การเลือกกินหอยทากบกของตัวอ่อนหิ่งห้อย *Pyrocoelia tonkinensis* Olivier และพลวัตประชากร ในพื้นที่ป่าอนุรักษ์และสถานีวิจัยของจุฬาลงกรณ์มหาวิทยาลัย ตำบลไหล่น่าน อำเภอเวียงสา จังหวัดน่าน

นางสาวกมลชนก บริบูรณ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสัตววิทยา ภาควิชาชีววิทยา
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

LAND SNAIL PREFERENCE OF FIREFLY *Pyrocoelia tonkinensis* Olivier LARVAE AND THEIR POPULATION DYNAMICS AT THE CHULALONGKORN UNIVERSITY FOREST AND RESEARCH STATION, LAI NAN SUBDISTRICT, WIANG SA DISTRICT, NAN PROVINCE

Miss Kamolchanok Boriboon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Zoology
Department of Biology
Faculty of Science
Chulalongkorn University
Academic Year 2014

Thesis Title	LAND SNAIL PREFERENCE OF FIREFLY *Pyrocoelia tonkinensis* Olivier LARVAE AND THEIR POPULATION DYNAMICS AT THE CHULALONGKORN UNIVERSITY FOREST AND RESEARCH STATION, LAI NAN SUBDISTRICT, WIANG SA DISTRICT, NAN PROVINCE
By	Miss Kamolchanok Boriboon
Field of Study	Zoology
Thesis Advisor	Assistant Professor Duangkhae Sitthicharoenchai, Ph.D.
Thesis Co-Advisor	Thongchai Ngamprasertwong, Ph.D.

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

....Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

....Chairman

(Noppadon Kitana, Ph.D.)

....Thesis Advisor

(Assistant Professor Duangkhae Sitthicharoenchai, Ph.D.)

....Thesis Co-Advisor

(Thongchai Ngamprasertwong, Ph.D.)

....Examiner

(Nipada Ruankaew Disyatat, Ph.D.)

....External Examiner

(Angoon Lewvanich, Ph.D.)

กมลชนก บริบูรณ์ : การเลือกกินหอยทากบกของตัวอ่อนหิ่งห้อย *Pyrocoelia tonkinensis* Olivier และพลวัตประชากรในพื้นที่ป่าอนุรักษ์และสถานีวิจัยของจุฬาลงกรณ์มหาวิทยาลัย ตำบลไหล่น่าน อำเภอเวียงสา จังหวัดน่าน (LAND SNAIL PREFERENCE OF FIREFLY *Pyrocoelia tonkinensis* Olivier LARVAE AND THEIR POPULATION DYNAMICS AT THE CHULALONGKORN UNIVERSITY FOREST AND RESEARCH STATION, LAI NAN SUBDISTRICT, WIANG SA DISTRICT, NAN PROVINCE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.ดวงแข สิทธิเจริญชัย, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.ธงชัย งามประเสริฐวงศ์, 138 หน้า.

การศึกษาการเลือกกินหอยทากบก พฤติกรรมการกินอาหาร และพลวัตประชากรของตัวอ่อนหิ่งห้อย *Pyrocoelia tonkinensis* กับหอยทากบกที่เป็นเหยื่ออาหาร ตั้งแต่เดือนกุมภาพันธ์ 2556 ถึง กุมภาพันธ์ 2557 พบว่า ตัวอ่อนหิ่งห้อยมีจำนวนการโจมตีเฉลี่ยต่อหอยทากสยาม *Cryptozona siamensis* มากกว่าหอยขัดเปลือก *Sarika resplendens* อย่างมีนัยสำคัญทางสถิติ อีกทั้งพฤติกรรมการกินอาหารของตัวอ่อนหิ่งห้อยแบ่งออกเป็น 2 ระยะคือ ระยะหาเหยื่อและระยะจัดการกับเหยื่อ โดยลำดับพฤติกรรมการกินอาหารของตัวอ่อนหิ่งห้อยจะเริ่มตั้งแต่ ยืดหัว เดิน จู่โจม สัมผัส กัด โยกหัวขึ้น-ลง ขยับเขี้ยว ยืดส่วนหัวเข้าไปในเปลือกของเหยื่อ ทำความสะอาดและเดินไปมากระทั่งเหยื่อถูกกินจนหมด นอกจากนี้ยังพบว่าตัวอ่อนหิ่งห้อยใช้เวลาในการกินยาวนานที่สุดเมื่อเทียบกับพฤติกรรมอื่นอย่างมีนัยสำคัญทางสถิติ การศึกษาพลวัติประชากรพบว่า เดือนกันยายน 2556 เป็นเดือนที่พบประชากรตัวอ่อนหิ่งห้อยมากที่สุด สอดคล้องกับการที่พบประชากรหอยทากสยามมากที่สุดในเดือนกรกฎาคมและเดือนกันยายน 2556 ผลการวิเคราะห์ความสัมพันธ์พบว่าประชากรหอยทากสยามเป็นปัจจัยสำคัญที่มีความสัมพันธ์ต่อจำนวนประชากรตัวอ่อนหิ่งห้อยอย่างมีนัยสำคัญทางสถิติ การปกคลุมของพืชคลุมดิน เป็นปัจจัยสำคัญทางสิ่งแวดล้อมที่ส่งผลต่อประชากรตัวอ่อนหิ่งห้อย อีกทั้งความชื้นของดิน การปกคลุมของพืชเรือนยอดและการปกคลุมของพืชคลุมดิน เป็นปัจจัยสำคัญทางสิ่งแวดล้อมที่ส่งผลต่อจำนวนประชากรหอยทากสยามอย่างมีนัยสำคัญทางสถิติอีกด้วย นอกจากนี้ยังพบการกระพริบแสงของหิ่งห้อยตัวเต็มวัยเพศผู้ทั้งสิ้น 8 เดือน โดยพบการกระพริบแสงเฉลี่ยสูงสุดในเดือนเมษายนและพฤษภาคม 2556 ทั้งนี้หิ่งห้อยจะเริ่มกระพริบแสงในช่วงเวลาประมาณ 19:00 น. และกระพริบแสงสูงสุดในช่วงเวลา 21:00 น. ถึง 22:00 น. หลังจากนั้นหิ่งห้อยจะกระพริบแสงลดลงจนถึงช่วงเวลาประมาณ 6:00 น.

ภาควิชา	ชีววิทยา	ลายมือชื่อนิสิต	....
สาขาวิชา	สัตววิทยา	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2557	ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

5471905423 : MAJOR ZOOLOGY

KEYWORDS: FOOD FREFERENCE / FEEGING BEHAVIOR / POPULATION DYNAMICS

KAMOLCHANOK BORIBOON: LAND SNAIL PREFERENCE OF FIREFLY *Pyrocoelia tonkinensis* Olivier LARVAE AND THEIR POPULATION DYNAMICS AT THE CHULALONGKORN UNIVERSITY FOREST AND RESEARCH STATION, LAI NAN SUBDISTRICT, WIANG SA DISTRICT, NAN PROVINCE. ADVISOR: ASST. PROF. DUANGKHAE SITTHICHAROENCHAI, Ph.D., CO-ADVISOR: THONGCHAI NGAMPRASERTWONG, Ph.D., 138 pp.

Land snail preference, feeding behavior and population dynamics of firefly *Pyrocoelia tonkinensis* larvae and land snail prey were investigated from February 2013 to February 2014. The number of attacks of the firefly larvae on *Cryptozona siamensis* land snails was significantly higher than *Sarika resplendens*. Feeding behavior of firefly larvae were divided into two phases, namely, searching phase and handling phase. The larval feeding behavior began with head stretch followed by walking, sprinting and approaching, touching, biting, head shake, mandible chew, head insert, cleaning and walking around, respectively. Moreover, firefly larvae significantly spent their time on feeding unit more than the other behavioral units. The firefly larva population size was highest in September 2013. Similarly, *C. siamensis* land snail population size was highest in July and September 2013. Simple correlation analysis revealed that land snail population had a significant positive correlation on the firefly larva population. Environmental factors such as herbaceous coverage showed significant positive effect on the firefly larva population. Likewise, soil moisture, canopy coverage and herbaceous coverage showed significant positive effects on *C. siamensis* land snail population. Additionally, flashing displays of adult male *P. tonkinensis* firefly occurred in eight months. Average numbers of flashing displays were highest in April and May 2013. The flashing displays began at about 19:00 PM. The display numbers peaked from 21:30 PM to 22:00 PM. After peak, flashing displays gradually decreased until the end of flashing activity at about 06:00 AM.

Department:	Biology	Student's Signature	....
Field of Study:	Zoology	Advisor's Signature	....
Academic Year:	2014	Co-Advisor's Signature	....

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude and deep appreciation to Assistant Professor Dr. Duangkhae Sitthicharoenchai, my thesis advisor, for providing guidance, invaluable comments and gentle support throughout my academic year. I would like to thank Dr. Thongchai Ngamprasertwong, my thesis co-advisor for precious suggestions and kind support on this research. I gratefully acknowledge the valuable discussions and useful comments of the chairman, Dr. Noppadon Kittana and the thesis examiner, Dr. Nipada Ruankaew Disyatat. Furthermore, I would like to thank Dr. Angoon Lewvanich, the thesis external examiner for recommendation and firefly identification.

This thesis would not succeed without kind support from Assistant Professor Dr. Wichase Khonsue, Assistant Professor Dr. Chirasak Sutcharit, Dr. Chatchawan Chaisuekul, Mr. Suttisan Pimpasalee, Mr. Sunitsorn Pimpasalee, Miss Patchara Danaisawadi and all turtle laboratory members of Chulalongkorn University. Additionally, I would like to thank Miss Patthamas Yasang, Miss Chanyapatch Seanthetthanyawat, Mr. Kittipum Chansri, Mr. Khattapan Jantawongsri and all members of the Chulalongkorn University Forest and Research Station for friendship and field assistance.

This thesis was supported by Sponsorship for Graduate Student Research under CU Academic Network in the Region (CU-ANR-56-02) and the 90th anniversary of Chulalongkorn University fund (Ratchadaphiseksomphot Endowment fund).

Finally, I would like to express my deepest gratitude to my family for their infinite love, attention, understanding and encouragement.

CONTENTS

Page

Page

Page

LIST OF TABLES

Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Fireflies are nocturnal insects, members of *Lampyridae*, a family of winged beetles in the order Coleoptera. They have specialized light-emitting organs namely light organ in the abdominal segments for sexual communication, courtship behavior and mating behavior (Copeland and Moiseff, 1997; De Cock and Matthysen, 2003). As a consequence of their beautiful flashing lights, fireflies have been attracted the public interest since ancient times (Ballantyne and McLean, 1970; Bassot and Polunin, 1967; Lloyd, 1973a). Therefore, many research publications of fireflies have focused on their bioluminescence, flash communication and sexual selection mechanisms (Blum and Sannasi, 1997; Branham and Greenfield, 1996; Eisner et al., 1997; Lloyd, 1966; Ohba, 1983; 2004; Ohba and Hidaka, 2002; Vencl et al., 1994; Vencl and Carlson, 1998; Wang et al., 2007). However, only a few studies focused on the biology and ecology of these insects (Buschman, 1984a; b; Fu et al., 2005; Kaufman, 1965; Ohba and Sim, 1994; Williams, 1917).

Existing reports include biological characteristic of fireflies such as their life cycle, habitat and glowing behavior, not only in adult fireflies, but also in firefly larvae (Buschman, 1984b; Ho et al., 2010; Sommit, 2004; Wang et al., 2007). This might be because of the larval stages are the longest stage of firefly life cycle, which could be more than 300 days to grow up (Chunram and Lewvanich, 1996). In this stage, firefly larvae are predators feeding on a variety of small invertebrates, such as snails, slugs, earthworms and other arthropod larvae (Buschman, 1984b; Fu et al., 2005; Kaufman, 1965; Wang et al., 2007; Williams, 1917). Moreover, recent studies revealed that larvae of many firefly species showed specific diet preferences. For instance, *Photuris* larvae in Florida usually fed on snails, slugs, insect larvae and non-living food (Buschman, 1984b) while *Photinus stenocladius* and *Phosphaenus hemipterus* larvae specially fed on earthworms (LaBella and Lloyd, 1991; Majka and MacIvor, 2009; Suzuki, 1997).

Additionally, habitats of firefly larvae were different among species. They were found in habitats with varying level of environmental factors such as temperature, humidity and herbaceous cover. For instance, the larvae of aquatic firefly, *Pyractonema lucifera,* in North America were abundant in the water surface around cattail stands. Specifically, they were found in gaps between the leaves of the hyacinths and cattail plants. The terrestrial firefly larvae of genus *Photuris* in North America appeared on the surface and crawled or climbed up on stem at night. The larvae were abundant in the wet leaf litter particularly after a rain in the forest, ponds and streams where their land snail prey were frequently found (Buschman, 1984a).

However, a few studies of firefly larva food preference, feeding behavior, population dynamics and relationship with land snail prey have been published in firefly larvae. The larval foraging behavior in the numerous firefly species are undiscovered especially *Pyrocoelia tonkinensis* firefly larvae which is the oldest firefly species found in Thailand (published by Matichon newspaper, November, 15th 2005, Volume 28, Issue 10111, page 10).

Therefore, the objectives of this research concentrated on food preference and feeding behavior of *P. tonkinensis* firefly larvae on land snails; and also focused on the relationship between the firefly larva and land snail prey populations including the correlation of environmental factors on firefly larva and land snail prey populations. Furthermore, the research were to explore the flashing displays of adult male *P. tonkinensis* fireflies at nighttime all year round at the Chulalongkorn University Forest and Research Station, Lai Nan Subdistrict, Wiang Sa District, Nan Province.

CHAPTER II

LITERATURE REVIEWS

2.1 Classification and distribution

Firefly or lightning bug belongs to order Coleoptera, family Lampyridae (Barrows et al., 2008; Stanger-Hall et al., 2007). In the past, seven subfamilies and 92 genera of lampyrids were recorded: subfamily Lampyrinae, Photurinae, Luciolinae, Ototretinae, Mathetinae, Pterotinae and Rhagophthalminae (McDermott, 1966). Currently, more than 100 genera and over 2,000 species of lampyrids were classified and existed all around the world such as Asia, Europe, America and Australia (Lloyd, 2002; Stanger-Hall et al., 2007; Viviani, 2001).

The lampyrids species were habitually observed in the tropical zone (Branham and Greenfield, 1996). In Europe, more than 35 species of fireflies were identified which two species in the United Kingdom (Fu et al., 2009) and eleven species in Spain and Portugal (Guzmán Álvarez and De Cock, 2011). In the United States of America, 120 species of North American lampyrids were described (Lloyd, 1997a) and presently classified into four or five subfamilies such as Lampyrinae, Photurinae, Ototretinae and Cyphonocerinae or Pterotinae (Stanger-Hall et al., 2007). In Brazil, where there is the largest diversity of lampyrids in the world, more than 500 species of fireflies were described and conforming to 23 % of firefly species worldwide (Viviani and Santos, 2012). In Asia, the species of fireflies in the Indo-Pacific area were classified into 23 genera in subfamily Luciolinae (Ballantyne and Lambkin, 2013) and more than four genera were found in Malaysia (Jusoh et al., 2010a). Furthermore, several firefly species were regularly found in many countries such as Japan, China, including Thailand.

More than 100 species of fireflies have been discovered in Thailand, most of them (more than 90 species) belong to the genus *Luciola* (Chunram and Lewvanich, 1996). Eight genera and 55 firefly species were classified from the Central and Eastern Thailand (Chunram and Lewvanich, 2001). Moreover, the research of the firefly project under HM Queen Sirikit’s Initative, Botanical Garden Organization, Ministry of Natural

Resource and Environment, Thailand form 1997 to 2007 reported that firefly species in 34 provinces of Thailand separated into nine genera, namely, *Diaphanes, Lamprigera, Luciola, Pteroptyx, Pyrocoelia, Pyrophanes*, *Rhagopthalmus* and *Sternocladius*. However, Dr. Angoon Lewvanich who is the firefly taxonomist currently suggested that there were 12 genera of fireflies in Thailand, namely, *Abscondita, Asymmetricata, Colophotia*, *Curtos, Diaphanes, Lamprigera, Luciola, Pteroptyx, Pygoluciola, Pyrocoelia, Sternocladius* and *Vesta* (Personal communication).

The genus *Pyrocoelia* was established by Gorham (Gorham, 1880). *Pyrocoelia* species were found throughout the Oriental and Palearctic regions (Jeng et al., 1999). More than 60 species were recorded, of which approximately 20 species were collected in mainland China (McDermott, 1966). However, basic information on taxonomy and behaviors were rarely reported for this genera (Wang et al., 2007) including in firefly *Pyrocoelia tonkinensis* species. This species were described originally in 1886 and established by Olivier (McDermott, 1966). They distributed throughout Thailand especially in the northern area such as Chiang Mai and Nan Provinces (Sommit, 2004).

2.2 Morphology

2.2.1 Morphology of adult fireflies

As with all insects, the firefly body is divided into three main parts: head, thorax and abdomen (Figure 1).

1. Head

The firefly head is separated into two common parts with a pair of antennae and a pair of compound eyes. Most male fireflies have bigger eyes than females for searching mate pairs (Case, 1984; Demary et al., 2006; Lloyd, 1997b). Moreover, There are two types of antennae that are filiform and serrate (Kazantsev, 2012).

Filiform, the common type of antenna, has many segments that are more or less equal in size. This basic antenna form was mostly found in firefly species. The basic filiform structure was modified in a variety of antenna forms. The second form is serrate which the segments being angled on one side giving the appearance of a saw edge such as *Pyrocoelia praetexta* (Sommit, 2004).

2. Thorax

The firefly thorax is composed of three segments, namely, prothorax, mesothorax and metathorax, each contains one pair of legs. The prothorax of some firefly species is broad and plate-like, concealing most of the head and is usually edged with yellow, red or orange. Two pairs of wings are found on the mesothorax and metathorax segments.

The elytra or fore-wings are the hardened structures with thick and inflexible shaped. The fore-wings are not used for flight, but tend to cover the hind part of the body and protect the second pair of wings (Johansson et al., 2012).

The second pair of wings or hind-wings are thin, elastic, flexible and membranous structures. The hind-wings of lampyrids are the main appendage that assists the insect to fly (Sitorus et al., 2010). The flight structure places on the metathorax which folds and tucks under the elytra at rest (Geisler and Topczewska, 2012). However, some female firefly species have reduced wings (or wingless) which look like larval form such as *Lampyris noctiluca*, *Pyrocoelia rufa* (Hayashi and Suzuki, 2003) including in *Pyrocoelia tonkinensis*.

3. Abdomen

The abdomen consists of a number of segments (between 6 to 8 segments) which the light organs or firefly lanterns have been placed on the last two segments. This special organs have been used for sexual communication between males and females (Copeland and Moiseff, 1997). The male *Pyrocoelia tonkinensis* firefly has a light organ on the fifth and the sixth abdominal segments whereas the female has a light organ on the lateral side of the fifth and the sixth abdominal segments. However, the position of light organs on the ventral abdominal segments are different depending on species, sexes and developmental stages.

For instance, in the most common North American fireflies, adult male has light organs in the entire ventral surface of the sixth and seventh abdominal segments; and adult female has light organ only in the sixth abdominal segment. (Stansbury and Moczek, 2014). In *Pteroptyx valida* fireflies, adult male has light organs in the fifth and the sixth abdominal segments, but female has light organ only in the fifth abdominal segment (Ballantyne and Menayah, 2002). These types of light organs position in the fifth and the sixth abdominal segments have been found in *Luciola aquatilis* and *Pyrocoelia pectoralis* (Thancharoen, 2007; Wang et al., 2007) including in *Pyrocoelia tonkinensis,* the research species. Additionally, the diurnal activity in some adult firefly species are unqualified of bioluminescent in light organs or the light organs are absent such as *Lucidota atra, Pyropyga nigricans* and *Photinus indictus* in North America, *Phosphaenus hemipterus* in Europe and *Lucidina biplagiata* in Japan (Lewis and Cratsley, 2008).

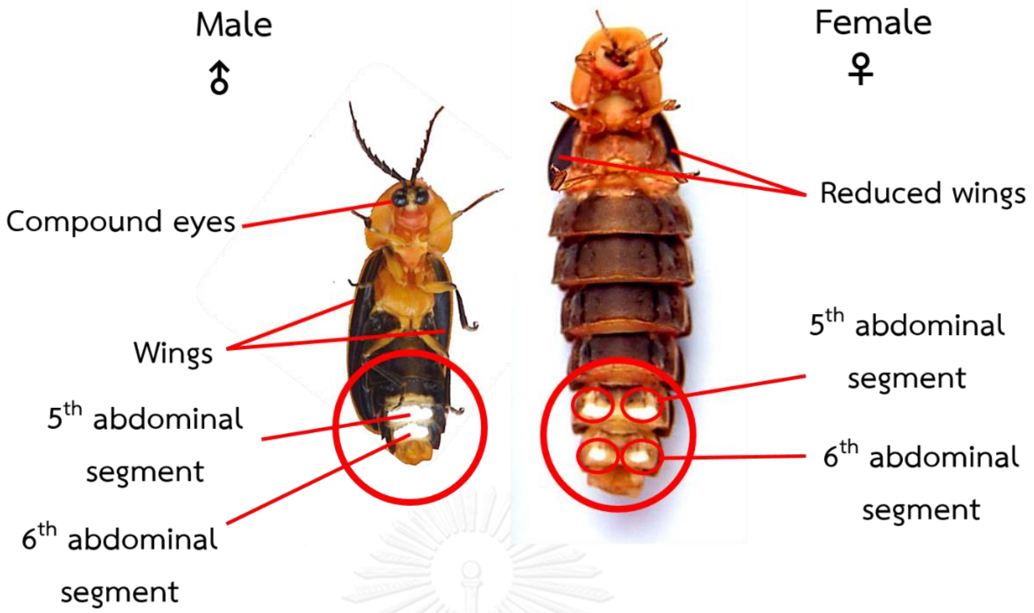


Figure 1 Morphology (ventral) of adult male and female *Pyrocoelia tonkinensis* fireflies.

2.2.2 Morphology of larval fireflies

Firefly larvae are usually elongate and slender with narrowing shape in front and behind. They have three thoracic segments and nine abdominal segments that usually in brown colors. The external dorsal plates or terga are more heavily sclerotized than ventral side of the body. All body segments have a dorsal medial logituinal line except the last segment (Fu et al., 2012) (Figure 2).

The head is concealed within prothorax but invisible under prothoracic tergum when the head is retracted. The firefly larva has the extensible neck membrane envelope around retracted head and antennae beside a small sense cone. The mandibles are well developed with strongly sclerotized, covered by hair along the outer margins. Moreover, there is the canal inside the mandible which opened on the outer margin at the apex (Fu et al., 2012).

The prothorax is usually longer than wide with the anterior side is rounded and containing retracted head within. The firefly larvae have six legs and each leg has a single apical claw at tip. Spiracles are found in the mesothorax or the metathorax in some species of firefly larvae. However, there were variability of spiracle number and function in the lampyridae larvae (Thancharoen et al., 2007).

The abdomens of terrestrial larvae are sclerotized plates with spiracles. The spiracles are replaced with the gills in some aquatic larvae. A pair of light organs are located in the eighth abdominal segment (Ohba and Sim, 1994). The abdomen terminates with the eversible filaments or pygypodia that function to supporting the larval body (Domagala and Ghiradella, 1984).

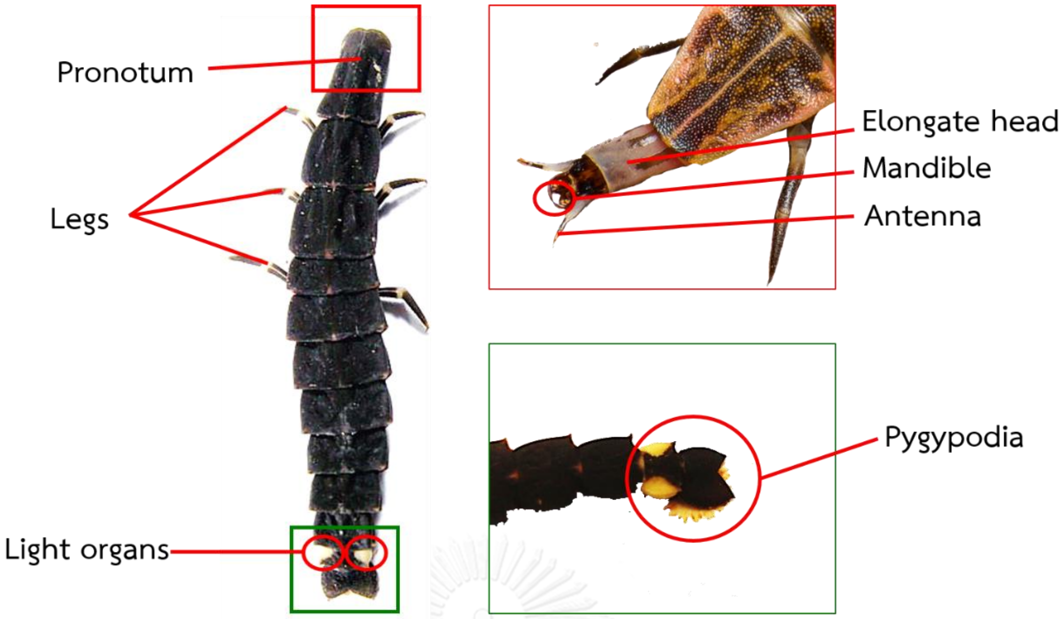


Figure 2 Morphology (ventral) of firefly *Pyrocoelia tonkinensis* larva.

2.3 Life cycle

Life cycle of firefly is a complete metamorphosis with four stages consisting of eggs, larvae, pupae and adults (Figure 3).

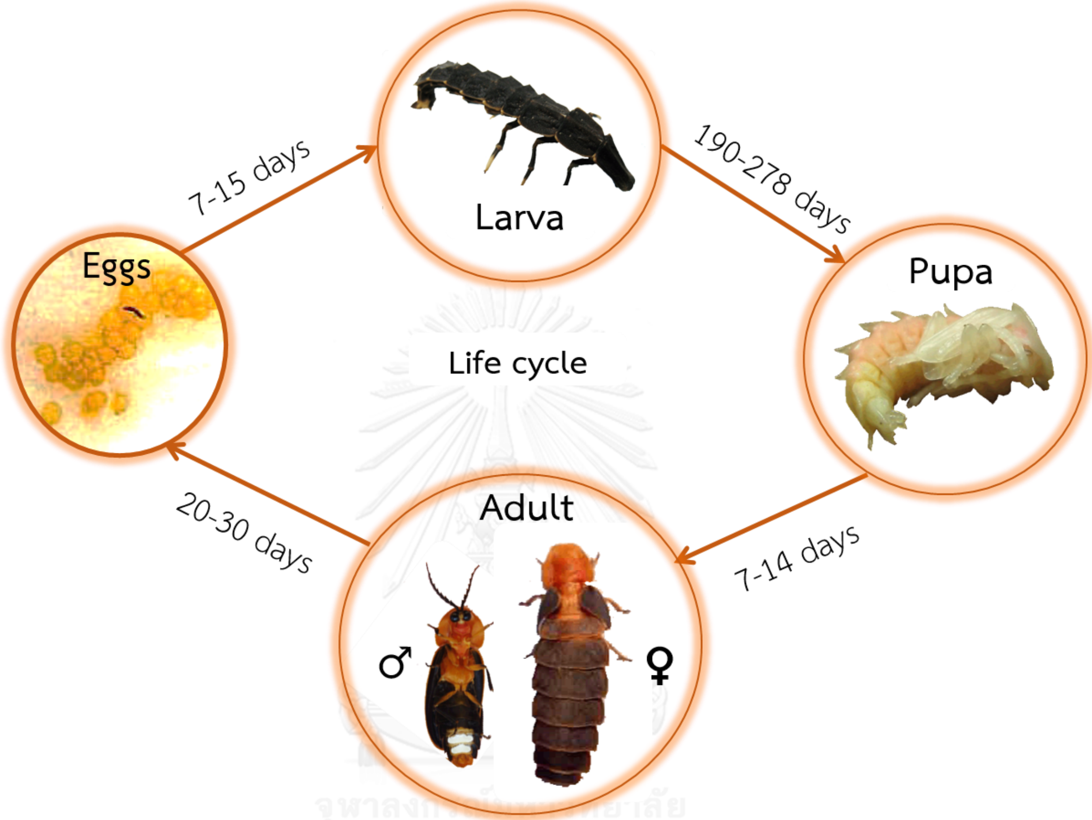


Figure 3 Life cycle of *Pyrocoelia tonkinensis* firefly modified from the closest relative species, *Pyrocoelia praetexta* in Chiang Mai Province, Thailand (Sommit, 2004)

2.3.1 Eggs

Firefly eggs are usually smooth, creamy white or milky yellow with oval or spherical in shape (Ho et al., 2010; Ohba and Sim, 1994; Sommit, 2004). Egg size is normally 0.5-1 mm in diameter and deposited singly or in groups at about 20-150 eggs (Buschman, 1984a; Chunram and Lewvanich, 1996; Fu et al., 2005; Ho et al., 2010). However, the egg size are varies depending on firefly species and relates to the size of larvae (Yuma, 1986), such as the egg size of *Luciola ficta* is smaller than the other *Luciola* spp. (Ho et al., 2010).

Eggs were laid depending on firefly species and their life history (Thancharoen, 2007). Terrestrial fireflies lay eggs in the soil while aquatic fireflies lay eggs on substrate under water. In several species, the eggs have light emitting after they were laid until hatched times such as *Pyractomena lucifera* (Buschman, 1984a) and the color of eggs changed from white to dark-colored before hatching (Ho et al., 2010).The egg incubation periods vary usually from 13 to 27 days (LaBella and Lloyd, 1991). However, the egg incubation periods are dependent on species, habitat and temperature (Ohba and Sim, 1994). In Japan *Luciola ficta* took 19 days to hatch (Ho et al., 2010), but *L. substriata* in China took 11 days (Fu et al., 2005) and *P. praetexta* in Chiang Mai Province, Thailand took four days to hatch (Sommit, 2004).

2.3.2 Larvae

Firefly larvae are generally found in the high humid environments. The aquatic fireflies such as *L. cruciate* in Japan (Suzuki, 1997; Yuma and Hori, 1981), *L. substriata* In China (Fu et al., 2005) were found in freshwater marshes, flooded grassland, rice paddies and ponds. The terrestrial fireflies such as *Photuris* and *Photinus* in North America were found under stone, on the ground and under leaf litter (Buschman, 1984b; McLean et al., 1972).

The numbers of larval stages are depended on species, sexes and timing of development (Ho et al., 2010). Minami (1961) reported that the Japanese aquatic firefly, *Luciola cruciata*, had eight larval stages, but there were six larval stages recorded form other researchers (Fu et al., 2006; Ohba, 1988). Buschman (1988) reported that female, *Lampyris noctulica*, firefly larva in Northcentral Florida had six larval stages and male had five larval stages in summer, whereas female had seven larval stages and male had six larval stages in winter. A terrestrial firefly, *Pyrocoelia analis* in Japan had four to seven stages which five to seven stages in females and four to six stages in males (Ho and Huang, 2003); and *Pyrocoelia praetexta* in Chiang Mai Province, Thailand had five larval stages in both males and females (Sommit, 2004).

Firefly larvae are actually predators and scavengers that feed on variety of small soft body organisms such as snails, slugs, small insects and earth worms (Thancharoen, 2007). The larvae of aquatic and semi-aquatic fireflies fed on freshwater snails, freshwater limpets and other aquatic insects (Chunram and Lewvanich, 1996; Fu et al., 2005). The terrestrial larvae fed on land snails, earthworm and soft body insects inhabited in the ground surface (Buschman, 1984b; Fu et al., 2005; Kaufman, 1965; Wang et al., 2007; Williams, 1917). Moreover, there are varieties of preys in each firefly species. For instance, *Photuris* larvae fed on snails, slugs, insect larvae and non-living food (Buschman, 1984b), but *Photinus, Stenocladius* and *Phosphaenus hemipterus* larvae specially fed on earthworm (LaBella and Lloyd, 1991; Majka and MacIvor, 2009; Suzuki, 1997). In Cuba, *Alecton discoidalis* was reported to be generalist predators of terrestrial snails (Rios and Quinta, 2010). Chinese firefly, *Pyrocoelia pectoralis,* larvae fed on two species of land snail, *Bradybaena similaris* and *B. ravida* (Wang et al., 2007). Moreover, *Pyrocoelia praetexta* larvae in Chiang Mai Province, Thailand generally fed on *Cryptozona siamensis* land snail (Sommit, 2004) which commonly found in Thailand (Sutcharit and Panha, 2008).

Firefly larvae are bioluminescence organisms which have a steady glow of light emission in several seconds at night (De Cock and Matthysen, 2003). They have a pairs of light organs on the eighth abdominal segment of the ventral-lateral surface (Fu and Meyer-Rochow, 2012; Thancharoen, 2007). The function of larval glow had been recorded as a mechanical stimulation when touched or vibrated or flash disturbed from adult fireflies (Lloyd, 1978). Additionally, the larvae had been tested in the hypothesis of the glow or bioluminescence function as the aposematic display or warning display (De Cock, 2009; Underwood et al., 1997). Nocturnal predators learned to associate a glow with the presented of distasteful in the preys or firefly larvae (De Cock and Matthysen, 2003; Sivinski, 1981; Underwood et al., 1997).

2.3.3 Pupae

Firefly pupae are generally milky white with a little yellow or pink color, but the pupae of *Pyractomena* become cryptically pigmented that differ from other lampyrids (Thancharoen, 2007). The pupa stage of aquatic firefly, *Luciola ficta*, began when the mature larvae started climb onto land. They searched for a place such as gap, hole or tunnel to arrange cocoons for pupation (Ho et al., 2010). Terrestrial fireflies such as *Photinus* and *Photuris* pupated underground. For the *Pyrocoelia pectoralis* fireflies, pupation started when the last stage of larvae moved to the holes underground. During pupation, the bodies of firefly larvae enlarged. Moreover, they could also glow when disturbed (Buschman, 1984b; Wang et al., 2007).

The pupal duration varies for several days to weeks depending on the firefly species (Buschman, 1984b; Chunram and Lewvanich, 1996; Fu et al., 2005; Kaufman, 1965; LaBella and Lloyd, 1991). The pupation of *Photuris* larvae were delayed when cool temperatures and short photoperiods occurred in their habitats (Buschman, 1984b). The pupation of *Pyractomena borealis* related to sunlight, tree height and thickness due to seasons (Lloyd, 1997a). Moreover, the male pupae used longer time than female pupae for developing to adult fireflies (Buschman, 1984b; Fu et al., 2005).

2.3.4 Adults

Adult fireflies are usually between 5-25 mm in length (Thancharoen, 2007). They are soft body beetles which have some differences between males and females. Some firefly species, the males always have well developed wings more than females which have short wings or wingless form or larviform (LaBella and Lloyd, 1991). The males have large eyes and two abdominal segments of light organs whereas in females which have only one segment (Case, 1984; Demary et al., 2006; Lloyd, 1966). They have short life span based on species and sexes which less than 30 days (Ohba and Sim, 1994). In aquatic firefly, *Luciola ficta*, male longevity was 18-19 days and female longevity was 10-12 days (Ho et al., 2010). In the terrestrial firefly such as *Pyrocoelia pectoralis*, male longevity was 7-19 days and female longevity was 4-5 days (Wang et al., 2007) and *Pyrocoelia praetexta,* male longevity was 22-24 days and female longevity was 27-30 days (Sommit, 2004). During daytime, adult fireflies rested on the underside of leaves or hid in shallow gaps in the soil to avoid the sunlight and fed only on nectar (Wang et al., 2007).

After emergence, adults have both functional larval and adult light organs for about 4-6 hours and then the larval light organs are disappeared. Also, the soft and light yellow color of the elytra is darkened and hardened (Ho et al., 2010; Wang et al., 2007). The emergence seasons of fireflies are different among species depending on the sexes and the climate (Thancharoen, 2007). In Japan, adult males of *Luciola cruciata* appeared from early June to early July and females emerged for a week after the males (Yuma and Hori, 1981) while the English fireflies, *Lampyris noctulica*, emerged in April or May. In Taiwan, the aquatic larvae emerged from March to July (Ho et al., 2010). In Japan, the terrestrial fireflies, *Pyrocoelia pectoralis,* emerged from late September to late October (Wang et al., 2007). Moreover, the adults *Luciola cruciata* in the different ages and different mating histories showed different behaviors for emerging. The virgin females were found near the ground where they emerged, but the aged females were found in broad-leaves trees (Yuma and Hori, 1990).

2.4 Habitats

Fireflies have been found throughout the world from temperate zone to tropical zone. Their habitats range from mountain to agricultural plantation, forests, scrublands and mangroves in the Southeast Asia (Branham and Greenfield, 1996). However, the firefly habitats are different among firefly species (Buschman, 1988). For instance, the habitats of aquatic fireflies are wetland and ponds (Ho et al., 2010), the semi-aquatic fireflies are mangroves (Jusoh et al., 2010a; Jusoh et al., 2010b) and the terrestrial fireflies are the forest and open field (Wang et al., 2007).

Japanese aquatic firefly, *Luciola ficta*, were found in wetlands, paddy fields and irrigation ditch environments which had a dominant species of water plants and common larval prey in these habitats (Ho et al., 2010). This information conversed in *L. hydrophila* that inhabited small streams or mountain ditches (Ho and Chiang, 1997; Ho and Chiang, 2002; Jeng et al., 2003) and *L. substriata* that lived in coastal marshes and field ponds with several dominant water plants (Fu et al., 2005; Ho et al., 1998).

For the semi-aquatic species, *Pteroptyx* firefly lived in the mangrove forests, the *Sonneratia caseolaris* trees were chosen for the display trees of firefly abundance in Malaysia (Jusoh et al., 2010a). This result showed that an individual tree such as height, crown size, leaf density and trunk diameter were the factors of flashing displays in this firefly species (Nallakumar, 2002).

The terrestrial firefly, *Aspisoma lineatum* are very common in open field and variety of habitats including pastures, marshy areas and garden (Viviani, 2001). The larvae lived on the environment where their snail preys were observed in Sao Paulo, Brazil (Viviani et al., 2012). In Brazil, most firefly species lives in dense forest and follows by the borders of forest, open field, respectively. Moreover, Photurinae species were a common fireflies lived in humid forest (Viviani and Santos, 2012). For the *Pyrocoelia pectoralis* in mainland China, they were found in cotton vegetable fields, small jungles with lichen and grasslands near the rivers, stream or ditches (Wang et al., 2007).

However, fireflies are highly sensitive in the course of the environment which height of pollution and chemicals especially in their larval stage. For instance, the increased river flows during the heavy rain restricted the feeding activities in small firefly *Luciola cruciata* larvae in Japan (Yuma, 2007). In this case, the heavy rain was the negative effect on populations of the small invertebrate preys of firefly larvae (Holomuzki and Biggs, 1999; Nallakumar, 2002; Yuma, 2007).

In addition, the artificial lights are the negative effect on the adult fireflies. The light pollution prolonged courtship signals and dorsal mounting behavior in mating times (Thancharoen, 2007). For instance, the common European glow-worm, *Lampyris noctiluca,* the males have wings, but do not glow. The males searched for the larval form females in the dark areas, but the abilities of males for located females were disturbed and delayed by the artificial night light (Ineichen and Rüttimann, 2012).

2.5 Biology of firefly larvae

2.5.1 Firefly larvae observation

The firefly larvae were found in specific environmental factors such as habitat used, temperature, humidity and other factors. Moreover, the observation and behavior of larvae were different among larval species.

Buschman (1984a) reported that the larvae of aquatic firefly, *Pyractonema lucifera,* in North America were abundant around cattail stand and stayed in the water surface. They were found in gaps between the leaves of the hyacinths and cattail plants. The larvae glowed at night and the glowing was more frequently when they crawled. Moreover, they used the caudal grasping organ to grasp the substrate during locomotion.

Ho et al. (2010) indicated that the larvae of aquatic firefly, *Luciola ficta*, were found in ditch habitats which water 5-9 cm in depth. The surrounding environment was plentiful aquatic plants. Water came from a mountain spring and the benthic substrate was sand mud. The pH of water was 6.8-7.1, conductivity was 53.1-64.4 µS/cm, dissolved oxygen was 22.3 % to 24.6 % and turbidity was 0.08 NTU. Water snails *Thiara granifera*, *Corbicula fluminea* and *C. chinensis* were common larval preys in this habitat. The larvae were negative phototropic and moved only at night. They crawled and searched for food in the water by using luminescence light to attract prey.

For the terrestrial firefly in USA, McLean et al. (1972) reported that *Photuris* larvae were found in the soil during daytime. They appeared on the surface and crawled or climbed up on stem at night. The larvae were abundant at the wet leaf litter particularly after a rain in the forest, ponds and streams. Buschman (1984a) reported that the ground temperature and high microenvironment humidity were essential for their life. Larvae were more actives in the night that high humidity was observed and died in a few hours if they stayed in the dry container at room temperature. The large larvae or late stages of larvae were found in April and May which the warm spring rains started. The medium sized of larvae were found from mid-September to mid-October.

Wang et al. (2007) indicated that *Pyrocoelia pectoralis*, the terrestrial firefly in China, lived in the soil and occurred in November to next April. The suitable temperature for larval activity was about 18-30 °C and the humidity was more than 90%. They were abundant around the deserted farmlands where several land snails occurred. The larvae lived in crevices or under the leaves with the head retracted inside the pronotum. They used the caudal grasping organ to attach the substrate. After sunset, the light emissions of larvae were observed when they began to crawl and they stopped glowing in the morning. Moreover, Rios and Quinta (2010) reported that the *Alecton discoidalis* firefly larvae in Cuba mostly occurred and emitted light from 20:00 PM to 22:00 PM. They lived under the leaf litters and rocks. The suitable temperature and humidity for larval activity was 26-32 °C and 64-86 %, respectively.

2.5.2 Larval predation and preference

In the aquatic fireflies, the larvae of *Pyractomena lucifera* in North America appeared to prey on water snail, fresh water limpet, a small jumping spider, a damselfly nymph and leech. The water snails were the common prey which they most preferred such as *Physa pumilia* and *Pseudosuccinea columella* (Buschman, 1984a) and the other firefly species such as *P. gamma* and *P. limbicollis* larvae also fed on snails (Farnworth, 1973; Lloyd, 1973a). Moreover, the aquatic firefly *Luciola ficta* larvae in Taiwan fed on the water snail, *Cipangopaludina chinensis* (Ho et al., 2010).

For the terrestrial fireflies, the larvae of *Alecton discoidalis* in Cuba preferred the land snail in family Helicinidae and Potamiidae as their food which most abundant in the study areas. Moreover, the spiral and elongate shapes of other land snails were not eaten by the larvae (Rios and Quinta, 2010). In China, the larvae of *Pyrocoelia pectoralis* fed on two species of land snails, *Bradybaena similaris* and *B. ravida.* Likewise, the larvae were found in the same place where they searched and captured snails prey (Wang et al., 2007). In the biological study of *Pyrocoelia praetexta* in Chiang Mai Province, the *Cryptozona siamensis* land snail was used to feed the firefly larvae in the laboratory for studying their life cycle (Sommit, 2004).

In addition, Fu and Meyer-Rochow (2012) presented the tests of firefly larval predation preference on freshwater snails in China. The purpose of this study was to investigate the morphological and behavioral adaptation of the firefly larvae to prey upon freshwater snails that were the intermediate hosts for the liver fluke. The fifth larval stage of *Aquatica leii* and three species of adult freshwater snails such as *Ballamya purificata*, *Lymnaea stagnalis* and *Hippeutis* sp. were used in the observation. The plastic containers were used to place the three species of freshwater snails and the fifth stage larva together. The results showed that *L. stagnalis* was the snail prey that the larvae preferred as their food. However, the larvae avoided *B. purificata* and *Hippeutis* sp. as a prey because of the largest sizes and the operculum used as defense mechanism of these two freshwater snails.

2.5.3 Feeding behavior

In the aquatic firefly, Buschman (1984a) indicated that larvae of *Pyractomena lucifera* in North America, attacked the prey above and below water. The larvae spent time in the water edge and climbed backwards with the caudal grasping organ and pulled the snail up the emergent vegetation. Snails were frequently observed crawling on the surface film and under water on aquatic plants. Larvae attacked snails by climbing onto the shells. They bit and chewed at the soft body of snails and also fed on the same prey in the laboratory.

For the terrestrial firefly, Buschman (1984b) described that the *Photuris* larvae in Northcentral Florida, were very sensitive to the injured caterpillars and other foods. They walked directly to foods that placed in their containers. Many larvae joined on the prey together after an injured caterpillar was attacked by one of larva. The larvae attacked the prey in 4-6 times and captured the prey to continue chewing for several minutes. When the prey was paralyzed, the larvae released it and crawled for several minutes before return to the prey and continue feeding.

Wang et al. (2007) reported that the *Pyrocoelia pectoralis* in China, attacked the land snail by climbing upon them and used their pygypodia or the caudal grasping organ clinging tightly to the snail's shell and bit into the snail's head. The snails rolled their shell to avoid the attack, but these responses are usually ineffective. The larvae used their elongate head to insert the mouthparts inside the shell to continue biting and chewing on the soft body of snail. Moreover, the snail was attacked by several larvae in the laboratory.

Clench and Jacobson (1968) suggested that the firefly larvae waited for the snail to relax its operculum and attacked them. They attacked on the active or recently active snails. Sometimes, the snails released their foam when the larvae attacked on them. This behavior is a defense mechanism in order to avoid the attack by the firefly larvae (Wang et al., 2007).

In the *Lampyris noctiluca* larvae, they followed the snail trails and attacked the snails at the head region to inject a toxin for paralyzing the snails. They followed the snail and continued biting and chewing on the snail's body (Schwalb, 1960). Additionally, Copeland (1981) reported that the larval hemolymph and midgut extracts produced a potent inhibitory as a paralyzing toxin when applied to the heart of snails. The toxin was produced in the larva's intestine and was able to digest protein. The larvae injected brown toxin fluid through their hollow fang-like mandibles into the body of snails. The number of bites depends on their relative sizes. A single bite from a well grown larva might paralyze the snail in a few minutes (Tyler, 1994).

2.6 Flash signal of adult fireflies

2.6.1 Bioluminescent signals

Many fireflies use the bioluminescence of flash light for species recognition and mating location (Carlson and Copeland, 1985; De Cock and Matthysen, 2003; Lewis and Cratsley, 2008; Long et al., 2012). This information created the simple of signaling system which flying males attracted to a slowly glow light emitted of flightless females or larviform females such as *Lampyris noctiluca*, the European glow-worm (Branham and Wenzel, 2003) and *Pyrocoelia praetexta*, the common species in northern Thailand (Sommit, 2004).

However, the characteristics of light flashing are different among firefly species (Aprille et al., 2004). In the groups that adult males and females produced flash light signal such as *Photinus* and *Pyractomena* (Lloyd, 1971; 1979). Males were the primary signalers which emited flashing advertisement during flight times. A female responded to a male by flashing back with courtship dialogue that the flash signals were exchanged between the male and the female. The courtship dialogues were continued until the copulation was occured (Lewis and Cratsley, 2008). In the flash signals of *Luciola* species, they had short bioluminescent flashes which more complex pattern and variation (Lloyd, 1972; Lloyd, 1973a; Lloyd, 1973b; Ohba, 2004). Finally, the flash synchronous system of several *Pteroptyx* species such as *P. malaccae* that males congregated in agreement to emit the courtship flashes attract females (Buck, 1988; Jusoh et al., 2010a).

2.6.2 Flashing display

The flashing displays or flight seasons of fireflies are varied among the firefly species (Barrows et al., 2008). For instance, *Photinus greeni* in USA, males began the advertisement flashes in 15 minutes after sunset. The flight periods occurred for 45 minutes and decreased in 90 minutes after sunset (Demary et al., 2006). In the synchronous firefly *Photinus carolinus* in Michigan*,* the flashing displays began at about 37-43 minutes after sunset (Lloyd, 1966) and appeared in three hours at the peak night (Faust, 2010). Flashing displays of males *Pyractomena lucifera* in North America began at about 32 minutes after sunset and peaked at about five minutes later. Flash display intense for 15 minutes then rapidly decreased at about 30 minutes after the flash began (Buschman, 1984a). Flashing activity of adults *Pyrocoelia pectoralis* firefly in China occurred from late September to late October which emergence of peak found in mid-October (Wang et al., 2007). Moreover, the study of firefly abundances in six genera at the Dyke Marsh Wildlife Preserve, Virginia reported that flashing display of *Ellychnia corrusca* occurred in April, *Lucidota atra* occurred in June to July, *Photinus pyralis* occurred in June to August, *Photinus* spp. occurred in May to August, *Pyractomena lucifera* occurred in June to July and *Pyropyga decipiens* occurred in June to October. The results suggested that flashing displays varied among the firefly taxa even they lived in the same habitat (Barrows et al., 2008).

Additionally, many firefly researchers suggested that the flashing behavior in firefly communication was disturbed by the artificial light which the courtship behavior was expanded and unsuccessful (Thancharoen, 2007). Moreover, Dreisig (1975) tested that the flashing activities in fireflies were based on the environmental control such as the circadian rhythm and ambient illumination in Florida. The result showed that the flashing display occurred in the place which low illumination and the different flashing activity in firefly species depended on the duration of twilight. Moreover, fireflies occurred at the lower light pollution in low temperature than high temperature and started the flashing activity earlier at higher temperature than at low temperature (Buck, 1937). In addition, flashing activity was inhibited if the brightness remains above 10 lux. Finally, flashing activity was shorter if the light was rapidly decreased.

2.6.3 Mechanism of flash production

Light production in fireflies is a type of chemical reaction called bioluminescence. This light occurs in the specialized cell called photocytes that placed in the light-emitting organs called light organs or lanterns. The firefly lanterns are located on the ventral abdominal segments which covered by the translucent cuticle (Timmins et al., 2001).

Firefly produces their bioluminescence in a two steps of reactions (Lewis and Cratsley, 2008). The reaction is controlled by the central nervous system for catalyzing the enzyme luciferase in photocytes (Timmins et al., 2001) and requires ATP and oxygen. In the first step, luciferase enzyme catalyzes the Mg-ATP-dependent adenylation of the substrate luciferin. The reaction of adenylation produces luciferyl-adenylate, an enzyme bound intermediate. The second step is a sequence of oxygen-dependent reactions that releases a photon of light and oxyluciferin returns to the ground state (Marques and Esteves da Silva, 2009).

Moreover, Aprille et al. (2004) suggested that nitric oxide, a soluble gas, played a role in the flash control. In the dark mode, oxygen in the air delivered through the lantern tracheolar system. This oxygen was extracted by respiration in mitochondria that placed in the tracheolar end cell. This process kept the oxygen away from the oxidation reaction in the peroxisomes. The oxidative phosphorylation in mitochondria produced ATP that necessary to activate luciferyl-adenylate intermediate which accumulated in the absence of oxygen. In the flash mode, the reaction began with the octopamine activated nitric-oxide synthase to inhibit oxygen in the mitochondrial reaction. This process made the oxygen deliver to the cell by tracheoles. For this process, the delivered oxygen activated the luciferyl-adenylate intermediate in the peroxisome for the light production.

$$\text{ATP + luciferase + luciferin} \longrightarrow \text{Luciferase-luciferin-AMP + PPi} \quad (1)$$

$$\text{Luciferase-luciferin-AMP} + O_2 \longrightarrow \text{Luciferase + Oxyluciferin} + CO_2 + \text{AMP + light} \quad (2)$$

CHAPTER III

STUDY AREA

The study was conducted at the 300-ha Chulalongkorn University Forest and Research Station located at Lai Nan Subdistrict, Wiang Sa District, Nan Province in the northern of Thailand (UTM zone 47Q: 2051960-2054260N and 0688400-0690360E) (Figure 4 and Figure 5). The station has been established more than ten years. Most of the areas were secondary deciduous dipterocarp due to earlier human disturbances such as farming and burning.

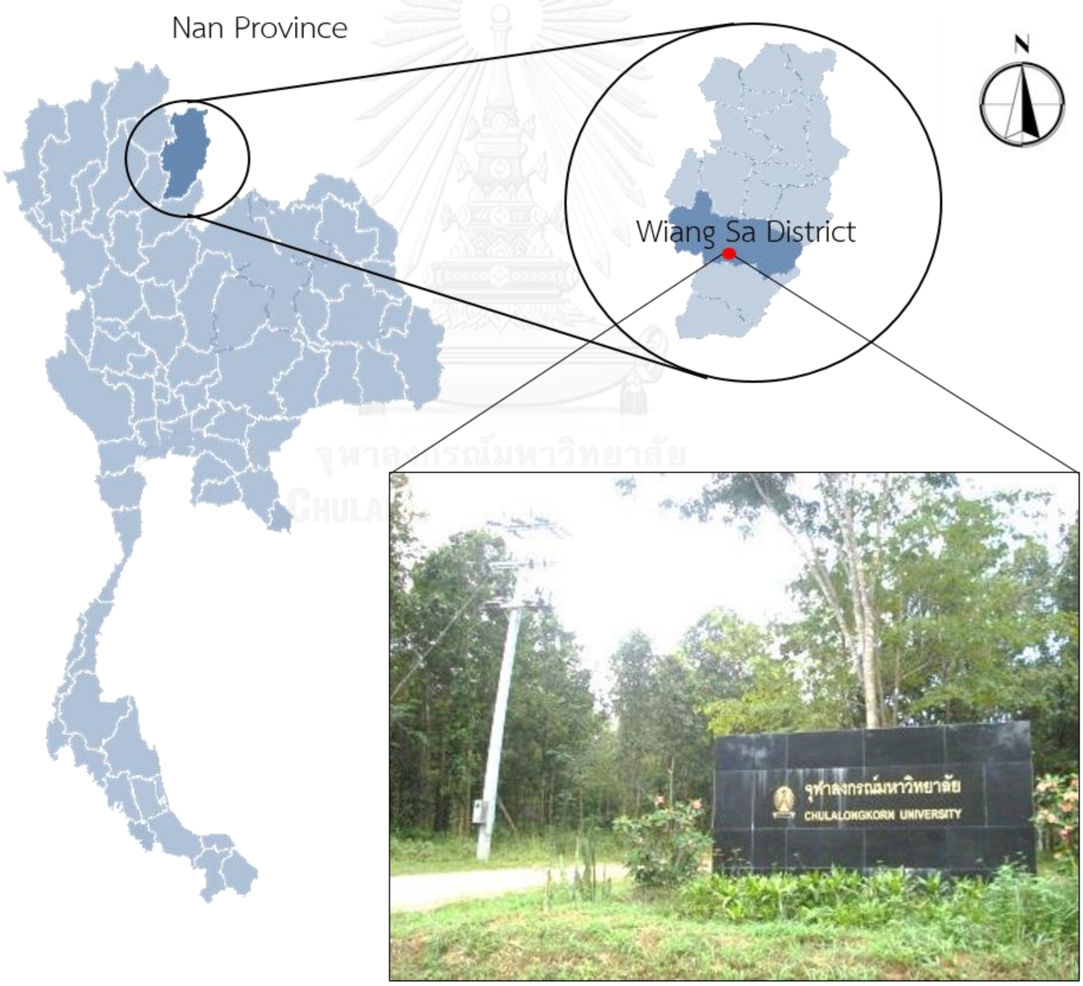


Figure 4 Map of Wiang Sa District at Nan Province in the northern of Thailand.

Figure 5 The study area (circle) at the Chulalongkorn University Forest and Research Station, Wiang Sa District in Nan Province of northern Thailand (https://www.google.co.th/maps/place/Lai+Nan,Wiang+Sa+District,Nan).

The researches were conducted in the different habitats of five areas around the Chulalongkorn University Forest and Research Station (Figure 6 and Figure 7).

1. Area 1 or A1: The area beside the Wiang Sa 1 building (900 m^2) which covered by most of tree canopy, shrub and herbaceous. High Relative humidity and soil moisture are usually observed in this area.
2. Area 2 or A2: The area placed in plants nursery (300 m^2) which covered by grass with a few of tree canopy and shrub. High relative humidity and soil moisture are usually observed in this area but less than A1.
3. Area 3 or A3: The area along the both sides of the road trail 300 m (600 m^2). One side of the trail close the reservoir. This area covered with a few of herbaceous and tree canopy with most sunlight and dry. Soil texture is

loamy sand. Relative humidity and soil moisture were less than the other area.

4. Area 4 or A4: The area in front of the Wiang Sa 1 building (160 m^2). This area covered by grass with a few of tree canopy and shrub. High relative humidity and soil moisture are usually observed in this area.
5. Area 5 or A5: The area placed on the other side of Wiang Sa 1 building (160 m^2). This area covered by grass with a few of tree canopy and shrub. High relative humidity and soil moisture are usually observed in this area but less than A4.

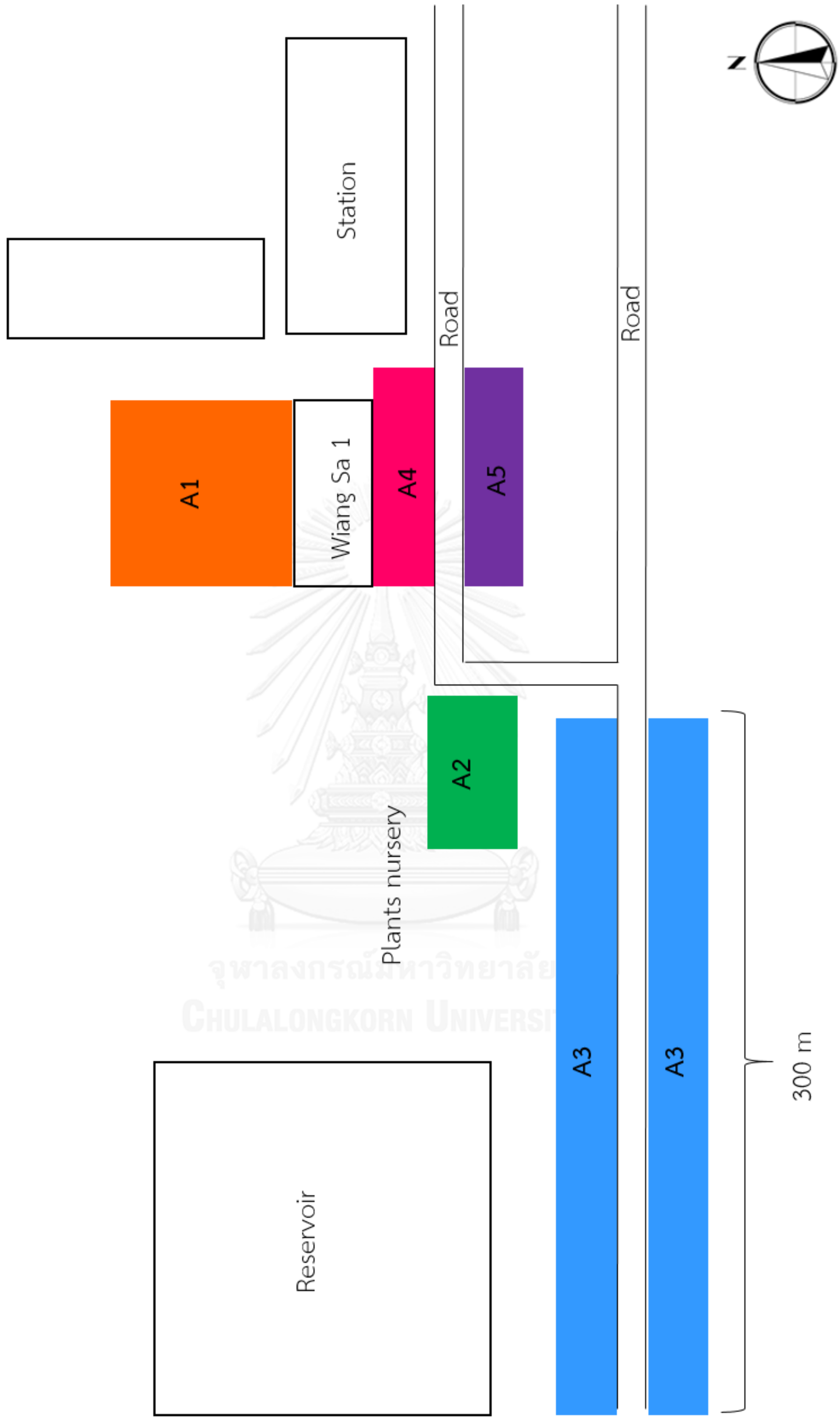


Figure 6 The diagram of five study sites in the Chulalongkorn University Forest and Research Station, Lai Nan Subdistrict, Wiang Sa District, Nan Province.

Figure 7 The study sites; A1, A2, A3, A4 and A5 in the Chulalongkorn University Forest and Research Station, Lai Nan Subdistrict, Wiang Sa District, Nan Province.

CHAPTER IV

LAND SNAIL PREFERENCE AND FEEDING BEHAVIOR OF FIREFLY *Pyrocoelia tonkinensis* LARVAE AT THE CHULALONGKRON UNIVERSITY FOREST AND RESEARCH STATION, LAI NAN SUBDISTRICT, WIANG SA DISTRICT, NAN PROVINCE

4.1 Introduction

Generally, finding and consuming food while simultaneously avoiding becoming food for the other organisms are most essential behaviors of most insects. They usually spend most of time to search and feed for their survivorship and reproductive outputs. In terms of food, many insects appear to prefer only one type of food. Moreover, a large proportion of insects, such as silk moths, bot flies, mayflies and including fireflies do not feed at all throughout their entire adult stage. In this case, the larvae act as a feeding machine for storing huge quantities of reserves for adult stage.

The firefly larval stages are the longest stage which could be more than 300 days in their life cycle (Chunram and Lewvanich, 1996). In this stage, fireflies are truly predators feeding on a variety of small invertebrates such as snails, slugs, earthworm and other arthropod larvae (Buschman, 1984b; Fu et al., 2005; Kaufman, 1965; Wang et al., 2007; Williams, 1917). Moreover, recent study revealed that many firefly larvae species showed specific difference in their diets. For instance, *Photuris* larvae fed on snails, slugs, insect larvae and non-living food (Buschman, 1984b), but *Photinus stenocladius* and *Phosphaenus hemipterus* larvae fed specially on earthworm (LaBella and Lloyd, 1991; Majka and MacIvor, 2009; Suzuki, 1997). The larvae of *Pyrocoelia pectoralis* in China fed on two species of land snail; *Bradybaena similaris* and *B. ravida* (Wang et al., 2007). Furthermore, *Pyrocoelia praetexta* larvae generally fed on *Cryptozona siamensis* land snail (Sommit, 2004) which commonly found in Thailand (Sutcharit and Panha, 2008).

However, only a few studies of food preferences and feeding behavior of firefly larvae have been published. The larval foraging behavior in the numerous firefly species are undiscovered especially firefly *Pyrocoelia tonkinensis* larvae which is the habitual species in the northern of Thailand. Thus, the small invertebrates in the study site at the Chulalongkorn University Forest and Research Station such as earthworms (*Lumbricus terrestris*), slugs (*Semperula siamensis*) and land snails (*Cryptozona siamensis* and *Sarika resplendens*) were used in the preliminary study to select the diets for the *P. tonkinensis* larvae food preference study. The results revealed that most of two land snail species (*Cryptozona siamensis* and *Sarika resplendens*) were killed and eaten by firefly larvae while earthworms and slugs were not selected. Consequently, the two land snail species; *C. siamensis* and *S. resplendens* where selected as diet item in the food preference study. As a result, the objectives of this research were to investigate the food preference of firefly *P. tonkinensis* larvae on two land snail species which mostly abundant species in the study area and also to observe feeding behavior in the laboratory to create a behavioral ethogram for describe the firefly feeding behavior.

4.2 Materials and methods

4.2.1 Preliminary observation of firefly larva diet

One of *P. tonkinensis* firefly larvae and three individauls of *C. siamensis* land snail, *S. resplendens* land snail, earthworms (*Lumbricus terrestris*) and slugs (*Semperula siamensis*) in Area 1 or A1 (30 x 30 m^2) were collected and identified during 19:00 PM to 22:00 PM from July 2012.

The experiments were conducted in the laboratory under the natural photoperiods at 25 °C air temperatures. A 9-cm-diameter of transparent circular petri dish was filled with soil litters and sprayed with water regularly in order to keep the high humidity.

All of sample species (firefly larvae (n = 1) and *C. siamensis* land snail (n = 3), *S. resplendens* land snail (n = 3), earthworms (n = 3) and slugs (n = 3)) were placed together in the experimental dish. The investigations were started from August 8^{th} – August 29^{th}, 2012 (3 weeks).

The results revealed that most of two land snail species, *Cryptozona siamensis* (n = 2) and *Sarika resplendens* (n = 1) were killed and eaten by firefly larvae while earthworms and slugs were not selected. Consequently, the two land snail species; *C. siamensis* and *S. resplendens* where selected as diet item in the food preference study.

4.2.2 Specimen preparation

Pyrocoelia tonkinensis larvae

Eight firefly larvae were identified and collected in Area 1 or A1 (30 x 30 m^2) during 19:00 PM to 22:00 PM from August to September 2013 and named which were PT1, PT2, PT3, PT4, PT5, PT6, PT7 and PT8. All of the collected larvae were separated into two sizes (based on *Pyrocoelia praetexta*, the closest species of *P. tonkinensis* (Sommit, 2004)); medium size which was the third and the forth instar (lengths = 2.40 ± 0.39 cm, weights = 0.03 ± 0.00 g, n = 3) and large size which was the fifth instar (lengths = 3.37 ± 0.33 cm, weights = 0.03 ± 0.00 g, n = 5) (Table 1). The collected firefly larvae were starved for a week under a natural photoperiod in the glass aquarium (40 x 30 x 20 cm^3) filled with 5-cm depth of soil. The aquarium was sprayed with the fresh water regularly before use in the experiments.

Table 1 Morphological characteristics of the firefly larvae.

Sizes	Names	Collected Date	Lengths (cm)	Weights (g)	Larval stage
Medium	PT1	20/8/2013	2.23	0.03	3
	PT4	26/9/2013	2.86	0.03	4
	PT7	29/9/2013	2.13	0.02	3
		(Mean)	**2.40**	**0.03**	
		(SD)	**0.39**	**0.00**	
Large	PT2	21/8/2013	3.56	0.04	5
	PT3	22/8/2013	3.15	0.03	5
	PT5	27/9/2013	3.13	0.03	5
	PT6	28/9/2013	3.10	0.03	5
	PT8	30/9/2013	3.94	0.04	5
		(Mean)	**3.37**	**0.03**	
		(SD)	**0.33**	**0.00**	

Remark: PT = *Pyrocoelia tonkinensis*

Land snails species

Two dominant species of land snails; *Cryptozona siamensis* and *Sarika resplendens* were used in the experiment (Figure 8). The two land snail species were collected from the study site A1 during 19:00 PM to 22:00 PM from August to September 2013. Eight land snail samples of each species were measured and calculated for the averages weighted (*C. siamensis*: shell diameters = 1.43 ± 0.04 cm, weights = 0.84 ± 0.07 g and *S. resplendens*: shell diameters = 1.43 ± 0.06 cm, weights = 0.82 ± 0.06 g) (Table 2). All of the collected land snails were maintained under natural photoperiod in the glass aquarium (40 x 30 x 20 cm^3) with 5-cm depth of soil and sprayed with the fresh water regularly before conducting the experiment.

Table 2 Shell size and weights of the land snails used in this study.

NO.	*Cryptozona siamensis*		*Sarika resplendens*	
	Shell diameters (cm)	Weights (g)	Shell diameters (cm)	Weights (g)
1	1.43	0.90	1.50	0.89
2	1.50	0.94	1.47	0.93
3	1.50	0.92	1.44	0.89
4	1.43	0.90	1.49	0.80
5	1.47	0.94	1.42	0.90
6	1.40	0.85	1.40	0.80
7	1.40	0.73	1.36	0.70
8	1.34	0.72	1.32	0.79
Mean	**1.43**	**0.84**	**1.43**	**0.82**
SD	**0.04**	**0.07**	**0.06**	**0.06**

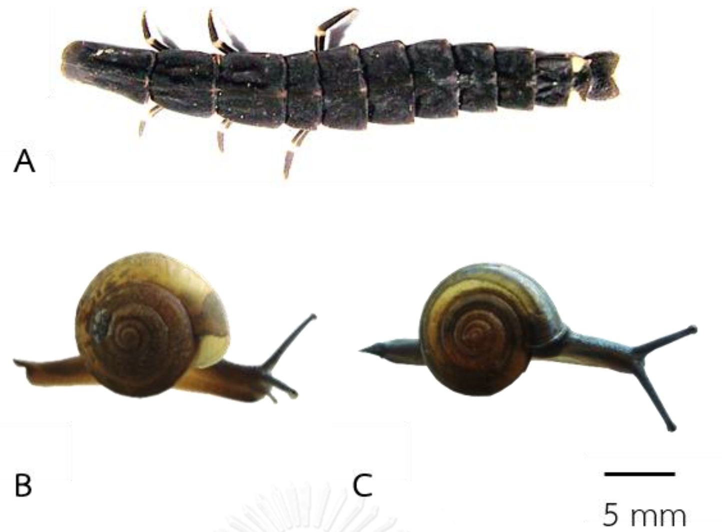


Figure 8 Experiment specimens (A) firefly *Pyrocoelia tonkinensis* larva and two species of land snail, (B) *Cryptozona siamensis* and (C) *Sarika resplendens*.

4.2.3 Food preference experiment

The experiments were conducted in the laboratory under the natural photoperiods at 25 °C air temperature. A 9-cm-diameter transparent circular petri dish was filled with soil and sprayed with water regularly in order to keep the high humidity (≈ 80 %).

The experiments were performed from 19:00 PM to 00:00 PM. Land snail samples (one sample of *C. siamensis* and one sample of *S. resplendens*) were placed randomly at equal distance around the circumference of the dish and one sample of firefly larva was placed in the center of the dish (Figure 9). The number of attacks of firefly larvae (used the antennae to touch the soft body or shell of land snails) on the two land snail species were recorded every minute until the land snails were paralyzed or retracted themselves into their shell.

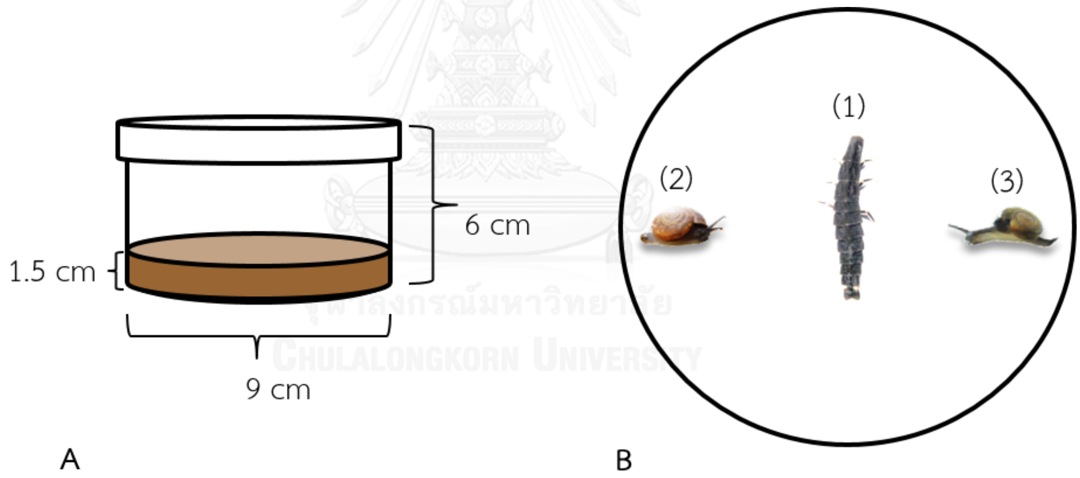


Figure 9 A transparent circular petri dish and experimental design for the study of firefly larvae preference on land snail.

A. Nine-cm-diameter of transparent circular petri dish were filled with soil.

B. Experimental design: a firefly larva was placed in the center of the dish and two species of land snails were placed randomly at equal distance around the circumference of the dish; (1) firefly *Pyrocoelia tonkinensis* larva, (2) *Cryptozona siamensis* and (3) *Sarika resplendens* land snails.

4.2.4 Nutritional test of the two land snail species

Composition sample of land snail (20 individuals in each species) was kept in the -20 °C and the snail's soft bodies without digestive organ were used to test for protein and fat contents by Inhouse method based on AOAC(2012).991.20 and AOAC(2012).954.02 at the Food Research and Testing Laboratory at Chulalongkorn University.

4.2.5 Feeding behavior of firefly larvae on land snails

To develop an ethogram for feeding behavior, ten feeding experiments from eight firefly larvae was recorded during their active period from 19:00 PM to 00:00 PM. The digital video cameras (Sony-HDR) were set up to record all details of feeding activities. Following the focal animal observation method, behavioral acts of firefly larvae that directly attacked on land snails were recorded and sequences of acts based on an all occurrences sampling method were described (Altmann, 1974). In addition, to visual observations, the videos and the visual records were systematically analyzed and noted the details of feeding behavior.

4.2.6 Data analyses

Food preference of firefly larvae on land snails

The mean number of attacks of firefly larvae were analyzed and compared between the two land snail species using *t*-test to indicate the most preference land snail species. The mean number of attacks of two different sizes firefly larvae were analyzed and compared between the two land snail species using Mann-Whitney *U*-test.

Feeding behavior of firefly larvae on land snails

The ethogram and the behavioral sequence of feeding behavior were described by using the observation record, hand writing and video analysis. The behavioral units were calculated and analyzed by Microsoft excel 2013. The mean numbers of times in each behavioral units were analyzed and compared using Kruskal-Wallis test and pairwise multiple comparison. The mean numbers of times in the behavioral units were analyzed and compared between the two different sizes of firefly larvae using Mann-Whitney *U*-test. Moreover, the mean numbers of times were compared between searching phase and handling phase using *t*-test.

4.3 Results

4.3.1 Food preference

Total numbers of the two land snail species killed by firefly larvae indicated that the firefly larvae preferred *C. siamensis* as their food more than *S. resplendens* (Figure 10). The comparison of the mean attacking numbers of firefly larvae on the two species of land snails revealed that there was significant difference between the attacking numbers of firefly larvae on the two land snail species (t-test, $P \leq 0.05$) (Figure 11).

However, the comparison of mean number of attacks by two different sizes of firefly larvae; medium size and large size on *C. siamensis* land snails indicated that there was no significant difference between the larval sizes and the number of attacks on the land snail preys (Mann-Whitney U-test at $P \leq 0.05$) (Figure 12).

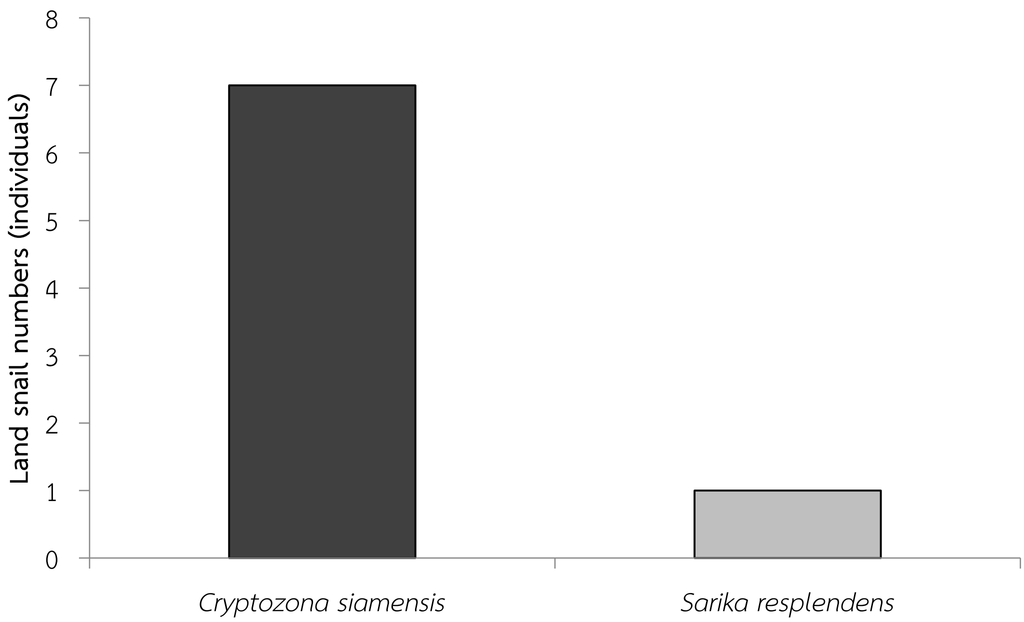
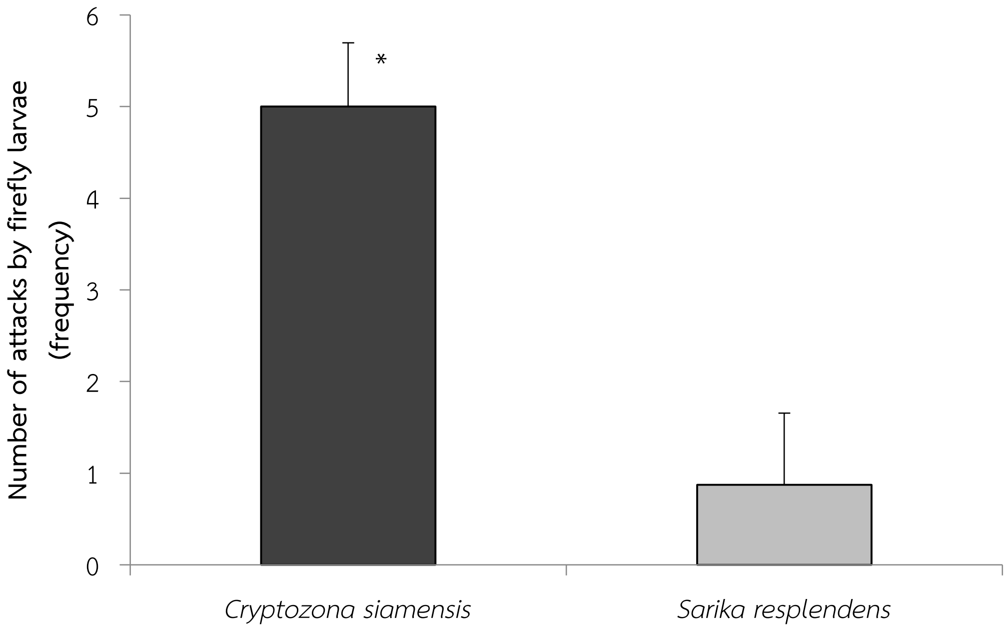


Figure 10 Total numbers of the two land snail species killed by eight firefly larvae during 19:00 PM to 00:00 PM from September to October 2013 in the laboratory at the Chulalongkorn University Forest and Research Station.



(*) indicates a significant difference of attacking numbers between the land snails species.

Figure 11 The mean (± SD) number of attacks of firefly larvae on the two species of land snails: *Cryptozona siamensis* and *Sarika resplendens*.

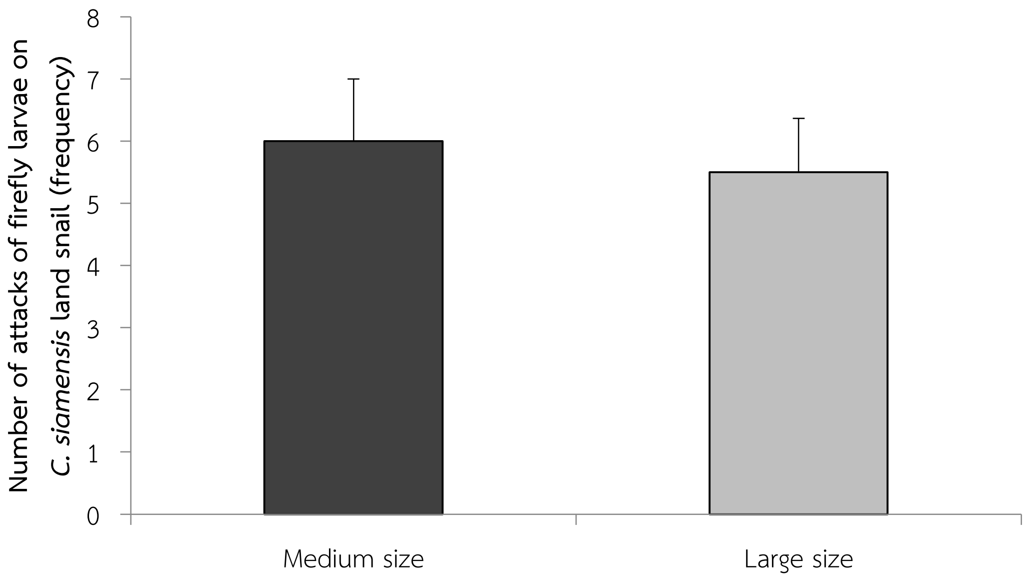


Figure 12 The means (± SD) number of attacks on *Cryptozona siamensis* land snails by two different sizes of firefly larvae; medium size and large size in the laboratory.

Additionally, the testing of nutrition in the two species of land snails from Food Research and Testing Laboratory at Chulalongkorn University suggested that *C. siamensis* and *S. resplendens* land snails had similarity in protein contents and total fat from their soft body. However, the results indicated that they had protein proportion more than fat in their soft body (Table 3).

Table 3 The protein content and total fat analysis of *Cryptozona siamensis* and *Sarika resplendens* land snails by Inhouse method.

Land snail species	Test items	Test Results (g / 100 g)
Cryptozona siamensis	Protein	10.92
	Total Fat	1.78
Sarika resplendens	Protein	13.27
	Total Fat	1.38

4.3.2 Feeding behavior

4.3.2.1 Ethogram of feeding behavior

Feeding behavior of *Pyrocoelia tonkinensis* larvae were recognized by observing in the movement and posture of the head, antenna, legs and other parts of the body of each larva during the experimental encounters. The results showed that the feeding behavior of *P. tonkinensis* firefly larvae was separated into 11 steps (Table 4).

1. Head stretch: stretch the head outside the prothorax and move the head slowly when snail prey closed up.

2. Walking: walk into the prey with alternately moving of legs and pygypodial apparatus.

3. Sprinting: fast forward to the prey directly with more frequency in pygypodial moving.

4. Approaching: approach to the prey by using labrum and antennae touch the substrate or snail's shell.

5. Touching: use the antenna and labrum to touching around the soft body of the prey.

6. Biting: bite the lateral side of snail's soft body from anterior portion.

7. Head shake: move the head, mouthparts and prothorax vertically after biting.

8. Mandible chew: use the mandibles to cut the soft body of land snail into smaller pieces.

9. Head insert: insert the head into snail's shell to continue chewing.

10. Cleaning: move outside the shell and use pygypodia against the head, mouthparts, legs and the antennae.

11. Walking around: walk outside the shell with pygypodial moving pattern and move around the prey alternately with body cleaning during feeding time.

In addition, these behavioral units were separated into four functional categories as described below:

1. Searching: head stretch, antennal move and walk with the movement of the legs and the pygypodial moving pattern, regularly alternating between walking and pygypodial moving.

2. Approaching: sprint the legs with the pygypodial moving pattern and used the mouthparts or labrum to inspect small particles in the ground (e.g. soil, snail's mucus) until reaching the attack distance.

3. Attacking: use the antennae and labrum to touch around the body of the prey and then move to the lateral side of the prey. Seizing and biting at the anterior head of the prey with the mandibles.

4. Feeding: use the mandibles to cut the soft body of land snail. When the snail's soft part was retracted into the shells, larvae inserted the elongate heads to used their mouthparts continued bite and chew inside the snail's shell.

Moreover, the feeding behavior in *P. tonkinensis* larvae was divided into two phases:

1. Searching phase

In the initial state, the elongate head of every larva were retracted inside the head capsule (prothorax). The pygypodia which is the terminal appendages at the abdomen of firefly larvae was not motivated. In the second state, the larvae's head were stretched outside the prothorax. The head was turned with the slightest motion of the antennae. Larvae used the antennae and labrum to inspect small particles in the ground (e.g. soil, snail's mucus). The antennae and labrum were used to approach by touching around the prey.

2. Handling phase

Attacking

In the critical attack distance, larvae closed to the lateral side of land snails. The prey was bitten at the anterior head with the mandibles where the paralyzing toxin was released. Larvae moved the head, mouthparts and prothorax alternately between up and down for 3-4 times after biting with mandibles (n=8/10).

Feeding

Mandibles were used to cut the soft body of land snail into smaller pieces and digestive juices (secreted from mandible tubes) digested the soft body to be more easily consumed. Moreover, elongated head of firefly larva was inserted inside the snail’s shell to bite and chew the soft body when the land snail’s head retracted into the shell.

During feeding, larvae moved outside the shell. They turned the head and walked around the prey for a moments (11.90 ± 8.90 minutes). Pygypodia were rubbed against the head, mouthparts, legs and the antennae to clean themselves before turning to the paralyzed prey and continuous chewing alternatively until the end of feeding time (land snail’s soft body were absent). Moreover, glowing behavior discontinuously occurred in some firefly larvae (n = 4/10) during feeding. In the end of feeding, the elongate head of firefly larvae moved inside the head capsule (prothorax). They changed their resting position and turned the body or moved a few steps for some time (Figure 13).

Figure 13 Feeding behavior of firefly *Pyrocoelia tonkinensis* larvae.

(A) Firefly *Pyrocoelia tonkinensis* larva fed on *Cryptozona siamensis* land snail in the laboratory.

(B) Firefly larva used their pygypodia against the head, mouthparts, legs and the antennae.

(C) The pygypodia of firefly larva.

(D) The snail released their foam as a defense mechanism in order to avoid the attack by the firefly larvae.

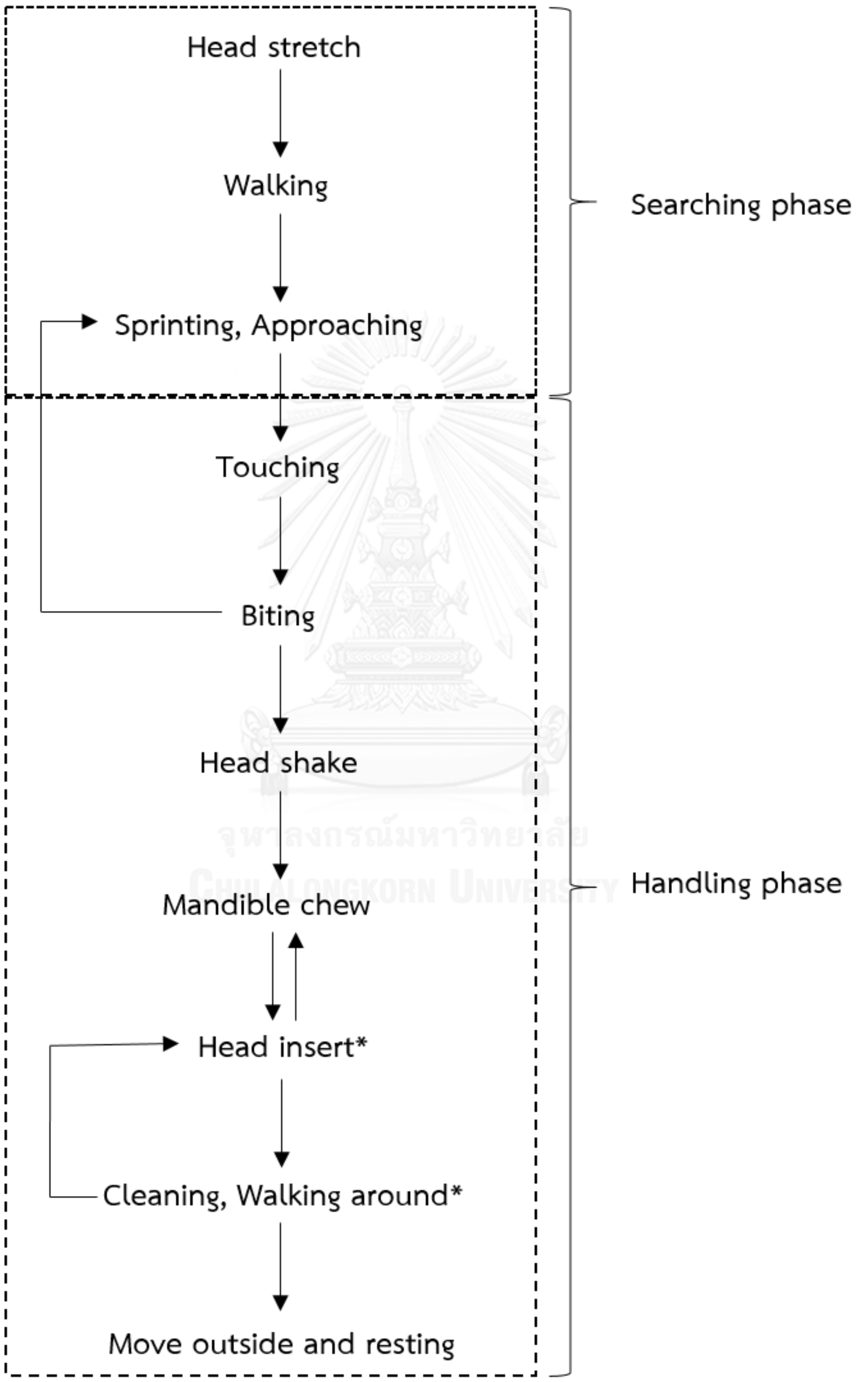
Table 4 Feeding behavioral ethogram, a catalogue or inventory of behaviors exhibited, in firefly *Pyrocoelia tonkinensis* larvae (n=10).

Feeding phase	Functional category	Behavioral step	Description
Searching phase	Searching	Head stretch	Stretch the head outside the prothorax and move the head slowly when snail prey closed up.
		Walking	Walk around the prey with alternate moving of legs and pygypodial apparatus.
	Approaching	Sprinting	Fast forward to the prey directly with more frequency pygypodial moving.
		Approaching	Approach to the prey by using labrum and antennae touch the substrate or snail's shell.
Handling phase	Attacking	Touching	Using the antennae and labrum to touching around the soft body of the prey.
		Biting	Bite lateral side of snail's soft body from anterior portion.
		Head shake	Move the head, mouthparts and prothorax vertically after biting.
	Feeding	Mandible chew	Using the mandibles to cut the soft body of land snail into small pieces.
		Head insert*	Insert the head into snail's shell to continue chewing.
		Cleaning*	Move outside the shell and use pygypodia rubbing against the head, mouthparts, legs and the antennae.
		Walking around*	Walk outside the shell with the pygypodial moving pattern and move around the prey alternately with body cleaning during feeding time.

Remark: *Glowing behavior which occurred intermittently in the handling phase (Head insert, cleaning and walking around)

4.3.2.2 Behavioral sequence

The feeding behavioral experiments of firefly larvae on land snail presented the sequence of feeding behavior from searching until the end of feeding (Figure 14).



(*) = Glowing behavior which occurred intermittently in the handling phase (Head insert, cleaning and walking around).

Figure 14 Behavioral sequences of feeding behavior in *Pyrocoelia tonkinensis* larvae.

4.3.2.3 Time budget

All activities were grouped into seven major behavioral units: standing, searching, attacking, glowing, feeding, cleaning and walking around. The term of standing was preferred to the resting stage and after feeding, including the initial stage. The functional system of searching was mainly recruited from the duration of searching phase (head stretch, walking, sprinting and approaching). Touching, biting and head shake were grouped into the attacking system. Moreover, the glowing behavior occurred discontinuously after attacking was recorded in the time budget. Mandible chew and head insert were grouped into the feeding system. Also, cleaning and walking around that occurred in every larva were recorded in the time budget (Figure 15). In addition, the follow diagram was given the video translation (Figure 16-20) and the total time that was spent by the larvae in the different functional categories (Figure 21).

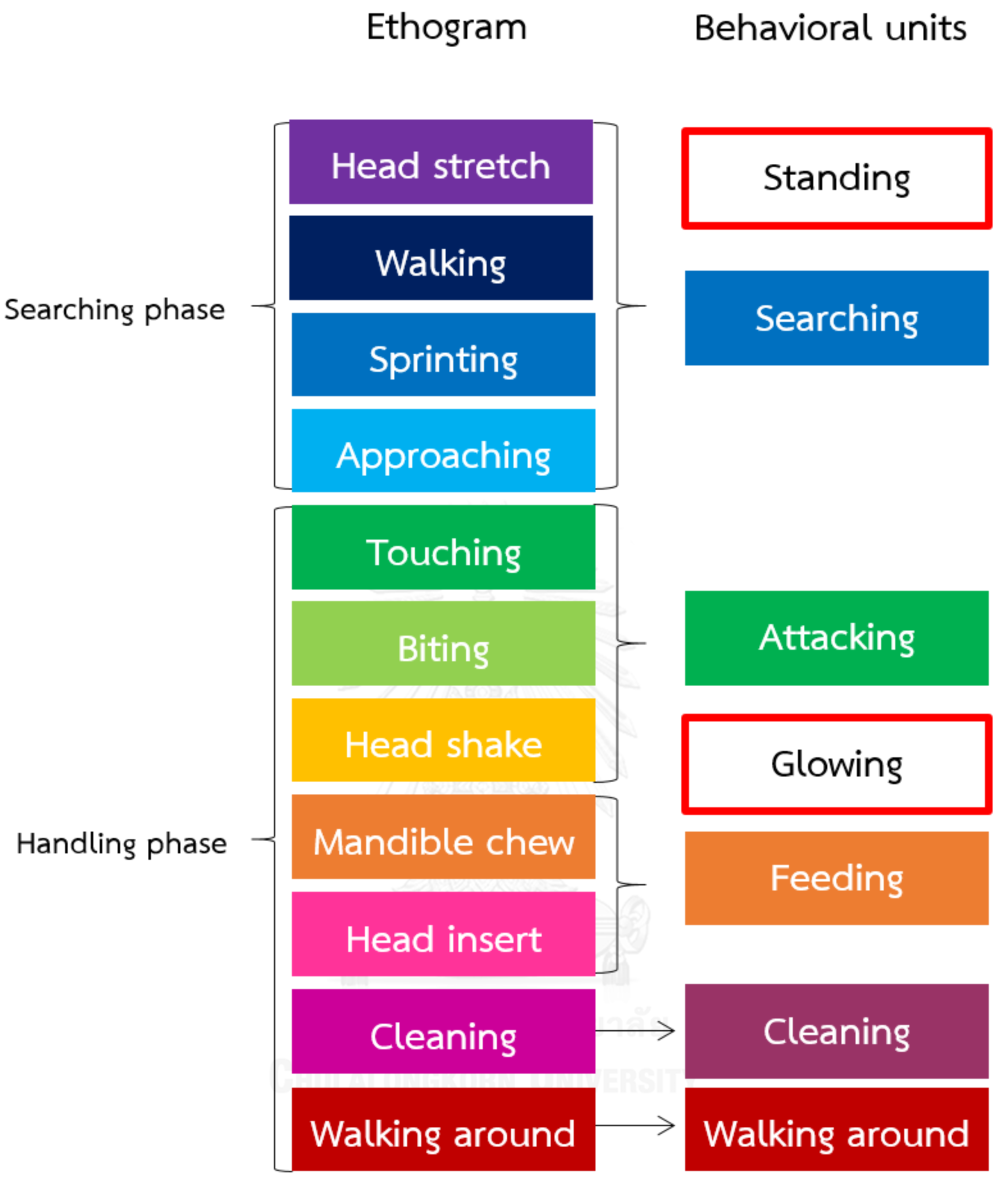


Figure 15 The seven behavioral units in the time budget which grouped from 11 behaviors in the ethogram of firefly *Pyrocoelia tonkinensis* larvae.

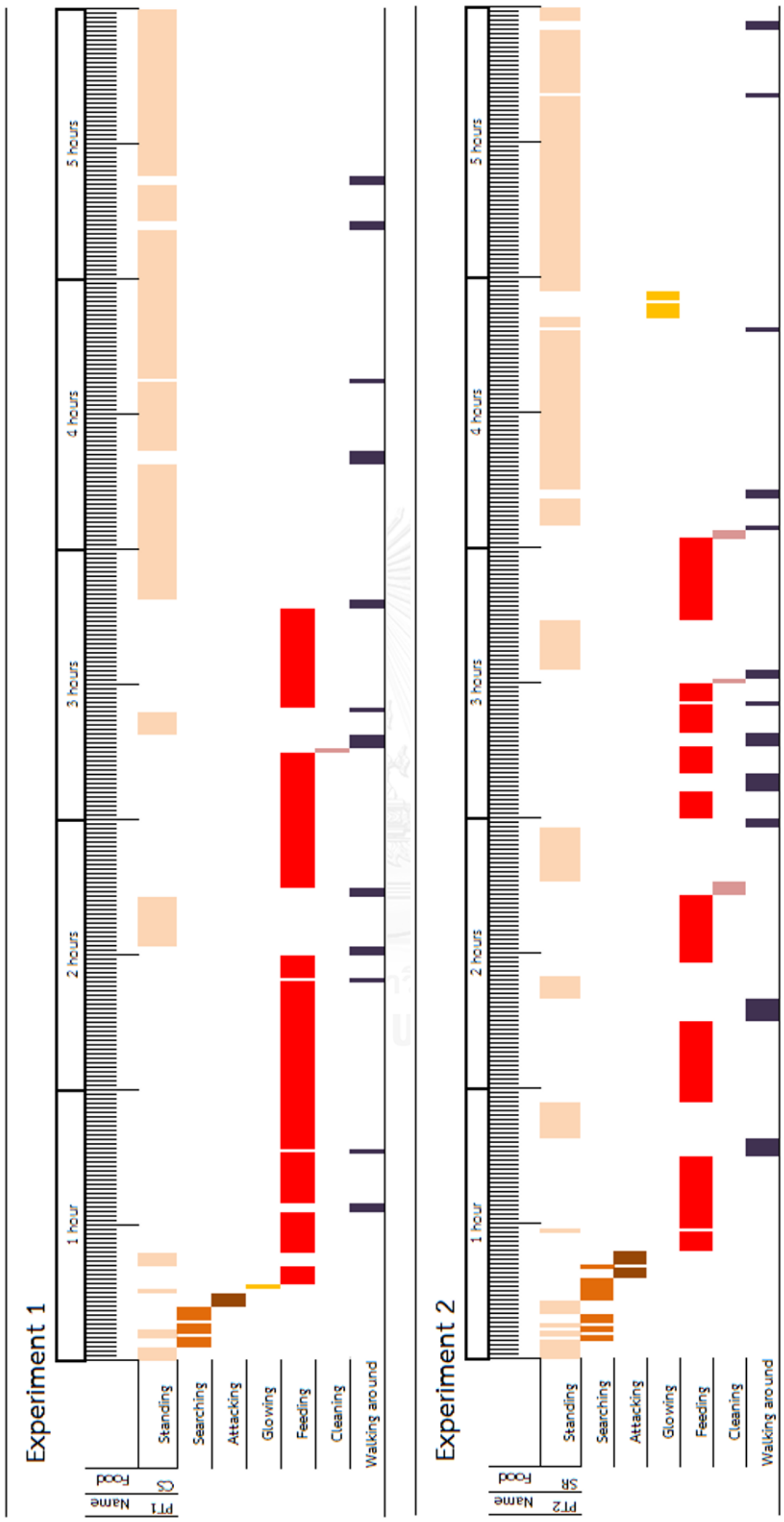


Figure 16 Video translation of firefly *Pyrocoelia tonkinensis* larva feeding behavior (experiment 1 and 2).

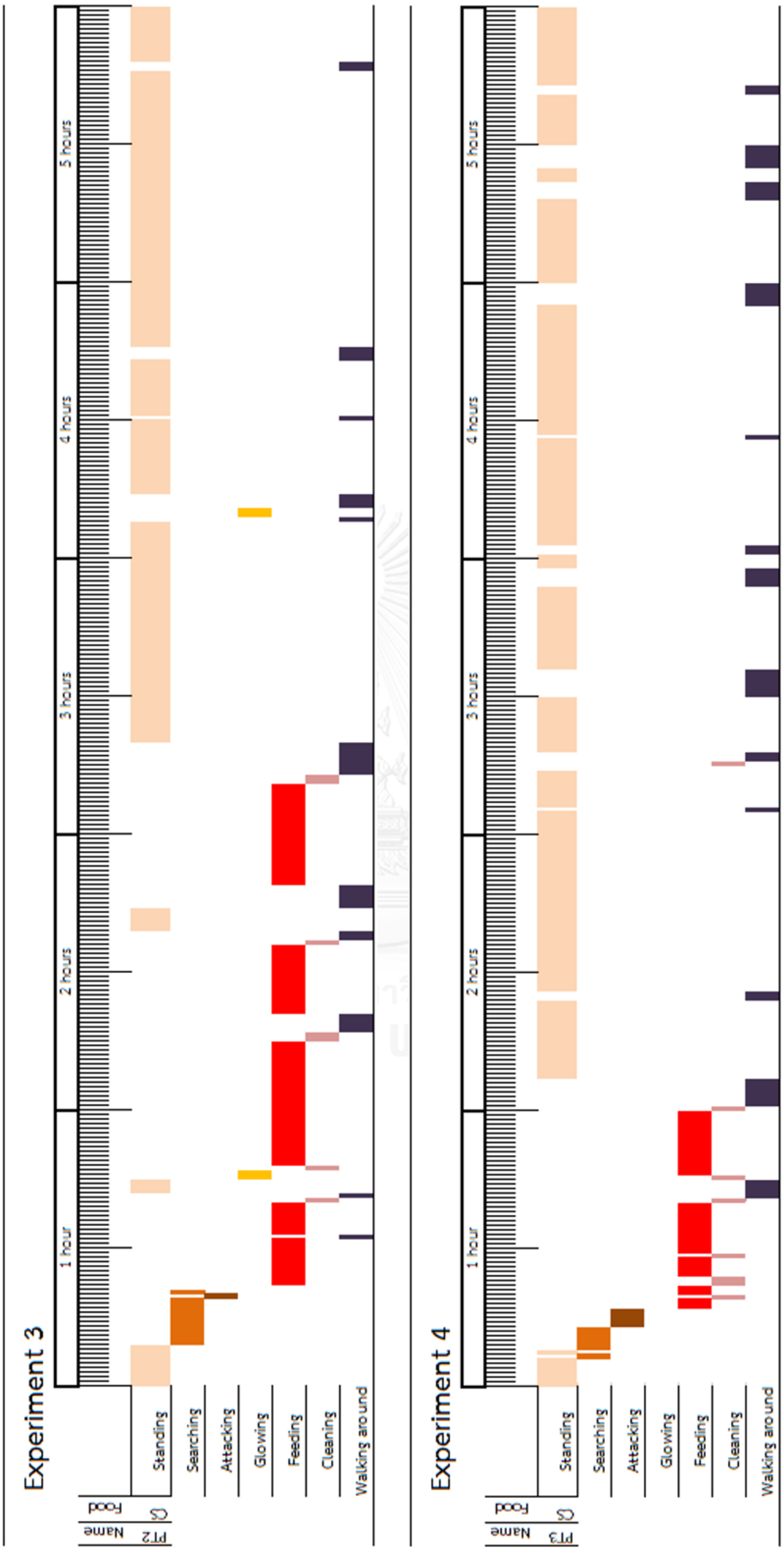


Figure 17 Video translation of firefly *Pyrocoelia tonkinensis* larva feeding behavior (experiment 3 and 4).

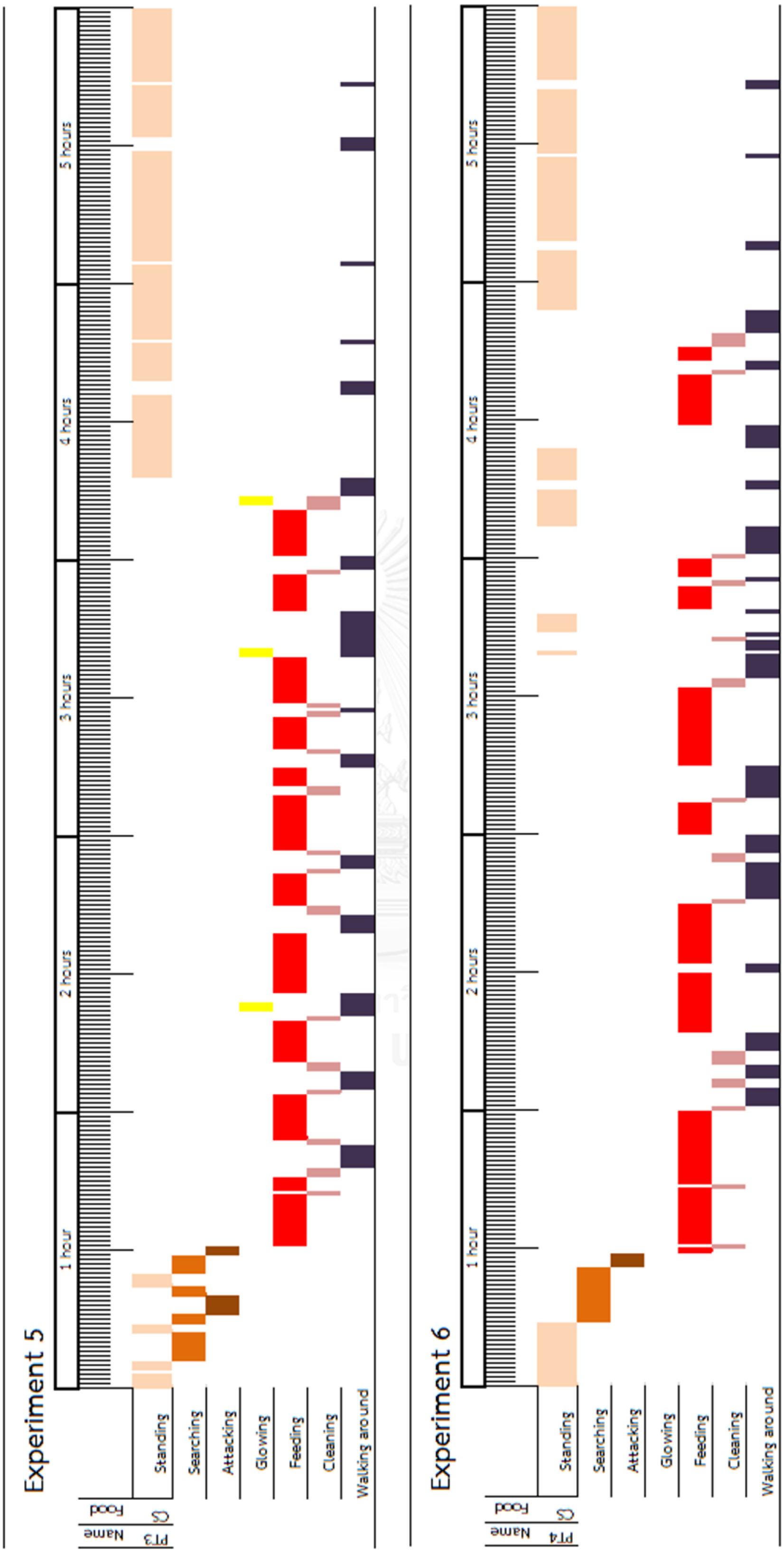


Figure 18 Video translation of firefly *Pyrocoelia tonkinensis* larva feeding behavior (experiment 5 and 6).

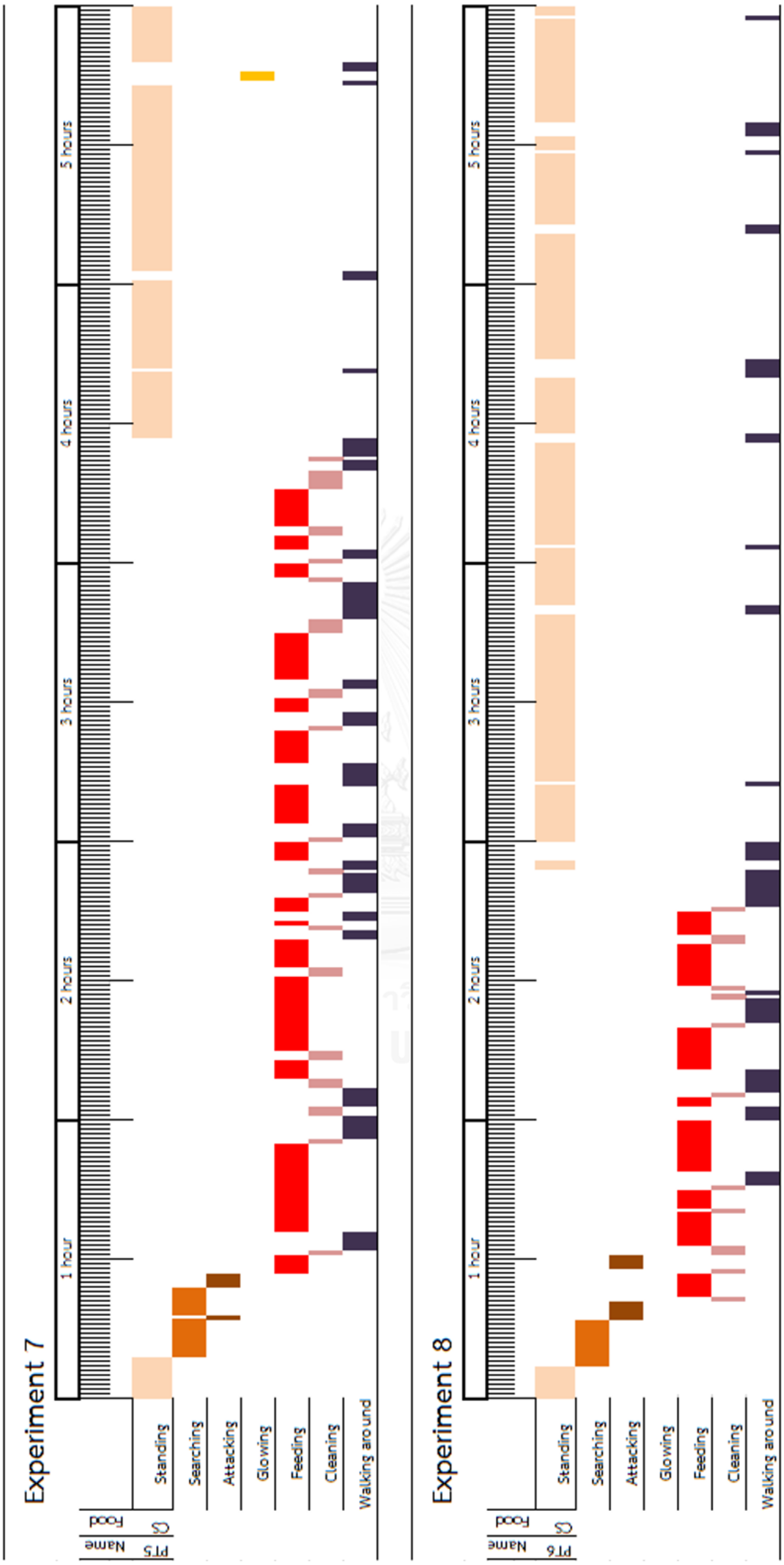


Figure 19 Video translation of firefly *Pyrocoelia tonkinensis* larva feeding behavior (experiment 7 and 8).

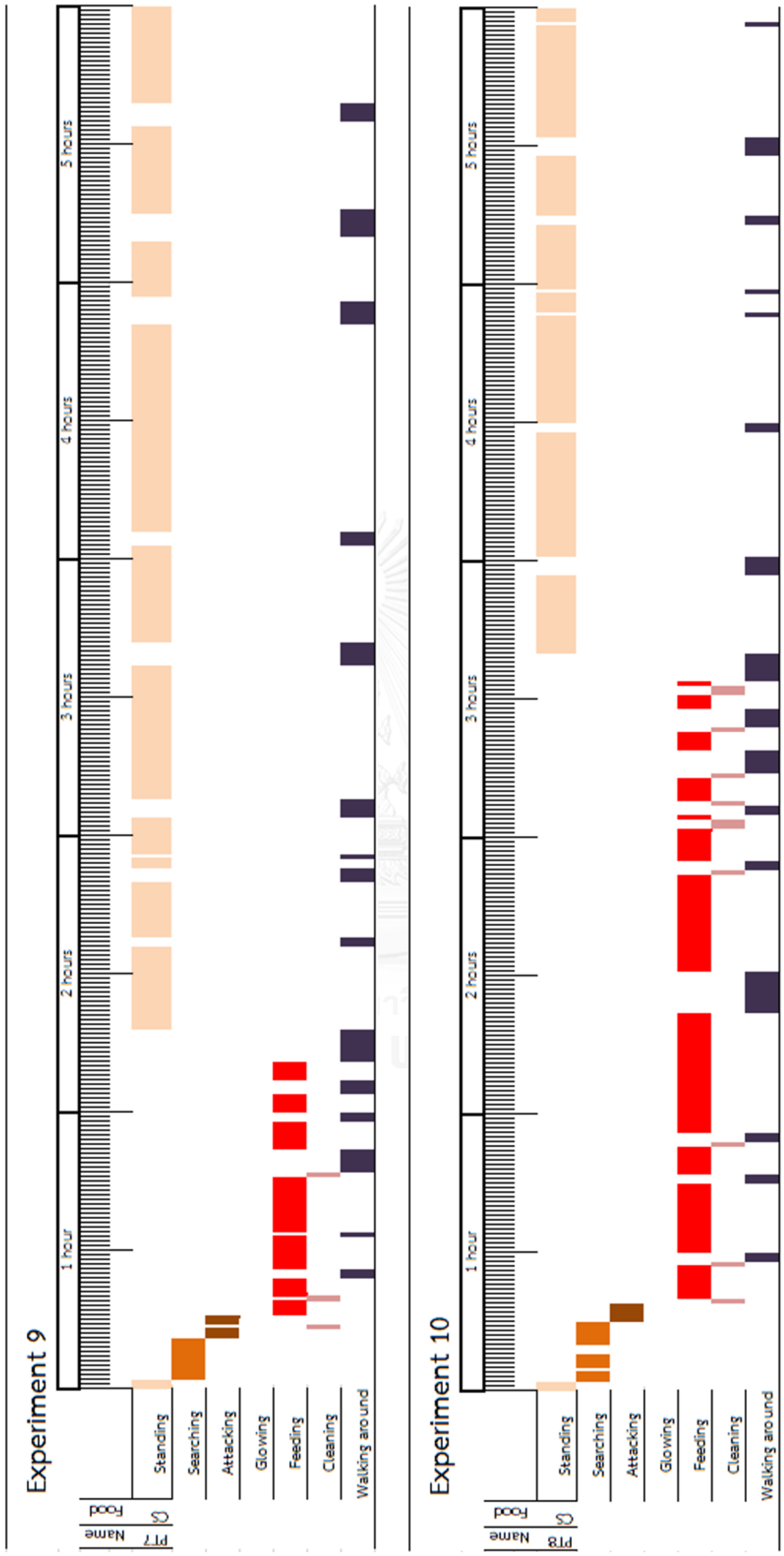


Figure 20 Video translation of firefly *Pyrocoelia tonkinensis* larva feeding behavior (experiment 9 and 10).

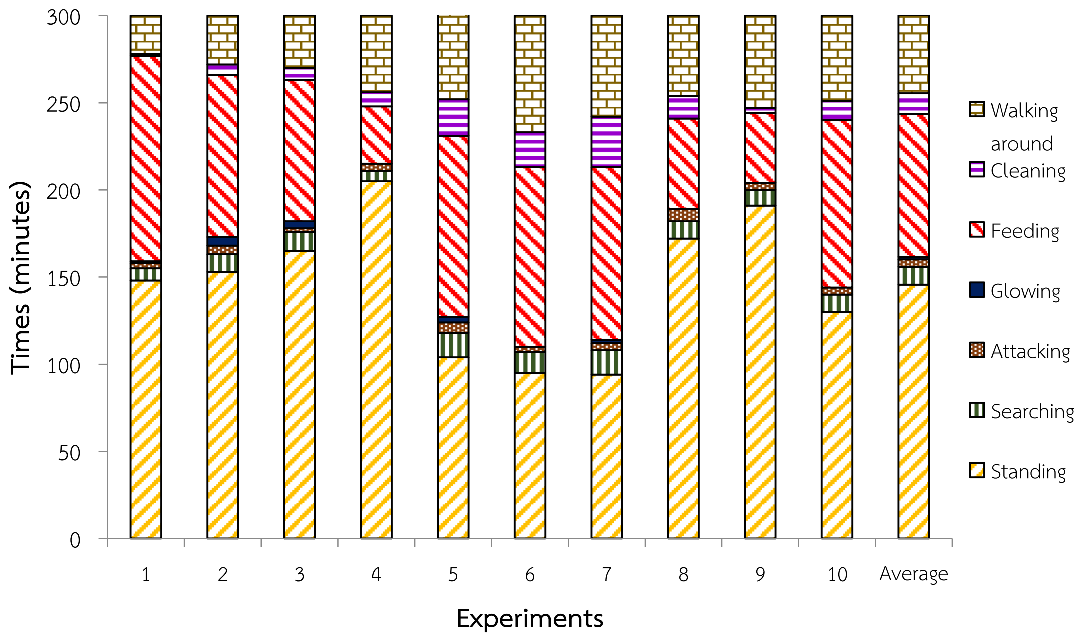
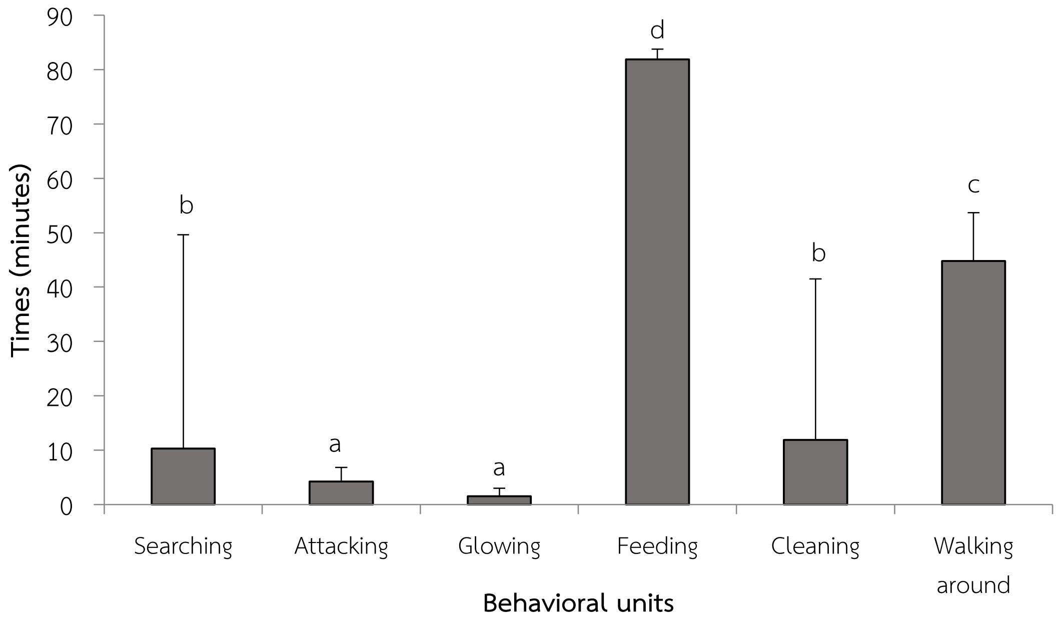


Figure 21 Duration of times spent in different functional activities of firefly *Pyrocoelia tonkinensis* larvae feeding on *Cryptozona siamensis* land snail (only experiment 2 which firefly larva fed *Sarika resplendens* land snail). The results were given for individual behavioral units for each experiment.

4.3.2.4 Behavioral units

For the mean differences, all of the behavioral units were used to calculate accepted standing which was preferred to the resting stage or after feeding (including the initial stage). However, the means (± SD) of six behavioral units were significant difference. The mean comparison of the six behavioral units revealed that the firefly larvae spent most of times to feed during five hours of the experiments followed by walking around. Moreover, firefly larvae spent their times to searching and cleaning in the equal proportion followed by attacking and glowing, respectively (Kruskal-Wallis test; *F-value* = 62.423, df = 5, *p-value* < 0.0001, Pairwise multiple comparison at $P \leq 0.05$) (Figure 22).



*Different letters (a, b, c and d) indicates a significant difference of times among the six behavioral units.

Figure 22 Average time numbers (means ± SD) of the six behavioral units in feeding behavior during five hours in ten experiments.

Additionally, the comparison of the means (± SD) in the two different sizes firefly larvae; medium size (n = 3) and large size (n = 5) indicated that there were no significant difference in the times that the two sizes firefly larvae spent in the six behavioral units of the feeding behavior (Mann-Whitney *U*-test at $P \leq 0.05$) (Figure 23). However, the mean comparison of times indicated that there was significant difference between searching phase (searching) and handling phase (attacking, glowing, feeding, cleaning and walking around) of the firefly larval feeding behavior (*t*-test, $P \leq 0.05$) (Figure 24).

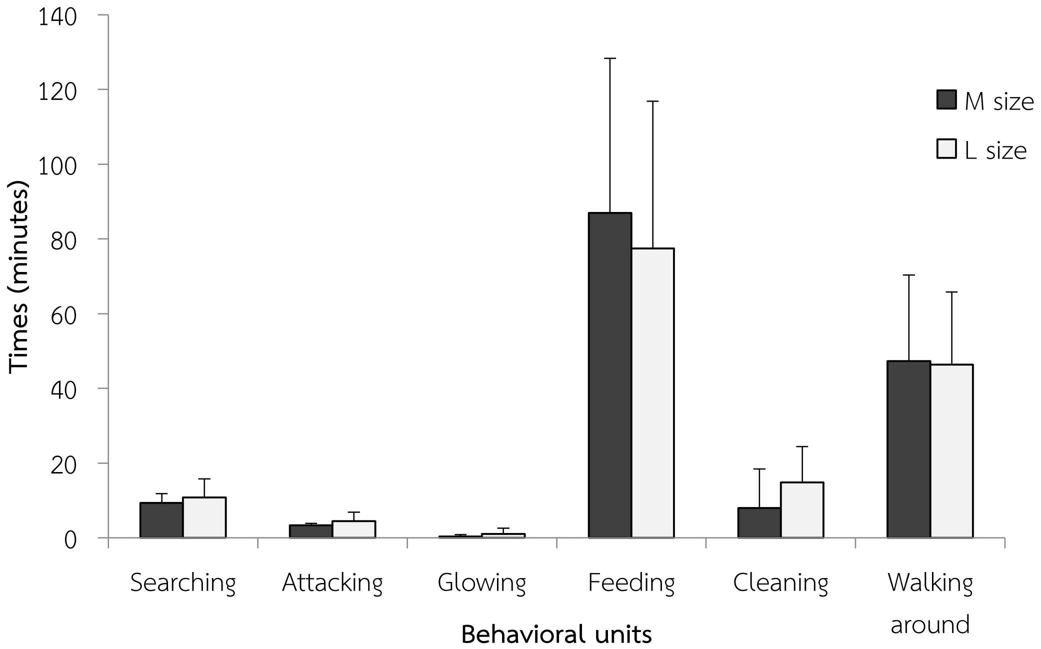
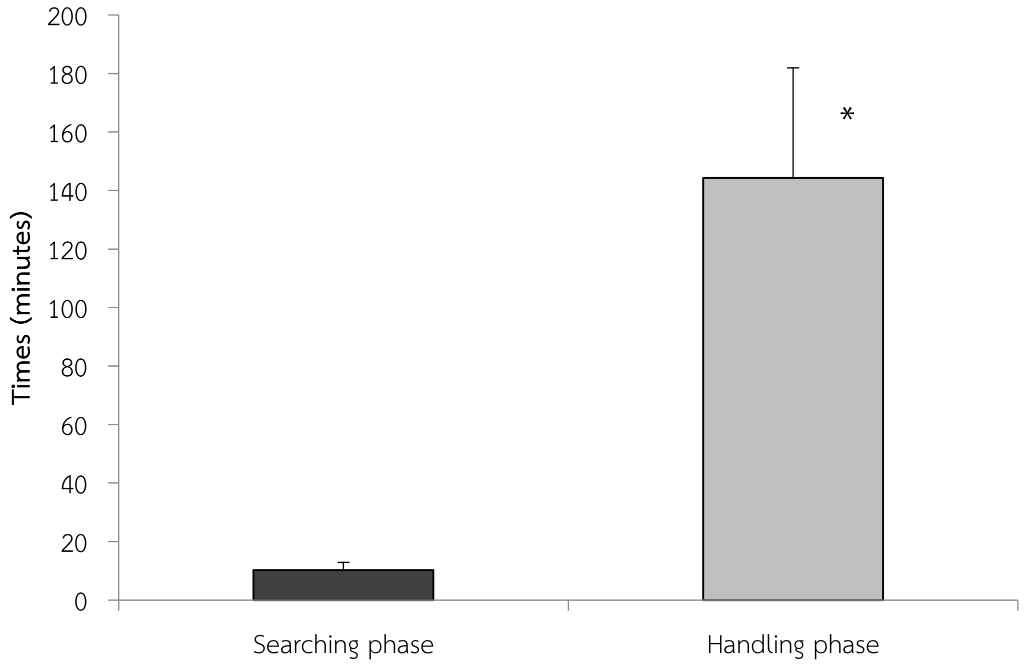


Figure 23 Average time numbers (means ± SD) of medium and large size firefly larvae in the six behavioral units during five hours of ten experiments recorded in the laboratory.



(*) indicates a significant difference of times between searching phase and handling phase.

Figure 24 Average time numbers (means ± SD) between searching phase (searching) and handling phase (attacking, glowing, feeding, cleaning and walking around) of the firefly larval feeding behavior.

4.4 Discussion

4.4.1 Food preference

The results of firefly larvae predation preference on the land snail revealed that firefly *Pyrocoelia tonkinensis* larvae significantly preferred *C. siamensis* land snails as their prey. Under the experimental conditions, there was no significant difference between the larval size and the number of attacks on their prey. The medium size and large size of firefly larvae greatly consumed *C. siamensis* land snails more than *S. resplendens* in the eight individual investigations. However, there were important reasons which greatly maintenance the results of food preference indication such as the defense mechanism and the population density of land snails.

In terms of defense mechanism of land snail, the individual of *S. resplendens* land snail seemed to be more readily than *C. siamensis* to crawl away or remove themselves from the encounter during the experiments. Moreover, *S. resplendens* land snail species have a specialize organ called "mantle appendage". This organ extends from the snail's soft body to cover a few parts of the snail's shell and rubs the shell with their mucus (Sutcharit and Panha, 2008). Although this appendage was used for clean the snail's shell, it might be troubled the attack of predators or probably use for detect and avoid their predators as well as firefly larvae.

Moreover, the population density of land snails indicated that *C. siamensis* mostly found in the study sites more than *S. resplendens* land snail (*C. siamensis*; 17.04 ± 1.25 and *S. resplendens*; 1.08 ± 0.08) (appendix A, Figure A-1) because of *C. siamensis* land snail can spread and produced the offspring more than *S. resplendens* land snail in the same area (Srihata et al., 2010). There were more chance which *C. siamensis* land snail were met and fed by firefly larvae. Thus, it was the possible reason why *C. siamensis* land snail was the preference food of *P. tonkinensis* firefly larvae.

However, the result was similar to the study of *Pyrocoelia praetexta* in Chiang Mai Province which used the *C. siamensis* land snails to feed the firefly larvae in the laboratory to study the life cycle of *P. praetexta* firefly (Sommit, 2004). Moreover, previous studies suggested that the firefly larvae fed on the land snail which was the most abundant species in the study area and the firefly larvae were found in the same place where their snail prey lived. (Buschman, 1984b; Fu and Meyer-Rochow, 2012; McLean et al., 1972; Rios and Quinta, 2010; Wang et al., 2007). For instance, *Alecton discoidalis,* the firefly larvae in Cuba preferred two land snails species in family Helicinidae and Poteamiidae inhabited the same area (Rios and Quinta, 2010). Moreover, *P. pectoralis* larvae there were high specificity to the land snail species that found in the same habitats (Wang et al., 2007).

Additionally, the nutritional compositions of two land snail species indicated that *C. siamensis* and *S. resplendens* were similar in protein and fat contents (high proteins and low fats). So, the consequence suggested that the firefly larvae preferred proteins in the feeding experiments. The results might be because the protein nutrients extended longevity and reproduction in a broad range of animal species (Fontana et al., 2010; Mair and Dillin, 2008; Nakagawa et al., 2012). For example, *Drosophila melanogaster* males, a protein-rich in larval diets increased capacity to resist heat and desiccation in their development (Anderson et al., 2005). Moreover, the brown toxin fluids that firefly larvae injected to the snail's soft body were able to digest protein more than the other nutrition (Tyler, 1994). The toxin produced an effective inhibition as a paralyzing toxin when applied to the heart of snails (Copeland, 1981). Therefore, the protein nutrients might be the essential composition to produce the toxin fluids (paralyzing toxin) for feeding activity and also extend longevity of firefly larvae.

4.4.2 Feeding behavior

Searching phase

In the initial stage, the elongate head of every sampling firefly larva was retracted inside the head capsule or pronotum for a few minutes. This behavior might be the first reflection of the defense mechanism because the firefly larvae always retracted their head when they were disturbed. Moreover, the head retracted behavior of firefly larvae could be observed during the day for hiding the sunlight in the crevices or under the leaves and they used the caudal grasping organ to attach the substrate (Wang et al., 2007). However, firefly larvae stretched their head outside the pronotum in a few minutes and started searching their prey. They used the antennae to explore the ground in front of them and sprinting to the prey with the pygypodial movement. Tyler (1994) suggested that the firefly larvae touched the substrate with a short sight by using the antennae to search the ground and they stretched the antennae out to touch anything round them. A biological study of *Pyractomena lucifera* firefly in Florida, USA indicated that the larvae were attracted by chemicals released from the prey (Buschman, 1984a). Moreover, Schwalb (1960) reported that the firefly larvae in *Lampyris noctiluca* species could follow the snail trails.

Handling phase

Firefly larvae attacked the land snail preys by approaching close to the snails and used the antennae to touch around the body of snail preys. They bit lateral side of snail's soft body by the mandibles and released the paralyzing toxin into their prey. The head of firefly larvae moved up and down for 3-4 times after biting. They took several times to keep biting and chewing at the snail's soft bodies. Moreover, firefly larvae usually cleaned themselves by using the pygypodia to clean the mucus of land snails from their head, pronotum and abdomen during feeding.

Feeding behavior of *P. tonkinensis* firefly larvae in the laboratory was similar to several previous studies. For instance, Buschman (1984a) suggested that *Pyractomena lucifera* larvae in Florida, USA attacked the snails by climbing onto the snail's shell then bit and chewed into the snail's body. There was no specific parts which the larvae preferred to bite their preys. However, Schwalb (1960) reported that *Luciola noctiluca* larvae attacked the snails in the head region to inject their toxin into the snails and then followed the snail to continue chewing.

Buschman (1984b) reported that *Photuris* larvae in USA were observed directly to the food items that placed in their containers in the laboratory. They usually attacked the snail prey for 4-6 times. After they captured the snails, they continued chewing for several minutes. When the snails were paralyzed, the larvae released them for several minutes and then returned to the snails and continue feeding. Moreover, the *Pyrocoelia pectoralis* larvae attacked the snails by climbing upon them and used the pygypodia clinging to the snail's shell. The larvae bit the exposed heads of the snails. The snails swung their shells to avoid their predators, but the response was usually ineffective. When the snail's head was retracted into the shell, firefly larvae used their elongate head to insert the mouthparts inside the shell and continued chewing into the snail's soft body (Wang et al., 2007). Tyler (1994) reported that the number of bites which the larvae needed to paralyze their snails depended on their relative size. Accordingly, a single bite from the well-grown firefly larva might paralyzed the snail in a few second. After biting, the firefly larvae waited for the effect of the poison. The larvae climbed to the entrance of the snail's shell and checked whether the snails were ready. At first, snails tried to defend themselves by covering their body with their mucus or began to foam when the firefly larvae attacked them (Rios and Quinta, 2010).

Time budgets

In the study, feeding of firefly larvae were the main behavioral units which the larvae spent a lot of times to finish their feeding activity. Tyler (1994) reported that the paralyzing toxin took effect as long as sixteen hours because most of the snails were still alive when paralyzed for several hours. Wang et al. (2007) indicated that feeding on the snail prey by firefly larvae was often lasts for 4-11 hours in the field. Moreover, the digestive process of firefly larvae took a large time to use the brown toxin to digest the protein for chewing and consuming their prey (Tyler, 1994). Therefore, all of these reasons could explain why the firefly larvae spent long time to feed on the land snails in the laboratory.

However, there was no significant difference between the medium size and large size of firefly larvae feeding behavior. This result might be because of the similarity of the body length in the both sizes of firefly larvae and the small number of the larvae in the experiments. Moreover, all behavioral units were exclusively defined except for glowing that occurred in four larvae during the experiments. The glowing was scored exceptionally as occurring discontinuously, since this behavior was observed in the different times with several kinds of movements during the experimental encounters. Usually, larval light emitted from the light organ and more frequently when they moved (Wang et al., 2007) or touched or vibrated or flash disturbed from adult fireflies (Lloyd, 1978). Additionally, the firefly larvae had been tested in the hypothesis of the glow or bioluminescence function as the aposematic display or warning display (De Cock, 2009; Underwood et al., 1997). However, there are still not enough evidences to explain why the firefly larvae glowed during feeding in the laboratory.

4.5 Conclusion

The third, the forth and the fifth instars of firefly *Pyrocoelia tonkinensis* larvae significantly preferred *Cryptozona siamensis* land snail as their prey. Feeding behavior of firefly larvae were divided into two phases. The first phase was searching phase which consisted of searching and approaching. The second phase was handling phase which consisted of attacking and feeding. Behavioral sequence of firefly larva feeding behavior began with head stretch followed by walking, sprinting and approaching, touching, biting, head shake, mandible chew, head insert, cleaning and walking around, respectively until the end of feeding time (move outside and resting). The time budget of feeding behavior was separated into seven units such as standing, searching, attacking, glowing, feeding, cleaning and walking around. Additionally, firefly larvae significantly spent their time to feed more than other behavioral units.

CHAPTER V

POPULATION DYNAMICS OF FIREFLY *Pyrocoelia tonkinensis* LARVAE AND THEIR LAND SNAIL PREY IN THE CHULALONGKORN UNIVERSITY FOREST AND RESEARCH STATION, LAI NAN SUBDISTRICT, WIANG SA DISTRICT, NAN PROVINCE

5.1 Introduction

The larval stages are the longest stage which could be more than 300 days to grow up (Chunram and Lewvanich, 1996). In this stages, firefly larvae are truly predators feeding on small invertebrates such as snails, slugs and other arthropod larvae (Buschman, 1984b; Fu et al., 2005; Kaufman, 1965; Wang et al., 2007; Williams, 1917). Moreover, the larval fireflies are usually found crawling on the ground surface, on stem of plants, fences, low walls and similar places while searching for and capturing their prey.

The habitats of firefly larvae are different depending on species. The larvae are found in the variety of the environmental factors such as temperature, humidity and herbaceous cover. For instance, the larvae of aquatic firefly, *Pyractonema lucifera,* in North America were abundant around cattail stand and stayed in the water surface. They were found in gaps between the leaves of the hyacinths and cattail plants. The larvae of aquatic firefly in Japan, *Luciola ficta*, were found in ditch habitats surrounding by plentiful aquatic plants. In this habitat, the water snails *Thiara granifera*, *Corbicula fluminea* and *C. chinensis* are common preys of the firefly larvae (Ho et al., 2010). For the terrestrial firefly in USA, *Photuris* firefly larvae were appeared on the surface and crawled or climbed up on stem at night. The larvae were abundant at the wet leaf litter particularly after a rain in the forest, ponds and streams where their land snails were frequently found (Buschman, 1984a). In China, the larvae of *Pyrocoelia pectoralis* fed on two species of land snails; *Bradybaena similaris* and *B. ravida.* Moreover, the larvae were found in the same place where they searched and captured snails prey (Wang et al., 2007).

However, the study of land snail preference and feeding behavior of *Pyrocoelia tonkinensis* larvae in the Chapter IV indicated that the larval of *P. tonkinensis* firefly preferred *Cryptozona siamensis* land snail as their prey. Therefore, the objectives of this research were to study the relationship between the population of *P. tonkinensis* firefly larva and the population of *C. siamensis* land snail, and also to study the effects of the environmental factors on the firefly larva and land snail populations in the Chulalongkorn University Forest and Research Station, Nan Province.

5.2 Materials and methods

5.2.1 Preliminary study of firefly larva population

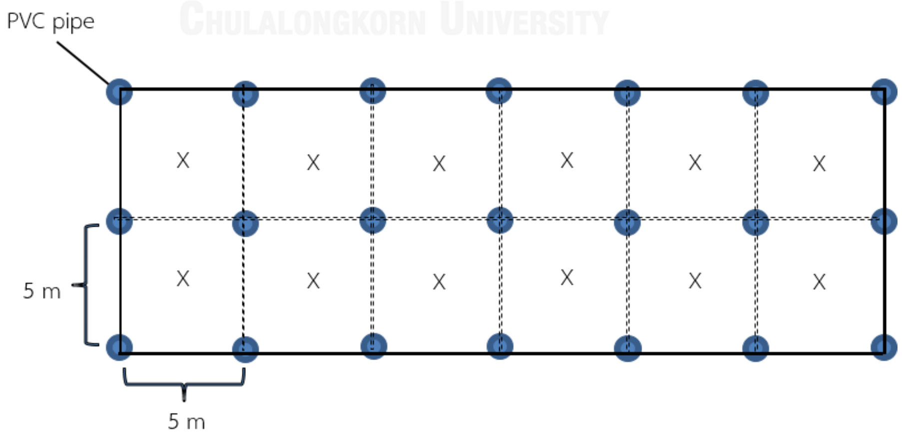
The firefly *Pyrocoelia tonkinensis* larvae were counted in all quadrats (25 m²) (Figure 25) during 20:00 PM to 22:00 PM (by counting the yellow-green light flash of the firefly larvae) in four study sites from April 2012 to October 2012. One minutes were spent in each observation point. Data were collected for one night per month in each study site.

5.2.2 Study sites and study periods

Study sites A2 = 300 m^2, A3 = 600 m^2, A4 = 160 m^2 and A5 = 160 m^2 were used to study the population of firefly larvae and land snails. The study was carried out during 19:00 PM to 22:00 PM in 13 months from February 2013 to February 2014.

5.2.3 Firefly larva population

The firefly *Pyrocoelia tonkinensis* larvae were counted in all quadrats (25 m²) (Figure 25) during the study period (by counting the yellow-green light flash of the firefly larvae) in four study sites. One minutes were spent in each observation point. Data were collected for three nights per month in each study site.



Remark: X = Observation point

Figure 25 The sample diagram of study site A2 (30 x 10 m²) placed with PVC pipes every five-meters for the study of firefly larva population at the Chulalongkorn University Forest and Research Station.

5.2.4 Land snail population

Cryptozona siamensis land snails were counted by quadrat sampling method during the study period in four study sites. The quadrats (1 m^2) were sampled in the study sites using a random table which were 12 quadrats in study site A2, 24 quadrats in A3, six quadrats in A4 and six quadrats in A5 (number of quadrats depended on sizes of each area). Data were collected for three nights per month in each study site (Figure 26).

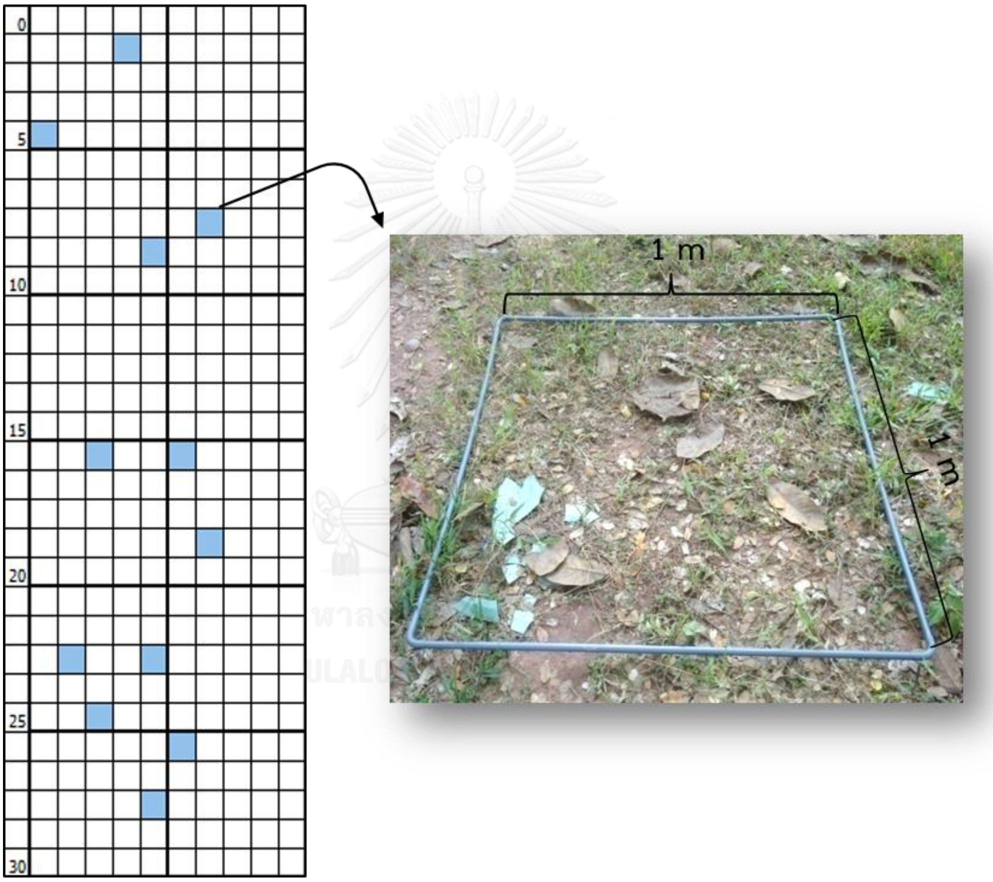


Figure 26 The sample diagram of random sampling method with 1 m^2 plots / quadrats in study site A2 (30 x 10 m^2) for the study of land snail population at the Chulalongkorn University Forest and Research Station.

5.2.5 Environmental factors

5.2.5.1 Physical factors

Air temperature and relative humidity

Thermo-hygrometers were used to measure air temperature and relative humidity for three times randomly during the study period in four study sites.

Soil temperature

Thermometers were used to measure soil temperature for three times randomly during the study period in four study sites.

Moisture content

The soils samples (100 g) were collected randomly for three times during the study period in four study sites. The collected soils were oven-dried at 105 ºC for 24 hours to calculate the moisture content in the laboratory.

$$\text{Moisture Content} = \frac{\text{Original weight} - \text{Oven-dried weight}}{\text{Oven-dried weight}} \times 100\ \%$$

Rainfall

The rainfall data in four study sites were supported by the Meteorological Department of Thailand at the Wiang Sa District Station, Nan Province.

5.2.5.2 Biological factors

Canopy coverage

The sampled quadrats from the study of land snail population (A2 = 12 quadrats, A3 = 24 quadrats, A4 = 6 quadrats and A5 = 6 quadrats) were measured for four times in each quadrat to calculate the percentile of canopy coverage in four study sites using a convex mirror, engraved with 25 squares (Rabinowitz, 1997).

Herbaceous coverage

The sampled quadrats (A2 = 12 quadrats, A3 = 24 quadrats, A4 = 6 quadrats and A5 = 6 quadrats) were captured by digital camera and engraved with 25 squares to calculate the percentile of herbaceous coverage in four study sites.

Herbaceous height

The sampled quadrats (A2 = 12 quadrats, A3 = 24 quadrats, A4 = 6 quadrats and A5 = 6 quadrats) were measured for three times randomly in each quadrat to calculate the herbaceous height in four study sites using a ruler and a tape rule.

5.2.6 Data analyses

Firefly larva and land snail populations

The mean of firefly larva and land snail numbers in four study sites were analyzed and compared among 13 months using Kruskal-Wallis test and pairwise multiple comparison.

The relationship between the population of firefly larva and land snail

The total numbers of firefly larvae and the mean numbers of land snails (numbers/ 1 m^2) in four study sites were calculated and analyzed using Spearman's rank correlation.

The correlation of environmental factors with firefly larva and land snail populations

The total numbers of firefly larvae, the mean numbers of land snails (numbers/ 1 m^2) and the mean of environmental factors in four study sites were calculated and analyzed using Spearman's rank correlation and multiple regression analysis.

5.3 Results

5.3.1 Preliminary study of firefly larva population

The total numbers of firefly larvae in four study sites among seven months indicated that the firefly *Pyrocoelia tonkinensis* larvae were found in every months which the firefly larva population was highest in July 2012 (Figure 27).

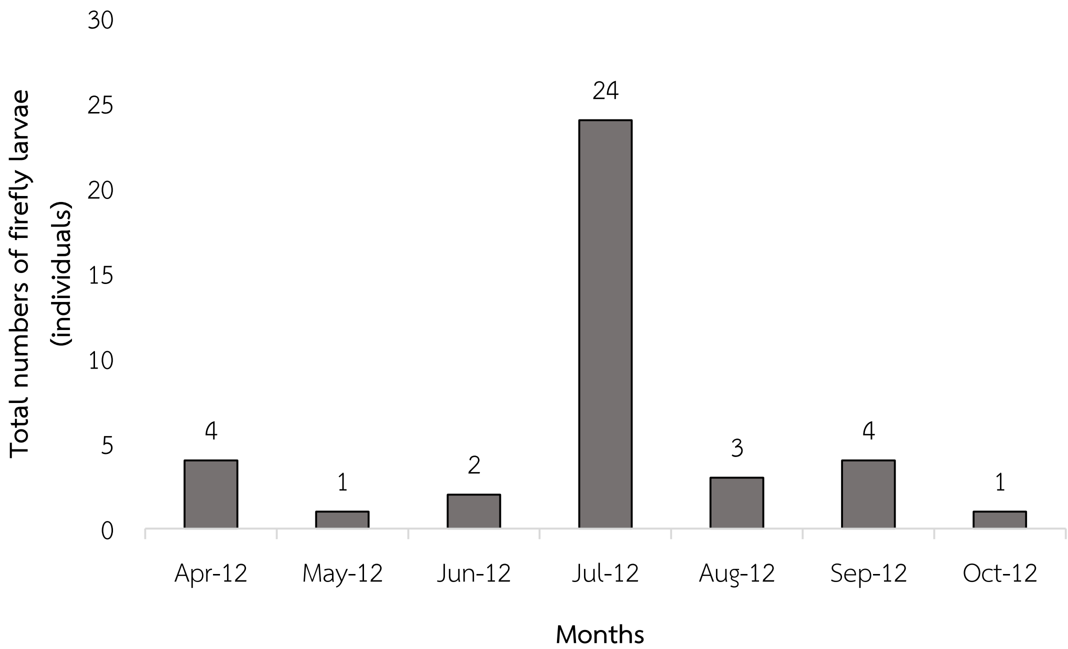


Figure 27 The total numbers of firefly *Pyrocoelia tonkinensis* larvae of the preliminary study in four study sites during 19:00 PM to 22:00 PM from April 2012 to October 2012 at the Chulalongkorn University Forest and Research Station.

5.3.2 Firefly larva population

The total numbers of firefly larvae in four study sites among 13 months indicated that the firefly *Pyrocoelia tonkinensis* larvae were found in July, August and September 2013 (Figure 28).

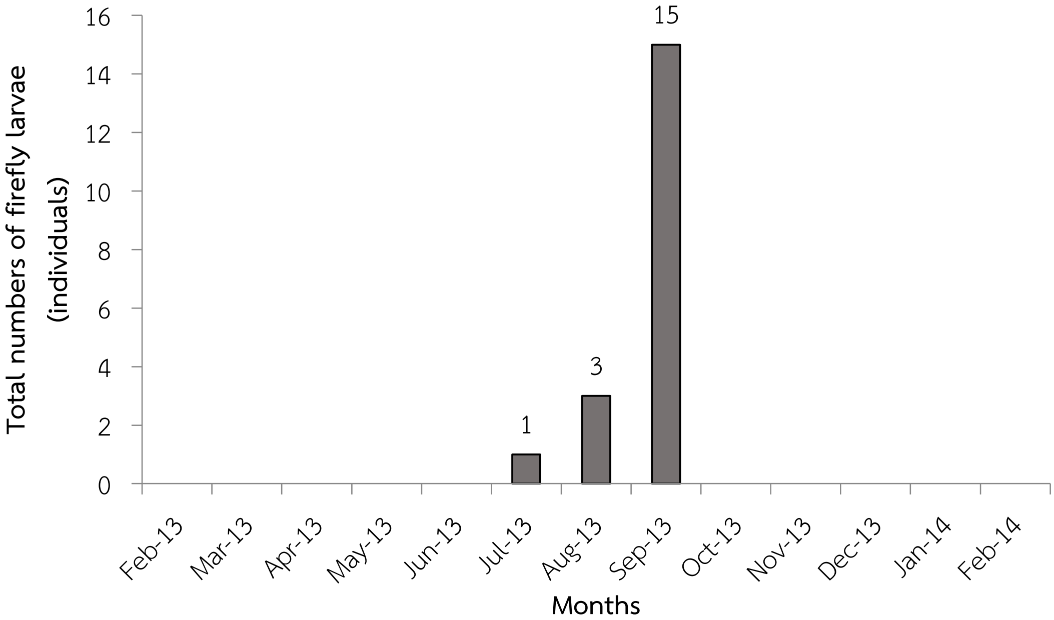
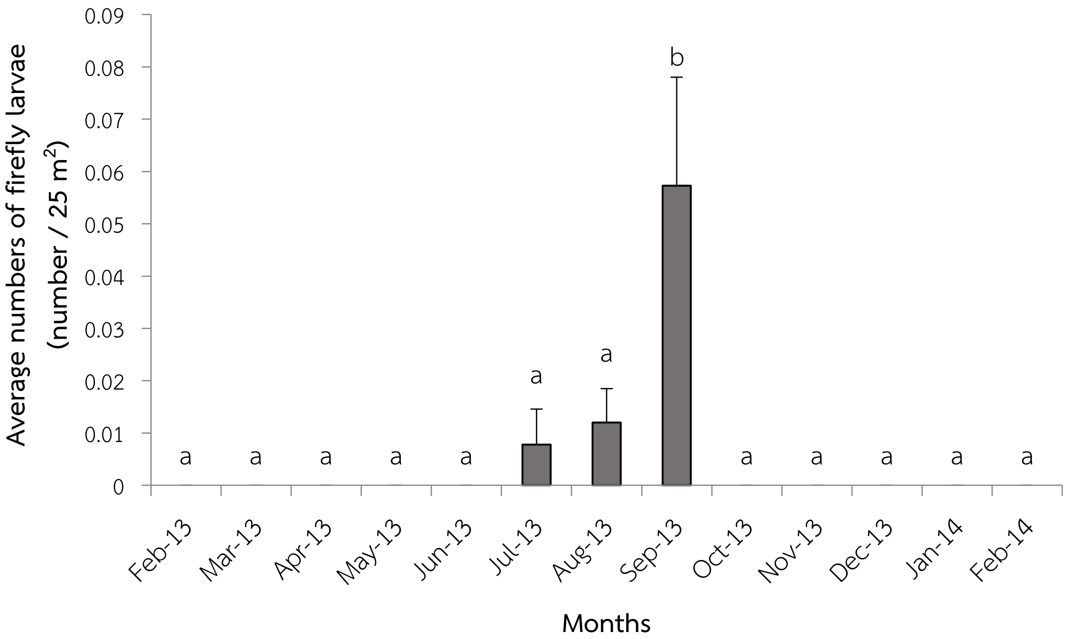


Figure 28 Total numbers of firefly *Pyrocoelia tonkinensis* larvae in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Moreover, the comparison of the mean larval numbers per 25 square meters revealed that there were significant differences in the larval numbers among 13 months, and the population of firefly larva significantly peaked in September 2013 (Kruskal-Wallis test; *F-value* = 64.543, df = 12, *p-value* < 0.0001, Pairwise multiple comparison at $P \leq 0.05$) (Figure 29 and Table 5).



*Different letters (a and b) designate significant differences of mean numbers.

Figure 29 The comparison of the mean (± SE) larval *Pyrocoelia tonkinensis* numbers per 25 square meters (numbers / 25 m^2) in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Table 5 The comparison of the mean (± SE) larval *Pyrocoelia tonkinensis* numbers per 25 square meters (numbers / 25 m²) in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	**Larval numbers (Mean ± SE)**
February 2013	0.000 ± 0.000^{a}
March 2013	0.000 ± 0.000^{a}
April 2013	0.000 ± 0.000^{a}
May 2013	0.000 ± 0.000^{a}
June 2013	0.000 ± 0.000^{a}
July 2013	0.008 ± 0.007^{a}
August 2013	0.012 ± 0.007^{a}
September 2013	0.057 ± 0.021^{b}
October 2013	0.000 ± 0.000^{a}
November 2013	0.000 ± 0.000^{a}
December 2013	0.000 ± 0.000^{a}
January 2014	0.000 ± 0.000^{a}
February 2014	0.000 ± 0.000^{a}

*Different letters (a and b) designate significant differences of mean numbers.

5.3.3 Land snail population

The total number of *Cryptozona siamensis* land snails in four study sites among 13 months indicated that land snail populations were frequently observed from July to October 2013 (Figure 30).

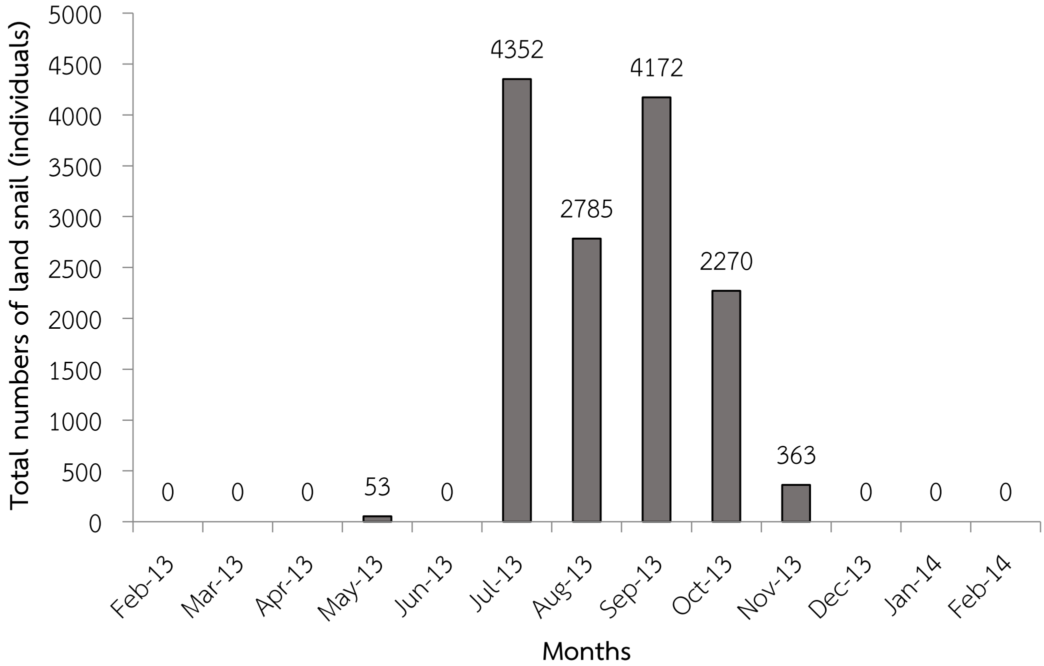
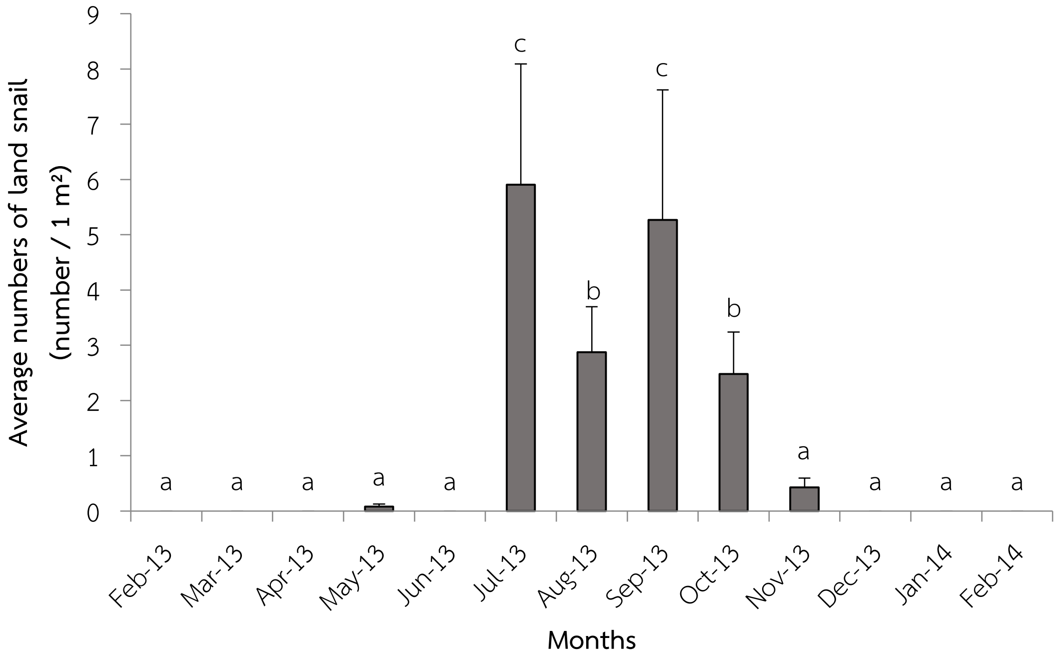


Figure 30 The total numbers of *Cryptozona siamensis* land snails in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

The comparison of the mean (± SE) land snail numbers in four study sites revealed that there were significant differences in the land snail numbers among 13 months, and the population of land snail significantly peaked in July and September 2013 (Kruskal-Wallis test; *F-value* = 137.655, df = 12, *p-value* < 0.0001, Pairwise multiple comparison at $P \leq 0.05$) (Figure 31 and Table 6).



*Different letters (a, b and c) designate statistical significances of group differences.

Figure 31 The comparison of the mean (± SE) *Cryptozona siamensis* land snails numbers per square meters (numbers / 1 m²) in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Table 6 The comparison of the mean (± SE) *Cryptozona siamensis* land snail numbers per square meters (numbers / 1 m²) in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Land snail numbers (Mean ± SE)
February 2013	0.00 ± 0.00^{a}
March 2013	0.00 ± 0.00^{a}
April 2013	0.00 ± 0.00^{a}
May 2013	0.08 ± 0.04^{a}
June 2013	0.00 ± 0.00^{a}
July 2013	5.91 ± 2.18^{c}
August 2013	2.88 ± 0.82^{b}
September 2013	5.27 ± 2.35^{c}
October 2013	2.48 ± 0.76^{b}
November 2013	0.43 ± 0.17^{a}
December 2013	0.00 ± 0.00^{a}
January 2014	0.00 ± 0.00^{a}
February 2014	0.00 ± 0.00^{a}

*Different letters (a, b and c) designate statistical significances of group differences.

5.3.4 Environmental factors

5.3.4.1 Physical factors

Air temperature and relative humidity

Average air temperature in four study sites was 24.29 ± 4.33 °C which was highest in June 2013 (29.33 ± 1.38 °C) and was lowest in January 2014 (14.57 ± 1.09 °C) (Figure 32A). Average relative humidity in four study sites was 73.02 ± 11.59 % which was highest in July 2013 (87.92 ± 0.28 %) and was lowest in February 2014 (54.83 ± 0.99 %) (Figure 32B).

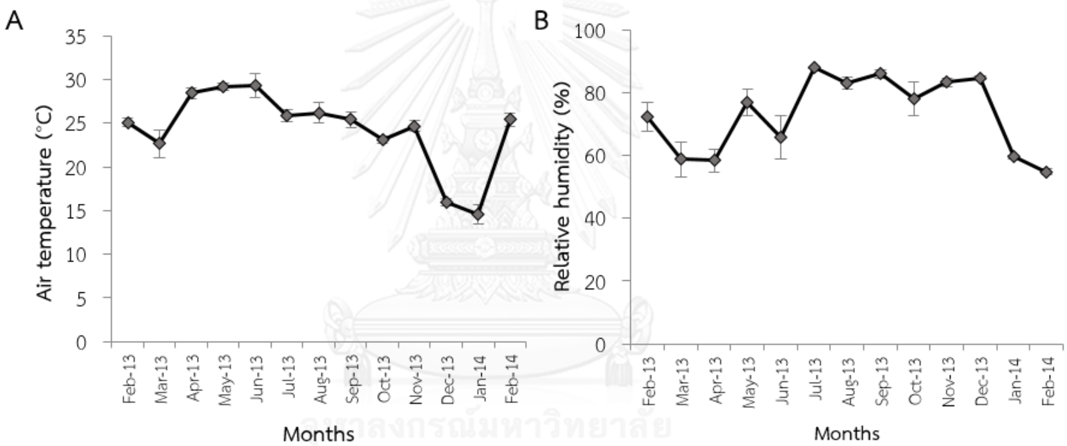


Figure 32 The means (± SD) of air temperature (A) and relative humidity (B) in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Soil temperature and soil moisture

Average soil temperature in four study sites was 27.38 ± 3.36 °C which was highest in June 2013 (31.83 ± 0.75 °C) and was lowest in January 2014 (20.71 ± 1.10 °C) (Figure 33A). Average soil moisture in four study sites was 5.61 ± 3.67 % which was highest in July 2013 (13.26 ± 4.21 %) and was lowest in January 2014 (1.53 ± 0.61 %). (Figure 33B).

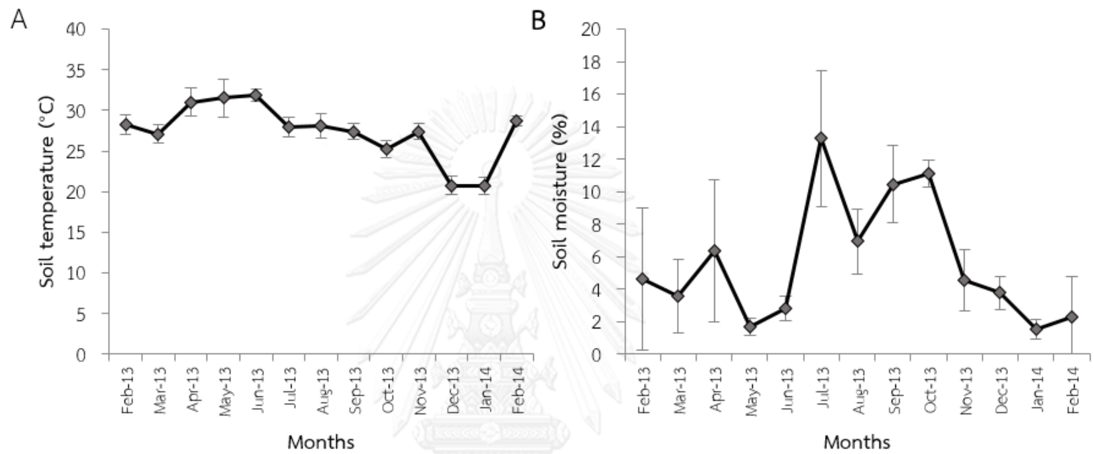


Figure 33 The means (± SD) of soil temperature (A) and soil moisture (B) in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Rainfall

Average rainfall in the study area was 1.78 ± 1.23 mm which was highest in October 2013 (7.13 ± 0.91 mm) and was lowest in February, December, January 2013 and February 2014 (0.00 mm). (Figure 34).

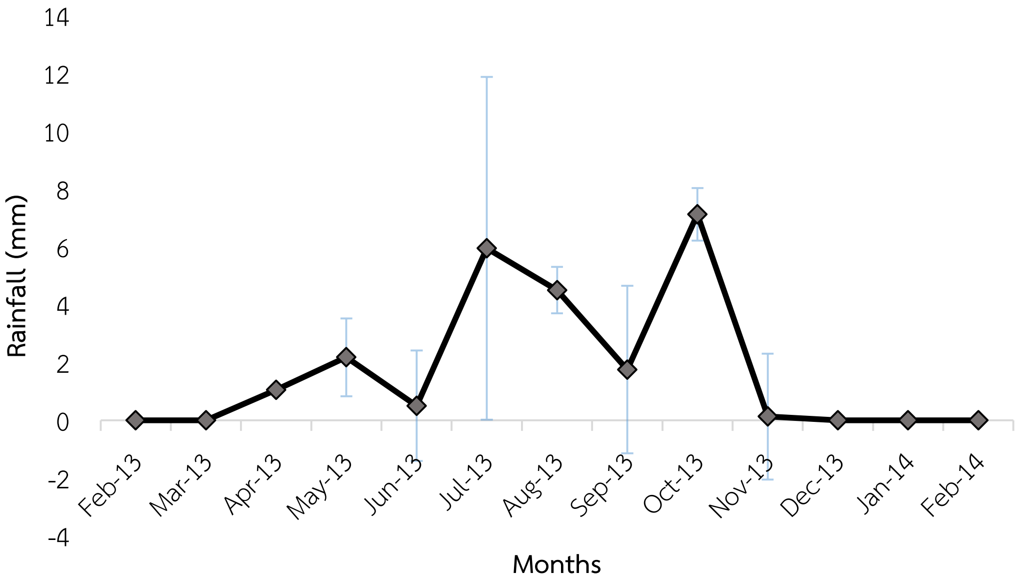


Figure 34 The means (± SD) of rainfall from February 2013 to February 2014 recorded by the Meteorological Department at the Wiang Sa District Station, Nan Province.

5.3.4.2 Biological factors

Canopy coverage

Average canopy coverage in four study sites was 62.02 ± 14.29 % which was highest in June 2013 (73.54 ± 19.28 %) and was lowest in February 2014 (21.84 ± 9.80 %). (Figure 35).

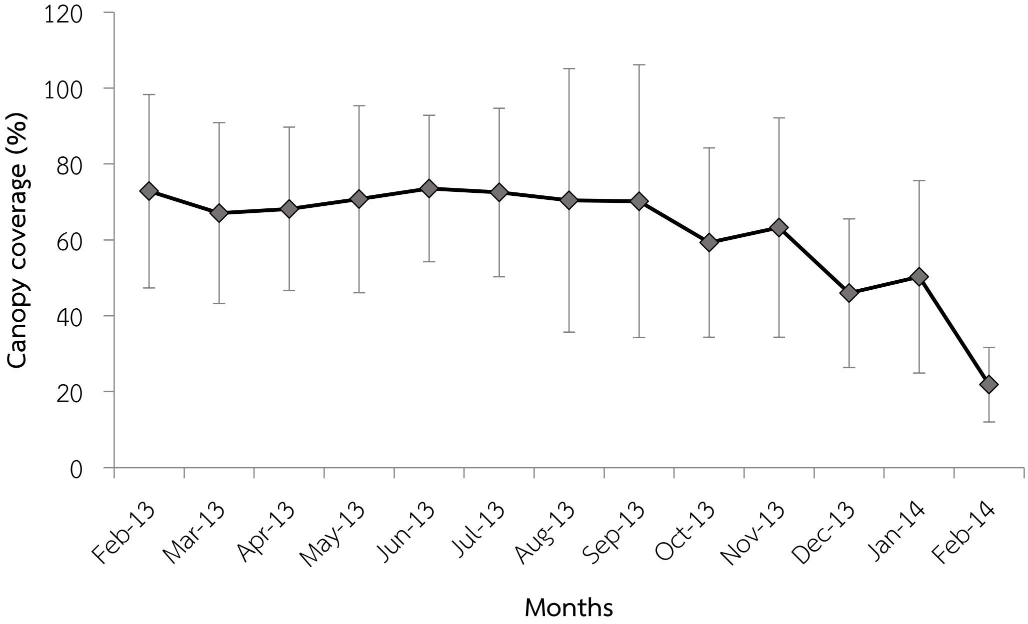


Figure 35 The means (± SD) of canopy coverage in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Herbaceous coverage

Average herbaceous coverage in four study sites was 69.75 ± 13.54 % which was highest in July 2013 (90.86 ± 0.46 %) and was lowest in February 2014 (40.87 ± 13.64 %) (Figure 36).

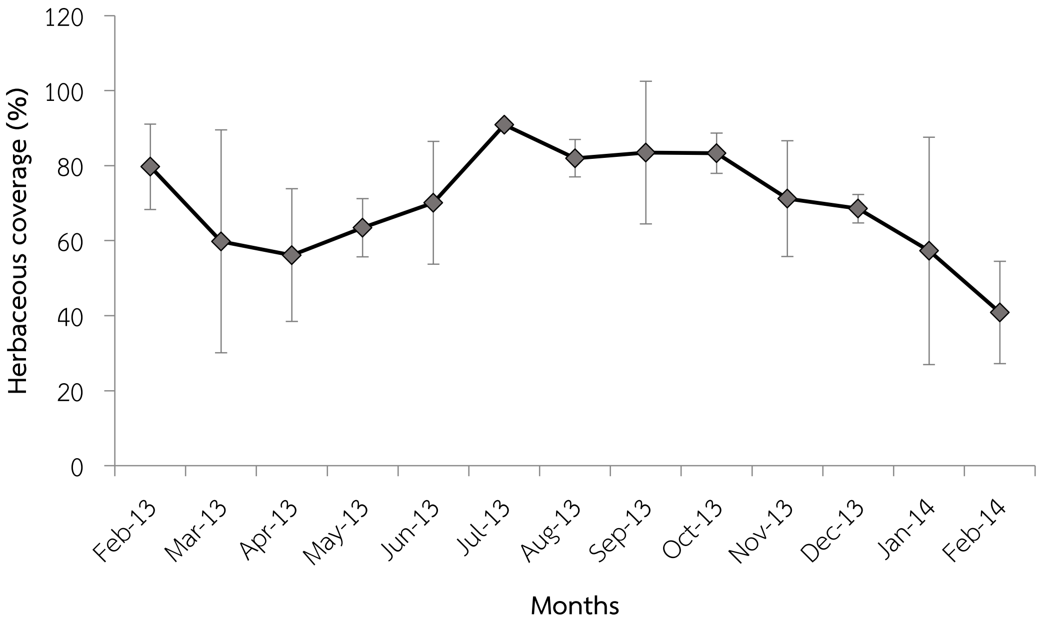


Figure 36 The means (± SD) of herbaceous coverage in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

3. Herbaceous height

Average herbaceous height in four study sites was 9.85 ± 5.13 cm which was highest in August 2013 (22.78 ± 24.66 cm) and was lowest in February 2014 (3.86 ± 2.50 cm) (Figure 37).

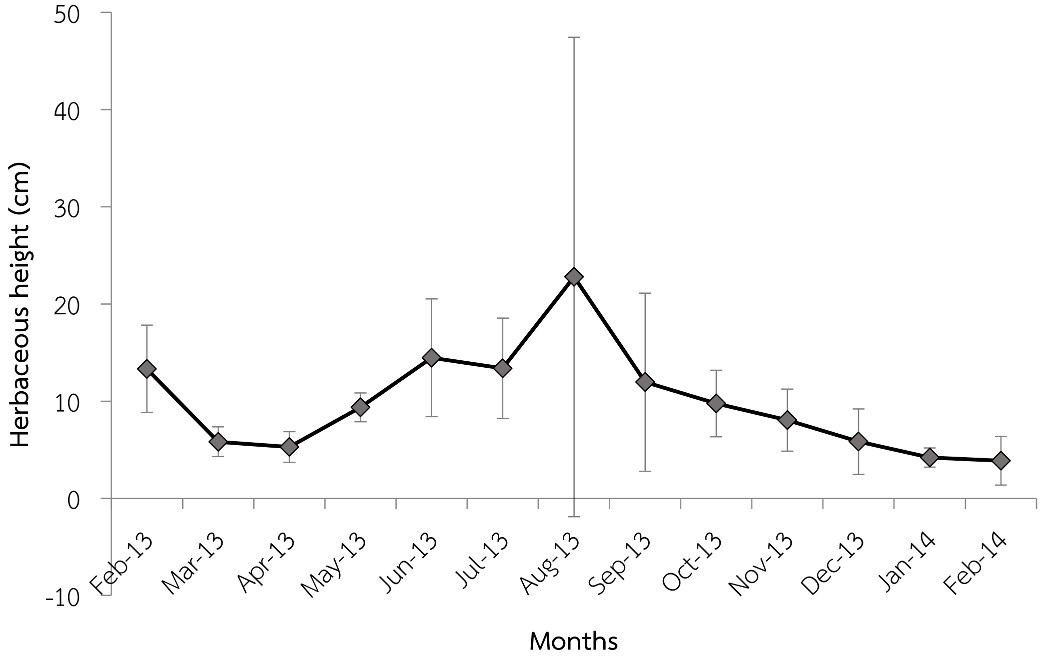


Figure 37 The means (± SD) of herbaceous height in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

5.3.5 Relationship between the firefly larva and land snail populations

The total numbers of firefly larvae and the mean numbers of land snails (numbers / 1 m²) in four study sites showed that there were similarities in term of their population dynamics (Figure 38).

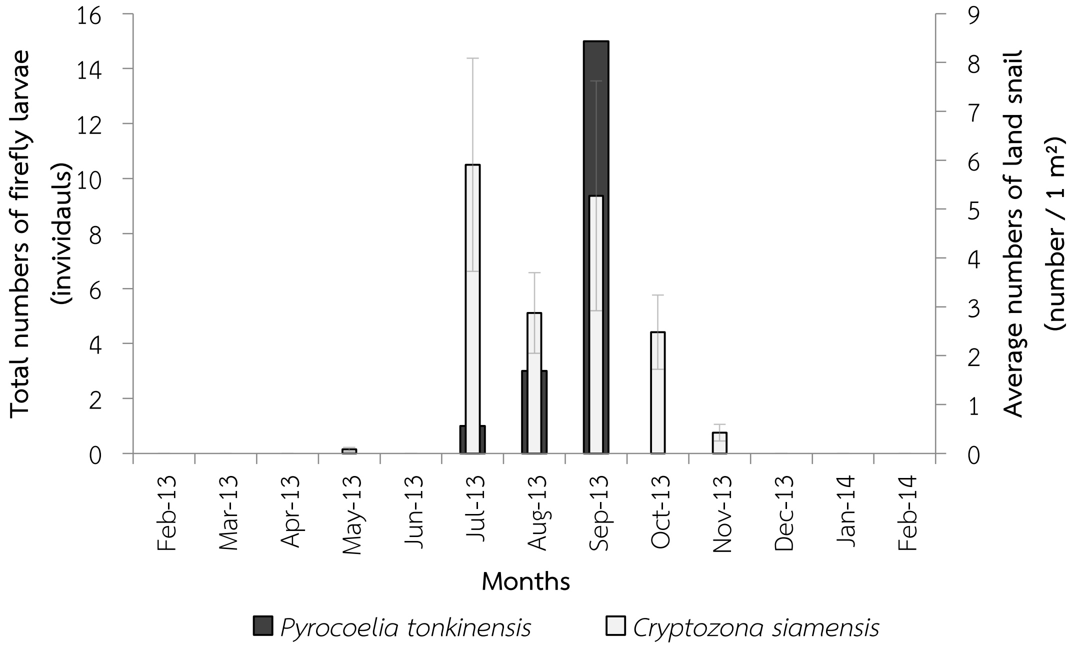


Figure 38 The relationship between the total numbers of firefly larvae and the mean numbers (± SE) of land snails (number / 1 m^2) from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

In addition, the simple correlation analysis between the firefly larva and land snail populations revealed that land snail populations were significant positive correlated with the population size of firefly larvae (Spearman's rank correlation coefficient, $P \leq 0.05$) (Table 7).

Table 7 The simple correlation between the firefly larva and land snail populations in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

			Firefly larvae	**Land snails**
Spearman's rho	**Firefly larvae**	Correlation	1.000	0.354**
		Sig. (2-tailed)	0.000	0.000
		N	156	156
	Land snails	Correlation	0.354**	1.000
		Sig. (2-tailed)	0.000	0.000
		N	156	156

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

5.3.6 Correlation of environmental factors with firefly larva population

The simple correlation analysis between the firefly larva population and the environmental factors (physical factors and biological factors) revealed that relative humidity, soil moisture, rainfall, herbaceous coverage and herbaceous height were significant positive correlated with the population size of firefly larva (Spearman's rank correlation coefficient, $P \leq 0.05$) (Table 8).

The correlated environmental factors (resulted from simple correlation analysis) which were relative humidity, soil moisture, rainfall, herbaceous coverage and herbaceous height in seven months (June, July, August, September, October, November and December) were used to analyze in the multiple linear regression. The significant environmental factors (resulted from multiple regression analysis ($P < 0.05$)) such as relative humidity and herbaceous coverage were selected to reanalyze again in the multiple regression analysis. However, the results revealed that relative humidity and herbaceous coverage were the positive effects on the population sizes of firefly larva while the strong factor influencing the firefly larva population was herbaceous coverage (*p-value* = 0.0070) (Multiple linear regression, R = 0.337*, p-value* = 0.0070).

Multiple linear regression $y = c + a_1x_1 + a_2x_2$

$y = -1.808 + 0.010x_1 + 0.016x_2$

y = Firefly larva population

c = Constant

x_1 = Air humidity

x_2 = Herbaceous coverage

Table 8 The simple correlation between the firefly *Pyrocoelia tonkinensis* larva population and the environmental factors in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

			Larvae	Airtemp	Rel.humid	Soiltemp	Soilmoisture	Canopy	Herbheight	Herbcover	Rainfall
Spearman's rho	Larvae	Correlation	1.000	-.001	.299**	-.007	.199*	-.020	.212**	.361**	.258**
		Sig. (2-tailed)		.987	.000	.932	.013	.804	.008	.000	.001
		N	156	156	156	156	156	156	156	156	156
	Airtemp	Correlation	-.001	1.000	-.085	.767**	-.017	.290**	.237**	-.034	.394**
		Sig. (2-tailed)	.987		.291	.000	.830	.000	.003	.673	.000
		N	156	156	156	156	156	156	156	156	156
	Airhumid	Correlation	.299**	-.085	1.000	-.199*	.485**	.080	.411**	.427**	.541**
		Sig. (2-tailed)	.000	.291		.013	.000	.323	.000	.000	.000
		N	156	156	156	156	156	156	156	156	156
	Soiltemp	Correlation	-.007	.767**	-.199*	1.000	-.170*	.091	.263**	-.016	.272**
		Sig. (2-tailed)	.932	.000	.013		.034	.256	.001	.839	.001
		N	156	156	156	156	156	156	156	156	156
	Soilhumid	Correlation	.199*	-.017	.485**	-.170*	1.000	.323**	.236**	.522**	.462**
		Sig. (2-tailed)	.013	.830	.000	.034		.000	.003	.000	.000
		N	156	156	156	156	156	156	156	156	156
	Canopy	Correlation	-.020	.290**	.080	.091	.323**	1.000	-.041	.236**	.334**
		Sig. (2-tailed)	.804	.000	.323	.256	.000		.608	.003	.000
		N	156	156	156	156	156	156	156	156	156
	Herbheight	Correlation	.212**	.237**	.411**	.263**	.236**	-.041	1.000	.597**	.667**
		Sig. (2-tailed)	.008	.003	.000	.001	.003	.608		.000	.000
		N	156	156	156	156	156	156	156	156	156
	Herbcover	Correlation	.361**	-.034	.427**	-.016	.522**	.236**	.597**	1.000	.534**
		Sig. (2-tailed)	.000	.673	.000	.839	.000	.003	.000		.000
		N	156	156	156	156	156	156	156	156	156
	Rainfall	Correlation	.258**	.394**	.541**	.272**	.462**	.334**	.667**	.534**	1.000
		Sig. (2-tailed)	.001	.000	.000	.001	.000	.000	.000	.000	
		N	156	156	156	156	156	156	156	156	156

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

*. Correlation is significant at the 0.05 level (2-tailed).

5.3.7 Correlation of environmental factors with land snail population

The simple correlation analysis between the land snail population and the environmental factors (physical factors and biological factors) revealed that relative humidity, soil moisture, rainfall, canopy coverage, herbaceous coverage and herbaceous height were the significant positive effects on the population size of land snail (Spearman's rank correlation coefficient, $P \leq 0.05$) (Table 9).

The correlated environmental factors (resulted from simple correlation analysis) which were relative humidity, soil moisture, rainfall, herbaceous coverage and herbaceous height in seven months (June, July, August, September, October, November and December) were used to analyze in the multiple linear regression. The significant environmental factors (resulted from multiple regression analysis ($P < 0.05$)) such as soil moisture, rainfall, canopy coverage and herbaceous coverage were selected to reanalyze again in the multiple regression analysis. However, the result revealed that soil moisture, rainfall, canopy coverage and herbaceous coverage were the positive effects on the population sizes of land snail while the strong factors influencing the land snail populations were soil humidity (*p-value* = 0.015), herbaceous coverage (*p-value* = 0.041) and canopy coverage (*p-value* = 0.001), respectively (Multiple linear regression, R = 0.604, *p-value* < 0.0001).

Multiple linear regression $y = c + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4$

$$y = -2.179 + 0.062x_1 + 0.025x_2 + 0.015x_3 + 0.018x_4$$

y = Land snail population

c = Constant

x_1 = Soil humidity

x_2 = Rainfall

x_3 = Canopy coverage

x_4 = Herbaceous coverage

Table 9 The simple correlation between the *Cryptozona siamensis* land snail population and the environmental factors in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

			C. siamensis	Airtemp	Rel.humid	Soiltemp	Soilmoisture	Canopy	Herbheight	Herbcover	Rainfall
Spearman's rho	*Cryptozona siamensis*	Correlation	1.000	.013	.602**	-.093	.605**	.237**	.310**	.523**	.623**
		Sig. (2-tailed)		.876	.000	.248	.000	.003	.000	.000	.000
		N	156	156	156	156	156	156	156	156	156
	Airtemp	Correlation	.013	1.000	-.085	.767**	-.017	.290**	.237**	-.034	.394**
		Sig. (2-tailed)	.876		.291	.000	.830	.000	.003	.673	.000
		N	156	156	156	156	156	156	156	156	156
	Airhumid	Correlation	.602**	-.085	1.000	-.199*	.485**	.080	.411**	.427**	.541**
		Sig. (2-tailed)	.000	.291		.013	.000	.323	.000	.000	.000
		N	156	156	156	156	156	156	156	156	156
	Soiltemp	Correlation	-.093	.767**	-.199*	1.000	-.170*	.091	.263**	-.016	.272**
		Sig. (2-tailed)	.248	.000	.013		.034	.256	.001	.839	.001
		N	156	156	156	156	156	156	156	156	156
	Soilhumid	Correlation	.605**	-.017	.485**	-.170*	1.000	.323**	.236**	.522**	.462**
		Sig. (2-tailed)	.000	.830	.000	.034		.000	.003	.000	.000
		N	156	156	156	156	156	156	156	156	156
	Canopy	Correlation	.237**	.290**	.080	.091	.323**	1.000	-.041	.236**	.334**
		Sig. (2-tailed)	.003	.000	.323	.256	.000		.608	.003	.000
		N	156	156	156	156	156	156	156	156	156
	Herbheight	Correlation	.310**	.237**	.411**	.263**	.236**	-.041	1.000	.597**	.667**
		Sig. (2-tailed)	.000	.003	.000	.001	.003	.608		.000	.000
		N	156	156	156	156	156	156	156	156	156
	Herbcover	Correlation	.523**	-.034	.427**	-.016	.522**	.236**	.597**	1.000	.534**
		Sig. (2-tailed)	.000	.673	.000	.839	.000	.003	.000		.000
		N	156	156	156	156	156	156	156	156	156
	Rainfall	Correlation	.623**	.394**	.541**	.272**	.462**	.334**	.667**	.534**	1.000
		Sig. (2-tailed)	.000	.000	.000	.001	.000	.000	.000	.000	
		N	156	156	156	156	156	156	156	156	156

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

*. Correlation is significant at the 0.05 level (2-tailed).

5.4 Discussion

5.4.1 Firefly larva population

The preliminary study of firefly *P. tonkinensis* larva population in four study sites during 19:00 PM to 22:00 PM from April 2012 to October 2012 indicated that the firefly larvae were found in seven months (n = 39) and the firefly larva population was highest in July 2012 (n = 24) (Figure 27). Total number of the firefly larva populations during 19:00 PM to 22:00 PM from February 2013 to February 2014 showed that the larvae were found in July, August and September 2013. Moreover, the comparison of the mean larval numbers revealed that the population of firefly larva was significantly peaked in September 2013 (n = 15).

The results showed that the population of larval *P. tonkinensis* firefly decreased during two year (2012-2014). The small numbers of firefly larvae might be because of the disturbances in all study sites from February 2013 to February 2014 such as the artificial lights, the heavy rain and the human activities (Thancharoen, 2007) including wildfire in the study sites (Figure 39). Additionally, the cold weather might be the negative effect on the larval firefly population because the larval of *P. pectoralis* fireflies were discovered in the holes under the ground or soil over winter season (Wang et al., 2007).

In other firefly species such as the terrestrial firefly, *Pyrocoelia praetexta,* in Chiang Mai, Thailand, the firefly larvae were frequently found in July (12.23 ± 9.91 individuals/ 9 m^2) while the adult male firefly were frequently found in June (25.75 individuals/ 1,600 m^2) (Sommit, 2004). In China, the biological study of the terrestrial firefly *Pyrocoelia pectoralis* indicated that the larval fireflies were consistently found from May to September. The larvae were discovered in the holes under the ground or soil over winter season from November to next April. However, the adult *P. pectoralis* fireflies were frequently observed from the end of September to the end of October which peaked in mid-October (Wang et al., 2007). In Taiwan, the study of the aquatic firefly *Luciola ficta* life cycle indicated that the first larval instars were found in July and the seventh larval instars peaked in February while the adult fireflies were frequently observed from April to May (Ho et al., 2010).

Figure 39 Natural phenomenon (A) heavy rainfall and water trapped in a study area and human activities (B) camping and (C and D) burning grass in all study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station, Wiang Sa District, Nan Province.

5.4.2 Relationship between the firefly larva and land snail populations

The results of the firefly larva and land snail populations in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 indicated that there was synchronization in term of their population dynamics as a predator and prey relationship (Figure 38). For instance, the land snail population increased from July to November 2013 followed by the firefly larva population which gradually increased from July to September 2013.

Moreover, the simple correlation analysis between the firefly larva and land snail populations among 13 months from February 2013 to February 2014 indicated that *C. siamensis* land snails were the major contributor to the positive correlation on the population size of firefly larvae (Table 7). Therefore, there was probably indicated that *C. siamensis* land snail was the major prey of firefly *P. tonkinensis* larvae because they were frequently found in the study area at the Chulalongkorn University Forest and Research Station.

Additionally, the other firefly larva species was actually reported about feeding on the land snail lived in the same place. For instance, the larvae of *Alecton discoidalis* in Cuba preferred the land snail family Helicinidae and Potamiidae as their food which were the most abundant in the study areas (Rios and Quinta, 2010). In China, the larvae of *Pyrocoelia pectoralis* fed on two species of land snails, *Bradybaena similaris* and *B. ravida.* Similarly, the larvae were found in the same place where they searched and captured snails prey. The larval fireflies were abundant in and around farmland where many land snails occurred (Wang et al., 2007). In aquatic firefly, *L. stagnalis* was the snail prey lived in the pond that the *Aquatica leii* firefly larvae were found and preferred them as food. Moreover, an aquatic firefly *Luciola ficta* larvae in Taiwan fed on the water snail *Cipangopaludina chinensis* which was the most abundant species in the study area (Ho et al., 2010).

5.4.3 Correlation of environmental factors with firefly larva population

The simple correlation analysis between the firefly larva population and environmental factors (physical factors and biological factors) among 13 months indicated that relative humidity, soil moisture, rainfall, herbaceous coverage and herbaceous height were the significant positive correlation on the population size of firefly larvae. Additionally, the multiple regression analysis of the environmental factors and the firefly larva population in seven months showed that herbaceous coverage was a significant factor that positively affected on the increase of firefly larva population.

From the results, the suitable environmental conditions for the firefly *P. tonkinensis* larvae in the peaked month (September 2013) was 85.92 ± 0.74 % of relative humidity, 10.45 ± 1.06 % of soil moisture, 1.75 ± 2.90 mm of rainfall, 11.95 ± 3.38 cm of herbaceous height and 83.50 ± 9.79 % of herbaceous coverage. However, the loss number of the firefly larva population after the peaked month (September 2013) might be because of the heavy rainfall in October 2013. Also, soil moisture and rainfall rapidly decreased in from November 2013 to the next June 2014.

In the other terrestrial firefly, *Photuris* firefly larvae were abundant on the wet leaf litter particularly after raining in the forest, ponds and streams (Buschman, 1984b; McLean et al., 1972). Moreover, the suitable ground temperature and high humidity were essential for their life. Larvae were more actives in the night that high humidity was observed and died in a few hours if they stayed in the dry container at room temperature (Buschman, 1984a). Wang et al. (2007) indicated that *Pyrocoelia pectoralis*, the terrestrial firefly in China, lived in the soil and occurred in November to next April. The suitable of temperature for larval activity was 18-30 °C and the humidity was more than 90 %. Moreover, Rios and Quinta (2010) reported that the *Alecton discoidalis* firefly larvae in Cuba, mostly occurred and emitted light from 20:00 PM to 22:00 PM. They lived under the leaf litter and under rocks. The suitable temperature and humidity for larval activity were 26-32 °C and 64-86 %, respectively.

5.4.4 Correlation of environmental factors with land snail population

The simple correlation analysis between the land snail population and environmental factors (physical factors and biological factors) among 13 months indicated that relative humidity, soil moisture, rainfall, canopy coverage, herbaceous coverage and herbaceous height were the significant positive correlation on the population size of land snail. Additionally, multiple linear regression of land snail population and the environmental factors in seven months revealed that soil moisture, canopy coverage and herbaceous coverage were significant factors that positively influenced the land snail population.

From the results, the suitable environmental condition for the *C. siamensis* land snail in the peaked month (July 2013) was 87.91 ± 2.21 % of relative humidity, 13.27 ± 1.37 % of soil moisture, 5.95 ± 5.93 mm of rainfall, 72.49 ± 11.20 % of canopy coverage, 13.38 ± 6.05 cm of herbaceous height and 90.88 ± 7.20 % of herbaceous coverage. Moreover, the loss number of the population of land snail after the peaked month (July 2013) might be because the percentage of soil moisture and rainfall rapidly decreased in those months from November 2013 to the next June 2014.

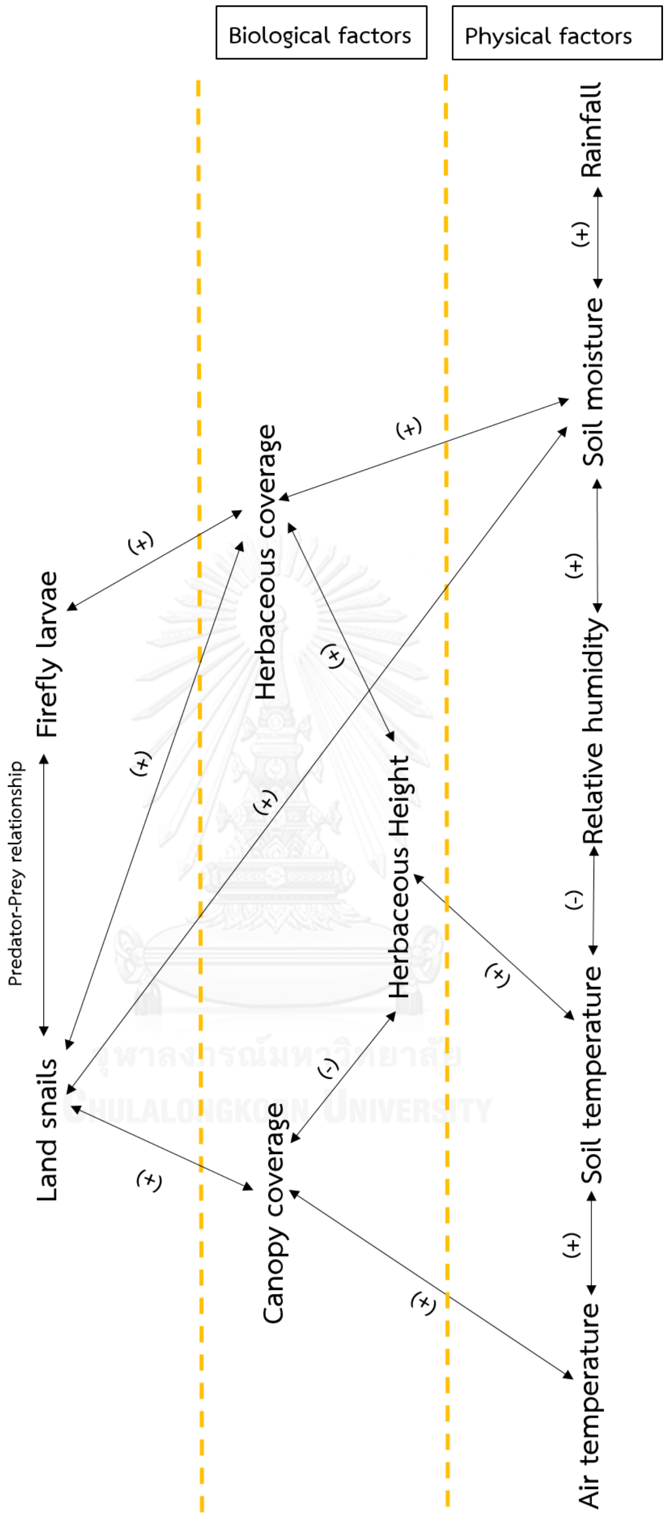
Moreover, the study of relationship between the firefly larva and land snail populations among 13 months indicated that *C. siamensis* land snail was the principle factor that positively correlated with the population size of larval fireflies. Accordingly, the environmental condition of land snails and firefly larvae were similar in the physical and biological factors. For instance, the land snails were mainly nocturnal and hid during the day. The main factors of their nocturnal habitat were the moisture or soil humidity and they were closed the shell aperture with a mucus flap in the adverse climatic conditions (Sallam and El-Wakeil, 2012). The populations of land snails were gradually increased when the temperature and humidity in the study areas were suitable (Kady et al., 1983). Land snail activity were influenced by percentage of moisture but inhibited by water lack. They were returned to the upper soil where it was cool and shady (Godan, 1983).

5.4.5 Correlation of environmental factors with firefly larva and land snail populations

From the results, multiple regression analysis of the environmental factors on the firefly larva population in seven months revealed that herbaceous coverage was the significant positive effect on the firefly larva population. Also, multiple linear regression analysis of the environmental factors on the land snail population in seven months revealed that soil moisture, canopy coverage and herbaceous coverage were significant factors that influenced the land snail population.

Moreover, the three environmental factors (soil moisture, canopy coverage and herbaceous coverage) had significant positive effects on the firefly larva (only herbaceous coverage) and land snail populations. These three factors also complicatedly related with the other environmental factors. For instance, soil moisture positively related with relative humidity, herbaceous coverage and rainfall. Canopy coverage positively related with air temperature and relative humidity; but was the negatively related with soil temperature and herbaceous height. Furthermore, herbaceous coverage positively related with relative humidity, soil temperature, soil moisture, herbaceous height and rainfall (Figure 40).

Consequently, the study probably revealed that the dynamics of firefly larva and land snail populations depended on the interaction of the physical and biological factors like as soil moisture, canopy coverage and herbaceous coverage which were the significant positive effects on the firefly larva (only herbaceous coverage) and land snail populations in the study area.



Remark: (+) = Positive relationship, (-) = Negative relationship

Figure 40 Correlation between environmental factors (physical factors and biological factors) and the firefly larva and land snail populations from February 2013 to February 2014 at the Chulalongkorn University Forest and Research station.

5.5 Conclusion

Firefly *Pyrocoelia tonkinensis* larva population significantly peaked in September 2013 and *Cryptozona siamensis* land snail population significantly peaked in July and September 2013 at the Chulalongkorn University Forest and Research Station, Wiang Sa District, Nan Province.

Land snail population was responsible for the positive correlation with firefly larva population. The suitability of environmental conditions for the larval *P. tonkinensis* firefly in the peaked month (September 2013) were 85.92 ± 0.74 % of relative humidity, 10.45 ± 1.06 % of soil moisture, 1.75 ± 2.90 mm of rainfall, 11.95 ± 3.38 cm of herbaceous height and 83.50 ± 9.79 % of herbaceous coverage. Moreover, the suitability of environmental conditions for the *C. siamensis* land snail in the peaked month (July 2013) were 87.92 ± 2.21 % of relative humidity, 13.27 ± 1.37 % of soil moisture, 5.95 ± 5.93 mm of rainfall, 72.49 ± 11.20 % of canopy coverage, 13.38 ± 6.05 cm of herbaceous height and 90.88 ± 7.20 % of herbaceous coverage.

CHAPTER VI

FLASHING DISPLAYS OF *Pyrocoelia tonkinensis* FIREFLY AT THE CHULALONGKORN UNIVERSITY FOREST AND RESEARCH STATION, LAI NAN SUBDISTRICT, WIANG SA DISTRICT, NAN PROVINCE

6.1 Introduction

In adult stage, fireflies have specialized light-emitted organs namely light organs or lanterns in the abdominal segments (Copeland, 1981; De Cock and Matthysen, 2003) which the positions of the light organs were different among firefly species and sexes. For instance, the male *Pyrocoelia tonkinensis* firefly has a light organ in the last two body segments, the fifth and the sixth abdominal segments, whereas the female has a light organ in the lateral side of the fifth and the sixth abdominal segment. Moreover, they use the bioluminescence of flash light in the light organs for species recognition and mating location (Carlson and Copeland, 1985; De Cock and Matthysen, 2003; Lewis and Cratsley, 2008; Long et al., 2012).

Typically, males were the primary signalers which emitted flashing advertisement during flight times. A female responded a male by flashing back with courtship dialogue that the flash signals were exchanged between the male and the female (Lewis and Cratsley, 2008). Some species, flying males attracted to a slowly glow light emitted of flightless females or larviform females such as *Lampyris noctiluca*, the European glow-worm (Branham and Wenzel, 2003) and also in *P. tonkinensis*, the interested species in this study. Moreover, the flashing displays or flight seasons of fireflies were varied among the firefly species (Barrows et al., 2008). For instance, the flashing displays of the synchronous firefly, *Photinus carolinus*, began at about 37 to 43 minutes after sunset (Lloyd, 1966) and flashing behavior appeared for three hours in the peak night (Faust, 2010). The courtship observation of males, *Photinus greeni*, reported that they began the advertisement flashes in 15 minutes after sunset. The flight periods were occurred for 45 minutes and decreased in 90 minutes after sunset (Demary et al., 2006). Furthermore, the flashing activity of adults

Pyrocoelia pectoralis firefly occurred from late September to late October which emergence of peak in mid-October (Wang et al., 2007).

However, flashing displays have not been recorded in *P. tonkinensis* firefly species. Because flightless females or larviform females are rare in the study site, individuals flashing are probably mostly adult male fireflies. Therefore, the purpose of this research was to investigate the flashing displays of adult male *P. tonkinensis* fireflies at nighttime in all year round at the Chulalongkorn University Forest and Research Station, Wiang Sa District, Nan Province.

6.2 Materials and methods

6.2.1 Study site

A 300-meters trail site in the study site A3 was used for conducting the adult male firefly flashing activity. Along the trial site, three study points were set with equal distance from each other; point 1 was at 50 meters, point 2 was at 150 meters and point 3 was at 250 meters (Figure 41).

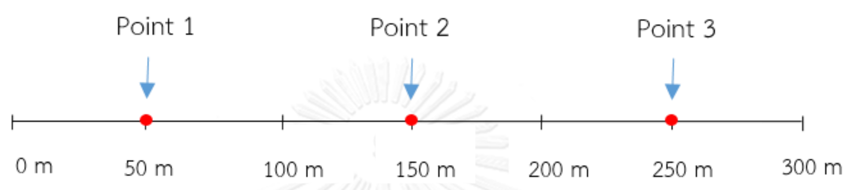


Figure 41 The study points diagram in a 300-meters trial site at the Chulalongkorn University Forest and Research Station, Nan Province, Thailand

6.2.2 Flashing activity

Flashing numbers of adult males *Pyrocoelia tonkinensis* fireflies were recorded using a counter by counting the flash displays at about 50 meters around each study point during 18:00 PM to 06:00 AM from February 2013 to February 2014. Moreover, three nights of each month were spent in this study (one night per one study point).

The duration of each study night was divided into three intervals; 18:00–23:00 interval, 23:00–01:00 interval and 01:00–06:00 interval, by the occurrence of flash light which usually intense in one or two hours after sunset. The first interval, flashing displays were counted every 15 minutes. The second interval, flashing displays were counted every 30 minutes and the third interval, the flashing display were counted every hour. About two-minutes was spent to count the flash displays in each time.

6.2.3 Data analyses

The means of flashing numbers were analyzed and compared among 13 months using Kruskal-Wallis test and pairwise multiple comparison to select the months that flashing displays generally occurred.

The mean flashing numbers of the peaked months were calculated and analyzed using Kruskal-Wallis test and pairwise multiple comparison to investigate the time interval that flashing displays were frequently found.

6.3 Results

6.3.1 Flashing displays of adult male firefly all year round

The total flashing display numbers of adult male *P. tonkinensis* firefly in the study site A3 during 18:00 PM to 6:00 AM from February 2013 to February 2014 indicated that the flashing numbers of adult male *P. tonkinensis* fireflies were frequently observed in April and May 2013 (Figure 42).

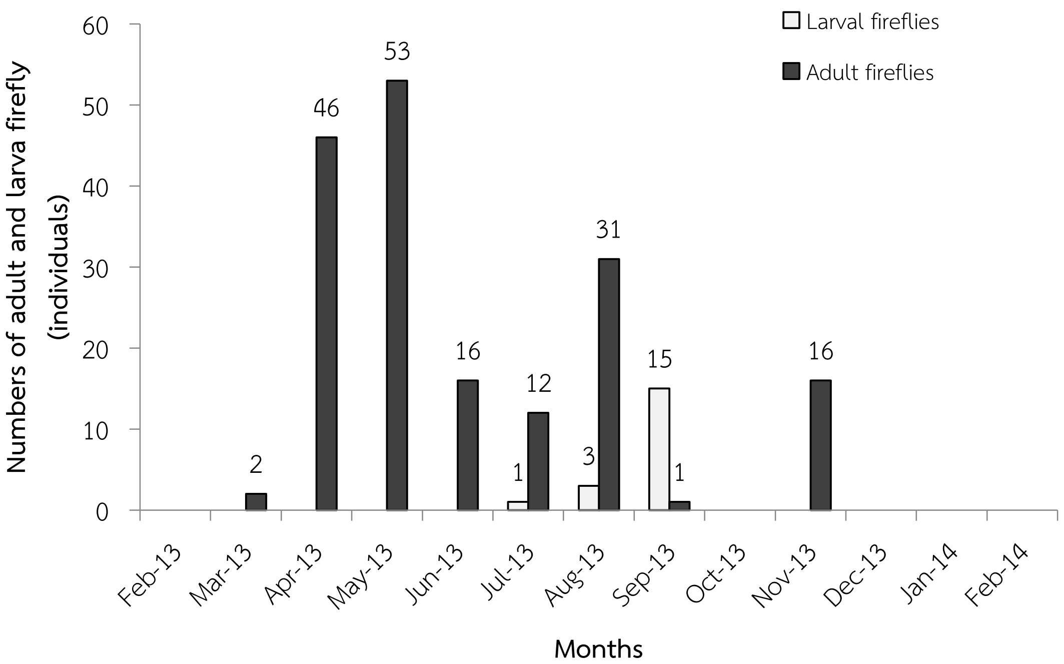
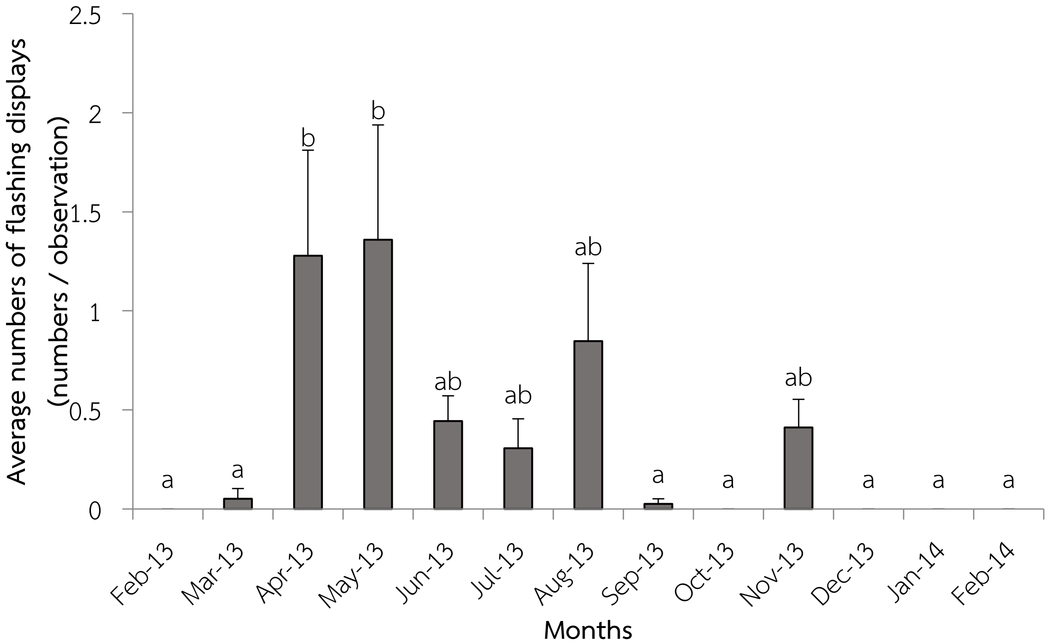


Figure 42 The total numbers of adult male *Pyrocoelia tonkinensis* firefly flashing displays in three nights during 18:00 PM to 6:00 AM in the study site A3 and the total number of firefly *Pyrocoelia tonkinensis* larvae in four study sites during 19:00 PM to 22:00 PM (resulted from Chapter V) from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

The mean ± SE of flashing numbers in adult male *P. tonkinensis* firefly were significant differences among months and the comparison of the mean flashing numbers indicated that April and May 2013 had higher flash numbers when compared with the other months (Kruskal-Wallis test *F-value* = 33.643, df = 12, *p-value* < 0.0001, Pairwise multiple comparison at $P \leq 0.05$) (Figure 43 and Table 10).



*Different letters (a and b) designate significant differences of mean numbers.

Figure 43 The comparisons of the mean number (± SE) flashing displays of adult male *Pyrocoelia tonkinensis* fireflies in the study site A3 during 18:00 PM to 6:00 AM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

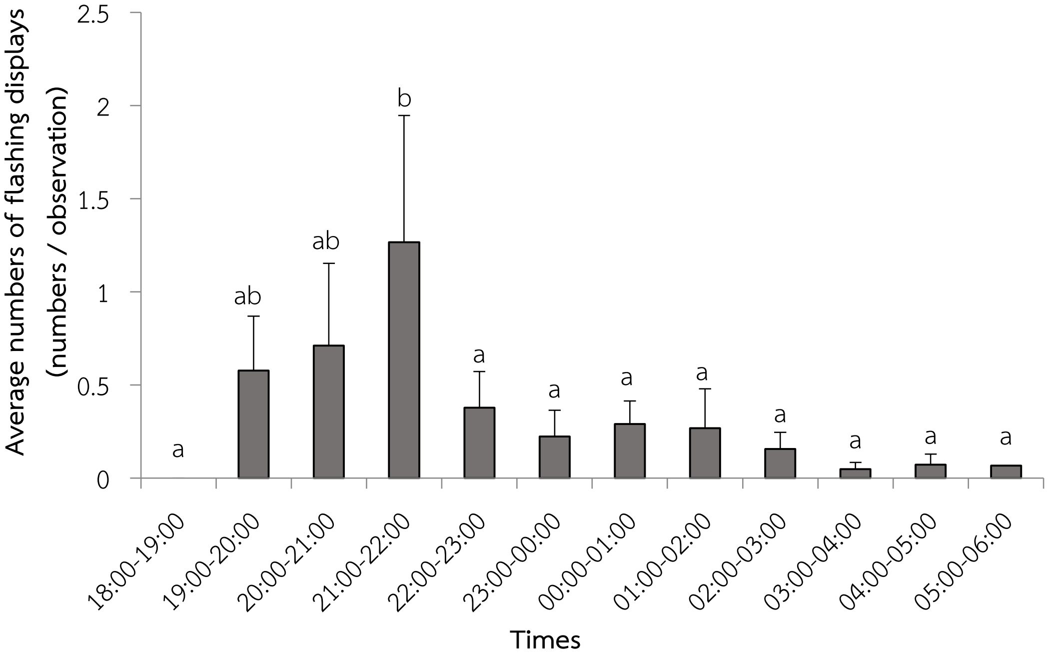
Table 10 The comparisons of the mean (± SE) flashing numbers of adult male *Pyrocoelia tonkinensis* fireflies in the study site A3 among 13 months from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Flashing Numbers (Mean ± SE)
February 2013	0.00 ± 0.0^{a}
March 2013	0.05 ± 0.0^{a}
April 2013	1.18 ± 0.5^{b}
May 2013	1.36 ± 0.6^{b}
June 2013	0.41 ± 0.1^{ab}
July 2013	0.31 ± 0.1^{ab}
August 2013	0.85 ± 0.4^{ab}
September 2013	0.03 ± 0.0^{a}
October 2013	0.00 ± 0.0^{a}
November 2013	0.41 ± 0.1^{ab}
December 2013	0.00 ± 0.0^{a}
January 2014	0.00 ± 0.0^{a}
February 2014	0.00 ± 0.0^{a}

*Different letters (a and b) designate significant differences of mean numbers.

6.3.2 Flashing display of adult male firefly at nighttime

In all study nights, there were significant differences of the mean flashing numbers of adult male *Pyrocoelia tonkinensis* firefly in each one hour interval and the comparison of the mean flashing numbers of each period indicated that the significant period of the highest flashing number of adult male *P. tonkinensis* firefly was from 21:00 PM to 22:00 PM (Kruskal-Wallis test *F-value* = 51.773, df = 11, *p-value* < 0.0001, Pairwise multiple comparison at $P \leq 0.05$) (Figure 44).

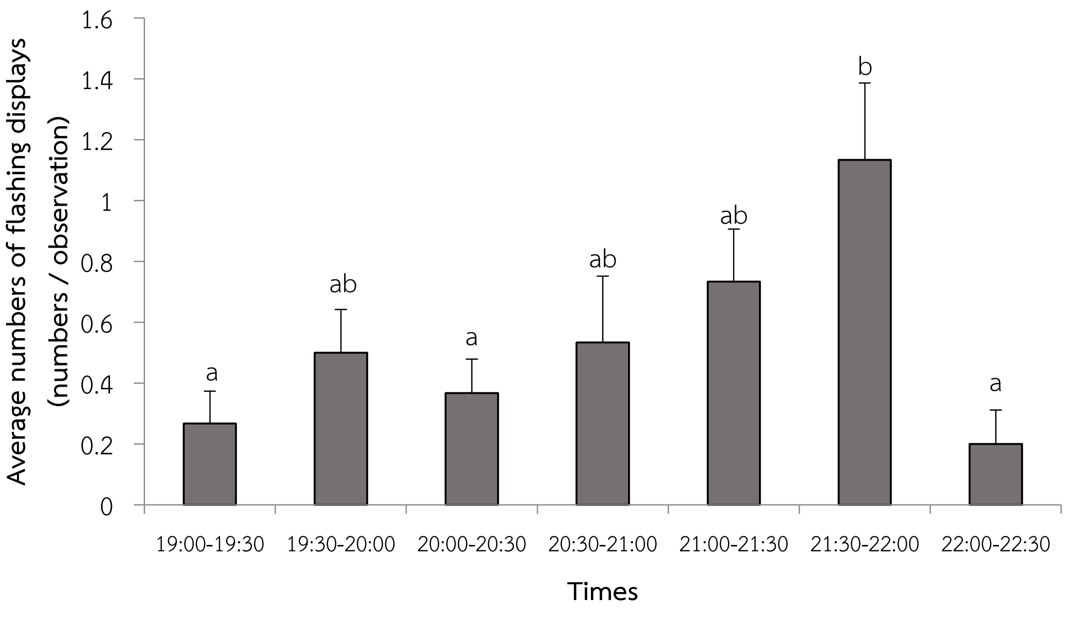


*Different letters (a and b) designate statistical significance of group differences.

Figure 44 The comparisons of the means (± SE) flashing numbers of adult male *Pyrocoelia tonkinensis* fireflies every hour from 18:00 PM to 6:00 AM in the study site A3 at the Chulalongkorn University Forest and Research Station.

From the results of flashing display in adult male *P. tonkinensis* firefly all year round and flashing display in adult male *P. tonkinensis* firefly at nighttime, five months; April, May, June, July and August 2013 were selected to calculate the flashing display numbers of adult male *P. tonkinensis* firefly in every 30 minutes from 19:00 PM to 22:30 PM.

In addition, the mean flashing numbers of adult male *P. tonkinensis* firefly at the peaked months (April, May, June, July and August 2013) from 19:00 PM to 22:30 PM were significant differences in each time and the time period from 21:30 PM to 22:00 PM was the significant period which most of firefly flashing activity was found in the study site A3 at the Chulalongkorn University Forest and Research Station. (Kruskal-Wallis test *F-value* = 18.950, df = 6, *p-value* = 0.0040, Pairwise multiple comparison at $P \leq 0.05$) (Figure 45).



*Different letters (a and b) designate statistical significance between group differences.

Figure 45 The mean (± SE) flashing numbers of adult male *Pyrocoelia tonkinensis* fireflies in every 30 minutes from 19:00 PM to 22:30 PM at the peaked months (April, May, June, July and August) in the study site A3 at the Chulalongkorn University Forest and Research Station.

6.4 Discussion

In the study of flashing display all year round, the flashing numbers of adult male *P. tonkinensis* firefly were frequently observed in April and May 2013. However, the absence of flashing numbers in October 2013 while the displays occurred in November 2013 might be because of the heavy rainfall of all three study nights in this month (average rainfall = 7.13 ± 0.46 mm, data obtained from the Department of Meteorology at Wiang Sa District Station).

In addition, the display numbers of adult male *P. tonkinensis* firefly from March 2013 to November 2013 might be the best information to indicate the numbers of firefly larvae when compared with the firefly *P. praetaxta* (the closest species) (Sommit, 2004) life cycle. For instance, in the firefly life cycle, they spent long time (might be 100 to 300 days) to grow up in larval stage from the first instar to the last instar (the fifth instar) (Chunram and Lewvanich, 1996) while spent 20 to 30 days for mating in the adult stage. Likewise, adult male *P. tonkinensis* fireflies were frequency observed or mated in April and May 2013. 2, firefly larvae were found in July 2013 or one month after mating season (Figure 40). This information probably suggested that the larval fireflies should be observed more than three months as the resulted (Chapter V).

Nevertheless, the results of flashing display of *P. tonkinensis* fireflies were diverse to flashing displays in *Pyrocoelia praetexta* in Chiang Mai Province, Thailand, which the displays regularly found in June (Sommit, 2004). On the other hand, the flashing displays of *Luciola cruciata* in Japan peaked from July to August (Yuma and Hori, 1981). The results indicated that the variation of firefly species produced the difference periods of firefly flashing displays (Barrows et al., 2008). For instance, flashing activity of adults *Pyrocoelia pectoralis* firefly occurred from late September to late October which emergence of peak in mid-October (Wang et al., 2007). Moreover, the study of the firefly abundances in six genera in the Dyke Marsh Wildlife Preserve, Virginia reported that flashing display of *Ellychnia corrusca* occurred in April, *Lucidota atra* occurred in June to July, *Photinus pyralis* occurred in June to August, *Photinus* spp. occurred in May to August, *Pyractomena lucifera* occurred in June to July and *Pyropyga decipiens* occurred in June to October. The results suggested that flashing displays vary among the firefly taxa even they lived in the same habitat (Barrows et al., 2008).

For the flashing display at nighttime, the results indicated that the time period from 21:30 PM to 22:00 PM was the significant period which the most of firefly flashing activity was found in the study sites A3. Moreover, the results of flashing numbers in *P. tonkinensis* fireflies at nighttime were related to the study in the biology of *P. praetexta* in Chiang Mai Province, which the flashing displays peaked at about 20:00 PM to 21:00 PM (Sommit, 2004). Moreover, the study of flashing displays in *Photinus greeni* firefly indicated that the displays began at about 15 minutes after sunset and ceased at about 90 minutes after sunset in Lincoln, USA (Demary et al., 2006). However, the differences in flashing numbers during nighttime might be because of the results of natural fluctuations in their population site due to the weather (Barrows et al., 2008) and the other factors such as firefly species and habitats. Moreover, the rapidly decrease of flashing display after peak might be related to the energy that a firefly used to flight and produced the light emission. Also, adult fireflies regularly hide in the safety place such as under leaves for avoiding their predators after mating (Kirton et al., 2012).

6.5 Conclusion

Overall, the flashing displays of adult male *Pyrocoelia tonkinensis* firefly species in the Chulalongkorn University Forest and Research Station, Wiang Sa District, Nan Province statistically peaked in May 2013. The period of time at about 21:00 PM to 22:00 PM was the significant period that the most of flashing displays was found. Last of all, the suitable time for sighted the beautiful natural creation of firefly flashing display in this area is practically from 19:00 PM to 21:00 PM.

CHAPTER VII

CONCLUSION AND RECOMMENDATION

7.1 Conclusion

Food preference of firefly *Pyrocoelia tonkinensis* larvae on land snails indicated that the third, the forth and the fifth instar larvae significantly preferred *Cryptozona siamensis* land snail as their prey. Accordingly, this result should be the primary data which firefly *P. tonkinensis* larvae might possibly use as a biological agent to control the *C. siamensis* land snail population at the study area in the future.

Feeding behavior of firefly *P. tonkinensis* larvae revealed that the behavior were divided into two phases; first, searching phase which consisted of searching and approaching and second, handling phase which consisted of attacking and feeding. The six behavioral units were found in this study such as searching, attacking, glowing, feeding, cleaning and walking around. Multiple comparison of behavioral times indicated that firefly larvae significantly spend their time to feed more than the other behavioral units. However, the glowing behavior of firefly larvae during feeding time was interesting as well as they usually fed the snail prey together after the snail was attacked in the field (Buschman, 1984b). These recorded might indicate specific behavior of firefly larvae during feeding. Therefore, the feeding behavior experiment should be concerned and considered about these records in the future.

Firefly *P. tonkinensis* larva population was largest in September 2013 and *C. siamensis* land snail population was highest in July and September 2013 at the Chulalongkorn University Forest and Research Station, Wiang Sa District, Nan Province. Spearman rank correlation between the firefly larva and land snail populations indicated that land snail populations were responsible for the majority of the significant positive correlated with the population size of firefly larvae.

The suitable environmental conditions for the firefly *P. tonkinensis* larvae in the peak month (September 2013) were 85.92 ± 0.736 % of relative humidity, 10.45 ±

1.06 % of soil moisture, 1.75 ± 2.90 mm of rainfall, 11.95 ± 3.38 cm of herbaceous height and 83.5 ± 9.79 % of herbaceous coverage. Moreover, the suitable environmental conditions for the *C. siamensis* land snails in the peaked month (July 2013) were 87.92 ± 2.21 % of relative humidity, 13.27 ± 1.367 % of soil moisture, 5.95 ± 5.93 mm of rainfall, 72.49 ± 11.20 % of canopy coverage, 13.38 ± 6.05 cm of herbaceous height and 90.88 ± 7.20 % of herbaceous coverage.

Multiple regression analysis of the environmental factors and the firefly larva population in seven months revealed that herbaceous coverage was the majority of the significant positive effect on the firefly larva population. Similarly, the interaction of soil moisture, canopy coverage and herbaceous coverage were the significant positive effects on the land snail population.

Additionally, flashing displays of adult male *P. tonkinensis* firefly statistically peaked in May 2013 at the Chulalongkorn University Forest and Research Station, Wiang Sa District, Nan Province. The period of time at about 21:00 PM to 22:00 PM was the significantly period that the most of flashing displays was found. As a results, the suitable time for sighted the beautiful natural creation of firefly flashing display in this area is practically from 19:00 PM to 21:00 PM.

7.2 Recommendation

1. Land snail pest as *C. siamensis* land snail can be destroyed in the several different ways but the chemical control measures probably damaged their predator population or firefly larva population. So, the mechanical control, agricultural control measures or biological control should be the best ways for land snail pest control.

2. The long term monitoring of firefly larva including land snail populations should consider the environmental factors such as relative humidity, soil moisture, canopy coverage especially herbaceous coverage that influence the local firefly larvae including land snail prey populations.

3. The firefly flashing display were disturbed by artificial light which the courtship behavior was expanded and unsuccessful (Thancharoen, 2007). So, the firefly mating area should not have light pollutions including deforestation and forest fire.

REFERENCES

Altmann, J. 1974. Observational study of behavior: Sampling methods. Behaviour 49: 227-267.

Anderson, A.R., Hoffmann, A.A., McKechnie, S.W., Umina, P.A. and Weeks, A.R. 2005. The latitudinal cline in the In(3R)Payne inversion polymorphism has shifted in the last 20 years in Australian *Drosophila melanogaster* populations. Molecular Ecology 14: 851-8.

Aprille, J.R., Lagace, C.J., Modica-Napolitano, J. and Trimmer, B.A. 2004. Role of nitric oxide and mitochondria in control of firefly flash. Integrative and Comparative Biology 44: 213-219.

Ballantyne, L.A. and Lambkin, C.L. 2013. Systematics and phylogenetics of Indo-Pacific Luciolinae fireflies (Coleoptera: Lampyridae) and the description of new genera. Zootaxa 3653: 1-162.

Ballantyne, L.A. and McLean, M.R. 1970. Revisional studies on the firefly genus *Pteroptyx* Olivier (Coleoptera: Lampyridae: Luciolinae: Luciolini). Transactions of the American Entomological Society 96: 223-305.

Ballantyne, L.A. and Menayah, R. 2002. A description of larvae and redescription of adults of the firefly *Pteroptyx valida* Olivier in Selangor, Malaysia (Coleoptera: Lampyridae: Luciolinae), with notes on Luciolinae larvae. Raffles Bulletin of Zoology 50: 101-109.

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.

Bassot, J.M. and Polunin, I.V. 1967. Synchronous flashing fireflies inthe Malay Penisula. Science Report of Yokosuka City Museum 13: 18-23.

Blum, M.S. and Sannasi, A. 1997. Reflex bleeding in the lampyrid *Photinus pyralis*: Defensive function. Insect Physiology 20: 451-460.

Branham, M.A. and Greenfield, M.D. 1996. Flashing males win mate success. Nature 381: 745-746.

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. 1988. Synchronous rhythmic flashing of fireflies. II. The Quarterly Review of Biology 63: 265-289.

Buck, J.B. 1937. Studies on the firefly. I. The effects of light and other agents on flashing in *Photinus pyralis,* with special reference to periodicity and diurnal rhythm. Physiological Zoology 10: 45-58.

Buschman, L.L. 1984a. Biology of the Firefly *Pyractomena lucifera* (Coleoptera: Lampyridae). The Florida Entomologist 67: 529-542.

Buschman, L.L. 1984b. Larval biology and ecology of *Photuris* fireflies (Lampyridae: Coleoptera) in northcentral Florida. Journal of the Kansas Entomological Society 57: 7-16.

Buschman, L.L. 1988. Larval development and its photoperiodic control in the firefly *Pyractomena lucifera* (Coleoptera: Lampyridae). Annals of the Entomological Society of America 81: 82-90.

Carlson, A.D. and Copeland, J. 1985. Flash communication in fireflies. The Quarterly Review of Biology 60: 415-436.

Case, J.F. 1984. Vision in mating behavior of fireflies. In: Insect Communication. Academic Press, New York.

Chunram, S. and Lewvanich, A. 1996. Behavior and life cycle of fireflies, *Luciola* sp. Journal of Agriculture 4: 44-55.

Chunram, S. and Lewvanich, A. 2001. Biodiversity and ecology of fireflies in central and eastern Thailand. Nontree.

Clench, W.J. and Jacobson, M.K. 1968. Monograph of the Cuban genus *Viana* (Mollusca: Archaeogastropoda: Helicinidae). Breviora 298: 1-25.

Copeland, J. 1981. Effects of larval firefly extracts on molluscan cardiac activity. Experientia 37: 1271-1272.

Copeland, J. and Moiseff, A. 1997. The effect of flash duration and flash shape on entrainment in *Pteroptyx malaccae*, a synchronic Southeast Asian firefly. Insect Physiology 43: 965-971.

De Cock, R. 2009. Biology and behaviour of European lampyrids. In Meyer-Rochow, V.B. (ed.), In Bioluminescence in Focus-a Collection of Illuminating Essays, pp. 161-200. Research Signpost, Kerala, India.

De Cock, R. and Matthysen, E. 2003. Glow-worm larvae bioluminescence (Coleoptera: Lampyridae) operates as an aposematic signal upon toads (*Bufo bufo*). Behavioral Ecology 14: 103-108.

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.

Domagala, P. and Ghiradella, H. 1984. Structure and function of the terminal abdominal appendages (pygypodia) of *Photurid* firefly larvae. Biological Bulletin 166: 299-309.

Dreisig, H. 1975. Environmental control of the daily onset of luminescent activity in glowworms and fireflies (Coleoptera: Lampyridae). Oecologia 18: 85-99.

Eisner, T., Goetz, M.A., Hill, D.E., Smedley, S.R. and Meinwald, J. 1997. Firefly "femmes fatales" acquire defensive steroids (lucibufagins) from their firefly prey. Proceedings of the National Academy of Sciences of the United States of America 94: 9723-9728.

Farnworth, E.G. 1973. Flashing behavior, ecology and systematics of Jamican lampyrid fireflies. Ph.D. dissertation, University of Florida.

Faust, L.F. 2010. Natural history and flash repertoire of the synchronous firefly *Photinus carolinus* (Coleoptera: Lampyridae) in the Great Smoky Mountains National Park. Florida Entomologist 93: 208-217.

Fontana, L., Partridge, L. and Longo, V.D. 2010. Extending healthy life span—from yeast to humans. Science 328: 321-326.

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

Fu, X. and Meyer-Rochow, V.B. 2012. An investigation into the morphological and behavioral adaptations of the aquatic larvae of *Aquatica leii* (Coleoptera:

Lampyridae) to prey upon freshwater snails that serve as intermediate hosts for the liver fluke. Biological Control 62: 127-134.

Fu, X., Meyer-Rochow, V.B., Tyler, J., Suzuki, H. and De Cock, R. 2009. Structure and function of the eversible organs of several genera of larval firefly (Coleoptera: Lampyridae). Chemoecology 19: 155-168.

Fu, X., Nobuyoshi, O., Zhang, Y. and Lei, C. 2006. A rearing apparatus and diet for the aquatic firefly *Luciola leii* (Coleoptera: Lampyridae). The Canadian Entomologist 138: 399-406.

Fu, X., Ohba, N., Vencl, F. and Lei, C. 2005. Structure, behavior, and the life cycle of an aquatic firefly, *Luciola substriata*, in China. Canadian Entomologist 137: 83-90.

Geisler, T. and Topczewska, S. 2012. Analysis of the structure and mechanism of wing folding and flexion in *Xylotrupes gideon* beetle (l. 1767) (Coloptera, Scarabaeidae). Acta Mechanica et Automatica 6: 37-44.

Godan, D. 1983. Pest slugs & snails –Biology & control. Springer Verlag Berlin Heidelberg 73: 287-293.

Gorham, H.S. 1880. Materials for a revision of the Lampyridae. Transactions of the Entomological Society of London 1880: 83-112.

Guzmán Álvarez, J.R. and De Cock, R. 2011. The biology and distribution of glow-worms (Coleoptera: Lampyridae) in Spain. Lampyrid 1: 22-31.

Hayashi, F. and Suzuki, H. 2003. Fireflies with or without prespermatophores: Evolutionary origins and life-history consequences. Entomological Science 6: 3-10.

Ho, J.Z. and Chiang, P.H. 1997. Two firefly species with aquatic larvae in Taiwan. Nature and Conservation Quarterly 17: 42-46.

Ho, J.Z. and Chiang, P.H. 2002. Shadows of firefly glowing on the water. the conservation and recovery of aquatic fireflies: Taiwan Endemic Species Research Institute, Nantou, Chichi.

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.

Ho, J.Z., Chu, C.S. and Chu, C.C. 1998. A discovery of the aquatic larvae of *Luciola substriata.* Nature and Conservation Quarterly 22: 47-51.

Ho, J.Z. and Huang, S.W. 2003. Effects of temperature and egg size on egg duration, hatching rate, and starvation tolerance of first instar larvae of the firefly *Pyrocoelia analis.* Formosan Entomology 24: 305-312.

Holomuzki, J.R. and Biggs, B.J.F. 1999. Distributional responses to flow disturbance by a stream-dwelling snail. Oikos 87: 36-47.

Ineichen, S. and Rüttimann, B. 2012. Impact of artificial light on the distribution of the common European glow-worm, *Lampyris noctiluca* (Coleoptera: Lampyridae). Lampyrid 2: 31-36.

Jeng, M.L., Lai, J., Yang, P.S. and Sato, M. 1999. On the validity of the generic name *Pyrocoelia* Gorham (Coleoptera, Lampyridae, Lampyrinae), with a review of Taiwanese species. Japanese Journal of systematic Entomology 5: 347-362.

Jeng, M.L., Yang, P.S. and Lai, J. 2003. Notes on the genus *Luciola* (Coleoptera, Lampyridae, Lampyrinae) of Taiwan. Specialist Bulletin Japanese Society Coleopterology 6: 247-262.

Johansson, L.C., Engel, S., Baird, E., Dacke, M., Muijres, F.T. and Hedenstrom, A. 2012. Elytra boost lift, but reduce aerodynamic efficiency in flying beetles. Journal of the Royal Society, Interface 9: 2745-8.

Jusoh, W.F.A.W., Hashim, N.R. and Ibrahim, Z.Z. 2010a. Distribution and abundance of *Pteroptyx* fireflies in Rembau-Linggi Estuary, Peninsular Malaysia. Environmental Asia 3: 56-60.

Jusoh, W.F.A.W., Hashim, N.R. and Ibrahim, Z.Z. 2010b. Firefly distribution and abundance on mangrove vegetation assemblages in Sepetang estuary, Peninsular Malaysia. Wetlands Ecology and Management 18: 367-373.

Kady, M.M., Ghanim, A.A., El-Adl, A.A. and Nassar, O.A. 1983. Seasonal abundance of the terrestrial snail *Monacha obstructa* & its chemical control. Journal of AgriculturalScience Mansoura University 8: 377-383.

Kaufman, T. 1965. Ecological and biological studies on the West African firefly *Luciola discicollis* (Coleoptera: Lampyridae). Annals of the Entomological Society of America 58: 414-426.

Kazantsev, S.V. 2012. New omethid and lampyrid taxa from the Baltic Amber (Insecta: Coleoptera). Zootaxa 3186: 59-63.

Kirton, G.L., Nada, B., Khoo, V. and Phon, C.K. 2012. Monitoring populations of bioluminescent organisms using digital night photography and image analysis: a case study of the fireflies of the Selangor River, Malaysia. Insect Conservation and Diversity 5: 224-250.

LaBella, D.M. and Lloyd, J.E. 1991. Lampyridae. In Stehr, F.W. (ed.), Immature Insects pp. 427-428. Kendall Hunt.

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

Lloyd, J.E. 1966. Studies on the flash communication system of *Photinus* fireflies. Museum of Zoology, University of Michigan Miscellaneous Publications

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 26: 155-163.

Lloyd, J.E. 1973a. Fireflies of Melanesia: bioluminescence, mating behavior, and synchronous flashing (Coleoptera: Lampyridae). Environmental Entomology 2: 991–1008.

Lloyd, J.E. 1973b. Model for the mating protocol of synchronously flashing fireflies. Nature 245: 268-270.

Lloyd, J.E. 1978. Insect bioluminescence In Herring (ed.), Biolominescence in Action, pp. 241-272. Academic Press. New York.

Lloyd, J.E. 1979. Sexual selection in luminescent beetles. In Blum, M.S. and Blum, N.A. (ed.), In sexual selection and reproductive competition in insects, pp. 293-342. New York Academic Press.

Lloyd, J.E. 1997a. Firefly mating ecology, selection and evolution. In Choe, J.C. and Crespi, B.J. (ed.), The evolution of mating systems in insects and arachnids., pp. 184-192. Cambridge University Press. Cambridge.

Lloyd, J.E. 1997b. On research and entomological education, and a different light in the lives of fireflies (Coleoptera: Lanpyridae; *Pyractomena*). Florida Entomologist 81: 120-131.

Lloyd, J.E. 2002. Lampyridae. In Arnett, R.A., et al. (ed.), American Beetles, pp. 187-196. Boca Raton: CRC Press.

Long, S.M., Lewis, S., Jean-Louis, L., Ramos, G., Richmond, J. and Jakob, E.M. 2012. Firefly flashing and jumping spider predation. Animal Behaviour 83: 81-86.

Mair, W. and Dillin, A. 2008. Aging and survival: the genetics of life span extension by dietary restriction. Annual Review of Biochemistry 77: 727-54.

Majka, C. and MacIvor, J. 2009. The European lesser glow worm, *Phosphaenus hemipterus* (Goeze), in North America (Coleoptera, Lampyridae). ZooKeys 29.

Marques, S.M. and Esteves da Silva, J.C. 2009. Firefly bioluminescence: a mechanistic approach of luciferase catalyzed reactions. IUBMB Life 61: 6-17.

McDermott, F.A. 1966. Lampyridae. In Steel, W.O. (ed.), Coleopterorum Catologus Supplementa, pp. 149. Netherland.

McLean, M., Buck, J. and Hanson, F.E. 1972. Culture and larval behavior of *Photurid* fireflies. American Midland Naturalist 87: 133-145.

Minami, K. 1961. A study of fireflies. Scientist Press, Tokyo.

Nakagawa, S., Lagisz, M., Hector, K.L. and Spencer, H.G. 2012. Comparative and meta-analytic insights into life extension via dietary restriction. Aging Cell 11: 401-9.

Nallakumar, K. 2002. Management and conservation of fireflies in Peninsular Malaysia. Ph.D. Thesis, University of London.

Ohba, N. 1983. Studies on the communication systems of Japanese fireflies. Science Reports of the Yokosuka City Museum 3: 1-62.

Ohba, N. 1988. Gaji firefly. Bun-ichi Sogo Shuppan.

Ohba, N. 2004. Flash communication systems of Japanese fireflies. Integrative and Comparative Biology 44: 225-33.

Ohba, N. and Hidaka, T. 2002. Reflex bleeding of fireflies and prey-predator relationship. The Yokosuka City Museum 45: 45-50.

Ohba, N. and Sim, S.H. 1994. The morphology, behaviour and life cycle of *Pteroptyx valida* (Coleoptera: Lampyridae) in Singapore. Science Report of the Yokosuka City Museum 42: 1-11.

Rabinowitz, A. 1997. Wildlife field research and conservation training manual. 2nd. New York: WCS.

Rios, O.M. and Quinta, M.H. 2010. Larval feeding habits of the Cuban endemic firefly *Alecton discoidalis* Laporte (Coleoptera: Lampyridae). Psyche 2010.

Sallam, A. and El-Wakeil, N. 2012. Biological and ecological studies on land snails and their control. Integrated Pest Management and Pest Control – Current and Future Tactics.

Schwalb, H.H. 1960. Beitrage zur Biologie der einheimischen Lampyriden *Lampyris noctulica* and *Phausis splendidula* and experimentelle Analyse ihres Beutefang and Sexualverhaltens. Zoologische Jahrbücher Systematik 88: 399-550.

Sitorus, P.E., Park, H.C., Byun, D., Goo, N.S. and Han, C.H. 2010. The role of elytra in beetle flight: I. generation of quasi-static aerodynamic forces. Journal of Bionic Engineering 7: 354-363.

Sivinski, J. 1981. The nature and possible functions of luminescence in Coleoptera larvae. The Coleopterists Bulletin 35: 167-179.

Sommit, W. 2004. Life History of Firefly *Pyrocoelia praetexta* Olivier. Master degree, Entomology Department Faculty of Agriculture, Chiang Mai University.

Srihata, S., Tumpeesuwan, C. and Tumpeesuwan, S. 2010. Species diversity, abundance and habitats of land snail in a square kilometer on Phu No, Kalasin Province. Journal of Science and Technology Mahasarakham University 29: 359-371.

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.

Stansbury, M.S. and Moczek, A.P. 2014. The function of Hox and appendage-patterning genes in the development of an evolutionary novelty, the *Photuris* firefly lantern. Proceedings of the Royal Society, Biological Sciences.

,

Sutcharit, C. and Panha, S. 2008. Land snails of Khao Nan National Park. Biological Resource Management (BRT), Thailand.

Suzuki, H. 1997. Molecular phylogenetic studies of japan fireflies and their mating system (Coleoptera: Cantharoidae). University of Bulletin Natural History 3: 1-53.

Thancharoen, A. 2007. The biology and mating behavior of an aquatic firefly species, *Luciola aquatilis* sp. nov. Thancharoen (Coleoptera: Lampyridae). Docter of Philosophy (Biology), Faculty of Graduate Studies Mahidol University.

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

Timmins, G.S., Robb, F.J., Wilmot, C.M., Jackson, S.K. and Swartz, H.M. 2001. Firefly flashing is controlled by gating oxygen to light-emitting cells. Journal of Experimental Biology 204: 2795-2801.

Tyler, J. 1994. Glow-worms. Tyler-Scagell.

Underwood, T.J., Tallamy, D.W. and Pesek, J.D. 1997. Bioluminescence in firefly larvae: a test of the aposematic display hypothesis (Coleoptera: Lampyridae). Journal of Insect Behavior 10: 365-370.

Vencl, F.V., Blasko, B.J. and A.D., C. 1994. Flash behavior of female *Photuris versicolor* fireflies in simulated courtship and predatory dialogues. Journal of Insect Behavior 7: 843-858.

Vencl, F.V. and Carlson, A.D. 1998. Proximate mechanisms of sexual selection in the firefly *Photinus pyralis* (Coleoptera: Lampyridae). Journal of Insect Behavior 11: 191-207.

Viviani, V.R. 2001. Fireflies (Coleoptera: Lampyridae) from Southeastern Brazil: Habitats, life history, and bioluminescence. Annals of the Entomological Society of America 94: 129-145.

Viviani, V.R., Rosa, S.P. and Martins, M.A. 2012. *Aspisoma lineatum* (Gyllenhal) (Coleoptera: Lampyridae) firefly: description of the immatures, biological, and ecological aspects. Neotropical Entomology 41: 89-94.

Viviani, V.R. and Santos, R.M.D. 2012. Bioluminescent Coleoptera of biological station of boracéia (Salesópolis, SP, Brazil): diversity, bioluminescence and habitat distribution. Biota Neotropica 12: 21-34.

Wang, Y., Fu, X., Lei, C., Jeng, M.-L. and Nobuyoshi, O. 2007. Biological characteristics of the terrestrial firefly *Pyrocoelia pectoralis* (Coleoptera: Lampyridae). The Coleopterists Bulletin 61: 85-93.

Williams, F.X. 1917. Notes on the life-history of some north American lampyridae. Journal of the New York Entomological Society 25: 11-33.

Yuma, M. 1986. Growth and size variations in the larvae of *Luciola cruciate* (Coleoptera: Lampyridae) in the relation to egg size. Physiology and Ecology Japan 23: 45-78.

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

Yuma, M. and Hori, M. 1981. Gregarious oviposition of *Luciola cruciata* Motschulsky (Coleoptera: Lampyridae). Physiology and Ecology Japan 181: 93-112.

Yuma, M. and Hori, M. 1990. Seasonal and age-related changes in the behavior of the Genji firefly, *Luciola cruciata* (Coloptera: Lampyridae). Japanese Journal of Entomology 58: 863-870.

APPENDICES

Appendix A

Table A-1 Land snail preference of firefly *Pyrocoelia tonkinensis* larvae

Firefly *Pyrocoelia tonkinensis* larvae						Land snail preference
No.	Co. Date	length	Weight	Stage	Size	
PT1	20/8/2013	2.231	0.025	3	M	*Cryptozona siamensis*
PT2	21/8/2013	3.562	0.037	5	L	*Sarika resplendens*
PT3	22/8/2013	3.145	0.031	5	L	*Cryptozona siamensis*
PT4	26/9/2013	2.855	0.028	4	M	*Cryptozona siamensis*
PT5	27/9/2013	3.125	0.030	5	L	*Cryptozona siamensis*
PT6	28/9/2013	3.100	0.027	5	L	*Cryptozona siamensis*
PT7	29/9/2013	2.125	0.022	3	M	*Cryptozona siamensis*
PT8	30/9/2013	3.940	0.039	5	L	*Cryptozona siamensis*

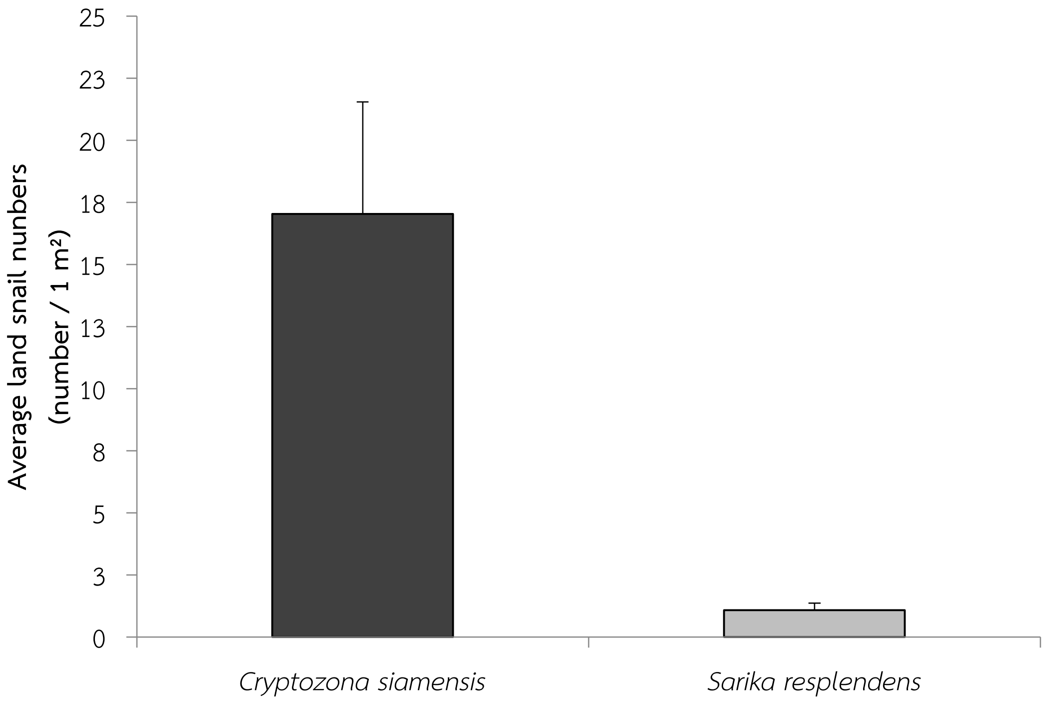


Figure A-1 The mean (± SE) of two land snails (*Cryptozona siamensis* and *Sarika resplendens*) per one square meters (number / 1 m^2) in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Appendix B

Table B-1 Total numbers of firefly *Pyrocoelia tonkinensis* larvae in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Times											
	19.00-20.00				20.00-21.00				21.00-22.00			
	A2	A3	A4	A5	A2	A3	A4	A5	A2	A3	A4	A5
Feb-13	0	0	0	0	0	0	0	0	0	0	0	0
Mar-13	0	0	0	0	0	0	0	0	0	0	0	0
Apr-13	0	0	0	0	0	0	0	0	0	0	0	0
May-13	0	0	0	0	0	0	0	0	0	0	0	0
Jun-13	0	0	0	0	0	0	0	0	0	0	0	0
Jul-13	0	0	0	0	0	0	0	0	0	0	1	0
Aug-13	0	0	0	0	0	0	0	0	0	2	0	1
Sep-13	5	3	0	1	2	2	0	1	0	1	0	0
Oct-13	0	0	0	0	0	0	0	0	0	0	0	0
Nov-13	0	0	0	0	0	0	0	0	0	0	0	0
Dec-13	0	0	0	0	0	0	0	0	0	0	0	0
Jan-14	0	0	0	0	0	0	0	0	0	0	0	0
Feb-14	0	0	0	0	0	0	0	0	0	0	0	0

Table B-2 Sampling numbers of *Cryptozona siamensis* land snails in four study sites during 19:00 PM to 22:00 PM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Times											
	19.00-20.00				20.00-21.00				21.00-22.00			
	A2	A3	A4	A5	A2	A3	A4	A5	A2	A3	A4	A5
Feb-13	0	0	0	0	0	0	0	0	0	0	0	0
Mar-13	0	0	0	0	0	0	0	0	0	0	0	0
Apr-13	0	0	0	0	0	0	0	0	0	0	0	0
May-13	0	0	1	1	0	0	0	0	0	0	0	0
Jun-13	0	0	0	0	0	0	0	0	0	0	0	0
Jul-13	13	2	18	20	16	5	16	24	9	0	20	23
Aug-13	18	3	6	5	26	6	11	4	15	5	6	4
Sep-13	14	9	13	34	12	8	8	27	11	3	3	18
Oct-13	12	13	5	12	7	6	4	11	5	3	3	7
Nov-13	1	0	2	0	1	1	1	0	1	2	3	2
Dec-13	0	0	0	0	0	0	0	0	0	0	0	0
Jan-14	0	0	0	0	0	0	0	0	0	0	0	0
Feb-14	0	0	0	0	0	0	0	0	0	0	0	0

Appendix C

Table C-1 Air temperature (°C) in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Study sites				Mean	SD
	A2	A3	A4	A5		
Feb-13	24.73	25.83	24.80	24.90	25.07	0.51
Mar-13	21.50	24.87	22.83	21.43	22.66	1.61
Apr-13	28.00	29.17	27.83	28.67	28.45	0.62
May-13	29.33	29.83	29.17	29.00	29.17	0.36
Jun-13	30.17	27.67	30.50	28.33	29.33	1.38
Jul-13	25.83	24.93	26.17	26.43	25.84	0.65
Aug-13	26.17	24.57	27.33	26.67	26.18	1.18
Sep-13	25.67	24.33	25.17	26.50	25.42	0.91
Oct-13	22.83	22.67	23.50	23.33	23.08	0.40
Nov-13	24.50	24.50	25.50	24.00	24.63	0.63
Dec-13	16.17	15.50	15.83	16.33	15.96	0.37
Jan-14	15.07	12.93	15.07	15.20	14.57	1.09
Feb-14	25.47	24.23	25.83	26.00	25.38	0.80
Mean	24.26	23.93	24.58	24.37		
SD	4.33	4.61	4.39	4.22		

Table C-2 Relative humidity (%) in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Study sites				mean	SD
	A2	A3	A4	A5		
Feb-13	67.00	79.67	72.00	70.67	72.33	4.61
Mar-13	53.67	68.00	56.67	56.67	58.75	5.48
Apr-13	55.67	54.00	61.67	62.33	58.42	3.64
May-13	79.33	69.33	79.33	79.33	76.83	4.33
Jun-13	60.33	77.67	62.33	63.33	65.92	6.87
Jul-13	87.67	88.33	87.67	88.00	87.92	0.28
Aug-13	81.00	86.00	82.33	82.33	82.92	1.86
Sep-13	85.00	87.67	84.67	86.33	85.92	1.19
Oct-13	73.67	87.00	75.33	76.00	78.00	5.27
Nov-13	81.33	83.00	84.33	84.33	83.25	1.23
Dec-13	84.00	84.33	85.00	85.33	84.67	0.53
Jan-14	59.67	58.33	60.00	60.00	59.50	0.69
Feb-14	54.33	53.67	56.33	55.00	54.83	0.99
Mean	70.97	75.15	72.90	73.05		
SD	12.40	12.52	11.48	11.74		

Table C-3 Soil temperature (°C) in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Study sites				Mean	SD
	A2	A3	A4	A5		
Feb-13	27.00	30.00	27.40	28.67	28.27	1.17
Mar-13	26.17	28.33	28.00	25.83	27.08	1.10
Apr-13	30.50	33.50	31.33	28.67	31.00	1.74
May-13	28.17	34.67	32.33	30.83	31.50	2.36
Jun-13	31.00	32.50	32.67	31.17	31.83	0.75
Jul-13	26.50	27.67	29.83	27.83	27.96	1.20
Aug-13	30.00	29.00	27.50	26.00	28.13	1.52
Sep-13	29.00	27.33	26.43	26.83	27.40	0.98
Oct-13	26.50	24.33	26.17	24.00	25.25	1.10
Nov-13	27.50	27.17	28.83	26.17	27.42	0.95
Dec-13	21.83	21.83	20.00	19.33	20.75	1.11
Jan-14	19.50	22.50	20.50	20.33	20.71	1.10
Feb-14	27.83	28.33	29.33	29.17	28.67	0.61
Mean	27.04	28.24	27.72	26.53		
SD	3.13	3.75	3.75	3.46		

Table C-4 Soil moisture (%) in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	**Study sites**				**Mean**	**SD**
	A2	A3	A4	A5		
Feb-13	3.82	0.92	11.92	1.84	4.63	4.34
Mar-13	1.58	1.29	4.76	6.69	3.58	2.25
Apr-13	3.52	0.88	9.36	11.71	6.37	4.35
May-13	1.76	0.75	2.14	2.07	1.68	0.55
Jun-13	2.31	2.86	4.07	2.02	2.81	0.78
Jul-13	10.93	20.48	11.66	10.00	13.26	4.21
Aug-13	7.07	3.96	7.12	9.57	6.93	1.99
Sep-13	7.79	9.99	14.27	9.76	10.45	2.36
Oct-13	12.55	10.95	10.40	10.56	11.12	0.85
Nov-13	4.22	2.80	7.69	3.51	4.55	1.88
Dec-13	4.33	2.33	3.38	5.01	3.76	1.01
Jan-14	2.09	0.59	2.07	1.38	1.53	0.61
Feb-14	1.16	0.41	6.62	0.97	2.29	2.51
Mean	4.86	4.48	7.34	5.78		
SD	3.52	5.68	3.82	3.91		

Table C-5 Rainfall (mm) from February 2013 to February 2014 recorded by the Meteorological Department at the Wiang Sa District Station, Nan Province.

Months	**Study dates**				**Mean**	**SD**
	Day 1	Day 2	Day 3	Day 4		
Feb-13	0	0	0	0	0.00	0.00
Mar-13	0	0	0	0	0.00	0.00
Apr-13	0	0	2.2	2	1.05	0.00
May-13	0	6.5	2.2	0	2.18	1.35
Jun-13	2	0	0	0	0.50	1.91
Jul-13	0	2.3	5.5	16	5.95	5.93
Aug-13	1	0	16	1	4.50	0.80
Sep-13	0	2	3.8	1.2	1.75	2.90
Oct-13	5.8	22	0.7	0	7.13	0.91
Nov-13	0	0.5	0	0	0.13	2.18
Dec-13	0	0	0	0	0.00	0.00
Jan-14	0	0	0	0	0.00	0.00
Feb-14	0	0	0	0	0.00	0.00

Table C-6 Canopy coverage (%) in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Study sites				Mean	SD
	A2	A3	A4	A5		
Feb-13	85.58	29.00	84.67	92.17	72.85	25.48
Mar-13	69.33	27.60	89.00	82.33	67.07	23.86
Apr-13	68.25	35.92	72.00	96.50	68.17	21.55
May-13	72.42	31.54	79.83	99.17	70.74	24.65
Jun-13	82.00	46.00	67.67	98.50	73.54	19.28
Jul-13	78.08	35.04	84.67	92.17	72.49	22.19
Aug-13	88.67	10.65	86.50	96.00	70.45	34.71
Sep-13	87.83	8.33	87.00	97.67	70.21	35.97
Oct-13	86.67	27.13	43.17	80.33	59.32	24.93
Nov-13	89.25	15.17	81.67	67.00	63.27	28.90
Dec-13	65.83	15.17	59.50	43.33	45.96	19.58
Jan-14	89.58	19.80	51.33	40.50	50.30	25.35
Feb-14	31.75	5.96	22.00	27.67	21.84	9.80
Mean	76.56	23.64	69.92	77.95		
SD	15.40	11.68	19.76	24.19		

Table C-7 Herbaceous coverage (%) in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	**Study sites**				**Mean**	**SD**
	A2	A3	A4	A5		
Feb-13	73.00	64.50	90.67	90.67	79.71	11.36
Mar-13	79.67	24.95	97.33	37.33	59.82	29.68
Apr-13	61.33	43.17	82.67	37.33	56.13	17.70
May-13	69.00	60.83	52.00	72.00	63.46	7.78
Jun-13	79.00	71.33	86.67	43.33	70.08	16.37
Jul-13	91.67	90.50	90.67	90.67	90.86	0.46
Aug-13	78.00	90.50	80.00	79.33	81.96	4.98
Sep-13	96.67	92.67	94.00	50.67	83.50	19.01
Oct-13	84.67	89.33	84.67	74.67	83.33	5.35
Nov-13	87.33	60.17	85.33	52.00	71.21	15.41
Dec-13	71.33	70.83	70.00	62.00	68.54	3.81
Jan-14	5.58	35.13	80.67	73.33	57.28	30.30
Feb-14	55.67	53.17	25.33	29.33	40.87	13.64
Mean	71.76	65.16	78.46	60.97		
SD	22.08	21.28	18.95	19.96		

Table C-8 Herbaceous height (cm) in four study sites from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	Study sites				Mean	SD
	A2	A3	A4	A5		
Feb-13	10.43	13.22	20.64	9.03	13.33	4.48
Mar-13	6.93	5.34	7.47	3.56	5.82	1.53
Apr-13	5.65	6.47	6.39	2.61	5.28	1.57
May-13	7.78	10.08	8.17	11.44	9.37	1.48
Jun-13	11.79	19.72	20.58	5.83	14.48	6.06
Jul-13	8.01	15.84	20.64	9.03	13.38	5.16
Aug-13	7.03	65.44	8.19	10.47	22.78	24.66
Sep-13	8.50	27.62	7.28	4.40	11.95	9.17
Oct-13	7.42	15.42	6.72	9.47	9.76	3.42
Nov-13	8.26	13.06	6.42	4.44	8.05	3.20
Dec-13	6.71	10.97	2.94	2.69	5.83	3.37
Jan-14	3.57	3.02	5.58	4.67	4.21	0.99
Feb-14	1.93	7.76	4.31	1.44	3.86	2.50
Mean	7.23	16.46	9.64	6.08		
SD	2.47	15.46	6.17	3.23		

Appendix D

Table D-1 Flashing numbers of adult male *Pyrocoelia tonkinensis* fireflies at study site A3 during 18:00 PM to 6:00 AM from February 2013 to February 2014 at the Chulalongkorn University Forest and Research Station.

Months	**Times**													**Total**
	18:00	19:00	20:00	21:00	22:00	23:00	0:00	1:00	2:00	3:00	4:00	5:00	6:00	
Feb-13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mar-13	0	0	0	0	0	0	2	0	0	0	0	0	0	2
Apr-13	0	1	6	20	3	5	2	8	1	0	0	0	0	46
May-13	0	10	17	16	5	0	4	0	0	0	1	0	0	53
Jun-13	0	1	1	4	1	0	1	3	3	0	2	0	0	16
Jul-13	0	6	1	1	0	1	0	1	1	1	0	0	0	12
Aug-13	0	5	1	15	6	1	0	0	2	1	0	0	0	31
Sep-13	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Oct-13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nov-13	0	3	4	1	2	3	3	0	0	0	0	0	0	16
Dec-13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jan-14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Feb-14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	26	30	57	17	10	13	12	7	2	3	0	0	

VITA

Miss Kamolchanok Boriboon was born on November 7, 1988 in Bangkok, Thailand. In 2009, she graduated with a Bachelor's degree from the Department of Biology, Faculty of Science, Kasetsart University. In 2011, she continued her Master degree of science, Zoology program in the Department of Biology, Faculty of Science, Chulalongkorn University and completed the program in 2015. Her research was supported by Sponsorship for Graduate Student Research under CU Academic Network in the Region (CU-ANR-56-02) and the 90th anniversary of Chulalongkorn University fund (Ratchadaphiseksomphot Endowment fund).

In the academic year, she presented the poster presentation in the 18th Biological Sciences Graduate Congress (BSGC) at the University of Malaya, Kualar Lumpur, Malaysia from January 6th – 7th, 2014. Moreover, she published her work and presented the oral presentation in the proceedings of the 40th Congress on Science and Technology of Thailand (STT40) at the Hotel Pullman Khon Kaen Raja Orchid, Khon Kaen Province, Thailand from December 2nd – 4th, 2014 (Proceedings book, pp. 693 – 698).