THE EFFECTS OF ARTIFICIAL ILLUMINATION ON THE DISPLAY BEHAVIOUR OF THE FIREFLY Pteroptyx tener Olivier (COLEOPTERA: LAMPYRIDAE)

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by

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ABSTRAK

Kajian kesan pencahayaan buatan terhadap perlakuan peragaan kelip-kelip *Pteroptyx tener* Olivier telah dijalankan di Sungai Selangor, Selangor. Kaedah di lapangan menggunakan fotografi pada waktu malam, dan pendedahan kelip-kelip di tapak kajian kepada lampu *spotlight* 400W untuk jangka masa pendek dan panjang. Tapak kawalan tidak didedahkan kepada cahaya buatan. Kajian jangka masa pendek melibatkan pendedahan cahaya untuk selang masa 30 minit dan kajian kedua melibatkan tempoh gelap untuk selang masa 1 minit sebanyak 3 minit selepas pendedahan kepada cahaya buatan. Hasil bagi kedua-dua kajian mendapati pencahayaan jangka masa pendek memberi kesan signifikan ke atas perlakuan peragaan kelip-kelip, akan tetapi pemulihan dapat dilihat secara beransur-ansur. Pendedahan cahaya *spotlight* berterusan selama tiga malam (jangka panjang) menunjukkan perbezaan signifikan. Kajian pendedahan pencahayaan buatan untuk tempoh jangka masa panjang mencadangkan kelip-kelip mungkin telah berpindah daripada kawasan yang telah didedahkan kepada pencahayaan buatan. Kajian di lapangan menunjukkan pendedahan kepada cahaya buatan yang mengeluarkan cahaya yang berintensiti rendah (0.4-1.6 lux) mendatangkan kesan kepada populasi kelip-kelip yang berkumpul untuk memperagakan cahaya mereka di habitat asal. Di makmal, kesan pencahayaan buatan berlainan warna dikaji terhadap corak paparan cahaya dan perlakuan kelip-kelip. Cahaya berwarna biru, hijau, putih, kuning dan

merah serta ujian kawalan (tanpa cahaya) digunakan. Hasil kajian mendapati lampu pencahayaan buatan tidak memberi kesan ke atas perlakuam kelip-kelip (*non-courting, courting and dorsal mounting*) tetapi mendatangkan kesan negatif terhadap corak paparan cahaya mereka. Corak paparan cahaya kelip-kelip betina tidak terjejas dengan cahaya buatan berwarna merah dan kuning, manakala kelip-kelip jantan pula tidak terjejas dengan warna merah. Cahaya buatan berwarna putih (spektrum warna yang pelbagai dengan output maksimum di kawasan berwarna hijau) mendatangkan kesan paling tinggi terhadap corak paparan cahaya kelip-kelip, diikuti dengan cahaya buatan berwarna biru dan hijau.

THE EFFECTS OF ARTIFICIAL ILLUMINATION ON THE DISPLAY BEHAVIOUR OF THE FIREFLY Pteroptyx tener Olivier (COLEOPTERA: LAMPYRIDAE)

ABSTRACT

A study on effects of artificial illumination on the *Pteroptyx tener* Olivier was carried out at Selangor River, Selangor. Studies in the field involved the use of digital night photography, and short and long term exposure to light from a 400W spotlight shining towards the congregating fireflies at the study site. A control site had no artificial light exposure. Short term studies involved exposure of light for 30-minute intervals. The second short term study included short 1-minute intervals of darkness for a total of 3 minutes to allow recovery. Short term exposure to artificial lights had a significant short term effect on flashing behaviour, but recovery took place over time. Continuous illumination for three nights (long term exposure) resulted in a significant long term effect. The long term study suggests that the fireflies moved away from the illuminated area. This suggests that the congregating fireflies are affected even by low light intensities (0.4-1.6 lux) in their natural habitat. Laboratory studies were conducted to determine the effect of different light colours on the firefly's display behaviour. Tests were carried out using blue, green, white, yellow and red lights and compared against a control (no light). Results indicated that firefly sexual behaviour (non-courting, courting and mounting) were not affected by the different lights but flash patterns were negatively affected. Females were not affected by red and yellow lights while males were not affected by red light. White

light (multi-spectrum with maximum output at the green region) had the greatest effect on the firefly's flash patterns followed by blue light and green light.

CHAPTER ONE

GENERAL INTRODUCTION

1.0 Introduction

Fireflies (Coleoptera: Lampyridae) are unique insects, as all known lampyrid larvae produce a faint glow, but adult lampyrids differ greatly in the absence, presence, location, shape and use of adult light organs (Branham & Wenzel, 2003; Stanger-Hall *et al.*, 2007). Some lampyrids produce lights as adults, while others mainly use chemical signals for mate attraction (Lloyd 1997, in Stanger-Hall *et al.*, 2007; Branham & Wenzel, 2003; Stanger-Hall *et al.*, 2007).

In general, fireflies occur in a range of habitats, and some are associated with mangroves and mangrove estuaries (Nagelkerken *et al.*, 2008). Among them, the genus *Pteroptyx* Olivier can be found within the range from South and Southeast Asia to New Guinea (Ballantyne & McLean, 1970; Ballantyne, 1987). They are recognised for their marvelous group displays on certain trees, usually along riverbanks (Lloyd, 1973; Buck & Buck, 1976; Lloyd *et al.*, 1989; Nallakumar, 1999; Zaidi & Wong, 2004). Some of them, i.e. *Pteroptyx tener* and *Pteroptyx malaccae* flash synchronously (Buck & Buck, 1968; Case, 1980), thus enabling countries such as Thailand and Malaysia to be firefly ecotourism destinations (Nallakumar, 1999; Wong, 2009; Saphakun, 2009).

Malaysia has a few firefly ecotourism sites in different states, such as, Penang, Perak, Selangor, Negeri Sembilan/ Melaka, Pahang, Terengganu, Kelantan, Johor, Sabah and Sarawak. However, the most popular firefly watching destination is undeniably along the lower reaches of the Selangor River near Kuala Selangor (Kirton & Nada, 2010). A total of 473,761 tourists visited the Kampung Kuantan Firefly Park for the years 2011-2016, and this contributed nearly RM6 million to the state’s economy (Majlis Daerah Kuala Selangor, 2017).

An increase in artificial light penetration to the firefly habitat could alter their flashing patterns. Light pollution is defined by Rich & Longcore (2006) as redundant artificial light from man-made sources that will inevitably affect nocturnal organisms’ habits, such as reproduction and migration of insects, amphibians, fish, birds, bats, and even plants. Intrusive illumination attributes to additional noise in the signals, thus making mating signals (bioluminescent emissions) less efficient to potential mates (Lloyd, 2006).

1.1 Problem Statement

Concerns about dwindling firefly population in Malaysia have been reported by the media (Salina, 2004; Devid & Teoh, 2004; Arziana, 2009; Perumal, 2009; Premananthini *et al.*, 2015). The major threats to the firefly population include habitat loss, salinity intrusion due to extraction of river water for agriculture and human consumption as well as pollution (Nada & Kirton, 2004; Nada *et al.*, 2010). Pollution comes in various forms, and one of them could be light pollution. Therefore, artificial night illumination could also be one of the factors that cause a reduction in the firefly population.

Presently, there is lack of experimental data that documents the actual impact of light pollution on fireflies (Thancharoen *et al.*, 2008). Lloyd (2008) stated that artificial light sources may be attractive to congregating and site-changing fireflies, and would be fatal for the *Pteroptyx* fireflies. However, there was no evidence to support the statement, and this study attempts to do so by investigating the effects of increasing usage of artificial night illumination in firefly habitats, in particular lights from adjacent jetties and housing. This study is also a first attempt to determine the possible effects of different coloured lights on firefly flashing in the laboratory. The courting behaviour of this species had been documented in Case (1980), but there have been no studies on artificial light and its implication towards their flashing behaviour.

The results from this study would be a valuable source of information as there will be data to corroborate the importance of minimising illumination in firefly habitats. It could also serve as recommendation to district councils on the planning of streetlights and spotlights adjacent to firefly habitats.

1.2 Research Questions and Objectives

Research questions for this study are as follows:

1. Will the firefly flash behaviour be affected by artificial light?
2. Will the fireflies resume flashing after being exposed to different durations of artificial light, and if so, how long will it take for them to do so?
3. How are the firefly flash patterns and behaviour affected when exposed to different coloured light sources?

The objectives of this study are to:

1. Determine the effects of artificial night illumination on firefly presence and firefly flash behaviour in the field.

2. Determine whether different wavelength spectrums of lights could affect the courtship behaviour and display patterns between male and female fireflies in the laboratory.

CHAPTER TWO

LITERATURE REVIEW

2.0 Fireflies of the World

The order Coleoptera is the largest order of insects and also the largest order in the animal kingdom. Beetles comprise approximately 40% of all species of insects and 25% of all animal species (Romoser & Stoffalano, 1988). Fireflies, which are also beetles, belong to the family Lampyridae, which is well represented and has been estimated to have about 2000 species belonging to 83 genera (Branham, 2010). The beetles have an elongate, flattened soft-body with a luminous organ at the apex of the abdomen, with a range of size from 4-30 mm (Ballantyne & Lambkin, 2000). They are found in forests, open fields and marshes (Viviani & Santos, 2012) and some species are restricted to mangroves (Nagelkerken *et al.*, 2008).

Approximately 120 species of described North American lampyrids are presently classified into four or five subfamilies (Lloyd, 1997), with many more waiting to be described. In Brazil, Viviani & Santos (2012) documented results on a 20-year survey at Biological Station of Boracéia, Salesópolis, which categorised 30 species belonging to three subfamilies. This area is one of the last and largest contiguous areas of Atlantic rainforest in Eastern São Paulo state. In other countries, there were less documented species; 7 species in Portugal and Spain, 12 species in France, 19 species in Italy and 14 species in Greece (Geisthardt 2007, in Geisthardt *et al.*, 2008). In Japan, the flash communication systems of 45 species had been studied by Ohba (2004).

Fireflies play an important role in the ecosystem. Fu & Meyer-Rochow (2012) suggested that the aquatic larvae of the firefly *Aquatica leii* could potentially be a bio-control agent of freshwater snails, as they prefer to feed on *Lymnaea stagnalis*, an intermediate host of parasites that are harmful to both humans and animals. Another study by Fu & Meyer-Rochow (2013) also suggested the possibility of terrestrial firefly larvae, *Pyrocoelia pectoralis* as a biological control agent on the *Bradybaena ravida*, a common land snail which is an agricultural pest commonly found in China, Japan, Korea and Russia (Chen 2004, in Fu & Meyer-Rochow 2013 ; Wang 2008, in Fu & Meyer-Rochow 2013).

Beetles especially dung beetles from the family Scarabaeidae (Nichols *et al.*, 2007; McGeoch *et al.*, 2002) and ground beetles from the family Carabidae (Ribera *et al.*, 2001; Rainio & Niemelä, 2003; Martinez *et al.*, 2009) are useful bioindicators for habitat changes. However, bioluminescent beetles are not used as indicators, although these light producing insects could be convenient bioindicators at night (Viviani & Santos, 2012). They could be effective to measure the impacts of artificial light intrusion in their habitats.

2.1 Fireflies in Malaysia

In Malaysia, there is lack of knowledge on the exact number of firefly species in the country; with synchronous and non-synchronous species. A study conducted by Nallakumar (2002) found fireflies from four genera, i.e. *Pteroptyx*, *Luciola*, *Colophotia*, and *Lychnuris* with a total of eight species identified. A most recent documentation from Ballantyne (2010) stated Malaysia has 11 species of fireflies under the sub-family Luciolinae alone. Another study by Jusoh (2015) documented an annotated checklist of 58 species distributed among four subfamilies and 15 genera.

The genus *Pteroptyx* Olivier can be found within the range from South and Southeast Asia to Papua New Guinea (Ballantyne & McLean, 1970; Ballantyne, 1987). In 2011, a new species had been found in Hong Kong, known as *P. maipo.* (Ballantyne *et. al*, 2011). Three more new species were found in Malaysia, namely *P. galbina, P. gombakia* and *P. sayangia* (Ballantyne *et. al,* 2015). This genus has males with the elytral apex (tip of the hardened wing) deflexed (Ballantyne, 2001). In general, *Pteroptyx* females are quite similar to the males, in terms of colouration. They vary primarily in the slightly smaller eyes, flatter (or even slightly convex) frons, absence of metathoracic femoral comb, and the light organ which is only confined to the sixth abdominal sternite (Ballantyne & McLean, 1970) [Figure 2.1]. *Pteroptyx* are recognised for their marvelous group displays on certain trees, usually along riverbanks (Lloyd, 1973; Buck & Buck, 1976; Lloyd *et al.,* 1989; Nallakumar, 1999; Zaidi & Wong, 2004). Ballantyne (2010) documented a total of eight species of *Pteroptyx* in Malaysia, which are found in Peninsular Malaysia, Sabah and Sarawak (Table 2.1).

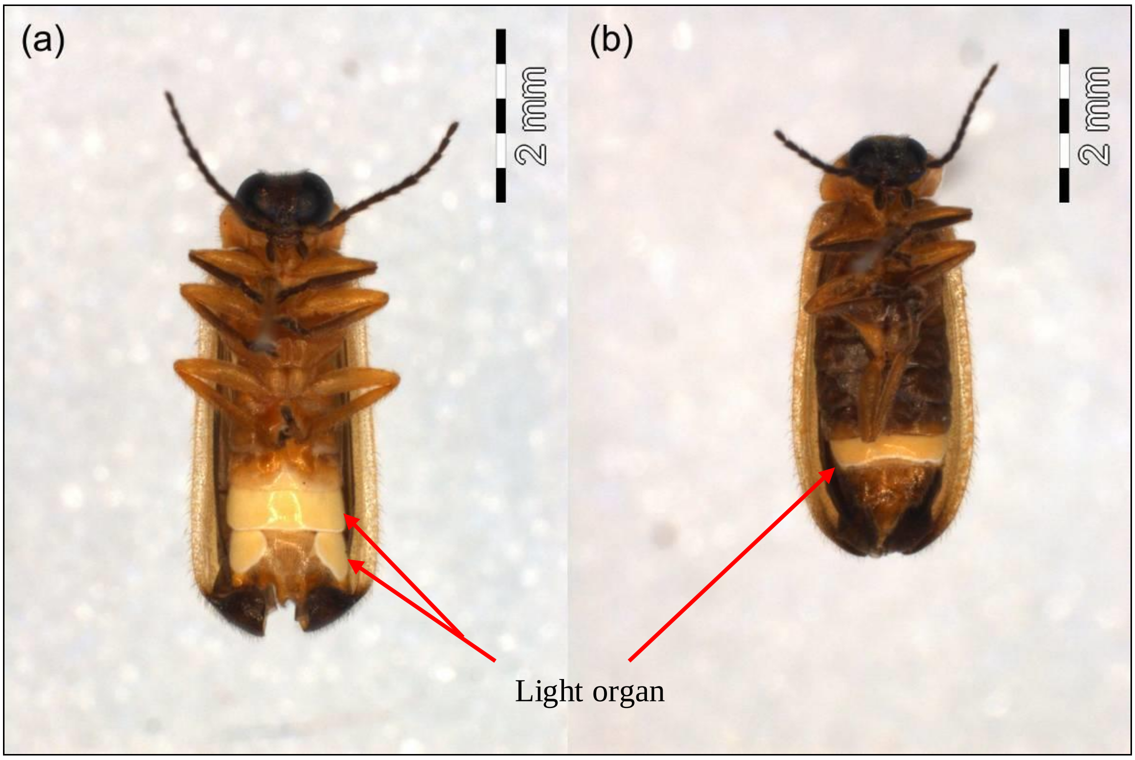

Figure 2.1. Ventral view of (a) male *Pteroptyx tener* and (b) female *Pteroptyx tener*. Males have light organs on the sixth and seventh abdominal sternite, while females only have light organ confined to the sixth abdominal sternite.

Table 2.1. List of *Pteroptyx* spp. found in Malaysia documented in Ballantyne (2010).

List of species	Peninsular Malaysia	Sabah	Sarawak
P. asymmetrica	/	-	-
P. bearni	/	-	/
P. decolor	-	-	/
P. gelasina	/	/	-
P. malaccae	/	-	/
P. similis	/	/	-
P. tener	/	/	/
P. valida	/	-	-

Note: (/) indicates the presence of the species and (-) indicates absence.

Based on Wong & Yeap (2012), six from the genera are confirmed to congregate on riverine vegetation (*P. bearni, P. gelasina, P. malaccae, P. similis, P. tener, P. valida*) while two are still unverified (*P. asymmetrica* and *P. decolor*).

2.2 *Pteroptyx* Fireflies, in Particular, *Pteroptyx tener* along the Selangor River

Pteroptyx tener, a synchronous flashing firefly that is 4.0-6.2 mm in body length (Ballantyne & McLean, 1970) is an iconic insect for the Selangor River, particularly in Kuala Selangor. This species is also found in mangroves in other states, based on compilations by Jusoh *et al.* (2013) namely, Johor (Benut, Kota Tinggi, Endau River), Negeri Sembilan (Kampung Sungai Timun, Rembau River), Perak (Sepetang River), Terengganu (Kerteh River, Chukai River), Sarawak (Kuching, Saratok) and in Sabah (Sandakan, Tawau, Kudat, Sipitang River).

This particular firefly has attracted thousands of visitors to its habitat as a result of its marvelous nightly displays (Perumal 1991, in Nada 2011; Ismail 1999, in Nada 2011). It is the dominant species along the Selangor River (Nallakumar, 2002), which inhabits the upper reaches of the river-estuary, an approximate ten-kilometer stretch (Malaysian Nature Society, 2002). Along this river, other species such as *P. malaccae* and *P. valida* can also be found, although in much lower numbers. *P. malaccae* were mostly found downstream (pers. obs., 2015). During the night, *P. tener* congregate in large groups on the mangrove trees, *Sonneratia caseolaris* (known locally as *berembang*) that can be found along the river, although not all trees have aggregations. Only the males display synchronous flashes (Case, 1980) beginning at dusk, whereby the flashes are brighter during early of the night and lasts till early hours of the morning, but with less flashing activities (Khoo & Kirton, 2012). They have been recorded to also display on other trees, although rare, such as *Durio zibethinus*, also known as durian (Ohba & Wong, 2004), *Mangiefera foetida* also known as horse mango (Nada & Kirton, 2004), bamboo from the sub-family *Bambuseae* (Nada, 2011) as well as *Nypa fruticans*, also known as nipah palms (Wong & Yeap, 2012).

This interesting species has a lifecycle that undergoes a complete metamorphosis, with a life span ranging from four to seven months. Almost 60% of its life is spent as a larva, feeding on the mangrove snail, *Cyclotopis carinata*. After several molts, the larva transforms into pupa, in which it lays inactive. The early stages of the firefly are spent behind the display trees, and once the pupa has developed into the adult stage, it will fly towards the trees lining the riverbank to continue the nightly flashing ritual, all for the purpose to attract potential mates (Nada & Kirton, 2004; Nada *et al.*, 2005).

2.3 Flash Communication in Fireflies

Flashing in fireflies is a means of communication and sexual attraction during the mating process (Carlson & Copeland, 1985). Flashing behaviour of the North American fireflies had been studied by various researchers, among them Lloyd (1966) who looked at the flash communication in the genus *Photinus*. He proposed the terminology for the flash patterns as follows (without indicating the intensity level): *Glow* – constant emission of light; *Flash pattern/phase* – unit of light emission of the male, which stimulates the female's *response flash* and is repeated at regular time intervals by the advertising males; *Pulse* – single emission of light (short duration).

Studies on the male *Photuris versicolor* documented that the central nervous system does not shape stimulated compound flashes, as it can be generated by individual lantern areas. The males can still produce a flash that is multi-peaked, which is the courtship flash for this species, although it had been decapitated (Carlson, 1981). Carlson & Copeland (1985) suggested that not all flashes are courtship flashes as some are used in predation, landing flashes (to light up the landing site) as well as an aid in finding suitable oviposition sites. Vencl *et al.* (1994) assessed the accuracy of the mimicry behaviour of the females of *Photuris versicolor* that preys on the males of the *Photinus* sp. Results from their study indicated that females seem to show considerable accuracy in their responses. Unmated *P. versicolor* females prefer to respond to conspecific patterns with variable latencies (brief intervals of non flashing), averaging one second. Females that have mated responded to heterospecific patterns more frequently.

Studies on their flash communication by Trimmer *et al.* (2001) revealed that light production by fireflies can be stimulated by nitric oxide (NO) gas in the presence of oxygen. This finding is a new discovery that aided in the understanding of light production in fireflies. Cratsley (2004) showed the female *Photinus* firefly prefer to respond to males that emit flashes with greater intensity and duration (in *Photinus* firefly species with single pulse flashes), and the males provide nuptial gifts (spiral, gelatinous spermatophore) to the females during mating. Synchronous fireflies were found to occur in North America, in which the species *Photuris frontalis* (the only species to synchronise in the genus) are intermittent synchronizers and do not congregate (Copeland & Moiseff, 2004). The evolution of North American fireflies' mating signals was observed using phylogenetic approach (Stanger-Hall *et al.*, 2007). Results from their study, which involved 26 species from 16 North American genera and one species from *Pterotus* that was removed from Lampyridae, showed that the use of light flashes as sexual signals evolved either two or three times.

For South East Asian fireflies, very few studies on flash communications have been carried out in the last 10 years (Lloyd, 1973; Case, 1980; Lloyd *et al.*, 1989; Ohba & Sim, 1994; Ohba & Wong, 2004). Lloyd (1973) described 22 flash mating signals in the genera *Luciola* and *Pteroptyx*. He postulated the reason of mass synchronization by the males and gave two possibilities: (1) By flashing in synchrony with adjacent males, there will be no disruption to the rhythm which is important as it conveys species information for female recognition, (2) Males that flash synchronously with neighbouring males may have the chance to interlope. A flying male that moves into the area will change his phase to a greater degree, and may have a better opportunity to mate with females which are in a progressing male-female dialogue.

Courtship behaviour of *Pteroptyx tener* had been described by Case (1980). His description is used in this study and had been detailed further in the methodology chapter. Studies by Lloyd *et al.* (1989) in Bangkok documented that *P. malaccae* flash in synchrony and remain at the same flashing site for long periods while *P. valida*, a non synchronous firefly moved about, flying from perch to perch. The flashing patterns for *P. malaccae* are rhythmically repeated at approximately half second intervals. Ohba & Sim (1994) carried out studies on *P. valida* in Singapore, in which the male's flash intervals gradually becomes shorter as the night progresses. The males were also seen to give off long flashes to the females which flew up from the undergrowth. Ohba & Wong (2004) conducted studies on *P. tener* in Kampung Kuantan and results from their study showed the males have an average flash interval of 0.28 sec.

Researchers have described the flash communication systems (Lloyd, 1983; Ohba, 1983; Branham & Wenzel, 2001; Lewis 2010, in Viviani & Santos 2012) and divided them into four: (System 1) Inactive females release pheromones to attract non-flashing flying males; (System 2; formerly System 1) Inactive females produce continuous glows sometimes with pheromones to attract non-flashing flying males; (System 3; formerly System 2) Males emit a signal to which inactive females respond and (System 4) are found in synchronous fireflies such as *Pteroptyx* in which males congregate in leks and emit synchronous flashes to attract flying females.

Some adult fireflies produce lights and congregate in large numbers (*P. tener, P. malaccae, P. bearni*) while others are solitary fliers (e.g. *Lamprigera* sp.). Those which congregate in large numbers produce flashes that are either synchronised or non-synchronised. In Malaysia, fireflies that flash synchronously have been reported to be found primarily on mangrove trees along brackish rivers, particularly mangrove-nipah swamps (Ohba & Wong, 2004). The congregating synchronous fireflies have been reported in tropical regions from Philippines and Papua New Guinea through Malaysia, Indonesia and Thailand, to Ceylon and Eastern India (Buck & Buck, 1978) and also in Japan (Ohba, 1984). Besides tropical regions, synchrony occurs occasionally in a few North American firefly species, *Photinus* *carolinus* (non-congregational ones) (Copeland & Moiseff, 1995; Moiseff & Copeland, 2000), as well as a new *Luciola* species in Europe (De Cock *et al.*, 2012). According to Buck (1988), there are different types of synchronies: Continuous or discontinuous synchrony as well as unison or wave synchrony. Fireflies with continuous synchrony have species-specific flashes that occur at a set interflash interval, without a gap in their flashing. For discontinuous synchrony, there would be alternate periods of non-flashing within the shorter periods of group synchrony. Hence, a synchronous group of fireflies will begin and end their flashing, with a pause occurring in the flashing, and then the beginning and the end of the synchrony happens again. A few of the Southeast Asian synchronised species (*Pteroptyx malaccae*, *P. tener*, and *P. cribellata*) exhibits continuous synchrony. The term unison synchrony implies that the whole firefly congregation flashes rhythmically all at once, while wave synchrony involves the spread of the flashes occurring at different times (metachronous), across the entire firefly congregation and its rhythmic cycle.

2.4 Flashing Behaviour of the *Pteroptyx tener*

Studies on flashing and courtship behaviour of the fireflies in Malaysia have been focused on *P. tener* (Case, 1980). In Case's study, he documented the frequency of the flashes of the males was about 3.7 flashes/sec, with only the males flashing synchronously. The males will usually perch at the tip of the leaf whilst flexing its abdomen so that its light would not be directed to the leaf surface but to showcase to nearby females as well as to compete with nearby males. Courtship begins when a synchronising male approaches a female (may or may not be flashing). The male firefly would climb onto the females back with both heads in the same direction. The male will hold its position using the first two pairs of legs, while twisting its light organ and held just over the female's head, and either continue its flashing or begin to flash. Courtship could last as long as half an hour in the dark room, and it could end up in copulation or separation of the pair. During the courtship process, the female may or may not flash and could be stationary or walking about. If copulation is successful, the pair would rotate to a tail-to-tail position and would remain in copulation for at least several hours. Flashing would quickly subside and the pair would seek a secluded area and resume their copulation.

The luminescence colour for both male and female is yellowish-green (Ohba & Wong, 2004) with a maximum luminescence at the wavelength of 575 nm (Isobe *et al.*, 1998). According to Ohba & Wong (2004), the flash patterns of synchronous flashing in the field comprises of a main peak and 1-3 small peaks continuously. Under the same conditions, the flash intervals between the main peaks and the small peak as well as between first and second small peak are constant between 0.11-0.12 seconds. However, the authors also noted that flash patterns of the 20 males placed in transparent plastic bags under laboratory conditions were not in synchrony.

2.5 Effects of Light on the Firefly Population

In recent times, light pollution had been highlighted by firefly researchers (Ohba & Wong, 2004; Lloyd, 2006; Thancharoen *et al.*, 2008, Ineichen & Rüttimann, 2012; Wong & Yeap, 2012). However, little research has been carried out to obtain concrete data on the effects of artificial light on the fireflies as light pollution remains a secondary threat with regards to habitat loss. Thancharoen *et al.* (2009) conducted an observation study on the courtship and mating behaviour of *Luciola aquatilis* when exposed to different light intensities of the fluorescent light in the laboratory, which resulted in prolonged courtship and mounting time. Thancharoen's study was focused on the duration of mating behaviour but did not discuss on the flash patterns. This study would look at the flashing patterns of *P. tener* when exposed to different coloured fluorescent lights. Findings from this study could form a basis to understand what coloured lights are least intrusive for the synchronous fireflies.

Field studies by Ineichen & Rüttimann (2012) in Switzerland showed that artificial night light causes hindrance for male *Lampyris noctiluca* in locating the females, but had no apparent influence on the spatial distribution of the glowing flightless females. Preliminary studies conducted in the field by Yiu (2012) on *Pteroptyx maipo* in Hong Kong showed that the number of flashes decreased when there is an increase in the intensity of illumination. Although the study sample was small (n=2), it was still evident that there was effect on the firefly's flashing behaviour. Bird & Parker (2014) conducted a study in Great Britain showed at low levels of light pollution, male *L. noctiluca* also failed to locate the females. Hagen *et al.* (2015) also conducted a study in Brazil that documented a reduction in firefly occurrence with the presence of artificial lighting. However, there is still a lack of experimental data that could document the actual impact of light pollution on fireflies (Thancharoen *et al.*, 2008).

With the presence of artificial light, flash communication may be disrupted as they are very dependent on the surrounding ambient light as an indication to begin their flashing (Thancharoen *et al.*, 2008). Therefore, if the artificial light source were to illuminate throughout the night, the fireflies would not begin their flashing, and the courtship and mating process would be delayed, and a negative impact on the firefly population ensues. Apart from that, artificial light sources are attractive to insects (moth, beetles), and this include the fireflies. The congregating and site-changing fireflies could interpret the artificial lights as the glow of combined emissions of fireflies in another "suitable" habitat. The *Pteroptyx* fireflies which have congregating behaviour would be the potential victim to the artificial illumination sources (Lloyd, 2008). This study which involved the usage of a spotlight as an illumination source in the field had attracted fireflies from nearby display trees to the spotlight (pers. observ. 2011, 2012). This observation has supported the statement suggested by Lloyd (2008). However, no studies have been conducted on the actual impact on the fireflies when they move away from the display trees and fly towards the artificial light sources. By changing sites, the fireflies may have wasted their energy to move their sites and this could be costly to their population.

Along the Selangor River, the major concern is artificial light sources from nearby jetty complex as well as spotlights and floodlights at the sluice gates, all which are in the immediate vicinity of the firefly display sites. At present, data for effects of artificial light on *P. tener* is still dearth. In Kampung Kuantan, Kuala Selangor, local villagers and boatmen informed media that visitors were using flash and torchlights during the boat excursion, thus causing the fireflies to shift their aggregations (Perumal 1996; 1997, in Nallakumar 2002). Results from this study could narrow the gaps of understanding the importance of minimising artificial light in the firefly habitat.

In Malaysia, there are a few places which carry out firefly watching tours along the river (i.e. Sungai Sepetang and Sungai Kerian in Perak, Sungai Chukai and Sungai Setiu in Terengganu, Cherating in Pahang) that most likely would have some problems with artificial light sources from nearby villages and townships. Sungai Sepetang is also facing another problem as the adjacent areas have many aquaculture ponds which use spotlights during the night. The light source from the spotlights, floodlights, streetlights, as well as households usually comes in colours of yellow and white. Thus it would be critical to carry out studies on the effects of different coloured light sources on the flashing behaviour of the fireflies.

2.6 Other Threats to the Firefly Population

Threats for fireflies come in various forms, with the major ones such as habitat loss, salinity intrusion due to river water extraction for agriculture (oil palm and banana) and human consumption as well as pollution (Nada & Kirton, 2004; Nada *et al.*, 2010). Conservation efforts on the firefly population were mainly conducted along the Selangor River.

Nada *et al.* (2010) and Khoo *et al.* (2009) reported land clearing activities for oil palm and banana plantations in Kuala Selangor, in place of natural sago (*Metroxylon sagu*) stands used by the firefly larva and its prey as their habitats. Previous studies conducted by Kirton *et al.*, (2006) mentioned the importance of riverine vegetation to ensure the survival of the firefly population. In areas where natural sago (*Metroxylon sagu*) stands occur, high densities of firefly larvae and snails were found, implicating the area as a suitable firefly breeding habitat. With the detection of land clearing, efforts were undertaken by the state government to gazette sections of the Selangor River in the District of Pasangan, Kuala Selangor as a Protection Zone under Section 48 of the Selangor Waters Management Authority Enactment 1999 (Khoo *et al.*, 2012).

Salinity intrusion would be another threat to the congregating firefly population along the estuary. Salinity intrusion occurs when there is less flow of freshwater from upstream that can be caused by the construction of dams (in the case of Selangor River) further upstream. The display trees, particularly in Kuala Selangor, consists majority of the mangrove tree species *Sonneratia caseolaris* which is less tolerant to high salinity levels thus resulting in stunted growth of the plant in height, leaf area and leaf length (MNS, 2002; Liao & Chen, 2007). During dry periods, there is a possibility of salinity intrusion exceeding the tolerance levels of the display trees. Hence, the fireflies would be without display and mating sites, and this in turn could negatively affect the sustainability of the firefly population.

Other firefly habitats are also not spared by threats, with the major threat being habitat degradation. In Sungai Chukai (Terengganu), Sungai Linggi (Port Dickson), and Sungai Kerian (Penang), river realignments were conducted for flood mitigation purposes. In Sungai Sepetang (Perak), the fireflies are affected by clearing of mangroves, aquaculture development and pollution. Unfortunately, there is a lack of data to corroborate its effects on the firefly population. Boat operators and visitors attest to the dwindling numbers of the firefly population in the areas (Premananthini *et al.*, 2015).

2.7 Firefly Research in Malaysia

Firefly research in Malaysia is still at its infancy stage. Previous researches carried out were mainly on fireflies in Kuala Selangor, focusing on the dominant species. The initial research consists of taxonomic work mainly conducted by Dr Lesley Ballantyne of Charles Sturt University, Australia, ecology (i.e. MNS, 2002; Nallakumar, 2002; Ohba & Wong, 2004; Kirton *et al.*, 2006) and some behavioral studies (i.e. Case, 1980; Ohba & Wong, 2004). For the latter, the focus has been mainly on the flashing, courtship and mating behaviour.

For local researchers and non-governmental organisations, the Malaysian Nature Society (MNS) was involved in a multi-component study to determine the impact of the construction of the Sungai Selangor Dam on the fireflies (MNS, 2002). MNS was also actively involved in surveys on the congregating fireflies around Peninsula Malaysia, and produced a map on the congregating firefly zones, known as CFZ (Wong & Yeap, 2012).

The Forest Research Institute Malaysia (FRIM) has been involved in firefly research for almost two decades in Kuala Selangor. FRIM researchers conducted studies on the biology and ecology of *P. tener* and presented a management plan for sustainable firefly-based ecotourism in Kuala Selangor (Nallakumar, 2002). Captive breeding studies were also carried out to further understand the developmental stages of the fireflies from eggs to adults (Nada *et al.,* 2005). Habitat requirement studies for the firefly larvae and snails were also carried out by Kirton *et al.* (2006) in which the snails and larvae were abundant in sago stands but scarce in oil palm plantations and village orchards. Another study which FRIM embarked on was to monitor the firefly population along the Selangor River using a non-destructive method, since 2006 (Kirton *et al.,* 2012). The findings from the firefly monitoring project have shown that there was a decrease of 38% on the firefly population throughout seven years of monitoring (Khoo *et al.,* 2016). Biological and ecological studies has shown that habitat loss is a major factor that causes a decline in the firefly population, although the topic is not widely studied. However, there is a need to also observe other factors that could have an impact on the firefly population, and among them would be the increase of artificial light sources in firefly habitats.

Other studies conducted by different researchers (i.e. Chey, 2004; Mahadimenakbar *et al.,* 2007; Abdul Latip *et al.,* 2009; Jusoh *et al.,* 2010) also on congregating fireflies, focused more towards the distribution and abundance. There is also not much research carried out on solitary or roving fireflies found in the forests of Malaysia. Malaysia still has a long way to go in firefly research, with many more interesting discoveries to be unearthed.

CHAPTER THREE

EFFECTS OF ARTIFICIAL LIGHT ILLUMINATION ON *PTEROPTYX TENER* IN THE FIELD

3.0 Introduction

Light pollution was hardly recognised as an important form of pollution previously. However, in this era, more are becoming aware of some of the potential effects of light pollution on living organisms (Thancharoen *et al.,* 2008). For firefly adults that produce light, the bioluminescent flashes play a vital role in their courtship behaviour (Lloyd, 1983; Branham & Greenfield, 1996; Underwood *et al.,* 1997) and, in some species, although not found in Malaysia, aggressive mimicry (Lloyd, 1975).

Along the Selangor River, Kuala Selangor, the dominant species is *Pteroptyx tener* Olivier (Nallakumar, 2002) and they congregate in large numbers, most often on the mangrove plant *Sonneratia caseolaris* (L.) Engl. (Nagelkerken *et al.,* 2008; Nada *et al.,* 2010). This species has been widely studied in the biological and ecological aspects (e.g. Motuyang, 1994; Rahmat; 1996; Nallakumar, 2002; Nada & Kirton, 2004). Many studies have also been conducted on the mechanisms and behaviour of the synchronous fireflies (e.g. Buck & Buck, 1968; Lloyd, 1973; Case, 1980; Ohba & Sim, 1994; Moiseff & Copeland, 1995; Copeland & Moiseff, 1997) but none on their behaviour towards artificial lightning.

The greatest concern at the study site are the effects of artificial light from sources in the immediate vicinity of the firefly display sites such as spotlights at the sluice gates and fluorescent lights at the jetty complex. Therefore, this study attempts to ascertain the effects of artificial light illumination on the fireflies aggregating on trees lining the riverbank.

3.1 Methodology

3.1.1 Selangor River Basin

The aggregating fireflies are located near the mouth of the Selangor River (Figure 3.1). They inhabit the upper reaches of the river estuary, about 12 km from the river mouth, along approximately 10 km stretch of the Selangor River (MNS, 2002). The Selangor River Basin is the third largest among the seven river basins in the state of Selangor, after the Bernam River Basin and Langat River Basin. This river basin flows through three main districts, namely, Kuala Selangor, Gombak and Hulu Selangor, as well as six major townships, which are Kuala Kubu Baru, Rawang, Serendah, Rasa, Bestari Jaya (Batang Berjuntai) and Kuala Selangor (Selangor Waters Management Authority, SWMA, 2010).

Selangor River originates from the foothills of Fraser's Hill and traverses the northeast regions of Selangor for approximately 110 km until it reaches the coastal areas. At the upper catchment areas, the river is constrained by rocky bands and steep riverbeds, with substrates comprising of rocks with little accumulation of sediment and organic matter. Therefore, during raining periods, water levels increase and flow rapidly. In the lower catchment, water velocity drops with an increase in the river width. Unlike the upper region, sediments accumulate in the flood plains. (Department of Irrigation and Drainage, DID, 2007).