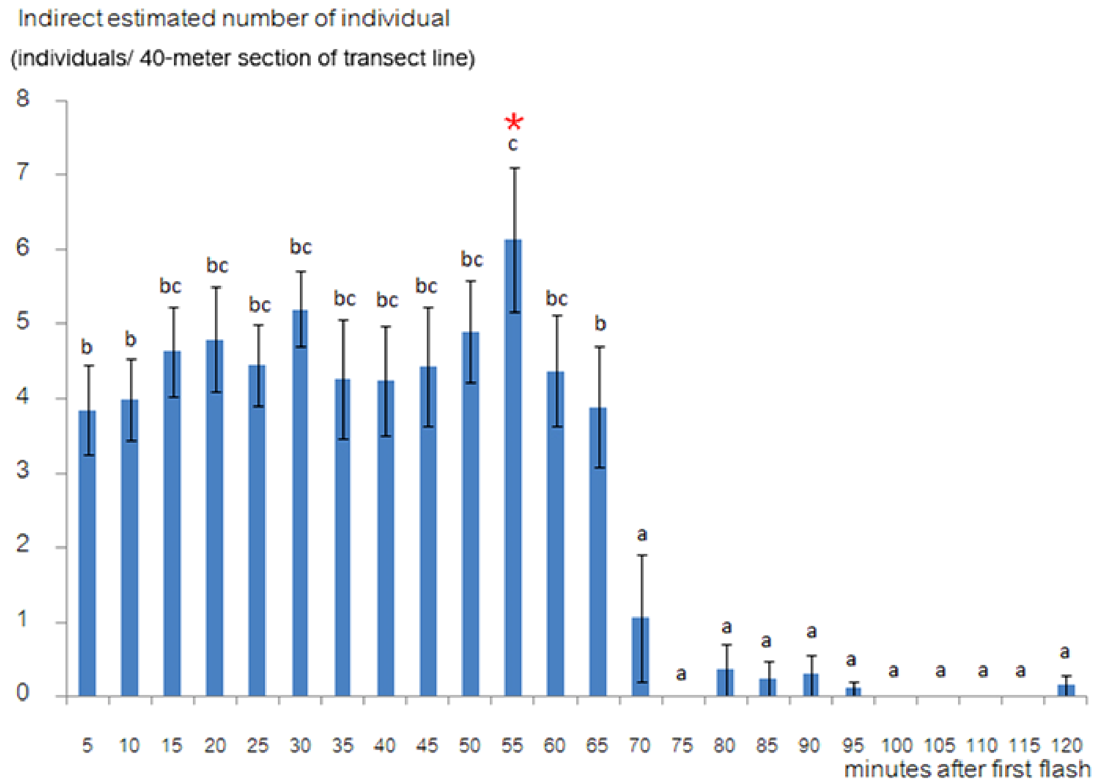


Figure 22 The indirect estimated number of individual, average monthly temperature and humidity of study sites from July 2014 to June 2015.

4.3.2 Peak flashing periods of *Luciola* sp. HK 1 firefly

In the study, the flashing displays peaked at about 55 minutes after the first flash (Wilcoxon signed-rank test, $p<0.05$)(Table 6 and Figure 23).



*Different letters in superscript following values indicated statistical significance

Figure 23 The averages of indirect estimated number of adult fireflies *Luciola* sp. HK 1 during peak flashing period from July to October 2014 and April to June 2015.

Table 6 The averages of indirect estimated number of individual (individuals/ 40-meter section of transect line; Mean±SE) recorded for 12 months from July 2014 to June 2015.

Min after first flash	Indirect estimated number of individual
5	3.85±0.60[b]
10	4.00±0.55[b]
15	4.63±0.60[bc]
20	4.80±0.70[bc]
25	4.45±0.54[bc]
30	5.20±0.51[bc]
35	4.27±0.81[bc]
40	4.25±0.74[bc]
45	4.44±0.80[bc]
50	4.91±0.68[bc]
55	6.14±0.97[c]
60	4.37±0.75[bc]
65	3.89±0.81[b]
70	1.05±0.85[a]
75	0.00[a]
80	0.36±0.35[a]
85	0.24±0.23[a]
90	0.29±0.28[a]
95	0.10±0.09[a]
100	0.00[a]
105	0.00[a]
110	0.00[a]
115	0.00[a]
120	0.14±0.14[a]

*Different letters in superscript following values indicated statistical significance

After data collecting, the real time periods were plotted as a graph in Figure 24, the data show in Table 7 and Table 8. *Luciola* sp. HK 1 fireflies started flashing estimately from 6:30-9:30 PM with the peak flashing period during 7:00-8:00 PM. So, tourists who would like to observe the behavior of firefly flash display from the starting moment, it is recommended to reach the site around 6:30 PM and the brightest flash display period is at about 7:00 PM.

Table 7 The averages of indirect estimated number of individual (individuals/ 40-meter section of transect line; Mean±SE) recorded 12 months from July to October 2014 and April to June 2015.

Time	Indirect estimated number of individual
18:25-18:30	1.07
18:30-18:35	0.97
18:35-18:40	1.23
18:40-18:45	1.14
18:45-18:50	0.81
18:50-18:55	1.99
18:55-19:00	1.85
19:00-19:05	0.56
19:05-19:10	2.04
19:10-19:15	2.20
19:15-19:20	3.06
19:20-19:25	2.91
19:25-19:30	4.26
19:30-19:35	2.45
19:35-19:40	4.05
19:40-19:45	4.86
19:45-19:50	3.38

Table 8 The averages of indirect estimated number of individual (individuals/ 40-meter section of transect line; Mean±SE) recorded 12 months from July to October 2014 and April to June 2015.

Time	Indirect estimated number of individual
19:50-19:55	3.58
20:00-20:05	3.33
20:15-20:20	3.12
20:20-20:25	0.71
20:25-20:30	0.40
21:30-21:35	0.00
21:35-21:40	0.00
21:40-21:45	0.36
22:45-22:50	0.33
22:50-22:55	0.00
22:55-23:00	0.00
23:00-23:05	0.00
23:05-23:10	0.00
23:10-23:15	0.00
23:15-23:20	0.00
23:20-23:25	0.00
23:25-23:30	0.00
23:30-23:35	0.10
23:35-23:40	0.00
23:40-23:45	0.00

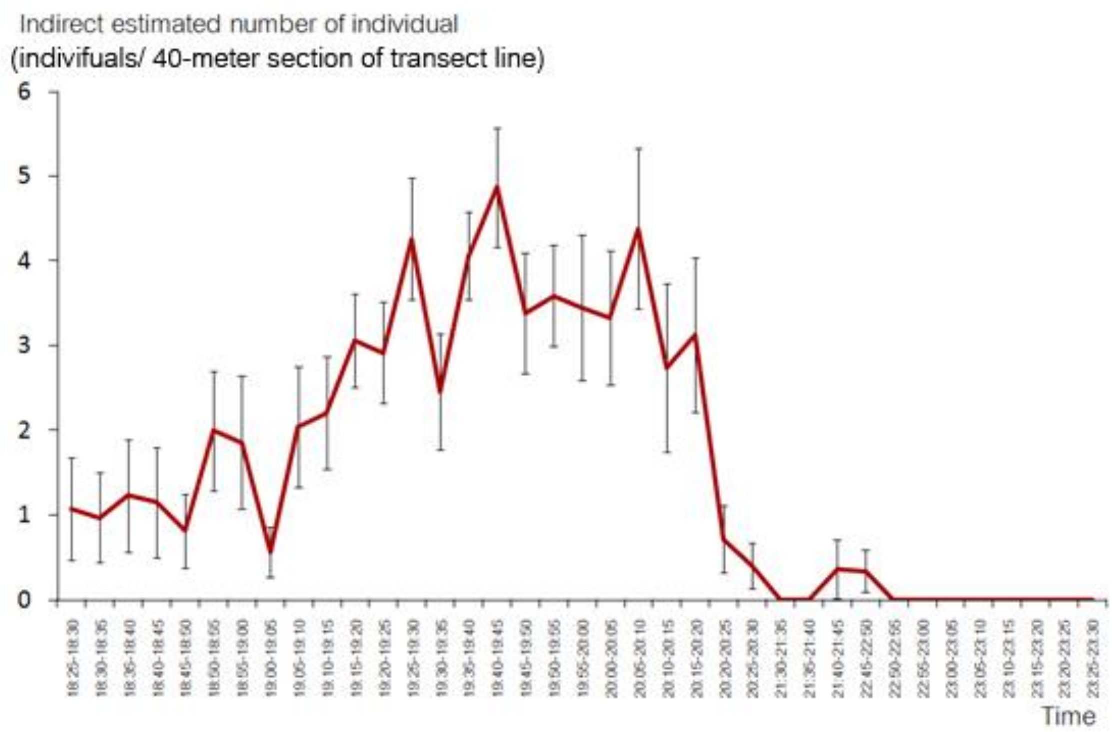


Figure 24 The average of indirect estimated number of individual of adult *Luciola* sp. HK 1 during peak flashing period (real time) from July to October 2014 and April to June 2015.

Fireflies started their flashing in different times and fireflies' start flashing times correlated with the sunset times (Spearman's correlation, p value=0.025; Table 9 and Figure 25).

From the study, the data also showed that the average flashing duration were varied among months (Mann-Whitney U-test, $p<0.05$) (Table 9 and Figure 26). The average flashing duration was longest in July 2014 and display was approximately at about 1 hour.

Table 9 The average flashing duration, start flashing times of adult fireflies *Luciola* sp. HK 1 and sunset times from July to October 2014 and April to June 2015.

Month	Flashing duration (minutes)	Start flashing times	Sunset times
July 2014	70.00±7.64	7:25 PM	7:03 PM
August 2014	68.33±1.67	7:05 PM	6:47 PM
September 2014	61.67±6.01	6:55 PM	6:22 PM
October 2014	60.00±2.89	6:30 PM	5:58 PM
April 2015	63.33±1.18	7:15 PM	6:42 PM
May 2015	65.00±2.04	7:05 PM	6:53 PM
June 2015	65.00±0.00	7:15 PM	7:03 PM

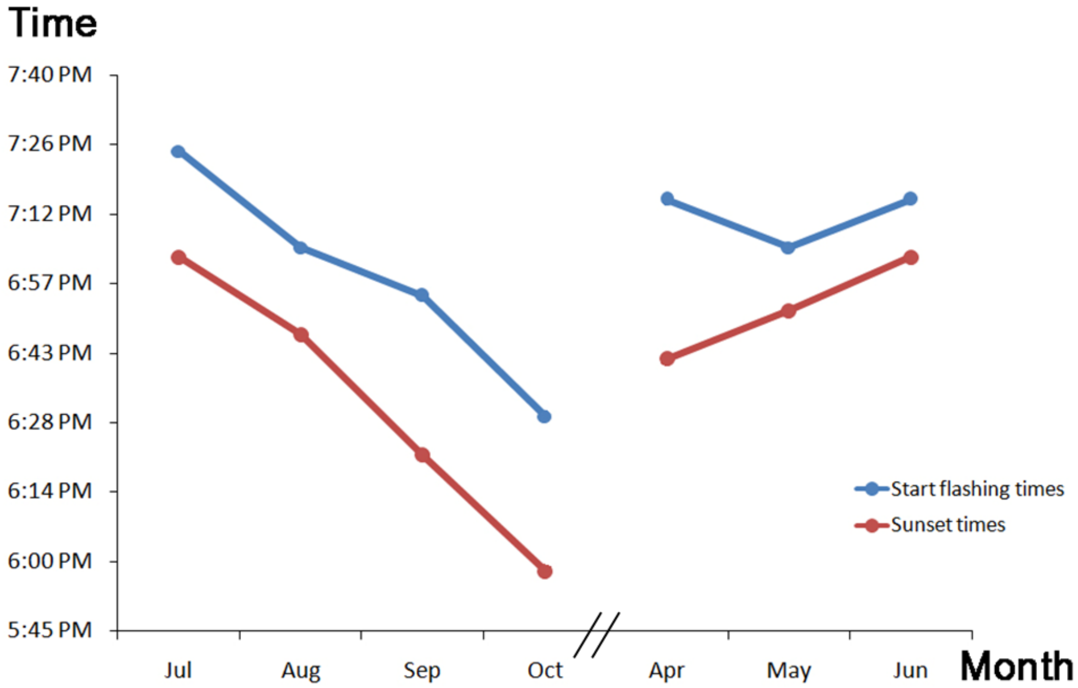


Figure 25 The fireflies' start flashing and sunset times from July to October 2014 and April to June 2015.

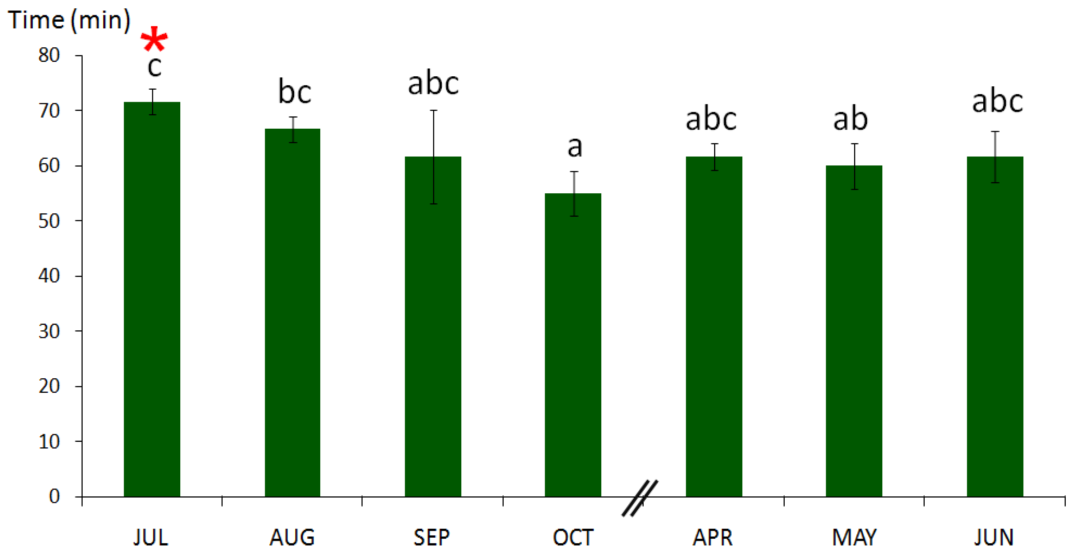


Figure 26 The averages of flashing duration of adult fireflies *Luciola* sp. HK 1 from July to October 2014 and April to June 2015.

In 1937, Buck discussed in details about flashing behavior of the males *Photinus pyralis*, they became active and flashed at 7:00 PM, and then at 9:00 PM, they became quiescent. The result is similar to this study as fireflies flash briefly in the dusk, then stop when the night grows darker (Buck, 1937). Furthermore, from previous study, the flashing display of fireflies in a mangrove at Welu Wetland, Chanthaburi Province, indicated that fireflies' flashing activity could be seen most of the night. Fireflies in that area are *Pteroptyx valida* and *P. malaccae*, semi-aquatic fireflies. They have different behaviors from *Luciola* sp. HK 1, an aquatic type, in the form that male semi-aquatic fireflies stay and flash on trees. During this period, they do not fly or change their location until they receive a flashing sign from female fireflies that they are ready for mating activity (Pongjun, 2013). In contrast, male aquatic fireflies fly while flashing their signal to females. When their signal is responded, they begin their mating activities on the ground. This behavior, flashing when flying, requires more energy than staying still.

From an unpublished data of Jarinthorn Winyuchoncharoen, who studied the abundance and peak flashing display of *Luciola aquatilis* at the Huai Hongkhrai Royal Development Study Center, Doi Saket District, Chiang Mai Province from July 2014 to March 2015, she found that the *L. aquatilis* fireflies started the flashing time after the *Luciola* sp. HK 1 fireflies around 15 minutes, also with a longer flashing duration (Winyuchoncharoen, 2015). The reason of differences between these two fireflies is probably due to the reproductive isolating process, which occurs from the temporal isolation of prezygotic isolation.

4.4 Conclusion

The indirect estimated numbers of individual was highest in April and adult firefly was not found from November 2014 to March 2015. T*he average of* flashing peak was at 55 minutes after first flash from July 2014 to June 2015. Display duration was about 1 hour. The recommended months for firefly-watching ecotourism are April, May and June and the recommended times are around 7:00-8:30 PM.

CHAPTER V

MOVING PATTERNS AND FLASHING PATTERNS IN COURTSHIP BEHAVIOR OF THE *Luciola* sp. HK 1 FIREFLY

5.1 Introduction

During the past five decades, fascinating courtship displays of fireflies have inspired scientists and naturalists to observe and examine. At present, firefly biology has been investigated in many behavior and ecology, evolutionary ecology and including predator-prey interaction (Branham and Wenzel, 2001; Cratsley and Lewis, 2003; Li et al., 2006; Lloyd, 1978) . Firefly light emission from laminating organ is very attractive to human and becomes very popular in firefly watching activity. The adult firefly flashing is well-known as communication between sexes during courtship. The flash displays and patterns are commonly specific depending on firefly species(Lewis and Cratsley, 2008). The aims of this research are to investigate moving and flashing patterns of adult Luciola sp. HK 1 firefly during courtship activity. The data obtained could be a basic knowledge for understanding courtship and mating behaviors and also evolutionary process of sexual selecting.

5.2 Materials and Methods

5.2.1 Moving patterns in courtship behavior

The study was conducted with adult fireflies in the laboratory during 6:00-8:00 PM by placing a couple specimen of one adult male and one adult female fireflies in a clear square box with lid; the box dimension was 15W x 30L x 10D cm (Figure 27). A male and a female were separated by a layer of a translucent plastic plate and a layer of opaque plastic plate. These plates were able to open-close from each side. The experiment started with randomly putting a male in one side of the box and a female in the other side. The male and female specimen were left for 5 minutes for getting as used to the container, then, started recording the moving of the couple specimen. The video was recorded in 3 phases (Figure 28).

Phase 1 - Before the couple saw each other with both translucent and opaque plates separating them.

Phase 2 - During the period that the couple saw each other via the translucent plant; the opaque plate was removed.

Phase 3 - The mating period between the couple specimen, as both plates were removed.

These processes were repeated 20 times with 20 different couples. The physical factors, including temperature and moisture, were recorded with the thermo-hygrometer.

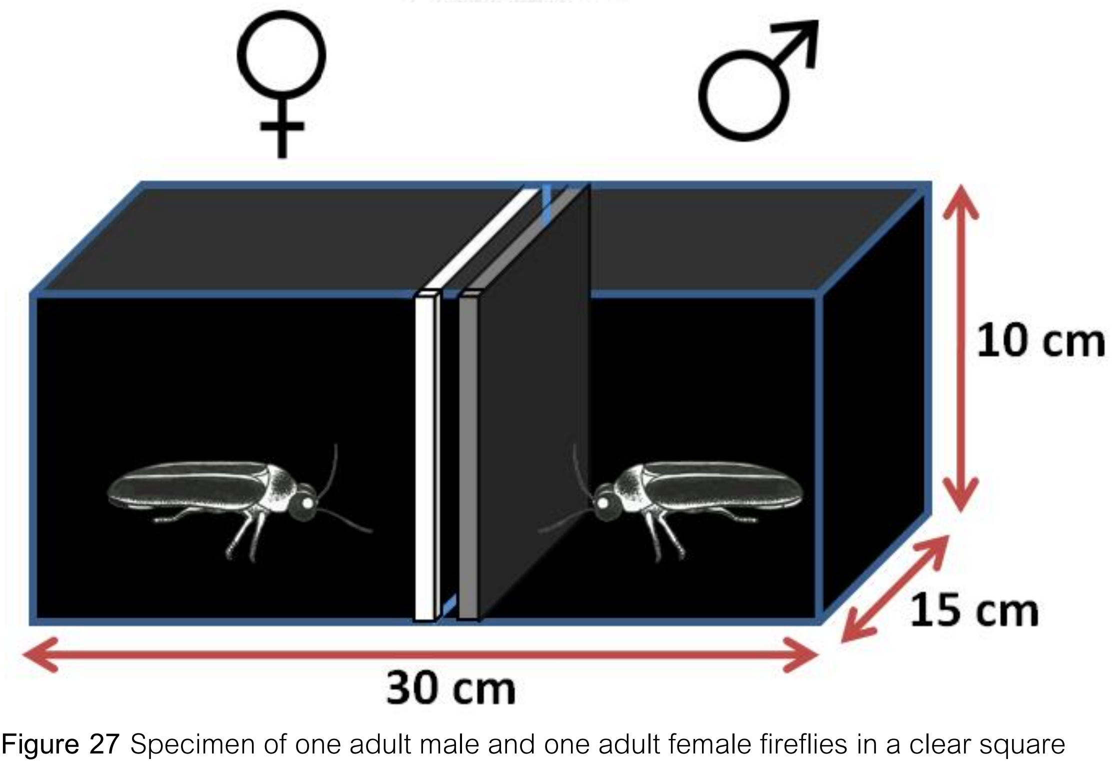


Figure 27 Specimen of one adult male and one adult female fireflies in a clear square box with lid; the box dimension was 15W x 30L x 10D cm. A male and a female were separated by a layer of a translucent plastic plate and a layer of opaque plastic plate.

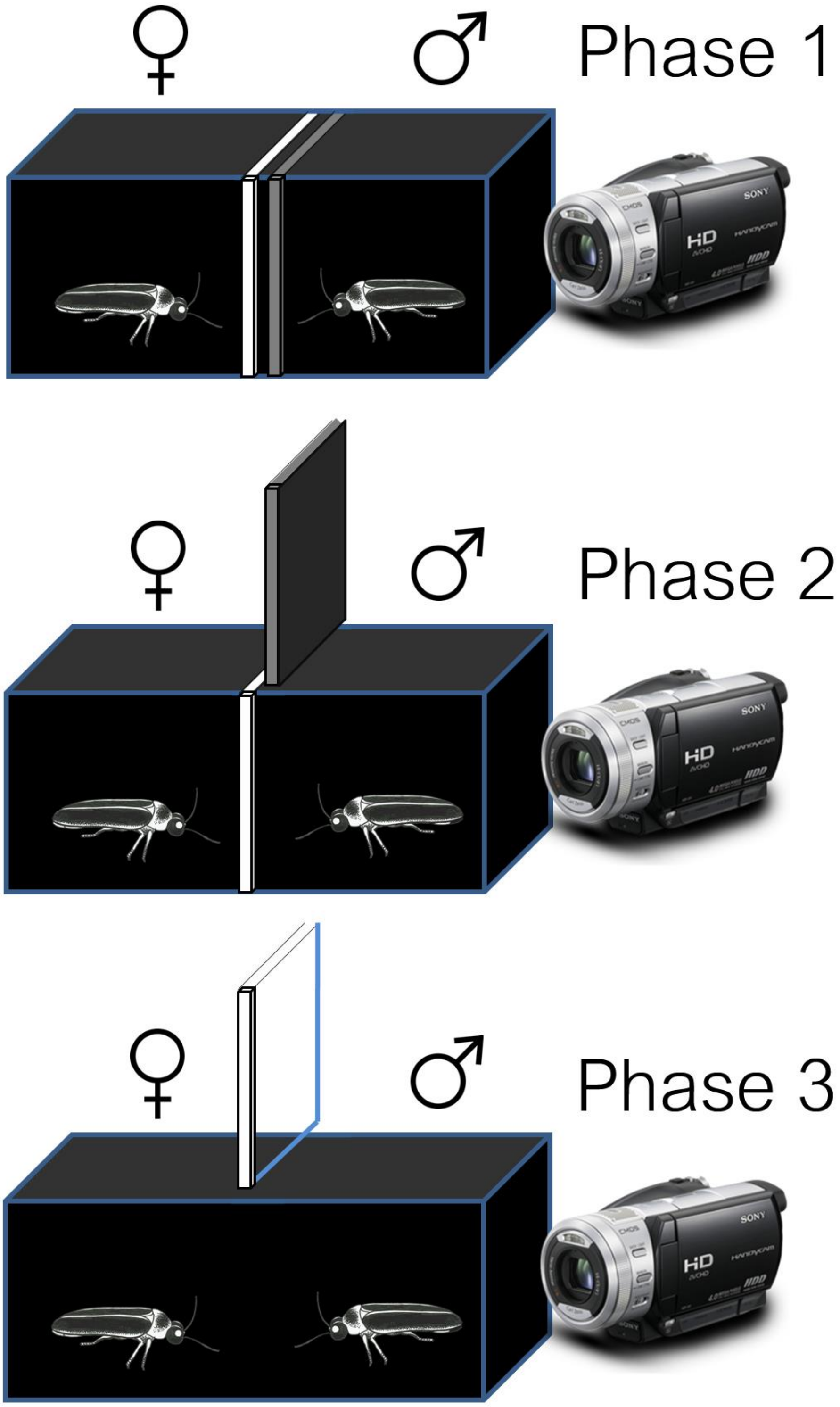


Figure 28 The video was recorded in 3 phases: before the couple saw each other with both translucent and opaque plates separating them, during the period that the couple saw each other via the translucent plant and the mating period between the couple specimen by removed both plastic plates.

Data Analysis

The moving patterns in courtship behavior were recorded in the video clips. To start the analysis, the clips were slow-motioned with a video editing program, Sony Vegas Pro 10 (Figure 29), afterwards the moving patterns between each phase were compared.

Figure 29 The video file was played in slow-motion mode via Sony Vegas 10.

5.2.2 Flashing patterns in courtship behavior

The experiment and data recording were used the same method as in 5.2.1

Data Analysis

The flashing patterns in courtship behavior were recorded in the video clips. To start the analysis, the clips were slow-motioned with a video editing program, Sony Vegas Pro 10. The method was to split 1 second in the clip into 30 frames of picture or 0.033 second per frame (Figure 30). The total frames with light (pulse duration) and dark (interpulse duration) of fireflies were counted by using the light intensity indicators (Figure 31), then, converted the light and dark frames of picture into the real time (Figure 32). Afterwards, the flashing patterns between each phase were compared.

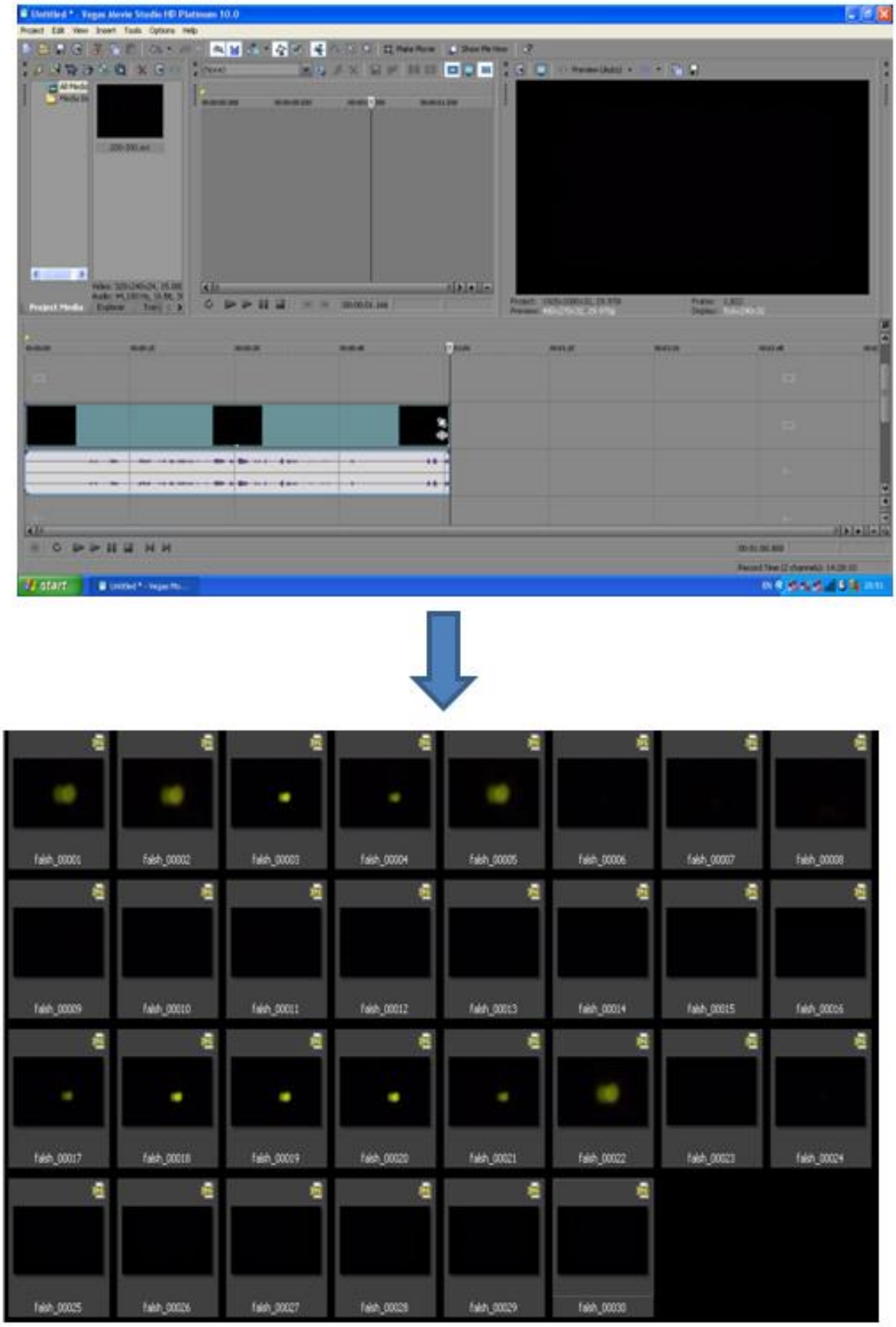


Figure 30 The moving patterns and flashing patterns in courtship behavior were recorded in the video clips at the speed of 0.033 second per frame and analysis in JPG.

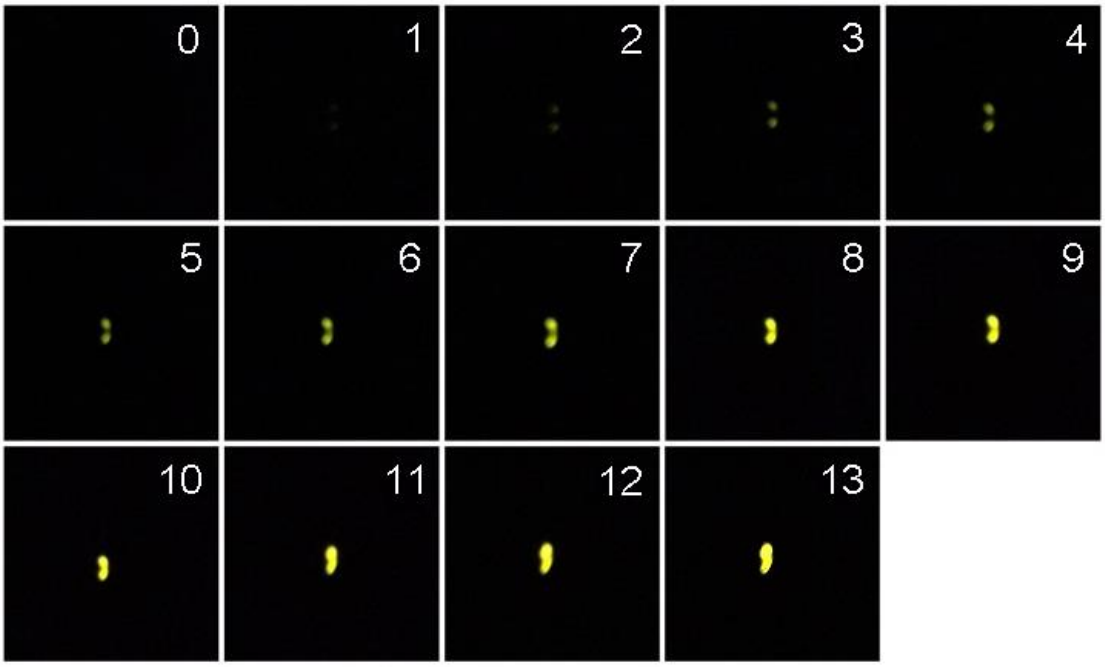


Figure 31 The light intensity indicator was created and generated into 14 levels for quantify firefly's flashes from the video observations.

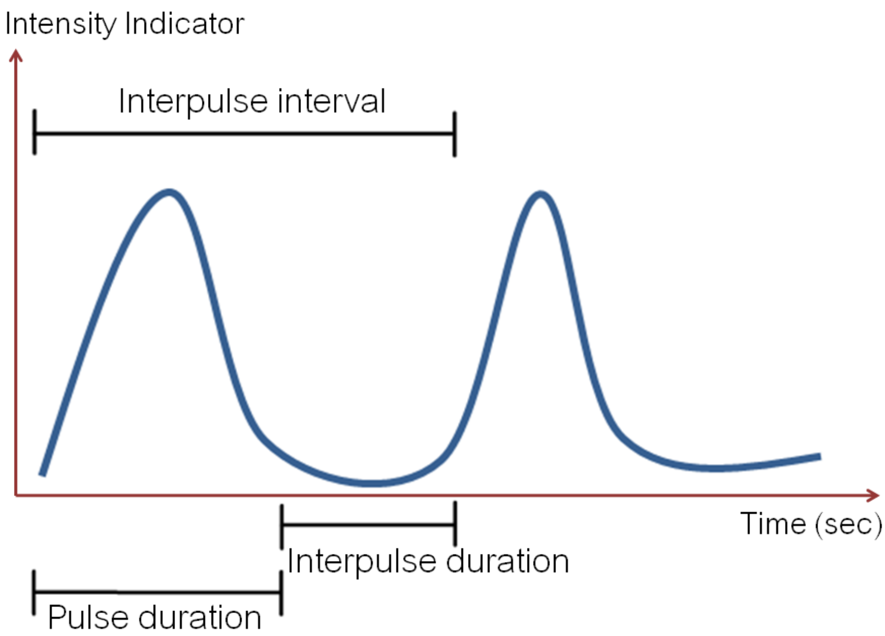


Figure 32 Terminology of firefly flash signals (Lewis and Cratsley, 2008 ; Lloyd, 1966; Thancharoen, 2007).

5.3 Results and Discussion

5.3.1 Moving patterns in courtship behavior

The 20 pairs of fireflies were observed in laboratory. The study was divided in 3 phases found that in phase 1, before the couple saw each other, males flashed rapidly and walked around container, while females stood still.

In phase 2, during the period that the couple saw each other, males became active and moved to the direction where females were. In the same time, females responded with flashes to males while stood still.

In phase 3, the mating period between the couple specimen, males moved closer to females while keeping communication via flashing. After this, males mounted the female dorsally and inserted the aedeagus into the female's genital chamber (Figure 33a). The couple remained in this position approximately 9-22 minutes, then males changed its position to the tail-to-tail position, pointing their butts to each other (Figure 33b). This stage took roughly 14-27 minutes, then the couple detached from each other and stayed still for a period of time. Afterward, males moved away from stilling females.

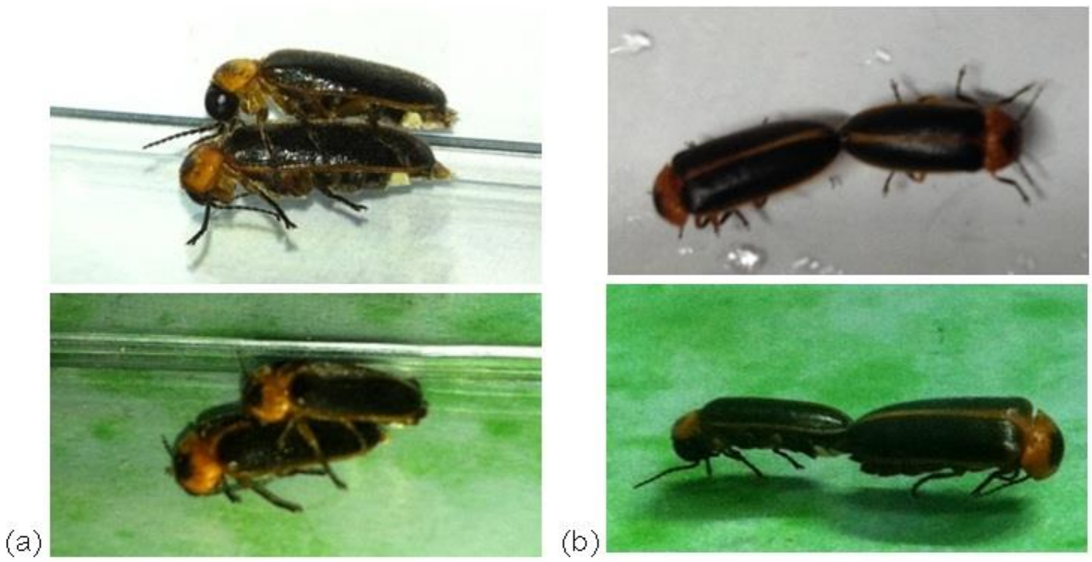


Figure 33 (a) The mounting position; (b) The tail-to-tail position in mating behavior

5.3.2 Flashing patterns in courtship behavior

The 20 pairs of fireflies were observed in laboratory. The study was divided in 3 phases found that in phase 1, before the couple saw each other, males walked around container and flashed rapidly also called as M0 flashes while females did not flash. The M0 flashes were continuous flashes with bright light and dim light periods (Figure 34). Interestingly, male MO flashes did not show dark periods, just only dim light.

In phase 2, during the period that the couple saw each other, males became active and flash faster than in phase 1, this flashes had the dark periods and call as M1 flashes. Females responded by flashing to males with the about half intensity light and call as F1 flashes (Figure 35). These synchronous flash periods overlapped between male (M1) and female (F1) flashes, and the M1-F1 flash pattern had both bright light and dark light periods.

In phase 3, the mating period between the couple specimen, males flashed faster than in phase 2, also called M2 flashes. Females responded with brighter and faster flashes to males, also called F2 flashes. These synchronous flashes did not have overlaps between male and female flashes, and the M2-F2 flash pattern had bright and dark light periods as in phase 2 (Figure 36). After copulation, males and females were not flash.

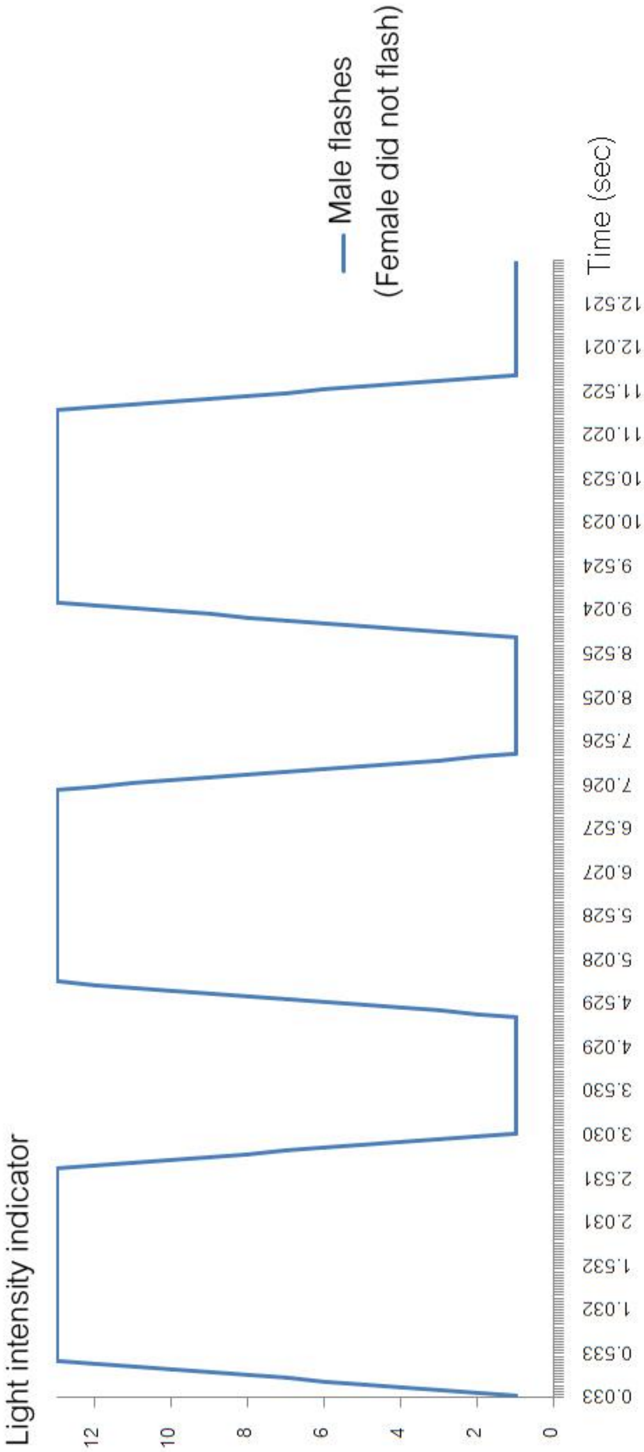


Figure 34 Male flashes of *Luciola* sp. HK 1 in phase 1, before the couple saw each other.

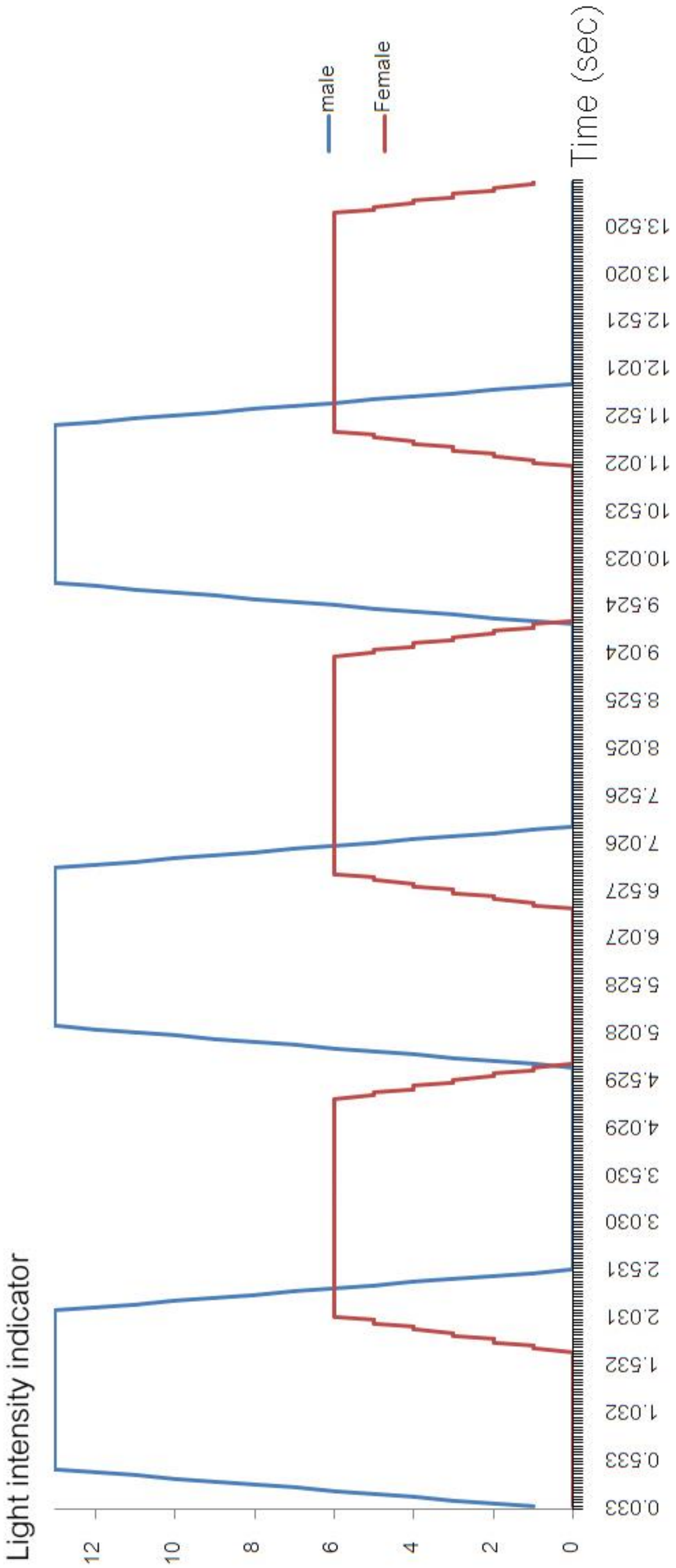


Figure 35 Males and females flash interaction of *Luciola* sp. HK 1 while the couple saw each other in phase 2.

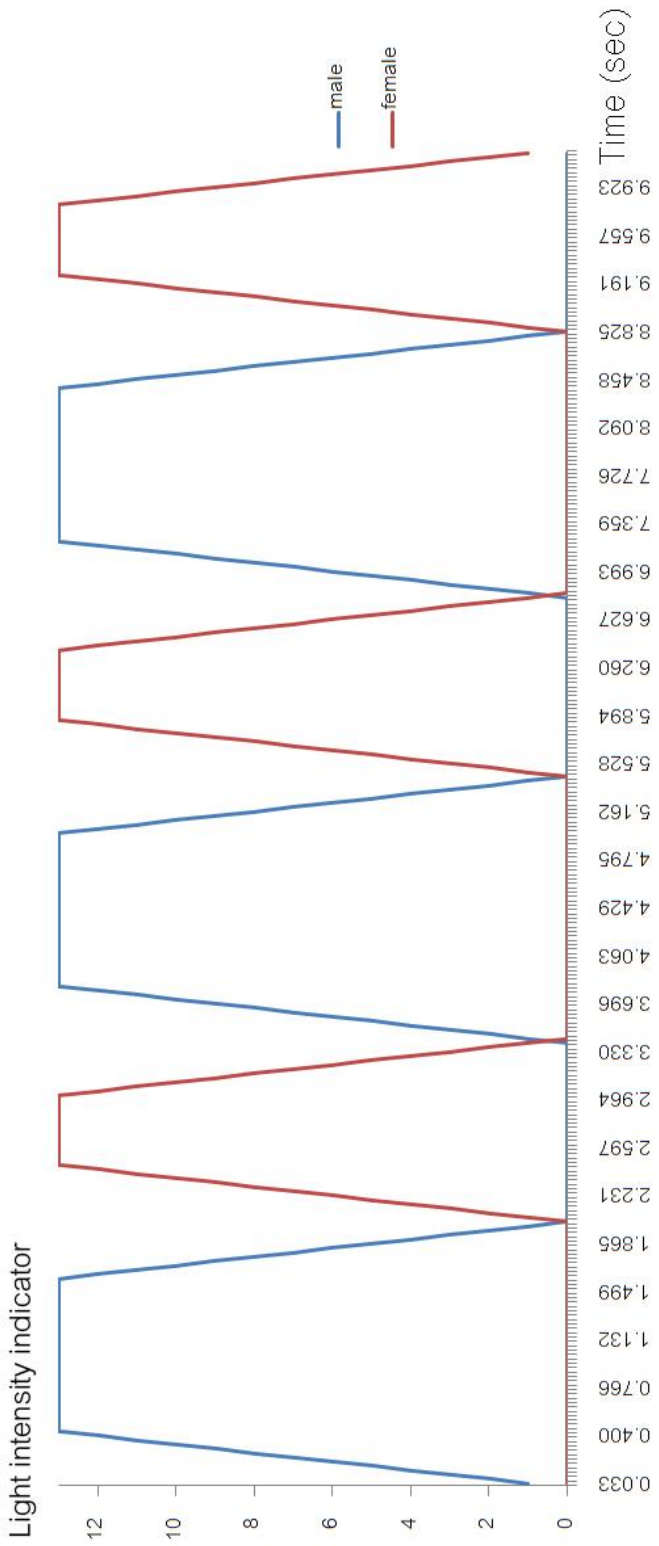


Figure 36 Males and females flash interaction of *Luciola* sp. HK 1 while the mating period between the couple specimen in phase 3.

The averages of pulse duration and interpulse duration of adult males and females *Luciola* sp. HK 1 in 3 phases (before the couple saw each other, during the period that the couple saw each other and the mating period between the couple specimen) were shown in Table 9. In phase 1, the male flash had the longest mean of pulse duration approximately 3.033±7.874 seconds, with dim light interpulse duration that took around 1.300±5.091 seconds. Females did not flash. In phase 2, males flashed faster than in phase 1, about 2.500±1.358 seconds in pulse duration mean and 2.167±1.356 seconds in interpulse duration mean. Females had long periods in pulse duration that took around 3.000±1.914 seconds, while interpulse duration were shot, approximately 1.667±0.476 seconds. In phase 3, males flashed fastest, took around 1.989±0.849 seconds in average pulse duration and 1.400±1.342 seconds in average interpulse duration. Females responded to males with brighter and faster light, pulse duration and interpulse duration were about 1.311±0.712 and 2.167±1.860 seconds, respectively.

Table 9 Averages of pulse duration and interpulse duration of males and females *Luciola* sp. HK 1 in second (unit), in 3 phases (before the couple saw each other, during the period that the couple saw each other and the mating period between the couple specimen).

Phase	Male		Female	
	Pulse duration (light)	Interpulse duration (dark/dim)	Pulse duration (light)	Interpulse duration (dark)
1	3.033±7.874	1.300±5.091 (dim)	-	-
2	2.500±1.358	2.167±1.356	3.000±1.914	1.667±0.476
3	1.989±0.849	1.400±1.342	1.311±0.712	2.167±1.860

In phase 1 (while male and female saw each other), males *Luciola* sp. HK 1 walked around container and flashed while females stood still and did not flash. In the same way, from the previous study of Thancharoen (2007), males *L. aquatilis* had become active after dusk and flashed continuously at a high rate without a dark period while female had a little data collected on the flash because the very low intensity of female flashes were extremely difficult to observe.

In phase 2 (while male and female saw each other), males *Luciola* sp. HK 1 walked to the females and flash while females responded to the male's flash with series of single pulse flash. Similar to *L. aquatilis* (Thancharoen, 2007), males walked to the females and flashed while females responded to the male's flash with series of single pulse flash.

In phase 3 (while male and female mating), males *Luciola* sp. HK 1 moved closer to females and communication via flash to females, while females responded with flashes to males. Similaly, *L. aquatilis* (Thancharoen, 2007), males walked to the females and flashed while females respond to the male's flash with series of single pulse flash.

On the other hand, after the males mounted the females dorsally and inserted the aedeagus into the female's genital chamber, both of male and female *Luciola* sp. HK 1 did not flash, but male and female *L. aquatilis* still communicated via flashes (Thancharoen, 2007).

Moreover, in this study, there was accidently evidence found that one of *Luciola* sp. HK 1 male copulated with a *L. aquatilis* female (Figure 37). From the unpublished data of Jarinthorn Winyuchoncharoen, who also studied the flashing display of *Luciola aquatilis* at The Huai Hongkhrai Royal Development Study Center, Doi Saket District, Chiang Mai Province from July 2014 to March 2015. She found that the *L. aquatilis* fireflies started the flashing time after the *Luciola* sp. HK 1 fireflies at about 15 minutes and also with a longer flashing duration (Appendix A: Table A-1 and Table A-2). These differences between these two fireflies are probably due to the reproductive isolating process, which occurs from the temporal isolation of prezygotic isolation.

Figure 37 The accidently male *Luciola* sp. HK 1 copulated with the female *L. aquatilis* in laboratory.

5.4 Conclusion

The study showed that before the couple saw each other, males flashed rapidly and walked around container, while females flashed slower and less active. During the period that the couple saw each other, males became active and moved to the direction where females were. After that, females responded with flash to males. In the mating period between the couple specimen, males moved closer to females while communicating by flashing, and dorsally mounted the female and mated. Then males changed its position to the tail-to-tail position. After that, the couple detached from each other and stayed still for a period of time. Afterward, males moved away from females (Figure 38 and 39). The study also indicated that there were 3 flash types; male flash, female respond and mating flash.

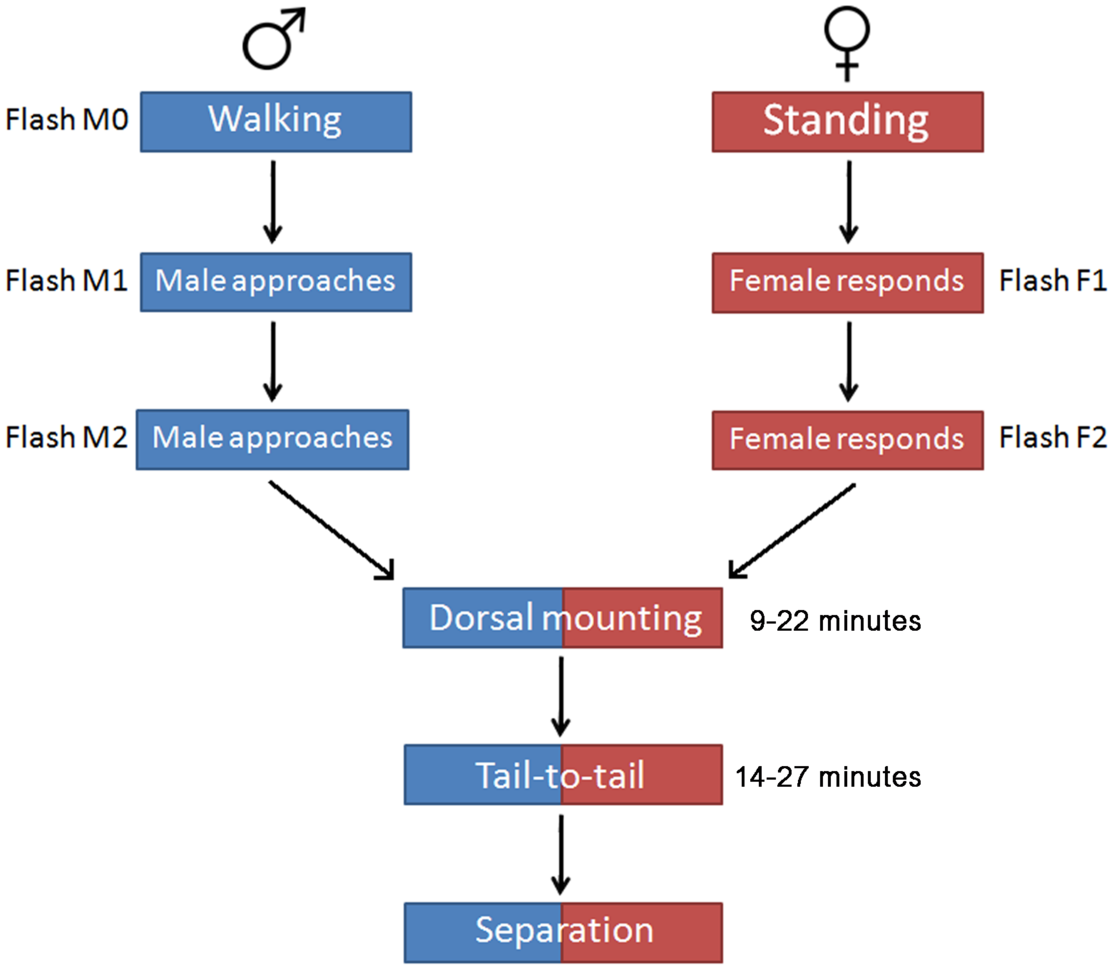


Figure 38 Schematic of *Luciola* sp. HK 1 mating behavior shows the positions and flash types.

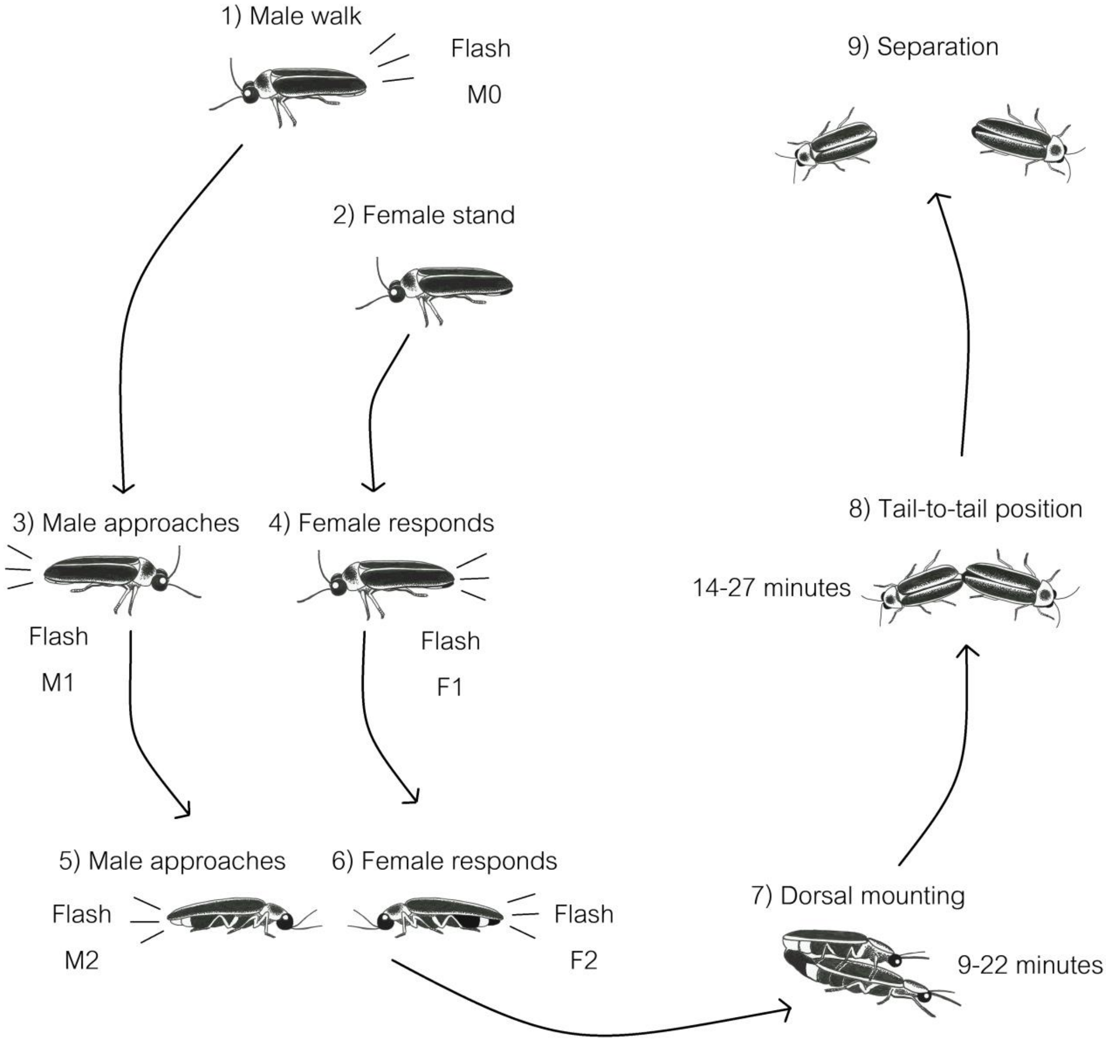


Figure 39 Schematic drawing of *Luciola* sp. HK 1 mating flashes and postures.

CHAPTER VI
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

From the study of abundance and peak flashing period of adult *Luciola* sp. HK 1 for 12 months, the indirect estimated numbers of individual were highest in April and adult firefly was not found from November 2014 to March 2015. T*he average of* flashing peak was at 55 minutes, after first flash from July 2014 to June 2015. The recommended months for firefly-watching ecotourism are April, May and June due to the high abundance of firefly displays and the possibility of rain is low. Tourists can reach to the watching site with ease. July-to-October is not suitable period because of low firefly population, and also there is a high possibility of rain. However, considering these factors, tourists still can visit the site during the latter period. The result shows that those fireflies do not exist from November to March, so it is recommended not to visit the site during this period. In addition, the recommended times for firefly-watching are around 7:00-8:30 PM due to the peak flashing period; but, if tourists would like to observe the behavior of fireflies from the starting moment, it is recommended to reach the site around 6:30 PM.

From the study of courtship behavior, the study was divided into 3 phases. In phase 1, before the couple saw each other, male flashed (M0) rapidly and walked around container, while females were not flash. In phase 2, during the period that the couple saw each other, males became active, flashed (M1) and moved to the direction where females were. In the same time, females responded with flashes (F1) to males. In phase 3, the mating period between the couple specimen, males moved closer to females while keeping communication via flashing (male flashed M2 and female flashed

F2). After this, males mounted the female dorsally and inserted the aedeagus into the female's genital chamber. Then males changed its position to the tail-to-tail position, pointing their butts to each other. After that, the couple detached from each other and stayed still for a period of time. Afterward, males moved away from the females.

6.2 Recommendations

1. Tourism can cause both environmental degradation and enhancement, so, the basic environmental knowledge should be provided to tourists for the best practice on sustainable tourism development, for example, limit the noise and torchlight usage because both of them disturb fireflies.

2. Aquatic firefly larvae commonly live in clean and clear natural fresh water habitats as their breeding sites. Maintenance of fresh water sources from human activities and dredging the natural water areas such as creeks and swamps are the best ways to protect firefly breeding habits. However, removal of excess aquatic plants affects directly to firefly larval population, so dredging water areas should be considered with the proper treatments and times.

REFERENCES

Abe, O. 2002. Interim Report FY 2001 IGES Environmental Education Project. [Online]. Available from: http://pub.iges.or.jp/modules/envirolib/upload/1519/attach/1003-fy2001.pdf

Baccetti, B. Crovetti, A. and Santini, L. 1987. Light-producing organs in *Keroplatus tipuloides* Bosc and *K. reaumuri pentophthalmus* Giglio-Tos (Diptera: Mycetophilidae). International Journal of Insect Morphology and Embryology 16: 169-176.

Bagnoli, P. Brunelli, M. Magni, F. and Musumeci, D. 1976. Neural mechanisms underlying spontaneous flashing and its modulation in the firefly *Luciola lusitanica*. Journal of Comparative Physiology 108: 133-156.

Ballantyne, L.A. and Buck, E. 1979. Taxonomy and behavior of *Luciola aphrogeneia*, a new surf firefly from Papua New Guinea. Transactions of the American Entomological Society (1890-) 105: 117-137.

Barrows, E.M. Arsenault, S.B. and Grenier, N.P. 2008. Firefly (Coleoptera: Lampyridae) flight periods, sex ratios, and habitat frequencies in a United States Mid-Atlantic freshwater tidal marsh, low forest, and their ecotone. Banisteria 31: 47-52.

Branham, M.A. and Wenzel, J.W. 2001. The evolution of bioluminescence in cantharoids (Coleoptera: Elateroidea). Florida Entomologist 565-586.

Branham, M.A. and Wenzel, J.W. 2003. The origin of photic behavior and the evolution of sexual communication in fireflies (Coleoptera: Lampyridae). Cladistics 19: 1-22.

Buck, J.B. 1937. Studies on the firefly. II. The signal system and color vision in *Photinus pyralis*. Physiological Zoology 10: 412-419.

Buschman, L.L. 1984. Biology of the firefly *Pyractomena lucifera* (Coleoptera: Lampyridae). Florida Entomologist 529-542.

Carlson, A.D. and Copeland, J. 1985. Flash communication in fireflies. Quarterly Review of Biology 415-436.

Carlson, A.D. Copeland, J. Raderman, R. and Bulloch, A.G. 1976. Role of interflash intervals in a firefly courtship (*Photinus macdermotti*). Animal Behaviour 24: 786-792.

Case, J. 1984. Vision in mating behaviour of fireflies. in Symposia of the Royal Entomological Society of London. pp.

Chunram, S. and Lewvanich, A. 1996. Behaviour and life cycle of firefly, *Luciola* sp. Thai Agricultural Research Journal (Thailand).

Chunram, S. and Lewvanich, A. 2000. Biology and behavior of firefly, *Pteroptyx malaccae* (Gorham). Entomology and Zoology Gazette (Thailand).

Cratsley, C.K. and Lewis, S.M. 2003. Female preference for male courtship flashes in *Photinus ignitus* fireflies. Behavioral Ecology 14: 135-140.

De Cock, R. and Matthysen, E. 2005. Sexual communication by pheromones in a firefly, *Phosphaenus hemipterus* (Coleoptera: Lampyridae). Animal behaviour 70: 807-818.

Demary, K. Michaelidis, C.I. and Lewis, S.M. 2006. Firefly courtship: Behavioral and morphological predictors of male mating success in *Photinus greeni*. Ethology 112: 485-492.

Erez, Y. and Huppert, D. 2010. Excited-state intermolecular proton transfer of the firefly's chromophore D-luciferin. The Journal of Physical Chemistry A 114: 8075-8082.

Fister, I. Yang, X.S. and Brest, J. 2013. A comprehensive review of firefly algorithms. Swarm and Evolutionary Computation 13: 34-46.

Fu, X. Ballantyne, L. and Lambkin, C. 2012. The external larval morphology of aquatic and terrestrial *Luciolinae* fireflies (Coleoptera: Lampyridae). Zootaxa 3405: 1-34.

Fu, X. Wang, Y. Lei, C. and Nobuyoshi, O. 2005. The swimming behavior of the aquatic larvae of the firefly *Luciola substriata* (Coleoptera: Lampyridae). The Coleopterists Bulletin 59: 501-505.

Fulton, B. 1941. A luminous fly larva with spider traits (Diptera, Mycetophilidae). Annals of the Entomological Society of America 34: 289-302.

Google. 2016. ศูนย์ศึกษาการพัฒนาห้วยฮ่องไคร้อันเนื่องมาจากพระราชดำริ. [Online]. Available from: https://www.google.co.th/maps/place/ศูนย์ศึกษาการพัฒนาห้วยฮ่องไคร้อันเนื่องมาจากพระราชดำริ [May 2016]

Hanson, F. 1962. Observation on the gross innervation of the firefly light organ. Journal of Insect Physiology 8: 105-111.

Harvey, E.N. 1952. Bioluminescence. n.p.: Academic Press.

Hastings, J.W. and Morin, J.G. 1991. Bioluminescence. New York: Wiley-Liss.

Ho, J. and Chu, J. 2002. Map for firefly watching in Taiwan. Taichung City: Morning Star Publisher.

Ho, J.Z. Chiang, P.H. Wu, C.H. and Yang, P.S. 2010. Life cycle of the aquatic firefly *Luciola ficta* (Coleoptera: Lampyridae). Journal of Asia-Pacific Entomology 13: 189-196.

Horridge, G. 1969. The eye of the firefly *Photuris*. Proceedings of the Royal Society of London B: Biological Sciences 171: 445-463.

Jeng, M.L. 2007. Description of *Luciola aquatilis* sp. nov., a new aquatic firefly (Coleoptera: Lampyridae: Luciolinae) from Thailand. Zootaxa 1611: 55-62.

Jeng, M.L. Engel, M.S. and Yang, P.S. 2007. *Oculogryphus*, a remarkable new genus of fireflies from Asia (Coleoptera: Lampyridae). American Museum Novitates 1-19.

Kato, K. 1953. On the luminous fungus gnats in Japan. The Science Reports of Saitama University 1: 59-63.

Lertsuchatavanich, P. 2005. Impact assessment of home stay tourism on firefly population at Ban Khog Kad Village, Samu Song Khram Province. Master. Science (Environmental Science), Bangkok, Chulalongkorn University.

Lewis, S.M. and Cratsley, C.K. 2008. Flash signal evolution, mate choice and predation in fireflies. Annual Review of Entomology 53: 293-321.

Li, X. Yang, S. Xie, M. and Liang, X. 2006. Phylogeny of fireflies (Coleoptera: Lampyridae) inferred from mitochondrial 16S ribosomal DNA, with references to morphological and ethological traits. Progress in Natural Science 16: 817.

Lloyd, J. 1978. Insect bioluminescence. Bioluminescence in Action 241-272.

Lloyd, J.E. 1966. Studies on the Flash Communication System in *Photinus* fireflies. Museum of Zoology, University of Michigan.

Lloyd, J.E. 1971. Bioluminescent communication in insects. Annual Review of Entomology 16: 97-122.

Lloyd, J.E. 1972. Mating behavior of a New Guinea *Luciola* firefly: A new communicative protocol (Coleoptera: Lampyridae). The Coleopterists' Bulletin 155-163.

Lloyd, J.E. 1983. Bioluminescence and communication in insects. Annual Review of Entomology 28: 131-160.

Longcore, T. and Rich, C. 2006. Ecological consequences of artificial night lighting. Island Press Washington, DC.

McDermott, F.A. 1964. The taxonomy of the Lampyridae (Coleoptera). Transactions of the American Entomological Society (1890-) 90: 1-72.

McDermott, F.A. 1966. Lampyridae. Coleopterorum Catalogus Supplementa,(ed. Secunda) 9.

McElroy, W. and DeLuca, M. 1978. Chemistry of firefly luminescence. Bioluminescence in action 109-127.

McElroy, W. Seliger, H. and White, E. 1969. Mechanism of bioluminescence, chemi-luminescence and enzyme function in the oxidation of firefly luciferin. Photochemistry and Photobiology 10: 153-170.

Meyer- Rochow, V. 2007. Glowworms: A review of *Arachnocampa* spp. and kin. Luminescence 22: 251-265.

Neuwirth, M. 1981. Ultrastructure of granules and immunocytochemical localization of luciferase in photocytes of fireflies. Tissue and Cell 13: 599-607.

Nurancha, P. Inkapatanakul, W. and Chunkao, K. 2013. Guidelines to the Management of Firefly Watching Tour in Thailand. Modern Applied Science 7: 8.

Oba, Y. Branham, M.A. and Fukatsu, T. 2011. The terrestrial bioluminescent animals of Japan. Zoological science 28: 771-789.

Ohba, N. 2004. Flash communication systems of Japanese fireflies. Integrative and Comparative Biology 44: 225-233.

Ohba, N. and Wong, C. 2004. External morphology and ecological study of the firefly, Pteroptyx tener at Kampong Kuantan, Selangor, Malaysia. Science Report of Yokosuka City Museum 51: 1-33.

Padelford, L. and Padelford, B. 2012. Firefly larva - Photinus. [Online]. Available from: http://bugguide.net/node/view/717427 [3 March 2013]

Pirk, C.W., et al. 2013. Statistical guidelines for *Apis mellifera* research. Journal of Apicultural Research 52: 1-24.

Pongjun, N. 2013. Species, abundance and flashing display of fireflies in a mangrove at Welu Wetland, Chanthaburi Province. Master of Science, Bangkok, Chulalongkorn University.

Rooney, J.A. and Lewis, S.M. 2000. Notes on the life history and mating behavior of *Ellychnia corrusca* (Coloeptera: Lampyridae). Florida Entomologist 324-334.

Seliger, H.H. and McElroy, W.D. 1960. Spectral emission and quantum yield of firefly bioluminescence. Archives of Biochemistry and Biophysics 88: 136-141.

Sherley, G. 2000. Invasive species in the Pacific. Samoa: South Pacific Regional Environment Programme.

Smith, D.S. 1963. The organization and innervation of the luminescent organ in a firefly, *Photuris pennsylvanica* (Coleoptera). The Journal of Cell Biology 16: 323-359.

Stanger-Hall, K.F. Lloyd, J.E. and Hillis, D.M. 2007. Phylogeny of North American fireflies (Coleoptera: Lampyridae): Implications for the evolution of light signals. Molecular Phylogenetics and Evolution 45: 33-49.

Tejon, R.C. 2015. Wildlife Profile: Beetles. [Online]. Available from: http://tejonconservancy.blogspot.com/2015/07/wildlife-profile-beetles.html [2 July 2015]

Thancharoen, A. 2007. The Biology and Mating Behavior of an Aquatic Firefly Species, *Luciola aquatilis* sp. nov. Thancharoen (Coleoptera: Lampyridae). Mahidol university.

Thancharoen, A. 2009. The story of firefly. Bangkok: Agricultural Technology Service Centre, Faculty of Agriculture, Kasetsart University.

Utarasakul, T. 2012. Environmental management system for tourist accommodations in Amphawa, Samut Songkram, Thailand. Waste Management 89: 10.6.

Williams, F. 1915. The luminous organ of the New Zealand glow-worm. Psyche 22: 36-43.

Winyuchoncharoen, J. Abundance and peak flashing display of *Luciola aqutilis* at The Huai Hongkhrai Royal Development Study Center, Doi Saket District, Chiang Mai Province Bangkok, 2015. (Unpublished Manuscript)

Wood, K.V. 1995. The chemical mechanism and evolutionary development of beetle bioluminescence. Photochemistry and Photobiology 62: 662-673.

Wyatt, T.D. 2003. Pheromones and Animal Behaviour: Communication by Smell and Taste. Cambridge: Cambridge University Press.

Yuma, M. 2007. Effect of rainfall on the long- term population dynamics of the aquatic firefly *Luciola cruciata*. Entomological science 10: 237-244.

Zuk, M. and Kolluru, G.R. 1998. Exploitation of sexual signals by predators and parasitoids. Quarterly Review of Biology 415-438.

APPENDIX

Appendix A

Table A-1 The average flashing duration of *L.* aqutilis fireflies from July to October 2014 and March 2015. The adult *L. aqutilis* firefly was not found from November 2014 to February 2015 (Unpubliched data of Jarinthorn Winyuchoncharoen).

Month	Flashing duration (minutes)
July 2014	80.00±0.00
August 2014	96.67±4.41
September 2014	86.67±8.33
October 2014	93.33±11.67
March 2015	68.33±3.60

Table A-2 The start flashing times of adult fireflies *L.* aqutilis and sunset times from July 2014 to March 2015 at Huai Hongkhrai Royal Development Study Center, Doi Saket district, Chiang Mai province (Unpubliched data of Jarinthorn Winyuchoncharoen).

Month	Start flashing times	Sunset times
July	7:35 PM	7:03 PM
August	7:20 PM	6:47 PM
September	7:05 PM	6:22 PM
October	6:45 PM	5:58 PM
November	-	5:46 PM
December	-	5:52 PM
January	-	6:09 PM
Febuary	-	6:28 PM
March	7:00 PM	6:35 PM

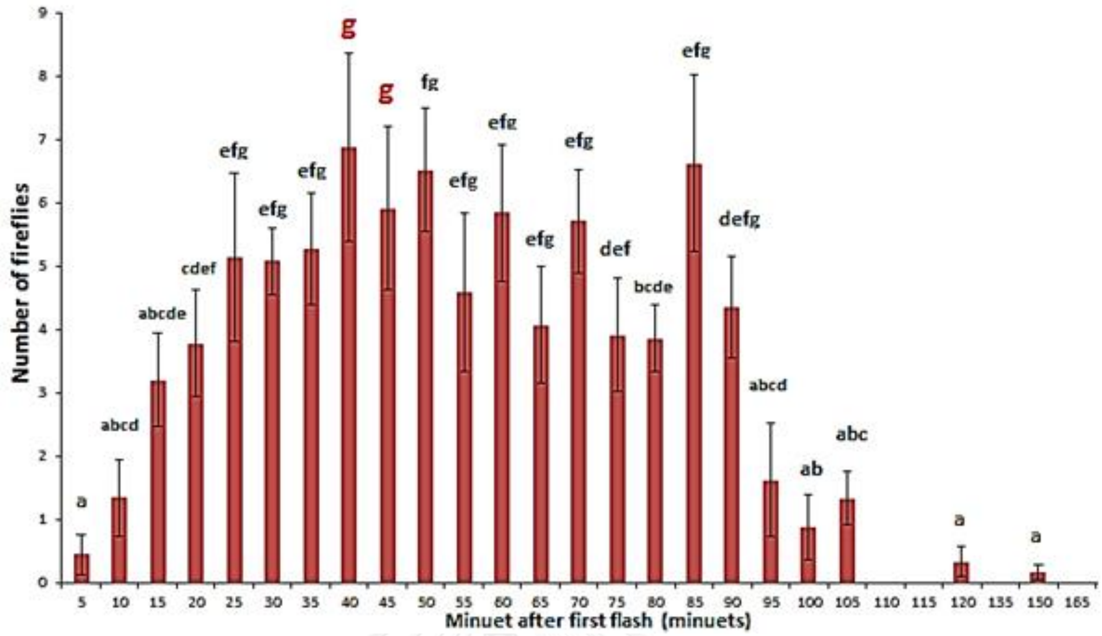


Figure A-1 The flash display peak of adult fireflies *L. aquatilis* at the Huai Hongkhrai Royal Development Study Center, Chiang Mai from July 2014 to March 2015 (Unpubliched data of Jarinthorn Winyuchoncharoen).

Appendix B

Table B-1 The indirect estimated number of individual of *Luciola* sp. HK 1 for study of abundance at the Huai Hongkhrai Royal Development Study Center from July to October 2014

min after first flash	JUL	AUG	SEP	OCT
5	0.83±0.62	2.33±2.05	6.87±0.24	8.00±1.41
10	2.83±1.03	1.67±2.36	4.10±1.10	8.07±1.41
15	2.67±0.29	1.33±1.89	5.80±2.07	7.90±2.30
20	5.77±0.66	4.43±4.16	4.33±3.55	7.37±0.61
25	3.50±3.34	8.57±4.17	8.83±2.95	5.17±0.29
30	3.73±2.64	3.53±0.76	6.83±5.72	8.33±2.59
35	2.10±2.97	3.77±1.23	0.00	4.00±2.83
40	1.67±2.36	3.40±0.70	1.10±1.56	3.33±2.49
45	2.43±3.44	5.60±1.37	5.83±4.90	2.57±1.82
50	5.43±4.13	3.67±0.78	3.50±2.48	4.43±4.16
55	2.33±3.30	2.60±1.84	0.00	1.67±2.36
60	0.00	0.00	1.67±2.36	1.67±2.36
65	0.00	2.77±1.96	1.50±2.12	0.00
70	7.50±5.76	1.83±2.59	2.67±3.77	0.00
75	2.83±4.01	1.67±2.36	0.00	0.00
80	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00

Table B-2 The indirect estimated number of individual of *Luciola* sp. HK 1 for study of abundance at the Huai Hongkhrai Royal Development Study Center from November 2014 to February 2015.

min after first flash	NOV	DEC	JAN	FEB
5	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00
35	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00
55	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00
65	0.00	0.00	0.00	0.00
70	0.00	0.00	0.00	0.00
75	0.00	0.00	0.00	0.00
80	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00

Table B-3 The indirect estimated number of individual of *Luciola* sp. HK 1 for study of abundance at the Huai Hongkhrai Royal Development Study Center from March to June 2015.

min after first flash	MAR	APR	MAY	JUN
5	0.00	4.58±1.59	2.50±1.08	2.00±0.82
10	0.00	6.43±1.82	4.78±0.16	5.00±0.82
15	0.00	5.78±1.31	5.33±0.54	6.89±3.27
20	0.00	10.33±2.62	7.00±1.52	6.71±1.38
25	0.00	8.57±1.23	6.57±1.11	6.88±1.29
30	0.00	8.79±3.40	8.07±4.22	10.81±3.80
35	0.00	6.60±1.41	6.17±0.49	6.64±3.85
40	0.00	8.00±0.00	8.00±0.33	4.83±1.15
45	0.00	8.58±1.85	5.78±0.57	5.25±0.89
50	0.00	6.50±1.08	7.64±1.09	5.33±3.77
55	0.00	7.72±2.37	6.58±2.18	6.52±1.95
60	0.00	3.17±2.25	5.83±4.13	4.08±2.97
65	0.00	1.50±2.12	2.83±4.01	3.73±2.88
70	0.00	0.00	0.00	0.00
75	0.00	0.00	0.00	0.00
80	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00

Correlations

		Number	Temp	Humidity	Rainfall
Pearson Correlation	Number	1.000	.878	.566	.372
	Temp	.878	1.000	.330	.285
	Humidity	.566	.330	1.000	.393
	Rainfall	.372	.285	.393	1.000
Sig. (1-tailed)	Number	.	.000	.028	.117
	Temp	.000	.	.148	.184
	Humidity	.028	.148	.	.103
	Rainfall	.117	.184	.103	.
N	Number	12	12	12	12
	Temp	12	12	12	12
	Humidity	12	12	12	12
	Rainfall	12	12	12	12

Figure A-2 The Spearman's correlation analysis of the indirect estimated number of individual of *Luciola* sp. HK 1, temperature, relative humidity and rainfall.

Correlations

		Sunset	Display
Sunset	Pearson Correlation	1	.891**
	Sig. (2-tailed)		.007
	N	7	7
Display	Pearson Correlation	.891**	1
	Sig. (2-tailed)	.007	
	N	7	7

**. Correlation is significant at the 0.01 level (2-tailed).

Figure A-3 The Spearman's correlation analysis of firefly first flash time and sunset time.

Appendix C

Table C-1 The indirect estimated number of individual of *Luciola* sp. HK 1 at the Huai Hongkhrai Royal Development Study Center from July to October 2014.

min after first flash	JUL	AUG	SEP	OCT
5	1.67±1.70	2.86±0.81	7.56±1.75	7.50±2.27
10	1.33±1.89	3.61±1.04	3.97±3.08	6.78±1.59
15	4.50±2.04	2.22±1.59	1.33±1.89	8.58±1.36
20	2.33±1.66	1.95±1.40	6.61±1.62	8.00±2.94
25	0.00	3.82±0.77	4.33±3.06	5.67±0.24
30	3.81±0.79	4.53±1.13	3.25±2.65	6.39±0.28
35	1.27±1.79	3.71±0.66	0.00	9.00±2.94
40	1.75±2.47	3.44±0.63	7.00±4.97	2.56±1.81
45	3.90±0.46	1.17±1.65	0.00	5.50±4.49
50	1.47±2.07	2.00±2.83	5.50±0.41	6.39±2.66
55	6.06±5.19	4.22±3.70	5.00±4.08	5.00±4.08
60	4.97±1.51	1.11±1.57	1.50±2.12	4.50±3.67
65	2.81±2.03	2.00±1.44	3.00±2.16	1.67±2.36
70	0.00	0.00	1.33±1.89	0.00
75	0.00	0.00	0.00	0.00
80	2.50±3.54	0.00	0.00	0.00
85	1.67±2.36	0.00	0.00	0.00
90	0.00	0.00	2.00±2.83	0.00
95	0.00	0.67±0.94	0.00	0.00
100	0.00	0.00	0.00	0.00
105	0.00	0.00	0.00	0.00
110	0.00	0.00	0.00	0.00

Table C-2 The indirect estimated number of individual of *Luciola* sp. HK 1 at the Huai Hongkhrai Royal Development Study Center from November 2014 to February 2015.

min after first flash	NOV	DEC	JAN	FEB
5	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00
35	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00
55	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00
65	0.00	0.00	0.00	0.00
70	0.00	0.00	0.00	0.00
75	0.00	0.00	0.00	0.00
80	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00
95	0.00	0.00	0.00	0.00
100	0.00	0.00	0.00	0.00
105	0.00	0.00	0.00	0.00
110	0.00	0.00	0.00	0.00
115	0.00	0.00	0.00	0.00

Table C-3 The indirect estimated number of individual of *Luciola* sp. HK 1 at the Huai Hongkhrai Royal Development Study Center from March to June 2015.

min after first flash	MAR	APR	MAY	JUN
5	0.00	3.00±0.82	2.17±1.31	2.17±0.62
10	0.00	5.44±1.23	4.67±1.09	2.17±2.09
15	0.00	5.92±2.12	5.11±0.63	4.78±1.02
20	0.00	7.60±1.01	4.67±1.52	2.44±3.46
25	0.00	6.72±0.86	6.54±0.90	4.05±0.69
30	0.00	8.22±0.31	3.83±2.75	6.40±0.99
35	0.00	8.58±0.61	3.78±2.73	3.52±2.52
40	0.00	5.91±1.43	7.56±0.71	1.53±2.17
45	0.00	9.67±2.36	6.33±1.25	4.50±0.92
50	0.00	7.33±0.94	7.39±1.49	4.27±3.29
55	0.00	6.50±2.55	6.17±4.37	10.01±4.06
60	0.00	2.56±2.04	7.94±1.16	8.03±2.20
65	0.00	3.17±2.32	6.00±4.24	8.62±4.42
70	0.00	0.00	6.00±8.49	0.00
75	0.00	0.00	0.00	0.00
80	0.00	0.00	0.00	0.00
85	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00
95	0.00	0.00	0.00	0.00
100	0.00	0.00	0.00	0.00
105	0.00	0.00	0.00	0.00
110	0.00	0.00	0.00	0.00
115	0.00	0.00	0.00	0.00

Table C-4 The indirect estimated number of individual of *Luciola* sp. HK 1 for study of peak flashing period at the Huai Hongkhrai Royal Development Study Center from July to October 2014.

	JUL	AUG	SEP	OCT
18:25-18:30	0.00	0.00	0.00	7.50±2.27
18:30-18:35	0.00	0.00	0.00	6.78±1.59
18:35-18:40	0.00	0.00	0.00	8.58±1.36
18:40-18:45	0.00	0.00	0.00	8.00±2.94
18:45-18:50	0.00	0.00	0.00	5.67±0.24
18:50-18:55	0.00	0.00	7.56±1.75	6.39±0.28
18:55-19:00	0.00	0.00	3.97±3.08	9.00±2.94
19:00-19:05	0.00	0.00	1.33±1.89	2.56±1.81
19:05-19:10	0.00	0.00	6.61±1.62	5.50±4.49
19:10-19:15	0.00	0.00	4.33±3.06	6.39±2.66
19:15-19:20	0.00	2.86±0.81	3.25±2.65	5.00±4.08
19:20-19:25	0.00	3.61±1.04	0.00	4.50±3.67
19:25-19:30	1.67±1.70	2.22±1.59	7.00±4.97	1.67±2.36
19:30-19:35	1.33±1.89	1.95±1.40	0.00	0.00
19:35-19:40	4.50±2.04	3.82±0.77	5.50±0.41	0.00
19:40-19:45	2.33±1.66	4.53±1.13	5.00±4.08	0.00
19:45-19:50	0.00	3.71±0.66	1.50±2.12	0.00
19:50-19:55	3.81±0.79	3.44±0.63	3.00±2.16	0.00
19:55-20:00	1.27±1.79	1.17±1.65	1.33±1.89	0.00
20:00-20:05	1.75±2.47	2.00±2.83	0.00	0.00
20:05-20:10	3.90±0.46	4.22±3.70	0.00	0.00
20:10-20:15	1.47±2.07	1.11±1.57	0.00	0.00
20:15-20:20	6.06±5.19	2.00±1.44	2.00±2.83	0.00
20:20-20:25	4.97±1.51	0.00	0.00	0.00
20:25-20:30	2.81±2.03	0.00	0.00	0.00
21:30-21:35	0.00	0.00	0.00	0.00
21:35-21:40	0.00	0.00	0.00	0.00
21:40-21:45	2.50±3.54	0.00	0.00	0.00
22:45-22:50	1.67±2.36	0.67±0.94	0.00	0.00

Table C-5 The indirect estimated number of individual of *Luciola* sp. HK 1, to study of peak flashing period at the Huai Hongkhrai Royal Development Study Center from November 2014 to February 2015.

	NOV	DEC	JAN	FEB
18:25-18:30	0.00	0.00	0.00	0.00
18:30-18:35	0.00	0.00	0.00	0.00
18:35-18:40	0.00	0.00	0.00	0.00
18:40-18:45	0.00	0.00	0.00	0.00
18:45-18:50	0.00	0.00	0.00	0.00
18:50-18:55	0.00	0.00	0.00	0.00
18:55-19:00	0.00	0.00	0.00	0.00
19:00-19:05	0.00	0.00	0.00	0.00
19:05-19:10	0.00	0.00	0.00	0.00
19:10-19:15	0.00	0.00	0.00	0.00
19:15-19:20	0.00	0.00	0.00	0.00
19:20-19:25	0.00	0.00	0.00	0.00
19:25-19:30	0.00	0.00	0.00	0.00
19:30-19:35	0.00	0.00	0.00	0.00
19:35-19:40	0.00	0.00	0.00	0.00
19:40-19:45	0.00	0.00	0.00	0.00
19:45-19:50	0.00	0.00	0.00	0.00
19:50-19:55	0.00	0.00	0.00	0.00
19:55-20:00	0.00	0.00	0.00	0.00
20:00-20:05	0.00	0.00	0.00	0.00
20:05-20:10	0.00	0.00	0.00	0.00
20:10-20:15	0.00	0.00	0.00	0.00
20:15-20:20	0.00	0.00	0.00	0.00
20:20-20:25	0.00	0.00	0.00	0.00
20:25-20:30	0.00	0.00	0.00	0.00
21:30-21:35	0.00	0.00	0.00	0.00
21:35-21:40	0.00	0.00	0.00	0.00
21:40-21:45	0.00	0.00	0.00	0.00
22:45-22:50	0.00	0.00	0.00	0.00

Table C-6 The indirect estimated number of individual of *Luciola* sp. HK 1 to study of peak flashing period at the Huai Hongkhrai Royal Development Study Center from March to June 2015.

	MAR	APR	MAY	JUN
18:25-18:30	0.00	0.00	0.00	0.00
18:30-18:35	0.00	0.00	0.00	0.00
18:35-18:40	0.00	0.00	0.00	0.00
18:40-18:45	0.00	0.00	0.00	0.00
18:45-18:50	0.00	0.00	0.00	0.00
18:50-18:55	0.00	0.00	0.00	0.00
18:55-19:00	0.00	0.00	0.00	0.00
19:00-19:05	0.00	0.00	0.00	0.00
19:05-19:10	0.00	0.00	2.17±1.31	0.00
19:10-19:15	0.00	0.00	4.67±1.09	0.00
19:15-19:20	0.00	3.00±0.82	5.11±0.63	2.17±0.62
19:20-19:25	0.00	5.44±1.23	4.67±1.52	2.17±2.09
19:25-19:30	0.00	5.92±2.12	6.54±0.90	4.78±1.02
19:30-19:35	0.00	7.60±1.01	3.83±2.75	2.44±3.46
19:35-19:40	0.00	6.72±0.86	3.78±2.73	4.05±0.69
19:40-19:45	0.00	8.22±0.31	7.56±0.71	6.40±0.99
19:45-19:50	0.00	8.58±0.61	6.33±1.25	3.52±2.52
19:50-19:55	0.00	5.91±1.43	7.39±1.49	1.53±2.17
19:55-20:00	0.00	9.67±2.36	6.17±4.37	4.50±0.92
20:00-20:05	0.00	7.33±0.94	7.94±1.16	4.27±3.29
20:05-20:10	0.00	6.50±2.55	6.00±4.24	10.01±4.06
20:10-20:15	0.00	2.56±2.04	6.00±8.49	8.03±2.20
20:15-20:20	0.00	3.17±2.32	0.00	8.62±4.42
20:20-20:25	0.00	0.00	0.00	0.00
20:25-20:30	0.00	0.00	0.00	0.00
21:30-21:35	0.00	0.00	0.00	0.00
21:35-21:40	0.00	0.00	0.00	0.00
21:40-21:45	0.00	0.00	0.00	0.00
22:45-22:50	0.00	0.00	0.00	0.00

VITA

Miss Nutta Pornthitichant was born on August 25, 1988 in Bangkok, Thailand. After completing her study at Triamudomsuksa pattanakarn School, in 2006, she entered Chulalongkorn University with a Bachelor's degree from the Department of Biology, Faculty of Science. In 2012, she continued her Master degree of Science, Zoology program in the Department of Biology, Faculty of Science, Chulalongkorn University and completed the program in 2016. Her research was supported by Sponsorship for Graduate Student Research under the 90th anniversary of chulalongkorn University fund (Rarchadaphiseksomphot Endowment fund).

In the academic year, she presented the poster presentation in the 20th Biology Science Graduate Congress (BSGC) at Chulalongkorn University, Thailand from December 9th-11th, 2015. In addition, she published her work and presented the oral presentation in the proceeding of the 8th National Science Research Conference at the University of Phayao, Thailand from May 30th-31th, 2016.